Programa de Doctorado en Bioingeniería
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Regulación del reciclaje del ribosoma citoplásmico por el gen *ABCE2* de Arabidopsis

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Regulación del reciclaje del ribosoma citoplásmico por el gen *ABCE2* de Arabidopsis

Trabajo realizado por la Graduada Carla Navarro Quiles, en la Unidad de Genética del Instituto de Bioingeniería de la Universidad Miguel Hernández de Elche, para optar al grado de Doctora.

Elche, 25 de junio de 2021

La presente Tesis Doctoral, titulada "Regulación del reciclaje del ribosoma citoplásmico por el gen *ABCE2* de Arabidopsis", se presenta bajo la modalidad de **tesis por compendio** de la siguiente **publicación**:

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JOSÉ LUIS MICOL MOLINA, Catedrático de Genética de la Universidad Miguel Hernández de Elche (UMH)

HAGO CONSTAR:

Que el presente trabajo ha sido realizado bajo mi dirección y recoge fielmente la labor desarrollada por la Graduada Carla Navarro Quiles para optar al grado de Doctora. Las investigaciones reflejadas en esta memoria se han desarrollado íntegramente en la Unidad de Genética del Instituto de Bioingeniería de la UMH, según los términos y condiciones definidos en el Plan de Investigación de la doctoranda, y cumpliendo los objetivos inicialmente previstos de forma satisfactoria y lo establecido en el Código de Buenas Prácticas de la UMH.

José Luis Micol Molina

Elche, 25 de junio de 2021

PIEDAD NIEVES DE AZA MOYA, Coordinadora del Programa de Doctorado en Bioingeniería de la Universidad Miguel Hernández de Elche por Resolución Rectoral 0169/17, de 1 de febrero de 2017

HACE CONSTAR:

Que da su conformidad a la presentación de la Tesis Doctoral de Doña Carla Navarro Quiles, titulada "Regulación del reciclaje del ribosoma citoplásmico por el gen *ABCE2* de Arabidopsis", que se ha desarrollado en el Programa de Doctorado en Bioingeniería bajo la dirección del profesor José Luis Micol Molina.

Lo que firmo en Elche, a instancias de la interesada y a los efectos oportunos, a veinticinco de junio de dos mil veintiuno.

Profesora PIEDAD NIEVES DE AZA MOYA
Coordinadora del Programa de Doctorado en Bioingeniería

A mon pare, ma mare i ma germana.

A David.

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I.- PREFACIO

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Siguiendo la normativa de la Universidad Miguel Hernández de Elche para la "Presentación de Tesis Doctorales por compendio de publicaciones", este documento se ha dividido en las partes siguientes:

I.- Este *Prefacio*.

II.- Un *Resumen* en español.

III.- Un *Summary* en inglés.

IV.- Una *Introducción*, en la que se presenta el tema de la Tesis y los antecedentes y objetivos del trabajo realizado.

V.- Un resumen de los *Materiales y métodos* de las publicaciones de la Tesis.

VI.- Un resumen de los *Resultados y discusión* de las publicaciones de la Tesis.

VII.- Un resumen de las *Conclusiones y perspectivas* del trabajo realizado.

VIII.- Una *Bibliografía de los apartados IV-VII*; algunas de las referencias que incluye se repiten en las bibliografías de los artículos incluidos en esta memoria.

IX.- Un apartado de *Publicaciones,* que incluye las dos siguientes, en las que se indica el factor de impacto [FI] del año correspondiente.

Navarro-Quiles, C., Mateo-Bonmatí, E., y Micol, J.L. (2018). ABCE proteins: from molecules to development. *Frontiers in Plant Science* **9**, 1-10 [FI: 4,106].

Navarro-Quiles, C., Mateo-Bonmatí, E., Candela, H., Robles, P., Martínez-Laborda, A., Ponce, M.R., y Micol, J.L. The Arabidopsis ATP-Binding Cassette E protein ABCE2 is a conserved component of the translation machinery. En preparación.

Los "Supplementary Data Sets" de este último artículo no se han incluido en esta memoria por su gran longitud. Las correspondientes hojas de cálculo se remitirán a los miembros del tribunal en formato electrónico.

X.- Un apartado de *Agradecimientos*.

Durante mi periodo predoctoral en el laboratorio de J.L. Micol también he presentado 16 comunicaciones a congresos que no se incluyen en esta Tesis, de las cuales, 9 son nacionales, y 7 internacionales, y soy primera autora en 9 de ellas.

II.- RESUMEN

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La mayoría de los genes que codifican proteínas implicadas en la traducción en *Arabidopsis thaliana* (en adelante, Arabidopsis) tienen parálogos cercanos. Sus alelos nulos son viables y causan un fenotipo morfológico débil pero distinguible del silvestre: generan hojas apuntadas e indentadas, con un patrón de venación aberrante, rasgo que se ha atribuido tradicionalmente a la perturbación de la homeostasis de la auxina.

Las secuencias de las proteínas ATP-Binding Cassette E (ABCE) están conservadas entre las arqueas y los eucariotas. Se ha demostrado en la arquea *Saccharolobus solfataricus*, la levadura *Saccharomyces cerevisiae* y la especie humana que las ABCE son esenciales para el reciclaje de los ribosomas citoplásmicos. En estas tres especies distintas y distantes, una proteína ABCE separa las dos subunidades del ribosoma tras la terminación de la traducción, y acompaña a la subunidad 30S/40S hasta la iniciación de un nuevo ciclo de síntesis de proteínas. No se ha obtenido evidencia experimental alguna de la implicación de una ABCE en la traducción en el reino vegetal.

En casi todos los genomas estudiados el gen *ABCE* es de copia única y solo los de algunos insectos, peces y plantas, como Arabidopsis, contienen dos, usualmente denominados *ABCE1* y *ABCE2*. En esta Tesis hemos estudiado el mutante *apiculata7-1* (*api7-1*) de Arabidopsis, que fue aislado en el laboratorio de J.L. Micol y es portador de un alelo puntual, hipomorfo, recesivo y viable del gen *ABCE2*. El fenotipo morfológico de *api7-1* es similar al causado por los alelos nulos viables de genes que codifican componentes de la maquinaria de la traducción. También hemos estudiado *api7-2*, un alelo insercional y letal recesivo de *ABCE2*.

Hemos caracterizado los fenotipos morfológico, histológico y molecular de *api7-1*, prestando especial atención al patrón de venación de sus órganos planos, concluyendo que está muy alterado en las hojas del primer nudo de la roseta, pero no tanto en los cotiledones, las hojas del tercer nudo y las caulinares, y que es silvestre en los sépalos y los pétalos. Dado que la biosíntesis, el transporte polar y la señalización de la auxina contribuyen a la formación del patrón de venación foliar, hemos obtenido plantas *api7-1 PIN1_{pro}:PIN1:GFP*, portadoras de una fusión traduccional de los genes que codifican el exportador de la auxina PIN-FORMED 1 (PIN1) y la proteína fluorescente verde (GFP), y *api7-1 DR5_{pro}:3XVENUS:N7*, portadoras de un transgén testigo de la señalización de la auxina. El estudio mediante microscopía confocal de las raíces de estas plantas transgénicas indicó que el transporte de la auxina está disminuido, y su percepción, incrementada. Hemos realizado un análisis global del transcriptoma de *api7-1*, concluyendo que los genes que codifican las enzimas de la ruta

principal de la biosíntesis de la auxina están desreprimidos. Nuestros resultados sugieren que una de las causas de las aberraciones de la venación foliar de *api7-1* es el exceso de auxina.

La ABCE2 de Arabidopsis contiene dos grupos hierro-azufre (FeS). Hemos constatado que los genes de respuesta a los déficits de hierro y azufre están desreprimidos en *api7-1*, posiblemente para compensar la insuficiencia de función de la ABCE2 mutante. El aumento de la concentración intracelular de hierro podría conllevar el de las especies reactivas de oxígeno, tal como sugiere la desrepresión de genes de respuesta a estrés oxidativo, que también se constata en nuestro análisis transcriptómico de *api7-1*.

Hemos obtenido un transgén *ABCE2pro:ABCE2:YFP*, que restablece el fenotipo silvestre en el mutante *api7-1*. Hemos demostrado, en un ensayo de coinmunoprecipitación, que la proteína de fusión ABCE2:YFP interacciona con varios componentes de la maquinaria de la traducción. Uno de ellos es EUKARYOTIC TRANSLATION INITIATION FACTOR 3J (eIF3j), cuyo papel como proteína accesoria de las ABCE durante el reciclaje de los ribosomas se ha demostrado en células de *Saccharomyces cerevisiae* y humanas. Esta observación sugiere que la ABCE2 de Arabidopsis —y por extensión, sus ortólogas vegetales— participa en el reciclaje del ribosoma citoplásmico.

Las regiones no traducidas 5′ (5′ UTR, de 5′ untranslated region) y 3′ UTR de los ARNm eucarióticos contienen secuencias que regulan postranscripcionalmente la expresión génica. La insuficiencia de la función de la ABCE1 de *Saccharomyces cerevisiae* y células humanas dificulta la disociación de los ribosomas, y propicia que se internen en la 3′ UTR, desplazando a su paso a los factores reguladores unidos a esta última, prolongando o acortando la vida media del ARNm. El mutante *api7-1* es un buen candidato para establecer si este fenómeno también ocurre en las plantas; de ser así, se obtendría por esta vía una confirmación adicional de que la ABCE2 de Arabidopsis participa en el reciclaje del ribosoma.

Hemos constatado que varias brasicáceas presentan dos genes *ABCE*. Nuestro análisis filogenético indica que la duplicación del correspondiente gen ancestral se produjo en las rósidas antes de la diversificación de las brasicáceas y sugiere que los genes *ABCE1* de las brasicáceas están sujetos a una menor presión selectiva que sus parálogos *ABCE2*. El transgén *ABCE2pro:ABCE1* —pero no el *ABCE1pro:ABCE2*— restablece parciamente el fenotipo silvestre en las plantas *api7-1*. *ABCE1* se expresa muy poco en Arabidopsis, siendo el órgano en que se detecta su mayor nivel la flor, cuya morfología es aparentemente silvestre en el mutante *api7-1*. Estas observaciones sugieren que las proteínas codificadas por los genes *ABCE1* y *ABCE2*, pero no sus promotores, son funcionalmente redundantes. *ABCE2* parece conservar su función ancestral, mientras que *ABCE1* está en vías de subfuncionalización o pseudogenización.

III.- SUMMARY

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Most of the genes that encode proteins involved in translation in *Arabidopsis thaliana* (hereafter referred to as Arabidopsis) have close paralogs. Their null alleles are viable and cause a morphological phenotype that is mild but distinguishable from the wild type: they produce pointed and dentate leaves, with an aberrant venation pattern usually ascribed to a perturbed auxin homeostasis.

The sequences of ATP-Binding Cassette E (ABCE) proteins are conserved among archaea and eukaryotes. It has been shown in the archaeon *Saccharolobus solfataricus*, the yeast *Saccharomyces cerevisiae* and humans, that ABCEs are essential for cytoplasmic ribosome recycling. In these three distinct and distant species, an ABCE protein dissociates the two ribosomal subunits after translation termination, and escorts the 30S/40S subunit to the initiation of a new cycle of protein synthesis. There is no experimental evidence of the involvement in translation of any ABCE from the plant kingdom.

In almost all studied genomes, *ABCE* is a single-copy gene, and only those from some insects, fishes and plants, like Arabidopsis, contain two, usually named *ABCE1* and *ABCE2*. In this Thesis, we studied the Arabidopsis *apiculata7-1* (*api7-1*) mutant, which was isolated in the laboratory of J.L. Micol, and carries a viable, recessive and hypomorphic allele of *ABCE2*. The morphological phenotype of *api7-1* is similar to those caused by viable null alleles of genes encoding components of the translation machinery. We also studied *api7-2*, an insertional, recessive lethal allele of *ABCE2*.

We characterized the morphological, histological and molecular phenotypes of *api7-1*, paying special attention to the venation pattern of its flat organs, concluding that it is severely perturbed in first-node rosette leaves, at a lesser extent in cotyledons, and third-node and cauline leaves, and wild-type in sepals and petals. Given that auxin biosynthesis, polar transport and signaling contribute to leaf venation patterning, we obtained *api7-1 PIN1_{pro}:PIN1:GFP* plants, which carry a translational fusion of the genes encoding the auxin efflux carrier PIN-FORMED 1 (PIN1) and the green fluorescent protein (GFP). We also obtained *api7-1 DR5_{pro}:3XVENUS:N7* plants, which carry a reporter transgene of auxin signaling. The study of the roots of these transgenic plants using confocal microscopy indicated that auxin transport and perception are decreased and increased, respectively. We performed an RNA-seq analysis of the *api7-1* transcriptome, concluding that the genes that encode enzymes involved in the main auxin biosynthesis pathway are upregulated. Our results suggest that an overproduction of auxin is partially responsible of the aberrations observed in the venation of *api7-1* leaves.

Arabidopsis ABCE2 contains two iron-sulfur (FeS) clusters. We observed that genes involved in the response to iron and sulfur starvation are upregulated in *api7-1*, possibly to compensate for the loss of function of the mutant ABCE2 protein. The increase in intracellular iron content might induce that of reactive oxygen species, as suggested by the upregulation of genes involved in the response to oxidative stress that we also observed in our RNA-seq of *api7-1*.

We obtained an *ABCE2$_{pro}$:ABCE2:YFP* transgene that restores the wild-type phenotype in the *api7-1* mutant. In a co-immunoprecipitation assay, we found that the ABCE2:YFP fusion protein interacts with several components of the translation machinery. One of these is EUKARYOTIC TRANSLATION INITIATION FACTOR 3J (eIF3j), whose role in ribosome recycling as an ABCE accessory factor has been described in *Saccharomyces cerevisiae* and human cells. This observation suggests that the Arabidopsis ABCE2 protein and, by extension, all its plant orthologs, participate in ribosome recycling.

The 5′ untranslated region (5′ UTR) and 3′ UTR of eukaryotic mRNAs contain sequences for the post-transcriptional regulation of gene expression. In *Saccharomyces cerevisiae* and human cells, ABCE1 loss of function hinders ribosome dissociation, and allows ribosomes to enter the 3′ UTR. In turn, the passage of the ribosomes through the 3′ UTR displaces the regulatory elements bound to that region, increasing or decreasing mRNA lifespan. The *api7-1* mutant is a suitable candidate to ascertain whether this process also occurs in plants; in that case, this would corroborate the involvement of Arabidopsis ABCE2 in ribosome recycling.

We found that several Brassicaceae species contain two *ABCE* genes. Our phylogenetic analysis indicates that the duplication of the ancestral gene occurred in Rosidae before the divergence of the Brassicaceae family. In addition, we determined that the *ABCE1* genes are under a lower evolutionary pressure than their *ABCE2* paralogs. The *ABCE2$_{pro}$:ABCE1* transgene, but not *ABCE1$_{pro}$:ABCE2*, partially restores the wild-type phenotype in *api7-1* plants. *ABCE1* expression is very low in Arabidopsis, with a maximum at the flower, whose morphological phenotype is indistinguishable from wild type in the *api7-1* mutant. These observations suggest that the proteins encoded by the *ABCE1* and *ABCE2* genes, but not their promoters, are functionally redundant. *ABCE2* seems to conserve its ancestral function, while *ABCE1* is undergoing subfunctionalization or pseudogenization.

IV.- INTRODUCCIÓN

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IV.1.- Estructura, función y clasificación de las proteínas ABC
IV.1.1.- Los transportadores ABC

No se conoce ningún ser vivo que carezca de proteínas ATP-Binding Cassette (ABC). La mayoría de los miembros de la familia ABC son proteínas de membrana y actúan como transportadores primarios de una amplia gama de sustratos, que varían tanto en tamaño como en naturaleza molecular (Theodoulou y Kerr, 2015). Los transportadores ABC contienen dos dominios transmembrana (TMD, de transmembrane domain) y dos de unión a ATP (NBD, de nucleotide-binding domain). Estos dominios corresponden a cuatro polipéptidos diferentes en las bacterias. La mayoría de los transportadores ABC eucarióticos, sin embargo, son una única proteína que contiene los cuatro dominios, o dos proteínas, cada una con un TMD y un NBD, que forman homo o heterodímeros (Higgins, 1992).

El sitio de unión al sustrato de las ABC está ubicado entre sus TMD. El transporte ocurre merced a un cambio de conformación del transportador, que acaba exponiendo su sitio de unión al sustrato hacia el lado de la membrana en el que este último es liberado (Locher, 2016). Este cambio de conformación se origina en los NBD, situados en la parte citoplásmica de la membrana, como consecuencia de su unión a dos moléculas de ATP y la ulterior hidrólisis de estas últimas, con la consiguiente liberación de ADP y fosfato inorgánico (Figura 1A, en la página 7). Los NBD tienen un origen filogenético común en todas las especies estudiadas y contienen varios motivos característicos, como los denominados Walker A y B, que también están presentes en otras proteínas de unión a ATP, y el ABC, que es exclusivo de la familia de proteínas del mismo nombre (Walker *et al.*, 1982; Holland y Blight, 1999).

Cada TMD incluye 6 hélices alfa, que atraviesan la membrana lipídica para facilitar el paso del sustrato. Aunque no están conservados filogenéticamente, los TMD comparten ciertos rasgos estructurales, en base a los cuales se ha clasificado a los transportadores ABC (Thomas *et al.*, 2020): los tipos I, II y III son importadores, y los VI y VII, exportadores y procarióticos. Los de los tipos IV y V están presentes tanto en los procariotas como en los eucariotas; en estos últimos actúan como exportadores, salvo algunas excepciones, como el importador ABCA4 humano y otros descritos en las plantas (Quazi *et al.*, 2012; Voith von Voithenberg *et al.*, 2019; Choi y Ford, 2021).

Las proteínas ABC eucarióticas también se han clasificado en subfamilias en base a sus relaciones filogenéticas y a la organización interna de sus dominios. Aunque esta
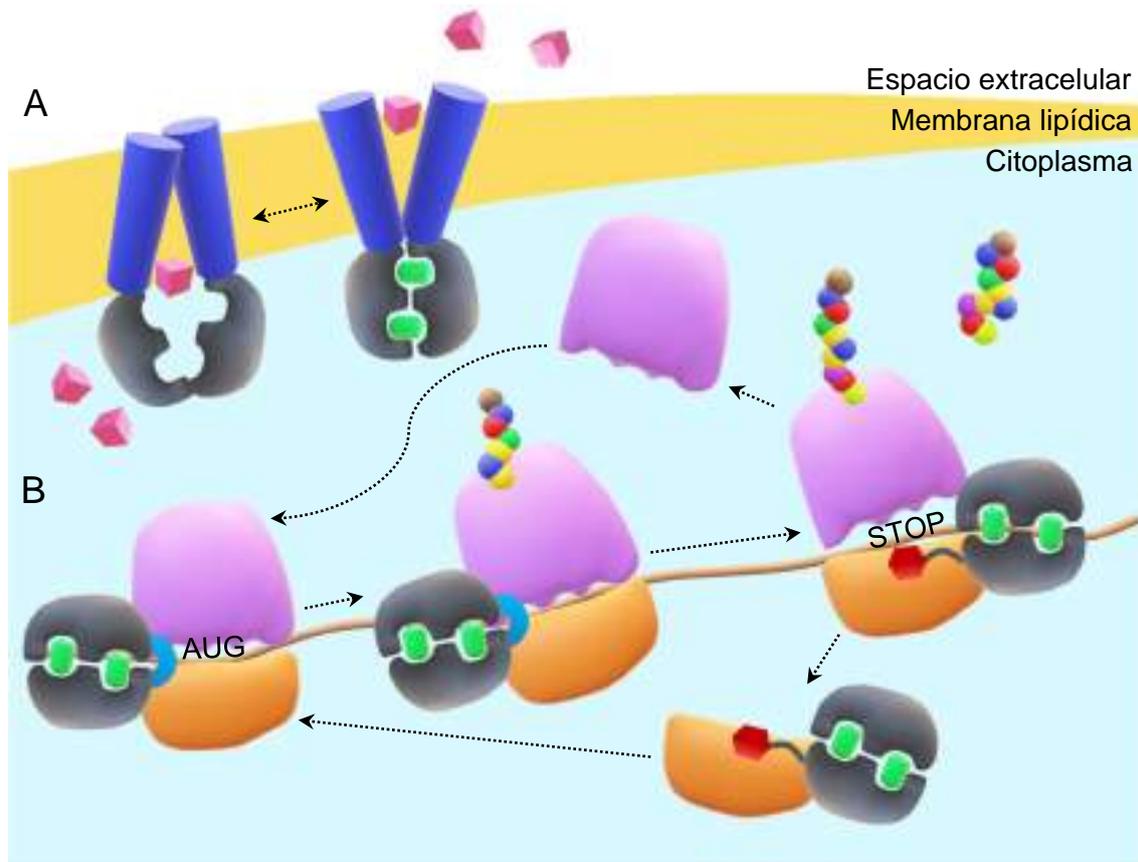
Figura 1.- Representación esquemática de las estructuras y funciones conservadas de varias proteínas ABC. (A) Transporte de un sustrato (cubos de color rosa) por un transportador ABC, cuyos TMD (cilindros azul oscuro) atraviesan la membrana lipídica (en amarillo), y los NBD (en gris) se sitúan en el citoplasma. Los NBD se cierran al unirse a dos moléculas de ATP (en verde), movilizando a los TMD, que acaban exponiendo al espacio extracelular su sitio de unión al sustrato, al que finalmente liberan. (B) Las proteínas ABC solubles participan en diferentes etapas de la traducción. Se representan dos proteínas ABCF con sus NBD unidos a dos moléculas de ATP y el motivo PtIM (en azul claro): una de estas dos proteínas (a la izquierda de la figura) facilita el reconocimiento del codón de iniciación de la traducción de un ARNm (AUG) por el ribosoma (la subunidad 60S se representa en rosa, y la 40S, en naranja), y la otra (en el centro de la figura) regula la traducción durante la síntesis de un polipéptido (cadena de esferas de varios colores). También se representan dos proteínas ABCE con sus NBD unidos a ATP y su dominio de unión a FeS (hexágono rojo): una (a la derecha de la figura) disocia un ribosoma tras el reconocimiento del codón de terminación (STOP), y la otra acompaña a una subunidad 40S para iniciar un nuevo ciclo de traducción. Las moléculas representadas no están a escala. Adaptado de (A) ter Beek *et al.* (2014) y (B) Hopfner (2016).

clasificación se propuso inicialmente para la especie humana e incluía 7 subfamilias (de la ABCA a la ABCG; Dean y Allikmets, 2001), su extensión a otras especies ha conllevado la creación de dos subfamilias más: la ABCH, que está representada en los artrópodos y los peces, y la ABCI, en las plantas (Dean y Annilo, 2005; Verrier *et al.*, 2008; Sturm *et al.*, 2009;

Popovic *et al.*, 2010). Se crearon posteriormente dos subfamilias más (ABCJ y ABCK), que incluyen transportadores descritos en las plantas no vasculares (Choi y Ford, 2021). Esta última clasificación, al contrario que la comentada en el párrafo anterior, no solo incluye a las proteínas ABC que son transportadores sino también a las que no lo son.

IV.1.2.- Las proteínas ABC solubles

Se denomina proteínas ABC solubles a las que carecen de TMD y no se asocian a membranas; se agrupan en las subfamilias ABCE y ABCF. Sus dos NBD están unidos por una región flexible, que permite su plegamiento durante la unión a dos moléculas de ATP y su hidrólisis subsiguiente. Las proteínas ABCE presentan un dominio de unión a dos grupos de hierro-azufre (FeS) de tipo $[4Fe-4S]^{2+}$ en su extremo amino (Barthelme *et al.*, 2007; Karcher *et al.*, 2008) y, las ABCF, un motivo de unión a ARNt (PtIM, de P-site tRNA interaction motif) situado entre sus dos NBD (Boël *et al.*, 2014). La subfamilia ABCE está presente en las arqueas y los eucariotas, y la ABCF, en las bacterias y los eucariotas. Ambas subfamilias suelen estar representadas por pocos genes en cada genoma, sus productos proteicos se localizan en el citoplasma, y sus funciones —conservadas en mayor o menor medida— están relacionadas con la síntesis de proteínas (Figura 1B).

Los genes *ABCE* son de copia única en la mayoría de las especies en las que se han estudiado, y solo algunos insectos, peces y plantas cuentan con dos (Liu *et al.*, 2013; Saha *et al.*, 2015; Lu *et al.*, 2016). Además, son esenciales en todos los organismos estudiados. La estructura y la función molecular de las proteínas ABCE se han estudiado fundamentalmente en la arquea *Saccharolobus solfataricus*, la levadura *Saccharomyces cerevisiae* y en células de mamíferos (Heuer *et al.*, 2017; Mancera-Martínez *et al.*, 2017; Nürenberg-Goloub *et al.*, 2018; Kratzat *et al.*, 2021). Los efectos de su insuficiencia de función sobre el desarrollo se han analizado principalmente en *Drosophila melanogaster* y *Cardamine hirsuta* (Andersen y Leevers, 2007; Kougioumoutzi *et al.*, 2013; Kashima *et al.*, 2014).

La función conservada de las ABCE es separar las dos subunidades del ribosoma tras la terminación de la traducción de una molécula de ARNm, y acompañar a la subunidad 30S/40S hasta el inicio de un nuevo ciclo de traducción. Además, las proteínas ABCE disocian los ribosomas en otros contextos, como tras el último paso de la maduración de la subunidad 40S en *Saccharomyces cerevisiae*, que ocurre en un ribosoma formado por una subunidad 60S madura y una pre-40S, en el citoplasma (Strunk *et al.*, 2012). También participan en el rescate de ribosomas inmovilizados en moléculas de ARNm aberrantes. Estos ARNm pueden contener estructuras secundarias que impiden el avance del ribosoma, o carecer de codón de terminación. Esta última circunstancia propicia que el ribosoma no se separe del ARNm y siga

recorriéndolo, internándose en la región 3′ no traducida (3′ UTR, de untranslated region) e incluso en la cola poli(A), en donde queda finalmente retenido (Inada, 2017). Aunque la relación de las proteínas ABCE con el reciclaje del ribosoma no está descrita en las plantas, se ha establecido que las proteínas ABCE1 humana y ABCE2 de Arabidopsis actúan como supresores endógenos del silenciamiento génico postranscripcional (Sarmiento *et al.*, 2006; Kärblane *et al.*, 2015; Mõttus *et al.*, 2020).

Mediante su interacción con el centro peptidil transferasa de la subunidad 50S/60S del ribosoma, las proteínas ABCF modulan diferentes etapas de la síntesis de proteínas (Fostier *et al.*, 2021). La subfamilia ABCF suele estar representada por 4 o 5 genes en cada especie estudiada (Murina *et al.*, 2019): por ejemplo, son 2 las proteínas ABCF en *Saccharomyces cerevisiae*, 3 en la especie humana y 5 en Arabidopsis. La ABCF humana más estudiada es ABCF1, que facilita el reconocimiento por el ribosoma del codón de iniciación de la traducción (Paytubi *et al.*, 2009; Stewart *et al.*, 2015). Una de las dos ABCF de *Saccharomyces cerevisiae*, A̱TP-Binding Cassette protein involved in Ṟibosome Ḇiogenesis (Arb1), actúa durante el rescate de ribosomas que han sintetizado polipéptidos aberrantes, promoviendo la liberación del peptidil-ARNt para su degradación (Su *et al.*, 2019). Además, la expresión heteróloga de la ABCF2 humana complementa el fenotipo de los mutantes *arb1* (Kachroo *et al.*, 2015), lo que demuestra la conservación de la función de Arb1 y ABCF2. La otra ABCF de *Saccharomyces cerevisiae*, ABCF3 (también llamada General Control Nonderepressible 20 [GCN20]), reduce junto con GCN1 la traducción en presencia de ARNt vacíos, causada por un déficit de aminoácidos (Marton *et al.*, 1997; Garcia-Barrio *et al.*, 2000).

Aunque las ABCF se han estudiado poco en Arabidopsis, se ha propuesto que ABCF3 (GCN20), ortóloga de la GCN20 de *Saccharomyces cerevisiae*, también participa en la adaptación a diferentes tipos de estrés mediante la regulación de la traducción (Izquierdo *et al.*, 2018). Las plantas portadoras de alelos nulos de los genes *GCN20* y *ABCF4* de Arabidopsis son más sensibles a las infecciones debido a que sus estomas no se cierran ante la presencia de patógenos, lo que sugiere una relación directa entre la regulación de la traducción y la respuesta al estrés biótico (Zeng *et al.*, 2011; Kaundal *et al.*, 2017).

IV.2.- Relación entre la formación de la venación foliar, la auxina y la traducción
IV.2.1.- Diferenciación del tejido vascular en Arabidopsis

Las venas de las gimnospermas y las angiospermas constituyen un sistema ininterrumpido de haces vasculares que distribuye agua, nutrientes y moléculas señalizadoras entre órganos distantes, y confiere resistencia mecánica a la planta. Estas venas incluyen tres elementos: el xilema, que transporta agua y minerales de las raíces a las hojas; el floema, que

distribuye nutrientes, hormonas y otras moléculas; y el procámbium, durante el crecimiento primario o longitudinal, o el cámbium, durante el secundario o en grosor, formados por células pluripotentes cuya diferenciación genera el xilema y el floema.

En Arabidopsis, el cámbium solo se forma en el hipocótilo, las raíces y la zona basal del tallo (Chaffey *et al.*, 2002; Mazur *et al.*, 2014). La disposición de estos tres elementos en el haz vascular varía según el órgano en que se encuentran, el estado de desarrollo, y la especie. Las venas de los órganos con simetría radial de Arabidopsis, como las raíces y el tallo, también adoptan esta simetría, con el xilema en la zona interna y el floema en la externa (Figura 2A, B, en la página 11). En los órganos con simetría bilateral, como las hojas, el xilema se sitúa en la región adaxial (o dorsal) y el floema, en la abaxial (o ventral; Figura 2C). En ambos casos, el procámbium se dispone entre el xilema y el floema (McConnell y Barton, 1995; Baum *et al.*, 2002).

Durante la embriogénesis de Arabidopsis, el procámbium se forma y da lugar a la venación del hipocótilo y las raíces, y tras la germinación, el meristemo apical del tallo genera las venas de los tejidos aéreos (Mähönen *et al.*, 2000; Scarpella *et al.*, 2004). En ambos casos, un aumento local en la concentración de auxina señala el inicio de la formación del tejido vascular (Mattsson *et al.*, 2003). La auxina activa al factor de transcripción AUXIN RESPONSE FACTOR 5 (ARF5), que a su vez desencadena una cascada de señalización en las células preprocambiales, que son morfológicamente indistinguibles de sus vecinas. En dicha cascada, ARF5 activa directamente la expresión de *ARABIDOPSIS THALIANA HOMEOBOX GENE 8* (*ATHB8*), que codifica un factor de transcripción que especifica la identidad procambial (Donner *et al.*, 2009). Durante la adquisición de la identidad procambial, estas células se alargan, adquieren la capacidad de drenar auxina de los tejidos colindantes y de dividirse periclinalmente, y finalmente se diferencian formando xilema o floema, aunque algunas conservan la identidad meristemática.

La diferenciación y la división de las células del procámbium se mantienen en equilibrio merced a la retroalimentación negativa causada por la señalización de la auxina y las citoquininas, que promueven la diferenciación del xilema, y la división celular y la identidad procambial, respectivamente. En el procámbium de la raíz, las citoquininas promueven la exportación de la auxina por el eflujo que llevan a cabo los transportadores PIN-FORMED (PIN) hacia las células que darán lugar al xilema. En estas últimas, la acumulación de la auxina activa simultáneamente la síntesis de las citoquininas y la transcripción de *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), cuyo producto proteico restringe la actividad de las citoquininas a las células del procámbium (Figura 2D; Mähönen *et al.*, 2006; Bishopp *et al.*, 2011; De Rybel *et al.*, 2014).
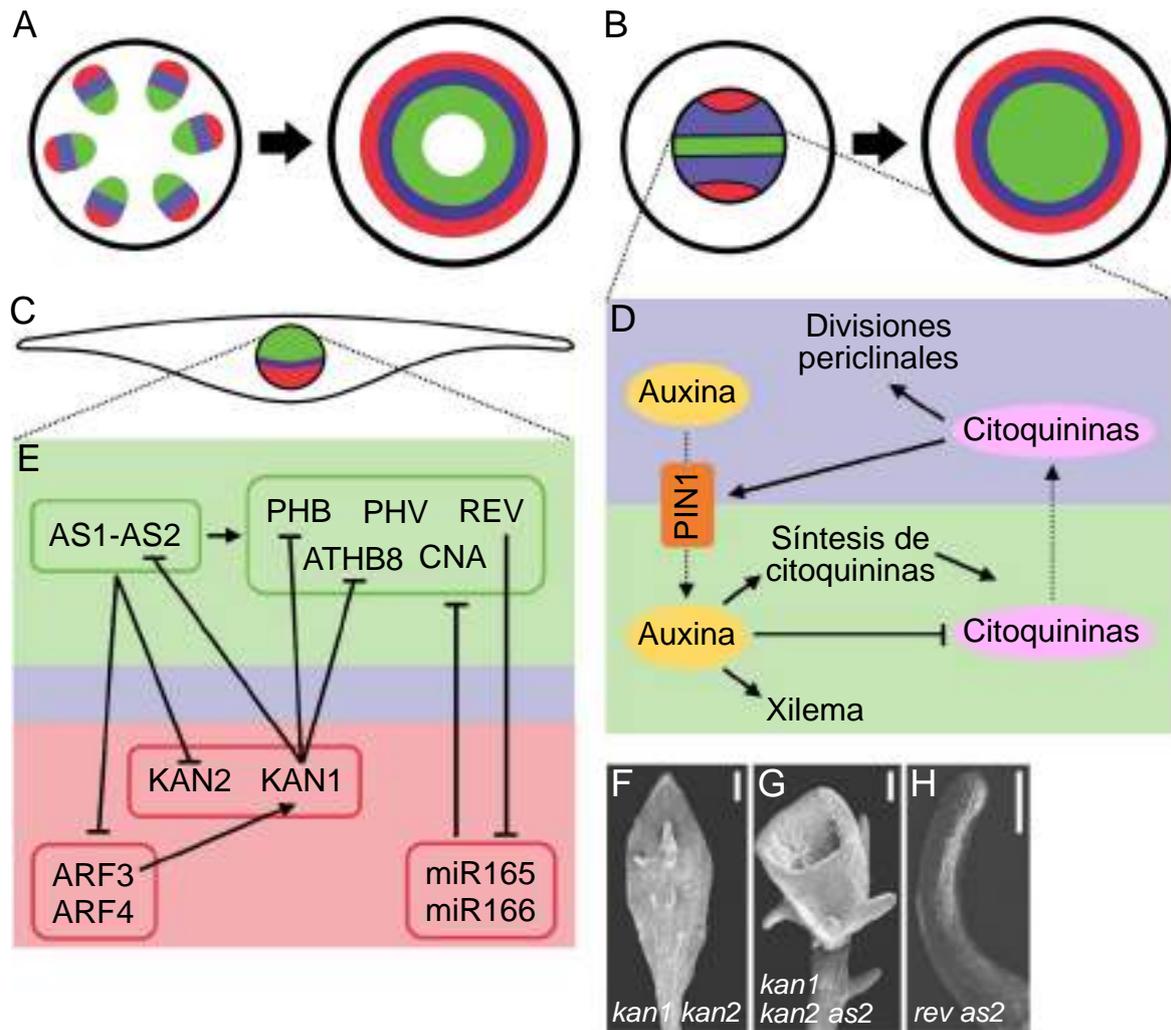
Figura 2.- Representación esquemática de la formación y disposición final de los elementos de los haces vasculares de la raíz, el tallo y las hojas de Arabidopsis. (A-C) Disposición del xilema (en verde), el floema (en morado) y el procámbium (en rojo), en cortes transversales de (A) la raíz, (B) el tallo y (C) la hoja. (A, B) Las flechas señalan la transición del crecimiento primario al secundario. (D, E) Interacciones moleculares entre los principales factores que regulan la formación del tejido vascular en (D) la raíz y (E) la hoja, en la que también se establece el eje dorsoventral. Los factores que se indican en este esquema, excepto los miARN, se representan sin cursiva porque pueden corresponder a una proteína, al gen que la codifica o a ambos. Las flechas punteadas indican flujo; las continuas terminadas en punta, activación; y las terminadas con una barra, represión de la expresión génica o, en el caso de los miARN, inhibición de su actividad. La auxina y las citoquininas se representan como elipses amarillas y rosas, respetivamente. (F-H) Pérdida de la dorsoventralidad foliar en los mutantes múltiples (F) *kan1 kan2*, (G) *kan1 kan2 as2*, y (H) *rev as2*. Las barras de escala indican (F, G) 1 mm y (H) 200 µm. Adaptado de (A-E) Campbell y Turner (2017), y (F-H) Fu *et al.* (2007).

La formación de la venación de las hojas de Arabidopsis ocurre a la vez que el desarrollo del primordio foliar, y depende de una compleja red de interacciones entre pequeños ARN, factores de transcripción de diferentes familias y otras proteínas que

contribuyen al establecimiento del eje dorsoventral de la hoja y a la disposición de los elementos vasculares a lo largo de dicho eje (Figura 2E; Iwasaki *et al.*, 2013; Ramachandran *et al.*, 2017). El núcleo de esta red de interacciones está formado por dos grupos de factores de transcripción, uno de los cuales promueve la identidad adaxial: la familia HOMEODOMAIN-LEUCINE ZIPPER III (HD-ZIP III), constituida por las proteínas REVOLUTA (REV), PHABULOSA (PHB o ATHB14), PHAVOLUTA (PHV o ATHB19), ATHB8 y CORONA (CNA o ATHB15; también llamado INCURVATA4 [ICU4]; Ohashi-Ito y Fukuda, 2003; Ochando *et al.*, 2006; Ramachandran *et al.*, 2017). El segundo grupo incluye a KANADI1 (KAN1) y KAN2, que promueven la identidad abaxial (Reinhart *et al.*, 2013).

En la red de interacciones mencionada en el párrafo anterior, ARF3 induce la expresión de *KAN1*, y el complejo formado por los factores de transcripción ASYMMETRYC LEAVES 1 (AS1) y AS2, la de *REV*, *PHB* y *PHV* (Fu *et al.*, 2007; Kelley *et al.*, 2012). A su vez, AS1 y AS2 inhiben a ARF3, ARF4 y KAN2; y KAN1 reprime a *AS2* (Iwakawa *et al.*, 2007; Wu *et al.*, 2008; Iwasaki *et al.*, 2013). Los genes de la familia HD-ZIP III son reprimidos postranscripcionalmente por los microARN (miARN) miR165 y miR166 (Zhou *et al.*, 2007), que están a su vez indirectamente reprimidos por REV (Figura 2E; Zhang y Zhang, 2012; Reinhart *et al.*, 2013). El resultado final de estas interacciones es la restricción de la expresión de los factores de identidad adaxial o abaxial a sus respectivos dominios, propiciando que la hoja se desarrolle como un órgano plano. La alteración del equilibrio entre las identidades adaxial y abaxial conduce a la pérdida de la dorsoventralidad de las hojas —y de su venación—, que en los casos más extremos adquieren una simetría radial, como en las hojas severamente abaxializadas del doble mutante *rev as2* (Figura 2H). Por el contrario, las hojas del doble mutante *kan1 kan2* se adaxializan y desarrollan protuberancias ectópicas en la cara abaxial, que se originan como consecuencia de un crecimiento perpendicular al plano mediolateral y proximodistal de la hoja (Figura 2G). El triple mutante *kan1 kan2 as2* presenta algunas hojas peltadas (con forma de copa), en las que la identidad adaxial se ve alterada y cuya cara exterior, de identidad abaxial, también desarrolla protuberancias ectópicas (Figura 2F; Fu *et al.*, 2007; Caggiano *et al.*, 2017).

