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Tian-Feng Lü, Xiao-Yu Chen, Nicola Trozzi, Wen He, Quan Yuan, Yu Han, Li-Min Lu, Chao-Bin Li, Jie Cheng, Adrien Sicard, Yao Zhang, Ya-Nan Su, Yan-Jun Zan, Michael Lenhard, Hong-Zhi Kong, Mateusz Majda, Lars Østergaard & Yang Dong

Title: Epigenetic regulation of fruit shape determination by *JAGGED* gene in *Capsella rubella*

Authors: Tian-Feng Lü[1,2,3#], Xiao-Yu Chen[1,2,3,4#], Nicola Trozzi[5#], Wen He[1,2,3,4], Quan Yuan[1,2,3,4], Yu Han[6], Li-Min Lu[1,2,3,4], Chao-Bin Li[1,2,3,4], Jie Cheng[1,2,3,4], Adrien Sicard[7], Yao Zhang[1,2,3,4], Ya-Nan Su[8], Yan-Jun Zan[9,10], Michael Lenhard[11], Hong-Zhi Kong[1,2,3,4], Mateusz Majda[5], Lars Østergaard[12] and Yang Dong[1,2,3,4]*

Affiliations:

[1]State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, China

[2]Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, China

[3]China National Botanical Garden, Beijing 100093, China

[4]University of Chinese Academy of Sciences, Beijing 100049, China

[5]The Mechanobiology Laboratory, Department of Plant Molecular Biology, University of Lausanne, CH-1015 Lausanne, Switzerland

[6]Department of Human Genetics, KU Leuven, Leuven, Belgium

[7]Uppsala Biocenter, Department of Plant Biology, BOX 7080, 750 07, Uppsala, Sweden

[8]College of Agronomy, Henan Agricultural University, Zhengzhou, 450046, China

[9]Department of Plant Physiology, Umeå Plant Science Center and Integrated Science Lab, Umeå University, Umeå, Sweden

[10]Tobacco Research Institute, Chinese Academy of Agricultural Sciences

[11]Institut für Biochemie und Biologie, Universität Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm, Germany

[12]Department of Biology, University of Oxford, South Parks Road, OX1 3EL, UK

[#]These authors contributed equally to this work.

*Correspondence: Yang.Dong@ibcas.ac.cn

Bluesky Accounts:

@yang-brassicaceae.bsky.social (Yang Dong)

Abstract

Fruits are a key feature defining angiosperms, yet how local growth coordinates during development to generate diverse fruits remains unclear. Here, we demonstrate that the *Capsella rubella* C2H2-zinc finger transcription factor JAGGED (CrJAG) controls fruit shape determination by promoting both cell division and anisotropic growth. At the molecular level, CrJAG physically interacts with the histone chaperones *Capsella rubella* MULTICOPY SUPPRESSOR OF IRA1 (CrMSI) members, increasing the chromatin accessibility and thereby sustaining the expression of genes involved in fruit morphogenesis. The resulting closed chromatin state in *Crjag* fruits is characterized by a reduction of the active histone marker (H3K18ac) and an increase of the repressive marker (H3K27me3). Further expression and pharmacological treatment analyses indicate that the developmental defects in *Crjag* fruits are largely attributable to down-regulation of the key cell-cycle regulator *CrAUR2*. Collectively, our findings suggest that fine-tuning the cell cycle via epigenetic modification represents an additional, essential layer of regulation critical to organ development and diversification.

Introduction

The evolutionary success of angiosperms is largely attributable to their ability to produce fruits[1,2]. The fruit is developed from the female reproductive organs, gynoecium, after pollination[3]. The fruits enclose, nourish and protect the developing seeds from harsh environments, and upon maturation, they facilitate seed dispersal and colonization of new habitats through diverse mechanisms[3]. Variations in fruit shape are associated with specific dispersal strategies[3]. Accordingly, the developmental program underlying fruit shape determination must be robustly regulated, as variations in this process would result in adaptive penalties due to limited dispersal[4,5]. Recently, the heart-shaped fruit of *Capsella* has been established as a model for understanding the developmental genetics of fruit shape determination[5]. We previously showed that development of

the heart shape is promoted by localized auxin biosynthesis at the apex of the valve tips[6,7]. This auxin maximum in the apical region reshapes the ovate spheroid gynoecium into a heart-shaped fruit through dynamic changes in cell growth and cell division[8]. However, how cell growth and division are coordinated during the heart-shape fruit development remain largely unknown.

Results and Discussion

The *Crjag* mutant produces deformed fruits with reduced cell divisions and cell growth

To identify the genetic components controlling the development of heart-shaped fruits in *Capsella*, we carried out a forward genetic screen of an EMS-induced *Capsella rubella* (Cr22.5) mutant population. One mutant, *EMS1196B*, was identified based on its strong defects in fruit development with compromised growth in the fruit valves (Figure 1a, b; Ref. 9). Bulked segregation analysis (BSA) revealed that the causal gene of the *EMS1196B* encodes a C2H2 zinc finger transcription factor (*Carub.0002s1499*), which is orthologous to the *Arabidopsis JAGGED* (*JAG*) gene[9-11]. We henceforth refer to the *EMS1196B* as *Capsella rubella JAGGED* (*CrJAG*) in the following sections.

Development of the heart-shaped fruit in Wild type *Capsella* (CrWT) starts from an ovate spheroid gynoecium soon after pollination at stage 12 (Supplementary Figure 1a). From stage 13 onwards, reshaping of fruit growth initiates from the apical parts of the valves and gradually leads to the formation of the heart shape by stage 14 (Supplementary Figure 1a and Ref. 6). In the *Crjag* mutant, however, this fruit reshaping process is abolished because valve outgrowth is significantly compromised, resulting in a fruit with an inverted triangular shape at stage 14 (Supplementary Figure 1b). To uncover the cellular basis of the *Crjag* phenotype, we performed a large-scale comparative live-imaging analysis between CrWT and *Crjag* fruits from developmental stage 12 (fertilization) to stage 14, when the most significant defects of the *Crjag* mutant are observed (Supplementary Figure 1a, b). Cell growth parameters, including growth rate (cell area expansion), cell proliferation, and anisotropy (direction of cell expansion), were visualized using lineage-tracking maps (Figure 1c, d). For each genotype, we conducted two biological replicates (see Methods). Analysis of the cell size heatmaps demonstrated a consistent distribution pattern

between replicates (Figure 1c, d and Supplementary Figure 2). Fruit morphogenesis in CrWT is characterized by the acropetal differentiation of epidermal cells in the valves in which cell expansion initiated from the base at stage 13 (Figure 1c). At stage 14, large cells are predominantly found at the base while smaller cells are distributed at the tip (Figure 1c and Supplementary Figure 1c and Ref. 8). In *Crjag* fruits, however, cell size increase occurs as early as stage 12 and ectopic large cells are observed in the apical and middle parts of the valves at stage 14 (Figure 1d and Supplementary Figure 1d). These data suggest that valve differentiation process is accelerated in *Crjag* fruits. During organ morphogenesis, cell growth contributes substantially to organ size once cells exit mitosis[12]. Analysis of areal growth rates reveals a pronounced acceleration of growth in CrWT valves from stage 13 to 14, consistent with the rapid increase in fruit size during this period (Figure 1e, f). In contrast, *Crjag* valves exhibit the opposite trend: growth rate is higher at stage 13 and decreases at stage 14 (Figure 1e, f). Comparative mapping and quantification of cell proliferation rates reveal a substantial reduction in cell division events (approximately 20-fold reduction in *Crjag* compared with CrWT) in the valve epidermis (Figure 1g, h and Supplementary Figure 1e). In addition to cell growth and division, a region of high anisotropic cell growth at the apical part of the fruit near the replum is critical for pushing the upper part of the valve outward to create the heart shape (Figure 1i, j). This pattern of anisotropic growth is completely lost in the upper part of *Crjag* fruits, with only residual anisotropic growth detected in the lower part of stage-13 *Crjag* fruits (Figure 1i, j). In summary, this comparative organ-wide cellular analysis suggests that *CrJAG* controls fruit shape determination by coordinating the differentiation process in the valves.

