



Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability

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The SWItch/Sucrose Non-Fermenting (SWI/SNF) complexes are evolutionarily conserved, ATP-dependent chromatin remodelers crucial for multiple nuclear functions in eukaryotes. Recently, plant BCL-DOMAIN HOMOLOG (BDH) proteins were identified as shared subunits of all plant SWI/SNF complexes, significantly impacting chromatin accessibility and various developmental processes in Arabidopsis. In this study, we performed a comprehensive characterization of *bdh* mutants, revealing the role of BDH in hypocotyl cell elongation. Through detailed analysis of BDH domains, we identified a plant-specific N-terminal domain that facilitates the interaction between BDH and the rest of the complex. Additionally, we uncovered the critical role of the BDH β -hairpin domain, which is phylogenetically related to mammalian BCL7 SWI/SNF subunits. While phylogenetic analyses did not identify BDH/BCL7 orthologs in fungi, structure prediction modeling demonstrated strong similarities between the SWI/SNF catalytic modules of plants, animals, and fungi and revealed the yeast Rtt102 protein as a structural homolog of BDH and BCL7. This finding is supported by the ability of Rtt102 to interact with the Arabidopsis catalytic module subunit ARP7 and partially rescue the *bdh* mutant phenotypes. Further experiments revealed that BDH promotes the stability of the ARP4-ARP7 heterodimer, leading to the partial destabilization of ARP4 in the SWI/SNF complexes. In summary, our study unveils the molecular function of BDH proteins in plant SWI/SNF complexes and suggests that β -hairpin-containing proteins are evolutionarily conserved subunits crucial for ARP heterodimer stability and SWI/SNF activity across eukaryotes.

SWI/SNF | chromatin | remodeling | Arabidopsis

Chromatin, the association of DNA with histones, enables strong genome compaction in eukaryotic nuclei and participates in numerous nuclear processes such as transcription, replication, repair, and recombination. The basic unit of chromatin is the nucleosome, where 147 base pairs of DNA are wrapped around a histone octamer composed of H2A-H2B dimers and an H3-H4 tetramer (1, 2). These nucleosomes facilitate chromatin compaction and reduce accessibility for DNA-binding proteins, thereby influencing their ability to reach their targets (3). Additionally, nucleosomes can incorporate various histone posttranslational modifications and histone variants, fine-tuning their properties and acting as key nuclear signaling elements recognized by diverse histone readers, which transduce this information into specific outputs (4). Therefore, controlling nucleosome positions and modifications is central to most nuclear processes, making their remodeling essential for precise genome function.

Among the various regulators of chromatin remodeling, ATP-dependent chromatin remodelers are critical for controlling nucleosome positioning and occupancy (5). These complexes utilize the energy from ATP hydrolysis to alter the interaction between DNA and histones, participating in nucleosome maturation, sliding, ejection, and composition (5). Consequently, their activity directly influences DNA accessibility, impacting diverse nuclear processes that require a DNA template. The SWItch/Sucrose Non-Fermenting (SWI/SNF) remodelers are an evolutionarily conserved family that play key roles in multiple processes, including the regulation of responses to internal and external signals and cell differentiation (6, 7). In plants, SWI/SNF complexes participate in multiple nuclear processes, such as transcription, DNA repair, gene silencing, and response to diverse signaling pathways (6, 8). Consequently, mutants in SWI/SNF components exhibit strong developmental defects, impaired growth, and altered stress responses (8–10).

The SWI/SNF family forms modular complexes with multiple subunits, assembled into functionally distinct configurations known as subclasses, defined by signature subunits

Significance

The SWI/SNF complex is an evolutionarily conserved chromatin remodeler formed by multiple subunits and involved in the control of chromatin accessibility. In this study, we characterized the function of a SWI/SNF subunit in plants called BDH, which shares sequence homology to the BCL7 SWI/SNF subunit in animals. Plants lacking BDH show multiple developmental defects, including longer hypocotyls. Although BDH does not share sequence similarity with the fungal SWI/SNF protein Rtt102, we found that BDH and Rtt102 share similar structural and functional roles, suggesting that this subunit is conserved across eukaryotes. Furthermore, through comprehensive characterization of BDH and its domains, we uncovered a role in promoting the stability of the complex's catalytic module.

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(6, 11, 12). In yeast, two main subclasses have been identified: RSC and SWI/SNF, while in mammals, there are three distinct assemblies known as BAF, PBAF, and ncBAF (5, 6, 13). Recent studies in plants have revealed the composition of three distinct subclasses: BRM-associated SWI/SNF complex (BAS), SYD-associated SWI/SNF complex (SAS), and MINU-associated SWI/SNF complex (MAS) (9, 10, 12, 14). All these subclasses evolved from a common ancestral SWI/SNF form (12) to meet the specific requirements of different organisms, highlighting their adaptive functions across a diverse range of species. Despite this subclass divergence, a common feature of all SWI/SNF complexes is their catalytic module, which incorporates a Snf2-like SWI/SNF ATPase and a heterodimer of ACTIN (ACT) and/or ACTIN-RELATED PROTEINs (ARP), which interact with the helicase-SANT-associated (HSA) domain of the ATPase (Snf2^{HSA}) (11, 15–17). Although the ATPase alone can remodel chromatin in vitro, interaction with an ACT-ARP heterodimer significantly enhances its remodeling activity (18). Notably, this ACT-ARP-Snf2^{HSA} assembly is conserved in other ATP-dependent chromatin remodelers like INO80 and SWR1 and the histone acetyltransferase NuA4 (19, 20). Additionally, in animals, the catalytic module of SWI/SNF complexes includes an additional subunit known as B cell lymphoma/leukemia protein 7 (BCL7), which is strongly implicated in cancer and is thought to assist the activity of the complex, possibly through its interaction with the ATPase and the nucleosome (11, 21–23). On the other hand, fungi incorporate the Regulator of Ty transposition protein 102 (Rtt102) protein into their SWI/SNF-RSC catalytic modules, which interacts with the Arp7/Arp9 heterodimer and promotes its compaction, enhancing the remodeling activity of the complex (17, 18, 24). However, no phylogenetic relationship has been found between animal BCL7 and fungal Rtt102, which are considered kingdom specific. Moreover, to our knowledge, no functional connection between them has been identified. More recently, immunoprecipitation followed by mass spectrometry (IP-MS) experiments identified the BCL-DOMAIN HOMOLOG (BDH) proteins (a.k.a. BCL7) in plants (9, 10, 12, 25). This was shown to interact with the catalytic module of the BAS complex, together with BRM, ARP4, and ARP7, likely through interaction with the BRM ATPase (26, 27). Furthermore, *bdh* mutants were shown to alter plant development and chromatin accessibility over hundreds of genes (9, 26, 27). BDH proteins are conserved across plants and contain an evolutionary conserved domain found in protozoan and metazoan proteins, including mammalian BCL7 (12), suggesting functional conservation. Importantly, no orthologs of BCL7 or BDH have been found in fungi (12), suggesting their loss upon divergence of animals and fungi. Apart from this conserved region, no other domains have been reported in BDH.

In this study, we performed a comprehensive characterization of BDH proteins in Arabidopsis, uncovering an evolutionary connection with animal and fungal SWI/SNF subunits. We thoroughly analyzed *bdh* mutants, revealing the role of BDH proteins as negative regulators of cell elongation in hypocotyls. Furthermore, we conducted a detailed functional analysis of conserved BDH domains, identifying two important domains for BDH interaction with the complex. Structural modeling revealed a strong connection between plant BDH, animal BCL7, and fungal Rtt102, despite the lack of sequence conservation of the latest. The functional conservation of Rtt102 and BDH was further supported by the Rtt102 ability to interact with Arabidopsis ARP7 and partially complement *bdh* mutants. In vivo studies showed that BDH stabilizes the ARP4-ARP7 heterodimer, resulting in a partial loss of ARP4 protein in the BDH-depleted SWI/

SNF complexes, providing a mechanistic explanation for the phenotypic and molecular defects observed in the absence of BDH.

Results

BDH Affects Plant Development and Hypocotyl Elongation.

The Arabidopsis genome contains two *BDH* paralogs, *BDH1* and *BDH2*, and T-DNA insertion mutants have been recently identified for these genes (9, 26). Consistent with recent reports, *bdh1* and *bdh2* single mutants had a wild-type (WT) appearance whereas double *bdh1/bdh2* mutants (from now on *bdh* mutants) exhibited significant developmental defects, indicating functional redundancy between BDH proteins in Arabidopsis (*SI Appendix, Fig. S1A*). The *bdh* mutants presented smaller rosettes with curled leaves and an early flowering phenotype in both long- and short-day conditions (*SI Appendix, Fig. S1 A–E* and *Dataset S1*). Additionally, the *bdh* mutants formed flowers with an increased number of petals compared to WT plants, although this occurred with low frequency (*SI Appendix, Fig. S1F*). Furthermore, these mutants had shorter siliques that produced fewer seeds, with the severity of this phenotype varying, as siliques of different sizes were found on the same stem (*SI Appendix, Fig. S1 G and H* and *Dataset S1*).

