SHORT COMMUNICATION

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Evidence that the Arabidopsis Ubiquitin C-terminal Hydrolases 1 and 2 associate with the 26S proteasome and the TREX-2 complex

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The 26S proteasome interacts with a number of different proteins, while the TREX-2 complex is an important component of the mRNA export machinery. In animals and yeast, members of the Ubiquitin C-terminal Hydrolase 37 (UCH37) family are found to associate with the 26S proteasome, but this has not been demonstrated in plants. The Arabidopsis UCH1 and UCH2 are orthologous to UCH37. Here, we show that UCH1 and UCH2 interact with the 26S proteasome lid subunits. In addition, the two UCHs also interact with TREX-2 components. Our data suggest that Arabidopsis UCHs may serve as a link between the 26S proteasome lid complex and the TREX-2 complex.

The yeast Ubiquiting C-terminal Hydroslase 2 (Uch2; UCH37 in humans) is the major deubiquitinating enzyme associated with the 26S proteasome, which contributes to and regulates 26S proteasome activity.[1] Yeast Uch2 and its orthologs in animals are physically associated with the 19S regulatory particle (RP) of the 26S proteasome.[1,2] Whether it is actually a subunit of the 19S RP is an unresolved issue,[3,4] since there are no uniformly applied criteria for defining a protein as the subunit of a complex.[3] In Arabidopsis, three genes encoding UCHs, designated *UCH1* to *UCH3*, have been identified.[5] The C-terminus of human UCH37 and yeast Uch2 orthologs have been reported to interact with the RPN12 and RPN10 subunits of the 26S proteasome, respectively.[1,6] Since the Arabidopsis UCH1 and UCH2 have a similar C-terminal extension, Yang et al. (2007) attempted but failed to detect a direct association of UCH1/UCH2 with the 26S proteasome, either by analyzing isolated 26S proteasome components or by yeast two hybrid (Y2H) assays.[5]

The transcription-export complex 2 (TREX-2) is an important component of mRNA export machinery, which targets genes that are being transcribed[7-9] and facilitates the export of mature mRNAs. The TREX-2 complex was first identified and characterized in yeast.[9-13] We reported recently the identification of the Arabidopsis homolog of the yeast TREX-2 complex[14] through the characterization of *essp1*, a mutant isolated in a genetic screen conducted to identify Arabidopsis mutants exhibiting ectopic expression of seed storage proteins (referred to as *essp*).[14-16] *ESSP1* encodes a then uncharacterized PCI domain-containing protein. Since the PCI domain is involved in protein-protein interactions, we performed a Y2H screen to look for its interacting partners. Genes encoding several putative TREX-2 components and a truncated version of UCH1 were identified by this Y2H screen.[14] We found that the Arabidopsis TREX-2 complex contains AtTHP1(ESSP1), AtSAC3A/AtSAC3B and AtCEN1/AtCEN2. It is tethered to the inner side of the nuclear pore complex (NPC) via AtNUP1 (also named NUP136) which is a plant-specific nucleoporin.[14,17] Similar TREX-2 homologs have also been identified in fruit flies[18] and humans.[19]

Here, we present evidence demonstrating a physical link between Arabidopsis UCH1/UCH2 and the 26S proteasome lid complex, as well as to the TREX-2 complex. As noted above, during the Y2H library screening to identify the interacting protein partners of AtTHP1,[14] a truncated version of UCH1 ($UCH1_{123-334}$) was uncovered. This truncated UCH1 lacks part of the conserved catalytic domain (UCH-domain) (**Fig. 1A**). Since UCH1 and UCH2 are highly similar, we made a similar truncated version of UCH2, $UCH2_{123-330}$. We systematically tested the physical interactions of the full length UCH1 and UCH2, their truncated versions ($UCH1_{124-334}$ and $UCH2_{123-330}$), as well as their C-terminal extensions ($UCH1_{215-334}$ and $UCH2_{209-330}$), with various components of the TREX-2 (AtTHP1, AtSAC3A/B, AtCEN1/2, AtNUP1) and 26S proteasome (RPN3s, RPN10, RPN12s, AtDSS1s) using both directed Y2H analysis and bimolecular fluorescence complementation (BiFC) assays. Please note that many subunits of the 26S proteasome RP complex in Arabidopsis are encoded by more than one gene.[20]

