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Review

Paradigm and Framework of WUS-CLV Feedback Loop in Stem Cell Niche for SAM Maintenance and Cell Identity Transition

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Abstract: Shoot apical meristem (SAM) consists of stem cells that act as a reservoir for the aerial growth. It plays an important role in the differential architectural development in plants. SAM actively performs parallel functions by maintaining the pluripotent of stem cells and continuous organogenesis throughout the plant's life cycle. Molecular mechanisms regulating the signaling networks of this dual function of the SAM have been progressively understood. In the SAM, the feedback loop of WUSCHEL (WUS)-CLAVATA (CLV) has been found to be the key regulator in stabilizing stem cell proliferation and differentiation. In general, WUS migrates into central zone (CZ) from organizing center (OC) and activates the expression of *CLV3* by binding to the promoter elements. CLV3 acts as a ligand to interact with the CLV1, leucine rich repeats (LRR) receptor-like kinase (RLK) and LRR receptor-like protein CLV2, and protein kinase coryne (CRN) (CLV2/CRN) to restrict *WUS* transcription to the OC. Evolution of *CLV3* is one of the main factors contributing to the transformation of two dimensional (2D) to 3D plants. WUS-CLV loop is involved in several pathways and networks that integrate on meristem maintenance and cell identity transition. WUS-CLV maintains stem cells with simultaneous differentiation signals by the spatial-temporal signaling of the phytohormones. WUS-CLV loop has an interaction with reactive oxygen species (ROS), an important signaling molecules regulating cell proliferation and developmental transition. WUS also forms feedback loop with AGAMOUS (AG) for differentiation, proliferation, and termination of floral meristem. These loops might also involve in interaction with vernalization and its regulatory factors that oversees the precise timing of flowering after exposure to cold temperatures. In this review, we highlight the evolutionary and developmental importance of the WUS-CLV feedback loop on SAM maintenance and cell identity transition for inflorescence and floral meristem development.

Keywords: evolution; shoot apical meristem (SAM); development; regulation; transition

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1. Introduction

Plants encompass a small population of stem cells at both shoot apical meristem (SAM) and root apical meristem (RAM) throughout their life cycle. Stem cells are pluripotent and self-renewing [1]. Stem cells have the ability to differentiate into various types of cells; meanwhile, daughter cells will be maintained as stem cell in the 'niche'. Stem cells present in both the shoot and root apices are specified during the embryogenesis stage. In early stages, it is mostly in an ordered pattern, whereas, in the later developmental stages, stem cell fate has more of a spatial and a regulatory-dependent pattern [2]. Stem cells has the ability to renew themselves. One set of cells remains as stem cells (undifferentiated) and other set differentiate into organs such as leaves, flowers, and stems indicate intrinsic complex networks of the stem cell niche [3]. All pathways responsible for formation or maintenance of stem cells are extensively regulated by phytohormones, such as cytokinin

(CK), auxin, transcription factors (TFs), and external environmental stimuli [1]. These cells are distributed in different kinds of meristems, including the embryonic and post-embryonic meristem. SAM and RAM are specified in the embryogenesis [4] and activated at favorable conditions. The post-embryonic meristem is the tissue formed after the development of the embryo, including the intercalary meristem and lateral meristem. SAM and RAM are the primary meristems, forming stem cells responsible for formation of the whole plant. Whereas, intercalary and lateral meristems are the secondary meristems, accountable for internode, internal tissue layers and other lateral growth of the plant [2,3].

Stem cells with various differentiation potencies are present at the shoot and root apex. RAM gives rise to entire root system whereas SAM consists of a pool of pluripotent stem cells that give rise to the above-ground parts of the plant [1]. Stem cells attain different identities due to the interactive effects of various gene regulatory pathways. SAM is structurally organized into three clonal layers: L1 (epidermal) layer, L2 (sub-epidermal) layer, and L3 (innermost corpus) layer(s). Outer L1 and L2 layers are called as tunica. Zones that are allocated radially are named as the central zone (CZ), containing pluripotent stem cells; peripheral zone (PZ), containing transit-amplifying stem cells; and organizing center (OC) that organizes and maintains the stem cell population [1,3]. The CZ contains pluripotent stem cells and the transit amplifying cells within the PZ are recruited to form the organ (Figure 1).

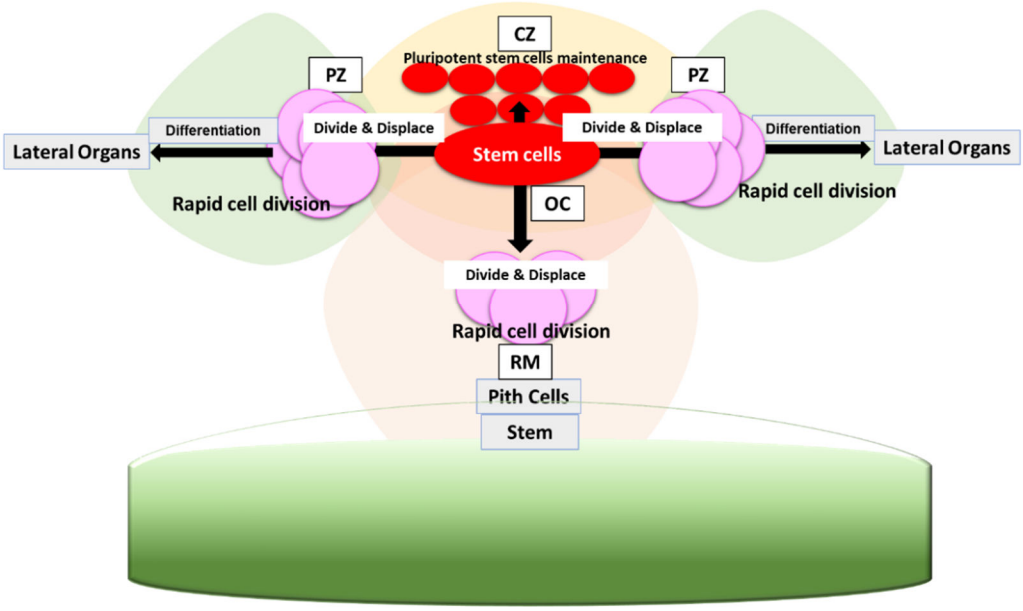

Figure 1. Stem cell organization in SAM. Stem cells present in the central zone (CZ) provides mainly two subsets of daughter cells. The one subset of cells rarely divides and maintains the stem cell pool. The other subset of cells rapidly divides and differentiates in peripheral zone (PZ) to give rise to lateral organs. Organizing center (OC) maintains stem cell population. Cells that are displaced basally to the rib meristem (RM) give rise to pith cells, eventually forming stem.