We also found that *bdh* mutants presented longer hypocotyls in different light conditions (continuous light, long-, and short-days) (*Fig. 1A* and *Dataset S2*), as well as under a gradient of light intensities and qualities (white light and blue, far-red, and red monochromatic lights) (*SI Appendix, Fig. S2 A–D* and *Dataset S3*). Furthermore, etiolated hypocotyls, which germinate and grow in darkness, also showed more elongated hypocotyls, suggesting that BDH affects hypocotyl elongation mechanisms independent of light (*Fig. 1A, SI Appendix, Fig. S2 A–D*, and *Datasets S2 and S3*). Measurements of hypocotyl cell number and length revealed that the longer hypocotyl of *bdh* mutants was due to an increase in cell length throughout the entire hypocotyl (*SI Appendix, Fig. S2 E and F* and *Dataset S3*). This suggests that BDH negatively regulates hypocotyl cell elongation rather than affecting cell division. To further gain information about the impact of BDH on etiolated hypocotyl growth, we performed a time-course experiment analyzing the growth of WT and *bdh* etiolated hypocotyls during the first 10 d after germination in the dark. Results showed that the *bdh* hypocotyls grew at a same speed than WT until the 4th day after which *bdh* continued growing at a faster rate for two additional days (*Fig. 1B* and *Dataset S2*). To shed light into the processes altered in the *bdh* mutant that led to increased hypocotyl elongation, we performed an RNA-seq experiment comparing 5-d-old WT and *bdh* etiolated seedlings. Differential expression analyses indicated hundreds of differentially expressed genes (DEGs) that were up- or down-regulated (1,100 and 856, respectively, |Fold change| > 1.5, p.adj < 0.05) (*Fig. 1C* and *Datasets S4 and S8*). An analysis of enriched gene ontologies revealed several categories overrepresented in *bdh* mutants like “Suberin biosynthetic process” and “Glucosinolate/glycosinolate/S-glycoside catabolic process” (*SI Appendix, Fig. S3A* and *Dataset S5*). Interestingly, the “Xyloglucan metabolic process” category was also highly enriched due to the upregulation of 14 *Xyloglucan endotransglucosylase/hydrolase (XTH)* genes in the *bdh* mutant (*Fig. 1D, SI Appendix, Fig. S3B*, and *Datasets S5 and S6*). XTHs are key enzymes controlling the arrangement of the cell wall and play an important role in promoting cell elongation (28) and, therefore, their general upregulation could explain the *bdh* elongated hypocotyls in the dark. Since this phenotype was also found in light-grown *bdh* seedlings, we searched for upregulation of *XTH* genes in a published RNA-seq comparing light-grown *bdh* and WT

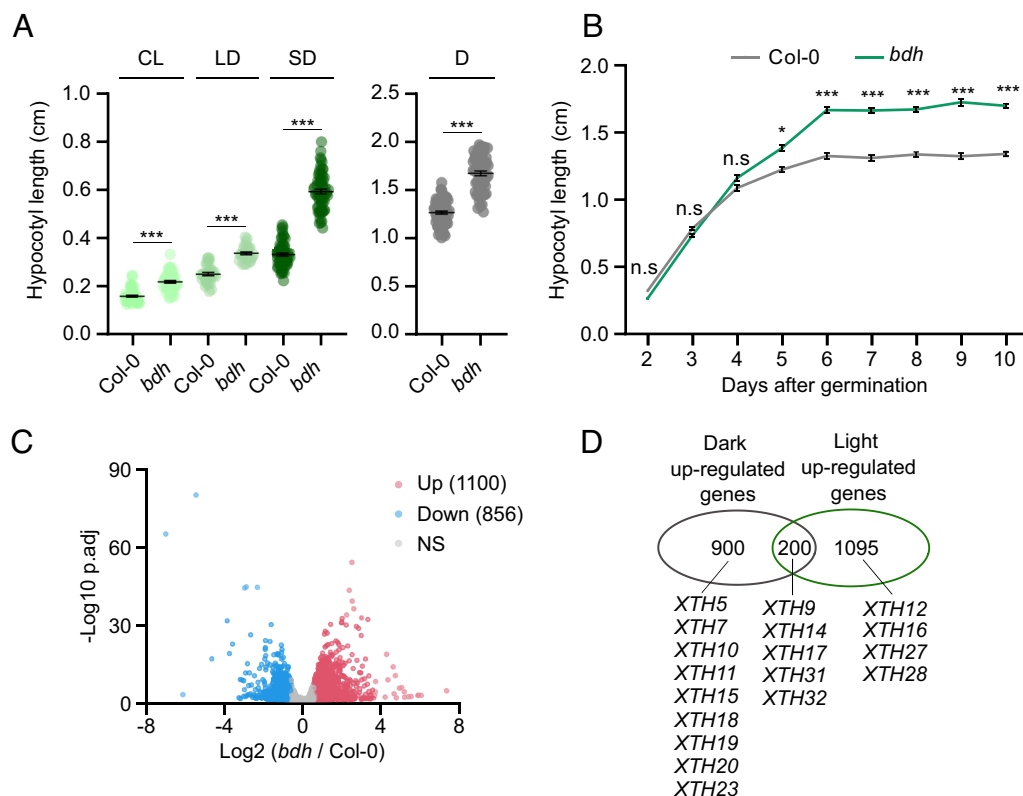


Fig. 1. BDH affects plant development and hypocotyl elongation. (A) Hypocotyl length measurements of 7-d-old Col-0 and *bdh* under Continuous Light (CL), 16 h light/8 h dark (LD), 8 h light/16 h dark (SD), and darkness (D) conditions (n = 28 to 63). Error bars represent Mean $\pm$ SEM. Asterisks denote statistically significant differences between mean values, as assessed by Student's *t* test ($***P < 0.001$). Two independent biological replicates were conducted with similar results (Dataset S2, Replicate 2). (B) Time course analysis of hypocotyl length in Col-0 and *bdh* mutant in darkness. Error bars represent Mean $\pm$ SEM, n = 38 to 64. Two independent biological replicates were conducted with similar results (Dataset S2, Replicate 2). Student's *t* test ($*P < 0.05$, $***P < 0.001$, n.s., not significant). (C) Volcano plot showing differential expression between 5-d-old *bdh* and WT seedlings grown in darkness determined by RNA-seq. Genes showing an adjusted *P*-value (p.adj) lesser than 0.05 and an absolute fold change over 1.5 were considered as DEGs. (D) Venn Diagram showing the overlap between up-regulated DEGs detected in etiolated (this work) and light-grown (9) *bdh* mutants, depicting the *XTH* genes present in each set and the overlap between them.

seedlings (9) (Datasets S7 and S8). Notably, nine *XTH* genes were also up-regulated in this condition being five commonly up-regulated in dark and light (Fig. 1D and Dataset S6). A visual inspection of a published BDH1 and BDH2 ChIP-seq in light-grown seedlings (10) indicated that these shared *XTH*s are direct SWI/SNF targets (SI Appendix, Fig. S3C). Interestingly, the overall overlap of DEGs in the light and dark RNA-seq experiments was not very high indicating that BDHs are generally mobilizing distinct transcriptomes in the different growth conditions (SI Appendix, Fig. S3D and Datasets S8 and S9). In summary, these results suggest that BDHs act as negative regulators of hypocotyl elongation possibly through the repression of *XTH* genes. Future studies will further investigate the functional connection between SWI/SNF complexes and *XTH* expression.

Functional Dissection of Conserved BDH Domains. BDH proteins were initially identified through a series of IP-MS experiments in plants using distinct SWI/SNF subunits as baits (9, 12, 14, 25, 26). However, a comprehensive analysis of BDH domain composition and their function remains unreported. Recently, we reported the evolutionary conservation of BDH, identifying homology between a BDH region, here named BCL domain, and a region in the mammalian SWI/SNF subunit BCL7 (12) (Fig. 2A and SI Appendix, Fig. S4A). Structural prediction analyses using AlphaFold2 (29) for Arabidopsis BDH and Human BCL7 indicated the formation of two antiparallel β -sheets (β -hairpin) in these conserved regions (Fig. 2B and SI Appendix, Fig. S4B). Furthermore, detailed phylogenetic analysis across multiple

plant species revealed a conserved domain in the most amino-terminal (N-term) region of BDH proteins, which we named N domain (SI Appendix, Fig. S5 and Dataset S10). However, structural modeling did not reveal any specific fold in this region (Fig. 2B and SI Appendix, Fig. S4B). Interestingly, despite the lack of sequence conservation, structural modeling predicted a conserved downstream alpha-helix fold across multiple plant species, named Alpha domain (Fig. 2B and SI Appendix, Fig. S4B and C). Downstream of this domain, sequence conservation is very low even among paralogs (SI Appendix, Fig. S5). An analysis using DISOPRED3 (30) predicted that the identified domains are ordered regions within overall disordered domains in BDH1 (SI Appendix, Fig. S4D).

To perform in vivo functional studies of BDH1, we generated a 3xFLAG-tagged BDH1 construct driven by its own promoter and expressed it in *bdh1* single mutants. The resulting transgenic lines were used to profile the BDH1 interactome and genomic targets in inflorescences via IP-MS and ChIP-seq, respectively. Using BDH1-3xFLAG as bait allowed the purification of all subunits from the three described SWI/SNF subclasses, confirming that BDH1 is a common SWI/SNF subunit and suggesting that the generated 3xFLAG-tagged BDH1 transgene was correctly incorporated in the complexes (SI Appendix, Fig. S6A and Dataset S11). Notably, the subunits identified were consistent with those found in similar experiments performed in seedlings (9, 27), indicating subunit conservation in the three SWI/SNF subclasses across tissues. Furthermore, as