IV.2.2.- Papel de la síntesis, el transporte y la señalización de la auxina en la formación del patrón de venación foliar

La complejidad del patrón de venación foliar de Arabidopsis es superior a la de los órganos con simetría radial. En efecto, además de organizar internamente sus haces vasculares respecto al eje dorsoventral de la hoja, la venación debe extenderse por todo el limbo foliar para asegurar una distribución uniforme de recursos y aportar resistencia

mecánica al órgano (Scarpella, 2017). A pesar de haber sido estudiado durante décadas (Nelson y Dengler, 1997; Berleth *et al.*, 2000; Scarpella *et al.*, 2010; Biedroń y Banasiak, 2018; ten Tusscher, 2021), son muchas la preguntas sin responder sobre la formación del patrón de venación foliar. No obstante, el desarrollo de herramientas moleculares (Baima *et al.*, 1995; Heisler *et al.*, 2005; Liao *et al.*, 2015; Yoshimoto *et al.*, 2016; Herud-Sikimić *et al.*, 2021; Pařízková *et al.*, 2021) y computacionales (Dhondt *et al.*, 2012; Bühler *et al.*, 2015; Xu *et al.*, 2021) ha permitido establecer que algunos de los procesos celulares que lo modulan son la biosíntesis, el transporte polar y la señalización de la auxina (Figura 3).
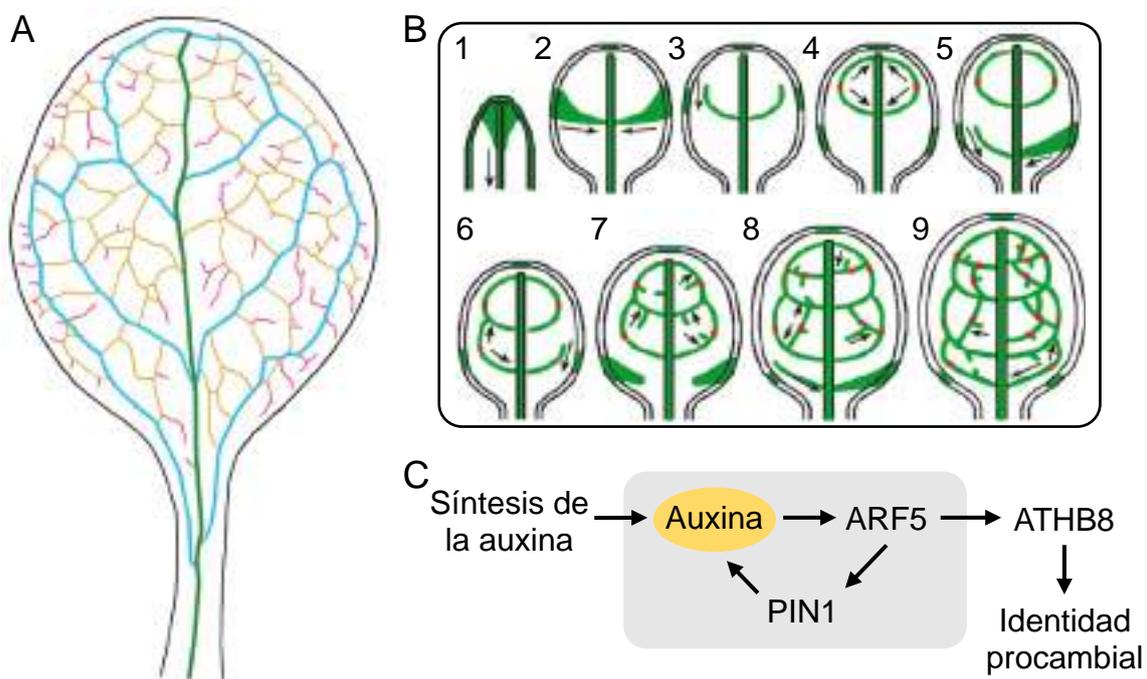
Figura 3.- Representación esquemática de la formación del patrón de venación de la hoja del primer nudo de Arabidopsis. (A) Diagrama de una hoja del primer nudo de la estirpe silvestre Landsberg *erecta* (L*er*). El margen se representa en negro; la vena primaria, en verde; las secundarias, en azul; las de órdenes superiores conectadas por sus dos extremos, en amarillo, y las de terminación libre, en rosa. (B) Dominios de expresión de PIN1 (en verde) durante la formación del patrón de venación foliar. Las flechas indican la polarización de PIN1 en la membrana plasmática de las células, y en consecuencia, la dirección en la que efluye la auxina. Los puntos rojos indican células en las que PIN1 se distribuye de forma bipolar. Los números indican el orden en que se suceden las fases. Adaptado de Biedroń y Banasiak (2018). (C) Modelo molecular que integra la síntesis de la auxina, el bucle de retroalimentación positiva entre la señalización (ARF5) y el transporte polar (PIN1) de la auxina (rectángulo gris), y la inducción de la diferenciación del tejido vascular en el primordio foliar, mediada por ATHB8.

La hoja del primer nudo de Arabidopsis ha sido la más usada como modelo para el estudio del desarrollo de la venación foliar. Su patrón de venación (Figura 3A) incluye una

vena primaria situada en el centro de la hoja, que la recorre a lo largo de su eje proximodistal, desde el ápice del limbo hasta el peciolo, en donde se conecta con la venación del tallo. De la vena primaria emergen, lateralmente y por pares, las secundarias, que se vuelven a unir a la primaria formando arcos, y de estas últimas, otras de órdenes superiores. La manifestación de las venas de sucesivos órdenes ocurre de forma gradual durante el desarrollo del primordio foliar (Candela *et al.*, 1999).

El primordio foliar del primer nudo comienza a emerger del meristemo apical del tallo unos dos días después de la germinación. En este momento también se inicia la diferenciación vascular en la zona que ocupará la vena primaria, con la generación de un máximo de auxina en el tejido subepidérmico del ápice del primordio, que se extiende en sentido basípeto, es decir, desde el ápice hacia la base (Mattsson *et al.*, 2003). Aunque se propuso que la auxina acumulada en el tejido interno procedía de la epidermis por el eflujo llevado a cabo por PIN1 (Scarpella *et al.*, 2006), se ha demostrado recientemente que la presencia de este transportador en las células epidérmicas no tiene efecto alguno sobre la formación del patrón de venación (Govindaraju *et al.*, 2020). Alternativamente, se ha propuesto que el máximo de auxina antes mencionado se debe a la síntesis local de la hormona (Kneuper *et al.*, 2021).

El incremento en la concentración de la auxina en el tejido subepidérmico activa la expresión de *ARF5*, que a su vez induce la de *PIN1* (Wenzel *et al.*, 2007; Krogan *et al.*, 2016). PIN1 se localiza en la membrana basal de estas células, que ya han adquirido identidad preprocambial, y dirige el flujo de la auxina hacia el tejido vascular preexistente del meristemo apical del tallo (fase 1 de la Figura 3B; Scarpella *et al.*, 2006). La hipótesis de la canalización de la auxina (Sachs, 1969; 1981) propone que el transporte polar de esta hormona induce la diferenciación en tejido provascular de las células que la perciben. Esta diferenciación, a su vez, incrementa la capacidad de transportar auxina de estas células y de drenarla de sus vecinas. Como consecuencia, las células preprocambiales con mayor capacidad de transportar auxina se diferencian en tejido vascular, mientras que las restantes vuelven a un estado indiferenciado para formar finalmente parte del mesófilo (Sachs, 1981; Scarpella *et al.*, 2004; Donner *et al.*, 2009). Según la hipótesis de la canalización, las células con mayores concentraciones de PIN1 transportan más auxina, de modo que el flujo de la hormona se va restringiendo a un número cada vez menor de filas de células. El aumento en la concentración de la auxina en estas últimas incrementa la expresión de *ARF5*, y a su vez, la de *PIN1*, creando un bucle de retroalimentación positiva (Wenzel *et al.*, 2007). Finalmente, tal como se ha descrito en el apartado IV.2.1, en la página 9, ARF5 induce la expresión de *ATHB8*, que especifica la identidad procambial y, posteriormente, la diferenciación vascular (Figura 3C; Donner *et al.*, 2009).

El proceso descrito en el párrafo anterior se repite en las venas de órdenes superiores (fase 2 y siguientes de la Figura 3B), con una peculiaridad: dado que durante la especificación de la identidad vascular de una célula, PIN1 está polarizado en su membrana hacia la vena preexistente adyacente, las que están conectadas por sus dos extremos se forman en dos etapas consecutivas. En efecto, PIN1 se polariza en sentidos opuestos y siempre hacia la vena preexistente más cercana en cada etapa (Scarpella *et al.*, 2006). Por ejemplo, el primer par de venas secundarias, las más apicales, comienza a manifestarse un día después que la vena primaria. En una primera etapa, la aparición de un máximo de auxina a cada lado del margen foliar induce la expresión de *PIN1*, que se extiende de forma basolateral hasta la vena primaria, iniciando la diferenciación vascular en las células que formarán la parte basal de la secundaria; en estas últimas células, PIN1 está polarizado hacia la vena primaria (fase 2). En una segunda etapa, la expresión de PIN1 en el margen se hace indetectable (fase 3) y comienza a manifestarse gradualmente en sentido acrópeto, en la zona en la que se formará la parte apical de la vena secundaria. En las primeras células en las que se expresa PIN1 en esta segunda etapa, esta proteína está polarizada hacia la vena secundaria en formación; cuando su dominio de expresión se acerca a la vena primaria, PIN1 se polariza hacia esta última. De esta forma, las células provasculares que darán lugar a la vena secundaria se agrupan en dos dominios, en los que PIN1 está polarizado en sentidos opuestos, y que están unidos por una única célula en la que el transportador se localiza de forma bipolar (fase 4 de la Figura 3B; Scarpella *et al.*, 2006).

Aunque son muchas las observaciones que apoyan la hipótesis de la canalización de la auxina, aún quedan preguntas por responder sobre la formación de las venas. Una de ellas es cuál es el mecanismo que dirige a PIN1 hacia un lado concreto de la membrana plasmática de cada célula, que además coordina su polarización entre células vecinas. Se sabe que la auxina induce la transcripción de *PIN1*, y se han descrito procesos que modulan la actividad de PIN1 y su presencia en la membrana plasmática, pero se desconoce cómo la auxina distribuye polarmente a esta proteína (ten Tusscher, 2021).

Paradójicamente, se ha demostrado también que el transporte polar de la auxina mediado por las proteínas PIN es innecesario para la formación de un patrón de venación foliar reproducible (Verna *et al.*, 2019). En ausencia de los transportadores PIN, el patrón de venación es más denso que el silvestre cerca del margen y a lo largo de la vena primaria, cuyos elementos vasculares se disponen correctamente a lo largo del eje proximodistal. Esta observación sugiere que el transporte polar de la auxina no es necesario para que las células vasculares se alineen formando venas (Ravichandran *et al.*, 2020). Tampoco parece necesaria la señalización de la auxina, pues su inhibición parcial solo conlleva una reducción

en el número de venas de órdenes superiores, manteniéndose el patrón de venación silvestre (Verna *et al.*, 2019). Sin embargo, cuando se inhiben simultáneamente la señalización de la auxina de forma parcial y su transporte polar mediado por los transportadores PIN, aparece un tercer patrón de venación, también reproducible, con agrupaciones de células vasculares desorganizadas en los puntos de convergencia entre venas (Verna *et al.*, 2019). Esto sugiere que la señalización y el transporte polar de la auxina actúan de modo redundante en la formación del patrón de venación, ya que solo bloqueándolos a la vez se perturba la orientación de sus células.

Para explicar la formación de un patrón de venación en ausencia de los transportadores PIN, se ha propuesto que la auxina podría transportarse de forma polar mediante difusión facilitada por los plasmodesmos (Verna *et al.*, 2019; Ravichandran *et al.*, 2020), o que la síntesis local de la hormona desempeña un papel más relevante del que se le había atribuido (Kneuper *et al.*, 2021). Por otro lado, y dado que la señalización de la auxina es inherentemente no direccional, se desconoce cómo contribuye a la orientación de las células vasculares durante la formación de las venas (Ravichandran *et al.*, 2020).

IV.2.3.- Papel regulador de las regiones no traducidas (UTR) de los ARNm eucarióticos
IV.2.3.1.- Regulación mediada por las uORF de las 5′ UTR

Algunos ARNm cuentan con pautas de lectura abierta (ORF, de open reading frame) en su 5′ UTR, a las que se denomina uORF (de upstream ORF). Las uORF participan en la regulación postranscripcional de la expresión génica, ya que dificultan la traducción de la ORF principal (mORF, de main ORF). Tras la traducción de una uORF, la de la mORF adyacente requiere la así denominada reiniciación de la traducción: la terminación de la traducción de la uORF se interrumpe antes de que la subunidad 40S del ribosoma se separe del ARNm, y se unen al complejo de la traducción un nuevo metionil-ARNt y factores de iniciación; estos últimos hacen posible que la subunidad 40S reinicie la lectura del ARNm en busca del codón de iniciación de la mORF (von Arnim *et al.*, 2014).

Se ha estimado que un 35% de los genes de Arabidopsis contienen una o más uORF, algunas de las cuales codifican péptidos de secuencia conservada (Kim *et al.*, 2007; von Arnim *et al.*, 2014). La presencia o ausencia de las uORF en los ARNm parece estar relacionada con la naturaleza del producto de la mORF de estos últimos. Los ARNm cuyas mORF codifican proteínas implicadas en rutas de señalización suelen contener uORF, que no aparecen en la mayoría de los que codifican proteínas domésticas (Kim *et al.*, 2007; Hu *et al.*, 2016). Se han descrito en Arabidopsis dos mecanismos por los que una uORF puede regular

la traducción de la mORF adyacente: según la secuencia del péptido que codifica la uORF o su longitud. En el primer caso, el péptido naciente detiene el avance del ribosoma durante la fase de elongación de su traducción, al establecer interacciones con el túnel de salida del ribosoma (Ebina *et al.*, 2015; Hayashi *et al.*, 2017). El péptido naciente también puede interaccionar con metabolitos como la sacarosa, el ascorbato o algunas poliaminas que impiden que el ribosoma finalice la traducción, regulando así su propio metabolismo (Laing *et al.*, 2015; Yamashita *et al.*, 2017; van der Horst *et al.*, 2020). En el segundo caso, se ha estimado que la traducción de las uORF de más de 48 nt evita la reiniciación de la traducción de la mORF situada aguas abajo, como consecuencia de la pérdida progresiva de factores de iniciación (David-Assael *et al.*, 2005; von Arnim *et al.*, 2014; Schepetilnikov y Ryabova, 2017).

El establecimiento del eje dorsoventral de las hojas y la formación de su patrón de venación acusan especialmente los efectos de la disfunción de la traducción en Arabidopsis (Tabla 1). Muchas mutaciones viables en genes que codifican proteínas ribosómicas reducen el tamaño de la roseta, cuyas dos primeras hojas se hacen apuntadas, y las restantes, indentadas (Byrne, 2009). También alteran el patrón de venación foliar, aparentemente como consecuencia de la perturbación de la homeostasis de la auxina. Las combinaciones de estas mutaciones con alelos de insuficiencia de función de los genes *AS1* y *AS2* rinden fenotipos

Tabla 1.- Genes que codifican proteínas relacionadas con la traducción, cuyas mutaciones alteran la regulación de la expresión génica mediada por las uORF y la formación del patrón de venación foliar

Gen (código AGI)	Transcritos con uORF cuya traducción se ve alterada	Referencias
*EIF3H** (At1g10840)	*ARF2, ARF3, ARF4, ARF5, ARF6, ARF7, ARF11, CLV1*	1, 2, 3, 4
RACK1 (At1g18080)	*SAC51*	5, 6
RPL4A (At3g09630)	*SAC51*	5, 6
RPL10A (At1g14320)	*SAC51*	5, 7
*RPL24B** (At3g53020)	*ARF3*	2, 8, 9
*RPL4D** (At5g02870)	*ARF3, ARF5, ARF7*	10, 11
*RPL5A** (At3g25520)	*ARF3, ARF5, ARF7*	9, 10, 11, 12
*RPL5B** (At5g39740)	-	9, 10, 13
*RPL7B** (At2g01250)	-	10
*RPL28A** (At2g19730)	-	9
*RPS6A** (At4g31700)	-	10

*Sus alelos de insuficiencia de función rinden fenotipos sinérgicos en combinaciones dobles mutantes con los de *AS1* o *AS2*. [1]Kim *et al.* (2007); [2]Zhou *et al.* (2010); [3]Schepetilnikov *et al.* (2013); [4]Zhou *et al.* (2014); [5]Imai *et al.* (2006); [6]Kakehi *et al.* (2015); [7]Imai *et al.* (2008); [8]Nishimura *et al.* (2005); [9]Yao *et al.* (2008); [10]Horiguchi *et al.* (2011); [11]Rosado *et al.* (2012); [12]Pinon *et al.* (2008); y [13]Van Minnebruggen *et al.* (2010).

sinérgicos: los dobles mutantes presentan diferentes grados de pérdida de la dorsoventralidad foliar, similares a los causados por los alelos hipomorfos y nulos de los genes de identidad dorsoventral (Figura 2F-H, en la página 11; Nishimura *et al.*, 2005; Pinon *et al.*, 2008; Yao *et al.*, 2008; Van Minnebruggen *et al.*, 2010; Horiguchi *et al.*, 2011; Rosado *et al.*, 2012).

Los mutantes *ribosomal protein l4d* (*rpl4d*) y *rpl5a* sirven de ejemplo de que la pérdida de función de algunas proteínas ribosómicas altera la regulación postranscripcional mediada por las uORF de los ARNm que codifican factores de transcripción de respuesta a la auxina. Se han obtenido construcciones en las que las uORF de los genes *ARF3*, *ARF5* o *ARF7* se han dispuesto aguas arriba de un gen testigo, cuya traducción dificultan, efecto que se potencia en los mutantes *rpl4d* y *rpl5a* (Rosado *et al.*, 2012). La mutación *rpl24b* también disminuye la eficacia de la reiniciación de la traducción de la mORF de *ARF3*. Además, la expresión de una variante truncada del ARNm de *ARF3*, que carece de su uORF, restablece parcialmente el fenotipo silvestre en el mutante *rpl24b* (Nishimura *et al.*, 2005).

Otro ejemplo de que la traducción de una uORF disminuye la eficacia de la maquinaria de la traducción lo proporcionan las mutaciones en el gen *EUKARYOTIC TRANSLATION INITIATION FACTOR 3H* (*EIF3H*) de Arabidopsis. La proteína eIF3h es necesaria para que la auxina promueva la traducción de moléculas de ARNm que contienen uORF. La auxina activa a la quinasa TARGET OF RAPAMYCIN (TOR), que a su vez fosforila a eIF3h. Esta fosforilación mantiene al complejo eIF3 unido al ribosoma durante la traducción de una uORF, propiciando así la reiniciación de la traducción en la mORF adyacente (Kim *et al.*, 2007; Schepetilnikov *et al.*, 2013). No obstante, la pérdida de función de eIF3h no solo altera la traducción de los ARNm que codifican ARF, ya que los dos alelos hipomorfos de *EIF3H* que se han descrito perturban severamente el desarrollo: un 75% de las plantas homocigóticas para dichos alelos no alcanza la etapa reproductiva, y las que lo consiguen presentan una fertilidad reducida (Kim *et al.*, 2004; Zhou *et al.*, 2014). La mitad de las plantas *eif3h* presentan hojas radializadas, como consecuencia del desarrollo aberrante del meristemo apical del tallo que a su vez se debe, al menos en parte, a una traducción insuficiente de la mORF del ARNm de *CLAVATA1* (*CLV1*), que contiene cuatro uORF; la proteína CLV1 regula la actividad del meristemo apical del tallo (Clark *et al.*, 1997; Zhou *et al.*, 2014).

La termoespermidina es una poliamina producida por la termoespermidina sintasa ACAULIS 5 (ACL5; Knott *et al.*, 2007) e inhibe la diferenciación del tejido vascular, al contrario que la auxina. Su implicación en la regulación de la diferenciación vascular se descubrió mediante una mutagénesis de segundos sitios, que se realizó con el objetivo de encontrar supresores extragénicos del fenotipo del mutante *acl5-1*, que presenta una proliferación excesiva del xilema, causada por la desrepresión de los cinco genes HD-ZIP III (Clay y Nelson,

2005; Imai *et al.*, 2006). Tras dicha mutagénesis se aislaron una mutación dominante, *suppressor of acaulis 51* (*sac51-d*), y tres semidominantes (*sac52-d*, *sac53-d* y *sac56-d*), en cuatro genes diferentes, todas las cuales rescatan parcialmente el fenotipo vascular de *acl5-1* y restablecen los niveles de expresión de los genes HD-ZIP III (Imai *et al.*, 2006).

El gen *SAC51* codifica un factor de transcripción de la superfamilia bHLH (basic helix-loop-helix; Imai *et al.*, 2006). La mutación *sac51-d* crea un codón de terminación prematuro en una de las cinco uORF del ARNm de *SAC51*. Las mutaciones *sac52-d*, *sac56-d* y *sac53-d* dañan genes que codifican dos proteínas ribosómicas (RPL10A y RPL4A) y un componente de la subunidad 40S (RECEPTOR FOR ACTIVATED C KINASE 1 A [RACK1]), respectivamente. Estas cuatro mutaciones facilitan la traducción de la mORF de *SAC51* en el mutante *acl5-1* (Imai *et al.*, 2008; Kakehi *et al.*, 2015), lo que indica que la termoespermidina producida por ACL5 facilita la traducción de la mORF de *SAC51*, y que el efecto inhibidor de las uORF de este último se ve contrarrestado por las perturbaciones de la maquinaria de la traducción. Dado que la termoespermidina sintetizada por ACL5 promueve la traducción del factor de transcripción SAC51, se ha propuesto que este último es un intermediario de la represión de los genes HD-ZIP III por ACL5 (Takahashi, 2018). ATHB8, a su vez, induce la expresión de *ACL5*, estableciendo un sistema que regula la diferenciación del tejido vascular (Figura 4; Baima *et al.*, 2014).
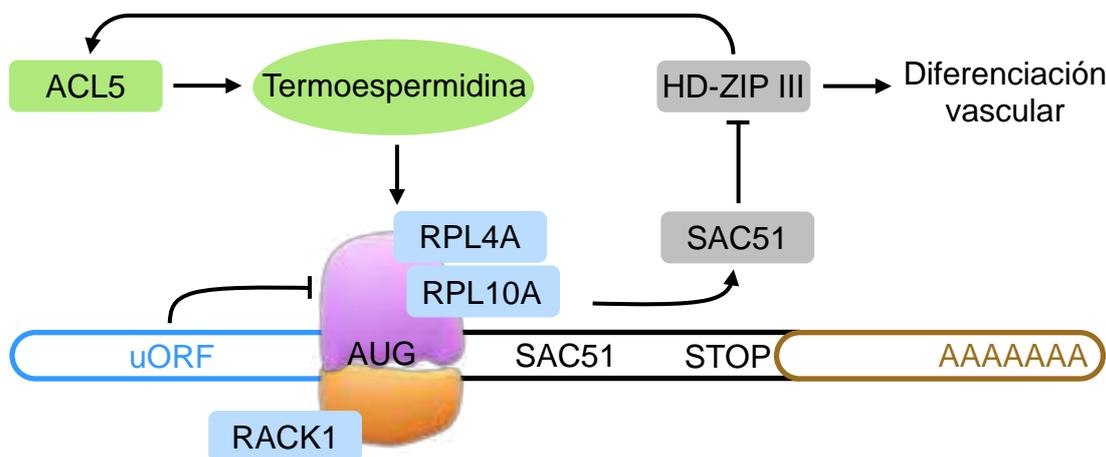
Figura 4.- Representación esquemática del mecanismo de acción de la termoespermidina sobre la regulación postranscripcional de *SAC51* y la diferenciación vascular. La termoespermidina (elipse verde) sintetizada por ACL5 (rectángulo verde) promueve la traducción de la mORF de *SAC51* (región negra del ARNm), que es a su vez inhibida por la de una de las cinco uORF situadas aguas arriba (región en azul). El efecto inhibidor de estas uORF depende del correcto funcionamiento del ribosoma, en concreto, de las proteínas RPL4A, RPL10A y RACK1 (rectángulos en azul). El factor de transcripción SAC51 inhibe la expresión de los cinco genes HD-ZIP III, cuyos productos proteicos promueven la diferenciación vascular, así como la expresión de *ACL5*.

IV.2.3.2.- Regulación mediada por las 3′ UTR

El único mecanismo molecular conocido que vincula las alteraciones de la maquinaria de la traducción con las aberraciones de la venación foliar en Arabidopsis es la regulación postranscripcional mediada por las uORF (Tabla 1, en la página 17). No obstante, las 3′ UTR también participan en mecanismos de regulación postranscripcional que modulan la vida media de los ARNm y, en consecuencia, sus niveles y los de los productos de su traducción. Se ha descrito en Arabidopsis que la presencia de N^6-metiladenosinas (m^6A) en la 3′ UTR de los ARNm incrementa su vida media, mientras que la unión de complejos de unión de exones (EJC, de exon-junction complex) la disminuye (Anderson *et al.*, 2018; Ohtani y Wachter, 2019; Wu *et al.*, 2020). Este segundo proceso ocurre mediante la degradación del ARNm mediada por mutaciones sin sentido (NMD, de nonsense-mediated decay), y está conservado en los eucariotas (Ohtani y Wachter, 2019). Un ejemplo de NMD se da en el ARNm del *EUKARYOTIC RELEASE FACTOR 1* (*eRF1*) de Arabidopsis, que codifica un factor de terminación de la traducción que regula su propia traducción. La presencia de un EJC en la 3′ UTR del ARNm de *eRF1* reduce su vida media, y también su traducción. Cuando los niveles de la proteína eRF1 disminuyen en exceso, también lo hace la eficacia de la terminación de la traducción de su ARNm, por lo que el ribosoma tiende a no disociarse. Como consecuencia, el ribosoma se interna en la 3′ UTR y desplaza al EJC, incrementando la vida media del ARNm de *eRF1* y a su vez el número de proteínas eRF1 (Nyikó *et al.*, 2017).

La proteína eRF1 está conservada en todos los eucariotas y las arqueas (aRF1), y requiere la actividad de una proteína ABCE para llevar a cabo la terminación de la traducción (Nürenberg y Tampé, 2013). De hecho, en *Saccharomyces cerevisiae* y en células humanas la insuficiencia de la función de ABCE1 conlleva la reiniciación de la traducción en la 3′ UTR, y que el ribosoma desplace los factores reguladores que encuentra a su paso en esta región, alterando la regulación postranscripcional (Young *et al.*, 2015; Zhu *et al.*, 2020).

No se ha descrito relación alguna entre la regulación postranscripcional mediada por las 3′ UTR y el desarrollo de la venación foliar. Sin embargo, el mutante *simple leaf3* (*sil3*) de la brasicácea *Cardamine hirsuta*, portador de una mutación en un gen que codifica una proteína ABCE, presenta alteraciones en la morfología y la venación de sus hojas, similares a las de los mutantes de Arabidopsis cuya maquinaria de la traducción está alterada. Aunque las hojas del tipo silvestre de *Cardamine hirsuta* son compuestas, las del mutante *sil3* son simples y presentan un menor número de venas de órdenes superiores y de venas de terminación libre. Se cree que la causa es la alteración de la homeostasis de la auxina, hormona que no solo promueve la diferenciación del tejido vascular, sino también la formación de los foliolos durante el desarrollo del primordio foliar (Kougioumoutzi *et al.*, 2013).

Dado el alto grado de conservación de los mecanismos de terminación de la traducción y de reciclaje de ribosomas entre los eucariotas y las arqueas, es verosímil que el fenotipo morfológico y fisiológico del mutante *sil3* se deba, al menos en parte, a una alteración de la regulación postranscripcional mediada por las 3′ UTR, lo que indicaría que existe un mecanismo de regulación postranscripcional del desarrollo de la venación foliar adicional al mediado por las uORF.

IV.3.- Antecedentes y objetivos
IV.3.1.- Un abordaje mutacional al estudio del desarrollo foliar

La Organización de las Naciones Unidas declaró el 2020 como Año Internacional de la Sanidad Vegetal, a fin de concienciar a la población sobre la importancia de proteger la salud de las plantas. Entre otros, son dos los motivos principales por los que las plantas deberían recibir una gran atención: porque son la base de nuestra alimentación, y una herramienta imprescindible para reducir el paulatino incremento de la concentración de CO_2 en nuestra atmósfera como consecuencia del uso masivo de combustibles fósiles. En ambos aspectos las hojas desempeñan un papel clave ya que, mediante la fotosíntesis, fijan el CO_2 atmosférico y lo convierten en materia orgánica que la planta usará para su desarrollo y reproducción. De esta manera, el conocimiento generado por el estudio del desarrollo foliar sienta las bases para incrementar la capacidad fotosintética de las plantas y, en el caso de las especies de interés agronómico, su rendimiento (Micol, 2009).

Con el fin de realizar una disección genética del desarrollo foliar, se han llevado a cabo en el laboratorio de J.L. Micol varias búsquedas de mutantes de Arabidopsis que fueran viables y fértiles, y presentaran alteraciones en la morfología de sus hojas. Los mutantes identificados se agruparon en 19 clases fenotípicas en base a la forma, el tamaño y la pigmentación de sus hojas, y se asignaron a 116 grupos de complementación (Berná *et al.*, 1999; Robles Ramos, 1999; Serrano-Cartagena *et al.*, 1999). Desde entonces, se han identificado en el laboratorio de J.L. Micol las mutaciones causantes de los fenotipos de más de 60 de estos mutantes, que han resultado afectar a procesos tan diversos como el transporte o la señalización de la auxina (Pérez-Pérez *et al.*, 2010; Esteve-Bruna *et al.*, 2013; Karampelias *et al.*, 2016), la contribución del cloroplasto y la mitocondria al desarrollo foliar (Quesada *et al.*, 2011; Casanova-Sáez *et al.*, 2014b; Muñoz-Nortes *et al.*, 2017), la biogénesis del ribosoma y la traducción (Van Minnebruggen *et al.*, 2010; Horiguchi *et al.*, 2011; Casanova-Sáez *et al.*, 2014a), el transporte nucleocitoplásmico (Ferrández-Ayela *et al.*, 2013), o la regulación epigenética (Mateo-Bonmatí *et al.*, 2018).

IV.3.2.- Los mutantes *apiculata*

Los rasgos más característicos del fenotipo morfológico de los alelos mutantes de muchos de los genes que codifican componentes de la maquinaria de la traducción son las hojas de la roseta pequeñas, apuntadas e indentadas, a diferencia de las de los tipos silvestres de uso más común, que son más redondeadas y de margen total o casi totalmente liso. Comparten estos rasgos fenotípicos los mutantes aislados en el laboratorio de J.L. Micol que se incluyeron en las clases fenotípicas a las que se denominó Apiculata (Api) y Denticulata (Den; Figura 5). De hecho, 5 de los 6 genes *API* y *DEN* que fueron clonados posicionalmente con anterioridad a esta Tesis codifican proteínas ribosómicas (Horiguchi *et al.*, 2011; Casanova-Sáez *et al.*, 2014a) y, el sexto, un factor de biogénesis del ribosoma (Micol-Ponce *et al.*, 2020).
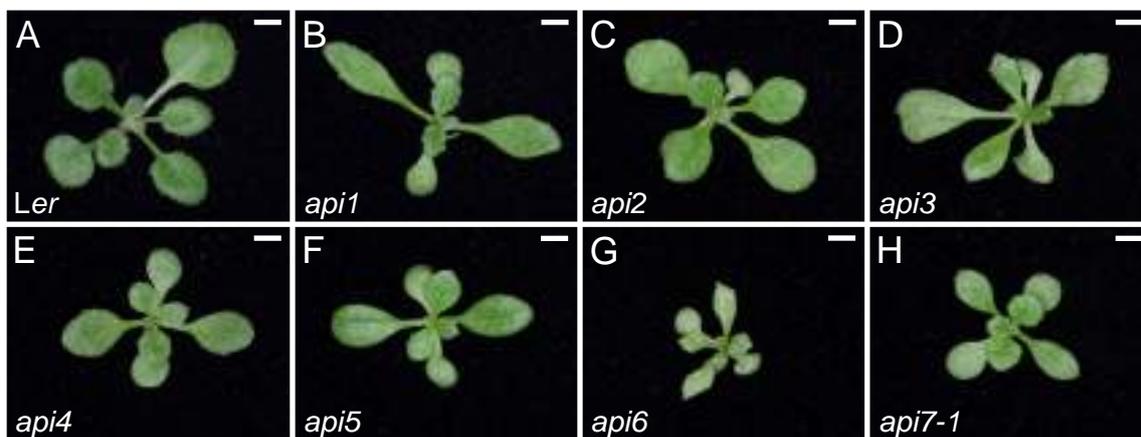
Figura 5.- Fenotipo foliar de los mutantes *apiculata*. Rosetas de (A) la estirpe silvestre L*er*, y de los mutantes (B) *api1*, (C) *api2*, (D) *api3*, (E) *api4*, (F) *api5*, (G) *api6* y (H) *api7-1*. Las fotografías se tomaron 14 dde. Las barras de escala representan 2 mm. Adaptado de Casanova Sáez (2014).

La clase fenotípica Apiculata incluye a 8 mutantes que fueron aislados tras una mutagénesis con metanosulfonato de etilo (EMS) del acceso Landsberg *erecta* (L*er*), que corresponden a 7 genes (*API1-API7*; Berná *et al.*, 1999). Aunque se conocen las posiciones de mapa de todos los genes *API*, solo se han clonado posicionalmente *API2* y *API7* (Robles y Micol, 2001; Casanova-Sáez *et al.*, 2014a). El gen *API2* (también denominado *RPL36a*) y su parálogo *RPL36aA* son funcionalmente redundantes: ambos codifican la proteína ribosómica RPL36a de la subunidad 60S del ribosoma citoplásmico, y la no complementación no alélica de los alelos hipomorfos *api2* y *rpl36aa* en plantas diheterocigóticas *API2/api2;RPL36aA/rpl36aa* rinde el mismo fenotipo que el de los mutantes simples homocigóticos (Figura 5C; Casanova-Sáez *et al.*, 2014a). El gen al que se denominó

inicialmente *API7* en el laboratorio de J.L. Micol es *ABCE2* y codifica una proteína que es esencial para el reciclaje del ribosoma citoplásmico en las arqueas y los eucariotas. El único alelo viable de *ABCE2* descrito es *api7-1*, cuyo fenotipo foliar es similar al de los alelos de insuficiencia de función de genes que codifican componentes de la maquinaria de la traducción (Figura 5H).

IV.3.3.- Objetivos de esta Tesis

Los dos objetivos generales definidos inicialmente para esta Tesis fueron completar la caracterización fenotípica del mutante *api7-1*, previamente iniciada en el laboratorio de J.L. Micol, y determinar, con el máximo grado de detalle posible, la función del gen *ABCE2* mediante abordajes genéticos y moleculares.

En concreto, nos propusimos: (1) revisar el estado del arte sobre la función de las proteínas ABCE en las especies en las que se hubiese estudiado; (2) confirmar que el gen *ABCE2* es el causante exclusivo del fenotipo del mutante *api7-1*, mediante la obtención de alelos adicionales de este gen y su análisis de complementación; (3) establecer la localización subcelular de la proteína ABCE2, mediante la construcción y visualización de la expresión de fusiones traduccionales del gen *ABCE2* y los de determinadas proteínas fluorescentes; (4) determinar el patrón de expresión espacial de *ABCE2* y *ABCE1*; (5) estudiar la eventual redundancia funcional de estos dos genes parálogos, mediante el intercambio de sus promotores; (6) llevar a cabo un análisis filogenético de *ABCE1* y *ABCE2*; (7) identificar proteínas interactoras de ABCE2 que aportasen información sobre la participación de esta última en la traducción; (8) completar el estudio del fenotipo de *api7-1* a niveles morfológico, histoquímico y molecular, (9) prestando especial atención al patrón vascular de sus hojas; (10) estudiar una posible alteración de la homeostasis de la auxina en *api7-1*, visualizando la expresión de transgenes testigo de su transporte y percepción; (11) identificar otros procesos biológicos potencialmente alterados en *api7-1* mediante la secuenciación masiva de su transcriptoma; y (12) proponer un modelo que explicase la relación entre la función molecular de ABCE2 y los efectos fenotípicos causados por la pérdida parcial de su función en el mutante *api7-1*.

V.- MATERIALES Y MÉTODOS

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Para la redacción de los apartados I a VII de esta memoria se han seguido las mismas pautas que en Tesis anteriores de los laboratorios de M.R. Ponce y J.L. Micol. En este apartado de Materiales y métodos se reproducen literalmente algunas frases procedentes de dichas Tesis. Se ha preferido usar los acrónimos castellanizados ADN y ARN —de uso común en los medios de comunicación españoles—, en lugar de los recomendados por la International Union of Pure and Applied Chemistry, DNA y RNA, para los ácidos desoxirribonucleico y ribonucleico, respectivamente. Esta elección no está basada en ningún argumento que se considere incontestable; ambas opciones son aceptadas por el *Diccionario de la Lengua Española* (vigesimotercera edición, 2014) de la Real Academia Española (RAE). Tal como recomienda la RAE en su *Ortografía de la lengua española* (2010), en esta memoria no se realiza el plural de las siglas añadiendo al final una s minúscula: se escribe "el ARN" y también "los ARN".

La nomenclatura que se aplica en esta memoria a genes, mutaciones y fenotipos nuevos se atiene a las pautas propuestas para Arabidopsis por Meinke y Koornneef (1997). No hemos traducido al español muchos de los nombres de genes y proteínas que se mencionan en esta memoria; en estos casos solo hemos usado la cursiva para los genes. Los transgenes se denotan según lo establecido en las instrucciones a los autores de la revista *Plant Cell.* Los genotipos completos, como *api7-1/api7-2*, en los que los alelos de un gen en cromosomas homólogos se separan con una barra, se han utilizado únicamente cuando fue imprescindible. Salvo que se indique lo contrario, los individuos que se describen en este trabajo son homocigóticos para la mutación que se menciona en cada caso. Hemos utilizado en algunos casos un punto y coma como separador entre mutaciones no alélicas.

Las estirpes de Arabidopsis, su manipulación y las condiciones de cultivo usadas en esta Tesis se describen en la página 53. Hemos realizado análisis histológicos y morfométricos, histoquímicos y de microscopía confocal de los mutantes a estudio (en las páginas 54-56). Hemos aislado ARN para su retrotranscripción seguida de PCR cuantitativa y para su secuenciación masiva (en las páginas 56 y 57). Hemos generado construcciones de intercambio de promotores, y fusiones traduccionales con los genes de las proteínas fluorescentes verde y amarilla (en la página 54). También hemos realizado experimentos de coinmunoprecipitación de proteínas (en la página 58) y alineamientos de secuencias aminoacídicas, por parejas y múltiples, así como otros análisis bioinformáticos y búsquedas *in silico* (en la página 59).

VI.- RESULTADOS Y DISCUSIÓN

VI.- RESULTADOS Y DISCUSIÓN

En esta Tesis se ha realizado una disección genética y molecular de la función del gen *ABCE2* de Arabidopsis. Hemos confirmado mediante ensayos de complementación que *api7-1* es un alelo hipomorfo y viable, y *api7-2*, nulo y letal recesivo, de dicho gen (en la página 60). Hemos determinado los patrones de expresión de *ABCE2* y de su único parálogo, *ABCE1*, concluyendo que *ABCE2* es de expresión constitutiva y generalizada y que *ABCE1* se expresa muy poco (en la página 62). Mediante un ensayo de intercambio de promotores hemos comprobado que el transgén *ABCE2pro:ABCE1*, pero no el *ABCE1pro:ABCE2*, restablece parcialmente el fenotipo silvestre en las plantas *api7-1* (en la página 62).

Hemos estudiado el fenotipo de *api7-1* mediante la determinación de la longitud de su raíz principal, que es más corta que la silvestre; la cuantificación de los pigmentos fotosintéticos y los niveles de ploidía de las hojas de su roseta, que están reducidos e incrementados, respectivamente; el análisis de la filotaxia de la roseta, que es indistinguible de la silvestre; y la disección y observación de sus frutos, estableciendo que la fertilidad de *api7-1* es similar a la silvestre (en la página 60). Además, hemos analizado el patrón vascular de sus cotiledones, hojas del primer y tercer nudos de la roseta, hojas caulinares, sépalos y pétalos; las mayores diferencias con los órganos silvestres se apreciaron en las hojas del primer nudo de la roseta (en la página 65). En estas últimas se observó una venación más densa en torno al hidatodo apical y menos venas de órdenes superiores en el mutante *api7-1* que en su tipo silvestre. Hemos visualizado el transporte y la percepción de la auxina en plantas portadoras de transgenes testigo, concluyendo que en *api7-1* están disminuido e incrementada, respectivamente (en la página 66). También hemos secuenciado masivamente el transcriptoma de *api7-1*, constatando la desregulación de procesos biológicos relacionados con el metabolismo y la señalización de la auxina, las respuestas al déficit de iones de hierro y azufre, y el estrés oxidativo (en la página 67).

Hemos demostrado que ABCE2 es una proteína citoplásmica, mediante la obtención de fusiones traduccionales *ABCE2:GFP* y *ABCE2:YFP* y la visualización de las proteínas híbridas correspondientes (en la página 63). Hemos realizado un ensayo de coinmunoprecipitación con la proteína de fusión ABCE2:YFP, identificado 20 presuntos interactores, la mayoría de los cuales están relacionados con la traducción (en la página 63), lo que sugiere que ABCE2 participa en este proceso (Figura 6, en la página 26). La interacción de ABCE2 con eIF3j que hemos observado en Arabidopsis también se ha constatado entre sus ortólogas en *Saccharomyces cerevisiae* y humanas; en estas especies se ha demostrado

que las ortólogas de ABCE2 disocian al ribosoma durante el reciclaje de los ribosomas (Young y Guydosh, 2019; Kratzat *et al.*, 2021).
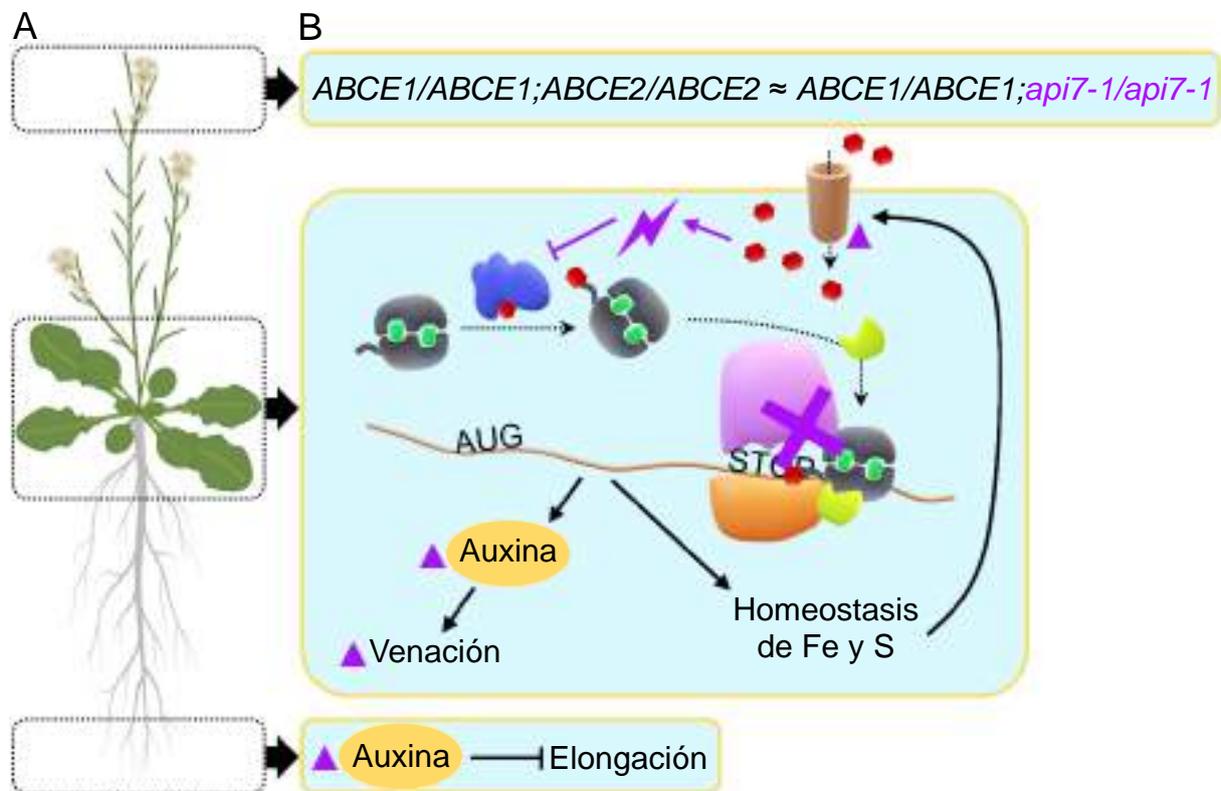
Figura 6.- Hipótesis sobre la función de la proteína ABCE2 y los efectos de la mutación *api7-1*. (A) Representación de la estructura de una planta adulta de Arabidopsis, con indicación, mediante rectángulos punteados, de los órganos del mutante *api7-1* estudiados en esta Tesis. Modificada a partir de Norris *et al.* (2021). (B) Modelo que proponemos para la función de ABCE2, representada como en la Figura 1, en la página 7. En una situación de insuficiencia de la función de ABCE2 como la que causa la mutación *api7-1*, ABCE1 podría mantener el fenotipo silvestre en las inflorescencias, pero no en las hojas o las raíces. La proteína codificada por At2g20830 (en azul) ensambla los grupos FeS en ABCE2. La subunidad eIF3j (en amarillo) contribuye con ABCE2 a la disociación del ribosoma. El ribosoma y el ARNm se representan como en la Figura 1; la auxina y las flechas, como en la Figura 2, en la página 11. Los elementos morados indican los efectos de la mutación *api7-1*: ✗: consecuencia directa; ▲: incremento; ⚡ : especies reactivas del oxígeno.

