

Supporting Information

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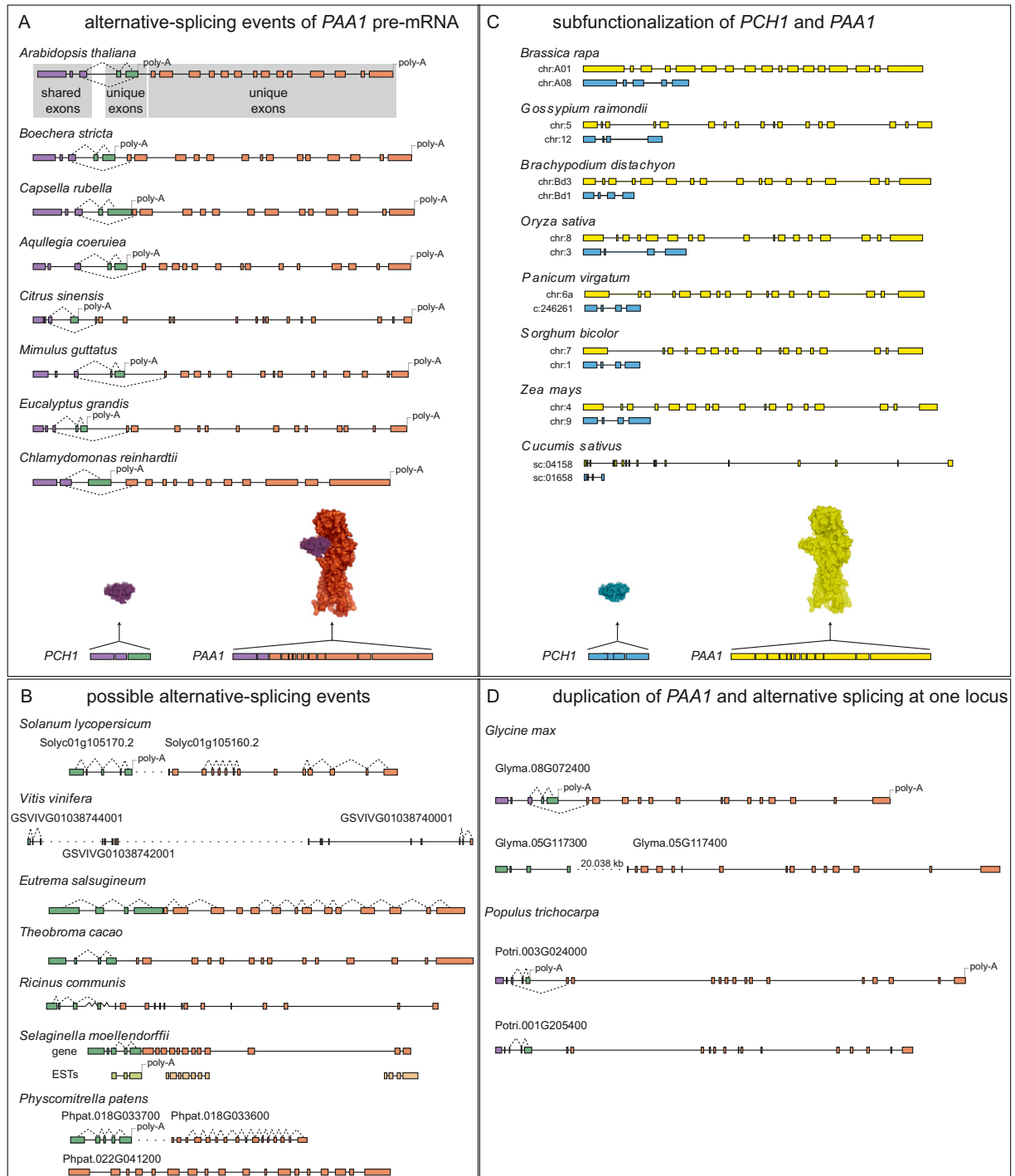


Fig. S1. Schematics of alternative-splicing events and gene duplications. (A) Gene models presented in Fig. 1A (Upper) and predicted structural models of *PCH1* and *PAA1* (Lower). The N-terminal HMBD of *PAA1* is colored purple to highlight identity with *PCH1*, also colored purple. (B) Gene models not presented in the main text, where alternative-splicing events producing both *PCH1* and *PAA1* are likely (because EST support for *PCH1* is available). (C) Gene models of *PAA1* and *PCH1* presented in Fig. 1B (Upper) and predicted structural models of *PCH1* and *PAA1* (Lower). (D) Gene models from *G. max* and *P. trichocarpa*, where the presence of two genes with alternative splicing is unclear. chr, chromosome; sc, scaffold.