Both the SAM and floral meristem (FM) determine the size, number, and shape of agronomic traits such as the flowers, fruits, seeds, kernels, etc [5]. A key regulatory pathway involved in the maintenance of the stem cell population and specification of stem cell identity in the SAM is the WUSCHEL (WUS)–CLAVATA (CLV) feedback loop. The WUS-CLV is a feedback loop/system that plays a crucial role in maintaining stem cell homeostasis as well as organ development [1–5]. Post-embryogenic development occurs from the apical meristem present in shoot and root tips. Leaves, inflorescences, and floral organs are differentiated from the SAM [4,5]. It is interesting to note that WUS-CLV pathway has been evolutionarily conserved across land plants [6]. This pathway is extensively studied in the model plant *Arabidopsis thaliana* and also in maize, rice, and tomato. WUS

activity is required for the identity, structural, and functional integrity of the SAM and FM. The *WUS* and *CLV* genes are mandatory for the progressing meristem cells toward organ initiation [7,8]. In tomato, orthologs of the *WUS* and *CLV* genes have been elucidated as the determining factor for the carpel number, floral growth, and fruit size [9,10]. A study on the WUS showed it plays a major role in the course of monocot and dicot evolution [11]. Previous reports on maize suggested that crop yield and plant architecture are strongly influenced by WUS-CLV orthologs [12–14]. The importance of WUS and CLV on floral organ numbers, subfunctionalization, and diversification was proved in rice [15–17].

The following table shows the orthologs of the *Arabidopsis* genes with their functions (Table 1).

Table 1. WUS, CLV, and AGAMOUS (AG) orthologs in tomato, maize and rice.

PLANT	GENES	ORTHOLOGS	REFERENCES
ARABIDOPSIS (dicot)	*WUS*		Ref. [7]
	CLV1		Ref. [8]
	CLV2		
	CLV3		
	AG		Ref. [18]
TOMATO (dicot)	*WUS*	*SlWUS*	Ref. [9]
	CLV1	*fasciated and branched (FAB)*	Ref. [10]
	CLV2	*SlCLV2*	Ref. [10]
	CLV3	*SlCLV3*	Ref. [10]
	AG	*Tomato Agamous1* (*TAG1*)	Ref. [19]
MAIZE (monocot)	*WUS*	*ZmWUS1 and ZmWUS2*	Ref. [11]
	CLV1	*THICK TASSEL DWARF1 (TD1)*	Ref. [12]
	CLV2	*FASCIATED EAR2 (FEA2)*	Ref [13]
	CLV3	*ZmCLE7, ZmCLE14*, and *ZmFON2-LIKE CLE PROTEIN1 (ZmFCP1)*	Ref. [14]
	AG	*Zea mays Agamous 1* (*ZAG1*)	Ref. [20]
RICE (monocot)	*WUS*	*TILLERS ABSENT1 (TAB1)*	Ref. [15]
	CLV1	*FLORAL ORGAN NUMBER 1 (FON1)*	Ref. [16]
	CLV2	-	
	CLV3	*FON2, FON4*	Refs. [17,21]
	AG	*OsMADS3* and *OsMADS58*	Ref. [22]

It is curious to know that, in the meristem, a small set of populations renew themselves and the rest undergoes differentiation to form the new organs (Figure 1). Though several studies were carried out in the model plants as well as crops, still some of the areas in stem cell research have to be addressed to open new avenues in the near future such as (i) transcriptional networks involved in defining the cell types, (ii) regulatory factors involved in determining stem cell fate, and (iii) cell identity transition.

In this review, we present the paradigm of the WUS-CLV feedback loop in the stem cell niche and framework of the identity transition, floral development, and phytohormones cross-talk. Importantly, ROS on regulating the SAM and also genetic network of vernalization in meristematic tissue are discussed.

2. WUS-CLV Feedback Loop in SAM Maintenance

WUS-CLV signaling is crucial for the development of meristem and considered to play a major role in crop domestication. Evolution, proliferation, and differentiation of stem cells in the SAM are synchronized by a primary auto-regulatory WUS-CLV signaling pathway [3]. *WUSCHEL-related homeobox (WOX)* encodes the homeodomain TF. Rodriguez et al. reported that last 63-aa stretch present at the C-terminus of *Arabidopsis* holds transcriptional regulatory domains namely acidic region, WUS-box and ethylene-responsive element binding factor-associated amphiphilic repression (EAR-like) domain. WUS-box is required for the nuclear retention of WUS, while EAR-like domain is responsible for its nuclear export, as it resembles the nuclear export signals (NESs). EAR-like domain determines high levels of the *WUS* inside the nucleus in the cells of the rib meristem (RM) whereas lower nuclear levels in adjoining cells. Additionally, WUS is constricted by the N-terminal DNA binding homeodomain and homodimerization sequence present in the central part of the protein which are responsible for homodimer formation [23]. WUS move between the cells via plasmodesmata. Subsequent to the migration to the outer layer with the activation of its negative regulator, WUS restrict its own accumulation [24].

