Supporting Information

Whitney et al. 10.1073/pnas.1420536112

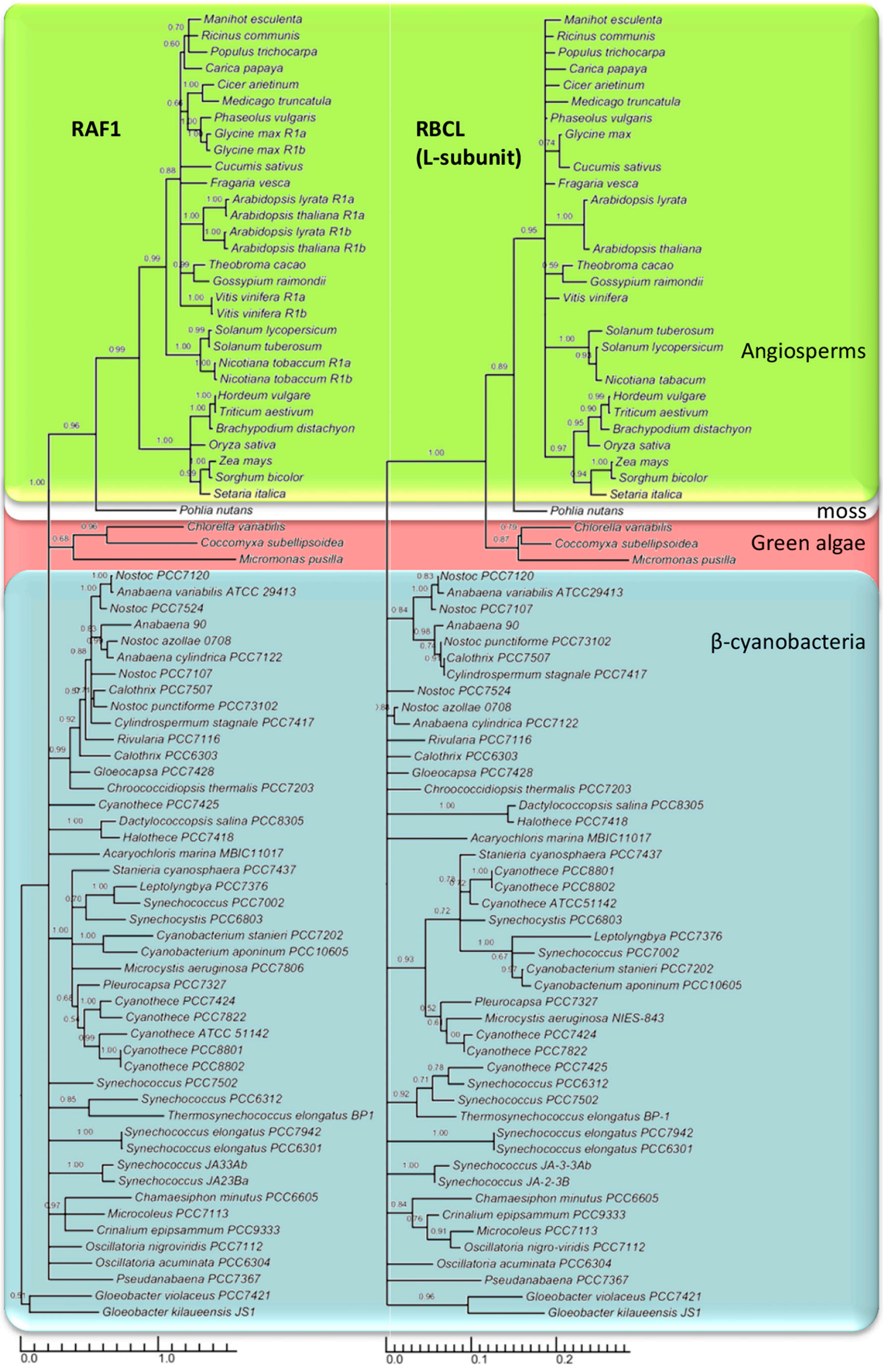

Fig. S1. RAF1 and Rubisco L-subunits phylogenies of plants, green algae, and β-cyanobacteria. (*A*) Maximum-likelihood trees assembled under the Dayhoff model implemented in RAxML v.8 (1) using translated amino acid sequences from the full length *raf1* and *rbc*L genes listed in Table S2. Posterior probability (PP) values are shown above tree branches; all clades with PP < 0.5 have been dissolved.

1. Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312–1313.