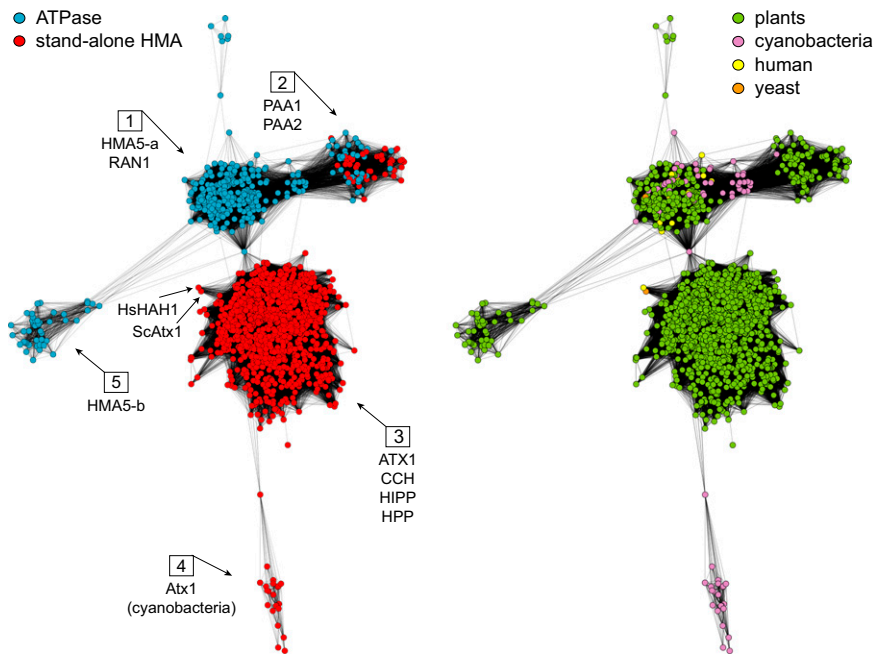


Fig. S2. PAA1/PAA2 protein cluster uniquely contains soluble proteins. The protein similarity network of the heavy metal associated (HMA) domains (CDD cI00207) in Cu^+ -ATPases and Cu^+ chaperones from plants, cyanobacteria, *Saccharomyces cerevisiae* (ScATX1), and *Homo sapiens* (HsHAH1) is shown. Nodes that represent the HMA domain from the Cu^+ -ATPases are colored blue, whereas the nodes that represent the HMA domain from identified and predicted chaperones are colored red. Proteins that are more similar to one another than to other proteins in the network will cluster, allowing the delineation of protein subgroups based on amino acid similarity. Each distinct cluster is given a number designation, and representative *A. thaliana* proteins from each cluster are shown. AtHMA5 contains two HMA domains, one in cluster 2 and the other in cluster 3. The same network, but colored by taxonomic group, is provided to the right. Atx1, antioxidant 1; CCH, Copper chaperone; HIPP, heavy metal associated isoprenylated plant proteins; HPP, heavy metal associated plant proteins; RAN1, responsive-to-antagonist 1.

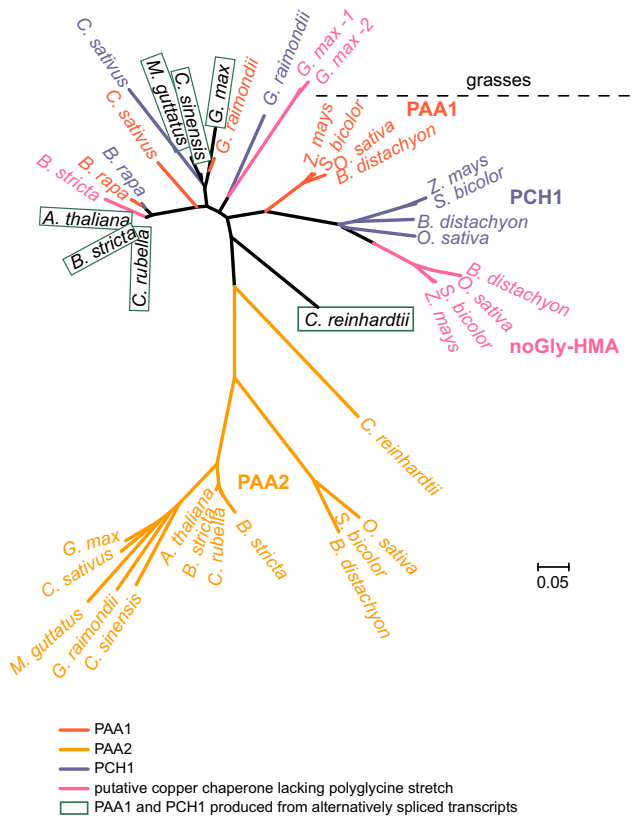


Fig. S3. Phylogenetic reconstruction (neighbor-joining tree) of the HMA domain from selected Cu^+ -ATPases (PAA1 in orange and PAA2 in yellow), PCH1 (lilac), and no-glycine stretch protein (pink) from the PAA1/PAA2 cluster identified in Fig. S2. The clade that represents grass-specific sequences is highlighted with a dashed line.