A study of *wus* mutants showed that the SAM failed to generate proper leaf primordial and FM terminate prematurely [7]. On the contrary, ectopic expression of *WUS* trigger pluripotency which results in altered SAM, activate shoot stem cell marker in root as well as induction of shoot tissue from root, and initiated new vegetative buds in *Arabidopsis* [25–27]. WUS and hairy meristem (HAM) family of GRAS domain transcriptional regulator controls stem cell production and ensure that *CLV3* transcription is activated only in the outermost apical layers of the SAM. Activation of *CLV3* by WUS occurs only in the absence of HAM [28].

CLV3 is a member of the clavata 3/embryo surrounding region (CLE) family of the polypeptide, formed as a pre-propeptide in the cells of L1 and L2 that encode 96 amino acid, processed into 12 amino acid or arabinosylate 13 amino acids peptides [29]. All members of the CLE protein family contain a conserved 14 amino acids sequence motif, termed as the CLE motif [30]. CLV1 encodes leucine-rich repeat (LRR) receptor-like kinase (RLK) and CLV2 encodes LRR receptors-like protein, and the CORYNE (CRN) protein encodes membrane-associated receptor-like cytoplasmic kinase (RLCK) (CLV2/CRN) form complex with the CLV3 peptide [4,5]. According to the signaling cascade, CLV1, CLV2/CRN receptors recognize the CLV3 peptide and are activated to restrict the expression of the *WUS* to a small subset of cells in the center (Figure 2).

The WUS acts as an inducer of the *CLV3* at its low concentration, while at high concentration it represses the *CLV3* by binding to the *cis*-regulatory elements of the *CLV3* gene [23,31]. WUS forms homodimers and inhibit CLV3 [31]. It was hypothesized that formation of homodimer could limit the mobility of WUS [32]. While in L1 and L2, due to the low WUS concentration, it remains in its monomeric form and binds to the *cis*-regulatory elements of the *CLV3* gene, but, this time, it activates *CLV3* expression. Therefore, the WUS gradient determines the activation or repression of the *CLV3*, thus forming the feedback loop [31]. Transcription of the *WUS* is inhibited by the activated CLV3 and its receptor complexes after the subsequent CLV3 signaling cascade formation. Fate of the stem cell is entirely governed by this regulatory feedback loop [33]. Loss of *clv* function leads to the enlarged meristem might be due to the increase in rates of cell division in the meristem and cells on the boundary of CZ-PZ, remains as CZ cells after division [34].

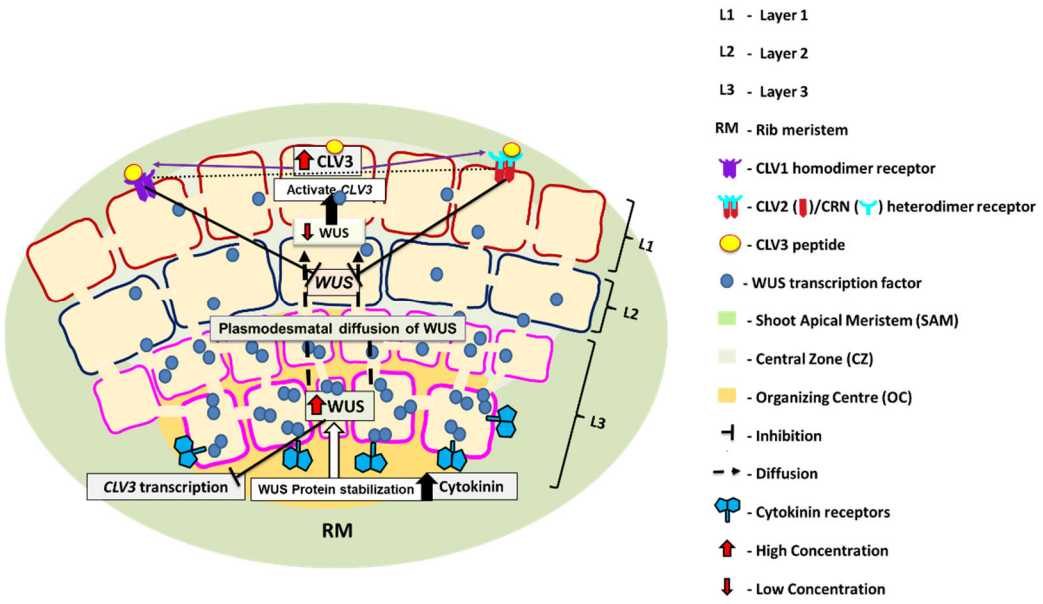

Figure 2. WUS-CLV feedback loop. *WUS* is mainly expressed in OC-containing cells of RM and L3. WUS acts non-autonomously in L1 and L2. High levels of WUS are maintained by high cytokinin concentration and absence of CLV3 suppression on *WUS* transcription. At higher concentration of WUS, it represses *CLV3* transcription in L3 and RM. WUS concentration decreases as it moves towards the apex because of the absence of cytokinin in L1 and L2. At low concentration of WUS, *CLV3* transcription is activated. CLV3 then represses the transcription of *WUS* in L1 and L2 by interacting with homodimer of CLV1 and heterodimer of CLV2/CRN, receptors of CLV3. In this way, WUS-CLV maintains a feedback loop. CLV, CLAVATA; WUS, WUSCHEL.

Therefore, the WUS-CLV is inevitable on the stem cell niche in SAM maintenance and inflorescence transition for reproductive development. There is further involvement of the WUS-CLV on the reactive oxygen species (ROS) signaling and vernalization for cell proliferation and organ development. This review gives an overview on the regulatory mechanisms of WUS-CLV in cellular differentiation and cell identity transition.