A Amino acid alignment of tobacco and *Arabidopsis* Rubisco L-subunits

```
  1  M S P Q T E T K A S V G F K A G V K E Y K L T Y Y T P E Y Q T K D T D I L A A F  Tobacco L
  1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . E . . . . . . . . . .  Arabidopsis L

 41  R V T P Q P G V P P E E A G A A V A A E S S T G T W T T V W T D G L T S L D R Y  Tobacco L
 41  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Arabidopsis L

 81  K G R C Y R I E R V V G E K D Q Y I A Y V A Y P L D L F E E G S V T N M F T S I  Tobacco L
 81  . . . . . H . . P . P . . E T . F . . . . . . . . . . . . . . . . . . . . . . .  Arabidopsis L

121  V G N V F G F K A L R A L R L E D L R I P P A Y V K T F Q G P P H G I Q V E R D  Tobacco L
121  . . . . . . . . . . A . . . . . . . . . . . . . T . . . . . . . . . . . . . . .  Arabidopsis L

161  K L N K Y G R P L L G C T I K P K L G L S A K N Y G R A V Y E C L R G G L D F T  Tobacco L
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Arabidopsis L

201  K D D E N V N S Q P F M R W R D R F L F C A E A L Y K A Q A E T G E I K G H Y L  Tobacco L
201  . . . . . . . . . . . . . . . . . . . . . . . . I . . S . . . . . . . . . . . .  Arabidopsis L

241  N A T A G T C E E M I K R A V F A R E L G V P I V M H D Y L T G G F T A N T S L  Tobacco L
241  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Arabidopsis L

281  A H Y C R D N G L L L H I H R A M H A V I D R Q K N H G I H F R V L A K A L R M  Tobacco L
281  S . . . . . . . . . . . . . . . . . . . . . . . . . . . M . . . . . . . . . . L  Arabidopsis L

321  S G G D H I H S G T V V G K L E G E R D I T L G F V D L L R D D F V E Q D R S R  Tobacco L
321  . . . . . . . A . . . . . . . . . D . E S . . . . . . . . . . . Y . . K . . . .  Arabidopsis L

361  G I Y F T Q D W V S L P G V L P V A S G G I H V W H M P A L T E I F G D D S V L  Tobacco L
361  . . F . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Arabidopsis L

401  Q F G G G T L G H P W G N A P G A V A N R V A L E A C V K A R N E G R D L A Q E  Tobacco L
401  . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . . . . . . . . . V .  Arabidopsis L

441  G N E I I R E A C K W S P E L A A A C E V W K E I V F N F A A V D V L D K .      Tobacco L
441  . . . . . . . . . . . . . . . . . . . . . . . . . T . . . P T I . K . . G Q E .  Arabidopsis L
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B Amino acid alignment of tobacco (*Nt*) and *Arabidopsis* (*At*) RAF1

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  1  M F S L T V N S P K P L S L S T P F L P S H H H P L P S - - I T H K P I L N P K P - - - - I T A L I  Nt-R1a
  1  . . . . . . . . . . . . . . . . . . . . T . P . . . . . - - . . . . . N . . . . . - - - - . . . . .  Nt-R1b
  1  . . . . K S - - - - - - L I . S . . T Q . T T . G . F T N P . . R P V N P L . R T V S F T V . . S M  At-R1a
  1  . L . . . A T T - - - - L S . S I . T Q . K T . G F F N - - - . R P V Y R K . F T - - - T . . S A L  At-R1b

 45  I P P S S G Q Q Q - - Q Y S T - - - Q Q Q Q L Y Q P F R P P P P P L P P K F R N L D T N A K L E V L  Nt-R1a
 45  . . . . . . . . . - - . . . . Q Q Q . . . . . . . . . . . . . . L . . . . . . . . . . . S . . . . .  Nt-R1b
 45  . . K R . S A N M I P K N P P A R - - - . . . . . . . . . . S S . I . T Q . . S . . S A G . I . I .  At-R1a
 41  . . A . N - - - - - - R Q A P P K - - - . . . . . . . . . . . S . I . . . . . S . . . A G . I . . .  At-R1b

 90  S N R L G L W Y E Y A P L I P Y L T S E G F T P S T L E E I T G L T G V E Q N R L V V A A Q V R D T  Nt-R1a
 93  A . . . . . . . . . . . . . . S . . R . . . . . . . . . . . . . . . . . D . . . . . . . . . . . . S  Nt-R1b
 92  A G . M A . . F . . . . . . S S . Y T D . . . . P . I . . L . . I S S I . . . . . I . G . . . . . S  At-R1a
 82  A D . . . . . F . . . . . . S S . Y T . . . . . P S I . . L . . I S . . . . . S . I . G . . . . . S  At-R1b

140  L V E S A A L D E E T L S Y F E S G G A E L L Y E I R L L S A R Q R T D A A T F L V K N G F D A K Q  Nt-R1a