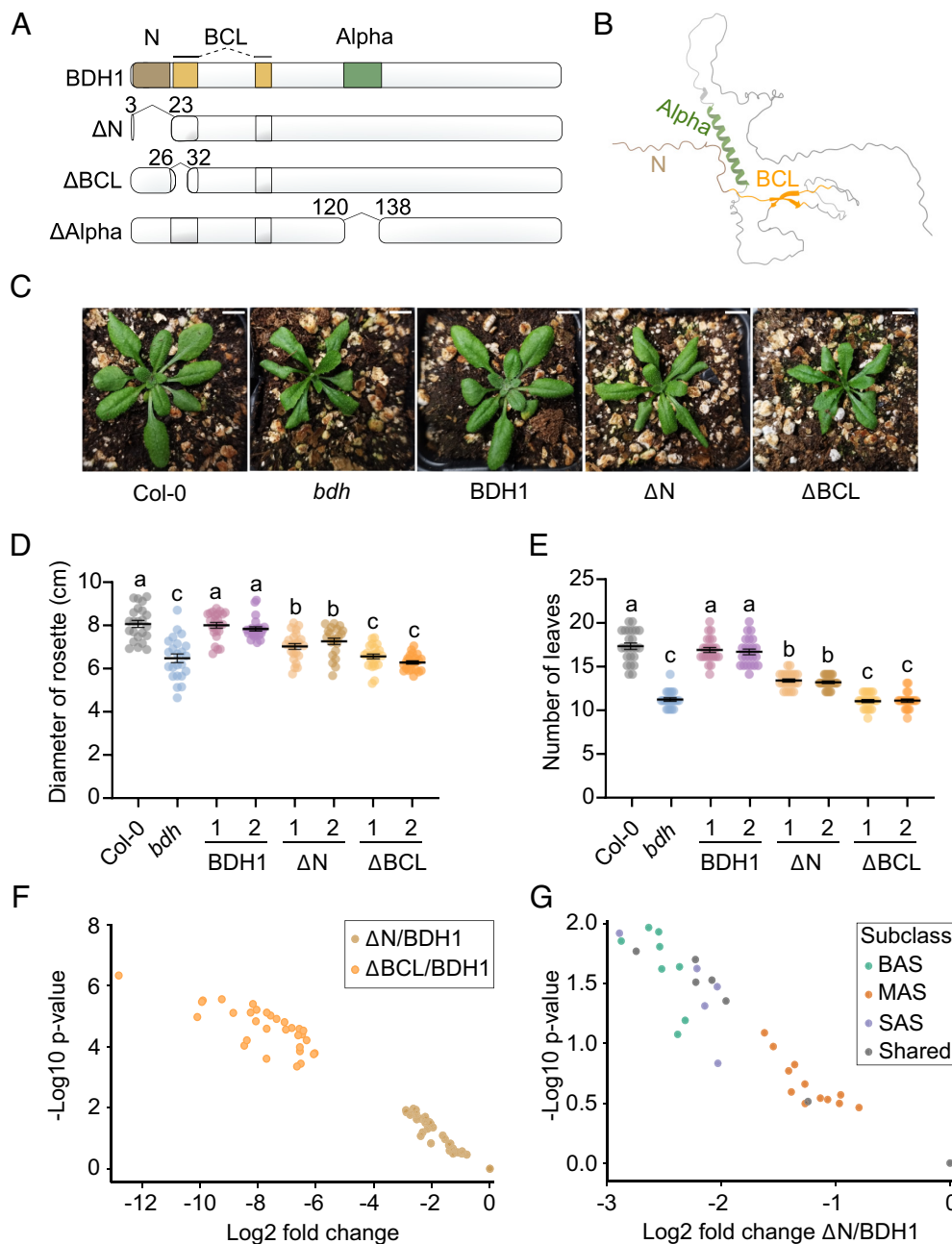


Fig. 2. Functional dissection of conserved BDH domains. (A) Schematic representation of the full-length BDH1 protein, depicting the three studied domains: N-domain (brown), BCL domain (orange; the conserved BCL domain (continuous black line) is separated in BDH1 by a nonconserved sequenced (dotted line)), and Alpha domain (green), and the truncated BDH1 versions depicting the first and last amino acids removed in each deletion. (B) Prediction of the BDH1 protein structure by AlphaFold2, highlighting the different protein domains following the color scheme described in panel A. (C) Pictures of representative 3-wk-old plants of the labeled backgrounds: WT, *bdh* double mutant, full-length BDH1-3xFLAG (BDH1), N-term-deleted BDH1-3xFLAG (Δ N), and BCL-deleted BDH1-3xFLAG (Δ BCL). (D) Length of the widest measured rosette diameter of the labeled backgrounds in 28-d-old plants grown in long-day conditions. (E) Number of total rosette and caulinar leaves after bolting in the labeled backgrounds. For (D) and (E), the different letters indicate significant differences ($P < 0.05$), as determined by ANOVA with Tukey's post hoc test. Error bars represent Mean $\pm$ SEM $n = 22$ to 24 . Two independent biological replicates were conducted with similar results (Dataset S16, Replicate 2). (F) Volcano plot depicting the log₂ fold change (Δ N/BDH1 and Δ BCL/BDH1) of the intensities of all the SWI/SNF subunits enriched after IP-MS experiments using the Δ N, Δ BCL, and BDH1 transgenic lines. (G) Volcano plot depicting the log₂ fold change (Δ N/BDH1) of the intensities of all the SWI/SNF subunits enriched after IP-MS experiments using the Δ N and BDH1 transgenic lines. BAS-, MAS-, and SAS-specific SWI/SNF subunits, as well as those shared in two or three subclasses are depicted in green, orange, purple, and gray, respectively. (F and G) The x axis depicts log₂ fold change of average intensities of IP experiments. The Y axis depicts significance -log₁₀ P-value.

expected for a pan-SWI/SNF subunit, ChIP-seq results revealed thousands (14265) of BDH1 targets distributed across the genome in inflorescences (Datasets S12 and S14). A comparison with a previously published BDH1 ChIP-seq in light-grown seedlings (10) (Datasets S13 and S14) showed a strong overlap of BDH1 targets, indicating that SWI/SNF remodelers share a

large proportion of targets across tissues (SI Appendix, Fig. S6B and Dataset S14). In line with this result, BDH1 colocalized with the three SWI/SNF ATPases in plants -MINU, BRM, and SYD- over the same genic region and across thousands of genes, despite these ATPase ChIP-seqs were done in light-grown seedlings (10) (SI Appendix, Fig. S6C).

To examine the roles of the BCL, N, and Alpha domains in BDH protein function, we generated BDH1 deletion mutants (Fig. 2*A*) and tested their ability to rescue the *bdh* mutant phenotypes. For the BCL domain, we removed its most conserved region, expected to disrupt the N-proximal β -sheet and prevent β -hairpin formation (Fig. 2*A* and *B* and *SI Appendix*, Figs. S4*A* and *B* and S5). For the N domain, we deleted the entire conserved region, while for the Alpha domain, we removed the segment predicted to form an alpha helix in BDH1 (Fig. 2*A* and *SI Appendix*, Figs. S4*B* and *C* and S5). Next, we expressed 3xFLAG-tagged full-length BDH1 driven by its own promoter, as well as 3xFLAG-tagged BCL-, N-, and Alpha-domain-deleted BDH1 transgenes (from now on Δ BCL, Δ N, and Δ Alpha, respectively) in *bdh* mutants. Importantly, the BDH1-3xFLAG genomic fragment was able to rescue all *bdh* mutant phenotypes (Fig. 2, *SI Appendix*, Fig. S7, and *Datasets S15* and *S16*). The deletion of BDH1 domains did not negatively affect protein accumulation, as the characterized lines expressed similar or slightly increased BDH1 levels compared to full-length BDH1 (*SI Appendix*, Fig. S7*A* and *Dataset S15*). Notably, Δ Alpha fully rescued defects in silique length in T1 plants and etiolated hypocotyl length in T2 plants to the same extent as full-length BDH1-expressing plants (*SI Appendix*, Fig. S7*B* and *C* and *Dataset S15*), suggesting no critical function of this domain under the tested conditions. Interestingly, Δ N expression partially rescued these phenotypes, while Δ BCL failed to complement the mutant (*SI Appendix*, Fig. S7*B* and *C* and *Dataset S15*). Based on these findings, we focused on the study of Δ N and Δ BCL as relevant regulators of BDH1 activity. A more comprehensive characterization of these lines in T3 generation, including their ability to rescue other phenotypes like rosette size and flowering time, showed that Δ N and Δ BCL were partially and fully required for BDH1 function (Fig. 2*C–E* and *Dataset S16*). Further analysis of hypocotyl length in various light conditions (CL, LD, SD, and darkness) and expression of *XTH* genes in etiolated seedlings revealed that Δ BCL failed to rescue the mutant phenotype or *XTH* expression under any condition, whereas Δ N lines showed varying degrees of complementation being more effective in complementing hypocotyl growth in CL and darkness compared to LD and SD (*SI Appendix*, Fig. S8*A–H* and *Dataset S17*).

We hypothesized that the N and BCL domains could be mediating BDH1's interaction with the SWI/SNF complex. Thus, we performed IP-MS experiments using two independent full-length BDH1, Δ N, and Δ BCL transgenic lines. Full-length BDH1 in the *bdh* mutant recovered subunits from all three SWI/SNF subclasses (*SI Appendix*, Fig. S7*D* and *Dataset S18*). Notably, Δ BCL failed to copurify SWI/SNF subunits, highlighting its requirement for complex interaction and explaining the lack of complementation in Δ BCL lines (Fig. 2*F* and *Dataset S18*). Conversely, Δ N recovered all three complexes, though with reduced efficiency, indicating an important, albeit not critical, role for the N domain in BDH complex interaction (Fig. 2*F* and *G* and *Dataset S18*). This observation aligns with the partial ability of Δ N plants to complement the *bdh* mutant. Interestingly, the reduced interaction was slightly more pronounced for the BAS and SAS subclasses compared to the MAS subclass (Fig. 2*G* and *Dataset S18*).

The β -Hairpin Fold Is an Evolutionary Conserved ACTIN/ARP Interacting Module in SWI/SNF Complexes. Plant BDH and mammal BCL7 proteins exhibit sequence homology within the BCL domain, sharing a similar β -hairpin fold as indicated by their predicted structures (*SI Appendix*, Fig. S4*A* and *B*). However, no additional structural comparisons have been done between these proteins in the context of the complex. Interestingly, yeast SWI/

SNF catalytic modules incorporate a subunit known as Rtt102 (17), which, despite lacking sequence homology with BDH or BCL7 (12), forms a β -hairpin fold that is reminiscent of the predicted folds of BDH1 and BCL7A BCL domains (*SI Appendix*, Fig. S9*A* and *B*). The crystal structure of Rtt102 in complex with the ATPase (Snf2) HSA domain and the Arp7/Arp9 heterodimer (PDB 4I6M) revealed the molecular mechanism of the interaction between these subunits (17). In the resolved complex, the HSA domain is formed by a long alpha helix which extensively interacts with the ARP heterodimer (Arp7/Arp9) through a central cleft and Rtt102 β -hairpin is positioned in one side of the complex and contacts both ARPs (Fig. 3*A*, *SI Appendix*, Fig. S9*C*, and *Dataset S19*). Strikingly, structural modeling with AlphaFold-Multimer (31) of the Arabidopsis and Human SWI/SNF catalytic modules, including BDH1 and BCL7A, respectively, together with their corresponding ACTIN/ARP heterodimers, and ATPase HSA domains (Arabidopsis MINU1 and Human BRG1), resulted in a complex structure resembling the one reported in yeast (Fig. 3*A–C*, *SI Appendix*, Fig. S9*C–E*, and *Dataset S19*). The Arabidopsis and Human predicted models showed good prediction values both at the per-residue (pLDDT score) and at the complex level (PAE, pTM, and ipTM scores) (*SI Appendix*, Fig. S10*A* and *B*). Furthermore, we carried out structural alignments of the experimental yeast complex structure with the predicted AlphaFold-multimer models for the Arabidopsis complex structures, which confirmed very little deviation of the position of the alpha carbons of the models compared to the experimental structure (*SI Appendix*, Fig. S10*C*). Like in the yeast complex, the predicted structures show that BDH1 and BCL7A β -hairpins are positioned in a cleft between the ACTIN/ARP heterodimer (Fig. 3*A–C*). The interactions established between these β -hairpins with their respective ACTIN/ARPs are supported by a hydrogen bond network and hydrophobic interactions similar to those described in the yeast complex (*SI Appendix*, Fig. S9*F–H*). Furthermore, supporting their conservation, structural alignments of the predicted BDH1 and BCL7A β -hairpins showed a very strong overlap and relative position of the side chains of conserved key amino acids (*SI Appendix*, Fig. S9*I*). Similarly, BDH1 and Rtt102 alignments predicted a conservation of the overall fold and side chain positions (*SI Appendix*, Fig. S9*J*). Notably, there is an equivalent interaction network between β -hairpins and ACTIN/ARPs where conserved tryptophans in the β -hairpins of Rtt102, BDH1, and BCL7A, similarly interact with a proline present in one of the two ACTIN/ARP subunits (Arp9, ARP7, and ACTB, respectively) (Fig. 3*D–F*). To experimentally validate these predictions, we performed Co-IP experiments to test the ability of BDH1 to interact with ARP4 and ARP7. As expected, using BDH1 as bait allowed to recover both ARP7 and ARP4 when expressed together (Fig. 3*G* and *Dataset S20*). Interestingly, BDH1 was able to interact specifically with ARP7 while no interaction was detected with ARP4 when expressed separately (*SI Appendix*, Fig. S11 and *Dataset S21*). The conservation of the tryptophan–proline interaction across organisms (Fig. 3*D–F*) suggests its crucial role in mediating the interaction between the β -hairpin-containing proteins and ACT/ARP. To investigate this, we mutated the two conserved tryptophans in BDH1 (W28 and W77) that are predicted to interact with ARP7 (Fig. 3*E*). We substituted tryptophans with glycines, as glycine's small, nonaromatic structure removes the bulky side chain and hydrophobic interactions provided by tryptophan, allowing us to directly assess the impact of losing these stabilizing features on the protein–protein interaction. Notably, CoIP experiments revealed that these mutations significantly weakened the BDH1–ARP7 interaction (Fig. 3*H* and *Dataset S20*), supporting the functional