3. WUS-CLV in Identity Transition

The WUS concentration plays a decisive role in stem cell homeostasis. CLE peptides are vital for SAM maintenance and differentiation. [30] WUS concentration regulates the transcription of *CLV3* through an activation or repression switch. The CLV3 inhibits the *WUS* transcript in upper layers. The WUS is restricted to the center of the OC while *CLV3* has a high expression in outermost layers. Regulation of *WUS* synthesis and its subcellular partitioning by the CLV3 maintains a nuclear WUS concentration in-turn regulating *CLV3* levels. This regulatory network fine-tunes the WUS accumulation [23,35]. Expression of *CLV3* in apex prevents conversion of CZ into PZ cells in the surrounding area of CZ [34]. This gradient-dependent expression or repression determines the maintenance and differentiation of stem cells, ultimately leading to the transition from the vegetative to reproductive phase.

Whitewoods et al. reported the role of the conserved CLV and receptor-like kinase pathways in the switch from the two-dimensional (2D) to 3D growth of land plants by comparing moss, *Physcomitrella,* and *Arabidopsis*. Modern mosses have leafy shoots that grow from a filamentous (2D) precursor. Genome data indicated the appearance of core elements of the CLV pathway in the last common ancestor of land plants, together with the evolutionary innovation of 3D growth. Their experiments showed that *Physcomitrella* CLE functions similarly to CLV3 in *Arabidopsis* in regulating growth and proliferation through the conserved receptor machinery [6]. Further comparative studies between plants with different inflorescence structures such as Arabidopsis, rice, and maize showed

the existence of homologs with similar functions for SAM maintenance and inflorescence meristem (IM) transition among plant lineages [36]. CLV3 is also recognized by the CLV1-related barely any meristem (BAM) LRR-RLKs and receptor-like protein kinase 2 (RPK2) via BAM1[5,6]. *Arabidopsis clv1/bam1/bam2/bam3* quadruple mutants showed strongly disordered planes in the stem cell niche and ground tissue layers of mutant roots [6]. BAM1, BAM2, and BAM3 promote shoot and flower meristem. It was suggested the BAM genes can play complementary or opposite role of CLV1 [37]. It is also clear that BAM regulated by CLV1 and interaction of CLV1 with *WUS* is distinct to each other [38]. During the *Physcomitrella* gametophore there was a high dynamic expression of *CLEs, CLVs*, and *RPK2* genes, and hence it can be deduced that CLEs also act via RPK2 in regulating the 3D growth [6]. However, still the inflorescence complexity needs to be explored by comparative analysis between different plant species.

In shoot meristem, CLV1/BAM1 and CLV2/CRN complexes act in separate but in the parallel pathways. For the fruit development, CLV1, CLV2, and CRN receptors all act in a linear pathway [39]. WUS also involved in FM determinacy through epigenetic mechanisms [40]. In this context, Sun et al. [41] recently showed that the *WUS* is repressed by AGAMOUS (AG) via KNUCKLES (KNU) through histone deacetylation at floral stage 6. An earlier study revealed that the *WUS* is expressed in immature stomium cells and is associated with anther development. As a result of this interaction, anthers of *wus* mutants had fewer and malformed lobes compared to the wild type, showing that the *WUS* is essential for normal anther development [42]. Another study in *Chrysanthemum morifolium* also indicated that the WUS interacts with cycloidea 2 (CYC2) TF to regulate reproductive organ development [43].

Flowering of plants starts with the transition of the SAM into the IM. Further differentiation of the FM is regulated by environmental cues. From the indeterminate shoot to the development of determinate floral organs such as sepal, petal, stamen, and carpel, cell identity transition and stem cell reprogramming occur with various level of developmental regulation in the apical region [44,45]. Therefore, the regulation of the FM by WUS, CLV, and AG is a dynamic switch that ensures successful reproduction.

4. WUS-CLV in Flower Development

Flowers are formed from the proliferating SAM where the meristem size depends greatly on the WUS-CLV feedback loop. Unlike *WUS*, *shoot meristemless* (STM) express throughout the meristematic region. STM is mainly required for maintenance of undifferentiated cells in SAM and FM. For SAM fate, *STM* can be used as marker gene as its expression is extinguished during the leaf primordia specification [46,47]. *Aintegumenta* (ANT) is specific to the floral organogenesis and patterning of other regulatory genes [48]. In floral primordia, expression of *ANT* is found in peripheral regions on stage 0 and 1 [47]. It was observed that mutant of *clv* showed supernumerary floral structure [49] and *wus* mutant terminate prematurely [7]. This observation suggests that the WUS-CLV loop plays an important role in floral organ differentiation rather than the transition.

Termination of the FM occurs at stage 6, in a WUS-AG dependent manner to ascertain proper development of the floral organs [50]. AG is a C-class MADS domain-containing TF, positively regulating FM determinacy [51]. The *ag* mutants exhibit indeterminate nature in FM containing interior flowers inside third whorl [52]. Induction of *AG* by the WUS is dependent on the leafy (LFY). It is mandatory that WUS required LFY to acts on cis-regulatory element of *AG* to confer floral identity in the FM after the transition from the IM. During the formation of the FM, WUS together with LFY binds sequences to activate *AG* expression. AG only mildly represses the *WUS* after floral stage 3 [50–53]. For the termination of the *WUS* at stage 6 after carpel initiated, KNU encodes C_2-H_2 zinc-finger TF plays a pivotal role in FM determinacy [53]. AG completely represses *WUS* transcription through *KNU* [54] and also directly through the terminal flower 2 (TFL2)-AG complex that brings chromatin loop formation at the *WUS* locus [55]. Thereby terminating stem cell activity and start determinate floral growth. *WUS* is required in developing stamens

at stages 7–8 [51] and in developing ovules [56]. It can be seen that the AG-WUS and WUS-CLV pathways function are at least partially independent since the effects of *ag* and *clv1* mutations on FM determinacy are additive. Consistently, *WUS* expression increased strongly in *ag-1 clv1-4* in comparison to *ag-1* [50]. Thus, this complex regulatory network is mandatory for FM development to produce flowers with specified numbers and whorls of floral organs.