143  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . .  Nt-R1b
142  I L Q . I H - E P . L I . A . D T . . . . . . . . . . . . . T T . . V A . . . . I I D R N I . S . G  At-R1a
132  . . Q . G . - K P . L I A A . D T N . . . . . . . . . . . N T T . . V A . . E Y I . D H . . . T . G  At-R1b

190  A Q D L A R A I K D Y P R R R V D Y G W D K F N G D S P G D C L A F M Y F R L A Q E Y A A A A S E D  Nt-R1a
193  . . . . . . . . . . . . . . . . E . . . . . . H . . . . . . . . . . . . . . . . . . . . . . . . . .  Nt-R1b
191  . . . . . . . . . . . . N . . G . V . . L D . D Y N L . . . . . S . L . Y . Q S R . N K N P S - - .  At-R1a
181  . G . . . . . . . . F . H . . G . V . L G D . D Y N L . . . . . S . . L Y . K S R . H R S P S - - E  At-R1b

240  L R R S S M E K A L E V V E S E S A R N L L V M E L E G R E V A K E S V L D - - - - - - - - D G V T  Nt-R1a
243  . . . . . . . . . . . . A . . . . . . . . . . . . . . . N . . . . . . . K . - - - - - - - - . . . .  Nt-R1b
239  Q . T . M L L Q . . G . A . . . K . K . R . N T . . Y . - D K E A . K E K E K K K K E E E V K A I R  At-R1a
229  I . T T L L . Q . . . T A V T . K . K K A V L R . . H . - . S E E . R . K E - - - - E E - I K I I R  At-R1b

282  V P L V R M K L G E V A E S T I V V V L P V C K A E G R D V E V E A A P W E C G G V G D F G I V E A  Nt-R1a
285  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Nt-R1b
288  I . V . . L . F . . . . . A . S . . . . . . . . . . E G E K K I L E . . M . I I A G . . . K V . . .  At-R1a
273  . . V . . L R F . . . . G A S S . . . . . . . . . . E G E E K L L E . . M . F E S G . E . . V . . .  At-R1b

332  E K D W R R W V V L P G W Q P I A G L E R G G V A V S F K S G - N F L P W R E K S K Y K Q E P V L V  Nt-R1a
335  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . . . . . . . . . . . . . . . . .  Nt-R1b
338  . . G . K . . . . . . S . N . V . A I G K . . . . . . . R D D R K V . . . D G . - - - - E . . L . .  At-R1a
323  . . . . S . . . . . . . . D . V V A V R K . - . . . . . S D D R E V . . . N G . - - - - G . A I M .  At-R1b

381  V A D R G R T E V A S E D G - F Y L V V D G G D G S N E E G L K V E R G S T L K K R G V E Q S L G I  Nt-R1a
384  . . . . . . . . . . . . . . - . . . . . . . . N . . . . . . . . . . . . . . . . . . . . . . . . . .  Nt-R1b
384  . . . . V . N V . E A D . . - Y . . . . A E N - - - - - - . . . L . K . . D . . A . E . K E . . . M  At-R1a
368  . I . . E K K T . E A D N . Y Y . . . . A D . - - - - - - . M . L D . . L V . . E K . . N E . . . M  At-R1b

430  V L I V V R P P R W E D E E - Q L G E E D W D .  Nt-R1a (GenBank Sequence Read Archieve SRP029184)
433  . . . . . . . . K . . N . D - . . . . . . . . .  Nt-R1b (Genbank Sequence Read Archieve SRP029184 )
427  . V L . . . . . . E D . D D W . T S H Q N . . .  At-R1a (Genbank accession NC_003074.8; TAIR:AT3G04560)
412  . V L . . . . . . D D . D . W . I N D . . . . .  At-R1a (Genbank accession NC_003076.8; TAIR:AT5G28500)
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C Amino acid sequence identity matrix (%)

Full length RAF1

	Nt-R1a	*Nt*-R1b	*At*-R1a	*At*-R1b
Nt-R1a		94.9	48.7	50.0
Nt-R1b	95.4		48.3	48.9
At-R1a	52.6	52.6		67.1
At-R1b	50.7	50.4	70.8	

Mature RAF1 (no transit peptide)

Fig. S2. Sequence comparison of the Rubisco L-subunit and RAF1 isoforms in tobacco and *Arabidopsis*. Alignment of (*A*) Rubisco L-subunits and (*B*) RAF1 homologs from *Arabidopsis thaliana* and *Nicotiana tabacum*. Tobacco *rbc*L (NC_001879) and *Arabidopsis rbc*L and *raf1* (ArthCp030, AT3G04550, AT5G28500) sequences were obtained from GenBank. The tobacco RAF1 sequences (*Nt-R1a* and *Nt-R1b*) were derived from the assembly of Illumina RNA-Seq transcriptome data of *N. tabacum* cv. K326 [Sequence Read Archive accession code SRP029184 (1)] using CLC Genomics Workbench 7.0.3 (http://www.clcbio.com) software. (*C*) Sequence identities of the different RAF1 homologs after Clustal W alignment both with and without (shaded gray) their predicted transit peptide coding sequences (highlighted red in *B*).