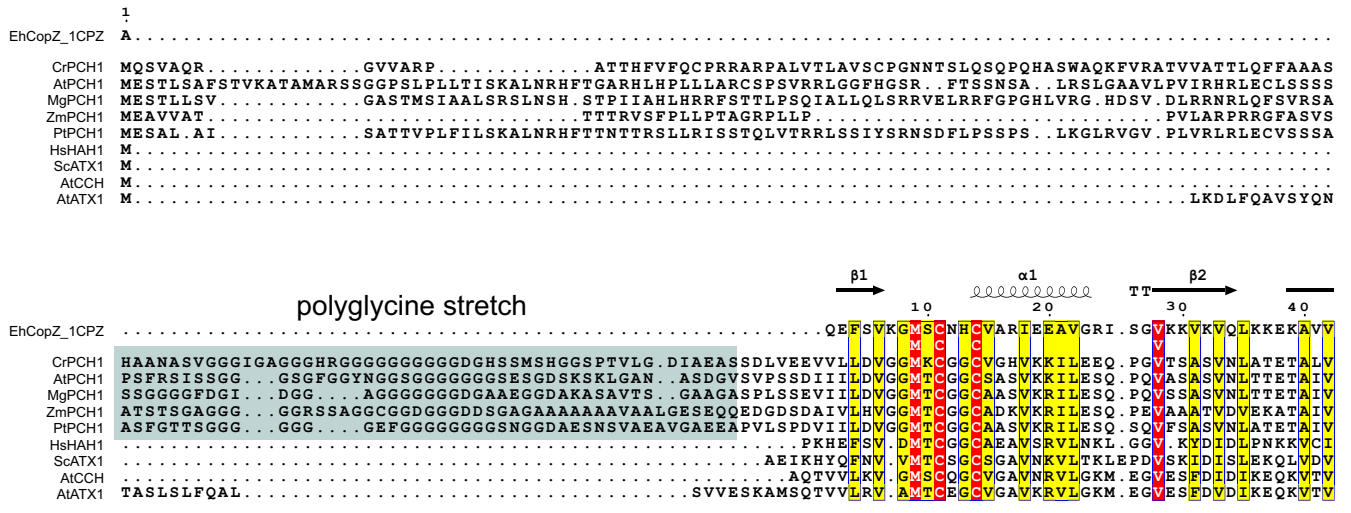


Fig. 54. Amino acid alignment of plant PCH1 sequences with the sequences of known Atx1-like chaperones. A cartoon representation of the aligned CopA (*Enterococcus hirae* Protein Data Bank ID code 1CPZ) secondary structure is shown above. The polyglycine stretch found near the N terminus of PCH1 is highlighted with a gray-blue box. At, *A. thaliana*; Cr, *C. reinhardtii*; Hs, *H. sapiens*; Mg, *M. guttatus*; Pt, *P. trichocarpa*; Sc, *S. cerevisiae*; Zm, *Z. mays*.