Ectopic cell expansion coincides with increases in endoreduplication in the *Crjag* fruits

In eukaryotic cells, the enlargement of cell volume is often associated with endoreduplication, a process in which chromosomal DNA is duplicated without undergoing mitosis[13]. In plant organs, cell size is strongly correlated with DNA ploidy levels[14,15]. In *Crjag* fruits, valve epidermal cells are ectopically expanded throughout the valves compared with CrWT (Figure 1c, d; mostly evident

in stage-14 fruits). To assess the contribution of endoreduplication to cell size changes and fruit shape development, we performed comparative flow cytometry of nuclei from CrWT and *Crjag* valves of stage-17 fruits. Compared to CrWT fruits, where most nuclei had a 2C DNA content, *Crjag* fruits exhibited a striking increase in the population of 4C and 8C nuclei (Supplementary Figure 3a, b). Moreover, quantification of the average number of endocycles per nucleus using the Endoreduplication Index (EI, Ref. 16) revealed a significant increase in EI in *Crjag* compared with CrWT (Supplementary Figure 3c). Altogether, these data indicate that the ectopic expansion of epidermal cells in the *Crjag* valves is accompanied by increase in endoreduplication events.

Genetic validation of *CrJAG*

The mutation responsible for the *Crjag* phenotype disrupts the donor site of the third intron that integrates this 244-bp intron with the fourth exon (Figure 2a; Ref.9). The resulting frameshift introduces a premature stop codon within the intron sequence, producing a truncated 115 amino acid (aa) protein instead of the 252-aa WT CrJAG protein (Figure 2a). To confirm the role of *CrJAG* in fruit shape development, we generated an independent mutant allele of *Carub.0002s1499* (*Crjag*^{ge}, "*ge*" indicating "genome editing") using the CRISPR/Cas9 system (Figure 2a and Supplementary Figure 4a). A single base-pair insertion in the second exon caused a frameshift that generated a 40-aa peptide (Figure 2a and Supplementary Figure 4a). The fruits of the *Crjag*^{ge} mutant perfectly phenocopied the *Crjag* fruit phenotype, and F_1 plants from a cross between *Crjag* and *Crjag*^{ge} showed no genetic complementation (Figure 2b). Moreover, a GFP-tagged CrJAG protein driven by the native CrJAG promoter (*pCrJAG:*CrJAG:GFP) effectively complements the *Crjag* fruits defects (Figure 2b and Supplementary Figure 4b). Collectively, these results provide compelling genetic evidence that the developmental defects observed in *Crjag* are caused by a loss-of-function mutation in Carub.0002s1499, which encodes a C2H2 transcription factor orthologous to *Arabidopsis JAG*.

***CrJAG* is dynamically expressed during fruit development**

To explore the expression pattern of the *CrJAG* gene during fruit development, we generated a *pCrJAG*:GUS reporter line by fusing a 4.5 kb promoter fragment to the β-glucuronidase (GUS) reporter gene. In agreement with the strong developmental defects in *Crjag* fruits, GUS expression was specifically localized to the lateral apical region of the ovary and to the valves during gynoecium patterning and fruit development (Figure 2c). Its expression in the ovary was initially detected at stage 8 and subsequently became stronger, reaching a maximum in the lateral valve regions at stages 12 and 13 (Figure 2c). After stage 14, when the heart shape is established, expression decreased gradually (Figure 2c). In addition, strong *CrJAG* expression was also detected in the valve margins from stage 12 to 14 (Figure 2c). Notably, the continuous and dynamic expression of *CrJAG* in *Capsella* valves contrasts with the expression of *JAG* in *Arabidopsis* gynoecium, where *JAG* displays transient valve-specific expression before stage 9, after which expression becomes restricted to the valve margins[11]. This divergence in expression patterns may reflect promoter differences between these orthologous genes, as seen for *INDEHISCENT*[6]. Indeed, the *Arabidopsis jag* mutant produces fruits similar to WT without obvious morphological alterations except that apical region of the fruit valves is slightly downturned (Supplementary Figure 5a-c; Ref.10).

CrJAG interacts with histone chaperones to regulate fruit development

To identify proteins that interact with CrJAG in fruit shape determination, we resorted to two independent approaches, i.e. immunoprecipitation followed by mass spectrometry (IP-MS) with a GFP antibody against CrJAG:GFP fusion proteins from *Crjag;pCrJAG:CrJAG:GFP* complemented fruits, and yeast two-hybrid (Y2H) library screening using CrJAG-BD as a bait on the library prepared from stage-10 to stage-13 fruits, when CrJAG are highly expressed (see Methods). Because the full-length CrJAG-BD exhibited strong auto-activation activity in the Y2H system, we performed a systematic truncation analysis to exclude the region responsible for auto-activation and used it for the library screening (Supplementary Figure 6). An overlap analysis of the proteins identified from these two independent approaches yielded a list of four high-

confidence candidate interactors (Figure 3a, b and Supplementary Data 1). Among these, the histone chaperone MULTICOPY SUPPRESSOR OF IRA1 3 (CrMSI3) was of particular interest, as its homologs have prominent roles in plant development[17–19]. The other three candidates are orthologous to *Arabidopsis LWD2* (*LIGHT-REGULATED WD2*), *ISI1* (*IMPAIRED SUCROSE INDUCTION1*), and a tetratricopeptide repeat (TPR)-containing protein, which have been implicated in light responses and physiological processes[20–22], respectively. These candidates were excluded for further analysis in this study.

MSI proteins belong to a family of WD40 histone chaperones conserved in yeast, mammals, and plants[23]. The *Capsella* genome encodes five closely related homologs, CrMSI1 to CrMSI5 (Figure 3c). While no interaction and only weak interaction were detected with CrMSI5 and CrMSI1, respectively (Figure 3d), CrJAG exhibits strong interaction with CrMSI2, CrMSI3, and CrMSI4 in the Y2H assays (Figure 3d). These interactions were further substantiated *in vivo* by BiFC and Co-IP experiments (Figure 3e, f and Supplementary Figure 7). Consistent with these interactions, CrJAG, CrMSI2, CrMSI3, and CrMSI4 were co-localized in the nucleus when transiently expressed in protoplasts (Supplementary Figure 8). Furthermore, promoter reporter analysis revealed that *pCrMSI2:GUS* and *pCrMSI3:GUS* showed strong expression in developing valves, overlapping with the *CrJAG* expression domain, whereas *CrMSI4* promoter activity is weakly detected in the valves (Figure 4a-c). To test whether CrMSI2 and CrMSI3 are required for *Capsella* fruit shape determination, we generated knock-out mutants by CRISPR/Cas9 (Supplementary Figure 9). Unexpectedly, neither single loss-of-function mutants in *Crmsi2*, *Crmsi3*, nor the *Crmsi2*;*Crmsi3* double mutant shows any detectable fruit defects (Figure 4d). Homozygous high-order *Crmsi* mutants could not be recovered, suggesting that further loss of *CrMSI* function may have led to embryo lethal[23,24]. Given the weak but detectable expression of *CrMSI4* in fruits, it may function redundantly with CrMSI2 and CrMSI3 proteins in regulating fruit shape development.