5. Phytohormones Crosstalk with WUS-CLV Regulatory Pathway in SAM

Plant hormone levels are modulated during various developmental stages causing differential expression of SAM maintenance, cell identity, and cell fate transition-related genes. Phytohormones play a major role in cell division and differentiation during organogenesis in plants. Spatial and temporal signaling of auxin is dynamic to specify distinct cell identities in the SAM and organogenesis in a concentration-dependent manner [4,5]. Auxin accumulates at the positions of floral anlagen through the polar transport by *pin-formed 1* (*PIN 1*) [57]. Auxin maxima then activates genes that lead to organogenesis. The model for auxin-mediated organogenesis in the SAM is based on the inhibitory field theory. According to this theory, pre-existing organs restrict the initiation of new organs in their vicinity by chemical inhibitors. Also, polar auxin transport deplete auxin around organs along to trigger organ initiation [58].

Auxin and CK play antagonistic roles in division and differentiation at the shoot apex. In the SAM, auxin maxima induce cellular differentiation and organ outgrowth at the site of the primordial formation, whereas CK maxima are found to undifferentiated state in SAM [59]. The RM-enriched CK stabilizes the WUS protein by acting on the nuclear retention signal. The WUS is stabilized through the CK signaling action on the acidic domain and the WUS-box. Stability of the WUS is achieved when the degron activity of WUS-box is masked by the combined effect of the nuclear retention machinery and the CK signaling to regulate CLV3 transcription [60]. The cells in the PZ have a high auxin concentration, and therefore they start to differentiate, whereas cells in the OC can maintain stem cell properties because of the presence of the WUS and CK [61]. WUS limits auxin signaling through histone acetylation, preventing cells at the CZ from differentiating [62]. Previously formed leaf primordia act as a sink of auxin. It can travel to certain distance for phyllotaxis position and protrusion [63]. The WUS represses the expression of type-A *ARR* genes such as *ARR5/6/7/15* to increase the CK activity in the OC [64]. WUS protein stabilization depends on the CK concentration until WUS can be independently stable [60]. The CK receptors such as *ARABIDOPSIS HISTIDINE KINASE 2/3/4* (*AHK2/3/4*) play an important role in CK response by sensing the environment [6,65]. Crosstalk between auxin and CK is necessary for SAM maintenance. Auxin response factor 3 (ARF3) inhibit CK for FM determinacy [66] whereas auxin response factor 5 (ARF5)/monopteros (MP) repress type-A ARRs such as ARR7/15, a negative regulator of CK [67]. This process fine tune auxin and CK level [67]. Moreover, by repressing the expression of target genes, WUS restricts auxin-mediated cell differentiation which enhances the CK-mediated *WUS* expression. Auxin through *ARF5/MP* represses (at least partly) the expression of the *dornroschen/enhancer of shoot regeneration 1* (*DRN/ESR1*). *DRN* strongly regulate CLV3 which proved to interfere with WUS-CLV feedback loop [61]. Changes in the auxin and CK signaling could affect the behaviour of stem cell fate. The interaction between the WUS-CLV loop and auxin and CK clearly highlights strong relations between stem cell maintenance and hormone signaling (Figure 3).