1. Sierro N, et al. (2014) The tobacco genome sequence and its comparison with those of tomato and potato. *Nat Commun* 5:3833.

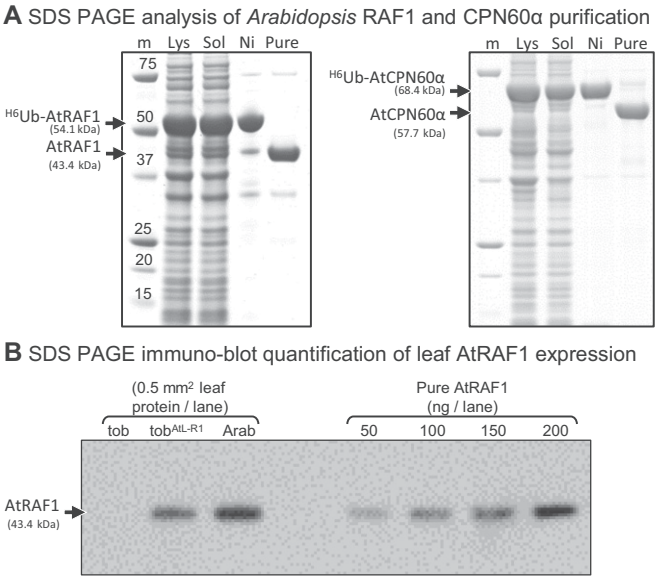


Fig. S3. CPN60α and ^{At}RAF1 purification and quantification by immunoblot analysis. The mature coding sequence CPN60α1 (GenBank NP_197383.1, At5g18820) from *Arabidopsis* (i.e., spanning amino acids 36–578 to exclude part or all of the chloroplast targeting sequence) was amplified by RT-PCR (SuperScript III Reverse Transcriptase, Life Technologies) using leaf RNA extracted using TRIzol Reagent (Life Technologies) and primers 5′SacIIAtCPN60α (5′-CCGCGGTGGAATGGGAGCTAAGAGAATACTATAC-3′) and 3′HindIII AtCPN60α (5′-AAGCTTATGATGTGGGTATGCCAGG-3′). The amplified 1637-bp SacII-HindIII product was cloned in frame with the N-terminal 6x-histidine (H_6)-Ub fusion peptide in plasmid pHue (1) to give plasmid pHueCPN60α. Similarly, the synthetic ^{At}*raf1* gene in pLEVAtL-RAF1 (Fig. 2*A*) was amplified with primers 5′SacIIAtRAF1 (5′-CCGCGGTGGAATGGCTCCTCTTAAATCTTTGATT-3′) and 3′HindIIIAtRAF1 (5′-AAGCTTCTCGAGATCCCAATTTTGATG-3′) and the 1,364-bp SacII-HindIII fragment cloned into pHue to give pHueAtRAF1. *Escherichia coli* BL21 (DE3) cells transformed with plasmids pHueAtRAF1 and pHueCPN60α were grown at 28 °C on a rotary shaker (150 rpm) in 0.5 L of Luria-Bertani medium containing 200 μg/mL ampicillin. At an A_{600} of 1.0 isopropyl-β-D-thiogalactopyranoside was added to 0.5 mM. After 6 h, the cells were harvested by centrifugation (3,300 × *g,* 10 min, 4 °C) and resuspended in 10 mL of ice-cold extraction buffer (0.1 M Tris·HCl, pH 8.0, 0.3 M NaCl, 1 mM PMSF, 5 mM mercaptoethanol) and lysed by passage through a prechilled French pressure cell at 140 MPa. The extract was centrifuged (33,000 × *g,* 10 min, 4 °C) and the (H_6)-Ub-RAF1 and (H_6)-UbCPN60α proteins purified by Ni^{2+}-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) chromatography, eluted in imidazole buffer (extraction buffer with 0.2M imidazole) and the (H_6)-Ub sequences removed with a (H_6)-Ub-protease as described (1) before dialyzing into storage buffer [40 mM EPPS-NaOH, pH8, 8 mM MgCl2, 0.8 mM EDTA, 20% (vol/vol) glycerol] and storing at −80 °C. (*A*) Protein samples during the purification were diluted with 0.25-volumes 4× SDS reducing buffer and analyzed by SDS PAGE as described previously (2). (*B*) The ^{At}RAF1 content in soluble protein from known leafs areas were calculated by immuno-blot densitometry analysis against known amounts of purified ^{At}RAF1 (quantified against BSA standards) separated in parallel by SDS PAGE.

1. Baker RT, et al. (2005) Using deubiquitylating enzymes as research tools. *Methods Enzymol* 398:540–554.
2. Whitney SM, Sharwood RE (2007) Linked Rubisco subunits can assemble into functional oligomers without impeding catalytic performance. *J Biol Chem* 282(6):3809–3818.