MSI proteins are histone chaperones without DNA binding domains, their target specificity is determined by the transcription factors they interact with[23-25]. To further unveil the functional

importance of the CrJAG-CrMSI2/3 protein complex in fruit development, we fused the DNA-binding domain (DBD) of CrJAG to either CrMSI2 or CrMSI3 with the idea that amino acid sequence outside the DNA-binding domain of CrJAG may function primarily to recruit MSI1-like proteins. We examined their ability to complement the *Crjag* fruit defects (Figure 4e). Whereas the control construct (*pCrJAG:*DBD:GFP) failed to complement the fruit defects, either *pCrJAG:*DBD-CrMSI2:GFP or *pCrJAG:*DBD-CrMSI3:GFP construct effectively rescued the *Crjag* fruit defects (Figure 4f). In summary, these expression and genetic analyses suggest that *CrJAG* plays a crucial role in fruit development by directly interacting with MSI1-like proteins in *Capsella*.

The *Crjag* phenotype is attributed to the down-regulation of its target genes

To dissect the molecular mechanism underlying CrJAG-regulated fruit development in *Capsella*, we first performed a comparative transcriptomic analysis of stage-13 fruits (when shape changes begin) between CrWT and *Crjag* (Supplementary Figure 1a, b). This analysis identified 3,574 differentially expressed genes (DEGs, |logFC|>1, FDR<0.05), of which 1,913 were up-regulated and 1,661 were down-regulated (Supplementary Figure 10a, b). Next, we performed a CUT&Tag experiment using a GFP antibody against CrJAG:GFP from *Crjag;pCrJAG*:CrJAG:GFP stage-13 fruits. This experiment identified 18,054 CrJAG:GFP binding peaks associated with 7,445 genes (see Methods). Integration of the RNA-seq and CUT&Tag datasets revealed 454 up-regulated DEGs and 373 down-regulated DEGs as CrJAG targeted genes (Supplementary Figure 10a, b and Supplementary Data 2). Gene Ontology (GO) enrichment analysis of the 454 up-regulated direct targets showed that these genes were enriched in GO terms related to response to stimulus (GO: 0050896), transmembrane transport (GO: 0055085) and other basic biological processes (Supplementary Figure 10c), whilst the 373 down-regulated direct targets were enriched in GO terms related to the cellular developmental process (GO: 0048869), cell cycle (GO:0007049), and microtubule-based process (GO: 0007017) (Supplementary Figure 10d). These results corroborate the live-imaging data and suggest that the *Crjag* fruit defects are primarily due to the down-

regulation of genes involved in cell cycle and differentiation, whereas the up-regulated genes appear to appear to have minimal phenotypic contribution.

The CrJAG–CrMSI2/3 complex governs fruit shape determination by sustaining the expression of *CrAUR2*

MSI1-like proteins bind to histones and recruit protein complexes that regulate chromatin accessibility, thereby modulating gene expression[19,24,25]. To pinpoint the target genes of the CrJAG–CrMSI2/3 complex in fruit shape determination, we performed comparative Assay for Transposase-Accessible Chromatin with sequencing (ATAC-seq) on stage 12–14 fruits from CrWT and *Crjag*. We focused on chromatin regions that were closed in *Crjag* compared with CrWT and integrated these data with the 373 directly down-regulated genes (Supplementary Data 2). This analysis revealed that 138 of the 373 down-regulated direct targets were associated with loss of chromatin accessibility in the *Crjag* fruits (Figure 5a, b and Supplementary Data 2). GO analysis of the 138 target genes identified genes related to the cell cycle (GO:0007049) that are significantly enriched (Supplementary Figure 11a).

Among the 10 genes associated with cell cycle (Supplementary Figure 11b), *CrAURORA2* (*CrAUR2*) caught our attention, as mutations in *AUR* orthologs in *Arabidopsis* result in defects in cell divisions, endoreduplication and deformed organs[26]. Using GUS reporter lines, we observed strong *pCrARU2:GUS* expression in developing valves, consistent with a role in valve morphogenesis (Supplementary Figure 12). Strikingly, this valve expression is almost abolished in *Crjag* fruits (Supplementary Figure 12), indicating that *CrJAG* is required for the transcriptional maintenance of *CrARU2* in valves. Indeed, using chromatin immunoprecipitation (ChIP) analysis, we further confirmed that CrJAG, CrMSI2 and CrMSI3 are co-associated with the identical regulatory regions within the *CrAUR2* locus (Figure 5c). Moreover, the DNA binding ability of CrMSI2 and CrMSI3 is dependent on CrJAG, as their ChIP enrichment is abolished in the absence of CrJAG proteins (Figure 5c). These data indicate that CrJAG is essential for recruiting CrMSI proteins to the *CrAUR2* locus, thereby sustaining its expression. In agreement, expression of

CrAUR2 was perfectly restored in *Crjag;pCrJAG*:DBD-CrMSI2:GFP and *Crjag;pCrJAG*:DBD-CrMSI3:GFP fruits, but not in *Crjag;pCrJAG*:DBD:GFP fruits (Figure 5d).

AUR kinase activity can be selectively blocked by an ATP-competitive inhibitor, Aurora Kinase Inhibitor II[27]. In line with a potential role of CrAUR2 in fruit development, treatment of the CrWT stage-12 fruits with Aurora Kinase Inhibitor II predominantly suppressed heart-shaped fruit development (Supplementary Figure 13a). Subsequent flow cytometric analysis indicates that the effect of Aurora Kinase Inhibitor II on fruit shape determination is associated with a significant increase in 4C nuclei and EI in the valve tissues and a decrease in 2C nuclei, in a pattern similar to that observed in the *Crjag* valves (Compare Supplementary Figure 13b-d with Supplementary Figure 3a-c). In contrast, *Crjag* mutant fruits failed to respond to Aurora Kinase Inhibitor II treatment (Supplementary Figure 13e-h), implying that Aurora Kinase activity is severely compromised in the mutant. Notably, the strong phenotypic effect of Aurora Kinase Inhibitor II on CrWT fruit shape development appears inconsistent with the limited cell division activity observed in the epidermis by live imaging analysis (Figure 1g, h). This discrepancy suggests that the key cell divisions driving heart-shaped fruit development may occur either prior to stage 12 or within the subepidermal layers, which were not accessible in time-lapse live-imaging experiment. Collectively, these expression and pharmaceutical results provide strong evidence that CrJAG-CrMSI complexes exert their function by directly regulating cell-cycle related genes, such as CrAUR2, in determining fruit shape.

In *Arabidopsis*, JAG directly represses the expression of the cell cycle inhibitors *KIP RELATED PROTEIN 4* (*KRP4*) and *KRP2*, thereby promoting the growth of the distal region of petals[28]. However, these genes were not identified in our analysis, indicating that CrJAG may regulates its target genes in an organ specific manner and further suggest a conserved role of JAG in plant organ development by regulating divergent components involved in the cell cycle machinery.