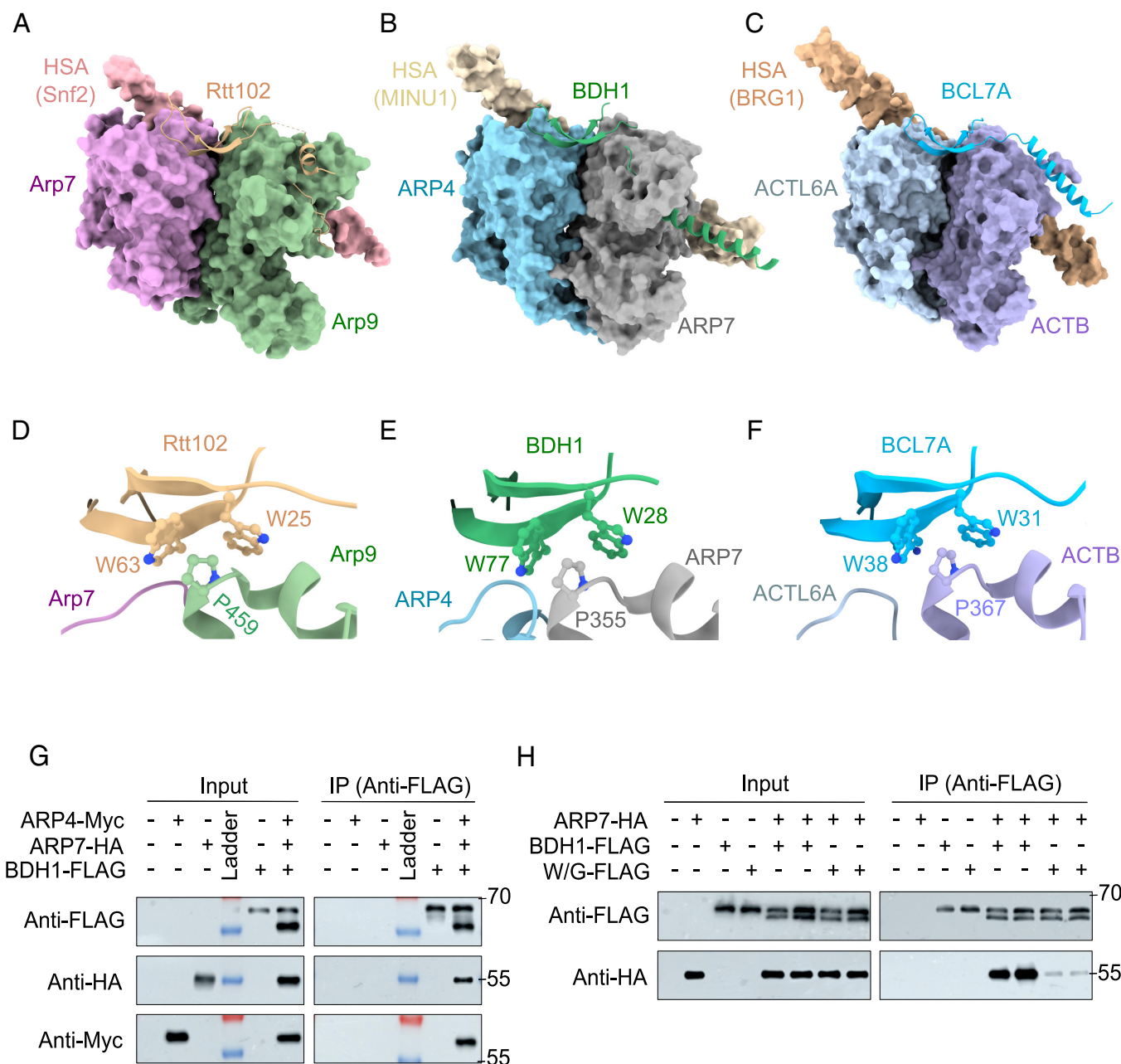


Fig. 3. The β -hairpin fold is an evolutionary conserved ACTIN/ARP interacting module in SWI/SNF complexes. (A) Representation of the crystal structure (4IGM) of the *Saccharomyces cerevisiae* SWI/SNF catalytic module depicting Arp7-Arp9-Snf2^{HSA}-Rtt102. (B and C) Representation of the predicted model of Arabidopsis ARP4-ARP7-MINU1^{HSA}-BDH1 (B) and Human ACTL6-ACTB-BRG1^{HSA}-BCL7A (C) catalytic modules. Detail of the tryptophan-proline interaction network observed between (D) Rtt102 and Arp9, (E) BDH1-ARP7, and (F) BCL7A-ACTB. (G) In vivo coimmunoprecipitation (CoIP) of transiently expressed BDH1-FLAG, ARP7-HA, and ARP4-Myc. BDH1-FLAG was IP using FLAG antibody and results were observed using anti-FLAG, anti-HA and anti-Myc antibodies. One representative replicate from two independent experiments is depicted. (H) In vivo CoIP of transiently expressed BDH1-FLAG, mutated BDH1-W28G/W77G-FLAG (W/G-FLAG), and ARP7-HA. BDH1-FLAG or W/G-FLAG were IP using FLAG antibody and results were observed using anti-FLAG and anti-HA.

importance of the tryptophan-proline interaction network. Consistent with the modeled N-term structure of BDH1 when calculated alone (Fig. 2B), modeling of the BDH1 N-term region (amino acids 1 to 23) in the complex only predicted a hydrogen bond interaction between the amino acid T22, proximal to the β -hairpin, and K347 from ARP7, while the rest of the domain could not be modeled (SI Appendix, Fig. S12 A and B). Thus, experimental validation is needed to shed light on the structure and interaction network of the N-term region with other SWI/SNF subunits. This information will be important to understand the impact of the N domain in the ability of BDH1 to interact with the complex. In summary, these results suggest the evolutionary

conservation across eukaryotes of the SWI/SNF catalytic module structure and composition.

Yeast Rtt102 Is A Distant Functional Homolog of Plant BDH proteins. To further explore the evolutionary conservation between BDH1 and Rtt102 proteins, we investigated whether Rtt102 could functionally replace BDH in plant cells. Structural modeling predicted the Rtt102 β -hairpin would interact with plant ARPs similarly to BDH1-ARP interactions (SI Appendix, Fig. S13 A and B). We expressed a 3xFLAG-tagged plant codon-optimized Rtt102 protein (Dataset S22) under the control of the constitutive *UBIQUITIN 10* promoter (*pUBQ10*) in *bdh* mutants and assessed

its ability to rescue *bdh* mutant phenotypes. As a control, BDH1 was also expressed under the *pUBQ10* in the *bdh* mutant. Both proteins were properly expressed in T1 plants (*SI Appendix, Fig. S13C* and *Dataset S23*). Interestingly, T1 plants expressing Rtt102 slightly rescued some *bdh* phenotypes like the silique defects (*Fig. 4A* and *Dataset S24*). Remarkably, four independent T2 lines expressing Rtt102 showed an almost complete rescue of the etiolated hypocotyl length defects, similar to full-length BDH1 complementation (*Fig. 4B* and *Dataset S24*). However, the rescue of other mutant phenotypes, such as rosette size, leaf shape, and flowering time, was significantly reduced (*Fig. 4C* and *D, SI Appendix, Fig. S13D*, and *Datasets S23* and *S24*). We hypothesized that the weaker complementation by Rtt102 could

be due to its reduced affinity for plant ARP proteins. Supporting their functional conservation, Rtt102 specifically interacted with ARP7, although the interaction was weaker than that of BDH1–ARP7, providing a molecular explanation for the partial rescue of *bdh* mutant phenotypes (*Fig. 4E* and *Dataset S24*). Overall, these data support the functional conservation between Rtt102 and BDH1 despite their lack of sequence conservation.