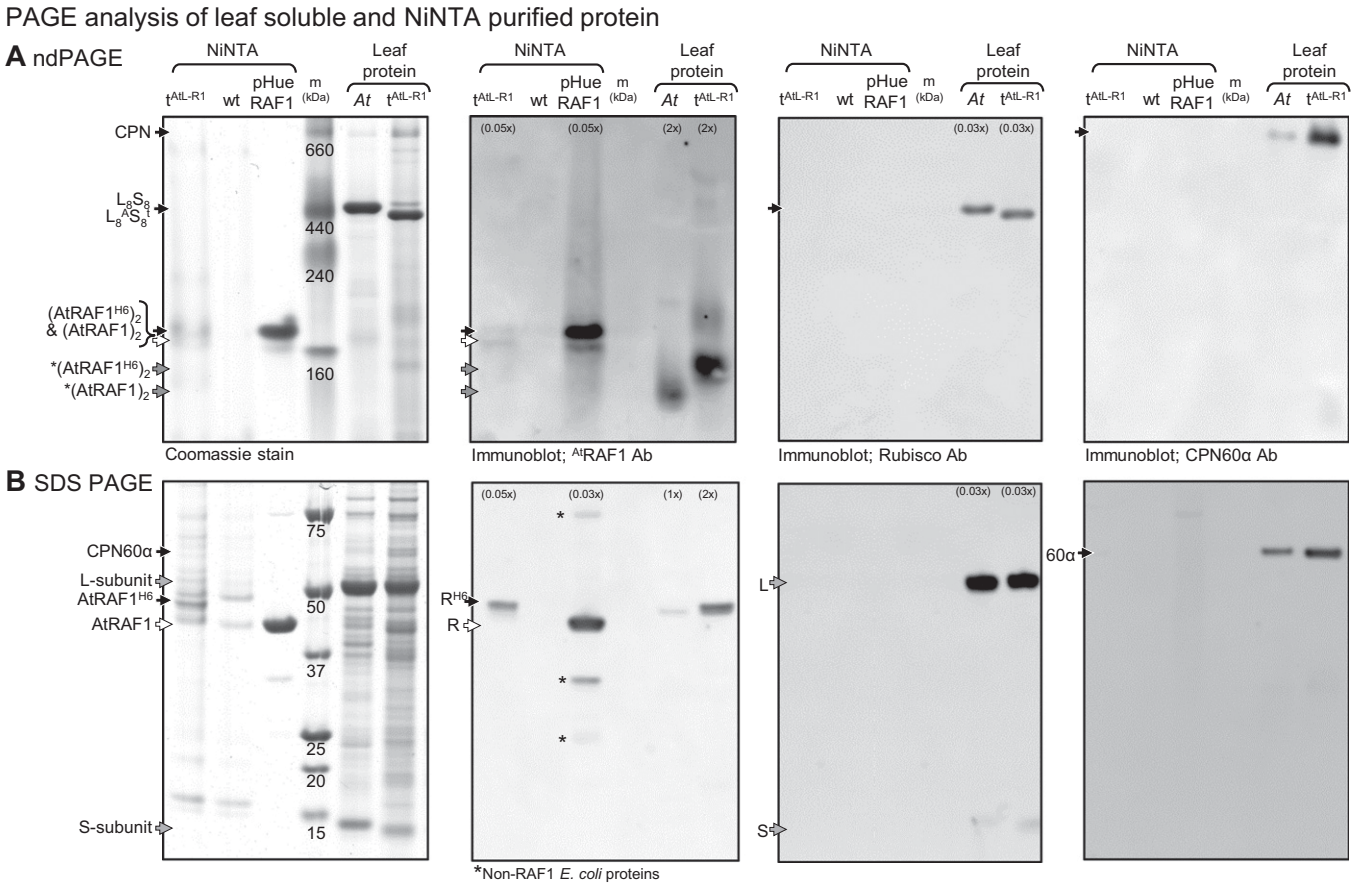
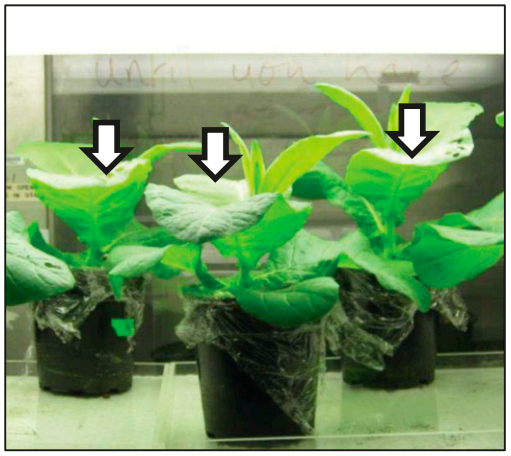


Fig. S4. PAGE analysis of NiNTA purified and total soluble leaf protein from *Arabidopsis* and the different tobacco genotypes. (*A*) ndPAGE and (*B*) SDS PAGE analysis of soluble leaf protein [from *Arabidopsis* (*At*), tob$^{AtL\text{-}R1}$ and tob^{AtL}] and Ni^{2+}-nitrilotriacetic acid agarose (Ni-NTA) purified protein from *E. coli*-pHueAtRAF1 cells (Fig. S3), tobacco (wild-type) and tob$^{AtL\text{-}R1}$ leaves. Variations in the amount of sample loaded per lane relative to the Coomassie-stained gel are shown in parentheses. For NiNTA purification ~2 g of tob$^{AtL\text{-}R1}$ and wild-type tobacco leaves were homogenized in 20 mL extraction buffer [0.1 M Tris·HCl, pH 8.0, 0.3 M NaCl, 5% (vol/vol) glycerol, 1% (wt/vol) PVPP, 1 mM PMSF, 5 mM mercaptoethanol] using 40 mL Wheaton glass homogenizers, then centrifuged (16,500 × *g*, 10 min, 2 °C). The soluble protein was transferred to a 10-mL Econo column (Promega) containing a 1-mL bed volume of Ni-NTA agarose (Qiagen). After the sample had passed through the resin, it was washed with 20 bed volumes of extraction buffer (no PVPP or mercaptoethanol). The bound protein was collected in 0.8 mL of elution buffer (0.1 M Tris·HCl, pH 8.0, 0.3 M NaCl, and 200 mM imidazole) and the proteins separated by PAGE, as described previously (1). Immunoblot analysis confirmed the ^{At}RAF1 purified from tob$^{AtL\text{-}R1}$ comprised two similar sized bands that matched the size of those purified from *E. coli*. In the *At* and tob$^{AtL\text{-}R1}$ soluble leaf protein samples the native ^{At}RAF1 and slightly larger recombinant ^{At}RAF1^{H6} products are seen as more diffuse bands