Changes in chromatin accessibility on CrJAG target genes are correlated with alterations in

H3K18ac and H3K27me3 levels

In the nucleus, open chromatin increases genome accessibility to transcription factors and the transcription machinery, thereby activating transcription, whereas closed chromatin represses transcription by limiting access[29]. During development, chromatin accessibility and gene expression are dynamically regulated by covalent histone modifications[30,31]. In *Arabidopsis*, MSI family proteins interact with HISTONE DEACETYLASE19 (HDA19) in a histone deacetylase complex and with CUL4–DDB1 in the Polycomb repressive complex 2 (PRC2), thereby dynamically modifying histone marks and regulating gene expression[19,32–35]. We next investigated whether the impact of the CrJAG–CrMSI2/3 complex on chromatin accessibility and target genes' expression is linked to alterations in histone modification patterns. To test this, we performed comparative CUT&Tag profiling of eight histone marks in a fruit sample between CrWT and *Crjag*, including the repressive mark H3K27me3 and seven active marks (H3K4me1, H3K4me3, H3K9ac, H3K14ac, H3K18ac, H3K23ac, and H3K27ac) [30]. In agreement with the reduced gene expression in the *Crjag* fruits, changes in chromatin accessibility are predominantly found in the regulatory regions of the 138 direct target genes (Figure 5b). Among the eight histone modifications profiled, six (H3K4me1, H3K4me3, H3K9ac, H3K14ac, H3K23ac, and H3K27ac) showed no consistent change in the *Crjag* mutant compared to CrWT across the 138 target genes (Supplementary Data 3). In contrast, H3K18ac and H3K27me3 exhibited a pronounced reciprocal pattern of enrichment (59 out of 138, with simultaneously reduced enrichment of H3K18ac and increased enrichment of H3K27me3) (Figure 5e and Supplementary Data 3). This shift in the epigenetic landscape is particularly evident around the proximal promoter region, where it coincides with altered chromatin accessibility (Figure 5b, f and Supplementary Figure 14). Notably, this coordinated change in histone modification and accessibility is observed in 3 (including *CrAUR2*) of the 10 genes involved in cell-cycle regulation (Figure 5f; Supplementary Figure 14; Supplementary Data 3). Thus, the shutdown of chromatin accessibility and gene expression in these target genes can be largely explained by reciprocal changes in covalent modification levels of H3K18ac and H3K27me3. However, how H3K18ac and H3K27me3 are modified on the target genes by the

CrJAG-CrMSI2/3 complex is an intriguing question that warrants investigation in future studies.

Concluding remarks

In this study, we dissected the mechanism by which the C2H2 zinc finger transcription factor CrJAG regulates fruit morphogenesis through coordinated cell division and expansion. During heart-shaped fruit development, CrJAG recruits histone chaperones CrMSI2/3 to sustain the expression of target genes associated with cell-cycle and differentiation process, such as *CrAUR2*, to promote fruit morphogenesis (Figure 6). The CrJAG-CrMSI2/3 complex modulates chromatin accessibility, likely by altering the deposition of H3K18ac and H3K27me3 histone markers, to facilitate their proper expression of the target genes during fruit shape development (Figure 6). While rewiring of developmental genes in new regulatory networks is known to be a primary mechanism for organ development, our work demonstrates that direct epigenetic modulation by a core developmental regulator represents an essential, additional layer of regulation for precise organ morphogenesis.

Methods

Plant materials, transformation and growth conditions

All *Capsella* materials used in the study were in the *Cr22.5* ecotype background. The *Arabidopsis* materials are in Col-0 ecotype. Seeds were sterilized with 5% sodium hypochlorite supplied with 0.1% Triton X-100 and germinated on MS medium with 10 mM GA3[36]. The 10-day-old seedlings were transplanted into soil in the controlled environment room (CER) at 22°C under long day conditions (16 h light / 8 h dark). The tobacco (*Nicotiana benthamiana*) plants used in this study were grown at 22°C under long day (16 h light / 8 h dark) conditions. 3-week-old seedlings were used for agroinfiltration.

Scanning electron microscopy (SEM) and phenotypic analysis

For SEM analysis, inflorescence samples were rapidly fixed with formalin-acetic acid-alcohol (FAA) for 48 hours at room temperature. Gynoecia or fruits at different developmental stages were dissected from the inflorescence under a microscope in 70% alcohol. Dehydration was performed with a series of graded ethanols (80%, 85%, 90%, 95%, 100%, each for 30 min) followed by critical point drying with CO_2. The samples were subsequently sputter-coated with gold and analysed on an S-4800 FESEM system (Hitachi, Japan). For whole-mount fruit images, stage-17 fruits of each genotype were collected and photographed using a Nikon D850 camera with a 105

mm prime lens.

Live-cell imaging and cell behavior analysis

To conduct live-cell imaging analysis, the *pUBQ10:acyl-YFP* reporter was introgressed into the *Crjag* background by crossing. The plants were cultivated on soil in the CER to the bolting stage. For both CrWT and *Crjag*, the stage-12 fruits were dissected and imaged, capturing YFP signals at 24-hour intervals using a Zeiss inverted laser confocal microscope (Zeiss LSM 980) equipped with a water immersion objective (×25/0.95). Confocal images were acquired at a resolution of 1024×1024, with 0.5 μm in the Z-stacks. Between each time point, the samples were grown on Petri dishes containing 1/2 MS medium supplemented with vitamins (PM1011, Coolaber) and 1% sucrose in the CER under long-day conditions (22°C, 16 hours light/8 hours dark) to allow development. The images were stitched and analyzed using MorphoGraphX software [37]. Heatmaps showing the differences between two consecutive time points were generated, with the heatmap being shown on the fruit stage at the later time point. To quantify growth anisotropy, cell area ratio and proliferation, the fluorescence was projected into a mesh, cell outlines were segmented, and relationships between cells were tracked across successive time points. For each genotype, two biological replicates were conducted, and representative growth or proliferation maps were obtained from a single experiment. To quantify cell division events, parameters were extracted using MorphoGraphX software and the Wilcoxon test was used to test significance.

Plasmid construction and plant transformation

To generate the promoter-GUS reporter plasmids, the promoter regions of *CrJAG* (*Carub.0002s1499*, 4549 bp upstream of ATG), *CrMSI2* (*Carub.0003s3060*, 560 bp), *CrMSI3* (*Carub.0007s0526*, 1043 bp), *CrMSI4* (*Carub.0003s3381*, 4267 bp), *CrAUR2* (*Carub.0004s0570*, 1354 bp)[8] were isolated using PrimeSTAR GXL DNA Polymerase (R050A, Takara) from genomic DNA. The DNA fragments were gel-purified and then inserted upstream of the *GUS* gene in the *pCambia*1301 vector. To generate the *pCrJAG*:*CrJAG*:*GFP* complementary plasmid, the promoter and coding sequences of *CrJAG* were recombined by PCR, and then the PCR products were purified and fused in-frame with the GFP sequence in the *pCambia*1302 backbone. To construct the chimeric complementary plasmids, the DNA-binding domain of *CrJAG* (DBD, 117 bp, plus start codon, 40 aa) was amplified from *pCrJAG*:*CrJAG*:*GFP* plasmids, then recombined with the coding sequences of *CrMSI2* or *CrMSI3* by PCR. The PCR products were gel-purified and inserted into the *pCambia*1302 backbone. To construct the CaMV 35S overexpression plasmids for subcellular localization, the coding sequence of the genes were amplified and inserted in-frame with GFP, driven by the 35S promoter, in the *pCambia*1302 vector. To generate the CRISPR/Cas9 gene editing plasmids, the gRNAs targeting the first or second exon of the target genes were designed using the CRISPR-P 2.0 tool[38]. The gRNAs were synthesised as oligonucleotides with Golden-gate cloning adapters and then were insert downstream of U6 promoters to generate the U6:gRNA plasmid using golden-gate cloning methods. The resulting U6:gRNA plasmids were then integrated with *pRPS5a:Cas9z:E9t* and *35S:HptII* (HYG) selection marker to produce the binary vectors. All vectors were verified by sequencing and introduced into *Agrobacterium*

tumefaciens strain GV3101. Details of the primers are listed in Supplementary Table S1.

The transformation of *Capsella* followed the floral dipping method[36]. Positive transformants were selected using 40 mg/L Hygromycin on MS medium. For each construct, at least 10 independent transgenic lines were produced for further analysis.