BDH Promotes ARP Heterodimer Stability. The predicted position of BDH1 between the two ARP proteins in the catalytic module suggested that it could promote ARP heterodimer interaction. To test this hypothesis, we conducted Co-IP experiments between ARP4 and ARP7 in the presence and absence of BDH1. Notably, the

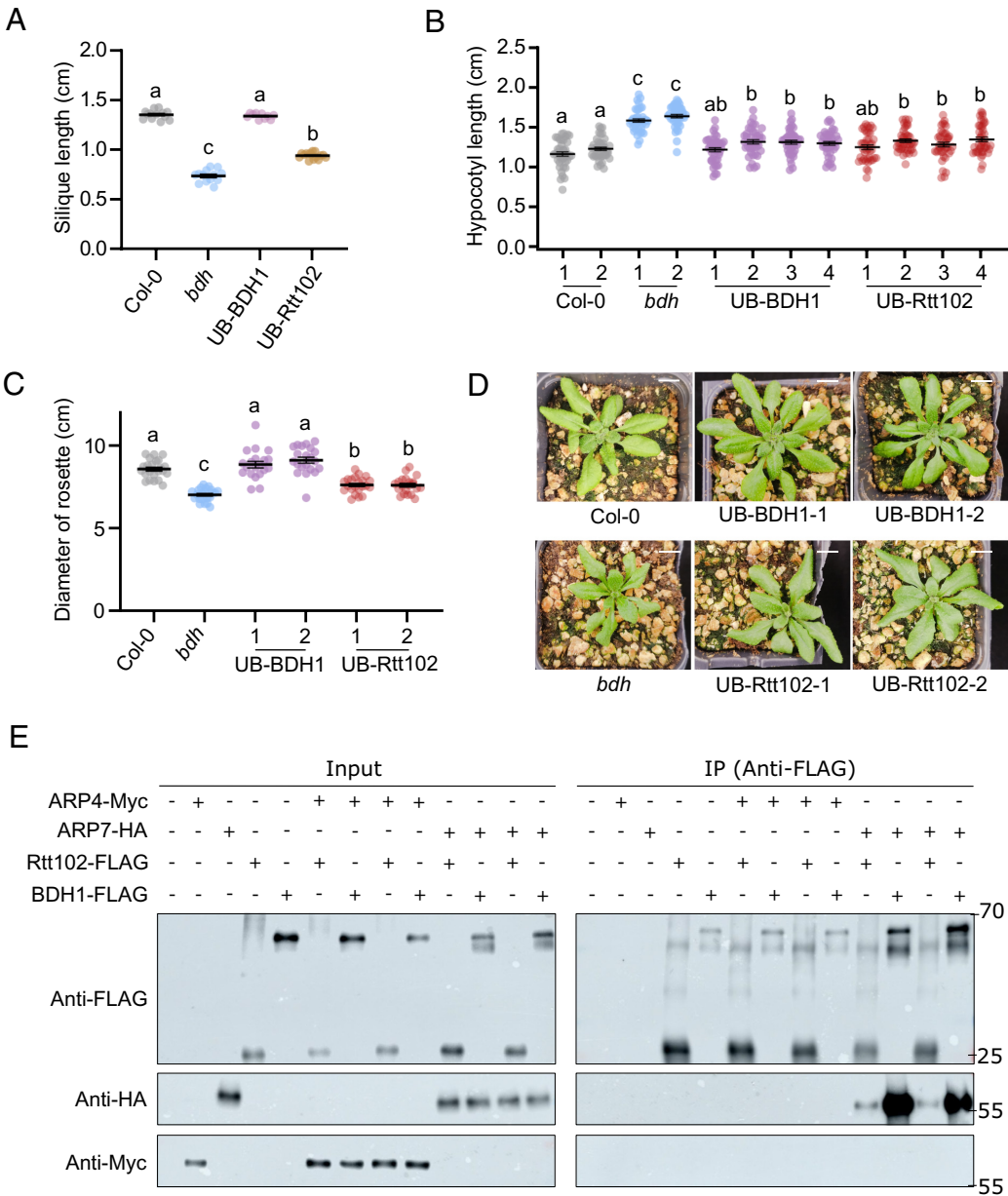


Fig. 4. Yeast Rtt102 is a distant functional homolog of plant BDH proteins. (A) Measurement of silique length from the main inflorescence of Col-0, *bdh*, and T1 plants expressing *pUBQ10::BDH1-6xHA* (UB-BDH1) and *pUBQ10::Rtt102-3xFLAG* (UB-Rtt102), *n* = 9 to 15. (B) Hypocotyl length measurements of 7-d-old etiolated seedlings of Col-0, *bdh*, and four independent T2 populations from UB-BDH1 and UB-Rtt102 lines, *n* = 40 to 42. (C) Diameter of the rosettes of 28-d-old plants from Col-0, *bdh*, and two independent T2 populations from UB-BDH1 and UB-Rtt102 lines grown under long-day conditions, *n* = 20 to 23. For (A–C), the different letters indicate significant differences (*P* < 0.05), as determined by ANOVA with Tukey's post hoc test. Error bars represent Mean ± SEM. (D) Top view of representative 3-wk-old Col-0, *bdh*, and two independent T2 plants expressing UB-BDH1 and UB-Rtt102. (Scale bar: 1 cm.) (E) In vivo coimmunoprecipitation (CoIP) of transiently expressed BDH1-FLAG, Rtt102-FLAG, ARP7-HA, and ARP4-Myc. BDH1-FLAG and Rtt102-FLAG were IP using FLAG antibody and results were observed using anti-FLAG, anti-HA, and anti-Myc antibodies. Two replicates of the same CoIP experiment are shown.

results showed that ARP4 was more efficiently immunoprecipitated by ARP7 when BDH1 was present, suggesting that BDH1 promotes ARP heterodimer formation or stability (Fig. 5A and Dataset S25). Next, we investigated whether this effect on ARP heterodimer impacted the overall complex composition. To address this, we generated transgenic plants expressing 6xHA-tagged BAF60B, a core subunit shared by all plant SWI/SNF subclasses, in both WT and *bdh* mutant backgrounds and performed IP-MS experiments using BAF60B-6xHA as bait. As anticipated for a plant pan-SWI/SNF subunit, BAF60B isolated subunits from all SWI/SNF complexes (Fig. 5B and Dataset S26). Importantly, a significant loss of ARP4 protein was observed when BAF60B was immunoprecipitated in the *bdh* mutant background compared to a similar experiment in WT plants (Fig. 5C and Dataset S26). Together with our previous findings, this result supports a model where BDHs promote ARP heterodimer stability, resulting in ARP4 becoming less stable or less incorporated into the complex. This selective loss of ARP4 could explain the molecular and phenotypical defects observed in the *bdh* mutant (9, 26, 27) (Fig. 1 and SI Appendix, Figs. S1 and S2).

Interestingly, we observed a modest increase in the abundance of MAS-specific subunits when BAF60B was immunoprecipitated in the *bdh* mutant compared to the WT. In contrast, the BAS- and SAS-specific subunits remained unchanged or showed a slight decrease (Fig. 5C). This increase in MAS-specific subunit abundance corresponded with the higher expression of some of these subunits in the *bdh* mutant (SI Appendix, Fig. S14A). Noteworthy, previous transcriptomic analyses in inflorescences showed that multiple MAS-specific subunits, and not BAS- or SAS-specific subunits, become up-regulated in the MAS-specific *tpf* and *minu* mutants (14). Using published RNA-seq data (9), we confirmed this trend in seedlings of *minu* (MAS-specific mutant) and *bdh* mutants, whereas *brm* (BAS-specific) nor *syd* (SAS-specific) mutants did not show such feedback regulation (SI Appendix, Fig. S14B). This indicates that this transcriptional response is uniquely related to MAS subclass malfunction. Thus, the slight increase in MAS subunit accumulation upon BAF60B immunoprecipitation in the *bdh* mutant likely reflects impaired function of the MAS complex as a consequence of BDH depletion.

Discussion

Decades of research on SWI/SNF chromatin remodelers in animal and fungal models have provided a thorough understanding of the distinct complex subclasses and their subunits (5, 6). Recent studies in plants have confirmed the presence and composition of three plant SWI/SNF subclasses and their equivalence to animal and fungal complexes (9, 10, 12, 14). However, the functions of these plant subclasses and their subunits are only beginning to be understood.

In this work, we characterized the BDH subunit, recently identified as a common component of all three plant subclasses and proposed as a distant ortholog of animal BCL7 proteins (9, 10, 12). We conducted a comprehensive phenotypical characterization to determine the processes affected by the absence of BDH proteins. Additionally, we examined the function of phylogenetically and structurally conserved domains and used modeling to predict the structural conservation of the SWI/SNF catalytic module across eukaryotes. Finally, we investigated the role of BDH proteins in the complex stability, proposing a model in which BDHs promote ARP heterodimer stability.

BDHs Have A Broad Effect on Plant Development and Participate in Hypocotyl Cell Elongation. We investigated the developmental defects caused by BDH loss of function. Consistent with previous

publications (26, 27), we report the redundant function of Arabidopsis BDH1 and BDH2 in controlling multiple phenotypes like the leave shape, flowering time, and silique length. Notably, defects in all these phenotypes were previously described in other plant SWI/SNF mutants but were more severe (9). For example, strong mutants in BRM and SYD ATPases present strong pleiotropic developmental defects that are more pronounced than in *bdh* mutants (33). Furthermore, double *brm* and *syd* mutants or strong *minu1/2* mutants are lethal (33, 34). This suggests that BDH fine-tunes the activities of SWI/SNF subclasses rather than playing an essential role.

Interestingly, we observed that *bdh* mutants grow longer hypocotyls compared to WT plants. This defect was observed in dark- and light-grown seedlings due to enhanced cell elongation. The difference in growth rate between WT and *bdh* mutant occurred after day 4 postgermination, indicating that BDHs facilitate the hypocotyl growth arrest that occurs at this moment of development. In line with these results, light-grown RNAi mutants of the SWI/SNF pan-subunit BAF60B also presented longer hypocotyls, while the opposite was found in BAF60 overexpressors in both light and dark conditions (35). This contrasts with the phenotypes found in mutants of different BAS complex subunits (BRM, BRIPs, BRDs) that showed shorter hypocotyls in the dark (36, 37). These results suggest that specific defects in SAS or MAS function, or the combined malfunction of MAS, SAS, and/or BAS, could be responsible for the enhanced hypocotyl cell elongation found in *bdh* and *baf60* mutants. Notably, MINU2 overexpression has been shown to slightly reduce hypocotyl length (38). Transcriptomic analyses revealed that BDH represses the expression of multiple *XTH* genes, which are key players in cell wall shaping and growth. Previous studies showed that overexpression of several Arabidopsis *XTHs* (being, for example, *XTH18* overexpressed just twofold compared to WT) was sufficient to promote growth of Arabidopsis etiolated hypocotyls (39, 40). These *XTHs* were among the up-regulated genes in our RNA-seq experiment, suggesting that the *XTH* overexpression in the *bdh* mutant could be responsible for the larger hypocotyls. Considering the relevance of *XTHs* in cell wall shaping and plant growth, understanding how SWI/SNF complexes regulate their expression could have biotechnological applications. While some of these genes are directly bound by BDHs, the mechanistic details about SWI/SNF-mediated regulation of *XTH* expression remain to be explored in future studies. Interestingly, BAF60 was shown to compete with the PIF4 transcription factor for binding to the *XTH15* (aka *XTR7*) promoter to regulate its expression (35).