Las proteínas ABCE tienen un dominio de unión a dos grupos FeS que las diferencia de las ABC solubles y es esencial para su función en la disociación de los ribosomas (Barthelme *et al.*, 2007). Nuestros resultados sugieren que ABCE2 también tiene un dominio de unión a FeS funcional. En efecto, ABCE2 interacciona con la proteína codificada por el gen At2g20830, que en otras especies es necesaria para el ensamblaje de grupos FeS (Zhai *et al.*, 2014; Paul *et al.*, 2015), y en el mutante *api7-1* está aparentemente activada la respuesta

al déficit de iones de hierro y azufre, quizás como consecuencia de la reducida actividad de la proteína ABCE2 mutante. Adicionalmente, un incremento intracelular de estos iones podría inducir la generación de especies reactivas del oxígeno (Briat *et al.*, 2010), tal como sugiere la activación de la respuesta al estrés oxidativo que hemos observado en *api7-1*. A su vez, el estrés oxidativo podría impedir el ensamblaje de grupos FeS en la proteína ABCE2 mutante, disminuyendo aún más su actividad (Alhebshi *et al.*, 2012; Sudmant *et al.*, 2018; Zhu *et al.*, 2020).

En el ápice de los primordios foliares se sintetiza la auxina de forma local, y su transporte polar basípeto induce la formación de los haces vasculares en todo el limbo (Linh *et al.*, 2018; Kneuper *et al.*, 2021). Un incremento en la síntesis de auxina en los primordios de *api7-1*, tal como sugieren los resultados de nuestro análisis transcriptómico, junto con una presunta disminución en su transporte polar, podrían ser las causas de la desigual diferenciación del tejido vascular observada a lo largo del eje proximodistal en las hojas de *api7-1*, y de su menor contenido en venas de órdenes superiores. Además, una producción excesiva de auxina en las raíces de *api7-1* podría inhibir su elongación (Fendrych *et al.*, 2018).

Por último, nuestros resultados sugieren que existe cierto grado de redundancia funcional entre *ABCE1* y *ABCE2*: *ABCE1* rescata parcialmente el fenotipo de *api7-1* cuando se expresa bajo el control del promotor de *ABCE2*. Que este rescate no sea completo puede deberse a que la secuencia de ABCE1, al igual que las de sus ortólogas en otras brasicáceas, está sujeta a una menor presión selectiva que sus parálogas ABCE2. No obstante, el órgano que muestra una mayor expresión de *ABCE1*, la flor, no presenta alteraciones evidentes en el mutante *api7-1*, que tampoco se aprecian en los patrones de venación de los sépalos y pétalos, lo que sugiere que *ABCE1* mantiene parte de su función ancestral.

VII.- CONCLUSIONES Y PERSPECTIVAS

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VII.1.- Las proteínas ABCE de las plantas participan en la traducción

Las proteínas ABCE, presentes en los eucariotas y las arqueas, llevan a cabo el reciclaje de los ribosomas citoplásmicos, según se ha demostrado en algunas arqueas, levaduras y animales. En esta Tesis se han obtenido por primera vez indicios de la implicación de una proteína ABCE vegetal, la ABCE2 de Arabidopsis, en la traducción. Se requerirán ensayos adicionales para confirmar dicha implicación, como la secuenciación masiva de segmentos de ARNm unidos a ribosomas de *api7-1*. Este ensayo consiste en la digestión mediante una RNasa de un extracto de polisomas, y la posterior secuenciación masiva de los fragmentos de las moléculas de ARNm que no hayan sido digeridas como consecuencia de su unión a un ribosoma. Pueden identificarse así las posiciones en las que se encuentran los ribosomas en todas las moléculas de ARNm de una muestra. Se ha demostrado de este modo en células humanas y de *Saccharomyces cerevisiae* que la insuficiencia de la función de ABCE1 dificulta el reciclaje de los ribosomas, que tras la terminación de la traducción se internan en la 3′ UTR, en la que desplazan los factores reguladores unidos al ARNm que encuentran a su paso.

Nuestros resultados también indican la existencia de redundancia funcional entre las proteínas ABCE2 y ABCE1, pero no entre los promotores de los genes que las codifican. La menor presión selectiva que parecen haber sufrido los genes *ABCE1* de las brasicáceas, así como el bajo nivel de expresión de *ABCE1* en Arabidopsis, sugieren que este último —y probablemente también sus ortólogos más próximos— ha sufrido un proceso de subfuncionalización o pseudogenización, mientras que ABCE2 mantiene la función ancestral. La obtención y el estudio de alelos mutantes de *ABCE1*, y de considerarse conveniente, la determinación de la localización subcelular de ABCE1 y la identificación de sus interactores, permitirían comprender su función, si es que la tiene.

VII.2.- La caracterización del mutante *api7-1* contribuye a la comprensión de la relación entre la traducción y el desarrollo

Una parte de esta Tesis es la revisión bibliográfica de los efectos sobre el desarrollo de la pérdida de la función de las proteínas ABCE, concluyéndose que estas proteínas son esenciales en todos los organismos estudiados, y que la mayoría de los alelos mutantes de los genes *ABCE* disponibles son nulos y letales recesivos, lo que dificulta el estudio de los efectos de la insuficiencia de función de estos genes durante el desarrollo. En esta memoria se describe un estudio de *api7-1*, un alelo hipomorfo del gen *ABCE2* de Arabidopsis, cuyo

fenotipo incluye algunos rasgos comunes a los asociados a la insuficiencia de función de genes que codifican componentes de la maquinaria de la traducción: una roseta pequeña con hojas apuntadas e indentadas y con una venación aberrante. Un fenotipo similar a este es el que causa *sil3*, un alelo hipomorfo del único gen *ABCE* identificado en *Cardamine hirsuta*, cuyas hojas son simples, mientras que las del tipo silvestre son compuestas, y presentan alteraciones en su venación. Autores anteriores han relacionado estos rasgos fenotípicos con la perturbación de la homeostasis de la auxina. La secuenciación masiva del transcriptoma de *api7-1* sugiere que sintetiza más auxina que el tipo silvestre. La cuantificación en *api7-1* del contenido en ácido indolacético —la auxina mayoritaria en las plantas— y sus metabolitos permitiría establecer si las alteraciones del transcriptoma que hemos constatado tienen consecuencias, tal como cabe esperar, en el metaboloma.

Además de las alteraciones relacionadas con la homeostasis de la auxina, nuestro análisis de enriquecimiento funcional del transcriptoma de *api7-1* identificó más de 200 procesos alterados en este mutante, entre los que destacan, por su relación con los grupos FeS de ABCE2, las respuestas a los déficits de hierro y azufre, y al estrés oxidativo. El gran número de procesos alterados en *api7-1* impide establecer cuáles son consecuencia directa de la pérdida de función de ABCE2. También en este aspecto la obtención de un perfil ribosómico de *api7-1* permitiría confirmar el papel de ABCE2 en la disociación de los ribosomas, así como identificar los ARNm más sensibles a la perturbación de este proceso.

VIII.- BIBLIOGRAFÍA
DE LOS APARTADOS IV-VII

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IX.- PUBLICACIONES

ABCE Proteins: From Molecules to Development

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Most members of the large family of ATP-Binding Cassette (ABC) proteins function as membrane transporters. However, the most evolutionarily conserved group, the ABCE protein subfamily, comprises soluble proteins that were initially denoted RNase L inhibitor (RLI) proteins. ABCE proteins are present in all eukaryotes and archaea and are encoded by a single gene in most genomes, or by two genes in a few cases. Functional analysis of *ABCE* genes, primarily in *Saccharomyces cerevisiae*, has shown that ABCE proteins have essential functions as part of the translational apparatus. In this review, we summarize the current understanding of ABCE protein function in ribosome biogenesis and recycling, with a particular focus on their known and proposed developmental roles in different species. The ABCE proteins might represent another class of factors contributing to the role of the ribosome in gene expression regulation.

Keywords: ABCE, ribosome, translation, development, RLI

ABC PROTEIN STRUCTURE, FUNCTION, AND CLASSIFICATION

The ATP-Binding Cassette (ABC) proteins, which are present in all living organisms, constitute one of the largest known protein families. Actually, in prokaryotes, the ABC genes constitute 1–3% of the genome (Tomii and Kanehisa, 1998). The *Saccharomyces cerevisiae* and human genomes encode 30 and 48 ABC proteins, respectively (Dean et al., 2001; Paumi et al., 2009; Vasiliou et al., 2009). By contrast, in plants like *Arabidopsis thaliana*, there are more than 100 genes encoding ABC proteins (Verrier et al., 2008). Such multiplication and functional diversification of ABC proteins is consistent with the sessile nature of plants and their adaptation to changing terrestrial environments, as well as with the history of whole-genome duplications in plant evolution (Hwang et al., 2016).

Most ABC proteins transport solutes across cell membranes. These solutes, referred to as allocrites (Holland and Blight, 1999), range from small inorganic and organic molecules to large organic compounds. ABC transporters contain two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs), otherwise known as ATP-binding cassettes, which are hallmarks of the ABC family. TMDs and NBDs can be contained together in a unique full-sized protein, as commonly found in eukaryotes, or can be separated into individual peptides (subunits), as observed in prokaryotes. ABC proteins can also occur as homo- or heterodimers formed by half-sized proteins, which contain one TMD and one NBD or consist only of fused NBDs (Hyde et al., 1990). TMDs are responsible for allocrite specificity and have polyphyletic origins. TMDs belonging to a specific transporter subtype display similar membrane topologies among distant species. Each TMD typically comprises 6–10 α-helices that span the cell membrane, thus generating a pore that is accessible from the cytoplasm or from the extracellular space (Wang et al., 2009; Zheng et al., 2013).

FIGURE 1 | Schematic representation of ABC proteins. **(A)** An ABC transporter, and **(B)** an ABCE soluble protein. A lipid bilayer is depicted in pale orange, and the cytoplasm, in pale blue. Transmembrane domains (TMD) are shown in purple and nucleotide binding domains (NBD), arranged in a head-to-tail orientation, in green. The two diamagnetic [4Fe-4S]$^{2+}$ clusters are depicted in brown. Adapted from Hopfner (2016).

In contrast to TMDs, NBDs are monophyletic with conserved sequences and structures (Higgins et al., 1986). The NBD regions that display the highest level of conservation are those that function specifically in ATP binding and hydrolysis, namely the Walker A and Walker B motifs; the LSGGQ signature or ABC motif; and the A-, D-, H-, and Q-loops, named after the conserved residues at their N- or C-termini (ter Beek et al., 2014). NBDs are arranged in a head-to-tail orientation, which allows them to bind two ATP molecules. These ATP molecules interact with motifs from both NBDs in a sandwich-like manner, bringing NBDs together in a closed conformation. ATP cleavage (hydrolysis) relaxes this conformation and drives the transport cycle by producing coupled conformational changes in the TMDs that recognize and translocate the allocrite across the membrane. Whether both NBDs stay in contact (continuous contact models) or totally separate (NBDs separation models) after ATP hydrolysis remains a matter of active debate. However, since there are several ABC transporter subtypes, it is reasonable to assume that more than one transport mechanism exists (Shi and Barna, 2015).

To facilitate ABC protein recognition and comparison among different species, a standardized nomenclature was proposed for all eukaryotes (Dean and Annilo, 2005; Verrier et al., 2008; Paumi et al., 2009; Xie et al., 2012; Dermauw and Van Leeuwen, 2014). Mammalian ABC proteins were divided into seven subfamilies (ABCA to ABCG) based on NBD sequence similarity (Dean et al., 2001). Two additional subfamilies were later proposed for

non-mammalian ABC proteins, specifically the ABCH subfamily, which is found in insects and osteichthyes (Dean and Annilo, 2005), and the ABCI subfamily, which is found exclusively in plants and contains prokaryotic-like ABC proteins, among others (Verrier et al., 2008). ABCA, ABCB, ABCC, ABCD, ABCG, and ABCH proteins function as transporters (**Figure 1A**; Hopfner, 2016).

By contrast, soluble ABC proteins belong to the ABCE and ABCF subfamilies, which lack TMDs but have the two NBDs in a single peptide chain (**Figure 1B**). ABCF proteins function in translation, as exemplified by eukaryotic elongation factor 3 (eEF3) (Andersen et al., 2006), or in chromosome segregation and DNA repair, as is the case for the Structural Maintenance of Chromosomes (SMC) proteins and the SMC-like protein Rad50 (Hirano, 2002). Here, we focus on the ABCE subfamily, which includes single-copy genes that, although initially thought to be specific to mammals (Bisbal et al., 1995), are found in both eukaryotes and archaea.

THE ABCE SUBFAMILY OF ABC PROTEINS

ABCE proteins display the highest level of conservation among the ABC subfamilies. For example, the yeast ABCE1/Rli1 shares 68 and 43% sequence identity with its human and archaeal (*Sulfolobus solfataricus*) orthologs, respectively (Kispal et al., 2005; Barthelme et al., 2007). The ABCE subfamily is represented in most genomes by a single-copy, essential *ABCE1* gene (Kerr, 2004). However, two *ABCE* paralogs, specifically *ABCE1/RLI1* and *ABCE2/RLI2*, exist in plants such as *Arabidopsis thaliana* and *Oryza sativa* (Sarmiento et al., 2006; Verrier et al., 2008), and in animals such as catfish (Liu et al., 2013) and the mosquitoes *Anopheles gambiae*, *Aedes aegypti*, and *Culex pipiens quinquefasciatus* (Lu et al., 2016).

Loss-of-function of *ABCE1* genes, either via null alleles or RNAi suppression, is associated with a lethal phenotype in all studied species, and hypomorphic *ABCE1* alleles result in slow-growth phenotypes (Amsterdam et al., 2004; Dong et al., 2004; Estévez et al., 2004; Zhao et al., 2004; Coelho et al., 2005a; Kispal et al., 2005; Chen et al., 2006; Barthelme et al., 2007; Broehan et al., 2013; Kougioumoutzi et al., 2013; **Table 1**). Conversely, in *Saccharomyces cerevisiae*, *RLI1* overexpression leading to accumulation of either the wild-type protein or mutated versions disrupted in conserved residues or lacking entire conserved domains caused a dominant negative effect on growth (Dong et al., 2004; Khoshnevis et al., 2010).

Determination of the crystal structure of archaeal ABCE1 (aABCE1) proteins (Karcher et al., 2005, 2008; Barthelme et al., 2011) showed that these proteins contain four conserved domains: (1–2) the two NBDs that are present in all ABC proteins; (3) a hinge region proposed to facilitate NBD orientation and function as a pivot point in the tweezer-like power stroke of NBDs following ATP binding (Karcher et al., 2005); and (4) an iron-sulfur (FeS) binding domain with eight cysteine residues that coordinate two diamagnetic [4Fe-4S]$^{2+}$ clusters present at the ABCE protein N-terminal region (**Figure 1B**; Barthelme et al., 2007; Karcher et al., 2008). The

TABLE 1 | Mutations affecting *ABCE* genes in different species.

Organism	Gene name	Loss of function caused by	Phenotype	References
Drosophila melanogaster	*pixie*	Strong hypomorphic alleles	Recessive lethal	Coelho et al., 2005a,b
		Weak hypomorphic alleles	Slow growth; disproportionate organ sizes; slender bristles; eye roughening	Coelho et al., 2005a,b
Caenorhabditis elegans	*abce-1*	RNAi	Slow growth; embryonic lethality	Kamath et al., 2003; Zhao et al., 2004
Danio rerio	*abce1*	Retroviral insertional allele	Small head and eyes; underdeveloped liver and gut; pericardial edema; lethal at 5 days post-fertilization	Amsterdam et al., 2004
Xenopus laevis	*abce1*	Antisense *ABCE1* morpholino oligonucleotides	Arrested growth at the gastrula stage	Chen et al., 2006
Cardamine hirsuta	*SIL3*; *ChRLI2*	Hypomorphic allele	Reduced growth; small and simple leaves; delayed leaf initiation; reduced auxin signaling; reduced cell proliferation; high rates of endoreplication	Kougioumoutzi et al., 2013
Nicotiana benthamiana	*RLIh*	Virus-induced gene silencing	Reduced growth; distorted leaves; whitened veins; reduced cell size and number	Petersen et al., 2004

latter domain plays an essential role in ABCE protein function (Kispal et al., 2005; Yarunin et al., 2005; Barthelme et al., 2007, 2011; Alhebshi et al., 2012). The high-level conservation among all ABCE amino acid sequences, in particular within their four conserved domains, allows to deduce the structure of eukaryotic ABCE orthologs based on that of aABCE1 (Karcher et al., 2005, 2008). Moreover, cryoelectron microscopy showed that *Pyrococcus furiosus* aABCE1 and yeast Rli1 associate similarly with ribosomes (Becker et al., 2012; Preis et al., 2014).

The function of the yeast ABCE1 protein Rli1 has been well characterized. Furthermore, based on the sequence conservation, ABCE1 protein function is likely to be well conserved among different organisms. Yeast Rli1 triggers the dissociation of ribosomes during different processes related to translation, as described in further detail below (**Figure 2**). Additional roles have been proposed for ABCE proteins in higher eukaryotes. For example, ABCE1 participates in the assembly of immature HIV-1 capsids in mammals (Zimmerman et al., 2002; Dooher and Lingappa, 2004), and ABCE proteins act as endogenous suppressors of RNA silencing in plants and humans (Sarmiento et al., 2006; Kärblane et al., 2015).

YEAST ABCE1/RLI1 FUNCTIONS IN RIBOSOME BIOGENESIS AND RECYCLING

The *Saccharomyces cerevisiae* genome encodes 30 ABC proteins, including one member of the ABCE subfamily, Rli1. *RLI1* encodes a canonical ABCE protein and contains two NBDs arranged in a head-to-tail manner (thus allowing the binding of two ATP molecules), and a FeS domain (Barthelme et al., 2007). The *Drosophila melanogaster* Rli1 ortholog, Pixie, localizes exclusively in the cytoplasm (Coelho et al., 2005a), whereas yeast Rli1 localizes in the cytoplasm and in the nucleus (Dong et al., 2004; Kispal et al., 2005; Yarunin et al., 2005).

Yeast Rli1 associates with eukaryotic translation initiation factors, 40S ribosomal subunits, 80S ribosomes, and polysomes. Suppression of *Rli1* strongly reduces polysome size and abundance, as well as translation rates (Dong et al., 2004; Kispal et al., 2005; Yarunin et al., 2005; Shoemaker and Green, 2011). These observations suggested that Rli1 participates in translation initiation by promoting assembly of the preinitiation complex. Equivalent observations were made for human ABCE1 (Chen et al., 2006) and *Drosophila* Pixie (Andersen and Leevers, 2007). Additionally, ABCE proteins in *Trypanosoma brucei* (Estévez et al., 2004) and *Caenorhabditis elegans* (Zhao et al., 2004) were also implicated in translation. A role for yeast Rli1 and mammalian ABCE1 in translation initiation was confirmed following analysis of the 48S initiation and the post-splitting complexes (Heuer et al., 2017; Mancera-Martínez et al., 2017).

The role of Rli1 as a ribosome biogenesis factor is supported by the nuclear accumulation of 40S and 60S ribosome subunits when Rli1 function is compromised (Kispal et al., 2005; Yarunin et al., 2005). Nevertheless, the most well-known Rli1 function is that of ribosome disassembly (**Figure 2**). Indeed, Rli1 facilitates ribosomal subunit dissociation through its direct interaction with the class I release factor Supressor 45 (Sup45), otherwise known as eukaryotic release factor 1 (eRF1) or its paralog Duplication of Multilocus region 34 (Dom34). Such interaction was confirmed by affinity pull-down, coimmunoprecipitation, and yeast two-hybrid analyses (Khoshnevis et al., 2010; Shoemaker and Green, 2011).

Yeast Rli1 Participates in Ribosome Recycling

Translation termination occurs when the stop codon of an mRNA enters the A site of the associated ribosome, after which the tRNA-mimicking eRF1 recognizes the stop codon via codon-anticodon recognition using its conserved NIKS (Asn-Ile-Lys-Ser) motif and occupies the A site of the ribosome (Song et al., 2000). Following this, the GTP bound to the eRF1-linked class II release factor Sup35/eRF3 is hydrolyzed, thus dissociating the post-termination complex.

During stop codon recognition, eRF3 has been proposed to facilitate the interaction between eRF1 and the ribosome.

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FIGURE 2 | Rli1 ribosome-dissociation activity has essential functions in diverse cellular processes, including ribosome maturation and release of stalled ribosomes. Schematic representation of a maturing ribosome formed by 60S (blue) and pre-40S (orange) subunits. Mature 40S subunits are depicted in pink. A faulty 40S subunit is represented by pink parallel lines. Class I release factors Dom34 (orange) and eRF1 (dark blue) dissociate ribosomes together with Rli1, whose NBDs are represented in green, and the FeS domain in brown. The "clamping" factor Stm1 is depicted in purple. The no-go decay mRNA contains a secondary structure represented by a red rhombus. The non-stop mRNA lacks an in-frame stop codon, depicted by a dashed line. Arrows indicate ribosome association-dissociation flow.

This function is thought to resemble delivery of aminoacylated tRNA to the ribosome A site during peptide elongation, which is performed by the eRF3 paralog eEF1α (Inagaki et al., 2003; Salas-Marco and Bedwell, 2004; des Georges et al., 2014). In an alternative scenario, DEAD-box protein 5 (Dbp5), an RNA helicase that participates in mRNA export, recruits eRF3·GTP to the ribosome following eRF1 stop codon recognition (Gross et al., 2007). Regardless of the exact protein-protein interactions, eRF3 must break down GTP and leave the ribosome to allow Rli1 binding (Shoemaker and Green, 2011; Preis et al., 2014).

The kinetic analysis of an *in vitro* reconstituted yeast translation system demonstrated that Rli1 induces eRF1 ribosome accommodation in an ATP-independent manner. Ribosome accommodation allows eRF1 to catalyze peptidyl-tRNA hydrolysis through its conserved GGQ (Gly-Gly-Gln) motif, which releases the newly synthesized peptide. ATP hydrolysis driven by Rli1 promotes ribosome subunit dissociation, demonstrating that eukaryotic translation termination and ribosome recycling are combined within the same release factor-mediated process. Such combination contrasts with the separation of the two processes observed in bacteria (Shoemaker and Green, 2011). In this manner, the 60S subunit is disassembled from the 40S subunit, which is

then released from the deacylated tRNA and mRNA molecules. During all this process of ribosome recycling, Rli1 remains bound to the 40S subunit and has been suggested to preclude 60S rejoining until a late-stage in the initiation complex (Heuer et al., 2017; Mancera-Martínez et al., 2017). In the case of archaea, it has been proposed that ribosome dissociation is caused by a conformational change following aABCE1-ribosome interaction, and that ATP hydrolysis is required to separate aABCE1 from the 30S subunit following ribosome dissociation (Barthelme et al., 2011; Kiosze-Becker et al., 2016).

Termination of translation can be inefficient. One of the known causes of inefficient translation termination is the continued association of ribosomes with defective mRNA molecules, which impairs translation and produces potentially deleterious peptides. To circumvent this, different mRNA surveillance pathways can degrade defective mRNA molecules and their translation products, and recycle the associated ribosomes. These mRNA surveillance pathways have been extensively reviewed (Franckenberg et al., 2012; Graille and Séraphin, 2012; Shoemaker and Green, 2012; Brandman and Hegde, 2016), so they are only briefly discussed here.

Each of the three primary mRNA surveillance mechanisms targets a different cause of ribosome stalling and Rli1 participates

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in all three mechanisms. In no-go decay (NGD), a physical obstruction slows down or stops ribosome progression on the mRNA molecule. Physical obstructions can include an inhibitory secondary structure, chemical damage, or a polybasic sequence within the nascent protein (Doma and Parker, 2006; Kuroha et al., 2010). Non-stop decay (NSD) occurs when the mRNA lacks a genuine stop codon, possibly due to truncation or premature polyadenylation of the mRNA molecule. In NSD, the ribosome continues translation until it encounters an in-frame stop codon on the 3′ UTR or comes to the poly(A) mRNA sequence (tail). Translation of the poly(A) tail generates a positively charged poly-lysine region that disturbs ribosome movement by interacting with its negatively charged translation tunnel (Frischmeyer et al., 2002; Ito-Harashima et al., 2007; Lu and Deutsch, 2008; Guydosh and Green, 2014). Lastly, non-functional 18S rRNA decay (18S-NRD) repairs errors in translation that are caused by dysfunctional ribosomes carrying an inactive or immature 40S subunit. 18S-NRD rapidly removes these faulty ribosomes that have initiated translation, but cannot produce an elongating peptide (LaRiviere et al., 2006; Soudet et al., 2010).

Each of these surveillance systems uses the same basic ribosome rescue machinery components. Ribosome rescue starts with recognition of the stalled ribosome by a ternary complex formed by Dom34 and Hsp70 subfamily B Suppressor 1 (Hbs1·GTP), which are paralogs of eRF1 and eRF3, respectively (Cole et al., 2009; Shoemaker et al., 2010; Tsuboi et al., 2012). Following this, Rli1 dissociates the ribosome into the 40S and 60S subunits via a similar mechanism as during the normal termination of translation. Dom34 lacks the conserved NIKS motif that is involved in stop codon recognition and the GGQ motif that catalyzes peptide release, which are characteristic features of eRF1 (Graille et al., 2008; Shoemaker et al., 2010).

The mRNA surveillance pathways appear to be conserved among all eukaryotes and archaea. For example, Pelota, the Dom34 paralog in *Drosophila melanogaster*, can restore NGD in Dom34-depleted yeast cells (Passos et al., 2009). Also, the human and fly Pelota-Hbs1 complex, together with ABCE1/Pixie, participates in NSD (Pisareva et al., 2011; Saito et al., 2013; Kashima et al., 2014). In archaea, the elongation factor aEF1α, an ortholog of eRF3, interacts with aRF1 during the normal termination of translation and aPelota during mRNA surveillance, resulting in ribosome dissociation via aABCE1 action (Saito et al., 2010; Barthelme et al., 2011; Becker et al., 2012).

Rli1 Is Required for Ribosome Biogenesis and Reactivation

Yeast ribosome biogenesis begins in the nucleolus where the 35S and 5S rDNAs are transcribed. Following this, pre-35S and pre-5S rRNAs are cotranscriptionally assembled with ribosomal proteins, ribosome biogenesis factors (RBFs), and small nucleolar ribonucleoproteins (snoRNPs) to form the 90S or small subunit (SSU) processome, which is the earliest ribosome precursor. Cleavage of the 35S pre-rRNA creates the pre-60S and pre-40S particles, and the maturation of these continues in the

nucleoplasm and the cytoplasm (Gerhardy et al., 2014). Once in the cytoplasm, RBFs prevent premature translation initiation on immature pre-60S and pre-40S particles (Gartmann et al., 2010; Strunk et al., 2011; Greber et al., 2012).

Maturation of the pre-60S particle is complete when the pre-6S rRNA is processed to form 5.8S rRNA, all ribosomal proteins are assembled, and the last RBFs are released (Lo et al., 2010). The last step in 40S subunit maturation is performed in a translation-like cycle, whereby the initiation factor eIF5B links a pre-40S particle to a mature 60S subunit to form an empty 80S-like ribosome, which is necessary for 20S pre-rRNA cleavage into mature 18S rRNA. This process also serves as an additional checkpoint that, together with those performed during subunit maturation, ensures functionality of the ribosome (Strunk et al., 2012; Karbstein, 2013). Finally, Rli1, together with Dom34 and possibly Hbs1, dissociates the ribosomal subunits, which are then ready to enter the translation cycle (Shoemaker and Green, 2011; Strunk et al., 2012). Likewise, human Pelota and ABCE1 can dissociate empty ribosomes *in vitro* (Pisareva et al., 2011).

Protein synthesis is a cyclic process in which ribosomal subunits dissociate once they have processed an mRNA molecule and subsequently can reinitiate translation. However, in yeast cells subjected to stress conditions, some ribosomal subunits are complexed into inactive 80S ribosomes to reduce translation rates and increase the probability of surviving (Ashe et al., 2000; Uesono and Toh, 2002). In yeast, these inactive ribosomes are stabilized by the Suppressor of ToM1 (Stm1) factor (Balagopal and Parker, 2011; Ben-Shem et al., 2011; Van Dyke et al., 2013). Ribosomal inactivation is reversed once stress conditions are relieved and the ribosomal subunits can reenter the translation cycle. In glucose-starved yeast cells, dissociation of Stm1-bound ribosome requires the combined action of the Dom34-Hbs1 complex and Rli1. When yeast cells are grown in glucose-deficient media, translation rates rapidly decrease, associated with a decline in polysome levels and the accumulation of Stm1-inactivated 80S ribosomes. With the addition of glucose, translation rapidly resumes; however, Dom34 or Hbs1 loss-of-function prevents the recovery of translation, which causes a cessation in growth (Ashe et al., 2000; van den Elzen et al., 2014).

THE ROLE OF ABCE PROTEINS IN DEVELOPMENT

Yeast Rli1 is the best-characterized ABCE protein. In yeast and the unicellular protist *Trypanosoma brucei*, *ABCE1* loss-of-function arrests growth (Dong et al., 2004; Estévez et al., 2004). Similar observations have been made in multicellular eukaryotes, showing that in some species, *ABCE1* loss-of-function and overexpression both result in impaired growth (**Table 1**; Amsterdam et al., 2004; Estévez et al., 2004; Zhao et al., 2004; Coelho et al., 2005a; Chen et al., 2006; Kougioumoutzi et al., 2013). Consistent with the fundamental role of ABCE1 in ribosome biogenesis and recycling, *ABCE1* expression is detected in most tissues and developmental stages in all studied organisms (Du et al., 2003; Zhao et al., 2004; Maeda et al., 2005; Sarmiento et al., 2006; Kougioumoutzi et al., 2013).

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Many of the effects caused by *ABCE1* loss-of-function in eukaryotes have been revealed through genetic screens. For example, the *pixie* alleles were identified in a screen for ethyl methanesulfonate-induced dominant modifiers of a small-wing phenotype in *Drosophila melanogaster* (Coelho et al., 2005b). Null *pixie* alleles are recessive lethal, whereas hypomorphic alleles produce a severe delay in growth (**Table 1**). Nevertheless, the final body size of *pixie* mutants is comparable to that of the wild type. The *pixie* mutant phenotype resembles the *Minute* phenotype, which is associated with mutant alleles of genes encoding ribosomal proteins. Adult *pixie* flies display short thoracic bristles, occasional eye roughening, and an increased wing size relative to body size (**Figures 3A,B**). The increased wing size relative to body size is proposed to be due to extra cell divisions that act as a compensation mechanism triggered by high-level apoptosis observed in the wing imaginal discs during the development of *pixie* larvae (Coelho et al., 2005a).

ABCE proteins also function in vertebrate development. In a large screen for essential genes in embryo and early larval development in *Danio rerio*, mutations in *ABCE1* were found to cause lethality 5 days after fertilization. These *abce1* mutants exhibited underdeveloped liver/gut, pericardial edema, and small heads. Like the hypomorphic *pixie* alleles, the zebrafish *ABCE1* alleles also limit eye development (**Table 1** and **Figures 3C,D**; Amsterdam et al., 2004). The *abce1* gene is also essential in *Caenorhabditis elegans* and *Xenopus laevis* as its suppression

by RNAi or antisense morpholino oligonucleotides, respectively, arrests growth (**Table 1**; Kamath et al., 2003; Zhao et al., 2004; Chen et al., 2006). The ability of the *ABCE1*-suppressed zebrafish and *Xenopus laevis* embryos to develop up to a certain point has been suggested to be due to the ABCE1 maternal supply to the egg. The growth arrest would therefore occur after depletion of the maternal supply (Amsterdam et al., 2004; Chen et al., 2006).

ABCE1 genes also have essential functions in plants. In *Cardamine hirsuta*, *SIMPLE LEAF3* (*SIL3,* also named *ChRLI2*) plays a role in leaf complexity. While wild-type *Cardamine hirsuta* plants display compound leaves, which are divided into leaflets, homozygous plants carrying the putatively hypomorphic *sil3* allele show a substantial decrease in leaflet but not leaf number (**Table 1** and **Figures 3E,F**; Kougioumoutzi et al., 2013). The *sil3* mutant has a reduced growth and its phenotype suggests alterations in auxin homeostasis at the whole-organism level. Further proof of perturbed auxin homeostasis was found by analyzing *sil3* leaves, which are small and exhibit aberrant venation patterns, as usually observed in mutants affected in auxin signaling. In addition, the lack of leaflets in *sil3* plants was explained by reduced cell proliferation on the regions where leaflets were expected to emerge. Nevertheless, leaf and leaflet initiation are controlled by the same general mechanisms that consist in the accumulation of auxin by its polarized flow through the PIN-FORMED1 (PIN1) transporters in the regions where leaf initiation occurs. Auxin accumulation
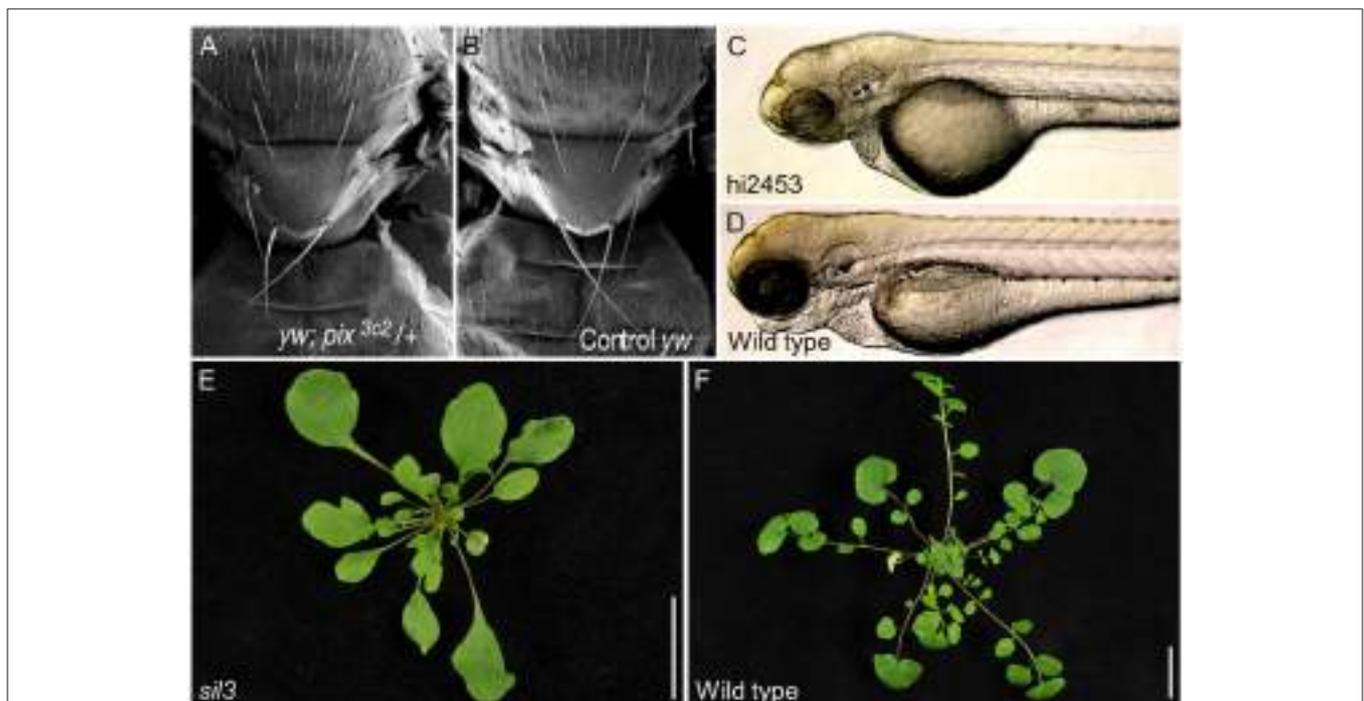
FIGURE 3 | Developmental effects of *ABCE* gene dysfunction in different species. **(A)** *Drosophila melanogaster pixie* mutants display thoracic bristles that are slenderer and shorter than those of **(B)** the wild type. **(C)** An insertional allele of *Danio rerio ABCE1* reduces head size compared with **(D)** wild type and produces pericardial edema and lethality at 5 days post-fertilization. **(E)** A hypomorphic allele of *Cardamine hirsuta SIL3* triggers loss of the leaflets that characterize **(F)** wild type leaves. Pictures of **(A,B)**, as well as **(C,D)**, were taken at the same scale. **(E,F)** Scale bars indicate 2 cm. Adapted with permission of authors and journals from **(A,B)** Coelho et al. (2005a), **(C,D)** Amsterdam et al. (2004) Copyright 2004 National Academy of Sciences, and **(E,F)** Kougioumoutzi et al. (2013).

then triggers leaf or leaflet initiation (Scarpella et al., 2010). Although the study of the *sil3* mutant is consistent with auxin homeostasis and signaling being sensitive to perturbation of ribosomal activity, the *sil3* mutant is interesting because leaflet number, but not leaf number, is reduced. To explain this observation, it has been suggested that the high energy demand from cells proliferating during leaflet development could not be satisfied in *sil3* plants due to suboptimal ribosome function (Kougioumoutzi et al., 2013). The two Arabidopsis *ABCE* genes have not been studied at a developmental level.

In addition, virus-induced gene silencing (VIGS) has been used to suppress expression of *RLIh* gene(s) in *Nicotiana benthamiana*, in which the number of *ABCE* paralogs remains to be established. For VIGS, 4-week-old plants were infected with potato virus X (PVX) or pea early browning virus (PEBV) vectors carrying partial *RLIh* cDNAs. *RLIh* silencing caused vein whitening, leaf distortion, and delayed growth, with silenced plants reaching only half of the height of controls, due to a reduction in cell size and number in shoot internodes (**Table 1**; Petersen et al., 2004). Again, these observations corroborate the important role of ABCE proteins in whole-organism development. Whether these developmental defects are due to the disruption of the ABCE function as a ribosome-dissociating factor (Franckenberg et al., 2012), an endogenous suppressor of RNA silencing (Sarmiento et al., 2006; Kärblane et al., 2015) or both, remains to be clarified.

CONCLUDING REMARKS

In this review, we have discussed the essential ribosome-dissociation activity of ABCE proteins, which is required for ribosome biogenesis and recycling. Furthermore, we have described the general growth defects associated with compromised ABCE protein function in all studied organisms. Most of these growth defects can be attributed to defective mRNA translation, which reduces protein levels and in turn prevents cells from generating the energy required for normal growth and/or proliferation. However, not all *abce* mutant phenotypes can be explained by a general depletion of cellular energy. As is the case for mutants affected in translation in several species, some phenotypes appear to be associated with compromised regulatory networks that remain uncharacterized. For instance, it is striking the fact that the *Cardamine hirsuta sil3* mutant shows a reduction in leaflet but not leaf number, given that both processes share common pathways. Future work is needed to clarify these and other questions and to determine whether there

are specific factors responsible for the differential requirements for ABCEs during development. Additional studies will also determine how ABCE dysfunction causes specific developmental aberrations in all studied organisms, as has been previously described for other proteins involved in ribosome biogenesis or function. In addition, the presence of more than one ABCE subfamily member in some genomes remains to be explained. Some plants and insects have two *ABCE* genes while other plants or *Drosophila melanogaster* have a single *ABCE* gene. The presence of two *ABCE* genes might be interpreted as an in-progress pseudogenization or a developing functional redundancy.

The ribosome has been proposed to represent a layer of post-transcriptional regulation of gene expression. Under the so-called filter hypothesis, the ribosome is viewed as a machine able to selectively influence or filter the translation of various mRNAs (Mauro and Edelman, 2002). There is increasing evidence of the existence of specialized ribosomes in different cell types, which are heterogeneous in either ribosomal protein composition, in their interactions with ribosome-associated factors, or both (Xue and Barna, 2012; Shi and Barna, 2015). These specialized ribosomes would differentially translate different mRNAs or mRNA groups. It follows from these assumptions that mutation of genes encoding discrete ribosomal proteins or ribosome-associated factors would render tissue- or organ-specific phenotypes. The ABCE proteins of *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Cardamine hirsuta* might represent one such factors.

