SUPPLEMENTAL INFORMATION

Supplemental Text. All amino acid sequences of the Arabidopsis (57), maize (47), rice (47) and *E. coli* (5) RH proteins used for phylogenetic analysis in supplemental Figure 1.

Supplemental Table 1. Protein accession numbers, nomenclature, annotation and clade assignment for the RH3 DEAD box helicase family in Arabidopsis, maize, rice used for phylogenetic analysis.

Supplemental Table 2. Primer sequences used for verification of Arabidopsis T-DNA insertions and RNA analysis.

Supplemental figure 1. Phylogenetic tree of the RH3 DEAD box helicase family in Arabidopsis, maize, rice and *E. coli* based on the core domains with major gaps removed. Different clades are assigned and proteins have modified DEAD domains are marked.

Supplemental figure 2. Multiple sequence alignment of ZmRH3A,B and AtRH3. The alignment was generated in ClustalW and BoxShade. Identical residues are shaded in black, and similar residues are shaded in gray. A predicted transit peptide cleavage site by stromal processing protease in AtRH3 is shown in black arrow. The conserved domains (Q-VI) are marked. The C-terminal GUCT and G-R-S enriched domains are showed in black lines with arrows. The red box represents the antigen for maize anti-RH3A. ZmRH3A corresponds to GRMZM2G415491_P01; ZmRH3B corresponds to GRMZM2G163072_P01; and AtRH3 corresponds to At5g26742. Blue: Identical residues. Yellow: Similar residues.

Supplemental figure 3. An albino seedling phenotype of hetero allelic *emb1138/rh3-4*. (A) Wild type (WT), *rh3-4*, and non-complementary plants *emb1138/rh3-4* were grown on 1/2 MS medium containing 2% sucrose for 21 days. The *emb1138/rh3-4* seedling is white. Bars = 5 mm. (B) PCR genotyping the progeny of the cross between *emb1138* and *rh3-4* confirms that the T-DNA insertion in *AtRH3* gene causes the pigment deficient phenotype. Primers used for the genotyping are shown top panel in (A). Genomic DNA was isolated from albino or wild-type phenotype resulted from cross between *rh3-4* and EMB1138/*emb1138* crossing. All of albino seedlings have T-DNA from both parents, whereas, wild-type phenotype has a T-DNA only from *rh3-4*.

Supplemental figure 4. PCR genotype of double knock down mutant rh3-4/clpr2-1.Primer pairs used for PCR genotyping are shown at the top panels with black arrows. Genomic DNA was isolated from albino, pale-green, wild type (WT) progenies from F2 generation. All of albino plants are double homozygous to rh3-4 and clpr2-1, whereas, pale-green mutants are homozygous to a single mutant. None of wild-type phenotypes are homozygote.

Supplemental figure 5. RH3 in wild type and *clpr2-1* mutant are in the same fraction in sucrose gradients. Arabidopsis wild type (WT) or *clpr2-1* stroma was sedimented through 10-40 % sucrose gradient. An equal portion of each fraction was detected by immunoblots by anti-RH3 antiserum. The blots stained with Ponceau S are showed the place of Rubisco at 550 kDa.

Supplemental Figure 6. RH3 functions and chloroplast splicing factors and their intron target in land plants. Introns in land plant chloroplasts are categorized to group I, subgroups IIA and IIB according to (Michel et al., 1989). RH3 functions both in group IIA intron splicing and ribosome biogenesis. Those found in *Arabidopsis* but not in maize are marked with asterisks. Nucleus-encoded splicing factors are annotated with their conserved domains. Solid arrows mark interactions supported by either genetic or co-IPs data, but not in both. Updated from (Barkan, 2011).