Functional Characterization of BDH Domains. BDH proteins have an evolutionary conserved sequence shared with human BCL7 proteins, that we named BCL domain (12). Our analyses revealed that the BCL domain is essential for BDH function, likely due to its role in mediating interaction with the ARP heterodimer, particularly with ARP7. Importantly, we only deleted the β -sheet closest to the N-terminal domain of BDH1, which includes the conserved W28 shown to mediate interaction with the conserved P355 in ARP7. This result suggests that the formation of an intact β -hairpin fold is required for BDH interaction with the ARPs. Additionally, BDHs have a highly conserved plant-specific region in the proximal N-term region, which is important but not critical for BDH function. Future structural analyses will help revealing how this region folds in the SWI/SNF structure and how it promotes BDH interaction with the complex. Furthermore, BDHs in multiple plant species also have a predicted alpha-helix fold downstream of the β -hairpin region, although this is not conserved at the sequence level. Interestingly, the N-terminal

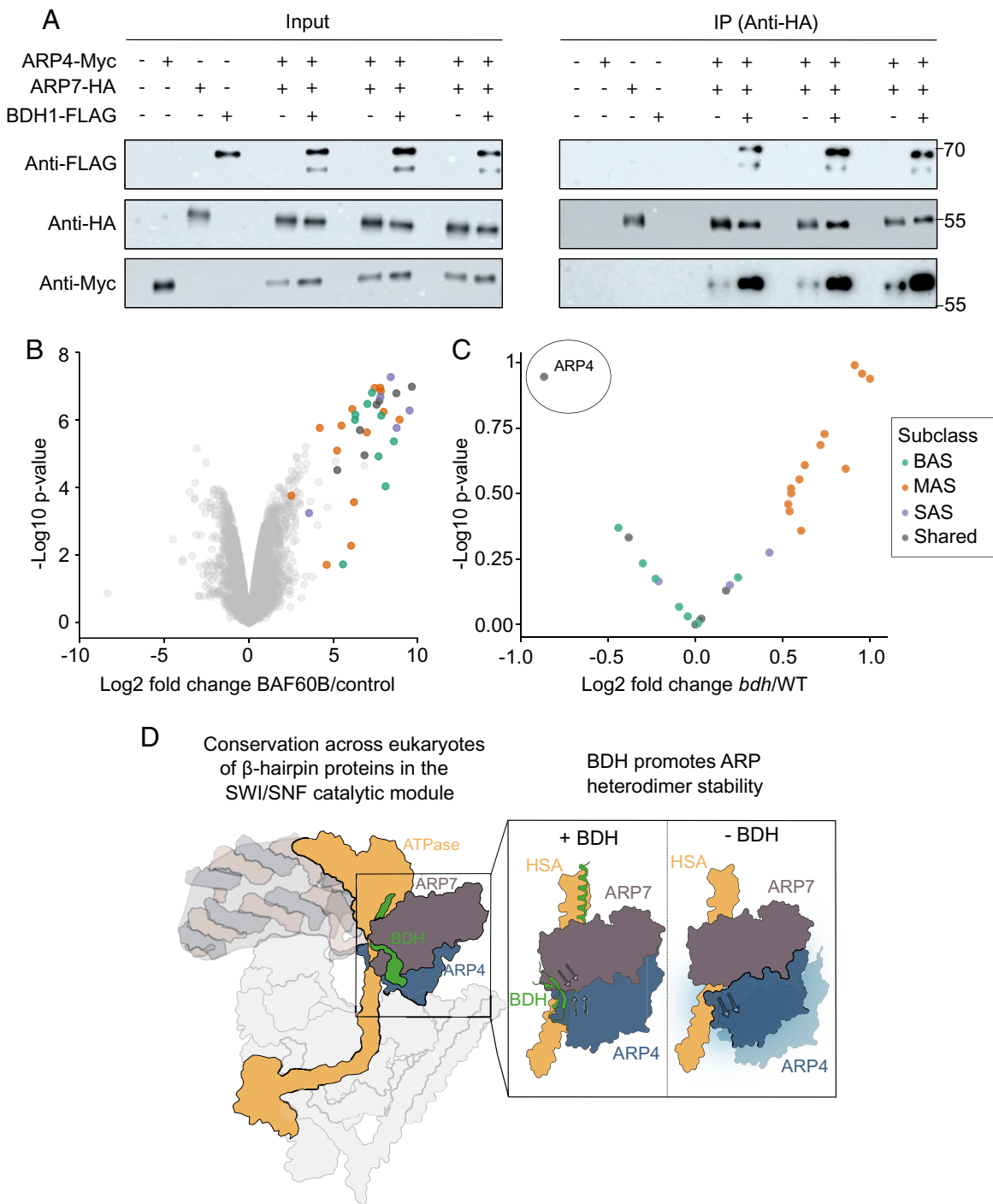


Fig. 5. BDH promotes ARP heterodimer stability. (A) In vivo coimmunoprecipitation (CoIP) of transiently expressed BDH1-FLAG, ARP7-HA, and ARP4-Myc. ARP7-HA was IP using HA antibody and results were observed using anti-FLAG, anti-HA, and anti-Myc antibodies. Three replicates of the same CoIP experiment are shown. (B) Volcano plot depicting the enrichment (log2 ratio) of all the identified SWI/SNF proteins in the BAF60B-6xHA IP-MS experiment in WT background. BAS-, MAS-, and SAS-specific SWI/SNF subunits, as well as those shared in two or three subclasses are depicted in green, orange, purple, and dark gray, respectively. Light gray dots represent non-SWI/SNF proteins identified in the experiment. The x axis depicts BAF60B-6xHA average intensities over Col-0 nontransgenic control. The y axis depicts significance $-\log_{10} P$ -value. (C) Volcano plot depicting the enrichment of SWI/SNF subunits identified in the IP-MS experiment using BAF60B-6xHA as bait in *bdh* mutant vs WT background. For clarity, ARP4 is enclosed in a circle. The x axis depicts log2 fold change of average intensities of IP experiments in WT and *bdh* backgrounds. The y axis depicts significance $-\log_{10} P$ -value. (D) Proposed model for the conservation of β -hairpin proteins in SWI/SNF catalytic modules and their function as promoters of ARPs heterodimer stability. The representation of the complex is based on the CryoEM structure of the BAF mammalian complex (32). The position of the ARP heterodimer and BDH protein was inferred from previous models and the prediction reported in our study.

regions of BCL7 and Rtt102 contain an alpha-helix fold which adopt a similar position within the heterotrimeric complex with ARPs as the one predicted for BDH's alpha helix, suggesting their functional conservation. However, while N-terminal deletion of BCL7 fully prevented its function and ability to interact with the

complex (41), deletion of the BDH alpha helix had no significant impact on BDH function according to mutant complementation analyses, at least under the conditions/phenotypes tested. This suggests that other regions in BDH, such as its N-terminal region, might help stabilizing the interaction of BDH with the complex

in the absence of the alpha helix domain. Overall, this functional analysis revealed two BDH domains important for its interaction with the complex.

Evolutionary Conservation of the β -Hairpin Domain as an ARP-Interacting Module in SWI/SNF Remodelers. The SWI/SNF complexes can be divided into core and catalytic modules (11). While the composition of the core module differs between SWI/SNF subclasses, depending on the incorporation of signature subunits, the catalytic module composition is the same across subclasses, featuring the Snf2-type ATPase and an ACTIN/ARP heterodimer (11, 16, 17). Mammal SWI/SNF complexes incorporate a fourth subunit in this module, BCL7, which has been shown to interact with the ATPase subunit and other complex subunits and the nucleosome (11, 21). Yeast SWI/SNF complexes incorporate Rtt102 in the catalytic module (17), which promotes a more compact ARP heterodimer conformation, enhancing the complex remodeling activity (18, 24). Furthermore, it has been shown to also interact with the ATPase (42). However, despite their similarities, no previous experimental connection had been made between BCL7 and Rtt102 proteins.

A comprehensive phylogenomic analysis across multiple eukaryotic species proposed plant BDH proteins as orthologs of animal BCL7. However, this analysis failed to identify any fungal BDH-BCL7 ortholog (12). In this study, we found a structural relationship between animal BCL7, fungal Rtt102, and plant BDH, based on their shared ability to interact with the ACTIN/ARP heterodimer through the formation of a conserved β -hairpin. The link between BDH and Rtt102 is further supported by BDH's ability to promote ARP heterodimer stability, reminiscent of Rtt102's function (24). Furthermore, Rtt102 can perform some of the functions of BDH, as demonstrated by *bdh* mutant complementation analyses. Interestingly, the complementation achieved with Rtt102 was similar to that observed with the N-domain-deleted BDH1 (Figs. 2 and 4, *SI Appendix*, Fig. S15, and *Dataset S27*). Both were able to rescue the etiolated hypocotyl defects and silique length while only weakly recovering the rosette size and flowering time. Notably, this recovery was to a greater degree than the BCL-deleted BDH1, which completely failed to recover any phenotype (Figs. 2 and 4, *SI Appendix*, Fig. S15, and *Dataset S27*). This functional connection between Rtt102 and N-domain-deleted BDH1 can be attributed to their weaker ability to interact with the complex compared with full-length BDH1 (Figs. 2 *F* and *G* and 4 *A* and *E*). The phenotype-dependent ability of Rtt102 or N-domain-deleted BDH1 to rescue the *bdh* mutant might reflect distinct modes of action or catalytic activities of the SWI/SNF complexes that may rely on BDH1 function to varying degrees, potentially acting on specific processes or genomic contexts.

Interestingly, a recent study reported the structural conservation of a similar ARP- β -hairpin interacting module in the context of the animal and fungal INO80 complexes (43). This study showed that the animal and yeast β -hairpin-containing proteins YY1 and Ies4, respectively, interact through two conserved tryptophans with a conserved proline in one of the two ARPs that heterodimerize in the animal and yeast complexes (ACTB-YY1 and Actin-Ies4). Notably, our study shows that BCL7, Rtt102, and BDH also conserve these tryptophans, which are predicted to interact specifically with one of the two ARPs in a similar fashion. Here, we show that the conserved tryptophans are pivotal for the BDH1-ARP7 interaction, providing a functional link for this conserved network. Notably, to our knowledge, no functional tests are available on the role of YY1 or Ies4 in the Actin/ARP heterodimer stability. Similarly, the Snf2^{HSA}-ARP heterodimer arrangement is also

conserved in the SWR1 and NuA4 complexes, both of which incorporate the β -hairpin-containing protein Swc4 (44, 45). What gives specificity to the incorporation of these β -hairpin proteins to each complex could be dictated by the stronger affinity of the β -hairpin protein to the complex-specific ARP protein. For example, BDH1 protein strongly interacts with the ARP subunit that is specific to the SWI/SNF complex, ARP7, and not with ARP4, also found in INO80, SWR1, and NuA4 complexes (44, 45). Similarly, Rtt102 showed a stronger interaction with the yeast SWI/SNF-specific ARP, Arp9 (24, 46). According to our modeling, this logic would predict that BCL7 interacts more strongly with ACTB. An alternative situation occurs in fungi where INO80 and SWI/SNF complexes incorporate the same actin-Arp4 heterodimer. In this case, a specific region in Ies4 was shown to provide complex specificity through the interaction with an additional INO80-specific subunit, ARP8, which is found near the actin-Arp4 module (43).