AUTHOR CONTRIBUTIONS

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The Arabidopsis ATP-Binding Cassette E protein ABCE2 is a conserved component of the translation machinery

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ABSTRACT

ATP-Binding Cassette E (ABCE) proteins dissociate cytoplasmic ribosomes after translation terminates, thus linking termination to reinitiation of translation. This function has been demonstrated in animals, yeast, and archaea, but not in plants. In most species, ABCE is encoded by a single-copy gene; by contrast, *Arabidopsis thaliana* has two *ABCE* paralogs, of which *ABCE2* seems to conserve the ancestral function. Indeed, our co-immunoprecipitation experiments showed that ABCE2 physically interacts with components of the translation machinery, including subunits of the EUKARYOTIC TRANSLATION INITIATION FACTOR 3 (eIF3) complex. We isolated *apiculata7-1* (*api7-1*)*,* a viable, hypomorphic allele of *ABCE2*, which has a pleiotropic morphological phenotype reminiscent of mutations affecting ribosome biogenesis factors and ribosomal proteins. We also studied *api7-2*, a null, recessive lethal allele of *ABCE2*. To explore the biological processes affected by ABCE2 depletion, we performed RNA-seq of the *api7-1* mutant, and observed increased responses to iron and sulfur starvation. We also found increased transcript levels of genes related to auxin signaling and metabolism. Our results indicate that the role of ABCEs in ribosome recycling is evolutionarily conserved in animal, fungal, plant, and archaeal lineages.

INTRODUCTION

The ATP-Binding Cassette (ABC) proteins are present in all living organisms and constitute one of the largest protein families known. Most ABC proteins consist of two transmembrane domains (TMDs), which transport solutes across cellular membranes, and two nucleotide binding domains (NBDs), which hydrolyze two ATP molecules to induce TMD movement and release of the cargo (1). In contrast to these typical ABC proteins, members of the ABCE and ABCF subfamilies of ABC proteins, as well as some members of the plant-specific ABCI subfamily, lack TMDs and are soluble (2). Human ABCE1 was first named RNASE L INHIBITOR (RLI) due to its ability to inhibit the activity of RNase L, an enzyme that is only present in mammals (3). Nevertheless, ABCE subfamily members are highly conserved among archaea and eukaryotes, and participate in ribosome recycling and translation reinitiation, as has been demonstrated for archaea, fungi, and animals, but not plants (4-10).

ABCE proteins contain an iron-sulfur cluster binding domain (FeSD), two NBDs (NBD1 and NBD2), and two hinge motifs (11-13). The first hinge motif is between the NBDs and allows NBD movement to bind and hydrolyze ATP. The second hinge motif, located at the C terminus, and a helix-loop-helix (HLH) motif within NBD1, allow the interaction of the ABCE protein with the ribosome. In this manner, occlusion of an ATP molecule favors ABCE binding to the translation termination complex. Next, the occlusion of another ATP molecule displaces the FeSD and promotes ribosome splitting. Finally, ATP hydrolysis allows ABCE detachment from the 30S/40S ribosome subunit (14-16). Alternatively, ABCE can retain the two ATP molecules and remain bound to the 30S/40S subunit in the post-splitting complex, where it is believed to prevent the premature recruitment of a 60S subunit until the late steps of translation initiation complex formation (5,6,10,15,17-20).

In most genomes, the ABCE subfamily is represented by a single-copy gene, usually named *ABCE1*, whose null alleles are lethal, while hypomorphic alleles result in developmental defects and slow-growth phenotypes in all studied organisms (21). *Arabidopsis thaliana* (hereafter referred to as Arabidopsis), however, has two *ABCE* paralogs named *ABCE1* and *ABCE2* (2,22). Arabidopsis ABCE2 has been studied for its RNA silencing suppression activity (23,24). In contrast, *Cardamine hirsuta,* a close relative of Arabidopsis with compound leaves has only one *ABCE* gene, *SIMPLE LEAF3* (*SIL3*), which is required for leaflet formation and leaf development. The leaves of homozygotes for the hypomorphic *sil3* mutation are simple and have vascular defects, probably caused by an aberrant auxin homeostasis (25).

The upstream open reading frames (uORFs) of the 5′ untranslated regions (5′ UTRs) of some mRNAs serve as regulatory elements that reduce the efficiency of translation of the main ORF (mORF) of these mRNAs. In Arabidopsis, translation of a long uORF prevents

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translation reinitiation on the adjacent mORF due to a progressive loss of initiation factors from the translating machinery (26,27). More than one third of Arabidopsis mRNAs contain uORFs, together with an mORF that usually encodes a transcription factor or other type of protein acting in a signaling pathway (27).

In fact, auxin signaling is known to be highly sensitive to perturbations in the translation machinery. Mutations in *RIBOSOMAL LARGE SUBUNIT 4 D* (*RPL4D*), *RPL5A*, *RPL24B*, and *EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT H* (*eIF3h*) reduce the ability of the translation machinery to reinitiate translation on mORFs, leading to low levels of several AUXIN RESPONSE FACTORS (ARFs) that are encoded by mRNAs containing uORFs (28-31). Among other developmental defects, these mutations cause aberrant leaf venation patterns, in agreement with a role for auxin in vascular patterning (32,33). In addition, auxin itself regulates translation by promoting reinitiation on the mORFs of the mRNAs of several *ARF* genes. Specifically, auxin induces the activation of TARGET OF RAPAMYCIN (TOR), a highly conserved growth regulator, which phosphorylates eIF3h, promoting translation reinitiation after the termination of the translation of a uORF (34).

Here, we describe a functional analysis of the Arabidopsis *ABCE1* and *ABCE2* genes. We studied two recessive alleles of *ABCE2*: the hypomorphic and viable *apiculata7-1* (*api7-1*) allele, and the null and lethal *api7-2* allele. The *api7-1* mutant exhibits the typical morphological phenotype caused by mutation of genes encoding ribosome biogenesis factors and ribosomal proteins, which includes aberrant leaf venation patterns. We found by co-immunoprecipitation that ABCE2 physically interacts with components of the translation machinery, and by RNA-seq that its partial loss of function triggers iron and sulfur deficiency responses related to FeS cluster biogenesis, as well as the upregulation of auxin biosynthesis genes. Our observations strongly support a conserved role for ABCE proteins in ribosome recycling in plants, as previously shown for the animal, fungal, and archaeal lineages.

4

MATERIALS AND METHODS

Plant materials, growth conditions, and crosses

The *Arabidopsis thaliana* (L.) Heynh. wild-type accessions Landsberg *erecta* (L*er*) and Columbia-0 (Col-0), and the *asymmetric leaves1-1* (*as1-1*; N3374; in the Col-1 genetic background) and *as2-1* (N3117; in ER) mutants were initially obtained from the Nottingham Arabidopsis Stock Center (NASC; Nottingham, United Kingdom) and then propagated in our laboratory for further analysis. We introgressed the *as1-1* and *as2-1* mutations into the Col-0 background by crossing to Col-0 three times. The NASC also provided seeds of the *api7-2* (GABI_509C06; N448798) (35) and *PIN1$_{pro}$:PIN1:GFP DR5$_{pro}$:3XVENUS:N7* (N67931) (36) lines. The *ATHB8$_{pro}$:GUS* line (N296) was kindly provided by Simona Baima (37). The *api7-1* line was isolated in the L*er* background after ethyl methanesulfonate (EMS) mutagenesis in our laboratory and then backcrossed twice to L*er* (38). Unless otherwise stated, all the mutants mentioned in this work are homozygous for the mutations indicated.

Seed sterilization and sowing, plant culture, crosses, and allelism tests were performed as previously described (38-40). In brief, plants were grown under sterile conditions on half-strength Murashige and Skoog medium (Duchefa Biochemie) with 0.75% plant agar (Duchefa Biochemie) and 1% sucrose (Duchefa Biochemie), at 20°C ± 1°C, 60−70% relative humidity, and continuous illumination at ~75 µmol·m^{-2}·s^{-1}.

Positional cloning and molecular characterization of *ABCE2* mutant alleles

Genomic DNA was extracted as previously described (41). The *ABCE2* gene was cloned as previously described (42). First, we mapped the *api7-1* mutation to a 123.5-kb candidate interval containing 30 genes using a mapping population of 273 F$_2$ plants derived from an *api7-1* × Col-0 cross, and the primers listed in Supplementary Table S1, as previously described (41,43). Then, the whole *api7-1* genome was sequenced by Fasteris (Geneva, Switzerland) using the Illumina HiSeq2000 platform. The bioinformatic analysis of the data was performed as previously described (42). The candidate mutation was verified by Sanger sequencing in an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems).

Discrimination between the wild-type *ABCE2* and *api7-1* mutant alleles was done by PCR with the api7-1_F and api7-1_R primers (Supplementary Table S1), followed by restriction with Eco57I (Thermo Fisher Scientific), as the *api7-1* mutation (CTCCAG→CTTCAG) creates an Eco57I restriction site. The presence and position of the *api7-2* T-DNA insertion in the GABI_509C06 line was confirmed by PCR amplification and Sanger sequencing, respectively, using gene-specific primers and the o8409 primer for the GABI-Kat T-DNA from the pAC161 vector (Supplementary Table S1).

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Gene constructs and plant transformation

All inserts were PCR amplified from Col-0 genomic DNA using Phusion High Fidelity DNA Polymerase (Thermo Fisher Scientific) and primers that contained *att*B sites at their 5′ ends (Supplementary Table S1). PCR products were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (Cytiva), and then cloned into the pGEM-T Easy221 vector, transferred to *Escherichia coli* DH5α, and subcloned into the pEarleyGate 101, pMDC83, or pMDC107 destination vectors (44,45) as previously described (46).

To obtain the *ABCE2pro:ABCE2* construct, a 4.54-kb segment of chromosome 4 was PCR amplified, including 1183 bp upstream of the start codon of *ABCE2*, and 489 bp downstream of the stop codon. For the *35Spro:ABCE2:GFP* and *35Spro:ABCE2:YFP* constructs, the 2.87-kb *ABCE2* transcription unit was amplified, excluding its stop codon. After purification, the PCR products were cloned into pGEM-T Easy221 and subcloned into pMDC83 and pEarleyGate 101, respectively.

For the promoter exchange assay, promoters and transcription units were separately PCR amplified and fused in a second amplification. For example, to obtain *ABCE1pro:ABCE2*, a genomic region of 1485 bp upstream of *ABCE1* was amplified using the primers ABCE1pro_F, which contained the *att*B1 site, and ABCE1pro_R, which included a 3′ sequence complementary to the first 25 nucleotides of the *ABCE2* transcription unit. The 2.87-kb *ABCE2* transcription unit was obtained with ABCE2tu_F, which is complementary to the ABCE1pro_R sequence, and ABCE2tu_R, which includes the stop codon and the *att*B2 site. Then, the same amounts of purified PCR products were combined in a second PCR mix, and fused using the ABCE1pro_F and ABCE2tu_R primers to obtain *ABCE1pro:ABCE2*. The *ABCE1pro:ABCE2* and *ABCE2pro:ABCE1* final PCR products were purified, cloned into pGEM-T Easy221, and subcloned into pMDC107.

All constructs were transferred to electrocompetent *Agrobacterium tumefaciens* GV3101 (C58C1 Rif^R) cells, which were used to transform L*er*, *api7-1*, or *API7/api7-2* plants by the floral dip method (47). Putative transgenic plants were selected on plates supplemented with 15 µg·ml⁻¹ hygromycin B (Thermo Fisher Scientific, Invitrogen).

Phenotypic analysis and morphometry

Photographs of Arabidopsis rosettes, inflorescences, flowers, and siliques were taken with a Nikon SMZ1500 stereomicroscope equipped with a Nikon DXM1200F digital camera. For larger specimens, four to five partial images from the same plant were taken and merged using the Photomerge tool of Adobe Photoshop CS3 software. Empty spaces resulting from the assembly were filled with black. To measure rosette size and root length, photographs of plants grown on Petri dishes, horizontally or vertically oriented, respectively, were taken with a Canon PowerShot S315 camera. For rosette size, rosette silhouettes were drawn on

the screen of a Cintiq 18SX Interactive Pen Display (Wacom) using Adobe Photoshop CS3, and their sizes were measured with the NIS Elements AR 3.1 image analysis package (Nikon). Root length was measured directly from photographs with the Freehand line tool from Fiji software (ImageJ; https://imagej.net/ImageJ) (48) on the screen of a Cintiq 18SX Interactive Pen Display. Rosette phyllotaxis was measured using the Fiji Angle tool on photographs taken from plants grown horizontally. The length of each root and the angles between consecutive leaves were measured per triplicate. Shoot length was measured *in vivo* with a millimeter ruler, from the soil to the apex of the main shoot.

Chlorophyll *a* and *b* and carotenoids were extracted as previously described (49), with some modifications. Each biological replicate contained 50−60 mg of fresh rosettes immediately frozen in liquid nitrogen after collection, to prevent pigment loss. After tissue homogenization with cold 80% (v/v) acetone, the cell debris was removed by centrifugation. Pigment content was spectrophotometrically determined as previously described (50), and normalized to the amount of collected tissue.

Sample preparation for flow-cytometry was performed as previously described (51). Nuclear DNA content was analyzed using a SH800S Cell Sorter (Sony Biotechnology) and the data were processed using the SH800 software (Sony Biotechnology). The endoreduplication index (EI) was calculated as previously described (52).

Differential interference contrast and bright-field microscopy, and GUS analyses

For differential interference contrast (DIC) and bright-field microscopy, all samples were cleared by immersion in a 2.67 g/ml chloral hydrate solution and mounted on glass slides with a solution of 2.67 g/ml chloral hydrate dissolved in 2:1 glycerol:water. DIC micrographs of epidermal and palisade mesophyll leaf layers were taken using a Leica DMRB microscope equipped with a Nikon DXM1200 digital camera. Micrographs of venation patterns from cotyledons, first- and third-node rosette leaves, cauline leaves, sepals and petals, and leaf primordia expressing *ATHB8$_{pro}$:GUS* were taken under bright field with a Nikon D-Eclipse C1 confocal microscope equipped with a Nikon DS-Ri1 camera, using the NIS-Elements AR 3.1 software (Nikon). When the organ did not fit into the objective field, the Scan Large Image tool from the NIS-Elements AR 3.1 software was used. Diagrams from leaf cells and venation patterns, and morphometric analysis of leaf cells were obtained as previously described (46,53). For venation pattern morphometry, the phenoVein (http://www.plant-image-analysis.org) (54) software was used. Leaf lamina circularity was calculated as $4 \cdot \pi \cdot area/perimeter^2$. Lamina area and perimeter were measured on diagrams from the leaf lamina with the Fiji Wand tool. GUS assays were performed as previously described (38).

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Confocal microscopy and fluorescence quantification

Confocal laser scanning microscopy images were obtained using a Nikon D-Eclipse C1 confocal microscope equipped with a Nikon DS-Ri1 camera and processed with the operator software EZ-C1 (Nikon). Visualization of the fluorescent proteins and dyes was performed on primary roots mounted with deionized water on glass slides. GFP, YFP, and VENUS fluorescent proteins were excited at 488 nm with an argon ion laser, and their emissions were collected with a 515/30 nm barrier filter. Nuclei were stained by immersing complete seedlings in a 0.2 µg·ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) solution (Sony Biotechnology) for 12 min, and the seedlings were then transferred to deionized water. DAPI was excited at 408 nm with a diode laser, and detected with a 450/35 nm filter. Cell walls were stained by immersing the seedlings in 10 µg·ml^{-1} propidium iodide (Sigma-Aldrich) for 8 min. Then, the seedlings were washed three times with deionized water before mounting their roots on glass slides. Propidium iodide was excited at 543 nm with a helium-neon laser, and detected with a 605/75 nm filter.

For fluorescence quantification of the *PIN1$_{pro}$:PIN1:GFP* and *DR5$_{pro}$:3XVENUS:N7* protein products, wild-type and *api7-1* seedlings homozygous for these transgenes were grown vertically on the same Petri dishes under identical conditions for 5 days. To further favor homogeneity between samples, wild-type and *api7-1* roots were mounted on the same slides in an alternating manner. Image acquisition was performed using a 40× objective with a 0.75 numerical aperture. The dwell time was set at 2.16 and 1.68 µs for PIN1:GFP and VENUS:N7, respectively. The fluorescent protein and propidium iodide images were consecutively acquired in different channels. Four images were acquired and averaged per optical section. Five optical sections encompassing 4 µm from the innermost root layers were photographed. Acquired images (.ids files) were used to generate flat images (.tiff files) with Fiji by stacking the optical sections from the fluorescent protein channel. Fluorescence quantification was performed using the Fiji Mean gray value measurement on the fluorescent regions from the flat images. The fluorescent regions were selected with a pre-established pixel intensity threshold.

RNA isolation, cDNA synthesis, and quantitative PCR

Samples for RNA extraction were collected on ice and immediately frozen for storage at −80°C until use. RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, Invitrogen) and its concentration was estimated using a Pearl NanoPhotometer (Implen) spectrophotometer. Removal of contaminating DNA, cDNA synthesis, and quantitative PCR (qPCR) were performed as previously described (46). For removal of contaminating genomic DNA, approximately 8 µg of RNA were treated with a TURBO DNA-free Kit (Thermo Fisher, Invitrogen) following the instructions of the manufacturer.

First-strand cDNA was synthesized from 1.8 µg of total RNA using random hexamers and the Maxima Reverse Transcriptase system (Thermo Fisher Scientific) in a final volume of 20 µl, using a Bio-Rad T100 Thermal Cycler (10 min at 25°C, 1 h at 50°C, and 5 min at 85°C). Absence of contaminating genomic DNA on cDNA samples was confirmed by PCR amplifying a region of the *ORNITHINE TRANSCARBAMYLASE* (*OTC*) gene, which contains an intron, using the OTC3D and OTCR primers (55) (Supplementary Table S1), and a genomic DNA sample from Col-0 plants as a control. For qPCR, *ACTIN2* (*ACT2*) was used as an internal control for relative expression analysis using the primers listed in Supplementary Table S1 (56). Three biological replicates, each with three technical replicates, were analyzed per genotype. PCR was performed using 7.5 µl of Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher), 5 µl of the corresponding primer pair (1.5 µM each), 1 µl of cDNA, and water in a total volume of 20 µl, on a Step One Plus System (Applied Biosystems). The qPCR was performed as follows: 2 min at 50°C, 10 min at 95°C, followed by 41 cycles of 15 s at 95°C and 1 min at 60°C, and a final step of 15 s at 95°C. Relative quantification of gene expression data was performed using the comparative C_T method ($2^{-\Delta\Delta CT}$) (57).

RNA-seq analysis

Total RNA was isolated from 100 mg of L*er* and *api7-1* rosettes collected 14 days after stratification (das) using TRIzol. Rosette samples were collected on ice and immediately frozen in liquid nitrogen. RNA concentration and quality were assessed using a 2100 Bioanalyzer (Agilent Genomics) with an RNA 600 Nano Kit (Agilent Technologies) as previously described (46). Three biological replicates per genotype, with more than 14 µg of total RNA per sample, and an RNA integrity number (RIN) higher than 7, were sent to Novogene (Cambridge, United Kingdom) for massive parallel sequencing. Sequencing libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs) and fed into a NovaSeq 6000 Illumina platform with a S4 Flow Cell type, which produced paired-end reads of 150 bp. Read mapping to the Arabidopsis genome (TAIR10) using the 2.0.5 version of HISAT2 (58), with default parameters, and the identification of differentially expressed genes between L*er* and *api7-1* with the 1.22.2 version of DESeq2 R package (59) were performed by Novogene. Genes with a *P*-value < 0.05 adjusted with the Benjamini and Hochberg's method, and with a fold change > 1.5 were considered differentially expressed. The gene ontology (GO) enrichment analysis of the differentially expressed genes was performed with the online tool DAVID (https://david.ncifcrf.gov/home.jsp) (60,61), using all the terms included in the GO Biological Process categorization (GOTERM_BP_ALL).

Co-immunoprecipitation assay

For protein extraction, 700 mg of whole *api7-1 35S_{pro}:ABCE2:YFP* seedlings were collected 10 das per biological replicate. The tissue was crosslinked with 1× phosphate-saline buffer containing 1% (v/v) formaldehyde as described in (62). For protein extraction, the tissue was ground to a fine powder with liquid nitrogen and then resuspended in a lysis buffer (50 mM Tris-HCl, pH 7.5; 0.1% [v/v] IGEPAL CA-630 [Sigma-Aldrich]; 2 mM phenylmethylsulfonyl fluoride [PMSF; Sigma-Aldrich]; 150 mM NaCl; and a cOmplete protease inhibitor cocktail tablet [Sigma-Aldrich]) using a vortexer. After incubation on ice for 10 min, the samples were centrifuged at 4°C and the supernatants were used as the protein extracts. Before co-immunoprecipitation, 20 µl from each extract were transferred to a new tube for use as inputs in a western blot.

Co-immunoprecipitation was performed with the µMACS GFP Isolation Kit (Milteny Biotec) following the instructions of the manufacturer, using proteins from three biological replicates. The immunoprecipitation of the ABCE2:YFP fusion protein was checked by western blotting using an anti-GFP-HRP antibody (Milteny Biotec), and the WesternSure chemiluminiscent substrate on a C-DiGit Blot Scanner (LI·COR).

The co-immunoprecipitates were analyzed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) at the Centro Nacional de Biotecnología (CNB) Proteomics facility (Madrid, Spain). Tandem mass spectra were searched against Araport11 using the MASCOT search engine (Matrix Science, http://www.matrixscience.com/). Peptide sequences identified with a false discovery rate (FDR) < 1% were considered statistically valid (Supplementary Data Set 1). Proteins identified with at least 2 peptides without overlapping sequences in at least 2 biological replicates (namely, at least 4 peptides) were considered as identified with high confidence. To search for potential ABCE2:YFP interactors, proteins whose subcellular localization was not predicted to be cytoplasmic by SUBA4 (https://suba.live/) (63,64) were discarded, with the exception of At2g20830, which is predicted to localize to mitochondria (see Results). To further discard potential false positive interactions, all the proteins identified in three other co-immunoprecipitations of GFP-fused proteins performed in our laboratory under identical conditions to that of ABCE2:YFP, but functionally unrelated, were used to create a subtract list. Proteins identified in ABCE2:YFP samples with at least twice the number of peptides assigned to the same protein in the subtract list were considered enriched. The rest of the proteins, which contained a more similar number of peptides between the ABCE2:YFP list and the subtract list, were considered false positives and discarded. In addition, there were few proteins that were solely identified in ABCE2:YFP samples.

Additional bioinformatic and statistical analyses

The identity and similarity values between conserved proteins were obtained from global pairwise sequence alignments performed with EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) (65) with default parameters and pair output format. The multiple sequence alignment of ABCE orthologs was obtained with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (65) with default settings and ClustalW output format, and shaded with BOXSHADE (https://embnet.vital-it.ch/software/BOX_form.html).

A TBLASTN search was performed to identify *ABCE* genes within eudicots (taxid:71240) against the sequences contained in the Nucleotide collection database at the National Center for Biotechnology Information BLASTP server (NCBI; https://blast.ncbi.nlm.nih.gov/ Blast.cgi) (66) using *Arabidopsis thaliana* ABCE2 protein as the query (NP_193656). Default parameters were used except for max target sequences, which was set at 1000, and word size, which was set at 2. Predicted *ABCE* coding sequences were downloaded from the NCBI Nucleotide database, except for *Eschscholzia californica ABCE*, which was downloaded from the Eschscholzia Genome DataBase (http://eschscholzia.kazusa.or.jp/index.html) (67) (Supplementary Table S2). The phylogenetic analysis was performed with MEGA X software (68): the multiple sequence alignment and the phylogenetic tree were obtained using codon recognition with Muscle (69,70), and the Neighbor-Joining method (71), respectively.

Hypothesis testing was performed as described in (46). The parametric Student's *t* test was used when the number of observations (*n*) was 10 or higher, under the assumption that most of the data followed a normal distribution. When the normality assumption could not be made ($n < 10$), the nonparametric Mann-Whitney *U* test for unpaired data was used. All *P*-values presented in this work are two-sided.

Accession numbers

Sequence data can be found at The Arabidopsis Information Resource (https://www.arabidopsis.org/) under the following accession numbers: *ABCE1* (At3g13640), *ABCE2* (At4g19210), *ACT2* (At3g18780), *ATHB8* (At4g32880), *OTC* (At1g75330), and *PIN1* (At1g73590).

RESULTS

The *api7-1* mutant exhibits a pleiotropic morphological phenotype

In a previous large-scale screen for EMS-induced mutations affecting leaf development, we identified 8 mutants with pointed rosette leaves and mild marginal indentations. The corresponding causal mutations were dubbed *apiculata* (*api*), and allelism tests demonstrated that they fell into 7 different complementation groups (*API1-API7*) (38). The phenotype of *api* plants is reminiscent of those of mutants carrying loss-of-function alleles of genes encoding ribosomal proteins or ribosome biogenesis factors (29,72-75). Indeed, *api2*, the only already characterized *api* mutant, carries a point mutation in the 5′ UTR of the *RPL36aB* gene, which encodes a 60S ribosomal subunit protein (76).

The *api7-1* mutant, which we initially named *api7* (38), exhibits slow growth and leaves with reduced size (Figure 1). Its pleiotropic morphological phenotype includes rosette leaves that are pointed, indented, with pale green lamina and yellowish margins, and a reduced content of photosynthetic pigments, compared to its wild-type L*er* (Figure 1A, B; Supplementary Figure S1A). DIC microscopy of *api7-1* and L*er* first-node leaves cleared with chloral hydrate uncovered a marked reduction in cell size in the abaxial and adaxial epidermal layers, but not in the palisade mesophyll (Supplementary Figure S2). The ploidy levels of the *api7-1* leaves from the first to the fourth nodes, determined by flow cytometry, were slightly higher than in the wild type, suggesting an increased number of endoreduplication cycles (Supplementary Figure S3) (77).

The projected area of *api7-1* rosettes is significantly smaller than that of wild-type plants throughout the vegetative phase (Figure 1D), while rosette leaf phyllotaxis seemed unaffected (Supplementary Figure S1B). In addition, the primary root is shorter in *api7-1* than in L*er* plants (Supplementary Figure S1C). Although the *api7-1* plants grow slower than the wild type, at the end of their life cycle the length of the main stem is similar to that of L*er* plants (Figure 1E). The *api7-1* inflorescences and siliques are seemingly normal (Supplementary Figure 1D-I), although *api7-1* siliques carry a higher percentage of aborted or unfertilized ovules (7.91%; n = 392) than L*er* siliques (4.22%; n = 403). Nevertheless, this reduced percentage of viable seeds per silique does not lead to a significant reduction in seed production: *api7-1* plants produced 479 ± 70 mg of seeds (mean ± standard deviation; n = 7) with similar sizes to those of L*er*, which produced 484 ± 66 mg (n = 6).

api7-1 is a viable mutant allele of the *ABCE2* gene

The *api7-1* mutation was previously mapped to chromosome 4 (78). To identify the mutated gene, we combined map-based cloning and next-generation sequencing, as previously described (42). First, we performed linkage analysis of an F_2 mapping population using the primers listed in Supplementary Table S1, as previously described (41,43), which allowed

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us to delimit a candidate interval encompassing 30 annotated genes (Figure 2A). We then sequenced the whole *api7-1* genome, and, after discarding all the putative L*er*/Col-0 polymorphisms, we identified 4 EMS-type nucleotide substitutions (3 G→A and 1 C→T) within the candidate interval (Supplementary Table S3; see Methods). Only one of these, a C→T transition in At4g19210, was predicted to be a missense mutation causing a Pro138→Ser substitution (Figure 2B).

The At4g19210 gene encodes ABCE2, a protein of 605 amino acids with a molecular mass of 68.39 kDa. The Pro138 residue, at the beginning of the HLH motif located within NBD1, is conserved across all eukaryotic ABCE proteins, except in *Caenorhabditis elegans*, in which it seems to have evolved more divergently (Supplementary Figure S4) (79). The conservation of this residue suggests that it is necessary for the proper function of ABCE proteins, specifically for the interactions with the ribosome and the post-splitting complex, which mainly occur through the HLH and hinge motifs (6,15,18).

To confirm that the mutation found in At4g19210 causes the phenotype of the *api7-1* mutant, we obtained the *ABCE2_{pro}:ABCE2* transgene, encompassing the whole At4g19210 transcriptional unit driven by its own promoter, which was transferred into *api7-1* plants. This transgene completely restored the wild-type rosette leaf shape and stem height (Figure 1C-E), as well as the photosynthetic pigment content (Supplementary Figure S1A), and percentage of aborted ovules (3.05%; *n* = 426). The *ABCE2_{pro}:ABCE2* transgene partially restored the root length, leaf epidermal cell sizes, and ploidy levels (Supplementary Figures S1C, S2, S3).

To provide further confirmation that *api7-1* is an allele of *ABCE2*, we performed an allelism test using GABI_509C06 plants, which were heterozygous for a T-DNA insertion in the 10th exon of At4g19210 (Figure 2B) (35). We named the insertional allele in GABI_509C06 *api7-2*. For the allelism test, we crossed *api7-1* to the *ABCE2/api7-2* heterozygous plants. In the F_2 population of this cross, no *api7-2/api7-2* plants were found, and *api7-1/api7-2* and *api7-1/api7-1* plants were phenotypically similar, confirming that these mutations are allelic and that loss of function of *ABCE2* is responsible for the phenotype of the *api7-1* mutant (Supplementary Figure S5A-C).

The absence of *api7-2/api7-2* plants derived from GABI_509C06 seeds and of ungerminated seeds in the F_1 progeny of selfed heterozygous *ABCE2/api7-2* plants, suggested an early lethality of this mutant allele. We dissected immature siliques from *ABCE2/api7-2* plants and found 21.95% aborted ovules (*n* = 328), which fits a 1:3 Mendelian segregation ratio (χ^2 = 1.63; *P*-value = 0.202; degrees of freedom = 1). Col-0 siliques showed 1.37% aborted ovules (*n* = 148; Supplementary Figure S5F, G). The lethality caused by *api7-2* suggests that it is a null allele of *ABCE2*, while *api7-1* is hypomorphic. We also obtained *api7-2/api7-2 ABCE2_{pro}:ABCE2* plants, which were viable and phenotypically wild-

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type (Supplementary Figure S5D, E).

The Arabidopsis genome contains two *ABCE* paralogs

Some animal (80,81) and plant (82,83) genomes, including that of Arabidopsis, encode two ABCE paralogs (2,22). To gather more information about the origin of Arabidopsis *ABCE* paralogs, we performed a phylogenetic analysis of *ABCE* coding sequences from some Rosidae species (rosids; Supplementary Figure S6). Among them, we found that other Brassicaceae genomes, namely *Arabidopsis lyrata*, *Capsella rubella*, *Eutrema salsugineum*, and *Brassica rapa*, also encode ABCE1 and ABCE2 proteins, but only *ABCE2* was identified in *Cardamine hirsuta*. Consistent with the whole-genome triplication in *Brassica rapa* (84), we found two and three *Brassica rapa ABCE1* and *ABCE2* sequences, respectively. All Brassicaceae *ABCE* genes clustered together in the phylogenetic tree that we obtained. Inside this clade and in all bootstrap replicates (1000 replicates), *ABCE1* genes grouped together and separately from their *ABCE2* paralogs, which formed other subclade. Additionally, rosids from other families contained only the *ABCE2* gene, suggesting that a duplication event of *ABCE* genes occurred before the divergence of Brassicaceae species. Although both *ABCE1* and *ABCE2* paralogs have been conserved, the phylogenetic tree shows that *ABCE1* orthologs have evolved more rapidly than their *ABCE2* paralogs, whose short evolutionary distances indicate that they are under strong evolutionary pressure, as expected for an essential gene.

As previously described (24,85), we observed that *ABCE2* is highly expressed throughout all Arabidopsis developmental stages. By contrast, the expression levels of its *ABCE1* paralog are very low in all studied organs, in which first-node leaves and flowers show the lowest and highest expression levels, respectively (Supplementary Figure S7A, B). The expression levels of *ABCE1* in *api7-1* plants were the same as in L*er*, showing that *ABCE1* cannot compensate for the partial loss of *ABCE2* function (Supplementary Figure S7C). The low expression levels of *ABCE1* and its unresponsiveness to decreased *ABCE2* function may indicate that *ABCE1* is undergoing pseudogenization. However, *api7-1* flowers, where we observed the highest *ABCE1* expression levels, do not show apparent aberrations (Supplementary Figure S1E), suggesting that *ABCE1* might play a role during flower development.

ABCE1 and ABCE2 proteins share 80.8% identity, suggesting that ABCE1 and ABCE2 might be functionally equivalent. To test this hypothesis, we performed a promoter swapping assay between the *ABCE1* and *ABCE2* genes (Figure 3). As expected from the lower expression levels driven by the *ABCE1* promoter, *api7-1 ABCE1~pro~:ABCE2* plants were indistinguishable from *api7-1* mutants, highlighting that correct protein levels are as important as the correct sequence for normal *ABCE2* function. In contrast, the

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ABCE2$_{pro}$*:ABCE1* transgene partially rescued the *api7-1* phenotype, showing that the ABCE1 and ABCE2 proteins are partially redundant.

ABCE2 is a cytoplasmic protein that physically associates with components of the translation machinery

ABCE proteins lack the TMDs found in most members of other ABC subfamilies and thus are expected to behave as soluble proteins (86). The single ABCE subfamily member in *Saccharomyces cerevisiae*, Rli1, is found at both the cytoplasm and the nucleoplasm (87-89), whereas its *Drosophila melanogaster* ortholog, Pixie, is exclusively cytoplasmic (90). To determine the subcellular localization of Arabidopsis ABCE2, we obtained an in-frame translational fusion of ABCE2 and GFP, driven by the constitutive *Cauliflower mosaic virus* 35S promoter: *35S*$_{pro}$*:ABCE2:GFP*. We then transferred this construct into L*er* plants. We visualized the ABCE2:GFP fusion protein in root cells treated with propidium iodide, which mainly stains cell walls, and detected fluorescence only in the cytoplasm (Figure 4A, C, E). To further confirm the nuclear exclusion of ABCE2, we constructed and transferred a *35S*$_{pro}$*:ABCE2:YFP* transgene into *api7-1* plants, which restored the wild-type phenotype (Supplementary Figure S8A-C). We also observed the ABCE2:YFP fusion protein in roots stained with the nucleoplasm fluorescent dye DAPI and confirmed the nuclear exclusion of ABCE2 (Figure 4B, D, F).

To further investigate ABCE2 function, we performed a co-immunoprecipitation assay using the ABCE2:YFP protein from a homozygous T$_2$ *api7-1 35S*$_{pro}$*:ABCE2:YFP* line. We checked the purification of the fusion protein by western blotting using an anti-GFP antibody (Supplementary Figure S8D-F). Using LC-ESI-MS/MS, we identified 20 putative interactors of ABCE2, of which 13 participate in translation (6 subunits of the eIF3 complex, eIF5B, RPL3B, and ROTAMASE CYP 1 [ROC1]) or in the regulation of translation (At5g58410, EVOLUTIONARILY CONSERVED C-TERMINAL REGION 2 [ECT2], ILITYHIA [ILA], and REGULATORY-ASSOCIATED PROTEIN OF TOR 1 [RAPTOR1] or RAPTOR2), and two others had previously been shown to interact with ABCE orthologs (At2g20830, and EXPORTIN 1A [XPO1A] or XPO1B). The functions of the remaining 5 proteins that co-immunoprecipitated with ABCE2 are unclear and these proteins were therefore set aside for future characterization (Figure 5; Supplementary Figure S9; Supplementary Tables S4, S5; Supplementary Data Set 1).

XPO1 proteins are conserved nuclear transporters that translocate their cargoes from the nucleus to the cytoplasm through nuclear pore complexes. In *Saccharomyces cerevisiae*, *Xenopus laevis*, and humans, XPO1 modulates the nucleo-cytoplasmic partitioning of hundreds of cargoes, preventing their unwanted, and sometimes accidental, presence in the nucleus, as is the case of ABCE proteins (91). The interaction between

ABCE2 and Arabidopsis XPO1A or XPO1B (XPO1A/B) supports a conserved role of Arabidopsis XPO1A/B in excluding ABCE2 from the nucleus, which is also in agreement with its cytoplasmic localization.

At2g20830 encodes a folic acid binding/transferase that shares 30.2% and 26.7% identity with human ORAL CANCER OVEREXPRESSED 1 (ORAOV1) and *Saccharomyces cerevisiae* Lto1 (named after "required for biogenesis of the large ribosomal subunit and initiation of translation in oxygen"), respectively. ORAOV1 (Lto1), together with YAE1 (Yae1), constitute an essential complex for FeS cluster assembly on ABCE1 (Rli1) (92-94). Despite the observation that At2g20830 protein was predicted to localize to mitochondria, the high conservation level of this protein with its yeast and human orthologs prompted us to consider At2g20830 an ABCE2 interactor. Indeed, At2g20830 may be necessary for FeS cluster assembly on ABCE2, suggesting that ABCE2 has a functional FeSD, which supports its conserved role in ribosome dissociation.

The presence of RPL3B and several eIF3 complex subunits among the identified interactors further supports the functional conservation of ABCE2. In higher eukaryotes, including plants, there are 13 eIF3 complex subunits (eIF3a to eIF3m) of which eIF3j is a non-stoichiometric subunit (95). Our co-immunoprecipitation assay suggested that Arabidopsis ABCE2 interacts with 6 of these eIF3 subunits: eIF3a, c, d, e, k, and j. The ABCE orthologs Rli1 and Pixie also interact with the eIF3a, c, and j subunits (15,87-89,96,97). Those interactions have been related to the presence of the ABCE protein in the 40S subunit after ribosome dissociation, where it may be impeding premature joining of the 60S subunit until late steps of initiation of a new cycle of translation (5,15,18). Interestingly, the interaction between eIF3j (High-Copy suppressor of Rpg1 [Hcr1] in *Saccharomyces cerevisiae*) and ABCE1 (Rli1) also occurs in humans and *Saccharomyces cerevisiae*. In these species, eIF3j acts as an accessory factor for ABCE1-mediated ribosome dissociation (18,98), a function that seems to be conserved in Arabidopsis.

eIF5B was also found among the proteins that co-immunoprecipitated with ABCE2. In humans and *Saccharomyces cerevisiae*, this translation initiation factor and ABCE1 perform opposite roles, joining and dissociating the ribosomal subunits, respectively (7,99,100). In addition, they cannot coexist on the ribosome during translation initiation, since both bind to the 40S GTPase-binding site on the interface of the subunits (5,15). However, our identification of eIF5B among the proteins that co-immunoprecipitated with ABCE2 suggests that the mechanisms of pre-40S maturation and/or translation initiation might occur differently in plants, where eIF5B and ABCE2 can coexist in the same complex.

Some of the proteins identified in our assay might not be direct interactors of ABCE2, such as ROC1, a cyclophillin that catalyzes cis-trans isomerizations of prolyl bonds during protein folding (101,102); ECT2, a reader of methylated *N6*-adenosines (m^{6}As) in 3′ UTRs

of mRNA molecules (103-105); and ILA, a HEAT repeat protein that inhibits translation in response to different stresses (106-108). Instead, they seem to co-immunoprecipitate as part of the translational complexes in which ABCE2 participates. Another candidate to be part of an indirect interaction is At5g58410, which is annotated in UniProt as encoding the E3 ubiquitin-protein ligase listerin (LTN1; UniProt code: Q9FGI1) whose function in Arabidopsis remains unexplored. However, in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and humans, both ABCE1 and Ltn1 are necessary during nonstop mRNA decay (NSD) and ribosome quality control (RQC), two parallel pathways that act simultaneously to degrade stop codon-less mRNAs and their products, respectively (4,109-111). We also identified the RAPTOR1 or RAPTOR2 (RAPTOR1/2) proteins, which belong to the TORC1 complex, further composed of TOR and LETHAL WITH SEC THIRTEEN 8-1 (LST8-1). This complex is highly conserved in eukaryotes and regulates translation and ribosome biogenesis in response to internal and external stimuli (112). The ABCE2 interaction with the TORC1 complex is also supported by its interaction through LST8-1 (113), but further research is needed to ascertain its biological meaning.

ASYMMETRIC LEAVES 1 (*AS1*) and *AS2* encode transcription factors involved in leaf dorsoventral patterning and double mutant combinations of *as1* or *as2* with mutations in genes encoding ribosomal proteins or components of the translation machinery usually produce synergistic phenotypes. These phenotypes are easily distinguished by the presence of trumpet-like (peltate) or radial leaves originated by partial or complete loss of dorsoventrality, respectively (56,73,76,114). We obtained *api7-1 as1-1* and *api7-1 as2-1* double mutants, introgressed into the Col-0 background by a cross and two additional backcrosses to Col-0; these double mutants exhibited additive and synergistic phenotypes, respectively (Supplementary Figure S10). The presence of radial leaves in *api7-1 as2-1* plants (Supplementary Figure S10G) further supports a role for ABCE2 in translation.