GUS staining and confocal microscopy

For GUS histochemical analysis, inflorescences containing stage 10-15 fruits were fixed in acetone for 20 mins at -80°C, then washed twice for 5 mins with 100 mM sodium phosphate buffer. The samples were then blocked with 100 mM sodium phosphate buffer containing 1mM $K_3Fe(CN)_6$, 1mM $K_4Fe(CN)_6$ at room temperature for 30 mins. Then. The samples were subjected to staining reaction by incubating at 37°C in the X-Gluc solution [100 mM sodium phosphate buffer, 10mM EDTA, 0.5 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$, 0.1% Triton X100 and 1 mg/ml of ß-glucoronidase substrate X-gluc (5-bromo-4-chloro-3-indolyl glucuronide, MELFORD) dissolved in DMSO]. After staining, samples were dissected, bleached with 70% ethanol, mounted in Chlorohydrate (Sigma) solution and analyzed using a Zeiss Axio Imager light microscope.

For confocal microscopic observation of CrJAG:GFP signals, stage 10-14 fruit samples were dissected under a microscope, and GFP signals were imaged using a laser scanning confocal microscope (Zeiss LSM 980) with excitation and emission wavelengths of 488 nm and 509 nm, respectively.

Yeast-two hybrid library construction and protein-protein interaction analysis

To construct the yeast-two-hybrid library for CrJAG interactor screening, fruits from stage-10 to stage-13 were collected and frozen immediately in liquid nitrogen for RNA isolation. Total RNA was isolated, and mRNA was purified using the Oligotex mRNA Midi Kit (70042, Thermo Fisher) according to the manufacturer's instructions. cDNAs were subsequently synthesized using the Revert Aid First Strand cDNA Synthesis Kit. A high-quality cDNA library was constructed using the CloneMiner II cDNA Library Construction Kit (A11180, Thermo Fisher). The cDNAs were ligated with a three-frame attB1 adapter by T4 ligase and then purified using the cDNA size fractionation columns. The purified cDNAs were inserted into the pDONR222 vector using BP Clonase (11789020, Thermo Fisher) and recombined into the pGADT7-DEST vector using LR Clonase (11791020, Thermo Fisher) to generate the yeast-two-hybrid cDNA library.

To avoid the autoactivating activity of *CrJAG*, a series of truncated versions of *CrJAG* were amplified from the *pCrJAG*:*CrJAG*:*GFP* plasmid and then inserted into pGBK-T7 (Clontech) to generate the bait plasmids. The respective plasmids were verified by sequencing and then transformed into the yeast strain Y2H gold with pGAD-T7 (Clontech). Positive transformants were selected on synthetic dropout (SD) medium without tryptophan and leucine (-WL). The autoactivating activity was tested by serial dilutions on SD medium without tryptophan, leucine, histidine, and adenine (-WLHA) according to the manufacturer's protocols. The yeast-two hybrid library screening was performed according to the manufacturer's instructions (630439, Clontech)

For verifying the CrJAG-CrMSIs interactions, the coding sequences of *CrMSI1*, *CrMSI2*, *CrMSI3*, *CrMSI4*, and *CrMSI5* were amplified from the *Capsella* inflorescences cDNAs and then

inserted into pGAD-T7 to generate prey plasmids. The interaction was tested as described above.

For the Biomolecular Fluorescence Complementation (BiFC) experiment, the full-length CDS without stop codon of *CrJAG* and *CrMSI2*, *CrMSI3*, and *CrMSI4* were amplified and inserted into pBI-cYFP and pBI-nYFP backbones, respectively. The constructs were verified by sequencing and then transformed into the *Agrobacterium* strain GV3101. Agrobacteria were inoculated in YEB medium overnight to OD600 of 1.0-1.2 and then resuspended with infiltration buffer (50 mM MES, pH 5.6, 0.5% glucose, 2 mM $NaPO_4$, and 15 mg/L acetosyringone) to OD600 of 0.4-0.5. A mixed *Agrobacterium* suspension containing nYFP and cYFP plasmids at a 1:1 ratio was infiltrated into 3-week-old *Nicotiana benthamiana* leaves. The plants were incubated under weak light for 36 hours before signal checking. Protein interaction was judged by the presence of YFP signal in the nucleus using a laser scanning confocal microscope (Zeiss LSM 980) with excitation at 488 nm and emission at 509 nm, respectively. When capturing the images, samples were scanned along the Z-axis with 5-μm distance. The results were then analyzed in ImageJ software.

For the Co-IP experiment, the full-length coding sequences without stop codon of *CrJAG*, *CrMSI2*, *CrMSI3* and *CrMSI4* were inserted into *pCambia*1300-cHA or *pCambia*1300-cFLAG backbones, respectively. The positive plasmids were verified by sequencing and then transformed into the *Agrobacterium* strain GV3101. They were co-infiltrated into *Nicotiana benthamiana* leaves as described above. The infiltrated leaves were collected 36 h after infiltration and ground into fine powder in liquid nitrogen. About 0.5 g sample was used for total protein isolation with 1 mL protein extraction buffer [50 mM Tris-HCl (pH=7.5), 150 mM NaCl, 1 mM EDTA, 2 mM NaF, 2 mM Na_3VO_4, 0.5% NP-40, 1 mM PMSF, 1 mM DTT and 1×Protease Inhibitor Cocktail]. 100 μL total protein mixture was kept as input and the remaining mixture was subjected to immunoprecipitation using DYKDDDDK (FLAG)-Nanoab-Magnetic beads (FNM-25-1000, Lablead) for 1 h at 4°C. The beads were then washed four times with buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, and 1 mM DTT. The input and IP samples were boiled for 10 mins in SDS loading buffer, and the eluates were analyzed by immunoblotting with anti-HA (MBL, Japan, 1:10000) or anti-FLAG (MBL, Japan, 1:10000) antibody.

Immunoprecipitation-Mass Spectrometry (IP-MS) analysis

About 3 g of fruits containing stage 10-13 from *Crjag; pCrJAG*:*CrJAG*:*GFP* and wild type were collected and crosslinked with 1% formaldehyde in PBS buffer (150 mM NaCl, 10 mM Na_2HPO_4, 2 mM KH_2PO4, 2.7 mM KCl, pH 7.4). The fruits samples were ground into fine powder in liquid nitrogen, and nuclei were isolated with Honda buffer [0.44 M sucrose, 1.25% Ficoll, 2.5% Dextran T40, 20 mM HEPES (KOH, pH 7.4), 0.5% Triton X-100, 10 mM $MgCl_2$ and 1×Protease Inhibitor Cocktail]. The nuclei were then resuspended in RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 5 mM DTT, and 1× Protease Inhibitor Cocktail) and sonicated for 24 cycles with 30 seconds on and 30 seconds off. The DNA was digested with Benzonase (E1014-25KU, Millipore) for 1 h at 4°C by gentle rotation. The protein extracts were then subjected to immunoprecipitation using Pierce Protein A/G Magnetic Beads (88847; Thermo Fisher), incubated with monoclonal anti-GFP antibody (ab290, abcam) at 4°C for 4 h. After immunoprecipitation, the

beads were washed twice with PBS buffer plus 0.5% NP-40, and once with PBS buffer and water, respectively. The beads were digested with 2 μg of Trypsin/P and in 50 mM NH_4HCO_3 solution overnight at 37°C. The peptides were purified with C18 stage tip and then processed for mass spectrometry on an Orbitrap Exploris 480 machine (Thermo Fisher).