BDH Promotes Stability of the ARP Heterodimer in the Catalytic Module.

A recent study reported genome-wide changes in chromatin accessibility and gene-body nucleosome occupancy in Arabidopsis *bdh* mutants (27). However, this study did not find substantial changes in the BAS complex composition or its recruitment to the chromatin in the absence of BDH, thus leaving unanswered how BDH depletion impaired SWI/SNF function. We propose that BDH impacts SWI/SNF function through the stabilization of the ARP heterodimer. We showed that ARP7-ARP4 interaction is enhanced in the presence of BDH1 and that BDH depletion triggers ARP4 destabilization in vivo. The partial loss of ARP4 observed in IP-MS experiments could reflect an overall weaker ARP heterodimer stability across all SWI/SNF subclasses. Notably, experiments in yeast showed that Rtt102 promotes a more compact Arp7/9 heterodimer conformation which, in turn, shortens the interaction surface of the heterodimer with the HSA domain, altering the network of interactions between distinct ATPase domains important for catalytic activity, like post-HSA and Protrusion 1 (24). Importantly, plant BDH, as well as BCL7 and Rtt102, can also interact with the ATPase which could also directly influence its activity (21, 27, 42). Future mechanistic studies will shed light on the specific impact of BDH on ARP compaction, ATP turnover, DNA translocation, and nucleosome remodeling in plants. Additionally, BCL7 proteins have been shown to interact with other proteins in the complex and the nucleosome, such as SMARCB1, H2B, and H2A (11, 21). Although we do not yet know whether BDHs interact with all these proteins, it is plausible that BDH1 depletion leads to altered subunit contacts within the complex and with the nucleosome, resulting in remodeling defects. Interestingly, transient coexpression of BDH1 with ARP7 in *Nicotiana benthamiana*, but not BDH1 alone or with ARP4, resulted in the detection of two BDH1 bands by Western blot (Figs. 3 *G* and *H*, 4*E*, and 5*A*), suggesting a potential modification triggered by the BDH1-ARP7 interaction. However, the additional band persisted even when W/G-mutated BDH1, which shows reduced interaction with ARP7, was coexpressed (Fig. 3*H*). In contrast, stably expressed BDH1 in transgenic Arabidopsis showed only a single band, despite confirmed interaction with ARP7 (*SI Appendix*, Fig. S7 *A* and *D*). Similarly, no band pattern differences were observed between full-length BDH1 and the Δ BCL variant, which lacks ARP7 interaction (Fig. 2*F* and *SI Appendix*, Fig. S7*A*). Currently, the reason for the extra BDH1 band in transient expression remains unclear, but it likely reflects an artifact of protein overexpression. Further functional assays will help clarify this observation.

Apart from the decrease in ARP4, we observed a slight increase in MAS-specific subunits isolated by BAF60B in the *bdh* mutant compared to WT. This might reflect a small increase in the accumulation of the MAS complex in the *bdh* mutant. Importantly, malfunction of the MAS complex leads to upregulation of MAS-specific subunits but not BAS- and SAS-specific ones (14) (*SI Appendix, Fig. S14B*). Thus, these results suggest that, whereas a small increase in MAS complex might occur in *bdh* mutants, these complexes are functionally impaired, leading to the transcriptional upregulation of MAS-specific subunits. On the contrary, we did not observe significant changes between WT and *bdh* in the accumulation of BAF60B-immunoprecipitated BAS- and SAS-specific subunits. In line with this result, a recent study found no differences in BAS-specific subunit accumulation when pulling from BRM in WT and *bdh* mutant (27). Interestingly, these IP-MS results showed a specific reduction in ARP4 peptides in the *bdh* mutant compared to other BAS subunits identified, supporting our observation that BDH alters ARP4 stability in the complex. Furthermore, this study showed that BDH affected chromatin accessibility in targets of the three subclasses (27). A clustering analysis of RNA-seq experiments conducted on multiple plant SWI/SNF mutants (9) revealed that DEGs in *bdh* mutants exhibit expression changes more closely aligned with BAS complex mutants (*brm*, *an3*, *brd1/2/13*, and *brip1/2*), followed by SAS (*syd*, *swi3d*, and *sys*), and then MAS complex mutants (*minu1/2* and *tpf1/2*) (*SI Appendix, Fig. S14C*), suggesting that BDH may have a more prominent regulatory role within the BAS complex than in the other SWI/SNF subcomplexes.

In summary, our findings suggest that BDHs influence the catalytic activity of plant SWI/SNF subclasses by stabilizing the pan-SWI/SNF ARP heterodimer module (Fig. 5D). Given the analogous role of yeast Rtt102 in Arp heterodimer compaction (24) and the structural conservation indicated by our study, we propose that BCL7, BDH, and Rtt102 are functionally equivalent subunits of the SWI/SNF catalytic modules conserved across eukaryotes.

Materials and Methods

Plant Materials and Growth Conditions. The *bdh1-1* (SALK_152173) and *bdh2-1* (SALK_029285) mutants used in this study, which were previously characterized (26), are in the Col-0 background and were requested from ABRC. A double *bdh1/bdh2*, called *bdh* in this manuscript, was obtained by genetic cross. Oligos for T-DNA genotyping are found in *Dataset S28*. More details on the plant materials and growth conditions are provided in *SI Appendix, Materials and Methods*.

Construction of Plasmids and Transgenic Lines. The *BDH1* genomic region, including 2,030 bp upstream of the start codon and all the gene body, including introns, until the stop codon, was amplified from genomic DNA of Col-0 plants and cloned into a pENTR/D vector (Invitrogen) to generate pENTR-gBDH1. The primers used for this and the following clonings can be found in *Dataset S28*. More details on the construction of plasmids and transgenic lines are provided in *SI Appendix, Materials and Methods*.

Transient Expression in *N. benthamiana*, WB, and Protein Coimmunoprecipitation. Details on the transient expression in *N. benthamiana* and WB are provided in *SI Appendix, Materials and Methods*. Agroinfiltrated Nicotiana leaves were ground to a fine powder in liquid nitrogen using a mortar and pestle and then resuspended in 1.5 mL of IP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 0.1% NP-40, 10% glycerol, 0.5 mM DTT, 1 mM PMSF, and 1× Complete Mini EDTA-Free Protease Inhibitor (Roche)). The extracts were clarified twice by centrifugation at 12,000 × g for 5 min at 4 °C. Total protein concentration was determined using the Bradford Protein Assay (Bio-Rad) and adjusted to 0.750 mg/mL. An amount between 0.5 and 1.5% of the extracts was set aside for protein level verification (input). For immunoprecipitations, the extracts were incubated with 1 µg of anti-FLAG M2 monoclonal antibody (Sigma) or anti-HA 3F10 monoclonal antibody (Roche) for 2 h

with gentle rotation at 4 °C. Subsequently, 20 µL of prewashed magnetic protein G Dynabeads (Invitrogen) in IP buffer were added to the samples and incubated for 1 h with gentle rotation at 4 °C. The samples were then washed three times with IP buffer, and the precipitated proteins were eluted by heating the beads at 95 °C for 2 min in 50 µL of 2× SDS-PAGE loading buffer. Finally, 5 and 20 µL of the eluate were separately subjected to Western blot analysis to detect the immunoprecipitated and coimmunoprecipitated proteins, respectively.

IP-MS. Immunoprecipitation coupled with mass spectrometry was conducted following established protocols (12, 14). For all the experiments, two independent transgenic lines of the indicated transgenes in the indicated backgrounds were used in parallel with untransformed Col-0 control. Details on the IP-MS data analysis are provided in *SI Appendix, Materials and Methods*.

RNA Extraction, qRT-PCR, and RNA-seq. Total RNA was isolated using the Direct-zol RNA Miniprep Kit (Zymo Research) according to the manufacturer's instructions. For *XTH* expression analyses, 1 µg of total RNA from 5-d-old etiolated seedlings of the indicated backgrounds was used for cDNA synthesis with the NZY First-Strand cDNA Synthesis kit (NZYTech) following the manufacturer's protocol. Primer details are provided in *Dataset S28*. Fold change was determined relative to the expression of the *PP2A* housekeeping gene using the $\Delta\Delta C_t$ method (47). For RNA-seq experiments, total RNA from 5-d-old etiolated seedlings of Col-0 and *bdh* backgrounds (three biological replicates per background) was submitted to the BGI company for the preparation of strand-specific mRNA libraries, which were sequenced using the DNBSEQ high-throughput platform as PE100 reads. Details on the RNA-seq data analysis are provided in *SI Appendix, Materials and Methods*.

Chromatin Immunoprecipitation and ChIP-seq. The chromatin immunoprecipitation (ChIP) protocol was conducted according to previously described methods with minor adjustments (14). Details on the chromatin immunoprecipitation and ChIP-seq data analysis are provided in *SI Appendix, Materials and Methods*.

Structural Modeling and Analysis. The Arabidopsis (MINU1^{HSA}-ARP4-ARP7-BDH1) and Human (BRG1^{HSA}-ACTL6-ACTB-BCL7A) complexes, as well as the chimeric Rtt102-Arabidopsis complex, were modeled with AlphaFold2 (v.2) (29) and AlphaFold-multimer (31) using a colab notebook running ColabFold (48) v1.5.5. More details on the structural modeling and analysis are provided in *SI Appendix, Materials and Methods*.

Statistical Analyses. All statistical analyses conducted in this manuscript are described above. Methods for statistical tests, sample sizes, and *P* values are provided in the figures. A two-tailed Student's *t* test was used to evaluate the differences between two groups. For multiple comparisons, a one-way ANOVA was conducted, followed by Tukey's honestly significant difference post hoc test. Statistical analyses were conducted using GraphPad Prism software (v.8) and RStudio. All experiments were conducted with a minimum of two replicates.

Figure Representation. Venn diagrams were drawn using eulerr (<https://eulerr.co/>). Heatmaps and metaplots were produced using the computeMatrix and plotHeatmap or plotProfile commands from deeptools v3.5.1 (49). Plots of RNA-seq data and IP-MS volcano plots were drawn using ggplot2. The protein alignments were performed using CLC Main Workbench 24 with a gap open cost of 10.0 and a gap extension cost of 1.0.