Leaf and vascular defects in *api7-1* plants

Mutations that disrupt the translation machinery usually alter leaf vascular development (29,72-75). The *ATHB8* (*ARABIDOPSIS THALIANA HOMEOBOX GENE 8*) gene is expressed in pre-procambial cells that will differentiate into veins (37). To determine if *api7-1* leaf venation was altered, we crossed *api7-1* plants to an *ATHB8pro:GUS* line, and studied the expression of the transgene in cleared first-node rosette leaf primordia of *api7-1 ATHB8pro:GUS* F₃ plants. Consistent with the slow growth phenotype of *api7-1* plants, we observed that they exhibit delayed first-node leaf emergence (Supplementary Figure S11). At similar early developmental stages in wild-type (Supplementary Figure S11A-C) and *api7-1* (Supplementary Figure S11K-L) emerged primordia, the GUS signal was intense at the apical lamina region. Shortly after the formation of the first vascular elements in the

midvein of wild-type primordia (Supplementary Figure S11C), the GUS signal became uniformly distributed along the lamina (Supplementary Figure S11E-G). In contrast, *api7-1* primordia retained high GUS activity at their apical region even after the formation of the whole midvein (Supplementary Figure S11M), suggesting that vascular differentiation is more intense in that region (Supplementary Figure S11N). Additionally, the formation of higher-order veins in *api7-1* primordia seemed to be reduced.

To further assess these vascular defects, we cleared fully expanded L*er* and *api7-1* first- and third-node leaves, which were examined under bright-field microscopy. We confirmed that *api7-1* first-node and, to a lesser extent, third-node leaves, contain fewer higher-order veins and more prominent indentations and vascularized hydathodes, in particular in the leaf apex, than L*er* leaves (Figure 6; Supplementary Figure S12). In contrast, these phenotypic traits seemed to be unaffected on *api7-1* cotyledons, cauline leaves, sepals, and petals (Supplementary Figure S13).

To further characterize the defects in the venation pattern of *api7-1* flat organs, we performed a morphometric analysis of *api7-1* cotyledons, first- and third-node leaves, cauline leaves, sepals, and petals. The cotyledons have a higher vein density, accompanied by an increase in the number of branching points and free-ending veins. In contrast, but in accordance with the reduced number of higher-order veins, *api7-1* first- and third-node leaves have less than half the number of branching points and free-ending veins than in L*er* plants. *api7-1* cauline leaves also show a slight decrease in branching points and free-ending veins. Regarding sepals and petals, we did not observe any remarkable differences between wild-type and *api7-1* plants (Supplementary Table S6). As expected from the rosette size measurements (Figure 1D), all *api7-1* organs, except sepals and petals, were smaller than those of L*er*. In addition, *api7-1* first- and third-node leaves are more pointed, i.e., less circular, than those of L*er*. In contrast, *api7-1* cauline leaves are more circular than in the wild type (Figure 6; Supplementary Figure S13; Supplementary Table S6).

Polar auxin transport and local auxin biosynthesis and signaling contribute to the establishment of leaf venation pattern (32,33). Therefore, the aberrant venation pattern observed in the *api7-1* mutant might be related to a potentially altered auxin homeostasis. The major player of auxin transport during venation patterning in leaf primordia is the PIN-FORMED1 (PIN1) exporter (115), while the main pathway for auxin biosynthesis is a two-step process that relies on the sequential action of TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) or TAA1-RELATED (TARs) and YUCCA (YUC) enzymes (116). Regarding auxin signaling, the synthetic auxin-responsive promoter *DR5* located upstream of the coding sequence of a reporter gene is widely used to assess auxin perception. To ascertain whether auxin transport or signaling are affected in the *api7-1* mutant, we crossed this mutant to plants carrying the *PIN1_{pro}:PIN1:GFP* and

DR5~pro~:3XVENUS:N7 constructs (36). After several rounds of selfing and selection, we obtained wild-type and *api7-1* plants homozygous for either *PIN1~pro~:PIN1:GFP* or *DR5~pro~:3XVENUS:N7*. Then, we visualized and photographed the fluorescent signals of GFP and VENUS on primary roots stained with the cell wall dye propidium iodide, using confocal microscopy. Quantification of the fluorescence observed indicated that PIN1:GFP signal decreased, and that of VENUS:N7 increased, in *api7-1* roots, compared to the wild type (Figure 7). The high auxin perception found in *api7-1* roots is in agreement with a potential increase in local auxin biosynthesis triggered to compensate for the scarce amount of auxin imported from above-ground tissues due to reduced PIN1 expression, as similarly observed when auxin transport is inhibited in naphthylphthalamic acid (NPA)-treated plants, which overexpress auxin biosynthesis genes in their primary roots (117).

Genes related to auxin and iron homeostasis are deregulated in *api7-1* plants

To gain insight into the biological processes affected in the *api7-1* mutant, we performed an RNA-seq analysis of L*er* and *api7-1* rosettes collected 14 das. We identified 3218 downregulated and 2135 upregulated genes in the *api7-1* mutant (Supplementary Data Set 2). A GO enrichment analysis performed separately for down- and upregulated genes showed that the downregulated genes were mainly related to responses to abiotic and biotic stresses and protein post-translational modifications. In contrast, upregulated genes grouped into more diverse Biological Process terms (Supplementary Data Set 3).

We found enriched terms referring to responses to several hormones among both the down- and the upregulated genes. Remarkably, the three terms related to auxin (response to auxin [GO:0009733], auxin-activated signaling pathway [GO:0009734], and auxin polar transport [GO:0009926]) were only enriched among the upregulated genes. In agreement with the increased response to auxin in the *api7-1* mutant, we observed that five out of seven genes that participate in the main auxin biosynthesis pathway in leaves were upregulated. They included the three genes encoding enzymes that convert tryptophan into indole-3-pyruvic acid (IPyA), *TAA1*, *TAR2*, and *TAR3*, and two *YUC* genes (*YUC2* and *YUC6*) encoding enzymes that turn IPyA into indole-3-acetic acid (IAA) (116). We also found that three genes involved in auxin inactivation, *IAA CARBOXYLMETHYLTRANSFERASE 1* (*IAMT1*), *GRETCHEN HAGEN 3.17* (*GH3.17*), and *DIOXYGENASE FOR AUXIN OXIDATION 2* (*DAO2*), were upregulated, and that three genes involved in auxin activation, *IAA-LEUCINE RESISTANT (ILR)-LIKE 2* (*ILL2*), *ILL3*, and *ILL4*, were downregulated, probably in response to high auxin levels (Supplementary Figure S14A).

In this manner, our results point to an increase in auxin biosynthesis in *api7-1* leaves, which might be partially compensated by inactivating auxin and preventing its reactivation. Nevertheless, the activation of these compensation mechanisms is insufficient to prevent a

phenotypic effect on *api7-1* plants. The potential high levels of active auxin during leaf primordia development in the *api7-1* mutant might be responsible for the dense vascularization observed in the apical region and hydathodes of *api7-1* leaves (Figure 6; Supplementary Figures S10; S11) (32,118). Accordingly, we observed that the genes encoding some members from the pathway that promotes provascular cell divisions, namely the transcription factors ARF5 and TARGET OF MONOPTEROS 5 (TMO5), and an enzyme involved in cytokinin biosynthesis (LONELY GUY 3 [LOG3]) (119-121), were upregulated in the *api7-1* mutant (Supplementary Figure S14A).

Interestingly, iron ion homeostasis and transport (GO:0055072 and GO:0006826), and response to iron and sulfur ion starvation (GO:0010106 and GO:0010438) terms were also enriched in the analysis of upregulated genes (Supplementary Data Set 3A). For instance, genes related to iron uptake, such as *IRON-REGULATED TRANSPORTER 1* (*IRT1*) and *FERRIC CHELATE REDUCTASE DEFECTIVE 1* (*FRD1*) (122,123), or to iron mobility, such as *NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 3* (*NRAMP3*) and *NRAMP6* (124,125), and several genes encoding transcription factors induced by iron and sulfur deficiencies were upregulated in the *api7-1* mutant. These pathways might be activated in *api7-1* plants to provide iron and sulfur for FeS cluster biogenesis, probably to compensate for the depletion in ABCE2 protein. Indeed, the gene that encodes the Arabidopsis NEET protein (termed after its conserved Asn-Glu-Glu-Thr sequence near its C-terminus) (126), which participates in FeS cluster transference during their biogenesis (127,128), was also upregulated (Supplementary Figure S14B).

Consequently, the iron content in *api7-1* cells might be higher than in the wild type, and might be inducing the formation of reactive oxygen species (ROS), as occurs in mutants affected in free iron storage (129). In agreement with this assumption, several terms related to oxidative stress responses were also enriched. Specifically, we found that *FERRITIN 2* (*FER2*) and *FER3*, which encode iron storage proteins in response to high iron levels to avoid oxidative damage (129,130), were upregulated (Supplementary Figure S14B). In addition, previous studies have shown that ROS prevent FeS cluster assembly into ABCE proteins, which is necessary for their activity in ribosome recycling (94,131,132). In this manner, *api7-1* plants might experience a positive feedback loop where a response to iron starvation due to reduced activity of ABCE2 increases iron levels, inducing the production of ROS which, in turn, further disturbs ABCE2 activity. Nevertheless, further studies are needed to ascertain a potential relation among ABCE2 activity, iron homeostasis, and oxidative stress, which were beyond the scope of our research.

DISCUSSION

Plant ABCE proteins participate in translation in a cross-kingdom conserved manner

In this work, we studied Arabidopsis ABCE2, one of the most conserved proteins among archaea and eukaryotes (86). Although the ribosome-recycling activity of plant ABCE proteins still has not been assessed, the molecular function of these proteins has been widely investigated in the archaeon *Saccharolobus solfataricus*, the yeast *Saccharomyces cerevisiae*, the insect *Drosophila melanogaster,* and humans. In these species, ABCEs dissociate cytoplasmic ribosomes into their 30S/40S and 50S/60S subunits at different translation events, like pre-40S maturation, translation termination, and ribosome rescue during NSD (133). After ribosome dissociation, an ABCE escorts the 30S/40S subunit until the late steps of translation reinitiation, preventing premature joining of the 50S/60S subunit into the preinitiation complex (5,6,15,18).

Here, we found that Arabidopsis ABCE2 is exclusively located in the cytoplasm, like its *Drosophila melanogaster* ortholog, Pixie (90). *Saccharomyces cerevisiae* Rli1 has also been found in the nucleoplasm, where it overaccumulates in the *xpo1-1* mutant (87-89). In fact, as previously mentioned, XPO1 nuclear exporters prevent the presence of cytoplasmic proteins like ABCEs in the nucleus (91). Accordingly, we identified Arabidopsis XPO1A/B as interacting partners of ABCE2 in our co-immunoprecipitation assay, strongly suggesting a conserved role for XPO1 beyond fungi and animals.

In addition, we found that ABCE2 co-immunoprecipitates with several proteins related to translation reinitiation, especially with subunits of the eIF3 complex. Among these, the identification of the eIF3j subunit points to a conserved role for both ABCE2 and eIF3j in ribosome recycling. In humans and *Saccharomyces cerevisiae*, eIF3j (Hcr1) has been proposed as an accessory factor of ABCE1 during ribosome dissociation, as it boosts ABCE1 ribosome-dissociating activity (18,98). Interestingly, *Saccharomyces cerevisiae rli1*- and *hcr1*-depleted cells show an increase of translation reinitiation events on 3′ UTRs, which in the latter case are restored to wild-type levels after *RLI1* overexpression. Indeed, *hcr1*-depleted cells overexpress *RLI1* and *LTO1*, which encodes a protein required for FeS cluster assembly on Rli1, together with genes required for FeS cluster biogenesis (92-94,98).

We observed similar trends in Arabidopsis. First, the responses to iron and sulphur starvation are activated in the hypomorphic *api7-1* mutant, suggesting that ribosome recycling and iron and sulphur homeostasis are also linked in Arabidopsis. Second, ABCE2 interacts with the At2g20830 protein, which is 26.7% and 30.2% identical to *Saccharomyces cerevisiae* Lto1 and its human ortholog, ORAOV1, respectively. Therefore, our observations suggest a novel function for the At2g20830 protein in FeS cluster assembly on cytoplasmic proteins like ABCE2. In turn, the presence of a functional FeSD in ABCE2 supports its role

in ribosome dissociation. Since iron is essential for photosynthesis and hence for crop yield, the relationship between the activity of ABCE proteins and iron homeostasis might lead to new ways to modulate plant growth in species with agronomic interest.

Although our co-immunoprecipitation assay did not allow us to discern direct from indirect ABCE2 interactors, the interactions with XPO1A/B, eIF3j, and At2g20830 are very likely to be direct, in agreement with previous studies (18,91-94,98). In contrast, the interactions observed with other eIF3 subunits, RPL3B, ROC1, ECT2, ILA, LTN1, and RAPTOR1/2, might occur indirectly as they are part of or interact with the translation machinery (4,101-108,111,134). However, the interaction between ABCE2 and eIF5B, which does not seem to occur in other species (5,15), will require further exploration.

Further supporting a role for ABCE2 in translation, we observed a synergistic interaction in the *api7-1 as2-1* double mutants, which show radial leaves, as previously described for double mutant combinations of loss-of-function alleles of *AS1* or *AS2* and other components of the translation machinery (56,73,76,114). In this manner, our results strongly suggest that Arabidopsis and, by extension, all plant ABCEs, participate in translation, probably by dissociating cytoplasmic ribosomes, as has been reported for species of other kingdoms (133). In addition, previous works also support a conserved role for the Arabidopsis ABCE2 and human ABCE1 proteins as suppressors of RNA silencing (23,24,85,135). However, we did not find any ABCE2 interactor potentially involved in this process, nor any enriched ontology term related to gene silencing in our RNA-seq assay. This might be due to the need for a cellular environment that triggers RNA silencing and exposes this novel function of ABCE proteins. Further research will help to assess a potential relationship between ribosome recycling and RNA silencing.

The developmental defects of the *api7-1* mutant link translation and auxin homeostasis

The essential function of ABCEs has been confirmed in several species, in addition to those mentioned above. Null alleles of *ABCE* genes in all studied organisms are lethal, while hypomorphic alleles cause severe growth aberrations (21). This is also the case for Arabidopsis. In this work, we describe the first hypomorphic and null alleles of Arabidopsis *ABCE2* gene, *api7-1* and *api7-2*, respectively (Figure 2). We showed that the *api7-2* mutation is lethal and that *api7-1* plants share developmental defects with other mutants affected in genes encoding ribosomal proteins or ribosome biogenesis factors. These phenotypic traits include a small rosette with pointed and dentate leaves, and a reduced growth rate. In addition, these mutants usually have small leaf epidermal cells and increased ploidy levels when compared to wild-type plants, as well as an aberrant leaf venation (73), as is the case for the *api7-1* mutant. Indeed, an allele of *SIMPLE LEAF3*, the

Cardamine hirsuta ABCE ortholog, also causes growth delay, increased leaf ploidy levels, and venation pattern defects related to an aberrant auxin homeostasis (25). Remarkably, both *sil3* and *api7-1* leaves contain a reduced number of branching points and free ending veins, and, consequently, fewer higher-order veins than their respective wild types (25).

In agreement with the involvement of local auxin biosynthesis, polar transport, and signalling in vascular development, we observed that auxin biosynthesis and auxin-induced genes were upregulated in the *api7-1* mutant (32,33,116). Among the auxin-induced genes, we found *ARF5* and its target, *TMO5*, which regulate vascular development. TMO5 promotes the expression of *LOG3*, which is also upregulated in the *api7-1* mutant, and encodes an enzyme involved in cytokinin biosynthesis. Cytokinin is known to induce cell divisions in the embryo and root procambium (119-121,136). The overexpression of these genes, together with the altered *ATHB8* expression in *api7-1* leaves, might be the cause of their hypervascularized hydathodes. Regarding auxin biosynthesis, the upregulation of this pathway in *api7-1* leaves, and the increased auxin perception in *api7-1* roots reported by *DR5$_{pro}$:3XVENUS:N7*, strongly suggest that auxin is being overproduced in the whole mutant plant. In this sense, our previous hypothesis about a local overproduction of auxin in *api7-1* roots to compensate for its reduced arrival due to decreased PIN1 levels, seems less likely. The reduced PIN1:GFP levels that we observed in *api7-1* roots might be a consequence, rather than a cause, of a general auxin homeostasis deregulation.

In agreement with results obtained from human and *Saccharomyces cerevisiae* ABCE1-depleted cells (8,94), the *api7-1* mutation might impede proper ABCE2-dependent ribosome dissociation, allowing ribosomes to reinitiate translation in the 3′ UTR. In turn, the passage of the ribosomes through the 3′ UTR would displace the regulatory elements found within that region, increasing or decreasing the mRNA lifespan. The inability to dissociate ribosomes in *api7-1* plants might also affect uORF-mediated post-transcriptional regulation, disturbing, among other biological processes, auxin homeostasis in similar ways to those described for other mutations in the translation machinery in Arabidopsis (29,30,34).

The Arabidopsis ABCE1 and ABCE2 proteins are functionally redundant

Arabidopsis has two ABCE paralogs, *ABCE1* and *ABCE2* (2,22). In agreement with previous literature (24,85), we observed that *ABCE1* expression levels are low in all studied organs and throughout development, in contrast to the high expression of *ABCE2*, and that ABCE2 disruption in the *api7-1* mutant does not trigger a compensation mechanism through *ABCE1* overexpression. These observations suggest that *ABCE1* is undergoing pseudogenization.

However, the wild-type phenotype of *api7-1* flowers, where we found the highest expression levels of *ABCE1*, and the ability of *ABCE2$_{pro}$:ABCE1* to complement the *api7-1*

mutant phenotype, indicate that ABCE1 contributes to translation in the reproductive tissues. In addition, our phylogenetic analysis showed that the *ABCE* duplication event occurred early during the evolution of Brassicaceae, and that at least five species from this clade conserved an *ABCE1* gene that evolved more rapidly than its *ABCE2* paralog, suggesting that *ABCE2* conserved the ancestral function, whereas *ABCE1* underwent subfunctionalization.

To conclude, although ABCE proteins are encoded by a single gene in most species, they are essential for archaea and eukaryotes (21). Due to their importance, the molecular mechanisms by which they participate in ribosome recycling have been deeply studied, and remain a subject of intense research (5,6,15,16,18). Nevertheless, the biological consequences of ABCE disruption are poorly understood in all organisms. In this sense, future research linking the molecular function of ABCEs with the phenotypic output of their disruption will contribute to determining the pathways through which translation modulates development, as we show here with the isolation and study of the hypomorphic and viable *api7-1* allele of the Arabidopsis *ABCE2* gene.

DATA AVAILABILITY

The raw data from genome resequencing and RNA-seq were deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) database under accession numbers SRP065876 and PRJNA719000, respectively.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

AUTHOR CONTRIBUTIONS

J.L.M. conceived, designed, and supervised the research, provided resources, and obtained funding. Several experiments were codesigned by C.N.-Q., E.M.-B., and J.L.M. C.N.-Q. performed most of the experiments. E.M.-B. obtained the *ABCE2$_{pro}$:ABCE2* and *35S$_{pro}$:ABCE2:GFP* transgenes, and contributed to the phenotypic analysis of *api7-1*. E.M.-B. and H.C. obtained the *api7-1 as* double mutants. C.N.-Q. and H.C. performed the phylogenetic analysis. H.C. and A.M.L. screened the Micol collection of leaf mutants for abnormal leaf venation patterns. P.R. performed preliminary morphometric analysis of cells and venation from *api7-1* leaves. M.R.P., H.C., and E.M-B. performed the mapping and cloning of the *api7-1* mutation. C.N.-Q. and J.L.M. wrote the manuscript. All authors revised and approved the manuscript.

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FIGURE LEGENDS

Figure 1. Morphological phenotype of the *api7-1* mutant. (A–C) Rosettes from (A) the wild-type L*er*, (B) the *api7-1* mutant, and (C) an *api7-1 ABCE2~pro~:ABCE2* mutant and transgenic plant. Pictures were taken 16 days after stratification (das). Scale bars indicate 2 mm. (D, E) Growth progression of (D) rosette area and (E) main stem length. Bars indicate (D) mean and (E) median values. Error bars represent (D) standard deviation and (E) median absolute deviation. Asterisks indicate a significant difference with L*er* in a (D) Student's *t* test ($10 < n < 17$) or (E) Mann-Whitney *U* test ($n = 8$) ($*P < 0.05$, $**P < 0.001$).

Figure 2. Fine mapping by linkage analysis of the *api7-1* mutation. (A) A mapping population of 273 F_2 plants derived from an *api7-1* × Col-0 cross allowed us to delimit a candidate region of 123.5 kb in chromosome 4, flanked by the T18B16 and g3883 markers. The names and physical map positions of the molecular markers used for linkage analysis are shown. All values outside parentheses indicate Mb. The number of recombinant chromosomes found (from a total of 546 chromosomes analyzed) is indicated in parentheses. (B) Structure of the At4g19210 (*ABCE2*) gene, located within the candidate region, with indication of the nature and position of the *api7* mutations studied in this work. Boxes and lines indicate exons and introns, respectively. White boxes represent UTRs. The arrow indicates the *api7-1* point mutation. The triangle indicates the *api7-2* T-DNA insertion (GABI_509C06).

Figure 3. Effects of the *ABCE2~pro~:ABCE1* and *ABCE1~pro~:ABCE2* transgenes on the morphological phenotype of the *api7-1* mutant. Rosettes from (A) L*er*, (B) *api7-1*, (C) *api7-1 ABCE2~pro~:ABCE1*, and (D) *api7-1 ABCE1~pro~:ABCE2* plants. Pictures were taken 14 das. Scale bars indicate 2 mm.

Figure 4. Subcellular localization of the ABCE2 protein in cells from the root elongation zone. Confocal laser scanning micrographs of (A, C, E) L*er 35S~pro~:ABCE2:GFP* and (B, D, F) *api7-1 35S~pro~:ABCE2:YFP* transgenic plants. Fluorescent signals correspond to (A) GFP, (B) YFP, (C) propidium iodide, and (D) DAPI staining, and (E, F) the overlay of (E) GFP and propidium iodide, and (F) YFP and DAPI. Pictures were taken (A, C, E) 14 and (B, D, F) 5 das. Scale bars indicate 20 μm.

Figure 5. Proteins identified in an ABCE2:YFP co-immunoprecipitation assay. Proteins were grouped within dashed boxes according to their annotated functions for Arabidopsis (names in black letters) or orthologous (names in blue letters) proteins. Green and blue boxes represent complexes that have been described in Arabidopsis and other species,

respectively. Proteins in striped boxes were not identified in our assay but have been included in this figure because they are known to belong to a given complex. Continuous and dashed lines connecting boxes indicate physical and genetic interactions described elsewhere for Arabidopsis (black) or other species (blue), respectively. For references, see Supplementary Table S5. Names in bold and plain letters indicate proteins unique to or enriched in ABCE2:YFP samples, respectively.

Figure 6. Venation pattern of *api7-1* first- and third-node leaves. Representative diagrams of mature (A, B) first- and (C, D) third-node leaves from (A, C) L*er* and (B, D) *api7-1* plants. Margins were drawn in black, primary veins in green, secondary veins in blue, higher-order connected veins in yellow, and higher-order disconnected veins in pink. Organs were collected 21 das. Scale bars indicate 1 mm.

Figure 7. Auxin transport and perception in *api7-1* roots. Visualization of the expression of reporter transgenes for auxin (A, B) transport and (C, D) perception, in (A, C) wild-type and (B, D) *api7-1* roots. Cell walls were stained with propidium iodide. Values indicate average fluorescence intensities ± standard deviation from (A, B) GFP and (C, D) VENUS, which are significantly different from the wild type in a Student's *t* test [(A, B) $P < 0.001$, n = 25; (C, D) $P < 0.05$, n = 27]. Pictures were taken 5 das. Scale bars indicate 50 µm.

Navarro-Quiles *et al.*, Figure 1

Navarro-Quiles *et al.*, Figure 2

A Chromosome 4

0.5 kb

Navarro-Quiles *et al.*, Figure 3

A — L*er*
B — *api7-1*
C — *api7-1 ABCE2$_{pro}$:ABCE1*
D — *api7-1 ABCE1$_{pro}$:ABCE2*
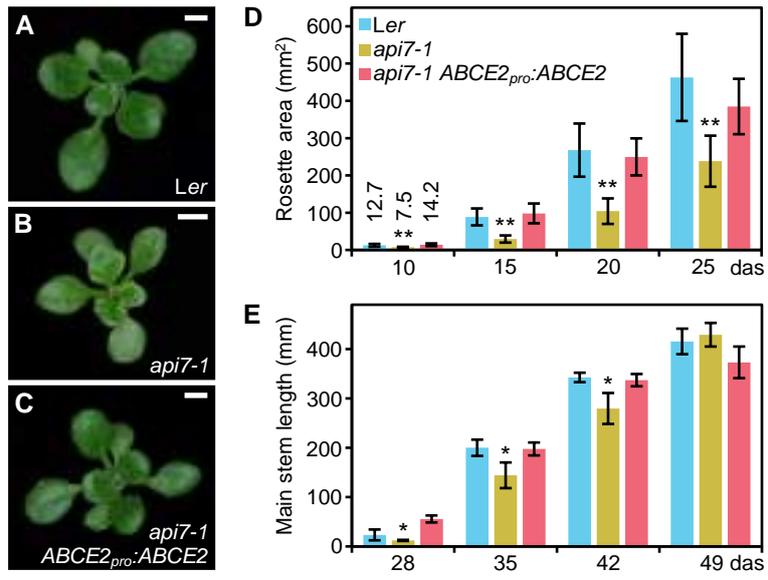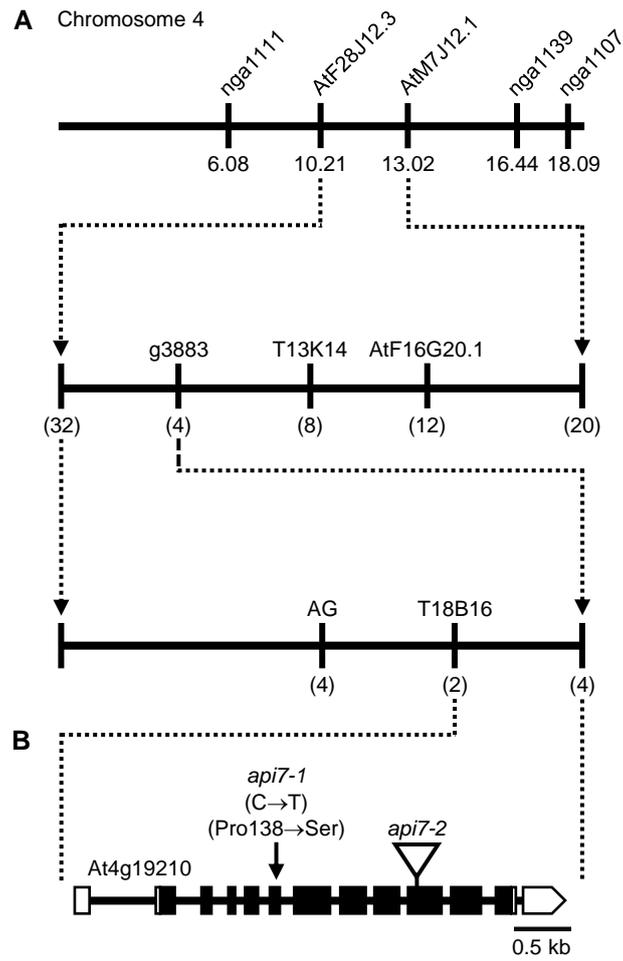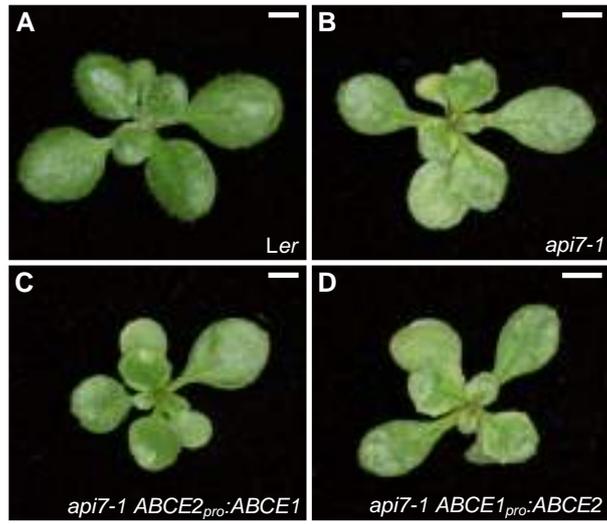
Navarro-Quiles *et al.*, Figure 4
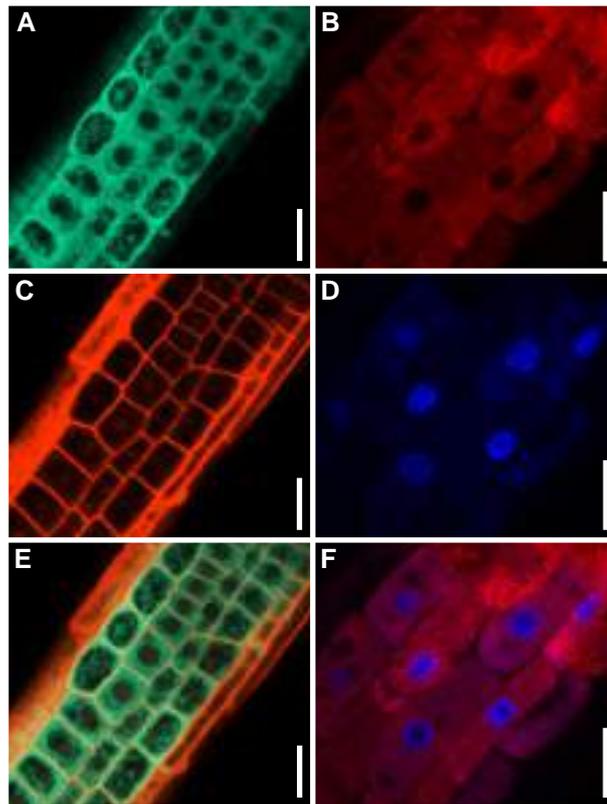
Navarro-Quiles *et al.*, Figure 5
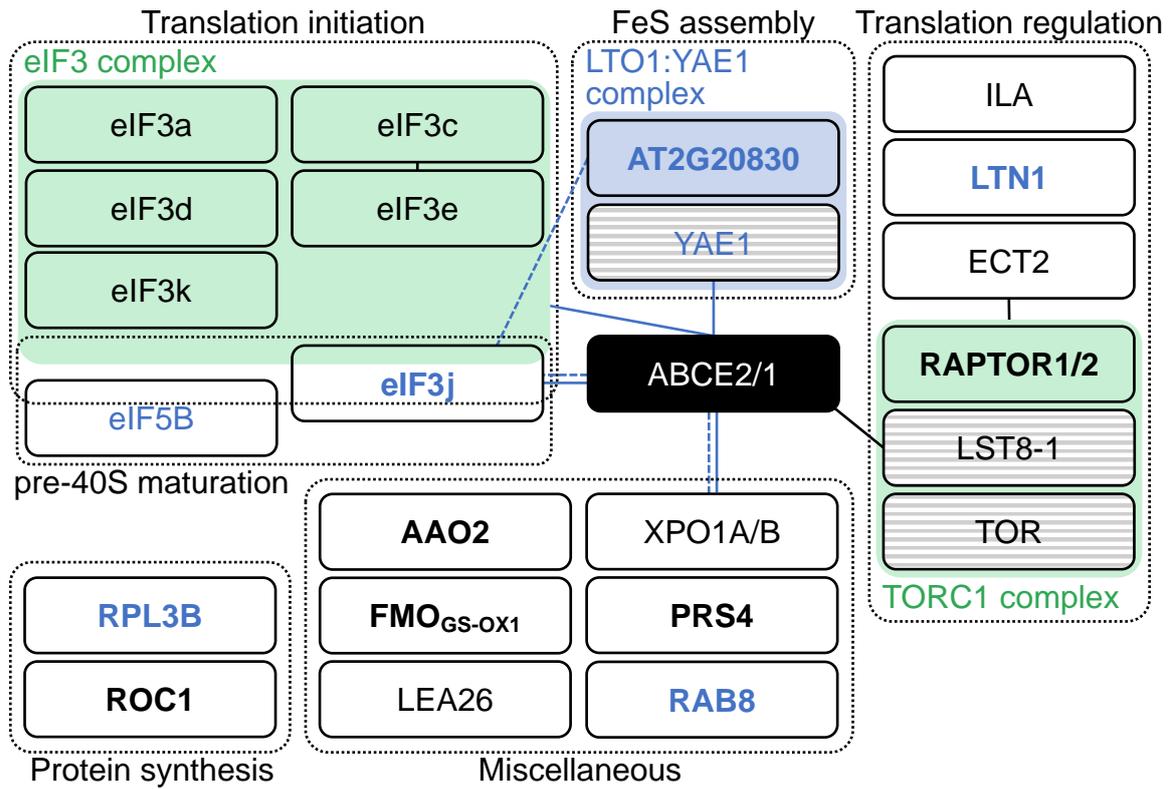
Navarro-Quiles *et al.*, Figure 6

Navarro-Quiles *et al.*, Figure 7

A   3953 ± 842
PIN1_{pro}:PIN1:GFP

B   2981 ± 451
api7-1 PIN1_{pro}:PIN1:GFP

C   7631 ± 1084
DR5_{pro}:3XVENUS:N7
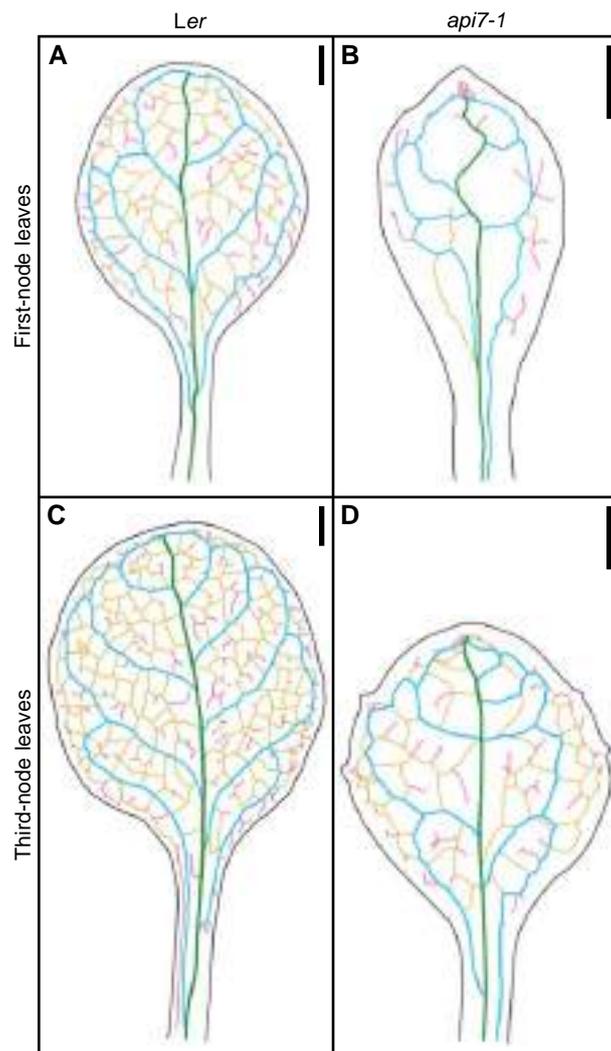
D   8535 ± 1707
api7-1 DR5_{pro}:3XVENUS:N7
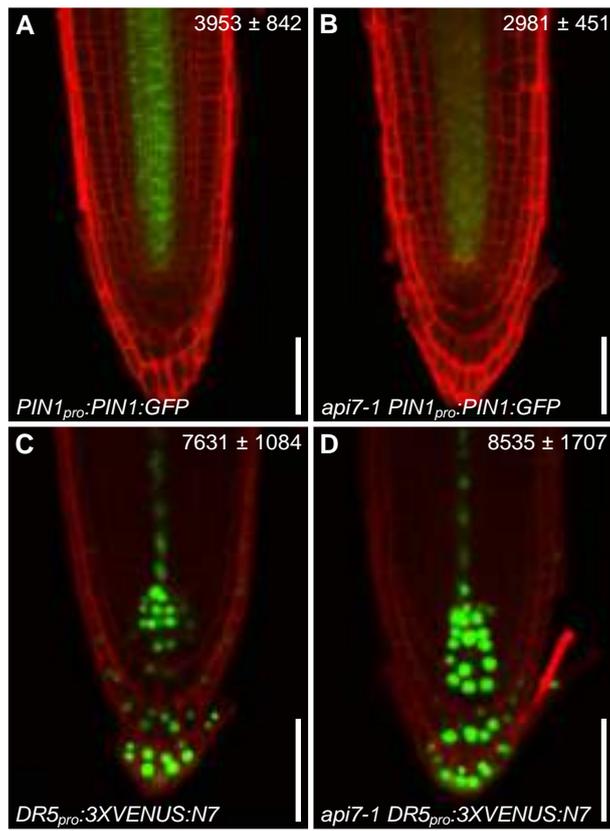
Arabidopsis ATP-Binding Cassette E2 is a conserved component of the translation machinery

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Pedro Robles, Antonio Martínez-Laborda, María Rosa Ponce,
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Supplementary Figures and Tables

Supplementary Data Sets not included in this file

Navarro-Quiles et al_Data Set 1.xlsx

Navarro-Quiles et al_Data Set 2.xlsx

Navarro-Quiles et al_Data Set 2.xlsx
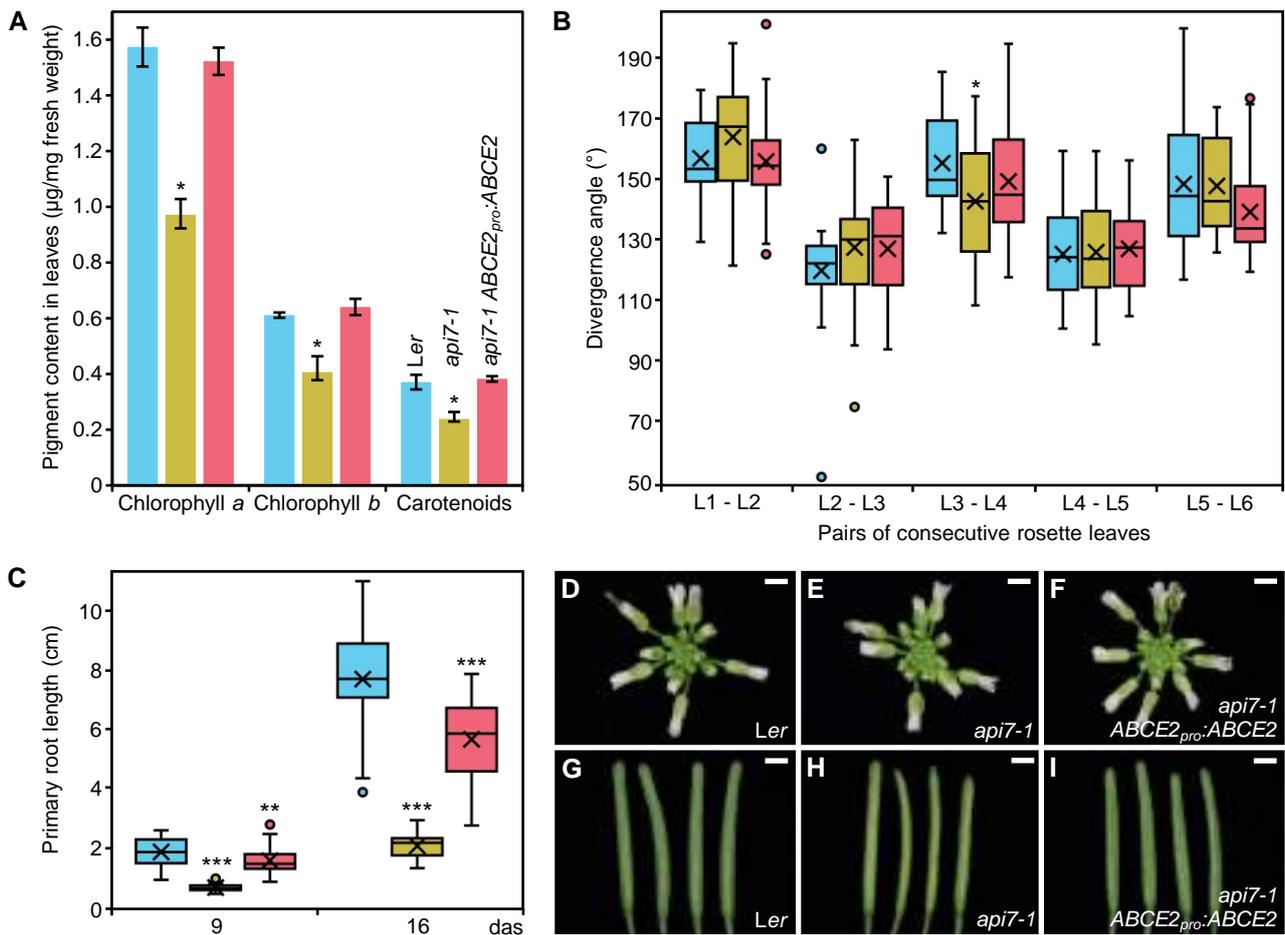
Supplementary Figure S1. Pigment content in leaves, rosette phyllotaxis, primary root length, and inflorescence and silique morphological phenotypes of L*er*, *api7-1*, and *api7-1 ABCE2pro:ABCE2* plants.