For data collection, MS1 spectra were collected in the Orbitrap every 0.6 s at a resolving power of 240,000 at m/z 200 over m/z 380–980. The MS1 normalized automatic gain control (AGC) target was set to 300% (3e6 charges) with a maximum injection time of 10 ms. DIA MS2 scans were acquired in the Astral analyzer over an m/z range of 380–980, with a normalized AGC target of 500% (5e4 charges), a maximum injection time of 3.5 ms, a higher-energy collisional dissociation (HCD) collision energy setting of 27%, and a default charge state of +2. Window placement optimization was enabled and isolation widths of 2 Th and an active gradient length of 23 minutes were used.

Label-free quantification was performed with DIA-NN software (version 1.9). The protein database of *Capsella rubella* (28,039 sequences) was downloaded from the UniProt Swiss-Prot database. MS/MS searches for the proteome datasets were performed with the following parameters: Oxidation of methionine and protein N-terminal acetylation as variable modifications; carbamidomethylation as fixed modification. A maximum of peptide charge was set to 6 and 1 missed cleavages per peptide was allowed. The mass tolerance for precursor ions was set to 20 p.p.m. for the first search (used for nonlinear mass re-calibration) and 7 p.p.m. for the main search. Peptide and protein FDRs were set to 1%. Proteins with at least two peptides coverage were considered as reliable targets. Label-free protein quantification was switched on, and unique and razor peptides were considered for quantification with a minimum ratio count of 1. Retention times were recalibrated based on the built-in nonlinear time-rescaling algorithm. MS/MS identifications were transferred between LC-MS/MS runs with the "Match between runs" option in which the maximal retention time window was set to 2 min. The quantification is based on the MS2 intensity. Statistical analyses were performed with a two tailed unpaired Student's t-test.

Cleavage under targets & tagmentation (CUT&Tag) experiment

The CUT&Tag experiment was performed following described methods with minor modifications[39]. Briefly, stage-10 to stage-13 fruits collected from either CrWT, *Crjag* or *Crjag;pCrJAG:CrJAG:GFP* rescue lines were chopped with a razor blade in HBM buffer (25 mM Tris-HCl pH 7.6, 0.44 M sucrose, 10 mM $MgCl_2$, 0.1% Triton-X-100, 0.2 M spermidine, 1× Protease Inhibitor Cocktail, 1 mM PMSF, 10 mM β-Mercaptoethanol). The nuclei suspension was filtered with a 40 μm cell strainer. The extracted nuclei were washed twice with HBB buffer (25 mM Tris-HCl pH 7.6, 0.44 M sucrose, 10 mM $MgCl_2$, 0.1% Triton-X-100, 10 mM β-Mercaptoethanol) and then incubated in 50 μL primary antibody buffer [2 mL wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM spermidine and 1× Cocktail inhibitor) supplemented with 8 μL 0.5 M EDTA and 6.7 μL 30% BSA] at 4°C overnight. 1 μL of primary antibody (1:50, anti-GFP, ab290, abcam; anti-H3K18ac, 07-354, Millipore; anti-H3K27me3, 07-449, Millipore) was used for each sample. The primary antibody was removed after centrifugation at 1200 g for 2 min. Subsequently, the nuclei were incubated with secondary antibody [1:100, Guinea pig anti-rabbit

IgG (Heavy & Light Chain) antibody, ABIN101961, Easybio] in 50 μL wash buffer at 4°C for 2 h. The nuclei were resuspended with 100 μL CT-300 buffer (20 mM HEPES pH 7.5, 450 mM NaCl, 0.5 mM spermidine and 1×Protease Inhibitor Cocktail) supplied with pA-Tn5 (1:150) and incubated at 4°C for 3 h. After incubation, the nuclei were washed twice with 600 μL CT-300 buffer, followed by incubation at 37°C for 1 h with 300 μL tagmentation buffer (1 mL CT-300 buffer plus 10 μL 1 M $MgCl_2$). To stop the tagmentation reaction, 10 μL 0.5 M EDTA, 3 μL 10% SDS and 2.5 μL 20 mg/mL Protinease K were added r and incubated at 50°C for 1 h. The DNA was extracted with phenol: chloroform: isoamyl alcohol (25:24:1), precipitated with ethanol, and then dissolved in ddH_2O.

RNA-seq, ATAC-seq, and CUT&Tag-seq data analysis

For RNA-sequencing analysis, stage-13 fruits from different genotypes were collected and frozen immediately in liquid nitrogen. Total RNA was isolated with the SV Total RNA Isolation System (Z3100, Promega) according to the manufacturer's instructions. The libraries were then constructed using the MGIEasy RNA Library Prep Kit and then sequenced on the DNBSEQ-T7 platform to generate 150 bp paired-end reads. Low-quality reads and adapter sequences were trimmed to generate clean reads using fastp (version 0.24.0)[40]. The sequences were then mapped and annotated using the *Capsella rubella* v1.1 genome sequence[41] with STAR (version 2.7.11b)[42]. Gene expression levels were calculated as fragments per kilobase of transcript per million fragments (FPKM) using an R script. Differentially Expressed Genes (DEGs) were identified as those with a fold change ≥ 2 and a False Detection Rate (FDR) < 0.05 using the R package DESeq2 (version 1.42.1)[43]. GO enrichment analysis of Biological Process (BP) on the DEGs was performed using the R packages enrichplot (version 1.22.0) and clusterProfiler (version 4.10.1)[44]. Boxplots were drawn using the R package ggplot2 (version 3.5.2)[45]. For the RNA-seq experiment, at least three independent biological replicates were conducted for each genotype.

The ATAC-seq experiment was performed following the protocol previously described[8]. Briefly, ~0.5 g of stage-12 to stage-14 *Crjag* fruits were harvested and ground into fine powder in liquid nitrogen. Approximately 50,000 nuclei were collected for DNA library construction. The library was sequenced on the Illumina Novaseq 6000 platform to generate 150 bp paired-end reads. The raw reads were filtered (reads shorter than 35 bp and bases with a quality value less than Q10) using fastp (version 0.24.0)[40] to generate clean FASTQ files. The sequences were then mapped back to the *Capsella rubella* v1.1 genome[41] using Bowtie2 (version 2.5.4)[46]. Duplicate reads were removed by sambamba (version 1.0.1)[47] and bedtools (version 2.31.1)[48]. Peaks were called using MACS2 (version 2.1.4)[49] with a screening criterion of FDR < 0.05. Differentially enriched peaks between CrWT and *Crjag* were identified using the R package DiffBind (version 3.12.0). Significant closed chromatin accessibility in *Crjag* was defined as fold change < 0.8 and FDR < 0.05. DeepTools (version 3.5.6)[50] was used to map the density distribution of sequencing reads in the upstream and downstream regions of the Transcription Start Site (TSS) and the Transcription End Sits (TES) of each gene. The profile plot was generated using the plotProfile function. For visualization, datasets were converted to bigwig format using bamCoverage in DeepTools (version 3.5.6) with a bin size of 1 bp and normalized by the RPKM method, then visualized using Integrative Genomics Viewer (IGV, version 2.4.14)[51]. Gene annotation was performed by ChIPseeker (version 1.38.0)[52] using a 2 kb promoter-proximal window. The ATAC-seq

experiments were performed with two independent biological replicates.

For CUT&Tag-seq data analysis, the library was constructed by amplifying 10–12 cycles using Q5 High-Fidelity DNA Polymerase (M0491S, NEB) and purified by AMPure XP beads (A63881, Beckman) according to the manufacturer's instructions. Primers used for library amplification are listed in Supplementary Table S1. The library was sequenced on the Illumina Nova X Plus platform to generate 150 bp paired-end reads. The raw reads were filtered using fastp (version 0.24.0) to generate clean FASTQ files. After filtering low-quality reads, the sequences were mapped back to the *Capsella rubella* v1.1 genome using Bowtie2 (version 2.5.4). Duplicate reads were removed by sambamba (version 1.0.1) and bedtools (version 2.31.1). Peaks were called using MACS2 (version 2.1.4) with a *p* value of 0.01. The bigwig files were generated by DeepTools (version 3.5.6) for visualization using IGV (version 2.16.2). Gene annotation was performed with a 2 kb promoter-proximal window using ChIPseeker (version 1.38.0)[52]. For each antibody and genotype, two biological replicates were conducted.