Data, Materials, and Software Availability. The ChIP-seq and RNA-seq data are available at GEO with accession numbers [GSE268510](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268510) (50) and [GSE268511](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268511) (51), respectively. The ChIP-seq data of BDH1, MINU2, BRM, and SYD in seedlings were obtained from GEO ([GSE218841](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE218841)) (52). The RNA-seq data of *bdh* vs. WT light-grown seedlings were obtained from GEO ([GSE193095](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193095)) (53). The IP-MS data are available at MassIVE ([MSV000094986](https://massive.ucsb.edu/ViewMain/MV000094986)) (54). Prediction models of Arabidopsis and Human SWI/SNF catalytic modules, as well as the chimeric Rtt102-Arabidopsis catalytic module, are available in Mendeley Data (<https://data.mendeley.com/datasets/37jzt2bgh/1>) (55). All other data are included in the manuscript and/or supporting information.

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- K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
- R. D. Kornberg, Chromatin structure: A repeating unit of histones and DNA. *Science* **184**, 868–871 (1974).
- S. L. Klemm, Z. Shipony, W. J. Greenleaf, Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* **20**, 207–220 (2019).
- G. Millán-Zambrano, A. Burton, A. J. Bannister, R. Schneider, Histone post-translational modifications—Cause and consequence of genome function. *Nat. Rev. Genet.* **23**, 563–580 (2022).
- C. R. Clapier, J. Iwasa, B. R. Cairns, C. L. Peterson, Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nat. Rev. Mol. Cell Biol.* **18**, 407–422 (2017).
- T. Bieluszewski, S. Prakash, T. Roulé, D. Wagner, The role and activity of SWI/SNF chromatin remodelers. *Annu. Rev. Plant Biol.* **74**, 139–163 (2023).
- C. Hodges, J. G. Kirkland, G. R. Crabtree, The many roles of BAF (mSWI/SNF) and PBAF complexes in cancer. *Cold Spring Harb. Perspect. Med.* **6**, a026930 (2016).
- J. Y. Shang, X. J. He, Chromatin-remodeling complexes: Conserved and plant-specific subunits in Arabidopsis. *J. Integr. Plant Biol.* **64**, 499–515 (2022).
- J. Guo *et al.*, Comprehensive characterization of three classes of Arabidopsis SWI/SNF chromatin remodeling complexes. *Nat. Plants* **8**, 1423–1439 (2022).
- W. Fu *et al.*, Organization, genomic targeting, and assembly of three distinct SWI/SNF chromatin remodeling complexes in Arabidopsis. *Plant Cell* **35**, 2464–2483 (2023).
- N. Mashtalir *et al.*, Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. *Cell* **175**, 1272–1288.e20 (2018).
- J. Hernández-García *et al.*, Comprehensive identification of SWI/SNF complex subunits underpins deep eukaryotic ancestry and reveals new plant components. *Commun. Biol.* **5**, 549 (2022).
- N. Mashtalir *et al.*, Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. *Cell* **175**, 1272–1288.e20 (2018).
- B. Diego-Martin *et al.*, The TRIPLE PHD FINGERS proteins are required for SWI/SNF complex-mediated +1 nucleosome positioning and transcription start site determination in Arabidopsis. *Nucleic Acids Res.* **50**, 10399–10417 (2022).
- H. Szerlong *et al.*, The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases. *Nat. Struct. Mol. Biol.* **15**, 469–476 (2008).
- Y. Han, A. A. Reyes, S. Malik, Y. He, Cryo-EM structure of SWI/SNF complex bound to a nucleosome. *Nature* **579**, 452–455 (2020).
- H. L. Schubert *et al.*, Structure of an actin-related subcomplex of the SWI/SNF chromatin remodeler. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 3345–3350 (2013).
- C. R. Clapier *et al.*, Regulation of DNA translocation efficiency within the chromatin remodeler RSC/Sth1 potentiates nucleosome sliding and ejection. *Mol. Cell* **62**, 453–461 (2016).
- C. R. Clapier, B. R. Cairns, The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* **78**, 273–304 (2009).
- R. B. Meagher, R. B. Deal, M. K. Kandasamy, E. C. McKinney, Nuclear actin-related proteins as epigenetic regulators of development. *Plant Physiol.* **139**, 1576–1585 (2005).
- N. Mashtalir *et al.*, A structural model of the endogenous human BAF complex informs disease mechanisms. *Cell* **183**, 802–817.e24 (2020).
- L. Wischhof *et al.*, BCL7A-containing SWI/SNF/BAF complexes modulate mitochondrial bioenergetics during neural progenitor differentiation. *EMBO J.* **41**, e110595 (2022).
- D. Diaz *et al.*, BCL7 proteins, metazoan-specific subunits of the mammalian SWI/SNF complex, bind the nucleosome core particle. *bioRxiv* [Preprint] (2023). <https://doi.org/10.1101/2023.03.17.532992> (Accessed 17 March 2023).
- B. Turegun, D. J. Kast, R. Dominguez, Subunit Rtt102 controls the conformation of the Arp7/9 heterodimer and its interactions with nucleotide and the catalytic subunit of SWI/SNF remodelers. *J. Biol. Chem.* **288**, 35758–35768 (2013).
- L. Vercruyssen *et al.*, ANGUSTIFOLIA3 binds to SWI/SNF chromatin remodeling complexes to regulate transcription during Arabidopsis leaf development. *Plant Cell* **26**, 210–229 (2014).
- P. Stachula *et al.*, BRM complex in Arabidopsis adopts ncBAF-like composition and requires BRD subunits for assembly and stability. *Int. J. Mol. Sci.* **24**, 3917 (2023).
- Y. Lei *et al.*, BCL7A and BCL7B potentiate SWI/SNF-complex-mediated chromatin accessibility to regulate gene expression and vegetative phase transition in plants. *Nat. Commun.* **15**, 935 (2024).
- J. M. Eklöf, H. Brumer, The XTH gene family: An update on enzyme structure, function, and phylogeny in xyloglucan remodeling. *Plant Physiol.* **153**, 456–466 (2010).
- J. Jumper *et al.*, Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
- D. T. Jones, D. Cozzetto, DISOPRED3: Precise disordered region predictions with annotated protein-binding activity. *Bioinformatics* **31**, 857–863 (2015).
- R. Evans *et al.*, Protein complex prediction with AlphaFold-multimer. *bioRxiv* [Preprint] (2022). <https://doi.org/10.1101/2021.10.04.463034> (Accessed 10 March 2022).
- S. He *et al.*, Structure of nucleosome-bound human BAF complex. *Science* **367**, 875–881 (2020).
- S. Bezhan *et al.*, Unique, shared, and redundant roles for the Arabidopsis SWI/SNF chromatin remodeling ATPases BRAHMA and SPLAYED. *Plant Cell* **19**, 403–416 (2007).
- Y. Sang *et al.*, Mutations in two non-canonical Arabidopsis SWI2/SNF2 chromatin remodeling ATPases cause embryogenesis and stem cell maintenance defects. *Plant J.* **72**, 1000–1014 (2012).
- T. Jégu *et al.*, The Arabidopsis SWI/SNF protein BAF60 mediates seedling growth control by modulating DNA accessibility. *Genome Biol.* **18**, 114 (2017).
- T. Zhu *et al.*, The BAS chromatin remodeler determines brassinosteroid-induced transcriptional activation and plant growth in Arabidopsis. *Dev. Cell* **59**, 924–939.e6 (2024).
- R. Archacki *et al.*, BRAHMA ATPase of the SWI/SNF chromatin remodeling complex acts as a positive regulator of gibberellin-mediated responses in Arabidopsis. *PLoS One* **8**, e58588 (2013).
- A. Folta *et al.*, Over-expression of Arabidopsis AtCHR23 chromatin remodeling ATPase results in increased variability of growth and gene expression. *BMC Plant Biol.* **14**, 76 (2014).
- E. Miedes *et al.*, Xyloglucan endotransglucosylase/hydrolase (XTH) overexpression affects growth and cell wall mechanics in etiolated Arabidopsis hypocotyls. *J. Exp. Bot.* **64**, 2481–2497 (2013).
- S. Dhar *et al.*, SHORT-ROOT controls cell elongation in the etiolated Arabidopsis hypocotyl. *Mol. Cells* **45**, 243–256 (2022).
- C. Balañs-Gavira *et al.*, Frequent mutations in the amino-terminal domain of BCL7A impair its tumor suppressor role in DLBCL. *Leukemia* **34**, 2722–2735 (2020).
- P. Sen *et al.*, Loss of Snf5 induces formation of an aberrant SWI/SNF complex. *Cell Rep.* **18**, 2135–2147 (2017).
- F. Kunert *et al.*, Structural mechanism of extranucleosomal DNA readout by the INO80 complex. *Sci. Adv.* **8**, eadd3189 (2022).
- L. Espinosa-Cores *et al.*, Insights into the function of the NuA4 complex in plants. *Front. Plant Sci.* **11**, 125 (2020).
- C. B. Gerhold, S. M. Gasser, INO80 and SWR complexes: Relating structure to function in chromatin remodeling. *Trends Cell Biol.* **24**, 619–631 (2014).
- B. Turegun, R. W. Baker, A. E. Leschziner, R. Dominguez, Actin-related proteins regulate the RSC chromatin remodeler by weakening intramolecular interactions of the Sth1 ATPase. *Commun. Biol.* **1**, 1 (2018).
- K. J. Livak, T. D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* **25**, 402–408 (2001).
- M. Mirdita *et al.*, ColabFold: Making protein folding accessible to all. *Nat. Methods* **19**, 679–682 (2022).
- F. Ramírez, F. Dündar, S. Diehl, B. A. Grüning, T. Manke, DeepTools: A flexible platform for exploring deep-sequencing data. *Nucleic Acids Res.* **42**, W187–W191 (2014).
- J. Candela-Ferre *et al.*, Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability. NCBI-Gene Expression Omnibus. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268510>. Deposited 28 May 2024.
- J. Candela-Ferre *et al.*, Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability. NCBI-Gene Expression Omnibus. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268511>. Deposited 28 May 2024.
- C. Li, W. Fu, Y. Yu, Organization, genomic targeting and assembly of three distinct SWI/SNF chromatin remodeling complexes in Arabidopsis. NCBI-Gene Expression Omnibus. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE218841>. Deposited 27 November 2022.
- X. He, Three classes of Arabidopsis SWI/SNF chromatin remodeling complexes differentially regulate development by affecting chromatin accessibility. NCBI-Gene Expression Omnibus. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193095>. Deposited 5 January 2022.
- J. Gallego-Bartolomé, Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability. MassIVE. <https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=2f76b69679cf42cca2c20866ffcd251c>. Deposited 11 June 2024.
- J. Candela-Ferre *et al.*, Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability. Mendeley Data. <https://data.mendeley.com/datasets/37jzt2bgth/1>. Deposited 27 December 2024.