(A) Chlorophyll *a* and *b*, and carotenoid content in wild-type L*er*, *api7-1* mutant, and *api7-1 ABCE2pro:ABCE2* mutant and transgenic rosettes collected 16 das. Median values are shown. Error bars represent median absolute deviation. (B) Rosette leaf phyllotaxis in plants of the genotypes mentioned in (A). The divergence angles between consecutive leaves are shown: L1 - L2, first- and second-node leaves; L2 - L3, second- and third-node leaves; L3 - L4, third- and fourth-node leaves; L4 - L5, fourth- and fifth-node leaves; L5 - L6, fifth- and sixth-node leaves. (C) Primary root growth progression between 9 and 16 das in plants of the genotypes mentioned in (A). Boxes in (B) and (C) are delimited by the first (Q1, lower hinge) and third (Q3, upper hinge) quartiles. Whiskers represent the values within the distribution that are immediately higher than $Q1 - 1.5 \cdot IQ$ (lower) and lower than $Q3 + 1.5 \cdot IQ$ (upper), where $IQ = Q3 - Q1$. ✕: Mean. —: Median. ○: Outlier. Measurements were made (A) 16, (B) 18, and (C) 9 and 16 das. Asterisks indicate a significant difference with L*er* in a (A) Mann-Whitney *U* (n = 5) or (B, C) Student's *t* test (B, n = 23; C, 28 < n < 35) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (D–F) Inflorescences and (G–I) siliques of (D, G) L*er*, (E, H) *api7-1*, and (F, I) *api7-1 ABCE2pro:ABCE2* plants. Pictures were taken 40 das. Scale bars indicate 2 mm.
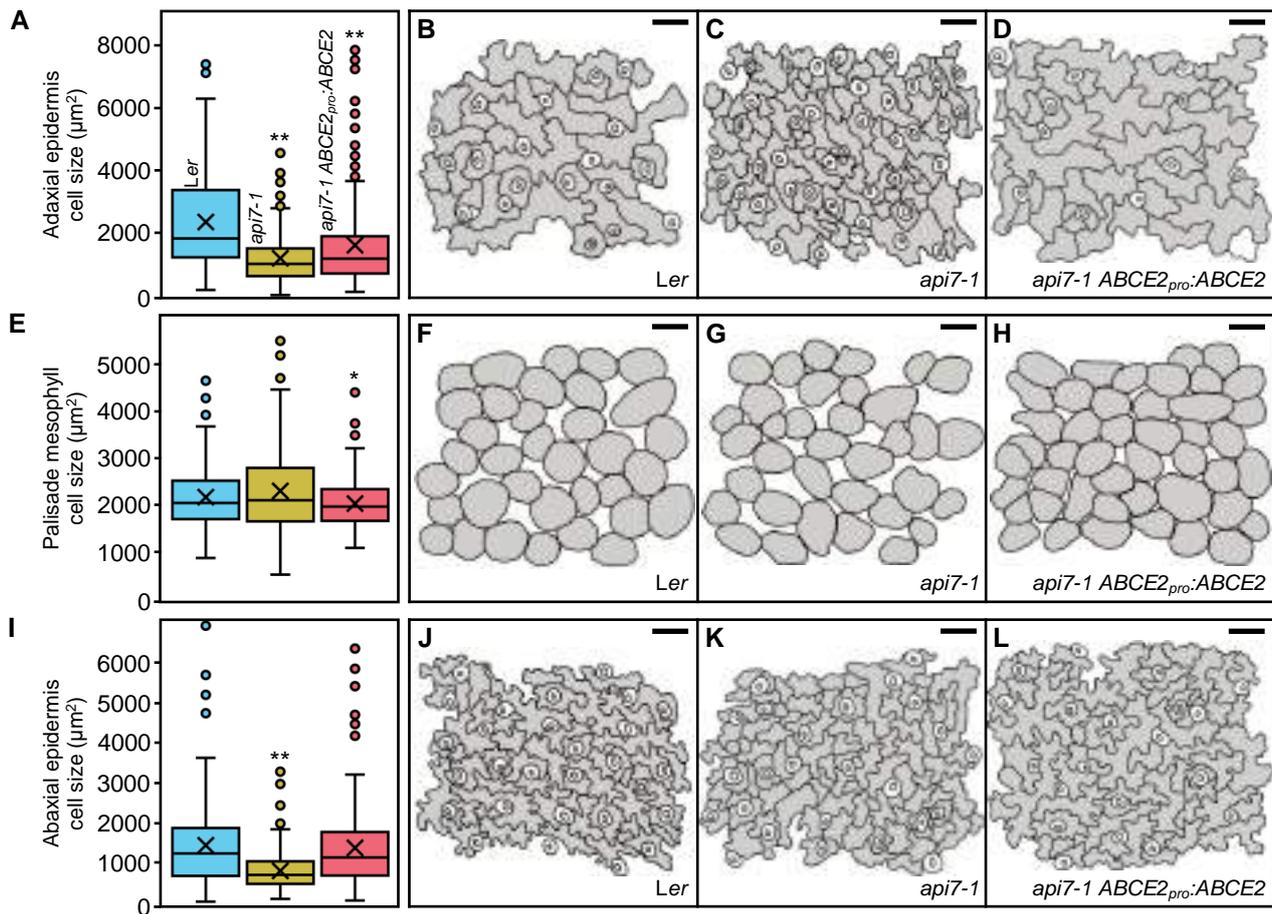
Supplementary Figure S2. Leaf cell phenotypes of L*er*, *api7-1*, and *api7-1 ABCE2_{pro}:ABCE2* plants.

(A, E, I) Boxplot distributions of cell area in the (A) adaxial epidermis, (E) subepidermal layer of palisade mesophyll, and (I) abaxial epidermis, from first-node leaves collected 21 das. Other details as described in the legend of Supplementary Figure S1 for its (B) and (C) sections. Between 144 and 351 cells were analyzed from at least 4 different samples. Asterisks indicate a significant difference with the L*er* wild-type in a Student's *t* test (*$P < 0.05$, **$P < 0.001$). (B–D, F–H, J–L) Representative diagrams of the (B–D) adaxial epidermis, (F–H) subepidermal layer of palisade mesophyll, and (J–L) abaxial epidermis, from (B, F, J) L*er*, (C, G, K) *api7-1*, and (D, H, L) *api7-1 ABCE2_{pro}:ABCE2* plants.
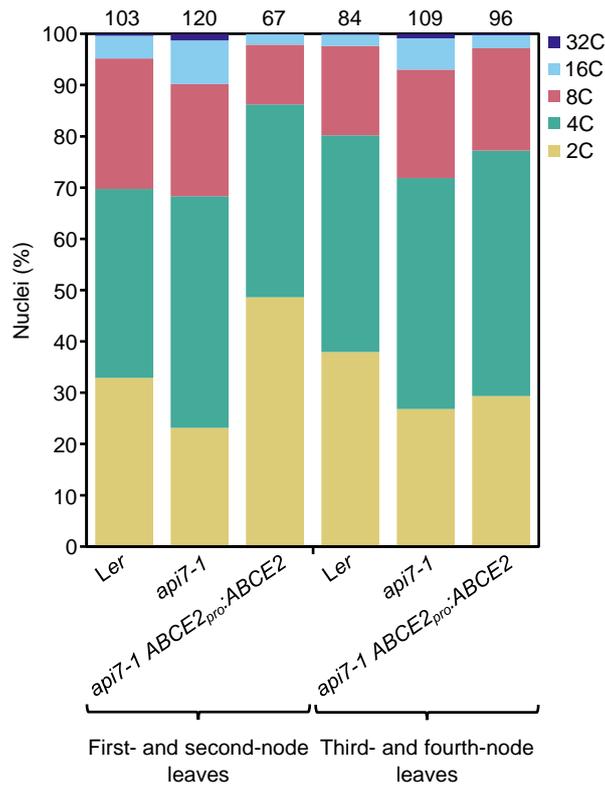
Supplementary Figure S3. Nuclear ploidy levels in L*er*, *api7-1*, and *api7-1 ABCE2_{pro}:ABCE2* rosette leaves.

DNA content was measured in nuclei from whole first- and second-node leaves, as well as third- and fourth-node leaves. The value at the top of each column indicates the mean endoreduplication index (EI) from three biological replicates. Each biological replicate was composed of either 6 first- and 6 second-node leaves or 6 third- and 6 fourth-node leaves. At least 20,000 events were analyzed in each biological replicate. Measurements were made 18 das.

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S. solfataricus      1 ----------------MRVAVINYDFCKPDKCNL-ECINFCPVDRSGGKAIELSEIVGK
P. furiosus          1 ----------------MRIAVIDYDKCNPDKCGHFLCERVCPVNRMGGEATIIDEE-NYK
C. elegans           1 MSRKGPLIKNNETDVPLRIAIVEKDRCKPKNCGL-ACKRACPVNRQGKQCIVVEAT-STI
S. cerevisiae        1 -----------MSDKNSRIAIVSADKCKPKKCRQ-ECKRSCPVVKTGKLCIEVTPT-SKI
O. sativa            1 -----------MAERLTRIAIVSEDKCKPKKCRQ-ECKKSCPVVKTGKLCIEVTPA-SKL
S. lycopersicum      1 -----------MSDRLTRIAIVSDDKCKPKKCRQ-ECKKSCPVVKTGKLCIEVTVG-SKI
A. thaliana          1 -----------MADRLTRIAIVSSDRCKPKKCRQ-ECKKSCPVVKTGKLCIEVTVG-SKL
C. hirsuta           1 -----------MADRLTRIAIVSSDRCKPKKCRQ-ECKKSCPVVKTGKLCIEVTVA-SKI
D. melanogaster      1 MSR---RKENEEVDKQTRIAIVSDDKCKPKRCRQ-ECKKTCPVVRMGKLCIEVTPT-SKI
H. sapiens           1 -----------MADKLTRIAIVNHDKCKPKKCRQ-ECKKSCPVVRMGKLCIEVTPQ-SKI
O. cuniculus         1 -----------MADKLTRIAIVNHDKCKPKKCRQ-ECKKSCPVVRMGKLCIEVTPQ-SKI
D. rerio             1 -----------MADKNTRIAIVNHDKCKPKKCRQ-ECKKSCPVVRMGKLCIEVSPQ-SKI
consensus            1        .  .. .*.*.. *.*.*..*. .*...***.. *...*...   ...

S. solfataricus     44 PVIYEETCIGCGICVKKCPYEAISIVNLPDELEGEVIHRYKVNGFKIFGLPTPKNNTILG
P. furiosus         44 PIIQEASCTGCGICVHKCPFKAISIVNLPEQLEEDCVHRYGVNAFVLYRLPVVKEGMVVG
C. elegans          59 SQISEILCIGCGICVKKCPYDAIKIINLPANLANETTHRYSQNSFKLHRLPTPRCGEVLG
S. cerevisiae       48 AFISEILCIGCGICVKKCPFDAIQIINLPTNLEAHVTHRYSANSFKLHRLPTPRPGQVLG
O. sativa           48 AFISEELCIGCGICVKKCPFDAIEIINLPKDLEKDTTHRYGPNTFKLHRLPVPRPGQVLG
S. lycopersicum     48 AFISEELCIGCGICVKKCPFEAIQIINLPKDLDKDTTHRYGPNTFKLHRLPVPRPGQVLG
A. thaliana         48 AFISEELCIGCGICVKKCPFEAIQIINLPRDLEKDTTHRYGANTFKLHRLPVPRPGQVLG
C. hirsuta          48 AFISEELCIGCGICVKKCPFEAIQIINLPRDLEKDTTHRYGPNTFKLHRLPVPRPGQVLG
D. melanogaster     56 ASLSEELCIGCGICVKKCPFEAITIINLPSNLEKHTTHRYSKNSFKLHRLPIPRPGEVLG
H. sapiens          48 AWISETLCIGCGICIKKCPFGALSIVNLPSNLEKETTHRYCANAFKLHRLPIPRPGEVLG
O. cuniculus        48 AWISETLCIGCGICIKKCPFGALSIVNLPSNLEKETTHRYCANAFKLHRLPIPRPGEVLG
D. rerio            48 VWISESLCIGCGICIKKCPFGALSIVNLPSNLEKETTHRYCANSFKLHRLPIPRPGEVLG
consensus           61 ....* .*.*****..***..*. *.***  *.....***  *.*.*..**..... ..*

S. solfataricus    104 VLGKNGVGKTTVLKILAGEIIPNFGDPNSKVGKDEVLKRFRGKEIYNYFKELYSNELKIV
P. furiosus        104 IVGPNGTGKSTAVKILAGQLIPNLCG--DNDSWDGVIRAFRGNELQNYFEKLKNGEIRPV
C. elegans         119 LVGTNGIGKSTALKILAGKQKPNLGNFQKEQEWTTIINHFRGSELQNYFTRILEDTLKCV
S. cerevisiae      108 LVGTNGIGKSTALKILAGKQKPNLGRFDDPEWQEIIKYFRGSELQNYFTKMLEDDIKAI
O. sativa          108 LVGTNGIGKSTALKVLAGKLKPNLGRFKNPPDWQEILTYFRGSELQNYFTRILEDNLKAI
S. lycopersicum    108 LVGTNGIGKSTALKVLAGKLKPNLGRFTNPPDWQEILTHFRGSELQNYFTRILEDNLKAI
A. thaliana        108 LVGTNGIGKSTALKILAGKLKPNLGRFTSPPDWQEILTHFRGSELQNYFTRILEDNLKAI
C. hirsuta         108 LVGTNGIGKSTALKVLAGKLKPNLGRFTNPPDWQEILTHFRGSELQNYFTRILEDNLKAI
D. melanogaster    116 LVGQNGIGKSTALKILAGKQKPNLGKYANPPDWTEILSYFRGSELQNYFTKILEDNLKAL
H. sapiens         108 LVGTNGIGKSTALKILAGKQKPNLGKYDDPPDWQEILTYFRGSELQNYFTKILEDDLKAI
O. cuniculus       108 LVGTNGIGKSTALKILAGKQKPNLGKYDDPPDWQEILTYFRGSELQNYFTKILEDDLKAI
D. rerio           108 LVGTNGIGKSTALKILAGKQKPNLGRFDAPPDWQEILAYFRGSELQNYFTKILEDDLKAI
consensus          121 ..*.**.**.*..*.***. .**....  ........  ***.*..***...... ....

S. solfataricus    164 HKIQYVEYASKFLKGTVNEI---LTKIDER--GKKDEVKELLNMTNIWNKDANILSGGGL
P. furiosus        162 VKPQYVDLIPKAVKGKVIEI---LKKADET--GKLEEVVKALELENVLDREIQHLSGGEL
C. elegans         179 IKPQYVDQIPRAAKGTVEKN---LTRKHDN--DNLNSVIDQMELRGILDREIDQLSGGEL
S. cerevisiae      168 IKPQYVDNIPRAIKGPVQKVGELLKLRMEKSPEDVKRYIKILQLENVLKRDIEKLSGGEL
O. sativa          168 IKPQYVDHIPKAVQGNVGQV---LDQKDER--GVKAEICVDLELNQVIDRNVGDLSGGEL
S. lycopersicum    168 IKPQYVDHIPKAVQGNVGQV---LDQKDER--DVKEKICVDLELNQVLDRNVGDLSGGEL
A. thaliana        168 IKPQYVDHIPRAVKGNVGEV---LDQKDER--DKKAEICADLELNQVIDRDVENLSGGEL
C. hirsuta         168 IKPQYVDHIPKAVRGNVGEV---LDQKDER--NKKAEICADLELNQVIDRDVENLSGGEL
D. melanogaster    176 VKPQYVDQIPKAVRGAVGDI---LDKKDER--ELQTKICEMLDLSHIRDREIAQLSGGEL
H. sapiens         168 IKPQYVDQIPKAAKGTVGSI---LDRKDET--KTQAIVCQQLDLTHIKERNVEDLSGGEL
O. cuniculus       168 IKPQYVDQIPKAAKGTVGSI---LDRKDET--KTQAIVCQQLDLTHIKERNVEDLSGGEL
D. rerio           168 VKPQYVDQIPKTVKGSVGAI---LSRKDDT--KTEELVCEQLDLHIRDRNVEDLSGGEL
consensus          181 .*.***. ......* *..  * ...  ..  .. ...  . ...  ****.*
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S. solfataricus   219 QRLLVAASLLREADVYIFDEPSSYLDVRERMNMAKAIRELLK-NKYVIVVDHDLIVLDYL
P. furiosus       217 QRVAIAAALLRNATFYFFDEPSSYLDIRQRLNAARAIRRLSEEGKSVLVVEHDLAVLDYL
C. elegans        234 QRFAIAMCCVQKADVYMFDEPSSYLDVKQRLKAAIIRERVSDTNYVVVVEHDLAVLDYL
S. cerevisiae     228 QRFAIGMSCVQEADVYMFDEPSSYLDVKQRLNAAQIIRSLPAPTKYVICVEHDLSVLDYL
O. sativa         223 QRFAIAVVAVQNAEIFMFDEPSSYLDVKQRLKAAQVVRSLLRPNSYVIVVEHDLSVLDYL
S. lycopersicum   223 QRFAIAVVAIQNAEIYMFDEPSSYLDVKQRLKAAQVVRSLLRPNSYVIVVEHDLSVLDYL
A. thaliana       223 QRFAIAVVAIQNAEIYMFDEPSSYLDVKQRLKAAQVVRSLLRPNSYVIVVEHDLSVLDYL
C. hirsuta        223 QRFAIAVVAIQNAEIYMFDEPSSYLDVKQRLKAAQVVRSLLRPNSYVIVVEHDLSVLDYL
D. melanogaster   231 QRFAIAMVCIQNADIFMFDEPSSYLDVKQRLNAALTIRSLHPTKFIIVVEHDLSVLDYL
H. sapiens        223 QRFACAVVCIQKADIFMFDEPSSYLDVKQRLKAAITIRSLINPDRYIIVVEHDLSVLDYL
O. cuniculus      223 QRFACAVVCIQKADIFMFDEPSSYLDVKQRLKAAITIRSLINPDRYIIVVEHDLSVLDYL
D. rerio          223 QRFACAVVCIQRADIFMFDEPSSYLDVKQRLRAAITIRSLISPDRYIIVVEHDLSVLDYL
consensus         241 **......  .. *....*********...*...*  .*....  . ....*.***.*****


S. solfataricus   278 TDLIHILYGESSVYGRVSKSYAARVGINNFLKGYLPAENMKIRPDEIKFMLKEVSD-LDL
P. furiosus       277 SDITHVVYGEPGVYGIFSQPKGTRNGINEFLRGYLKDENVRFRPYEIKFTKTGER----V
C. elegans        294 SDFICCLYGVPGVYGVVTLPSGVREGINMFLEGFIRTENMRFRESKLSFKTSEQQ----E
S. cerevisiae     288 SDFVCIIYGVPSVYGVVTLPASVREGINIFLDGHIPAENLRFRTEALQFRIADATEDL-Q
O. sativa         283 SDFICCLYGKPGAYGVVTLPFSVREGINIFLAGFVPTENLRFRDESLTFKIAET-QESAE
S. lycopersicum   283 SDFICCLYGKPGAYGVVTLPFSVREGINIFLAGFVPTENLRFRDESLTFKVAETPQEAAE
A. thaliana       283 SDFICCLYGKPGAYGVVTLPFSVREGINIFLAGFVPTENLRFRDESLTFKVAETPQESAE
C. hirsuta        283 SDFICCLYGKPGAYGVVTLPFSVREGINIFLAGFVPTENLRFRDESLTFKVAETPQESAE
D. melanogaster   291 SDFICCLYGVPGCYGVVTMPFSVREGINIFLDGFVPTENMRFRTESLTFKVSESAT--EE
H. sapiens        283 SDFICCLYGVPSAYGVVTMPFSVREGINIFLDGYVPTENLRFRDASIVFKVAETAN--EE
O. cuniculus      283 SDFICCLYGVPSAYGVVTMPFSVREGINIFLDGYVPTENLRFRDASIVFKVAETAN--EE
D. rerio          283 SDFICCLYGVPSAYGVVTMPFSVREGINIFLDGYVPTENLRFRETSLVFKVAETAA--EE
consensus         301 .*.....** . **........*.***.** *....**...*. .. *.....      .


S. solfataricus   337 SKDLKTKMKWTKIIKKLGDFQLVVDNGEAKEGEIIGILGPNGIGKTTFARILVGEITADE
P. furiosus       333 EIERETLVTYPRLVKDYGSFRLEVEPGEIKKGEVIGIVGPNGIGKTTFVKMLAGVEEPTE
C. elegans        350 DIKRTGNIKYPSMSKTLGNFHLDVEAGDFSDSEIIVMLGENGTGKTTMIKMMAGSLKPED
S. cerevisiae     347 NDSASRAFSYPSLKKTQGDFVLNVEEGEFSDSEILVMMGENGTGKTTLIKLLAGALKPDE
O. sativa         342 EIETYQRYKYPTMSKTQGNFKLTVVEGEFTDSQIVVMLGENGTGKTTFIRMLAGLLKPDT
S. lycopersicum   343 EIESYARYRYPTMTKTQGNFKLKVSEGEFTDSQIVVMLGENGTGKTTFIRMLAGLLKPDV
A. thaliana       343 EIQSYARYKYPTMTKTQGNFRLRVSEGEFTDSQIIVMLGENGTGKTTFIRMLAGLLKPDD
C. hirsuta        343 EVQSYARYKYPTMSKTQGNFRLRVSEGEFTDSQIIVMLGENGTGKTTFIRMLAGLLKPDE
D. melanogaster   349 EIKRMNHYVYPAMVKTLGKFELTVEKGHFSDSEILVLLGENGTGKTTFIRMLAGNLQPDG
H. sapiens        341 EVKKMCMYKYPGMKKKMGEFELAIVAGEFTDSEIMVMLGENGTGKTTFIRMLAGRLKPDE
O. cuniculus      341 EVKKMCMYKYPGMKKKMGEFELAIVAGEFTDSEIMVMLGENGTGKTTFIRMLAGRLKPDE
D. rerio          341 EVKKLCRYQYPNMKKSMGEFTLTITEGEFTDSEIMVMLGENGTGKTTFIRMLAGGLKPDG
consensus         361 ..        .... . *. * * * .  *............*.**.****......* .....


S. solfataricus   397 ----GSVTPEKQILSYKPQRIFPNYDGTVQQYLENASKDALSTSSWFFEEVTKRLNLHRL
P. furiosus       393 ----GKIEW-DLTVAYKPQYIKADYEGTVYELLSKIDASKLN-SNFYKTELLKPLGIIDL
C. elegans        410 E---NTELP-HVSISYKPQKISPKSETTVRFMLHDKIQNMYE-HPQFKTDVMNPLMMEQL
S. cerevisiae     407 ----GQDIP-KLNVSMKPQKIAPKFPGTVRQLFFKKIRGQEL-NPQFQIDVVKPLRIDDI
O. sativa         402 VEGTEIEIP-EFNVSYKPQKISPKFQHSVRHLLHQKIRDSYT-HPQFVSDVMKPLQIEQL
S. lycopersicum   403 VEGSDIEMP-EFNVSYKPQKISPKFQSTVRHLLHQKIRDSYQ-HPQFCSDVMKPLQIEQL
A. thaliana       403 TEGPDREIP-EFNVSYKPQKISPKFQNSVRHLLHQKIRDSYM-HPQFMSDVMKPLQIEQL
C. hirsuta        403 TEGPDREIP-EFNVSYKPQKISPKFQNSVRHLLHQKIRDSYM-HPQFMSDVMKPLQIEQL
D. melanogaster   409 ----EVELP-MLNISYKPQKISPKFQNHVRHLLHDKIRDAYV-HPQFIADVMKPMKIEEI
H. sapiens        401 ----GGEVP-VLNVSYKPQKISPKSTGSVRQLLHEKIRDAYT-HPQFVTDVMKPLQIENI
O. cuniculus      401 ----GGEVP-VLNVSYKPQKISPKSTGSVRQLLHEKIRDAYT-HPQFVTDVMKPLQIENI
D. rerio          401 ----GGDVP-ILNVSYKPQKISPKFKGSVRALLHDKIRDAYT-HPQFVTDVMKPMQIESI
consensus         421       ...    ....***.*....  .*. ... .... . .........  .
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6

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S. solfataricus   453  LESNVNDLSGGELQKLYIAATLAKEADLYVLDEPSSYLDVEERYIVAKAIKRVTRERKAV
P. furiosus       447  YDREVNELSGGELQRVAIAATLLRDADIYLLDEPSAYLDVEQRLAVSRAIRHLMEKNEKT
C. elegans        465  LDRNVKELSGGELQRVALALCLGKTASLYLIDEPSAYLDSEQRLHAAKVIKRFIMHAKKT
S. cerevisiae     461  IDQEVQHLSGGELQRVAIVLALGIPADIYLIDEPSAYLDSEQRIICSKVIRRFILHNKKT
O. sativa         460  MDQEVINLSGGELQRVAICLCLGKPADIYLIDEPSAYLDSEQRIVASKVIKRFILHAKKT
S. lycopersicum   461  MDQEVVNLSGGELQRVALALCLGKPADIYLIDEPSAYLDSEQRIVASKVIKRFILHAKKT
A. thaliana       461  MDQEVVNLSGGELQRVALTLCLGKPADIYLIDEPSAYLDSEQRIVASKVIKRFILHAKKT
C. hirsuta        461  MDQEVINLSGGELQRVALALCLGKPADIYLIDEPSAYLDSEQRIVASKVIKRFILHAKKT
D. melanogaster   463  MDQEVQNLSGGELQRVALVLCLGKPADVYLIDEPSAYLDSEQRLVAAKVIKRYILHAKKT
H. sapiens        455  IDQEVQTLSGGELQRVALALCLGKPADVYLIDEPSAYLDSEQRLMAARVVKRFILHAKKT
O. cuniculus      455  IDQEVQTLSGGELQRVALALCLGKPADVYLIDEPSAYLDSEQRLMAARVVKRFILHAKKT
D. rerio          455  IDQDVQNLSGGELQRVALALCLGKPADVYLIDEPSAYLDSEQRLMAARVIKRFILHAKKT
consensus         481  ....*  *******.......*...*..*..****.***.*.*... ............

S. solfataricus   513  TFIIDHDLSIHDYLADRIIVFKGEPEKAGLATSPVTLKTGMNEFLREIEVTFRRDAETGR
P. furiosus       507  ALVVEHDVLMIDYVSDRLMVFEGEPGKYGRALPPMGMREGMNRFLASIGITFRRDPDTGR
C. elegans        525  AFVVEHDFIMATYLADRVVVFEGQPSVKCTACKPQSLIEGMNRFLKMLDITFRRDQETYR
S. cerevisiae     521  AFIVEHDFIMATYLADKVIVFEGIPSKNAHARAPESLLTGCNRFLKNLNVTFRRDPNSFR
O. sativa         520  AFIVEHDFIMATYLADKVIVYEGRPSIDCTANAPQSLVSGMNKFLSHIDITFRRDPTNYR
S. lycopersicum   521  AFVVEHDFIMATYLADRVIVYEGTPSIDCVANAPQSLITGMNLFLSHLNITFRRDPTNYR
A. thaliana       521  AFVVEHDFIMATYLADRVIVYEGQPSIDCTANCPQSLLSGMNLFLSHLNITFRRDPTNFR
C. hirsuta        521  AFVVEHDFIMATYLADRVIVYEGQPSIDCTANCPQSLISGMNLFLSHLNITFRRDPTNFR
D. melanogaster   523  GFVVEHDFIMATYLADRVIVIEGQPSVKTTAFSPQSLLNGMNRFLELLGITFRRDPNNFR
H. sapiens        515  AFVVEHDFIMATYLADRVIVFDGVPSKNTVANSPQTLLAGMNKFLSQLEITFRRDPNNYR
O. cuniculus      515  AFVVEHDFIMATYLADRVIVFDGVPSKNTVANSPQTLLAGMNKFLSQLEITFRRDPNNYR
D. rerio          515  AFVVEHDFIMATYLADRVIVFDGIPSRTTNANAPQTLLAGMNKFLAQLEITFRRDPNNFR
consensus         541  .....**.....*..*...*..* *.     *  *.... *.*.**  . .*****. ..*

S. solfataricus   573  PRVNKIGSYLDRVQKERGDYYSMVLSTQ-
P. furiosus       567  PRANKEGSVKDREQKEKGEYYYIA-----
C. elegans        585  PRINKLDSVKDVDQKKSGQFFFLDDN---
S. cerevisiae     581  PRINKLDSQMDKEQKSSGNYFFLDNTGI-
O. sativa         580  PRINKLDSTKDREQKSAGSYYYLDD----
S. lycopersicum   581  PRINKLESTKDREQKSAGSYYYLDD----
A. thaliana       581  PRINKLESTKDREQKSAGSYYYLDD----
C. hirsuta        581  PRINKLESTKDREQKSAGSYYYLDD----
D. melanogaster   583  PRINKNNSVKDTEQKRSGQFFFLEDEACN
H. sapiens        575  PRINKLNSIKDVEQKKSGNYFFLDD----
O. cuniculus      575  PRINKLNSIKDVEQKKSGNYFFLDD----
D. rerio          575  PRINKLNSIKDVEQKKSGNYFFLDD----
consensus         601  **.**. * .* .**  * ......
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Supplementary Figure S4. Sequence conservation among ABCE orthologs.
Multiple sequence alignment of full-length ABCE proteins from the archaea *Saccharolobus solfataricus* (Q980K5) and *Pyrococcus furiosus* (I6V0C7), and the eukaryotes *Caenorhabditis elegans* (Q9U2K8), *Saccharomyces cerevisiae* (Q03195), *Oryza sativa* (A0A0P0Y344), *Solanum lycopersicum* (A0A3Q7H7H5), *Arabidopsis thaliana* (At4g19210), *Cardamine hirsuta* (L7VNS9), *Drosophila melanogaster* (Q9VSS1), *Homo sapiens* (P61221), *Oryctolagus cuniculus* (G1SG72), and *Danio rerio* (Q6TNW3). Full-length protein sequences were obtained from UniProt (https://www.uniprot.org/), except that of *Arabidopsis thaliana*, which was obtained from The Arabidopsis Information Resource (TAIR; https://www.arabidopsis.org/). The alignment was obtained with Clustal Omega 1.2.4 with default settings, and shaded with BOXSHADE with output format RTF_new. Identical and similar residues across at least eight out of the twelve sequences are shaded in black and gray, respectively. Asterisks and periods indicate identical and conserved residues, respectively. Numbers indicate residue positions. The conserved Pro138 residue, which is replaced by Ser in the *api7-1* mutant, is highlighted in red.

7
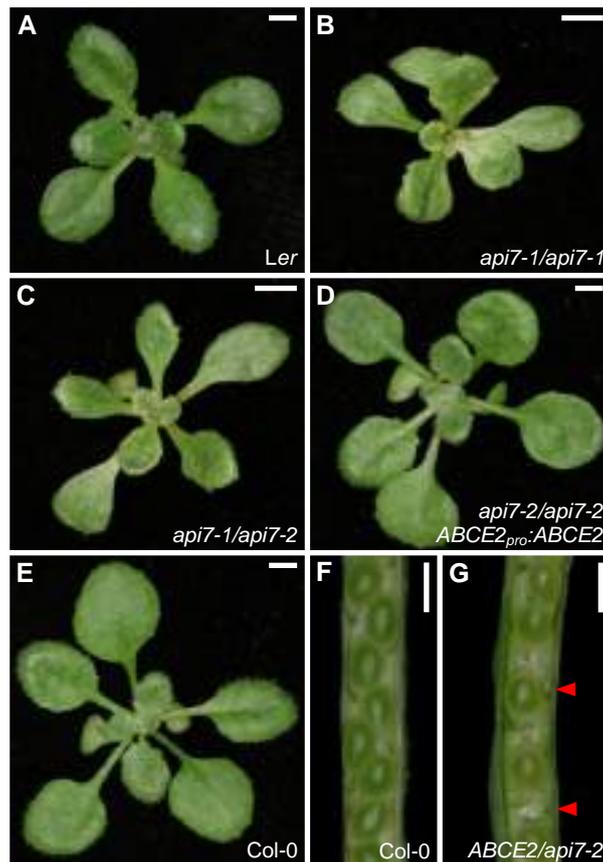
Supplementary Figure S5. *api7-2* is a lethal allele of *ABCE2*.
(A–E) Rosettes from (A) L*er*, (B) *api7-1/api7-1*, (C) *api7-1/api7-2*, (D) *api7-2/api7-2 ABCE2_{pro}:ABCE2*, and (E) Col-0 plants. (F, G) Dissected immature siliques from (F) Col-0 and (G) *ABCE2/api7-2* plants. Red arrowheads indicate aborted or unfertilized ovules. Pictures were taken (A–E) 16 and (F, G) 57 das. Scale bars indicate (A–E) 2 mm and (F, G) 500 μm.
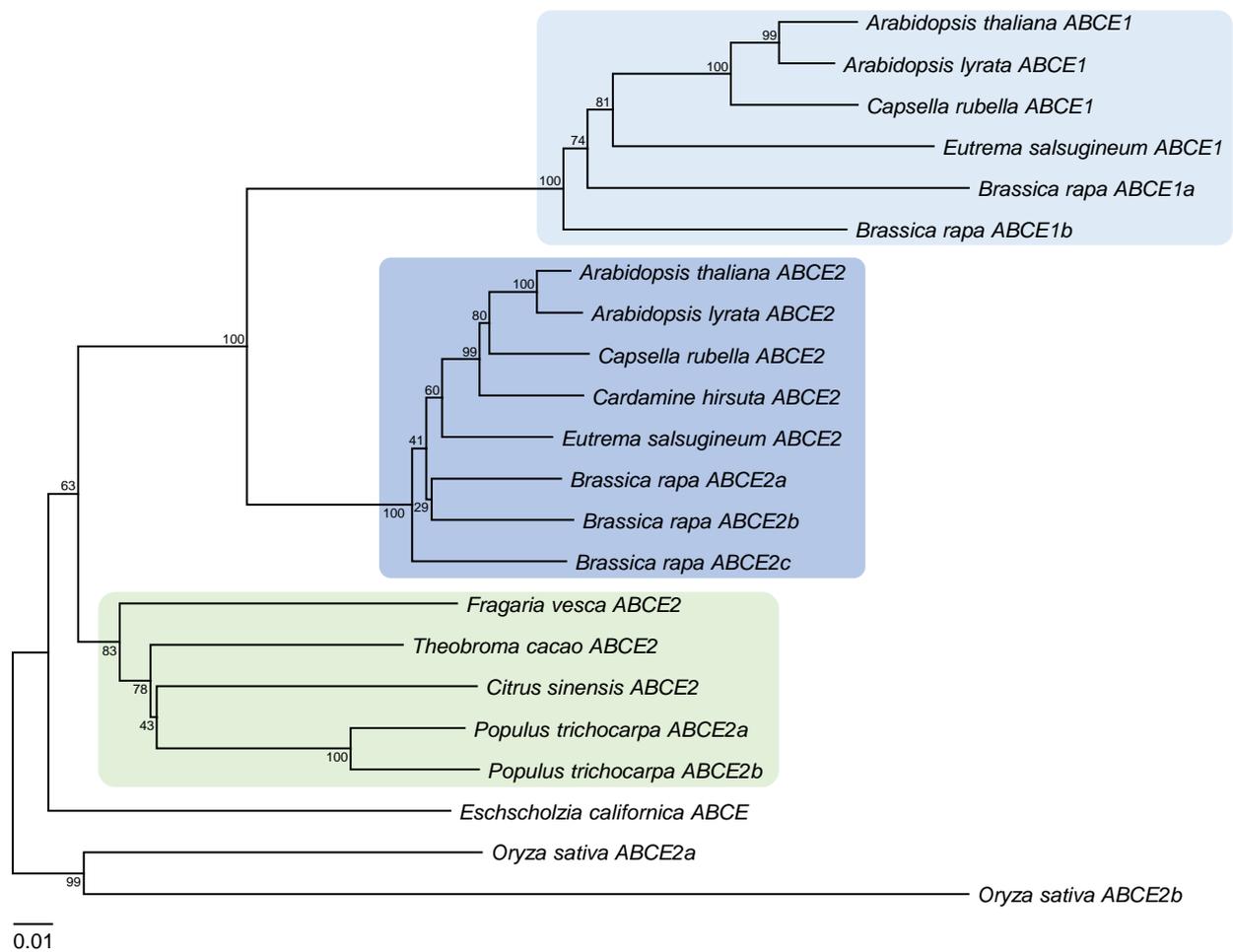
Supplementary Figure S6. Phylogenetic analysis of some plant *ABCE* genes.
Phylogenetic analysis of some Rosidae *ABCE* genes. Rectangles indicate Brassicaceae *ABCE1* (clear blue), *ABCE2* (dark blue), and other rosids *ABCE2* (green) genes. *Eschscholzia californica* and *Oryza sativa ABCE* sequences were used as outgroups. Multiple *ABCE1* or *ABCE2* genes from *Brassica rapa*, *Populus trichocarpa*, and *Oryza sativa* are distinguished with arbitrarily given *a*, *b*, and *c* designations. Refer to Supplementary Table S2 to see the NCBI Nucleotide codes of the sequences used during the multiple sequence alignment. The phylogenetic tree was obtained using the Neighbor-Joining method. All positions containing gaps and missing data were eliminated (complete deletion option). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was rooted on the midpoint. The scalebar indicates the evolutionary distance as the number of base substitutions per site, and was computed using the Tamura 3-parameter method.
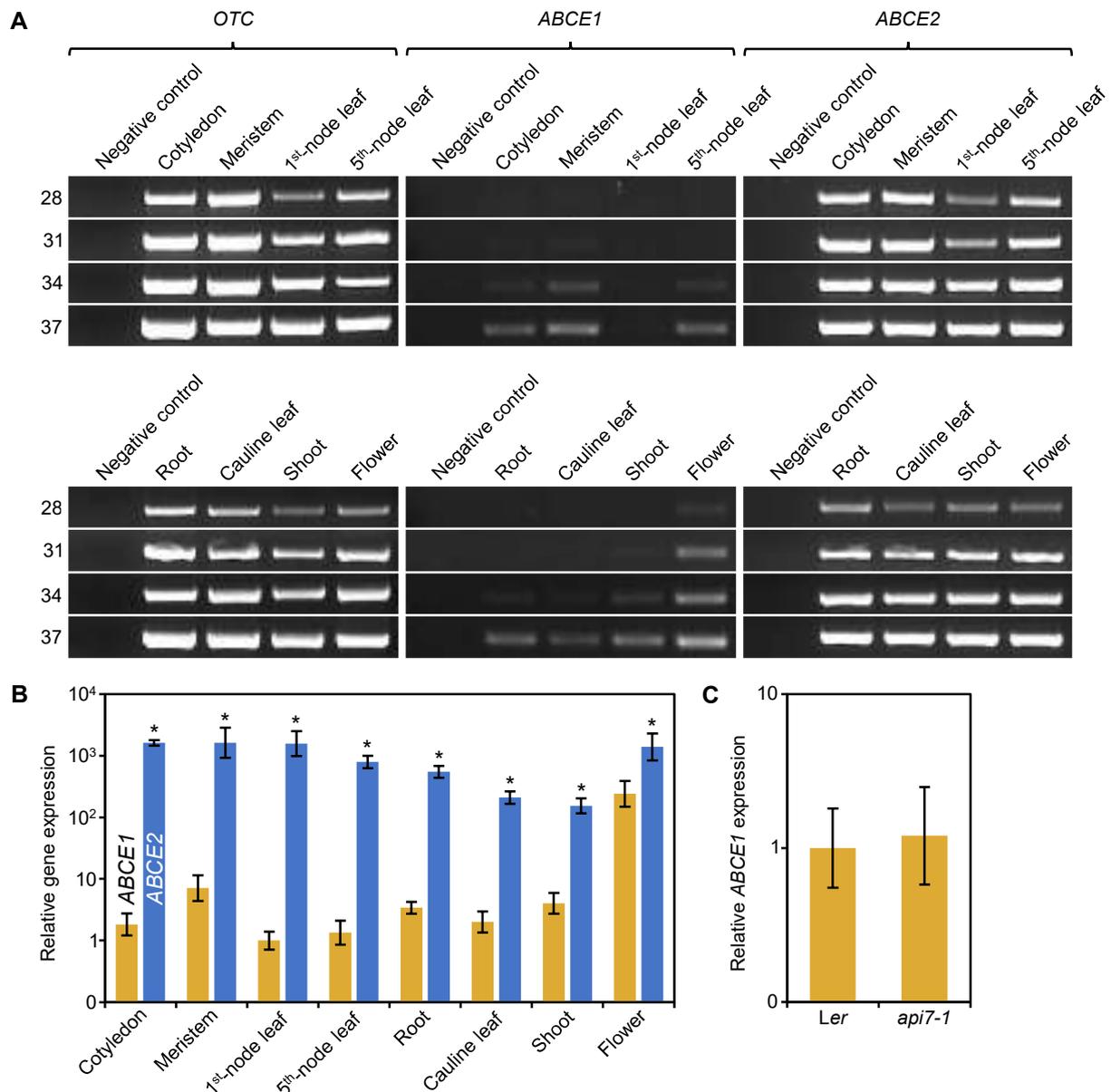
Supplementary Figure S7. *ABCE1* and *ABCE2* expression analyses.
(A) Semiquantitative and (B) quantitative PCR analyses of *ABCE1* and *ABCE2* expression in Col-0 plants. (A) The domestic *OTC* gene was used as a control. Negative control samples had no template. The PCR products were visualized after 28, 31, 34, and 37 amplification cycles. (A, B) Samples were collected 7 (cotyledons), 14 (meristems, first- and fifth-node leaves, and roots), or 28 (cauline leaves, shoots, and flowers) das. (C) *ABCE1* expression in wild-type L*er* and mutant *api7-1* first-node leaves. Samples were collected 14 das. (B, C) *ABCE1* expression levels in (B) first-node leaves and (C) L*er* were used as the reference value. Error bars indicate the interval delimited by $2^{-(\Delta\Delta CT \pm SD)}$, where SD is the standard deviation of the $\Delta\Delta C_T$ values. Note that the relative gene expression levels are in a logarithmic scale. (B) Asterisks indicate values significantly different between *ABCE1* and *ABCE2* in a Mann-Whitney *U* test (*$P < 0.001$). (B, C) Three different biological replicates were analyzed in triplicate.
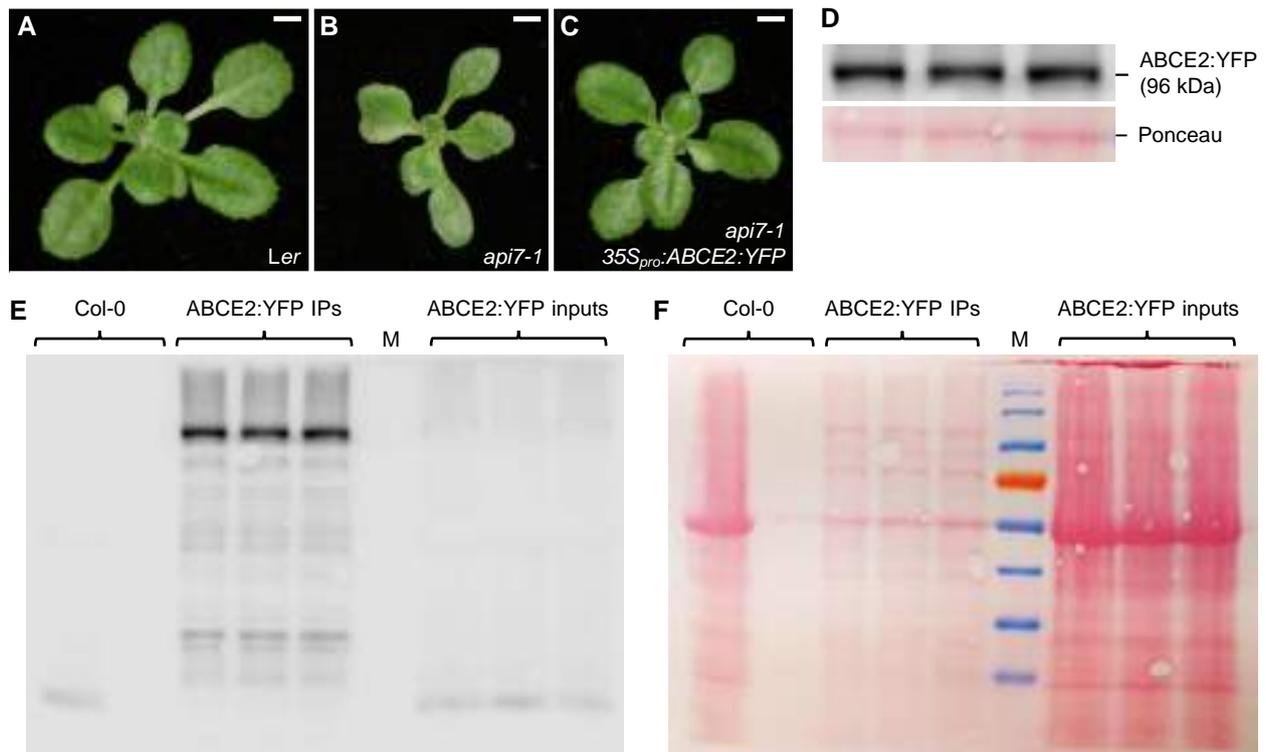
Supplementary Figure S8. The *35S~pro~:ABCE2:YFP* transgene fully restores the wild-type phenotype in *api7-1* plants.