For data integration, Venn diagrams were generated with the R package VennDiagram (version 1.7.3)[53].

Protoplast isolation, transient expression and ChIP-qPCR analysis

Protoplast isolation and transient protein expression were performed with the published protocols[54]. Briefly, leaves from 10-day-old young seedlings were sliced and digested with Enzyme buffer I (1.0 % cellulase R10, 0.5 % macerozyme R10, 0.5 % pectinase, 20 mM KCl, 20 mM MES, 0.6 M mannitol, 10 mM $CaCl_2$, 0.1 % BSA) rotating at 60 rpm and 25°C for 2.5 h. The protoplasts were collected by filtering with a 40 μm cell strainer. 35S:CrJAG/CrMSI2/CrMSI3/CrMSI4:GFP and AtUBQ10:NLS-mCherry plasmids were prepared using a QIAGEN Plasmid Maxi Kit according to the manufacturer's instructions. A total of 1000 ng plasmids (35S:CrJAG : AtUBQ10:NLS-mCherry = 1:1) were co-transformed into protoplasts with a PEG-mediated heat-shock method (42°C, 3mins). The protoplasts were incubated under weak light at 26°C for 18 h. The subcellular localization of CrJAG:GFP, CrMSI2:GFP, CrMSI3:GFP, and CrMSI4:GFP proteins was detected on a Zeiss 980 confocal microscope with AtUBQ10:NLS-mCherry as a nuclear-localized marker.

The ChIP-qPCR analysis was performed using modified protocols described[6]. Briefly, protoplasts transformed with *pCambia*1300-cFLAG/CrMSI2/CrMSI3:GFP and CrJAG-cFLAG/CrMSI2/CrMSI3:GFP were collected and crosslinked with 1% formaldehyde for 10 mins at room temperature and stopped with 125 mM glycine for 5 min. The protoplasts were resuspended in RIPA buffer and sonicated for 24 cycles with 30 s on and 30 s off. The samples were then subjected to immunoprecipitation using an anti-GFP antibody (1:1500, ab290, Abcam) or an anti-FLAG antibody (1:1500, F3165, Sigma-Aldrich) pre-incubated with Pierce Protein A/G magnetic beads (88847, Thermo Fisher) at 4°C for 4.5 h. The beads were washed, then reverse-crosslinked by adding 10% sodium dodecyl sulfate (SDS) and incubating at 65°C for 12 h. The proteins in the DNA complex were digested with Proteinase K at 45°C for 1 h. The DNA was extracted using phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol, then resuspended in water (W4502, Sigma-Aldrich). qPCR was performed on a qTOWER PCR System (Analytik Jena AG) using SYBR Premix Ex Taq.

Chemical treatment and cell flow cytometry

For chemical treatment, stage-12 fruits were collected and transferred onto 0.4% agar half-strength Murashige and Skoog (1/2 MS) medium plates containing 100 μM Aurora kinase inhibitor II (17541, Cayman) or mock solution (DMSO) for 5 days. The phenotypes were recorded using a Nikon D850 camera with a 105 mm prime lens.

For the cell-flow cytometry analysis, fruit valves were placed in 250 uL PVPK12-mGB2 buffer[30 mM sodium citrate, 45 mM $MgCl_2$, 20 mM MOPS, 20 mM NaCl, 20 mM EDTA $Na_2{\cdot}2H_2O$, 0.1% (v/v) Triton X-100, 0.5% (v/v) Tween-20, 10 μL/mL 1–2% PVPK12, pH 7.0], finely chopped and the resulting suspension was filtered through a 40-micron mesh to isolate nuclei. Nuclear suspensions were mixed, stained with 4 μg/mL DAPI, and analyzed on a BD LSR Fortessa flow cytometer with 405 nm excitation for detecting DAPI fluorescence. Approximately 20,000 nuclei were analyzed after gating. Quantitative analysis of ploidy level was conducted using FlowJo software (v10.10). EI was calculated from the ploidy histograms using the formula: EI = 4C% + 2 × 8C%. Statistical analysis was performed in GraphPad Prism (v10.1.2).

Quantification and statistical analysis

All statistics were collected and analyzed using Microsoft Excel and GraphPad Prism (v10.1.2). All data are presented as means ± SD with sample sizes marked by solid dots and described in the figure legends. Comparisons between groups were performed with Microsoft Excel and GraphPad Prism (v10.1.2) using a two tailed unpaired Student's t-test, and significance levels are marked as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Data availability

RNA-seq data generated in this study are deposited in the NCBI database under Bioproje ct PRJNA1420675 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1420675] accession num bers: SRR37212930 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212930], SRR37212931 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212931], SRR37212932 [https://www.ncbi.nl m.nih.gov/sra/?term=SRR37212932], SRR37212933 [https://www.ncbi.nlm.nih.gov/sra/?term= SRR37212933], SRR37212934 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212934], SR R37212936 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212936], SRR37212937 [https:// www.ncbi.nlm.nih.gov/sra/?term=SRR37212937], SRR37212938 [https://www.ncbi.nlm.nih.go v/sra/?term=SRR37212938], SRR37212939 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR372 12939], SRR37212940 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212940], SRR372129 41 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212941], SRR37212942 [https://www.ncb i.nlm.nih.gov/sra/?term=SRR37212942], SRR37212943 [https://www.ncbi.nlm.nih.gov/sra/?ter m=SRR37212943], SRR37212944 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212944], SRR37212945 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212945], SRR37212947 [http s://www.ncbi.nlm.nih.gov/sra/?term=SRR37212947], SRR37212948 [https://www.ncbi.nlm.nih. gov/sra/?term=SRR37212948]. ATAC-seq data generated in this study are deposited in the NCBI database under Bioproject PRJNA1420675 [https://www.ncbi.nlm.nih.gov/bioproject/P

RJNA1420675] accession numbers: SRR37212928 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212928], SRR37212929 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212929]. CUT&Tag data generated in this study are deposited in the NCBI database under Bioproject PRJNA1420675 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1420675] accession numbers: SRR37212922 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212922], SRR37212923 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212923], SRR37212924 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212924], SRR37212925 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212925], SRR37212926 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212926], SRR37212927 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212927], SRR37212935 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212935], SRR37212946 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212946], SRR37212949 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212949], SRR37212950 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212950], SRR37212951 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212951], SRR37212952 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212952], SRR37212953 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212953], SRR37212954 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212954], SRR37212955 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212955], SRR37212956 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212956], SRR37212957 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212957], SRR37212958 [https://www.ncbi.nlm.nih.gov/sra/?term=SRR37212958]. Live-cell imaging data [https://doi.org/10.6084/m9.figshare.31384462] and BiFC data [https://doi.org/10.6084/m9.figshare.32087253] analyzed in this study are deposited in Figshare platform. IP-MS data generated in this study are deposited in the ProteomeXchange partner repository iproX under project IPX0016837000 [https://www.iprox.cn/page/home.html]. Source data are provided in this paper.