of lower apparent molecular size. No Rubisco or CPN60α subunits were detected in the NiNTA purified protein from tob$^{AtL\text{-}R1}$ or wild-type. Only the ^{At}RAF1 protein was visually unique in the Coomassie-stained NiNTA purified protein from tob$^{AtL\text{-}R1}$ suggesting it does not stably interact with any other tobacco chloroplast protein to any significant extent, although this requires closer proteomic scrutiny.

1. Whitney SM, Sharwood RE (2007) Linked Rubisco subunits can assemble into functional oligomers without impeding catalytic performance. *J Biol Chem* 282(6):3809–3818.

A Plant phenotype and experimental setup for analyzing Rubisco synthesis and turnover in whole leaves by ^{35}S-Met pulse-chase



B Schematic of the leaf pulse-chase analysis abaxial infiltration and sampling régime

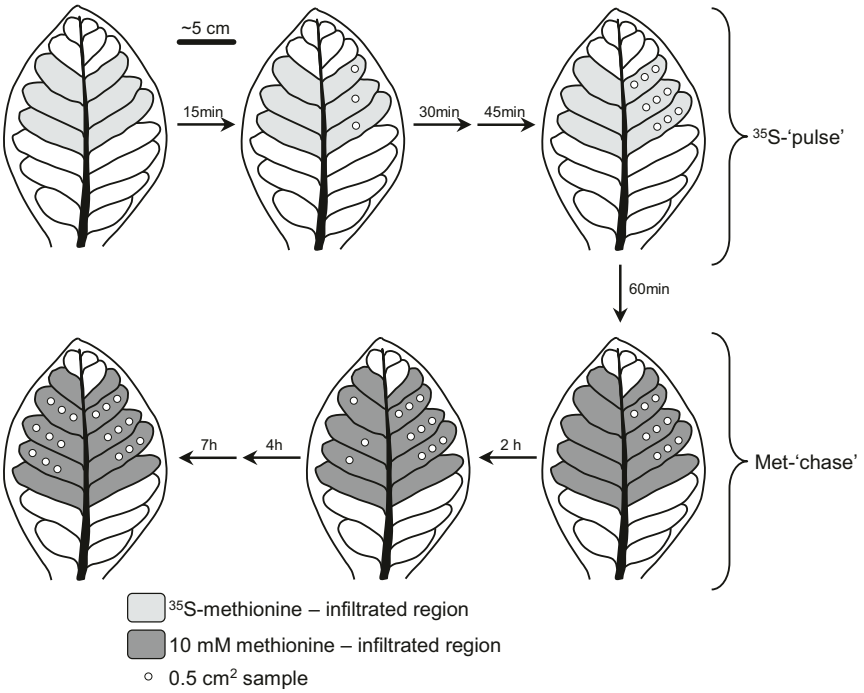


Fig. S5. ^{35}S-labeling of Rubisco in attached tobacco leaves by a direct infiltration approach. Because of significant variations in Rubisco expression down the canopy of tobacco (1), significant care was taken to perform the ^{35}S-infiltration experiments on leaves of comparable developmental status and positioning in the upper canopy. (*A*) The plants analyzed were all of comparable size with infiltration experiments performed on the youngest near fully expanded leaf (the fifth from the top of the canopy, indicated by white arrow) where the intercellular air spaces are optimally developed for fast and efficient liquid infiltration. (*B*) Showing the regions of the leaves toward the tip that were infiltrated in the experiment and the sampling protocol undertaken during both the [^{35}S]methionine labeling ('pulse') and ensuing 10-mM methionine "chase" period.