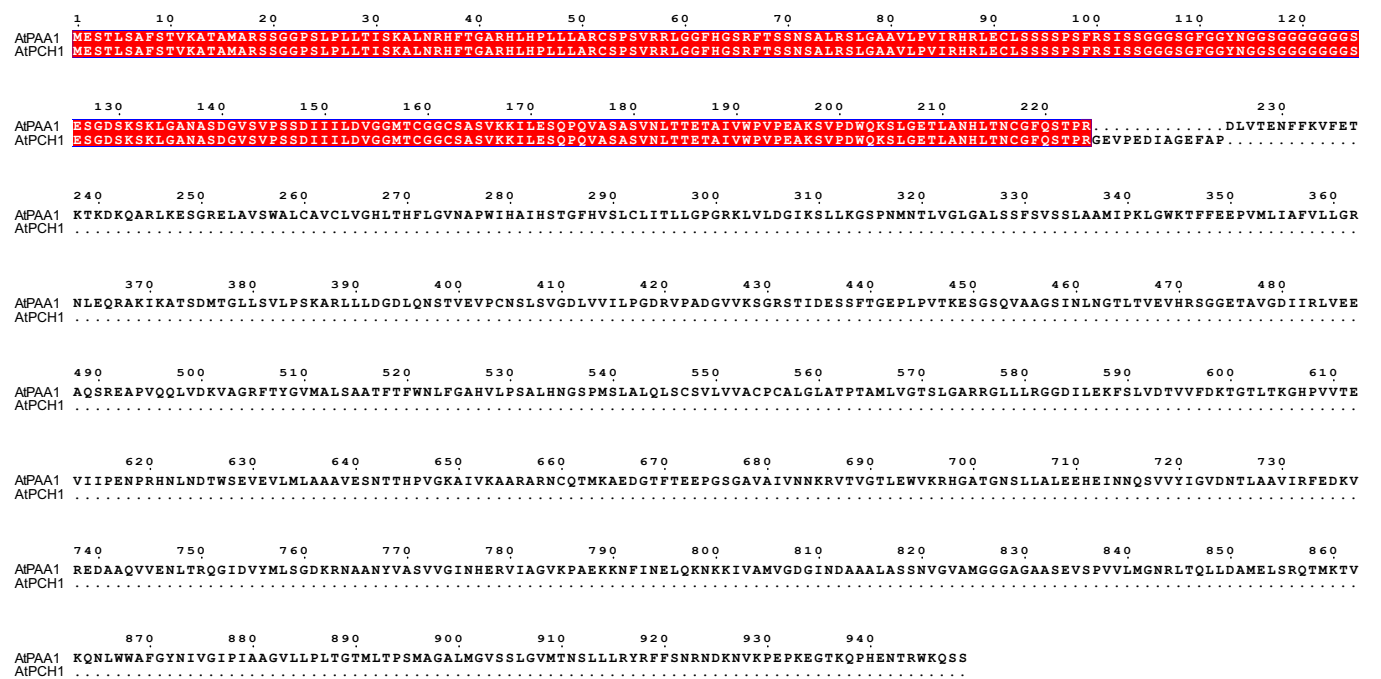


Fig. 55. Amino acid alignment of full-length AtPAA1 and AtPCH1. Identical amino acid residues are highlighted with a red background.

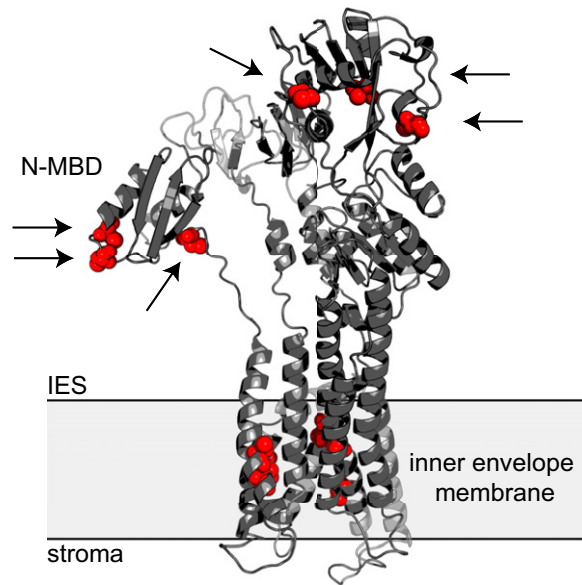


Fig. S6. Predicted structural model of the pea PAA1 ortholog. Each cysteine is shown as a red space-filled model.

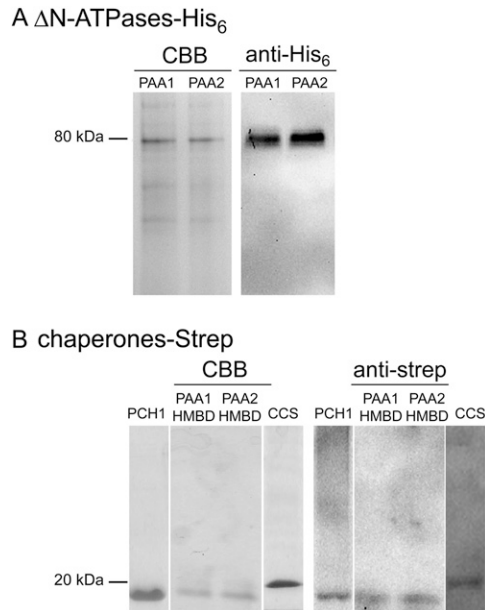


Fig. S7. Coomassie brilliant blue (CBB) staining and immunoblot of recombinantly expressed and purified Δ N-AtPAA1 and Δ N-AtPAA2 (A) and AtPCH1, AtPAA1-HMBD, AtPAA2-HMBD, and AtCCS (B).