In the Y2H assay, full length UCH1 and UCH2 interacted with each other as well as with AtDSS1(V) (**Fig. 1B**), a small protein that has been shown in our earlier work to be associated with both TREX-2 and the 26S proteasome, potentially serving

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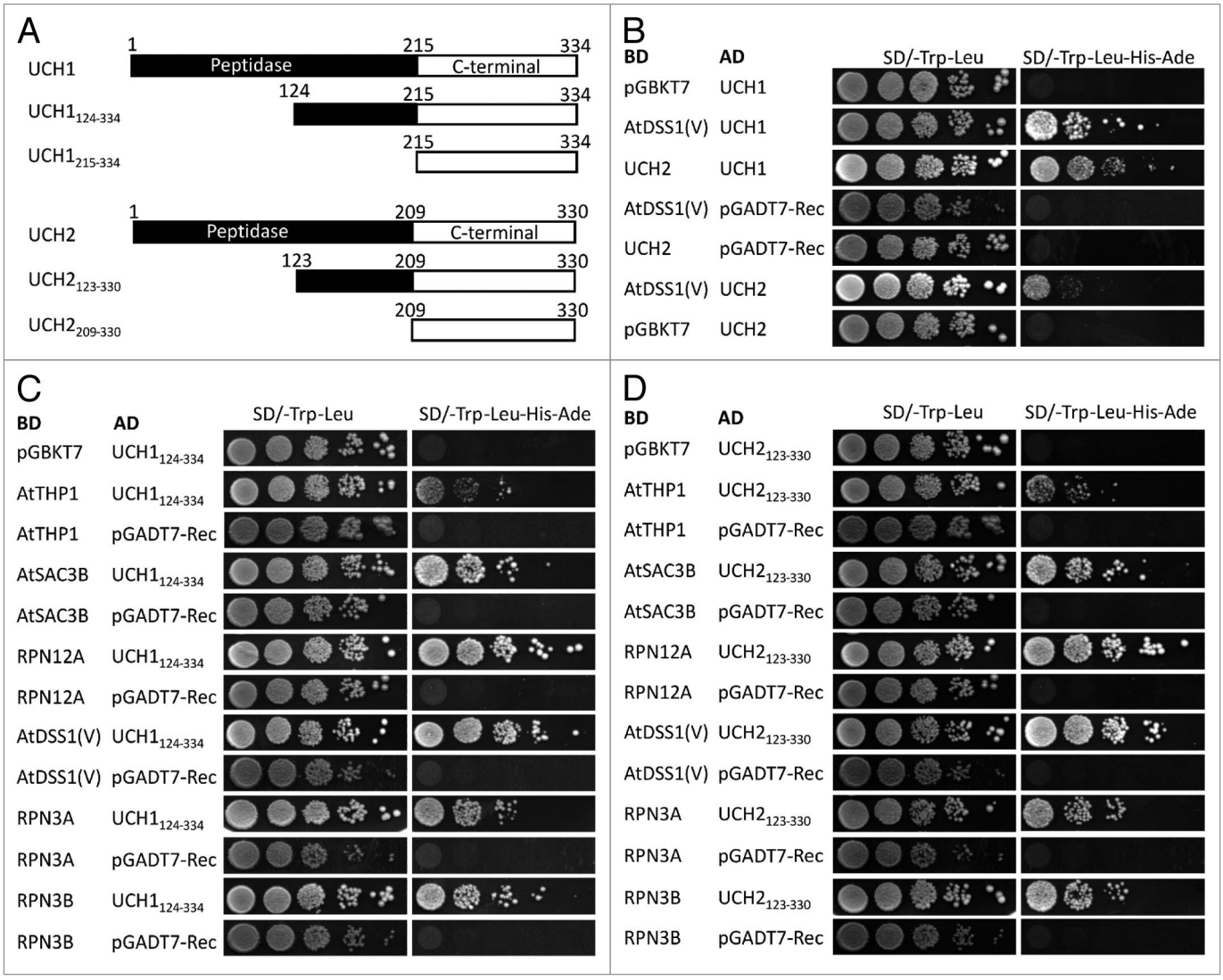