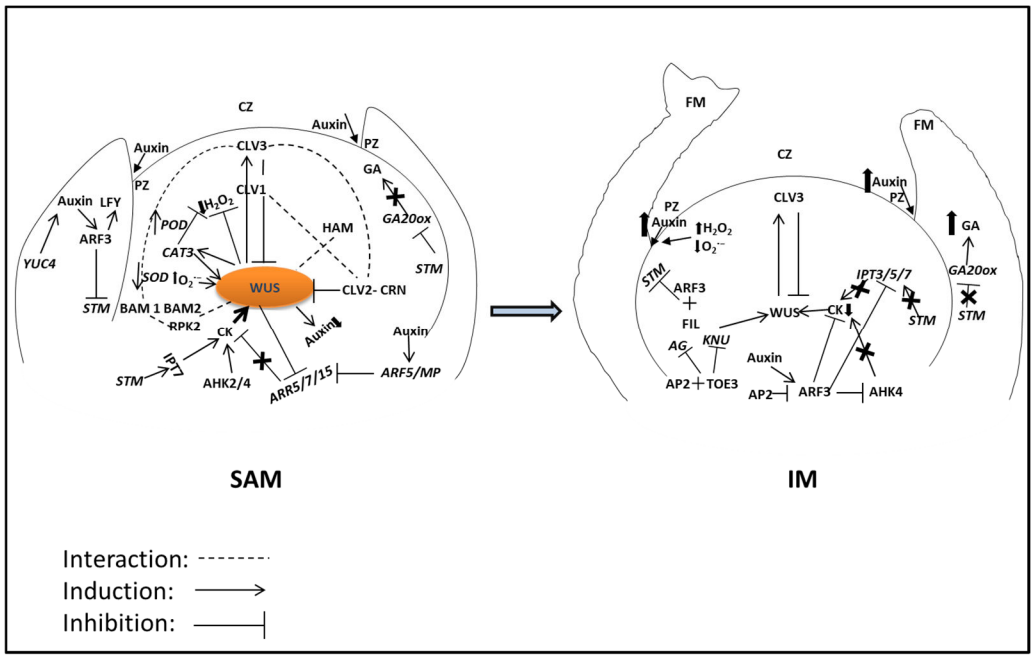

Figure 3. Regulatory network of WUS-CLV feedback loop in SAM maintenance and its transition to the inflorescence meristem (IM). Genes involved in the SAM, IM, and floral meristem (FM) are spatial and temporally regulated by several factors including phytohormones. AG, AGAMOUS; AHK, ARABIDOPSIS HISTIDINE KINASE; AP, APETALA; ARF, AUXIN RESPONSE FACTOR; ARR, ARABIDOPSIS RESPONSE REGULATORS; BAM, BARELY ANY MERISTEM; CAT, CATALASE; CK, CYTOKININ; CLV, CLAVATA; CRN, CORYNE; FIL, FILAMENTOUS; GA, GIBERELLIC ACID; GA20OX, GA20 OXIDASE; H_2O_2, HYDROGEN PEROXIDE; HAM, HAIRY MERISTEM; IPT, ISOPENTENYL TRANSFERASE; KNU, KNUCKLES; LFY, LEAFY; MP, MONOPTEROS; $O_2^{\bullet-}$, SUPEROXIDE; POD, PEROXIDASE; RPK, RECEPTOR-LIKE PROTEIN KINASE; SOD, SUPEROXIDE DISMUTASE; STM, SHOOT MERISTEMLESS; TOE, TARGET OF EAT; WUS, WUSCHEL; YUC, YUCCA. CZ, central zone; PZ, peripheral zone. Other regulatory factors/genes have been avoided due to its complexity and out of focus of this review.

Along with auxin, gibberellin (GA) is another key hormone in connection with cell growth and differentiation. Higher level of auxins and GA are required for outgrowth at the flanks of SAM. It regulates phyllotaxis, floral transition, and flowering [68]. Suppression of GA biosynthesis and induction of CK are carried out by KNOX TFs including KNOTTED1 (KN1) in maize, STM in *A. thaliana*, leading to the inhibition of differentiation in the CZ of the SAM [69]. *STM* also induces *isopentenyl transferase 7* (*IPT7*) which increase the cytokinin level for WUS maintenance [70]. Chang et al. reviewed the role of ARF3 in induction of *LFY* and *YUCCA4* (*YUC4*) to increase auxin that can leads to differentiation in PZ. MP and FIL indirectly and ARF4 and ARFs ETTIN (ETT)/ARF3 directly reduces expression of STM to promote flowering [71]. For FM initiation, APETALA 2 (AP2) and TARGET OF EAT (TOE3) inhibits *AG* for floral patterning [4,72]. GA level is reduced in SAM at CZ by KNOX. Suppression of GA signaling is achieved by two functions of the KNOX TF. Primarily, KNOX suppressing *GA20 oxidase* is involved in the biosynthesis of GA and, secondly, by enhancing the activity of *GA2 oxidase* which in turn is responsible for inactivation of active forms of GAs. The low levels of GA are required for undifferentiated vegetative meristematic activity, whereas its high level supports floral transition [73]. *AGAMOUS-LIKE15 (AGL15)* regulate endogenous GA levels by regulating *GA2ox6* [74]. During floral transition GA levels are generally high based on spatial- and –temporal expression of *GA20ox2* [69]. Interaction with brassinazole-resistant 1 (BZR1) and DELLA was required by both brassinosteroids and GA_3 to regulate each other's. DELLA mediates various hormonal and signaling pathways. [75]. Bao et al. [68] summarized GA signaling as the main component of the crosstalk between different hormones during FM develop-

ment and its interaction with *LFY*, *flowering locus d (FD), flowering locus T (FT)* and *suppressor of the overexpression of constans 1 (SOC1*), involved in the process. As the WUS interacts with LFY [52], these hormones may also have their effects on *WUS* expression directly or indirectly. Jasmonic acid (JA) is also involved in plant developmental processes but majorly in defense-related responses [76]. MYC2, MYC3, and MYC4 plays major role in most of the JA-dependent regulation processes [77]. For the JA-mediated inhibition of flowering above three TFs are most vital [78].

Ethylene is another vital hormone participating in the stress response, senescence, regulation of floral transition, and fruit ripening. JA and ethylene were found to induce the expression of *HISTONE DEACETYLASE 6* (*HDA6*) and *HDA19* [79,80]. Especially, HDA6 is vital for the deacetylation of *FLC* chromatin [79]. This chromatin remodeling is essential for repression of *FLC*. Abscisic acid (ABA) acts antagonistically to GA, as GA promotes seed germination, while ABA restricts it [81]. Abscisic acid (ABA)-insensitive mutant 5 (ABI5), a basic leucine zipper (bZIP) transcription factor, is involved in floral transition in *Arabidopsis*. Induction of *ABI5* could delay flowering initiation via upregulating *FLC* expression [82] whereas ABA-dependent GIGANTEA signaling induces expression of *FT* which accelerates flowering to escape drought conditions [83].

Only few reports are available on the direct interaction of phytohormones such as GA, ethylene, and ABA with the WUS-CLV pathway. Further research needs to be conducted with respect to hormonal signaling in the SAM involving cell identity genes which would fetch information on how hormonal levels and their signaling alter the SAM maintenance and development by affecting major genes involved the WUS and CLV.

6. ROS in SAM Regulation

Similar to the phytohormones, ROS are also vital for the stem cell maintenance and differentiation. Redox state decides the stem cell fate. Especially low proliferation of OC could be overlapped with the redox status [84]. ROS such as super oxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl ion ($^{\bullet}OH$), etc. are a natural by-product generated during the normal aerobic metabolism process. Excessive accumulation of ROS damages macromolecules and nucleic acids. Equilibrium of ROS generation and oxidation was maintained by the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) etc. [85]. ROS act as a secondary messengers in regulating the balance between cell division and differentiation. ROS status is one of the determining factors for cell fate.