56 Arabidopsis & 46 maize & 47 rice & 5 *E. coli*. Core domain (minus gaps) with some C-term extension



Os02g55260 - ZmG030873 DETD

100

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ZmRH3A	1	GWAAAGUT-LEALALALENPGAVRERAAAGRCWAEREEGWAAAGULASENSYMSEHEEREGOOG
ZmRH3B	1	WASEVILEAIAFSNPATASGAVRERAAAFRCWAERDEGWAVAANVSSDENSVISSEBAFREOOD
AtRH3	1	MASTVGVPSIVQVPHLEISKPNSKKRSNCLSLSMDKPPETPLSMVSITRRIHSSSLLVPSIVATENSVASEPASKSGAS
		Q
ZmRH3A	61	SDEDGEGPYGSDADEGFEAGEGDNEEMATAREGTEDEEVATMEKROLTELATELORDLORDLORDLORTA
ZmRH3B	64	SDDEDEEGFYGSDADEGFQGDEEELATARLGDEDELVATLEKRGITELFFIQRAVLIPALGGRDLIARAKTGTGKTL
AtRH3	81	DHDYDLDGDNNNVEADDGEHMAHSKUSGREESUAHSKUSGERAFIGEAAAAVORDI
		<u>la</u>
ZmRH3A	141	AFOTEMEROD <mark>I</mark> EOD-DORIIER-ORIFRVOVLAPTELAKOVEKEIKESAFKLOTVOVVGOVSYNVOQNALSROVDVVVG
ZmRH3B	141	AFGIPHIKOLHEGD-DGRSTRR-GRTPRVLVLAPTRELAKQVEKEIKESAPKLGTVCVYGGVSYNVQQHALSRGVDVVVG
AtRH3	155	AFOLFIITERATEEAGDYTAFRESGELE <mark>K</mark> FDVIAPTEEGAKOVEKELKESAFYLSTVCVYGOVSYT <mark>I</mark> QQSAL <mark>T</mark> RGVDVVVG
ZmRH3A	219	F 2 GRITIDITEN COSTO DE VOTEN DE ADONITAVO F E EDVETILO O F FACROSMIFSATMES AVIRES RATEN PLETEDAV
ZmRH3B	219	TPGRIIDLINGCSLCGEVCILVLDEADQNLAVGFEEDVETILQCLPADRQSNLFSATMPSNVKKLSRBILNPLTIDLV
AtRH3	235	WEGGOOD DE CREMENDED BUILDEN DE AUDERACION AND ELE AVERSEN EL AUDERACIONE DA COMPANSION DE LA COMPANSION DE LA
ZmRH3A	299	gdqdeklaegikl <mark>yaipli</mark> tskri <mark>v</mark> lsdlitvyakggktivytr <mark>rkk</mark> dadevslal <mark>tk</mark> sia <mark>sealegdisqeqrertan</mark>
ZmRH3B	299	GDQDEKLAEGIKL <mark>E</mark> AI PL TATSKRT <mark>I</mark> LSDLITVYAKGGKTIVFT <mark>R</mark> TK <mark>K</mark> DADEVSLAL <mark>TI</mark> SIA <mark>S</mark> EALEGDISQEQRERTLM
AtRH3	315	OTODEKAAEGUKAT <mark>IS</mark> HEKEM <mark>T</mark> ISDAUWAAKGOKAMATODAEVELAA <mark>S</mark> ISHA <mark>T</mark> EAREGOTEEVAA
		<u> </u>
ZmRH3A	379	GFRQGKFTVLVATDVAARGLDIPHVDL<mark>I</mark>IHTELPHDPETFVHRSGRTGRAGK<mark>AGT</mark>AILM<mark>F</mark>TSSQKRTV<mark>K</mark>SLERDVGC<mark>N</mark>FE
ZmRH3B	379	GFROGKFTVLVATDVAARGLDIPHVDLIIHIELPHDPETFVHRSGRTGRAGKAGTAILMFTSSOKRTVNSLERDVGCKFE
AUNHS	395	APROCKET/U/APD/ASCODDIEN/DD/ULDIE/PDD/ETP/EKBOKTOKAOPBOSALUAHESSOKKA/KSIDECD/OCEDE
		<u></u>
ZmRH3A	459	FISERSIEEVILESSAEHVIATLROVEFEETKYFLGAAEKLTEELOPHALASALAHLSOFSOFFESRELISHE QUVTLQL
ZmRH3B	459	FISPPSIEEVLESSAEHVIATLROVEPESTOVFLGAAEKLTEELOPHALASALAHLSOFSOPPSSRSLISTEOWVTLQL
AtRH3	475	FISPETVCDLLESSADQVVATLNGVEPDSIKFFSATAQKLTEEKGTDALAALAELSGFSQPPSSRSL <mark>LSEE</mark> XGWVTLQL
ZmRH3A	539	TREQGFCROFFSPRSVTOFLSDVCSAAADEVGKTYLTADENVQCAVFDLPEETARDIALTNELFPONTLTKTSKIAFALQDD
ZmRH3B	539	TREPGYGRGPFSPRSVTGFUSDVCSAAADEVGKIYITADENVQGAVFDLPEEIAKDMLTNEVPPONTUTKISKMPALQDD
AtRH3	555	IRDPTNARGFLSARSVTGFLSDLYRTAADEVGKIPLIADDRIGGAVFDAPEEIAKEMAEKDVPEONS <mark>AS</mark> MITAAPPLODD
		G-R-S
ZmRH3A	619	GEATDS YORFSHOROGRNNERSROOGAS KOROGNDT DE BDEFRROORSLES DNDSTSDDDMSGCORNSNESSS-FOSRES
ZmRH3B	619	SPATDSYORFSHDROSEN-RESECTORASECROCEDTDSEDR <mark>y</mark> rroceslesdnd8wsdddwsgcorksnes <mark>ss</mark> fgcr38
AtRH3	635	GINS SUN V() NYSSED-ENPERIOUS CONSTR
ZmRH3A	698	SYSERCEPSFOORSESFOCRESMREFSCACENCOESCERATOCEME
ZmRH3B	698	SYCERCEPSPSPOVRESSLOCRESSROPSCACTACCESCREA <mark>S</mark> DCPHR
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Suppl. Fig.2



WT rh3-4 emb1138/rh3-4

0.5 kb







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Suppl. Fig. 3



Suppl Fig. 4



Suppl. Fig. 5