Figure 1. Y2H assay showing interactions between UCHs and components of the 26S proteasome or the TREX-2 complex. (**A**) Schematic representations of full length UCH1 and UCH2 and their truncated versions. The ubiquitin hydrolase activity domain and C-terminal extension are shown as black and white boxes, respectively. The numbers indicate amino acid positions. (**B–D**) Y2H assay between (**B**) full length UCH1/UCH2, (**C**) $UCH1_{124-334}$ (**D**) $UCH2_{123-330}$ and their interacting partners. Yeast strains were either plated on SD -Trp/-Leu or SD -Trp/-Leu/-His/-Ade dropout medium to determine the ability to grow and to identify positive interaction, respectively. Series of 10-fold dilutions of the co-transformed yeast cultures were spotted and incubated at 30°C for 2–4 d. **BD** and **AD**, proteins fused with GAL4 DNA binding and activation domains, respectively. *pGBKT7* and *pGADT7-Rec* are vectors that only express the **BD** and **AD** domains, respectively, and are used as negative controls. Only data for positive interaction are shown, together with their controls.

as a physical link between the two complexes.[14] Interestingly, the truncated versions, $UCH1_{124-334}$ and $UCH2_{123-330}$, interacted with several additional components of the 26S proteasome lid complex, RPN3s, RPN12s and AtDSS1(V) (**Fig. 1C**). UCH1 and UCH2 interactions were also observed with the TREX-2 components AtTHP1, AtSAC3B and AtCEN1 (**Fig. 1C**). The C-terminal extensions ($UCH1_{215-334}$ and $UCH2_{209-330}$) alone did not interact with any of the proteins (data not shown).

In the BiFC assay, full length UCH1 and UCH2 interacted with TREX-2 components, displaying an YFP signal in the nucleus and cytoplasm with AtTHP1 and AtCEN1 (**Fig. 2A and C**), in the nucleus with AtSAC3B (**Fig. 2B**) and along the nuclear periphery with AtNUP1 (**Fig. 2D**). UCH1 also interacted with the 26S proteasome components RPN12s, AtDSS1s and RPN3B (**Fig. 2E–I**), producing YFP signals that were localized in the nucleus and cytoplasm. The observed BiFC YFP signal patterns are consistent with the localization pattern of the proteins tested. For example, the 26S proteasome is localized in both the cytoplasm and nucleoplasm, AtSAC3B in the nucleus and AtNUP1 along the nuclear periphery.[14] UCH2 showed similar interaction networks as those of UCH1 (data not shown).

It is generally accepted that Uch2 in yeast and its orthologs in animals are components of the 19S RP of the 26S proteasome.[3,4] Thus it is expected that the same physical association exists in Arabidopsis, but the experimental evidence to support this has been lacking.[5] Our interaction data presented here provide strong