1. Pengelly JJ, et al. (2014) Transplastomic integration of a cyanobacterial bicarbonate transporter into tobacco chloroplasts. *J Exp Bot* 65(12):3071–3080.

Table S1. Rubisco catalysis comparison

Plant source	Tobacco	*Arabidopsis*	tob$^{AtL\text{-}R1}$
k_C^{cat} (s^{-1})	3.1 ± 0.1	3.0 ± 0.2	2.3 ± 0.3*
K_C (μM)	9.7 ± 0.2	9.8 ± 0.3	8.6 ± 0.2*
K_O (μM)	174 ± 16	192 ± 17	221 ± 16
$k_C^{cat}/K_C^{21\%O2}$ (mM^{-1}/s^{-1})	138	125	126
$S_{C/O}$ (mol/mol^{-1})	82 ± 1	80 ± 2	80 ± 3

*Significance variation ($P < 0.05$) determined by *t*-test. $K_C^{21\%O2}$, the apparent K_m for CO_2 (K_C) at atmospheric [O_2] (assumed 252 μM at 25 °C) calculated as $K_C(1+[O_2]/K_O)$.

Table S2. List of species and accession numbers for the *raf1* and *rbc*L sequences from 26 plant, 3 algal, and 46 cyanobacteria genomes used to construct the maximum-likelihood trees in Fig. S1

Organism	*raf1*	*rbcL*	*matK*
Angiosperms			
Arabidopsis lyrata	XM_002882316; XM_002872267	XM_002888303	AF144342
Arabidopsis thaliana	BT015787; AY063107	U91966ATU91966	AF144378
Brachypodium distachyon	XM_003573939	194033128:54293–55723	133917479
Carica papaya	Phytozome: 162.24_CDS	EU431223:58728–60155	EU431223:2266–3786
Cicer arietinum	XM_004495508	197294093:5003–6430	197294093:2070–3599
Cucumis sativus	XM_004142526	DQ865976:57578–59005	68164782:1838–3376
Fragaria vesca	XM_004304718	325126844:56459–57886	AF288102
Glycine max	XM_003536095; XR137658	91214122:5312–6739	AF142700
Gossypium raimondii	Phytozome:013G120100.1_CDS	372290914:58642–60081	AF403559
Hordeum vulgare	AK353664	AY137453:111–1550	AB078139
Manihot esculenta	Phytozome:03614:2579552.0.2581338	169794052:58063–59496	EU117376:2063–3583
Medicago truncatula	BT141443	JX512024:117295–118722	AY386945
Nicotiana tobaccum	current study	NC_001879	81238323:2131–3660
Oryza sativa	115482237	AY522330:54082–55536	EU434287
Phaseolus vulgaris	KF033821	EU196765:70304–71734	AY582987
Populus trichocarpa	XM_002319615	134093177:55716–57143	134093177:1981–3513
Ricinus communis	XM_002521916	372450118:58961–60388	372450118:2387–3907
Setaria italica	XM_004982939	558603649:54628–56034	390607728
Solanum lycopersicum	XM004249865	544163592:56683–58116	544163592:2124–3653
Solanum tuberosum	565368659	DQ386163.2|:56531–57964	JF772171:2140–3669
Sorghum bicolor	XM_002448739	118614470:57693–59123	AF164418
Theobroma cacao	Phytozome: EG026242t1_CDS	JQ228389:59398–60852	AY321195
Triticum aestivum	AK334642	AY328025:60–1493	KJ592713:1678–3216
Vitis vinifera	FQ395584; FQ393164	91983971:59436–60863	91983971:2016–3524
Zea mays	226508017	11994090:56874–58304	11994090:1674–3215
Bryophyta			
Pohlia nutans		AY631193	AY522574
Green Algae			
Coccomyxa subellipsoidea	XM_005643171	HQ693844:164006–165433	323149147:70601–72805
Chlorella variabilis	XM_005847023	331268093:47431–48858	331268093:26130–28334
Micromonas pusilla	XM_003063100	FJ858267:20006–21433	FJ858269
β-Cyanobacteria			
Acaryochloris marina MBIC11017	CP000828:1771175–1772245	CP000828:1775408–1776838	
Anabaena cylindrica PCC 7122	CP003659:5732014–5733099	CP003659:34579–36009	
Anabaena sp 90	CP003284:2564028–2565113	CP003284:1480330–1481760	
Anabaena variabilis ATCC 29413	CP000117:1756144–1757229	CP000117:4857469–4858899	
Calothrix sp PCC 6303	CP003610:4364743–4365828	CP003610:3605242–3606672	
Calothrix sp PCC 7507	CP003943:5400132–5401217	CP003943:325257–326687	