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Author contributions

Y.D. initiated, conceived and designed the research with great support from L.Ø. Y.D. secured the funding and supervised this study. T.L. performed the research and collected the data with assistance from the other authors. X.-Y.C., Y.H. and Y.-J.Z. did the bioinformatic analysis on the RNA-seq, CUT&Tag-seq and ATAC-seq data. N.T., Y.Z. and M.M. conducted the live cell imaging data analysis. W.H. and Q.Y. did the SEM analysis and GUS staining. L.-M.L., C.-B.L., Y.-N.S. and C.J. were involved in large-scale genotyping analysis. A.S. and M.L. characterized the original *Crjag* mutant. H.-Z.K. was involved in routine discussion and provided valuable input to this research. Y.D. and T.L. outlined, drafted the manuscript and prepared the figure. All authors participated in the discussion of the data and contributed to the production of the final version of the manuscript.

Competing Interests

The authors declare no competing interests.

Figure Legends

Figure 1. Comparative live-cell imaging analysis reveals the cellular basis of the shape defects in *Crjag* fruits

a,**b**, Fruit morphology of CrWT (**a**) and *Crjag* (**b**) at stage 17. **c**,**d**, Quantitative depiction of valve development using heatmaps of cell sizes in the CrWT (**c**) and *Crjag* (**d**) fruit during 96 h of time-lapse live-cell imaging analysis, corresponding to developmental stages 12, 13 and 14 (0 h, 48 h and 96 h, respectively). Cell size is quantified by cell area (μm^2). In total, 10,481 and 6,738 cells were segmented for CrWT and *Crjag* fruits, respectively. **e**,**f**, Heatmaps of cell growth (the rate of cell area increase) of the CrWT (**e**) and *Crjag* (**f**) fruit at 48 h and 96 h time points. **g**,**h**, Heatmaps showing cell division status of CrWT(**g**) and *Crjag* (**h**) fruit at 48 h and 96 h time points. **i**,**j**, Heatmaps of cell anisotropy (the ratio of cell expansion in the maximum and minimum principal directions) of the CrWT (**i**) and *Crjag* (**j**) fruit at 48 h and 96 h time points. Scale bars, 5 mm (**a**,**b**)

and, 200 μm (**c**–**j**).

Figure 2. Genetic validation and expression analysis of CrJAG

a, Molecular characterization of *Crjag* and *Crjag^{ge}* alleles. The *Crjag^{ge}* was generated by CRISPR/Cas9 with a single-base pair insertion in exon 2, resulting in a frameshift that gave rise to a 40-amino-acid (aa) protein. The guide RNA (gRNA) and PAM sequences are indicated by red and blue characters, respectively. The red triangles indicate the target site of the gRNA. **b**, Fruit morphology of CrWT, *Crjag^{ge}*, *Crjag/Crjag^{ge}* and *Crjag;pCrJAG:CrJAG:GFP* complementary line at stage 17. **c**, GUS staining analysis of the *pCrJAG:GUS* reporter line showing the dynamic expression pattern of *CrJAG* during fruit development. Strong *CrJAG* expression is detected in the valves from stages 12 to 14. Scale bars, 5 mm (**b**) and 500 μm (**c**).

Figure 3. CrJAG interacts with histone chaperones CrMIS2 proteins

a, Venn diagram showing the overlap of protein interactors identified from IP-MS and Y2H library screening. **b**, Molecular identities of the four candidate proteins with the MSI1 protein shown in bold. **c**, Neighbor-joining tree of proteins encoded by the *MSI* genes from the *Capsella* and *Arabidopsis* genome, bootstrap values over 50% (1,000 replicates) are indicated for each branch. The *Damaged DNA Binding 1 (DDB1)-binding WD40* (*DWD1*) gene was used as an outgroup. **d**, Yeast-two-hybrid analysis of the interaction of CrJAG protein with CrMSI1-5 proteins. **e**,**f**, Verification of the protein interaction of CrJAG-CrMSI2, CrJAG-CrMSI3 and CrJAG-CrMSI4 using BiFC analysis (**e**) and Co-IP experiment after transient expression in tobacco leaves (**f**). For the BiFC and Co-IP analysis, two independent biological replicates were conducted. The results shown in **e** and **f** are representative of one biological replicate. with similar results. Scale bars, 50 μm (**e**).

Figure 4. The CrJAG-CrMSIs complex is required for fruit shape determination in *Capsella*

a-c, GUS staining analysis of *pCrMSI2:GUS* (**a**), *pCrMSI3:GUS* (**b**), and *pCrMSI4:GUS* (**c**) reporter lines showing the expression pattern of these genes during fruit development. **d**, Fruit morphology of *Crmsi2* and *Crmsi3* single, and *Crmsi2*; *Crmsi3* double mutants at stage 17. **e**, Schematic illustration of the chimeric constructs of CrJAG(DBD) and CrMSI proteins used for the complementation test. The total protein size is shown. **f**, Fruit morphology of *Crjag*, *Crjag; pCrJAG:DBD:GFP*, *Crjag; pCrJAG:DBD-CrMSI2:GFP* and *Crjag; pCrJAG:DBD-CrMSI3:GFP* at stage 17, showing that the DBD-CrMSI2 and DBD-CrMSI3 chimeric proteins successfully complement the *Crjag* fruit shape defects. Scale bars, 500 μm (**a-c**) and 5 mm (**d**,**f**).

Figure 5. The CrJAG-CrMSIs complex regulates fruit shape development via epigenetic mechanism

a, Venn diagram showing the 138 genes identified from *CrJAG*:*GFP* CUT&Tag, down-regulated DEGs in the *Crjag* fruits, and closed chromatin accessibility in *Crjag* fruits by ATAC-seq. **b**,

Metaplots displaying the ATAC-seq signals of the 138 *CrJAG* targeted genes, showing decreased ATAC-seq signals in the proximal promoter region and 3′regulatory region in the *Crjag* compared with CrWT. TSS, Transcription Starting Site; TES, Transcription Ending Site. **c**, ChIP-qPCR analysis in the CrWT protoplasts showing CrMSI2:GFP and CrMSI3:GFP association with the same DNA region as CrJAG:GFP on the *CrAUR2* locus, and that these associations depend on the presence of CrJAG:GFP protein. **d**, Gene expression analysis of *CrAUR2* in different genetic backgrounds. The expression level was quantified as FPKM from RNA seq data. **e**, Venn diagram showing the overlap of down-regulated targeted genes and changes in H3K18ac and H3K27me3 modification levels. **f**, Snapshots showing the chromatin binding peaks of *CrJAG*:*GFP*, ATAC-seq signals, H3K18ac and H3K27me3 modification signals in the genomic regions of *CrAUR2* between CrWT and *Crjag* fruit samples. The double arrowheads (A-C) indicate the regions used for ChIP analysis. The full epigenetic landscape is provided in Supplementary Figure 14. In **c**,**d**, the values are the values are mean ± SD (n=3 biological replicates). Statistical analysis was performed with a two tailed unpaired Student's t test. ns, not significant, *p < 0.05, **p < 0.01 (Student's t test).

Figure 6. The CrJAG–CrMSIs complex orchestrates fruit shape determination via epigenetic regulation

During fruit morphogenesis, CrJAG recruits CrMSI2/3 to downstream target genes. This complex locally reduces H3K27me3 and increases H3K18ac via an as-yet-unidentified mechanism (dashed line), thereby promoting chromatin accessibility and gene expression. A key target of CrJAG-CrMSIs in fruit shape determination, *CrAUR2*, exemplifies this regulatory logic. In the *Crjag* mutant, loss of CrJAG function abolishes CrMSI recruitment. Consequently, H3K27me3 enrichment rises while H3K18ac declines, leading to reduced chromatin accessibility, decreased target gene expression, and impaired fruit morphogenesis.

Editorial summary:

Here Lü *et al*. show that the CrJAG transcription factor interacts with histone chaperones to maintain chromatin accessibility and expression of cell cycle-related genes, thereby controlling fruit shape determination in *Capsella*.

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