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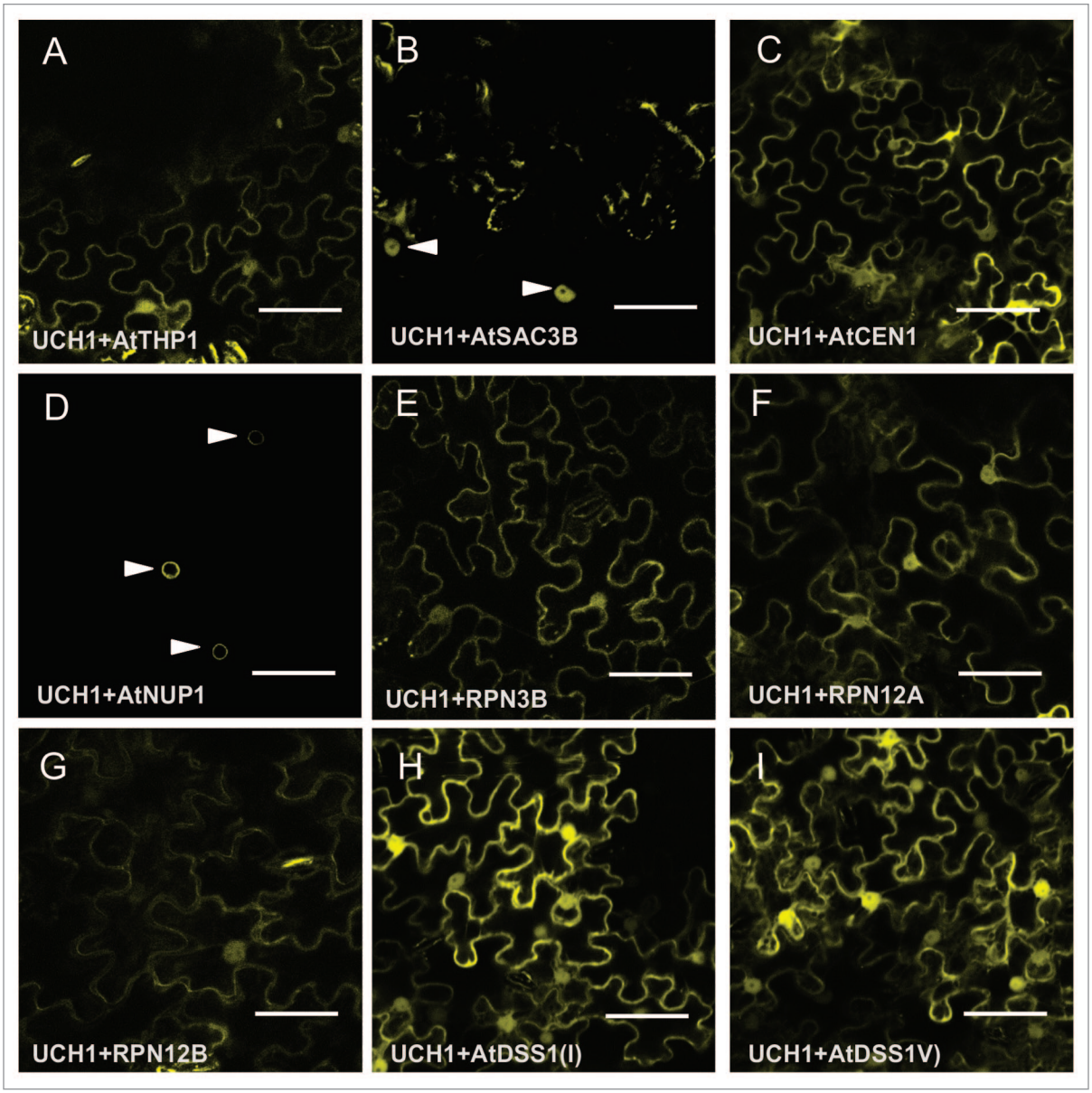

Figure 2. BiFC assay showing interaction between the full length UCH1 and its interacting partners in planta. *N. benthamiana* leaves were co-transformed with constructs expressing proteins fused to the N- and C-terminus of YFP, respectively. Images were recorded 48–72 h after infiltration using a Leica TCS SP2 confocal microscope as described previously.[14,24] Images are shown as confocal YFP images. (**A–D**) In planta interactions with TREX2 components. (**E–F**) In planta interactions with components of the 26S proteasome. The arrows indicate the YFP signal in nucleus (**B**) and along nuclear periphery (**D**). Scale bar = 40 μm. All negative controls (YFP-fused components with vectors expression half YFPs only) did not show an YFP signal and therefore are not shown.

new evidence that link UCH1/UCH2 to the 26S proteasome. First, we demonstrated that the full length UCH1/UCH2 interact with each other and both interact with AtDSS1(V). DSS1 is physically associated with the 26S proteasome lid complex subunits.[14,21-23] These observations suggest that UCH1/UCH2 are associated with the 26S proteasome, likely via AtDSS1(V). AtDSS1(V) is associated with more than one distinct complex. However, given the documented data so far in yeast and animal cells, the likelihood for UCH1/UCH2 to be associated with complexes other than the 26S proteasome lid is rather slim. Second, more direct evidence comes from the observation that a truncated version of UCH1/UCH2, which contains part of the N-terminal portion plus the C-terminal extension (the same fragment identified in the Y2H screening), interacts with 26S proteasome subunits RPN3s, RPN12s and AtDSS1s. Furthermore, interactions of UCH1/UCH2 with AtTHP1 and AtSAC3B were observed, suggesting a possible physical association network involving the TREX-2, NPC and 26S proteasome (**Fig. 3**). In addition, we observed that UCH1 and UCH2 interact with each other, likely forming heterodimers in vivo, but we did not observe self-interactions (homodimerization). The functional significance of this interaction remains to be studied.