Chamaesiphon minutus PCC 6605	CP003600:6052812–6053882	CP003600:694685–696115	
Chroococcidiopsis thermalis PCC 7203	CP003597:1959990–1961051	CP003597:5964292–5965722	
Crinalium epipsammum PCC 9333	CP003620:4318634–4319728	CP003620:4709290–4710720	
Cyanobacterium aponinum PCC 10605	CP003947:3620023–3621099	CP003947:800936–802342	
Cyanobacterium stanieri PCC 7202	CP003940:251659–252741	CP003940:126365–127771	
Cyanothece sp ATCC 51142	CP000806:1951795–1952787	CP000806:3281510–3282925	
Cyanothece sp PCC 7424	CP001291:3045110–3046189	CP001291:1503225–1504643	
Cyanothece sp PCC 7425	CP001344:4048780–4049862	CP001344:3372918–3374348	
Cyanothece sp PCC 7822	CP002198:3872031–3873092	CP002198:3223935–3225353	
Cyanothece sp PCC 8801	CP001287:819957–821021	CP001287:1677472–1678890	
Cyanothece sp PCC 8802	CP001701:819755–820819	CP001701:1666285–1667703	
Cylindrospermum stagnale PCC 7417	CP003642:6936516–6937604	CP003642:2391125–2392555	
Dactylococcopsis salina PCC 8305	CP003944:2505154–2506221	CP003944:1798755–1800176	
Gloeobacter kilaueensis JS1	CP003587:711901–712965	CP003587:713821–715245	
Gloeobacter violaceus PCC 7421	37508091:2309302–2310369	37508091:2307046–2308470	
Gloeocapsa sp PCC 7428	CP003646:1785908–1786993	CP003646:1141494–1142924	
Halothece sp PCC 7418	CP003945:2360587–2361660	CP003945:3829408–3830826	
Leptolyngbya sp PCC 7376	CP003946:2022725–2023804	CP003946:204758–206173	
Microcoleus sp PCC 7113	CP003630:771030–772124	CP003630:2675003–2676433	
Microcystis aeruginosa PCC 7806	159027328:13224–14216	166085114:4390428–4391843	
Nostoc azollae 708	CP002059:4390613–4391698	CP002059:2235547–2236977	
Nostoc punctiforme PCC 73102	CP001037:5521656–5522744	CP001037:5263600–5265030	
Nostoc sp PCC 7107	CP003548:2972009–2973094	CP003548:2119530–2120960	

Table S2. Cont.

Organism	*raf1*	*rbcL*	*matK*
Nostoc sp PCC 7120	47118302:6264560–6265645	47118302:1785970–1787400	
Nostoc sp PCC 7524	CP003552:4087403–4088488	CP003552:1290272–1291702	
Oscillatoria acuminata PCC 6304	CP003607:7273598–7274692	CP003607:1163939–1165369	
Oscillatoria nigro-viridis PCC 7112	CP003614:6651808–6652902	CP003614:6951541–6952971	
Pleurocapsa sp PCC 7327	CP003590:3516618–3517697	CP003590:357448–358863	
Pseudanabaena sp PCC 7367	CP003592:182052–183158	CP003592:1184484–1185896	
Rivularia sp PCC 7116	CP003549:6792297–6793388	CP003549:4304946–4306376	
Stanieria cyanosphaera PCC 7437	CP003653:1606913–1607992	CP003653:369045–370463	
Synechococcus elongatus PCC 6301	56684969:792692–793771	56684969:139920–141338	
Synechococcus elongatus PCC 7942	CP000100:827112–828182	CP000100:1479461–1480879	
Synechococcus sp JA-2-3Ba(2-13)	CP000240:535600–536703	CP000240:2682338–2683762	
Synechococcus sp JA-3-3Ab	CP000239:929252–930337	CP000239:1207204–1208628	
Synechococcus sp PCC 6312	CP003558:1545379–1546446	CP003558:1977136–1978563	
Synechococcus sp PCC 7002	CP000951:2467879–2468958	CP000951:1882749–1884164	

Two gene copies of *raf1* were found in five plant species (including tobacco and *Arabidopsis*; see Fig. S2*B*), and one copy in all other species. Accession numbers are also shown for the chloroplast *mat*K sequences that were used as a negative control when testing for putative *raf1* and *rbc*L coevolution by correlating their pairwise nonsynonymous (leading to amino acid substitutions) and synonymous (selectively neutral) distances across green plants and algae (see Fig. 1*B*).