(A–C) Rosettes from (A) L*er*, (B) *api7-1*, and (C) *api7-1 35S~pro~:ABCE2:YFP* plants. Pictures were taken 16 das. Scale bars indicate 2 mm. (D) The ABCE2:YFP fusion protein was detected from three independent immunoprecipitates in a western blot probed against GFP. Immunoprecipitates were obtained by immunoprecipitation with anti-GFP magnetic beads of whole-protein extracts from *api7-1 35S~pro~:ABCE2:YFP* plants collected 10 das. A band from the Ponceau staining of the membrane is shown as a loading control. (E, F) Full pictures of (E) the detection of ABCE2:YFP and (F) the membrane stained with Ponceau, from the whole-protein extracts previous to immunoprecipitation (inputs) and the immunoprecipitated samples (IPs). A protein extract from wild-type Col-0 seedlings was used as a control: input and immunoprecipitation were loaded in the left and right lanes, respectively. M: we used the EZ-Run Prestained *Rec* Protein Ladder (Thermo Fisher Scientific, Fisher BioReagents) molecular weight marker.

At4g19210 (ABCE2; 62%)

MADRLTRIAIVSSDRCKPKKCRQECKKSCPVVKTGKLCIEVTVGSKLAFISEELCIGCGICVKKCPFEAIQIINLPRDL
EKDTTHRYGANTFKLHRLPVPRPGQVLGLVGTNGIGKSTALKILAGKLKPNLGRFTSPPDWQEILTHFRGSELQNYFTR
ILEDNLKAIIKPQYVDHIPRAVKGNVGEVLDQKDERDKKAELCADLELNQVIDRDVENLSGGELQRFAIAVVAIQNAEI
YMFDEPSSYLDVKQRLKAAQVVRSLLRPNSYVIVVEHDLSVLDYLSDFICCLYGKPGAYGVVTLPFSVREGINIFLAGF
VPTENLRFRDESLTFKVAETPQESAEEIQSYARYKYPTMTKTQGNFRLRVSEGEFTDSQIIVMLGENGTGKTTFIRMLA
GLLKPDDTEGPDREIPEFNVSYKPQKISPKFQNSVRHLLHQKIRDSYMHPQFMSDVMKPLQIEQLMDQEVVNLSGGELQ
RVALTLCLGKPADIYLIDEPSAYLDSEQRIVASKVIKRFILHAKKTAFVVEHDFIMATYLADRVIVYEGQPSIDCTANC
PQSLLSGMNLFLSHLNITFRRDPTNFRPRINKLESTKDREQKSAGSYYYLDD

At3g13640 (ABCE1; 11%)

MSDRLTRIAIVSEDRCKPKKCRQECKKSCPVVKTGKLCIEVGSTSKSAFISEELCIGCGICVKKCPFEAIQIINLPKDL
AKDTTHRYGANGFKLHRLPIPRPGQVLGLVGTNGIGKSTALKILAGKLKPNLGRFNTPPDWEEILTHFRGSELQSYFIR
VVEENLKTAIKPQHVDYIKEVVRGNLGKMLEKLDERGLMEEICADMELNQVLEREARQVSGGELQRFAIAAVFVKKADI
YMFDEPSSYLDVRQRLKAAQVIRSLLRHDSYVIVVEHDLSVLDYLSDFVCCLYGKPGAYGVVTLPFSVREGINVFLAGF
IPTENLRFRDESLTFRVSETTQENDGEVKSYARYKYPNMTKQLGDFKLEVMEGEFTDSQIIVMLGENGTGKTTFIRMLA
GAFPREEGVQSEIPEFNVSYKPQGNDSKRECTVRQLLHDKIRDACAHPQFMSDVIRPLQIEQLMDQVVKTLSGGEKQRV
AITLCLGKPADIYLIDEPSAHLDSEQRITASKVIKRFILHAKKTAFIVEHDFIMATYLADRVIVYEGQPAVKCIAHSPQ
SLLSGMNHFLSHLNITFRRDPTNFRPRINKLESIKDKEQKTAGSYYYLDD

At4g11420 (eIF3a; 32%)

MANFAKPENALKRADELINVGQKQDALQALHDLITSKRYRAWQKPLEKIMFKYLDLCVDLKRGRFAKDGLIQYRIVCQQ
VNVSSLEEVIKHFLHLATDKAEQARSQADALEEALDVDDLEADRKPEDLQLSIVSGEKGKDRSDRELVTPWFKFLWETY
RTVLEILRNNSKLEALYAMTAHKAFQFCKQYKRTTEFRRLCEIIRNHLANLNKYRDQRDRPDLSAPESLQLYLDTRFDQ
LKVATELGLWQEAFRSVEDIYGLMCMVKKTPKSSLLMVYYSKLTEIFWISSSHLYHAYAWFKLFSLQKNFNKNLSQKDL
QLIASSVVLAALSIPPFDRAQSASHMELENEKERNLRMANLIGFNLEPKFEGKDMLSRSALLSELVSKGVLSCASQEVK
DLFHVLEHEFHPLDLGSKIQPLLEKISKSGGKLSSAPSLPEVQLSQYVPSLEKLATLRLLQQVSKIYQTIRIESLSQLV
PFFQFSEVEKISVDAVKNNFVAMKVDHMKGVVIFGNLGIESDGLRDHLAVFAESLSKVRAMLYPVPSKASKLAGVIPNL
ADTVEKEHKRLLARKSIIEKRKEDQERQQLEMEREEEQKRLKLQKLTEEAEQKRLAAELAERRKQRILREIEEKELEEA
QALLEETEKRMKKGKKKPLLDGEKVTKQSVKERALTEQLKERQEMEKKLQKLAKTMDYLERAKREEAAPLIEAAYQRRL
VEEREFYEREQQREVELSKERHESDLKEKNRLSRMLGNKEIFQAQVISRRQAEFDRIRTEREERISKIIREKKQERDIK
RKQIYYLKIEEERIRKLQEEEEARKQEEAERLKKVEAERKANLDKAFEKQRQREIELEEKSRREREELLRGTNAPPARL
AEPTVTPVGTTAPAAAAAAGAPAAPYVPKWKRQTTEVSGPSAPTSSETDRRSNRGPPPGDDHWGSNRGAAQNTDRWTS
NRERSGPPAEGGDRWGSGPRGSDDRRSTFGSSRPRPTQR

At3g56150 (eIF3c; 14%)

MTSRFFTQVGSESEDESDYEVEVNEVQNDDVNNRYLQSGSEDDDDTDTKRVVKPAKDKRFEEMTYTVDQMKNAMKINDW
VSLQENFDKVNKQLEKVMRITEAVKPPTLYIKTLVMLEDFLNEALANKEAKKKMSTSNSKALNSMKQKLKKNNKLYEDD
INKYREAPEVEEEKQPEDDDDDDDDDDEVEDDDDSSIDGPTVDPGSDVDEPTDNLTWEKMLSKKDKLLEKLMNKDPKEI
TWDWVNKKFKEIVAARGKKGTARFELVDQLTHLTKIAKTPAQKLEILFSVISAQFDVNPGLSGHMPINVWKKCVLNMLT
ILDILVKYSNIVVDDTVEPDENETSKPTDYDGKIRVWGNLVAFLERVDTEFFKSLQCIDPHTREYVERLRDEPMFLALA
QNIQDYFERMGDFKAAAKVALRRVEAIYYKPQEVYDAMRKLAELVEEEETEEAKEESGPPTSFIVVPEVVPRKPTFPE
SSRAMMDILVSLIYRNGDERTKARAMLCDINHHALMDNFVTARDLLLMSHLQDNIQHMDISTQILFNRTMAQLGLCAFR
AGMITESHSCLSELYSGQRVRELLAQGVSQSRYHEKTPEQERMERRQMPYHMHLNLELLEAVHLICAMLLEVPNMAAN
SHDAKRRVISKNFRRLLEISERQAFTAPPENVRDHVMAATRALTKGDFQKAFEVLNSLEVWRLLKNRDSILDMVKDRIK
EEALRTYLFTYSSSYESLSLDQLAKMFDVSEPQVHSIVSKMMINEELHASWDQPTRCIVFHEVQHSRLQSLAFQLTEKL
SILAESNERAMESRTGGGGLDLSSRRRDNNQDYAGAASGGGGYWQDKANYGQGRQGNRSGYGGGRSSGQNGQWSGQNRG
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At3g57290 (eIF3e; 40%)

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TLCSNLERSLSALWGKLASEILMQNWDIALEELNRLKEIIDSKSFSSPLNQVQNRIWLMHWGLYIFFNHDNGRTQIIDL
FNQDKYLNAIQTSAPHLLRYLATAFIVNKRRRPQLKEFIKVIQQEHYSYKDPIIEFLACVFVNYDFDGAQKKMKECEEV
IVNDPFLGKRVEDGNFSTVPLRDEFLENARLFVFETYCKIHQRIDMGVLAEKLNLNYEEAERWIVNLIRTSKLDAKIDS
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At1g64790 (ILA; 4%)

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MTSDIAPVIVDMIFQTLAIYDDRASRKAVDDLIVKGLGNVTFMKTFAAMLVQVMEKQLKFCFDTVCYRLLIWSCLLLEK
SQFATVSKNAFVRVASTQASLLRIIMESSFRMRRACKRFMFHLFSQSQAIYSLYMDEVKGSRIPYKDSPELLGLLLEFS
CSSPALFEQSKAIFVDIYVKDVLNSREKQKPNLSNCFKPLLQRLSHEEFQTVILPAAVKMLKRNPEIVLESVGFLLANV
NIDLSKYALELLPVILPQARHTDEDRRLGALSMVMCLSEKSSNPDTIEAMFASVKAIIGGSEGRLQSPHQRIGMLNAVQ
ELASAPEGKYIGSLSRTICSFLIACYKDEGNEDVKLSILSAVASWASRSSVAIQPNLVSFIAAGLKEKEALRRGHLRCV
RIICRNPDTISQISDLLSPLIQLVKTGFTKAVQRLDGIYALLIVSKIAACDIKAEDTMVKEKLWTLISQNEPSLVQITL
ASKLSSDDCVVCVDLLEVLLVEHSSRVLEAFSLKSLSQLLLFLLCHPSWNVRKTAYNSVTKIFLATSQLATTLLDEFSD
FLSITGDQIVSSRTSDADNPADHQAPFVPSVEVLVKALIVISSAAVAGPPSSWIVRAIFCSHHPSIVGTGKRDAVWKRL
QKCLKTCGFDVATFLSTNGESVCKSLLGPMGLTSAKTPEQQAAVYSLSTMMSLAPEDTFTVFKMHLQDLPDRLSHDMLS
ETDIKIFHTPEGMLLSEQGVYVAQTIGAKYTKQEPSSNHSLKKGLASRETANSGRRDTAKLTKKADKGKTAKEEARELM
LKEEASTRENVHRIQKSLSLVLHALGEMGLANPVFCHSQLPFLATFLDPLLRSPIVSAAAFENLVKLARCTVQPLCNWA
LEISTALRLIAIDEVDTSFDFRPSVDKAGKTYEGLFERIVNGLSISCKSGPLPVDTFTFIFPVLYHVLGVVPAYQASVG
PALNELCLGLQADDVANALYGVYSKDVHVRLACLNAVKCIPAVSKCSLPQNVKIATNIWIALHDPEKSVAESADDLWAR
YGHDLGTDYSGIFKALSHINLNVRLAAAEALADALHESPSSIQLSLSTLFSLYIRDATSGEDVFDAGWIGRQGIALALQ
SAADVLTTKDLPAVMTFLISRALADPNTDVRGKMINAGIMIIDKHGKENVSLLFPIFENYLNKEASDEEEYDLVREGVV
IFTGALAKHLARDDPKVHNVVEKLLEVLNTPSESVQRAVSTCLSPLVLSKQEEAPALFLRLLDKLMKSDKYGERRGAAF
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PKVQSAGQLALQQVGSVIKNPEISSLVPTLLLALTDPNEYTRHALDTLLQTTFVNSVDAPSLALLVPIVHRGLRERSSE
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QGRAIIDILGMDKRNEVLAALYMVRTDVSLSVRQAALHVWKTIVANTPKTLKEIMPILMSTLISSLASPSSERRQVAGR
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LAFSTLYKSAGLQAMDEIIPTLLEALEDDEMSTTALDGLKQIISVRTAAVLPHILPKLVHLPLSALNAHALGALAEVAG
AGFNTHLGTILPALLSAMGGENKEVQELAQEAAERVVLVIDEEGVETLLSELLKGVSDSQASIRRSSAYLIGYFFKSSK
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PSTIMTSSLFSSMLNSLKSSLKDEKFPLRESSTKALGRLLLKQLATDPSNTKVVIDVLSSIVSALHDDSSEVRRRALSS
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SESDDDNVSG
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At2g44060 (LEA26; 23%)

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MSTSEDKPEIISRVVHQEGDVEIVDRSQKDKDEEKEEGKGGFLDKVKDFIHDIGEKLEGTIGFGKPTADVSAIHIPKIN
LERADIVVDVLVKNPNPVPIPLIDVNYLVESDGRKLVSGLIPDAGTLKAHGEETVKIPLTLIYDDIKSTYNDINPGMII
PYRIKVDLIVDVPVLGRLTLPLEKCGEIPIPKKPDVDIEKIKFQKFSLEETVAILHVRLQNMNDFDLGLNDLDCEVWLC
DVSIGKAEIADSIKLDKNGSGLINVPMTFRPKDFGSALWDMIRGKGTGYTIKGNIDVDTPFGAMKLPIIKEGGETRLKK
EDDDDDDEE
```

At4g20980 (eIF3d; 13%)

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MVTEAFEFVAVPFNSDGWGPPDASDVSSSASPTSVAAANLLPNVPFASFSRSDKLGRVADWTRNLSNPSARPNTGSKSD
PSAVFDFSAFAIDEGFGLASSGGNPDEDAAFRLVDGKPPPRPKFGPKWRFNPHHNRNQLPQRRDEEVEAKKRDAEKERA
RRDRLYNNNRNNIHHQRREAAAFKSSVDIQPEWNMLEQIPFSTFSKLSYTVQEPEDLLLCGGLEYYNRLFDRITPKNER
RLERFKNRNFFKVTTSDDPVIRRLAKEDKATVFATDAILAALMCAPRSVYSWDIVIQRVGNKLFFDKRDGSQLDLLSVH
ETSQEPLPESKDDINSAHSLGVEAAYINQNFSQQVLVRDGKKETFDEANPFANEGEEIASVAYRYRRWKLDDNMHLVAR
CELQSVADLNNQRSFLTLNALNEFDPKYSGVDWRQKLETQRGAVLATELKNNGNKLAKWTAQALLANADMMKIGFVSRV
HPRDHFNHVILSVLGYKPKDFAGQINLNTSNMWGIVKSIVDLCMKLSEGKYVLVKDPSKPQVRIYEVPPDAFENDYVEE
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At5g44320 (eIF3d; 9%)

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MVFEAFEVGTVPFNSDGWGPPDASDTSSTSVAAANLLPNVPFASFSRSEKLGRVADWTRALSNPSARPHTGSKSDPSAI
FDFSAFAVDEGFGLTNSGGNADEDAAFRLVDGKPPPRPKFGPKWRFNQYHNRNQLPQRRDEEVEAKKREAEKDRARRDR
LYNNNRNNIHQQRREAAAFKSSVDIQPEWNMLEQIPFSTFSKLSFTVSEPEDLLLCGGLESYDRSFDRITPKADRRLER
FKNRSFKVTTSDDLVIRRLAKEDKATVFATDAILAALMCAPRSVYSWDLVIQRVGNKLFFDKRDGSPLDLLSVHETSQE
PLPEGKDDINSAHSLGLEAAYINQNFAQQVLVKNGKRETFDEPIPNVNEGEENASIAYRYRRWKLDDSMYLVARCELQS
TVDLNNQRSFLTLNALNEFDPKYSGVDWRQKLETQRGAVLANELKNNGNKLAKWTAQALLANADMMKIGFVSRVHPRDH
FNHVILSVLGYKPKDFAGQINLNTNNMWGIVKSIVDLCMKLSEGKYVLVKDPSKPQVRIYEVPADAFDNDYVEEPLPED
EQVQPPEENTDAGAETNGVSSTNVAVEDKKSEVEA
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At5g17020 (XPO1A; 6%)

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MAAEKLRDLSQPIDVGVLDATVAAFFVTGSKEERAAADQILRDLQANPDMWLQVVHILQNTNSLDTKFFALQVLEGVIK
YRWNALPVEQRDGMKNYISEVIVQLSSNEASFRSERLYVNKLNVILVQIVKHDWPAKWTSFIPDLVAAAKTSETICENC
MAILKLLSEEVFDFSRGEMTQQKIKELKQSLNSEFKLIHELCLYVLSASQRQDLIRATLSALHAYLSWIPLGYIFESTL
LETLLKFFPVPAYRNLTIQCLTEVAALNFGDFYNVQYVKMYTIFIGQLRIILPPSTKIPEAYSSGSGEEQAFIQNLALF
FTSFFKFHIRVLESTPEVVSLLLAGLEYLINISYVDDTEVFKVCLDYWNSLVLELFDAHHNSDNPAVSASLMGLQPFLP
GMVDGLGSQVMQRRQLYSHPMSKLRGLMINRMAKPEEVLIVEDENGNIVRETMKDNDVLVQYKIMRETLIYLSHLDHDD
TEKQMLRKLNKQLSGEEWAWNNLNTLCWAIGSISGSMAEDQENRFLVMVIRDLLNLCEITKGKDNKAVIASNIMYVVGQ
YPRFLRAHWKFLKTVVNKLFEFMHETHPGVQDMACDTFLKIVQKCKRKFVIVQVGENEPFVSELLTGLATTVQDLEPHQ
IHSFYESVGNMIQAESDPQKRDEYLQRLMALPNQKWAEIIGQARHSVEFLKDQVVIRTVLNILQTNTSAATSLGTYFLS
QISLIFLDMLNVYRMYSELVSTNITEGGPYASKTSFVKLLRSVKRETLKLIETFLDKAEDQPHIGKQFVPPMMESVLGD
YARNVPDARESEVLSLFATIINKYKATMLDDVPHIFEAVFQCTLEMITKNFEDYPEHRLKFFSLLRAIATFCFPALIKL
SSPQLKLVMDSIIWAFRHTERNIAETGLNLLLEMLKNFQQSEFCNQFYRSYFMQIEQEIFAVLTDTFHKPGFKLHVLVL
QQLFCLPESGALTEPLWDATTVPYPYPDNVAFVREYTIKLLSSSFPNMTAAEVTQFVNGLYESRNDPSGFKNNIRDFLV
QSKEFSAQDNKDLYAEEAAAQRERERQRMLSIPGLIAPNEIQDEMVDS
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At3g03110 (XPO1B; 2%)

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MAAEKLRDLSQPIDVVLLDATVEAFYSTGSKEERASADNILRDLKANPDTWLQVVHILQNTSSTHTKFFALQVLEGVIK
YRWNALPVEQRDGMKNYISDVIVQLSRDEASFRTERLYVNKLNIILVQIVKQEWPAKWKSFIPDLVIAAKTSETICENC
MAILKLLSEEVFDFSKGEMTQQKIKELKQSLNSEFQLIHELCLYVLSASQRQELIRATLSALHAYLSWIPLGYIFESPL
LEIILKFFPVPAYRNLTLQCLSEVASLNFGDFYDMQYVKMYSIFMNQLQAILPLNLNIPEAYSTGSSEEQAFIQNLALF
FTSFFKLHIKILESAPENISLLLAGLGYLISISYVDDTEVFKVCLDYWNSLVLELFGTRHHACHPALTPSLFGLQMAFL
PSTVDGVKSEVTERQKLYSDPMSKLRGLMISRTAKPEEVLIVEDENGNIVRETMKDNDVLVQYKIMRETLIYLSHLDHE
DTEKQMLSKLSKQLSGEEWAWNNLNTLCWAIGSISGSMVVEQENRFLVMVIRDLLSLCEVVKGKDNKAVIASNIMYVVG
QYSRFLRAHWKFLKTVVHKLFEFMHETHPGVQDMACDTFLKIVQKCKRKFVIVQVGESEPFVSELLSGLATIVGDLQPH
QIHTFYESVGSMIQAESDPQKRGEYLQRLMALPNQKWAEIIGQARQSADILKEPDVIRTVLNILQTNTRVATSLGTFFL
SQISLIFLDMLNVYRMYSELVSSSIANGGPYASRTSLVKLLRSVKREILKLIETFLDKAENQPHIGKQFVPPMMDQVLG
DYARNVPDARESEVLSLFATIINKYKVVMRDEVPLIFEAVFQCTLEMITKNFEDYPEHRLKFFSLLRAIATFCFRALIQ
LSSEQLKLVMDSVIWAFRHTERNIAETGLNLLLEMLKNFQKSDFCNKFYQTYFLQIEQEVFAVLTDTFHKPGFKLHVLV
LQHLFSLVESGSLAEPLWDAATVPHPYSNNVAFVLEYTTKLLSSSFPNMTTTEVTQFVNGLYESRNDVGRFKDNIRDFL
IQSKEFSAQDNKDLYAEEAAAQMERERQRMLSIPGLIAPSEIQDDMADS
```

At1g61580 (RPL3B; 12%)
MSHRKFEHPRHGSLGFLPRKRASRHRGKVKAFPKDDPTKPCRLTSFLGYKAGMTHIVRDVEKPGSKLHKKETCEAVTII
ETPPMVVVGVYGYVKTPRGLRSLCTVWAQHLSEELRRRFYKNWAKSKKKAFTRYSKKHETEEGKKDIQSQLEKMKKYCS
VIRVLAHTQIRKMKGLKQKKAHLNEIQINGGDIAKKVDYACSLFEKQVPVDAIFQKDEMIDIIGVTKGKGYEGVVTRWG
VTRLPRKTHRGLRKVACIGAWHPARVSYTVARAGQNGYHHRTEMNKKVYRVGKVGQETHSAMTEYDRTEKDITPMGGFP
HYGIVKEDYLMIKGCCVGPKKRVVTLRQTLLKQTSRLAMEEIKLKFIDAASNGGHGRFQTSQEKAKFYGRTIKA

At4g38740 (ROC1; 38%)
MAFPKVYFDMTIDGQPAGRIVMELYTDKTPRTAENFRALCTGEKGVGGTGKPLHFKGSKFHRVIPNFMCQGGDFTAGNG
TGGESIYGSKFEDENFERKHTGPGILSMANAGANTNGSQFFICTVKTDWLDGKHVVFGQVVEGLDVVKAIEKVGSSSGK
PTKPVVVADCGQLS

At3g13460 (ECT2; 11%)
MATVAPPADQATDLLQKLSLDSPAKASEIPEPNKKTAVYQYGGVDVHGQVPSYDRSLTPMLPSDAADPSVCYVPNPYNP
YQYYNVYGSGQEWTDYPAYTNPEGVDMNSGIYGENGTVVYPQGYGYAAYPYSPATSPAPQLGGEGQLYGAQQYQYPNYF
PNSGPYASSVATPTQPDLSANKPAGVKTLPADSNNVASAAGITKGSNGSAPVKPTNQATLNTSSNLYGMGAPGGGLAAG
YQDPRYAYEGYYAPVPWHDGSKYSDVQRPVSGSGVASSYSKSSTVPSSRNQNYRSNSHYTSVHQPSSVTGYGTAQGYYN
RMYQNKLYGQYGSTGRSALGYGSSGYDSRTNGRGWAATDNKYRSWGRGNSYYYGNENNVDGLNELNRGPRAKGTKNQKG
NLDDSLEVKEQTGESNVTEVGEADNTCVVPDREQYNKEDFPVDYANAMFFIIKSYSEDDVHKSIKYNVWASTPNGNKKL
AAAYQEAQQKAGGCPIFLFFSVNASGQFVGLAEMTGPVDFNTNVEYWQQDKWTGSFPLKWHIVKDVPNSLLKHITLENN
ENKPVTNSRDTQEVKLEQGLKIVKIFKEHSSKTCILDDFSFYEVRQKTILEKKAKQTQKQVSEEKVTDEKKESATAESA
SKESPAAVQTSSDVKVAENGSVAKPVTGDVVANGC

At4g33250 (eIF3k; 23%)
MGVEIQSPQEQSSYTVEQLVALNPFNPEILPDLENYVNVTSQTYSLEVNLCLLRLYQFEPERMNTHIVARILVKALMAM
PTPDFSLCLFLIPERVQMEEQFKSLIVLSHYLETGRFQQFWDEAAKNRHILEAVPGFEQAIQAYASHLLSLSYQKVPRS
VLAEAVNMDGASLDKFIEQQVTNSGWIVEKEGGSIVLPQNEFNHPELKKNTGENVPLEHIARIFPILG

At1g76810 (eIF5B; 4%)
MGRKKPSARGGDAEQQPPASSLVGATKSKKKGAQIDDDEYSIGTELSEESKVEEEKVVVITGKKKGKKGNKKGTQQDDD
DDFSDKVSAAGVKDDVPEIAFVGKKKSKGKKGGGSVSFALLDDEDEKEDNESDGDKDDEPVISFTGKKHASKKGKKGGN
SFAASAFDALGSDDDDTEEVHEDEEEESPITFSGKKKKSSKSSKKNTNSFTADLLDEEEGTDASNSRDDENTIEDEESP
EVTFSGKKKSSKKKGGSVLASVGDDSVADETKTSDTKNVEVVETGKSKKKKKNNKSGRTVQEEEDLDKLLAALGETPAA
ERPASSTPVEEKAAQPEPVAPVENAGEKEGEEETAAAKKKKKKKEKEKEKKAAAAAAATSSVEVKEEKQEESVTEPLQP
KKKDAKGKAAEKKIPKHVREMQEALARRQEAEERKKKEEEEKLRKEEEERRQEELEAQAEEAKRKRKEKEKEKLLRKK
LEGKLLTAKQKTEAQKREAFKNQLLAAGGGLPVADNDGDATSSKRPIYANKKKSSRQKGIDTSVQGEDEVEPKENQADE
QDTLGEVGLTDTGKVDLIELVNTDENSGPADVAQENGVEEDDEEDEWDAKSWGTVDLNLKGDFDDEEEEAQPVVKKELK
DAISKAHDSEPEAEKPTAKPAGTGKPLIAAVKATPEVEDATRTKRATRAKDASKKGKGLAPSESIEGEENLRSPICCIM
GHVDTGKTKLLDCIRGTNVQEGEAGGITQQIGATYFPAENIRERTKELKADAKLKVPGLLVIDTPGHESFTNLRSRGSS
LCDLAILVVDIMHGLEPQTIESLNLLRMRNTEFIVALNKVDRLYGWKTCKNAPIVKAMKQQNKDVINEFNLRLKNIINE
FQEQGLNTELYYKNKDMGDTFSIVPTSAISGEGVPDLLLWLVQWAQKTMVEKLTYVDEVQCTVLEVKVIEGHGTTIDVV
LVNGELHEGDQIVVCGLQGPIVTTIRALLTPHPMKELRVKGTYLHYKEIKAAQGIKITAQGLEHAIAGTALHVVGPDDD
IEAIKESAMEDMESVLSRIDKSGEGVYVQASTLGSLEALLEYLKSPAVKIPVSGIGIGPVHKKDVMKAGVMLERKKEYA
TILAFDVKVTTEARELADEMGVKIFCADIIYHLFDLFKAYIENIKEEKKKESADEAVFPCVLQILPNCVFNKKDPIVLG
VDVIEGILKIGTPICVPGREFIDIGRIASIENNHKPVDYAKKGNKVAIKIVGSNAEEQKMFGRHFDMEDELVSHISRRS
IDILKSNYRDELSLEEWKLVVKLKNIFKIQ

At3g53610 (RAB8; 18%)
MAAPPARARADYDYLIKLLLIGDSGVGKSCLLLRFSDGSFTTSFITTIGIDFKIRTIELDGKRIKLQIWDTAGQERFRT
ITTAYYRGAMGILLVYDVTDESSFNNIRNWIRNIEQHASDSVNKILVGNKADMDESKRAVPKSKGQALADEYGMKFFET
SAKTNLNVEEVFFSIAKDIKQRLADTDARAEPQTIKINQSDQGAGTSQATQKSACCGT

At5g37475 (eIF3j; 16%)
MDDWEAEDFQPLPSKVELKSNWDDEDVDENDIKDSWEEEDVSAPPPIVKPASEKAPKKPAVKAVEKKVKTVEAPKGTSR
EEPLDPIAEKLRMQRLVEEADYQSTAELFGVKTEEKSVDMLIPKSESDFLDYAELISQRLVPFEKSFHYIGLLKAVMRL
SVANMKAADVKDVASSITAIANEKLKAEKEAAAGKKKSGKKKQLHVDKPDDDLVSGPYDAMDDDDFM

At3g43600 (AAO2; 3%)
MSLVFAINGQRFELELSSVDPSTTLLEFLRYQTSFKSVKLSCGEGGCGACVVLLSKFDPVLQKVEDFTVSSCLTLLCSV
NHCNITTSEGLGNSRDGFHPIHKRLSGFHASQCGFCTPGMSVSLFSALLDADKSQYSDLTVVEAEKAVSGNLCRCTGYR
PIVDACKSFASDVDIEDLGLNSFCRKGDKDSSSLTRFDSEKRICTFPEFLKDEIKSVDSGMYRWCSPASVEELSSLLEA
CKANSNTVSMKLVAGNTSMGYYKDEREQNYDKYIDITRIPHLKEIRENQNGVEIGSVVTISKVIAALKEIRVSPGVEKI
FGKLATHMEMIAARFIRNFGSIGGNLVMAQRKQFPSDMATILLAAGAFVNIMSSSRGLEKLTLEEFLERSPLEAHDLVL
SIEIPFWHSETNSELFFETYRAAPRPHGSALAYLNAAFLAEVKDTMVVNCRLAFGAYGTKHAIRCKEIEEFLSGKVITD
KVLYEAITLLGNVVVPEDGTSNPAYRSSLAPGFLFKFLHTLMTHPTTDKPSNGYHLDPPKPLPMLSSSQNVPINNEYNP
VGQPVTKVGASLQASGEAVYVDDIPSPTNCLYGAFIYSKKPFARIKGIHFKDDLVPTGVVAVISRKDVPKGGKNIGMKI
GLGSDQLFAEDFTTSVGECIAFVVADTQRHADAAVNLAVVEYETEDLEPPILSVEDAVKKSSLFDIIPFLYPQQVGDTS
KGMAEADHQILSSEIRLGSQYVFYMETQTALAVGDEDNCIVVYSSTQTPQYVQSSVAACLGIPENNIRVITRRVGGGFG
GKSVKSMPVATACALAAKKLQRPVRTYVNRKTDMIMTGGRHPMKITYSVGFKSTGKITALELEILIDAGASYGFSMFIP
SNLIGSLKKYNWGALSFDIKLCKTNLLSRAIMRSPGDVQGTYIAEAIIENIASSLSLEVDTIRKINLHTHESLALFYKD
GAGEPHEYTLSSMWDKVGVSSKFEERVSVVREFNESNMWRKRGISRVPIIYEVLLFATPGRVSVLSDGTIVVEIGGIEL
GQGLWTKVKQMTSYALGMLQCDGTEELLEKIRVIQSDSLSMVQGNFTGGSTTSEGSCAAVRLCCETLVERLKPLMERSD
GPITWNELISQAYAQSVNLSASDLYTPKDTPMQYLNYGTAVSEVEVDLVTGQTTVLQTDILYDCGKSLNPAVDLGQIEG
SFVQGLGFFMLEEYIEDPEGLLLTDSTWTYKIPTVDTIPKQFNVEILNGGCHEKRVLSSKASGEPPLLLAASVHCATRQ
AVKEARKQLCMWKGENGSSGSAFQLPVPATMPVVKELCGLDIIESYLEWKLHDNSNL

At1g65860 (FMO GS-OX1; 4%)
MAPTQNTICSKHVAVIGAGAAGLVTARELRREGHTVVVFDREKQVGGLWNYSSKADSDPLSLDTTRTIVHTSIYESLRT
NLPRECMGFTDFPFVPRIHDISRDSRRYPSHREVLAYLQDFAREFKIEEMVRFETEVVCVEPVNGKWSVRSKNSVGFAA
HEIFDAVVVCSGHFTEPNVAHIPGIKSWPGKQIHSHNYRVPGPFNNEVVVVIGNYASGADISRDIAKVAKEVHIASRAS
ESDTYQKLPVPQNNLWVHSEIDFAHQDGSILFKNGKVVYADTIVHCTGYKYYFPFLETNGYININENRVEPLYKHVFLP
ALAPSLSFIGLPGMAIQFVMFEIQSKWVAAVLSGRVILPSQDKMMEDIIEWYATLDVLGIPKRHTHKLGKISCEYLNWI
AEECHCSPVENWRIQEVERGFQRMVSHPEIYRDEWDDDDLMEEAYKDFARKKLISSHPSYFLES

At2g20830 (Folic acid binding / transferase; 8%)
MSSGLNEDFLDCIVRLEETHVQQGFDEGYEEGLVSGREDARHLGLKLGFETGELIGFYRGCSALWNSALRIDPTRFSPQ
LHKHLNDFHVLLDKIPLLDPEDEAKDGIKDDLRVKFSIICASLGFSKKQFEWSEEMLREMLGCCKVYISEARNKTALEA
IERALKPFPPAAIVNKFEDAAYGRVGYTVVSSLANGSSSSLKNAVFAMVKTALDTINLELHCGSHPRLGVVDHICFHPL
SQTSIEQVSSVANSLAMDIGSILRVPTYLYGAAEKEQCTLDSIRRKLGYFKANREGHEWAGGFDLEMVPLKPDAGPQEV
SKAKGVVAVGACGWVSNYNVPVMSNDLKAVRRIARKTSERGGGLASVQTMALVHGEGVIEVACNLLNPSQVGGDEVQGL
IERLGREEGLLVGKGYYTDYTPDQIVERYMDLLNNS

At5g58410 (HEAT/U-box domain-containing protein; 1%)

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MGKPKGDAARSKARPSSSSLAASLLPSGSAAAVGFGGYVGSSRFQTSLSNEDSASFLDLDSEVAQHLQRLSRKDPTTKI
KALASLSELVKQKQGKELLPIIPQWTFEYKKLILDYSRDVRRATHDVMTNVVTGAGRDIAPHLKSIMGPWWFSQFDLAS
EVSQAAKSSFQVGSSFGNSVFLVEAAFPAQEKRLHALNLCSAEIFAYLEENLKLTPQNLSDKSLASDELEEMYQQMISS
SLVGLATLLDILLREPDNTGSANINSESKLASKARAVATSSAEKMFSSHKCFLNFLKSESPSIRSATYSLLSSFIKNVP
EVFGEGDVR[SLAPALLGVFR]ENNPTCHSSMWEAVLLFSKKFPQSWVYLNVHKSVLNHLWQFLRNGCYGSPQVSYPALIL
FLEVMPAQSVESDKFFVNFFKNLLAGRSMCESSSTDQLSLLRATTECFLWGLRNASRYCDVPNSIHDLQVDLIDKVLVK
ILWADFTELSKGSIPPNQRKSAENLGMGNSVSYLQELGRCILEILSGINLLEQNLLSFFCKAVQESFLNMLQQGDLEIV
AGSMRKMIDFLLLLERYSVLEGESWPLHQFMGPLLSKAFPWIRSSELLDGVKLLSVSVSVFGPRKVVPVLIDDIETSTL
LSVEKEKNMSPEKLIKVFQEIFIPWCMDGYDSSTAARQDLLFSLLDDECFTQQWSDVISYVFNQQHQGFNNLAAMK[MLL
EKAR]DEITKRSSGQELNQRIGSRPEHWHHTLIESTAISLVHSSSATTTSAVQFLCSVLGGSTQDSSISFVSRSSLVLIY
RGILEKLLSFIKQSPLCSVNDTCSSLIVEAIAFDSSSSVDVIVVAKFAAEVIDGSFFSLKSLSQDATLLTTVLSSIFII
DLENRMTSLVDNTLSESKEKRKDRNFVCDYVHAVCSKMDNQFWKSINYDVRKSSASTLAQFLRSVVLLEDDLQPFELTL
LCASRMTEVLEYLSLDQSDEENICGLLLLESDAWPIWVSPSSSASIDTHGMPVQLCELRKSKSQRYVSFIDSLIMKLGI
HRFIVGHKDHGFASQAWLSVEILCTWEWPGGKVQTSFLPNLVSFCKDEPSSGGLLNSIFDILLNGALVHVKDEEEGLGN
MWVDFNNNIVDVVEPFLRALVSFLHILFKEDLWGEEEAMAAFKMITDKLFIGEETSKNCLRIIPYIMSIIISPLRTKVK
SGGSGKDTLLPLEVLLRNWLERSLSFPPLVLWQSGEDIQDWFQLVISCYPVSDKAEEAKELQRHLSTEERTLLLDLFRK
QKQDPGASTVVTQLPAVQILLARLIMIAVSYCGNDFNEDDWDFVFSNLKRLIQSAVVVMEETSENVNDFISGVSSMEKE
KENDTLEGLGHIVFISDPSINSAQNALSAFSLLNALVNHKSVEGEDNLKSLADETWDPVKDRILEGVLRLFFCTGLTEA
IAASYSPEAASIVASFRVDHLQFWELVAHLVVDSSPRARDRAVRAVEFWGLSRGSISSLYAIMFSSNPIPSLQLAAYTV
LSTEPISRLAIVADLNAPLNDESLNDQDSSNAGLPSEDKLLLRDEVSCMVEKLDHELLDTDLTAPERVQTFLAWSLLLS
NVNSLPSLTQGRERLVQYIEKTANPLILDSLFQHIPLELYMGQSLKKKDGDIPSELSVVASAATRAIITGSSLSTVESL
WPIETGKMASLAGAIYGLMLRVLPAYVREWFSEMRDRSASSLIEAFTRTWCSPSLIKNELSQIKKADFNDESFSVSISK
AANEVVATYTKDETGMDLVIRLPVSYPLKPVDVNCAKSIGISEAKQRKWLMSMQMFVRHQNGALAEAIRIWKRNSDKEF
EGVEDCPICYSVIHIGNHSLPRRACVTCKYKFHKACLDKWFYTSNKKLCPLCQSPC
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At2g42910 (PRS4; 8%)

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MSENAANNIMETKICTDAIVSELQKKKVHLFYCLECEELARNIAAESDHITLQSINWRSFADGFPNLFINNAHDIRGQH
VAFLASFSSPAVIFEQISVIYLLPRLFVASFTLVLPFFPTGSFERMEEEGDVATAFTMAR[IVSNIPISRGGPTSVVIYD
IHALQER]FYFADQVLPLFETGIPLLTKRLQQLPETEKVIVAFPDDGAWKRFHKLLDHYPTVVCTKVREGDKRIVRLKEG
NPAGCHVVIVDDLVQSGGTLIECQKVLAAHGAVKVSAYVTHGVFPKSSWERFTHKKNGLEEAFAYFWITDSCPQTVKAI
GNKAPFEVLSLAGSIADALQI
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At3g08850 (RAPTOR1; 1%)

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MALGDLMVSRFSQSSVSLVSNHRYDEDCVSSHDDGDSRRKDSEAKSSSSYGNGTTEGAATATSMAYLPQTIVLCELRHD
ASEASAPLGTSEIVLVPKWRLKERMKTGCVALVLCLNITVDPPDVIKISPCARIEAWIDPFSMAPPKALETIGKNLSTQ
YERWQPRARYKVQLDPTVDEVRKLCLTCRKYAKTERVLFHYNGHGVPKPTANGEIWVFNKSYTQYIPLPISELDSWLKT
PSIYVFDCSAARMILNAFAELHDWGSSGSSGSSRDCILLAACDVHETLPQSVEFPADVFTSCLTTPIKMALKWFCRRSL
LKEIIDESLIDRIPGRQNDRKTLLGELNWIFTAVTDTIAWNVLPHELFQRLFR[QDLLVASLFR]NFLLAERIMRSANCNP
ISHPMLPPTHQHHMWDAWDMAAEICLSQLPQLVLDPSTEFQPSPFFTEQLTAFEVWLDHGSEHKKPPEQLPIVLQVLLS
QCHRFR[ALVLLGR]FLDMGSWAVDLALSVGIFPYVLKLLQTTTNELRQILVFIWTKILALDKSCQIDLVKDGGHTYFIRF
LDSSGAFPEQRAMAAFVLAVIVDGHRRGQEACLEANLIGVCLGHLEASRPSDPQPEPLFLQWLCLCLGKLWEDFMEAQI
MGREANAFEKLAPLLSEPQPEVRAAAVFALGTLLDIGFDSNKSVVEDEFDDDEKIRAEDAIIKSLLDVVSDGSPLVRAE
VAVALARFAFGHKQHLKLAAASYWKPQSSSLLTSLPSIAKFHDPGSATIVSLHMSPLTRASTDSQPVARESRISSSPLG
SSGLMQGSPLSDDSSLHSDSGMMHDSVSNGAVHQPRLLDNAVYSQCVRAMFALAKDPSPRIASLGRRVLSIIGIEQVVA
KPSKPTGRPGEAATTSHTPLAGLARSSSWFDMHAGNLPLSFRTPPVSPPRTNYLSGLRRVCSLEFRPHLLGSPDSGLAD
PLLGASGSERSLLPLSTIYGWSCGHFSKPLLGGADASQEIAAKREEKEKFALEHIAKCQHSSISKLNNNPIANWDTRFE
TGTKTALLHPFSPIVVAADENERIRVWNYEEATLLNGFDNHDFPDKGISKLCLINELDDSLLLVASCDGSVRIWKNYAT
KGKQKLVTGFSSIQGHKPGARDLNAVVDWQQQSGYLYASGETSTVTLWDLEKEQLVRSVPSESECGVTALSASQVHGGQ
LAAGFADGSLRLYDVRSPEPLVCATRPHQKVERVVGLSFQPGLDPAKVVSASQAGDIQFLDLRTTRDTYLTIDAHRGSL
TALAVHRHAPIIASGSAKQLIKVFSLQGEQLGIIRYYPSFMAQKIGSVSCLTFHPYQVLLAAGAADSFVSIYTHDNSQA
R
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At5g01770 (RAPTOR2; 1%)

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MALGDLMVSRLSQSSVTVVTNHLYDDDDNCASSAHDDSRVSIIASPRVASSSYENLSAATSMAYLPQTLVLCDLRHDDA
SDIVQPPRWRLKERMKTGCVALVMCLHITVDPPDVIKISPCARLECWIDPFSMFPPRRALEAIGQNLSIQYERWLARAR
YKVELDPTKDDVRKLCLSCRKYAKTERVLFHYNGHGVPKPTPNGEIWVYNKNFTQYIPLPVSELDSWLKTPTIYVFDCS
AARVILNAFAEGESSGPPKDCILLAACDVHETLPQSVEFPADVFTSCLTTPINIALKWFCRRSLLKEFIDESLIDRIPG
RQNDRKTLLGELNWIFTAVTDTIAWNVLPRELFQRLFRQDLLVASLFRNFLLAERIMRSGNCTPISHPMLPPTHQHHMW
DAWDMAAEICLSQLPQFFLDPNTEFQPSSFFTEQLTAFEVWLDHGSEHKKPPEQLPIVLQVLLSQCHRYRALVLLGRFL
DMGPWAVDLALSVGIYPCVVKLLQTTTIELRQILVFIWTKILALDKSCQVDLVKDRGHIYFIRFLDSSDAFPEQRAMAA
FILAVIVDGYKRGQESCLEANLIAVCLGHLEATQLCDPPPEPLFLQWLCLCLGKLWEDYLEAQIMGREANASENLIAGH
TNLLQVRAAAVFALGTLLDVGFDSGKGVCDEEFDDDENIVEDIIIKSLLDVVSDGSPLVRTEVAVALARFAFGHKQHLK
SVADSYWKPQNSLRTSLPSMAKFHDSGTSIVASSDMGSLTRASPDSQPVAREGRISSSLQEPFSGLMQGSPLADDSSLH
SDVGIIHDGVSNGVVHQPRPLDNAIYSQSVLAMFTLAKDPSPRIASLGRRVLSVIGIEQIVAKPSKSNGRPGEAASASH
TPLAGLVRSSSWFDMHTGHLPLTFRTPPVSPPQTSYLTGLRRVCSLELRPHLLGSPDSGLADPILGVSGSERSLLPQST
IYNWSCGHFSKPLLGGADANEEIAAQREEKKKFSLEHIAKCQHSSISGLSNIPIANWDTKFETGTKTALLHPFSPIVVA
ADENERIRVWNYEEATLLNGFDNNDFPDKGISNLCLVNELDDSLLLVASCNVPTLSRASFAIRIWKDYATKGRQKLVTG
FSSIQGQKPGASGLNAVVDWQQQSGYLYVSGESLSIMVWDLDKEQLVKSMPFESGCSVTALSASQVHGSQLAAGFADGS
VRLYDVRTPDFLVCATRPHQRVEKVVGLSFQPGLDPAKIVSASQAGDIQFLDLRRPKETYLTIDAHRGSLTALGVHRHA
PIIASGSAKQLIKVFSLKGEQLGIIKYHTSFMGQQIGPVSCLAFHPYQMLLAAGAAGSFVSLYTHHNTQLPR
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Supplementary Figure S9. Amino acid sequences of proteins identified by LC-ESI-MS/MS in co-immunoprecipitated ABCE2:YFP protein.