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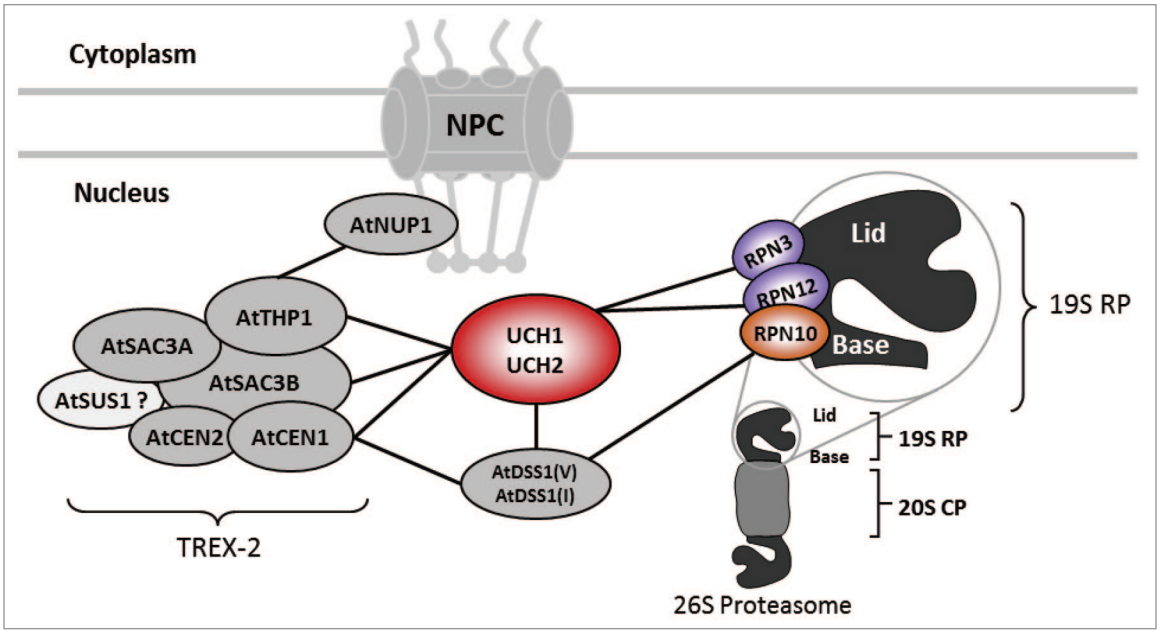

Figure 3. Schematic representation of the association of Arabidopsis TREX-2 complex and 26S proteasome mediated by UCH1 and UCH2.At the top is a schematic representation of a NPC embedded in the nuclear membrane and is shown together with cytoplasmic filaments and nuclear basket. The interaction between AtTHP1 and AtNUP1 links the Arabidopsis TREX-2 complex to the nuclear basket. UCH1 and UCH2 interact with TREX-2 components (AtTHP1, AtSAC3B, AtCEN1 and AtDSS1s) and 26S proteasome lid complex components (RPN3s, RPN12 and AtDSS1s), thus act as a linker between the TREX-2 complex and 26S proteasome. RP, regulatory particle; Lines, protein-protein interactions determined in this study.

We did not find any interaction between the full length UCH1/UCH2 and RPNs, nor their C-terminal extension alone in Y2H, which is consistent with Yang et al.[5] It is interesting that the Arabidopsis UCH1/UCH2 C-terminal extensions alone are not sufficient for interacting with 26S proteasome subunits, while the yeast and animal counterparts do interact. Apparently additional amino acid residues from the ubiquitin hydrolase activity domain are required. Future structural investigation is needed to determine the detailed roles of various regions of UCH1/UCH2 in mediating the association with the 26S proteasome. Such structural studies will also likely reveal the differences between Arabidopsis UCHs and their animal counterparts. Future study is also needed to unveil the puzzle of why the full length UCHs cannot interact with the 26S proteasome in Y2H. One possibility is that this might serve as a regulatory mechanism in vivo to allow the UCHs to bind the 26S proteasome only when the N-terminal region is occupied/covered by a signaling molecule specifically found only in certain cellular environments. In conclusion, our data strongly suggest that Arabidopsis UCH1/UCH2 interact with each other and are physically associated with the 26S proteasome and with the TREX-2 complex.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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