Table S1. Primers

Primer name	R.E. site	Sequence 5' → 3'
PAA1_His_For	NdeI	<i>aaaaaacatatgCCTCGAGATTTGGTGACAGAGAA</i>
PAA1_His_Rev	Sall	<i>aaaaaagtcgacAGAGCTTTGCTTCCATCTTG</i>
PAA2_His_For	NdeI	<i>aaaaaacatatgAAATGGAAAGAAATGGTTAGC</i>
PAA2_His_Rev	Sall	<i>aaaaaagtcgacCAGAGAATTTTGGCTGGTT</i>
AtPCH1_strep_For	NdeI	<i>aaaaaacatatgTCAAGTGGCGGCGGTTCT</i>
AtPCH1_strep_Rev	Sall	<i>aaaaaagtcgacAGGTGCAACTCTCCTGCAA</i>
CCS_strep_For	Asel	<i>atgcattaatATGGTGGATATGACATGTGAGG</i>
CCS_strep_Rev	Sall	<i>atgcgtcgacAACCTTACTGGCCACGAAATC</i>
PAA2HMBD_strep_For	NdeI	<i>aaaaaacatatgATCGAAAGCGTGAAAAGTATTACG</i>
PAA2HMBD_strep_Rev	Sall	<i>aaaaaagtcgacGGATTTGACCAGCAGGTCTTC</i>
PAA1HMBD_strep_For	NdeI	<i>aaaaaacatatgTCAAGTGGCGGCGGTTCT</i>
PAA1HMBD_strep_Rev	Sall	<i>aaaaaagtcgacGCGAGCCTGCTTGTCTTTTG</i>
PhoALacZ_For	BamHI	<i>gatcggatccCCTGTCTGGAAAACCGG</i>
PhoLacZ_Rev	XmaI	<i>gatccccgggCCATTGCCCATTGAGGCT</i>
PAA1_PhoLac_For	NcoI	<i>atgccccatggAGTCTACACTCTCAGCTTTC</i>
PAA1_PhoLac_Rev	NheI	<i>atgcgctagcCTAAGAGCTTTGCTTCCATCTTG</i>
PAA1_PhoLac_1Rev	BamHI	<i>atgcggatcccAACAGAGACACCATCACTTGCA</i>
PAA1_PhoLac_1For	AgeI	<i>atgcaccggtCCGTCGTGAGATATCATTATTCTC</i>
PAA1_PhoLac_2Rev	BamHI	<i>atgcggatcccGGGAGCATTAAACCCCTAGAAA</i>
PAA1_PhoLac_2For	AgeI	<i>atgcaccggtTGATTTCATGCGATCCATT</i>
PAA1_PhoLac_3Rev	BamHI	<i>atgcggatcccACCTTTTAAAGACTCTTGATACCATC</i>
PAA1_PhoLac_3For	AgeI	<i>atgcaccggtTCCCCAACATGAACACG</i>
PAA1_PhoLac_4Rev	BamHI	<i>atgcggatcccTGCTTCCAGCCCAATTTTG</i>
PAA1_PhoLac_4For	AgeI	<i>atgcaccggtTTTTTGGAGAACAGTTATGTTAA</i>
PAA1_PhoLac_5Rev	BamHI	<i>atgcggatcccACCATCAAGCAGAAGGCG</i>
PAA1_PhoLac_5For	AgeI	<i>atgcaccggtGATCTGCAGAATTCAACTGTTGA</i>
PAA1_PhoLac_6Rev	BamHI	<i>atgcggatcccAACATGTGCACCAATAGATTCC</i>
PAA1_PhoLac_6For	AgeI	<i>atgcaccggtCTCCTTCTGCCTTGCAATA</i>
PAA1_PhoLac_7Rev	BamHI	<i>atgcggatcccCCATTATGCAAGGCAGAAG</i>
PAA1_PhoLac_7For	AgeI	<i>atgcaccggtAGCCCAATGTCTTTGGCC</i>
PAA1_PhoLac_8Rev	BamHI	<i>atgcggatcccATTGTGTCTTGATTTTCAGGAA</i>
PAA1_PhoLac_8For	AgeI	<i>atgcaccggtTTGAATGATACTTGGTCAGAAGTAGA</i>
PAA1_PhoLac_9Rev	BamHI	<i>atgcggatcccTGTGTTGACAATTACGAGCCC</i>
PAA1_PhoLac_9For	AgeI	<i>atgcaccggtATGAAGGCAGAGGATGGAAC</i>

R.E., restriction enzyme.

Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)