The Arabidopsis Genome Initiative (AGI) gene identifier (AtNgNNNNN) is shown, together with the TAIR10 annotation or protein name and peptide coverage (in percentage) for each protein. Full-length protein sequences were obtained from TAIR. The unique peptides identified by LC-ESI-MS/MS are shaded in black; some unique peptide sequences overlap. Peptide coverage was calculated by dividing the total number of residues of each protein by that of those covered by the peptides.
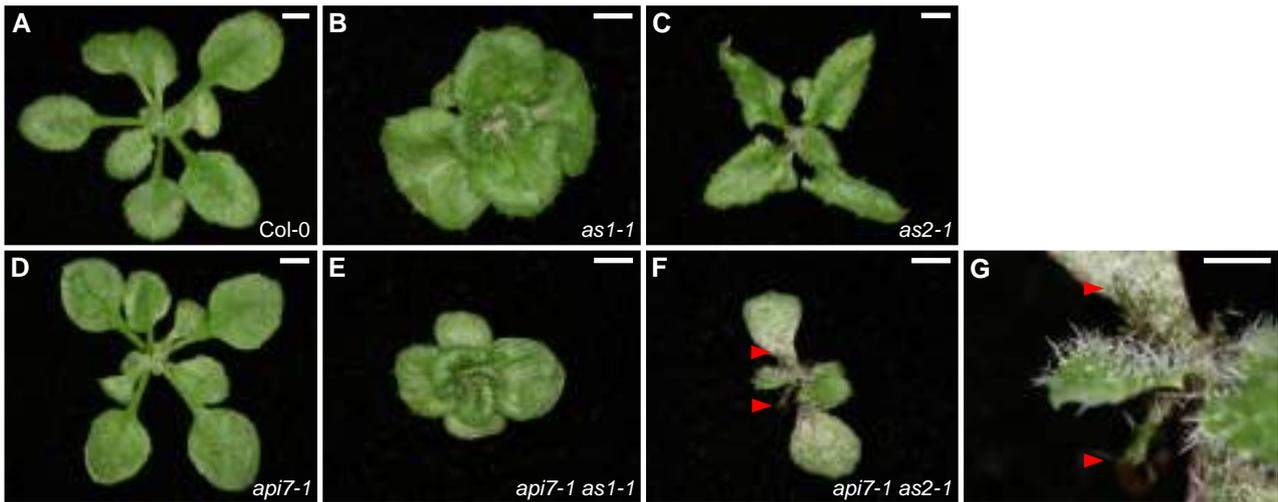
Supplementary Figure S10. Genetic interactions of *api7-1* with *as1-1* and *as2-1*.
(A–F) Rosettes from (A) the wild-type L*er*, the (B) *as1-1*, (C) *as2-1*, and (D) *api7-1* single mutants, and the (E) *api7-1 as1-1* and (F) *api7-1 as2-1* double mutants. (G) Close up of (F). Red arrowheads indicate radial leaves. Pictures were taken 16 das. Scale bars indicate (A–F) 2 mm and (G) 1 mm.
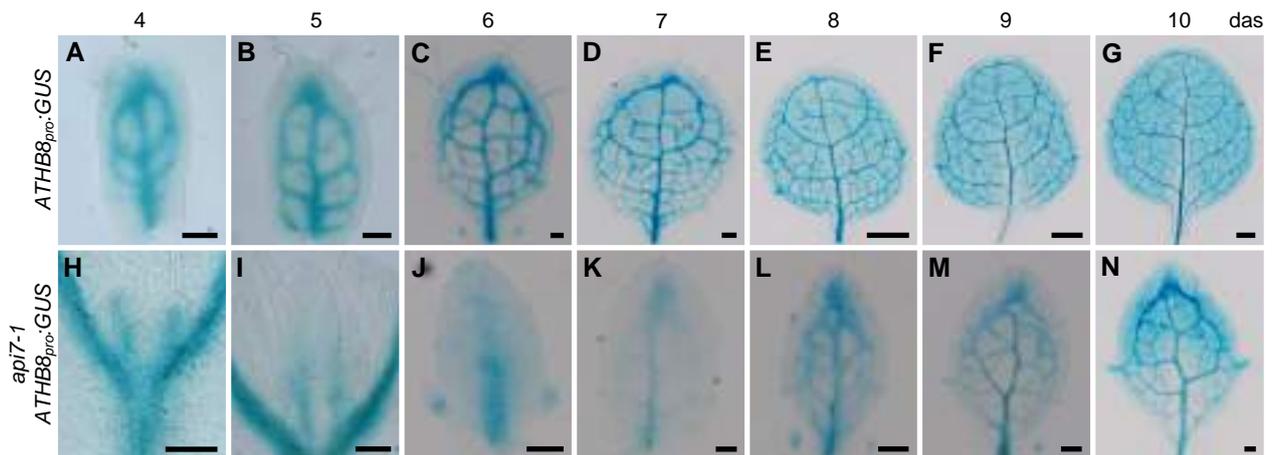
Supplementary Figure S11. Vascularization in *api7-1* leaf primordia.
Vascular fate specification is shown as *ATHB8pro:GUS* activity at expanding first-node leaf primordia in (A–G) L*er* and (H–N) *api7-1* backgrounds. Pictures were taken (A, H) 4, (B, I) 5, (C, J) 6, (D, K) 7, (E, L) 8, (F, M) 9, and (G, N) 10 das. Scale bars indicate (A–C, H–J) 50, (D, K–N) 100, and (E–G) 500 µm.
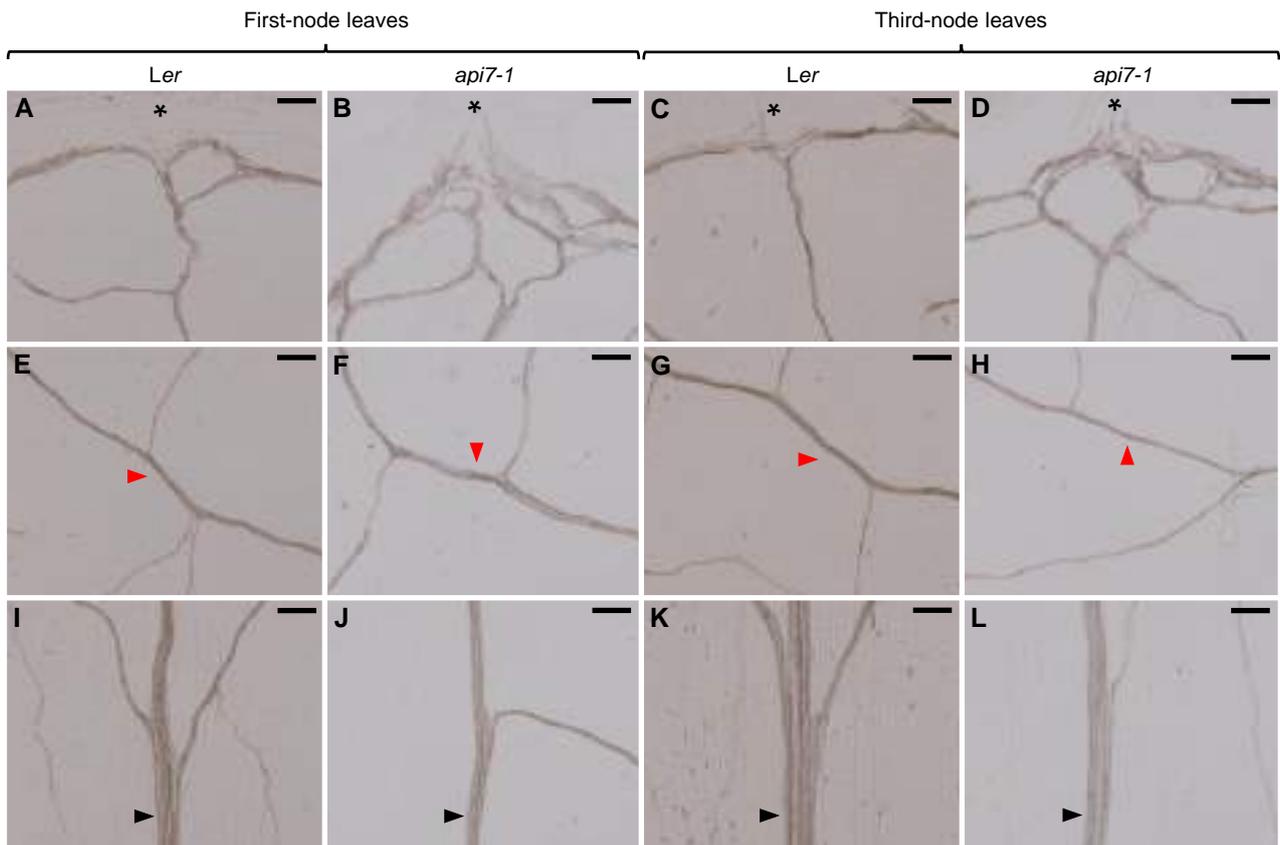
Supplementary Figure S12. Some details of the vascular phenotype of first- and third-node leaves from L*er* and *api7-1* plants.

Veins from (A, C, E, G, I, K) L*er* and (B, D, F, H, J, L) *api7-1* (A, B, E, F, I, J) first- and (C, D, G, H, K, L) third-node leaves. (A–D) Venation on the apical region (an asterisk indicates the most apical region) of the lamina, (E–H) a secondary vein (red arrowheads) bifurcating to render tertiary veins, and (I–L) the primary vein (black arrowheads), close to the base of the lamina. We observed 6 first- and third-node leaves from L*er* and 18 from *api7-1* with similar vascular phenotypes to the ones shown. Pictures were taken 21 das. Scale bars indicate 100 μm.

| | Cotyledons | Cauline leaves | Sepals | Petals |
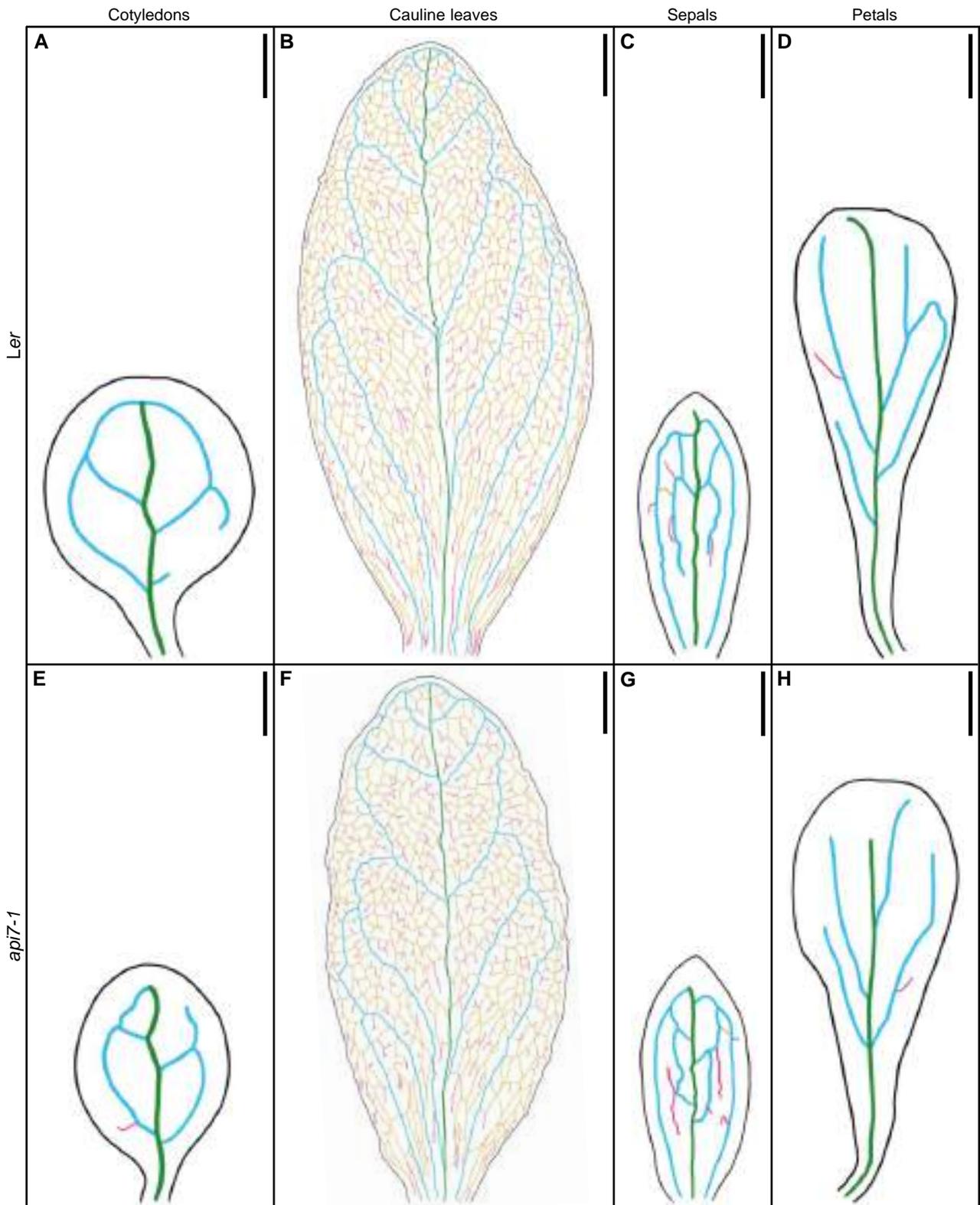
Supplementary Figure S13. Venation pattern of *api7-1* cotyledons, cauline leaves, sepals, and petals.

Representative diagrams of (A, E) cotyledons, (B, F) cauline leaves, (C, G) sepals, and (D, H) petals from (A–D) L*er* and (E–H) *api7-1* plants. Margins and veins were drawn as described in Figure 6. Organs were collected (A, E) 6 and (B–D, F–H) 35 das. Scale bars indicate (A, C–E, G, H) 0.5 and (B, F) 2.5 mm.
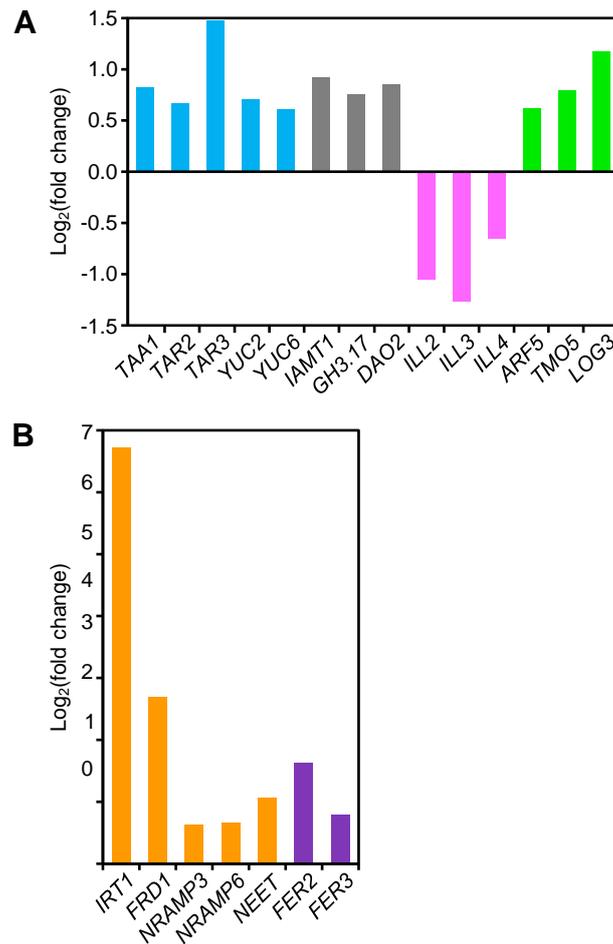
Supplementary Figure S14. Expression levels of some genes deregulated in *api7-1* plants. Expression levels of genes related to (A) auxin metabolism (biosynthesis, blue; inactivation, grey; activation, pink) and provascular cell division (green), and (B) iron homeostasis and FeS cluster biogenesis (orange), and response to oxidative stress (purple). Values are shown as the binary logarithm of the foldchange between *api7-1* and L*er* mean reads. Mean reads were calculated from three biological replicates.

Supplementary Table S1. Primer sets used in this work

Purpose	Name	Forward primer (F; 5′ → 3′)	Reverse primer (R; 5′ → 3′)
Linkage analysis	nga1111_F/R	GGGTTCGGTTACAATCGTGT	AGTTCCAGATTGAGCTTTGAGC
	AtF28J12.3_F/R	GCTCCGCCGTTGGATTCTG	GTTCGGGTTTAATTCTCGGGT
	AtM7J12.1_F/R	AGCAACTTGTGTTCTCATTT	TTATAGGGTACGACAACCAT
	nga1139_F/R	CTAGGCTCGGGTGAGTCAC	TTTTTCCTTGTGTTGCATTCC
	nga1107_F/R	GCGAAAAAACAAAAAAATCCA	CGACGAATCGACAGAATTAGG
	g3883_F/R	CATCCATCAAACAAACTCC	TGTTTCAGAGTAGCCAATTC
	T13K14_F/R	CTGAAACATATAAGAGAATCATCC	ACTCGTAGTTTGGTGTTGAGAC
	AtF16G20.1_F/R	TCAGTGTTACTATGTACCAAGTA	TAGGACGTAATATCCTTAGTTAC
	AG_F/R	CAACAGGTTTCTTCTTCTTCTC	CAAACACCATTTAATCTTGACA
	T18B16_F/R	TAACTTCTTGCAGCCTCTGAAG	TTCTATTGGGATGCTGCCCTC
Sequencing of *ABCE1* and *ABCE2*	ABCE1_F1/R1	GGTTAGCTAGTCCCTTTCAAAG	GAAGTATGCTAATGTGGCCC
	ABCE1_F2/R2	ACTACCTCTTGCGCAGACTC	GGCATCAGACTCACTTCATGA
	ABCE1_F3/R3	GGAAGTGAAGTCCTATGCAAGA	CTTGAAATCTCCAAGTTGCTTAGT
	ABCE1_R4		CTGCCAAGATTTGGTTTGAG
	ABCE2_F1/R1	TCGGTTCACCATTTTTATCTGAAG	CACCACAAGATGCTAACAATGAT
	ABCE2_F2/R2	AGCCTGCGGATATATACCTGAT	GGTCGTATCTTTCTCCAAGTCT
	ABCE2_F3/R3	GTTACACCGTATGGGCAAGAG	GGAGACTTACAGATAAGAAGAGA
	ABCE2_F4/R4	CTTAGAACAATCGGCACACG	AGAGAAATCGAGATTAGTACCTGAG
	ABCE2_F5/R5	GTTCTGATACCCTGTGCATG	ATCTCTTCAGCACTTTCTTGTG
Genotyping	api7-1_F/R	TGCCTCTAGAAATGGCACCT	GTATGGCAACAACAGCGATT
	GABI_509C06_LP/RP	TTCTTGGTCTGAAATTGGTGG	TGGCTGGATTTGTTCCTACAG
	o8409 (GABI-Kat lines)[1]	ATATTGACCATCATACTCATTGC	
	M13_F/R	TGTAAAACGACGGCCAGT	GGAAACAGCTATGACCATGATT
	GFP_R		CACGTATCCCTCAGGCATGG
	YFP_R		GACTTGAAGAAGTCGTGCTGC

Supplementary Table S1 (continued). Primer sets used in this work

Purpose	Name	Forward primer (F; 5′ → 3′)	Reverse primer (R; 5′ → 3′)
qRT-PCR	qABCE1_F/R	CCTAAATCTTCGGAAAGTGAAC	GGCCATGAACCAACTTACGC
	qABCE2_F/R	GACAACTACCAAGAGAATATAGG	CAACTCAGGAAGTACAAAGCC
	qACTIN2_F/R[2]	GCACCCTGTTCTTCTTACCG	AACCCTCGTAGATTGGCACA
	OTC3D/OTCR[3]	TCCTTGCCAAATCATGGCCG	GCATGCATGCGATTCTCCGC
Gateway cloning	ABCE2pro:ABCE2_F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCT TTTCTATCTTGTTATTCTTCGTTTTT	GGGGACCACTTTGTACAAGAAAGCTGGGT AGTTTTCCTATATCAGTGAGTTAG
	35Spro:ABCE2:GFP-YFP_F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCT GGATGGCAGATCGATTGACACGTA	GGGGACCACTTTGTACAAGAAAGCTGGGT CATCATCCAAGTAGTAGTATGAGC
	ABCE1pro_F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCT TACTTTTCTCTCGGCCGTACT	CAATACGTGTCAATCGATCTGCCATCTCTC TTGAGAATATTACATACAAG
	ABCE2tu_F/R	CTTGTATGTAATATTCTCAAGAGAGATGGC AGATCGATTGACACGTATTG	GGGGACCACTTTGTACAAGAAAGCTGGGT CTAATCATCCAAGTAGTAGTA
	ABCE2pro_F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCT TTTCTATCTTGTTATTCTTCGTTTTT	AATCCGCGTCAATCGATCTGACATCTCTCA ACAGACCTACAAAATACATG
	ABCE1tu_F/R	CATGTATTTTGTAGGTCTGTTGAGAGATGT CAGATCGATTGACGCGGATT	GGGGACCACTTTGTACAAGAAAGCTGGGT TCAATCGTCTAAGTAGTAGTA

Sequences were taken from [1](S1), [2](S2), and [3](S3).

Supplementary Table S2. NCBI accession numbers of the sequences used for phylogenetic analysis

Species	*ABCE1* gene	*ABCE2* gene
Arabidopsis thaliana	NM_112210.3	ABCE2 NM_118041.5
Arabidopsis lyrata subsp. *lyrata*	XM_021033623.1	XM_021033596.1
Capsella rubella	XM_006299733.2	XM_006285967.2
Cardamine hirsuta	-	JX097073.1
Eutrema salsugineum	XM_006418662.2	XM_006413929.2
Brassica rapa (*a*)	XM_033286408.1	XM_033276765.1
Brassica rapa (*b*)	XM_009119375.3	XM_009138752.3
Brassica rapa (*c*)	-	XM_018655198.2
Fragaria vesca subsp. *vesca*	-	XM_004291176.2
Theobroma cacao	-	XM_018117748.1
Citrus sinensis	-	XM_015531558.2
Populus trichocarpa (*a*)	-	XM_024599960.1
Populus trichocarpa (*b*)	-	XM_024597900.1
Eschscholzia californica	-	Eca_sc194497.1_g0120.1*
Oryza sativa (*a*)	-	XM_015762126.2
Oryza sativa (*b*)	-	XM_026023394.1

Multiple *ABCE1* or *ABCE2* genes from *Brassica rapa*, *Populus trichocarpa*, and *Oryza sativa* are distinguished with arbitrarily given *a*, *b*, and *c* designations. *Obtained from Eschscholzia Genome DataBase (http://eschscholzia.kazusa.or.jp/cgi-bin/list.cgi).

Supplementary Table S3. Mutations identified in the *api7-1* candidate interval

Mutation	Region affected	Predicted effect
G→A	At4g19185, 1st intron	-
G→A	At4g19185, 1st exon	Cys55→Cys (Synonymous)
C→T	At4g19210, 6th exon	Pro138→Ser
G→A	At4g19390, 2nd exon	Ile128→Ile (Synonymous)

Supplementary Table S4. ABCE2 interactors identified in a co-immunoprecipitation assay

AGI gene code	Protein		Peptides		Peptide coverage
	Abbreviation	Full name	Total[1]	Unique[2]	(%)
At4g19210	ABCE2	ATP-BINDING CASSETTE E2	153 (20)	26 (5)	62
At3g13640	ABCE1	ATP-BINDING CASSETTE E1			
-	YFP	Yellow fluorescent protein	59	10	-
At4g11420	eIF3a[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT A	48	24	32
At3g56150	eIF3c[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT C	29	11	14
At3g57290	eIF3e[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT E	25	15	40
At1g64790	ILA[3]	ILITYHIA	17	11	4
At2g44060	LEA26[3]	LATE EMBRYOGENESIS ABUNDANT 26	15	6	23
At4g20980	eIF3d[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT D	13 (8)	6 (4)	13
At5g44320	eIF3d[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT D			
At5g17020	XPO1A[3]	EXPORTIN 1A	12 (4)	5 (2)	6
At3g03110	XPO1B[3]	EXPORTIN 1B			
At1g61580	RPL3B	RIBOSOMAL PROTEIN L3 B	12	4	12
At4g38740	ROC1	ROTAMASE CYP 1	11	5	38
At3g13460	ECT2[3]	EVOLUTIONARILY CONSERVED C-TERMINAL REGION 2	8	6	11
At4g33250	eIF3k[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT K	8	4	23
At1g76810	eIF5B[3]	EUKARYOTIC TRANSLATION INITIATION FACTOR 5B	6	4	4
At3g53610	RAB8	RAB GTPASE HOMOLOG 8	6	3	18
At5g37475	eIF3j	EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT J	5	3	16
At3g43600	AAO2	ALDEHYDE OXIDASE 2	4	3	3
At1g65860	FMO GS-OX1	FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 1	4	3	4
At2g20830	-[4]	FOLIC ACID BINDING / TRANSFERASE	4	2	8
At5g58410	LTN1	E3 UBIQUITIN-PROTEIN LIGASE LISTERIN	4	2	1

Supplementary Table S4 (continued). ABCE2 interactors identified in a co-immunoprecipitation assay

AGI gene code	Protein		Peptides		Peptide coverage
	Abbreviation	Full name	Total[1]	Unique[2]	(%)
At2g42910	PRS4	PHOSPHORIBOSYL DIPHOSPHATE SYNTHASE 4	4	2	8
At3g08850	RAPTOR1	REGULATORY-ASSOCIATED PROTEIN OF TOR 1	4 (3)	2 (2)	1
At5g01770	RAPTOR2	REGULATORY-ASSOCIATED PROTEIN OF TOR 2			

Three biological replicates were assayed and we identified 20 candidate interactors. Translation initiation factors were named according to (S4). [1]Sum of the number of peptides identified in the three biological replicates. [2]Number of associated peptides with significantly different sequences from each other. Values within parentheses refer to the second protein of the paralogous group, whose peptides were also associated with the first protein in all cases. [3]Enriched proteins (identified with at least twice the number of peptides associated with the same protein in the control co-immunoprecipitations). The rest of the proteins were unique to ABCE2:YFP samples. [4]This protein was included after being first discarded due to its predicted mitochondrial localization.

Supplementary Table S5. Conservation level and described functions of putative ABCE2 interactors

| AGI gene code | Protein | Conservation between orthologs (%)[1] | | | Described molecular function(s) and available evidence of its interaction with ABCE proteins[2] |
		Arabidopsis and *S. cerevisiae*	Arabidopsis and *H. sapiens*	*S. cerevisiae* and *H. sapiens*	
At4g19210	ABCE2	68.9 (82.6)	75.4 (86.0)	68.3 (82.9)	In Arabidopsis: suppression of RNA silencing (S5,S6). In yeast (Rli1): ribosome dissociation (S7). In humans (ABCE1): inhibition of RNAse L, suppression of RNA silencing, and ribosome dissociation (S8,S9,S10).
At4g11420	eIF3a	26.3 (43.4)	25.7 (41.6)	18.3 (32.0)	In Arabidopsis, yeast (Rpg1), and humans (EIF3A): translation initiation (S11).
At3g56150	eIF3c	24.3 (41.7)	34.5 (49.9)	23.4 (39.2)	In Arabidopsis, yeast (Nip1), and humans (EIF3C): translation initiation (S11).
At4g20980	eIF3d	NC	39.0 (54.4)	NC	In Arabidopsis and humans (EIF3D): translation initiation (S11).
At3g57290	eIF3e	NC	50.1 (69.0)	NC	In Arabidopsis and humans (EIF3E): translation initiation (S11).
At5g37475	eIF3j	22.5 (37.0)	29.7 (45.1)	26.2 (40.9)	In yeast (Hcr1): translation initiation and, as a non-stoichiometric subunit of the eIF3 complex, participates in pre-40S maturation, and as an accessory factor for Rli1-mediated ribosome dissociation (S12,S13). In humans (EIF3J): start codon selection and eIF3 complex formation during translation initiation (S14,S15). It is also present in the 40S post-splitting complex with ABCE1 (S16).
At4g33250	eIF3k	NC	31.4 (50.7)	NC	In Arabidopsis and humans (EIF3K): translation initiation (S11) .
At1g76810	eIF5B	36.3 (50.1)	39.7 (55.6)	36.5 (54.1)	In yeast (Fun12): 40S and 60S joining during pre-40S maturation and translation initiation (S17,S18,S19,S20).
At1g61580	RPL3B	65.3 (79.6)	64.9 (80.4)	65.3 (80.0)	In Arabidopsis, yeast (Rpl3), and humans (RPL3): ribosomal protein. Translation (S21,S22).

Supplementary Table S5 (continued). Conservation level and described functions of putative ABCE2 interactors

AGI code	Protein	Conservation between orthologs (%)[1]			Described molecular function(s) and available evidence of its interaction with ABCE proteins[2]
		Arabidopsis and *S. cerevisiae*	Arabidopsis and *H. sapiens*	*S. cerevisiae* and *H. sapiens*	
At1g64790	ILA	27.6 (45.1)	32.4 (50.2)	27.7 (47.1)	In Arabidopsis: translation regulation through two pathways, one involving GCN2 and eIF2α, and the other involving GCN20 (S23,S24). In yeast (Gcn1): translation downregulation by binding to translating ribosomes with Gcn2 and Gcn20 (S25,S26).
At3g13460	ECT2	12.5 (19.6)	25.5 (35.9)	15.5 (24.6)	In Arabidopsis: m^{6}A reader that regulates 3′UTR processing in the nucleus and mRNA stability in the cytoplasm (S27,S28,S29). In yeast (Pho92) and humans (YTHDF2): m^{6}A reader that decreases mRNA stability (S30,S31).
At5g58410	LTN1	19.2 (36.1)	22.5 (37.9)	20.8 (36.8)	In yeast, *Drosophila melanogaster*, and humans: ubiquitination of nascent non-stop proteins for their degradation during ribosome quality control (S32,S33,S34).
At4g38740	ROC1	64.0 (74.4)	67.4 (80.2)	64.2 (74.5)	In Arabidopsis, yeast (Cpr1), and humans (PPIA): it is a cyclophilin that belongs to the peptidyl-prolyl cis-trans isomerase family and participates in protein folding (S35,S36,S37).
At3g08850	RAPTOR1	27.3 (42.1)	40.4 (54.7)	31.8 (45.3)	In Arabidopsis, yeast (Kog1), and humans (RAPTOR): it is part of the TORC1 complex, composed of TOR, RAPTOR, and LST8-1 proteins. It controls cellular growth in response to different signals through regulation of translation, as it promotes translation reinitiation and ribosome biogenesis (S38,S39). In Arabidopsis: ABCE2 has been shown to interact with LST8-1 (S40).
At2g20830	-	26.7 (47.2)	30.2 (43.4)	27.8 (50.9)	In Arabidopsis: not studied. A BLASTp search suggested homology to *Saccharomyces cerevisiae* Lto1 and human ORAOV1. In yeast and humans: FeS cluster assembly on Rli1 and ABCE1, respectively (S41,S42).

Supplementary Table S5 (continued). Conservation level and described functions of putative ABCE2 interactors

AGI code	Protein	Conservation between orthologs (%)[1]			Described molecular function(s) and available evidence of its interaction with ABCE proteins[2]
		Arabidopsis and *S. cerevisiae*	Arabidopsis and *H. sapiens*	*S. cerevisiae* and *H. sapiens*	
At5g17020	XPO1A	40.7 (61.7)	48.5 (67.6)	46.2 (65.8)	In Arabidopsis, yeast (Crm1), and humans (XPO1): nuclear export receptor (S43,S44). In yeast, humans, and *Xenopus laevis*: ABCE might be an XPO1 cargo (S45). In yeast: *xpo1-1* mutants accumulate Rli1 in nucleus (S46,S47).
At2g42910	PRS4	20.7 (38.2)	19.2 (40.0)	60.3 (76.6)	In yeast (Prs4) and humans (PRPS1): synthesis of phosphoribosylpyrophosphate (PRPP), which is required for nucleotide biosynthesis (S48).
At1g65860	FMO GS-OX1	NC	NC	NC	In Arabidopsis: synthesis of aliphatic glucosinolates (S49).
At3g53610	RAB8	51.1 (70.0)	58.7 (73.1)	48.9 (67.0)	In Arabidopsis: it might be involved in post-Golgi transport to the plasma membrane (S50,S51). In yeast (Sec4): involved in membrane trafficking during cytokinesis and autophagy (S52,S53).
At3g43600	AAO2	NC	29.6 (48.1)	NC	In Arabidopsis: it might be involved in ABA biosynthesis (S54,S55). In humans (AOX1): it is an oxidase with broad substrate specificity (S56).
At2g44060	LEA26	NC	NC	NC	In Arabidopsis: unknown.

[1]Identity and similarity (between parentheses) percentages were obtained by global pairwise sequence alignments between pairs of protein sequences using the Needle EMBOSS tool. Protein sequences were obtained from TAIR for Arabidopsis, *Saccharomyces* Genome Database (SGD; https://www.yeastgenome.org/) for *Saccharomyces cerevisiae,* and UniProt for *Homo sapiens* proteins. NC: not conserved. [2]The abbreviated names for *Saccharomyces cerevisiae* and *Homo sapiens* orthologs are indicated in parentheses. The full names of Arabidopsis proteins are provided in Supplementary Table S3.

Supplementary Table S6. Morphometry of the leaf venation pattern of the *api7-1* mutant

Organ	Genotype	Area (mm^2)	Circularity	Vein density	Vein branching points	Free-ending veins
Cotyledons	L*er*	2.9 ± 0.5	0.84 ± 0.03	2.7 ± 0.2	6.5 ± 0.5	2.1 ± 1.7
	api7-1	***1.9 ± 0.5***	0.85 ± 0.01	***3.1 ± 0.3***	*8.2 ± 2.4*	***4.4 ± 2.2***
First-node leaves	L*er*	33.9 ± 8.3	0.86 ± 0.02	3.1 ± 0.2	178.6 ± 35.6	77.8 ± 17.1
	api7-1	***11.2 ± 4.8***	***0.76 ± 0.05***	3.0 ± 0.3	***72.4 ± 24.6***	***35.9 ± 10.8***
Third-node leaves	L*er*	51.3 ± 11.2	0.85 ± 0.01	3.8 ± 0.3	361.7 ± 56.9	126.9 ± 24.4
	api7-1	***18.8 ± 4.0***	*0.84 ± 0.02*	*3.5 ± 0.4*	***145.2 ± 25.1***	***52.7 ± 10.6***
Cauline leaves	L*er*	200.6 ± 32.8	0.56 ± 0.23	3.9 ± 0.3	1307.6 ± 213.8	439.9 ± 66.1
	api7-1	***136.6 ± 50.5***	*0.69 ± 0.04*	4.2 ± 0.5	**1023.0 ± 201.1**	*373.3 ± 73.4*
Sepals	L*er*	1.4 ± 0.2	0.66 ± 0.05	6.6 ± 0.7	13.4 ± 4.2	9.5 ± 2.3
	api7-1	*1.3 ± 0.1*	0.65 ± 0.04	7.0 ± 1.0	14.7 ± 4.1	10.8 ± 4.8
Petals	L*er*	2.4 ± 0.4	0.70 ± 0.03	4.2 ± 0.6	6.4 ± 0.8	6.5 ± 1.8
	api7-1	2.2 ± 0.2	0.70 ± 0.03	4.0 ± 0.4	5.9 ± 1.1	7.7 ± 2.1

All values are means ± standard deviation from 12 measurements. Organs were collected 6 (cotyledons), 21 (first- and third-node leaves), and 35 (cauline leaves, petals, and sepals) das. Values in italics, bold, or bold and italics are significantly different from those of L*er* in a Student's *t* test, with *P* < 0.05, *P* < 0.01, or *P* < 0.001, respectively.

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