Recent findings by Zeng et al. [86] showed that equilibrium between the H_2O_2 and $O_2^{\bullet-}$ regulate *WUS* transcription. A high concentration of $O_2^{\bullet-}$ maintains *WUS* expression to inhibit differentiation of stem cells in OC, while higher signals of H_2O_2 in the PZ repress *WUS* for differentiation. It can be inferred that ROS direct stem cell fate, and the balance between $O_2^{\bullet-}$ and H_2O_2 is crucial for SAM maintenance and differentiation. Wang et al. established a putative model for ROS-mediated regulation of age-dependent developmental programmed cell death (dPCD) involving the WUS-CLV pathway. At the proliferative stage, *WUS* expression is maintained by $O_2^{\bullet-}$ and also WUS might promote *CAT3* to keep the H_2O_2 in lesser concentration. *WUS* expression averted the activation of the *ORESARA 1–BIFUNCTIONAL NUCLEASE 1* (*ORE1-BFN1*) senescence cascade, hence preventing dPCD of stem cell. Promoting the *CAT3* and repressing *acyl-CoA oxidase 1* (*ACX1*) helps to maintain low level of H_2O_2. Production of $O_2^{\bullet-}$ persuade WUS to maintain stem cells. In contrast, CLV3 could inhibit $O_2^{\bullet-}$ for proliferation [87]. ROS play a critical role in abiotic and biotic responses to plant development and maintaining homeostasis [88]. Recent studies mentioned that spatial distribution of the $O_2^{\bullet-}/H_2O_2$ balance serves as a key switch for stem cell maintenance and differentiation. Antioxidant enzymes such as SOD and POD plays a major role in keeping ROS under controlled level [86,87]. Decrease in the $O_2^{\bullet-}$ and increase in H_2O_2 could lead to the differentiation rather than stem cell maintenance [86]. These results reveal that the WUS-CLV participates in the determina-

tion of stem cell fate through ROS, as $O_2^{\bullet-}$ promotes WUS activity and stem cell maintenance, and H_2O_2 induces CLV for differentiation. Due to this interaction of the WUS-CLV with ROS, it can be stated that the pathway articulates cell proliferation and differentiation homeostasis and involved in thermomorphogenesis.

7. Vernalization

Ambient temperature is the most crucial for the precise timing of flowering. For instance, several Brassicaceae plants need to be exposed to low temperatures (few weeks) for flowering which is generally termed as vernalization. Appropriate vernalization triggers genetic and developmental changes in the SAM resulting in the transition to the IM and further development of either terminal or lateral FMs. Flowering in response to vernalization is not immediately occur in low temperatures instead it accelerates flowering when the low temperature is removed, i.e., in the warm temperatures of the following spring after winter [89].

The molecular genetic network of vernalization in *Arabidopsis* majorly involves suppression of *flowering locus C* (*FLC*) during cold exposure leads to flowering [90]. Frigida (*FRI*) repress the flowering is correlated with its induction of *FLC* gene. However, natural allelic variation exists between these two key genes contribute for difference in flowering time [91]. FRI act as a scaffold protein that holds the FRI-LIKE 1 (FRL 1), FRI essential 1 (FES 1), suppressor OF FRI 4 (SUF 4), and FLC expressor (FLX) to form a transcription activator complex for increasing the transcription of *FLC* [92]. FLC repress transcription of *flowering locus D* (*FD*), *FT,* and *SOC1* resulting in delayed flowering. It was reported that *FLC* repressed by the cold exposure [89,93]. It is involved with several pathways such as prolonged cold exposure, photoperiod via CONSTANS (CO), and autonomous pathways to suppress the *FLC* gene. In exposure to cold, activation of *VRN1* and increase in *VIN3* expression and association with *VIL1*/*VRN5* induce chromatin repression of *FLC* [93–95]. This silencing involves two repressive histone modifications such as trimethylation of Lys9 and Lys27 of histone H3 (H3K9me3 and H3K27me3) resulting in stable epigenetic repression of *FLC* in response to cold temperatures. Before vernalization, high levels of FLC block the floral transition in winter-annual strains of *Arabidopsis* as its chromatin is enriched active histone marks [93]. To prove that Yang et al. reported that H3K36me3 and H3K27me3 rarely coexist [96]. During vernalization, it can be considered that main factor of *FLC* gradual silencing could be due to increasing H3K9me3 and H3K27me3 (repressive histone marks) and decreasing H3K4me3 and H3K36me3 (active histone marks) at the nucleation level of *FLC* (Figure 4). Meanwhile, GA activates the promoter of LFY promoter [97]. Simultaneously, *SOC1* induced by both GA and FD and FT [93]. SOC1 and AGL24 possessed positive regulation to each other which is required for LFY activation [98].

Repression of *FLC* is stable even after the cold exposure is over. However, this repression is only stable throughout mitosis, and *FLC* is activated again. It transiently expressed during the gametogenesis, and mid-to-late embryogenesis [93,99,100]. During cold, stable silencing of *FLC* is mediated by H3K27me3. In the following warm period, epigenetic genetic process opposing the histone modification such as silencing H3K27me3 quantitatively based on the cold exposure period [101]. Active chromatin-modifying complex for epigenetic reprogramming and FRI-C and PAF1 complexes, are crucial for the reactivation of FLC [101,102].

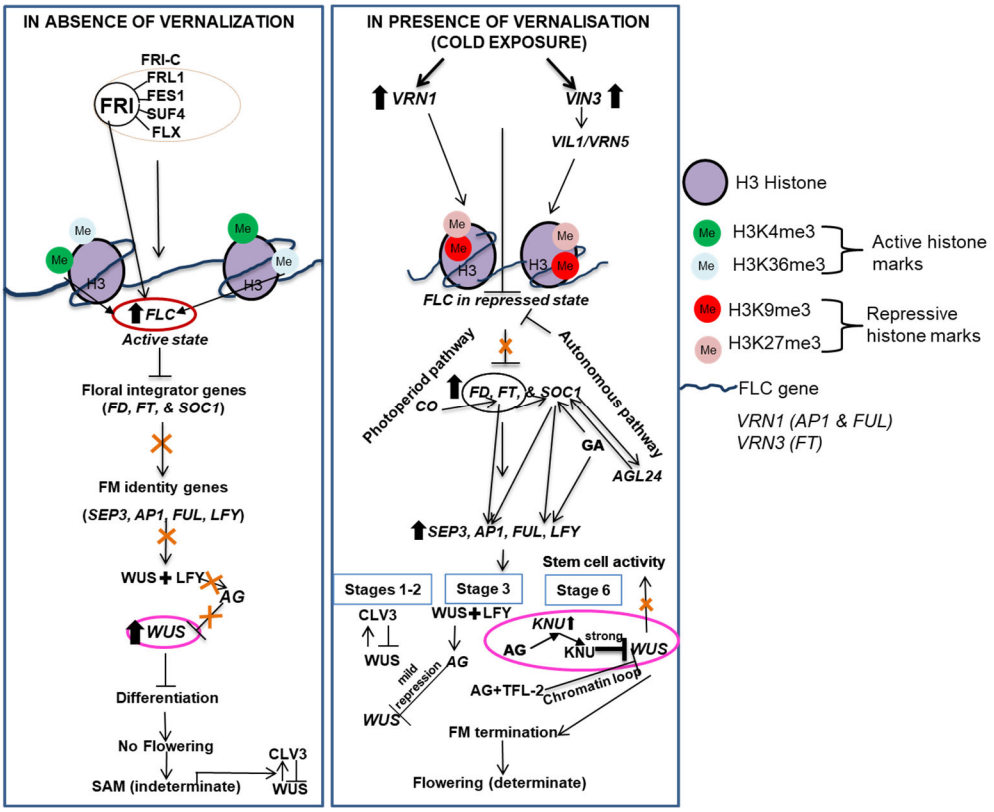

Figure 4. Simple heuristic model of the FRI-mediated FLC activation and vernalization-induced flowering pathway of *Arabidopsis*. In the absence of vernalization, *FLC* remains activated by a scaffold protein known as FRI. Because of the allelic variation in *FLC* and *FRI*, there is existence of natural variation in the requirement of vernalization. It must be noted that not all plants required to exhibit above mentioned model. AG, AGAMOUS; AGL24, AGAMOUS-LIKE 24; APETALA 1 (AP1); CLV, CLAVATA; CONSTANS (CO); FES 1, FRI essential 1; FLC, flowering locus C; FLOWERING LOCUS D (FD); FLX, FLC expressor; FRI, frigida; FRL 1, FRI-LIKE 1; FRUITFUL (FUL); FT, FLOWERING LOCUS T; LFY, LEAFY; SEPALLATA (SEP); SOC1, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1; SUF 4, suppressor OF FRI 4; TFL, TERMINAL FLOWER; VERNALIZATION 1 (VRN1); VERNALIZATION INSENSITIVE (VIN3); VRN5/VIN3-LIKE 1 (VIL1); WUS, WUSCHEL; H, Histone; K, Lysine; me3, trimethylation.

Vernalization oversees the precise timing of flowering majorly through *FLC* repression which activates the floral pathway integrator genes such as *FD*, *FT* and *SOC1*. This in turn upregulates the FM identity genes such as *SEPALLATA3 (SEP3), FRUITFUL (FUL), APETALA 1 (AP1)*, and *LFY* [93]. As the stages of flower development progress, stage 1–2 WUS-CLV loop is active, in stage 3 WUS induce *AG* along with the activated *LFY*, and, at stage 6, AG indirectly represses the *WUS* through KNU and also directly with TFL-2 to modulate chromatin loop for the FM termination [51]. Hence, it can be proposed that a direct/indirect relation can be established between vernalization and the WUS-CLV loop (Figure 4). The loop maintains stem cells in the SAM in vegetative state could also guides them for further organ development, transition, and termination. However, more research on this area needs to be conducted to elucidate the role of the WUS-CLV in vernalization, repression and reactivation of *FLC*.

8. Conclusions

Functional importance of the WUS-CLV feedback loop can be understood from the studies as this loop is active from the embryogenic stage to flower termination. According to the phylogenetic and functional analyses, the WUS-CLV feedback loop is conserved in plant lineages. These genes play pivotal role in embryo patterning, stem cell regulation,

SAM maintenance, organ differentiation, and cell identity transition. During this transition, other genes such as *LFY* become interlinked with the WUS loop and contribute to the cell identity transition for the IM and FM development. FM determinacy control requires a precise regulatory network of these genes to arrest floral stem cell activity for proper sepal, petal, stamen, and carpel development. Based on the environmental cues such as vernalization, photoperiod, phytohormones and ROS signaling interact with the WUS-CLV and WUS-AG for optimal flowering and reproduction growth. However, our knowledge of the gene regulatory networks on the stem cell niche for SAM maintenance and its transition mechanism on distinct floral development between plant species is still incomplete.

Our future investigation will be focused on the evolution of the flower, the conserved and distinct adaptation mechanism existing between the plant species during evolution and domestication. The results of our works can be utilized for plant architecture developmental studies and crop improvement programes.

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References

1. Gaillochet, C.; Lohmann, J.U. The never-ending story: from pluripotency to plant developmental plasticity. *Development* **2015**, *142*, 2237–2249. https://doi.org/10.1242/dev.117614.
2. Scheres, B. Stem cells: A Plant biology perspective. *Cell* **2005**, *122*, 499–504. https://doi.org/10.1016/j.cell.2005.08.006.
3. Weigel, D.; Jürgens, G. Stem cells that make stems. *Nature* **2002**, *415*, 751–754. https://doi.org/10.1038/415751a.
4. Chang, W.; Guo, Y.; Zhang, H.; Liu, X.; Guo, L. Same actor in different stages: Genes in shoot apical meristem maintenance and floral meristem determinacy in Arabidopsis. *Front. Ecol. Evol.* **2020**, *8*, 89. https://doi.org/10.3389/fevo.2020.00089.
5. Fletcher, J.C. The CLV-WUS Stem cell signaling pathway: A roadmap to crop yield optimization. *Plants* **2018**, *7*, 87. https://doi.org/10.3390/plants7040087.
6. Whitewoods, C.D.; Cammarata, J.; Nemec Venza, Z.; Sang, S.; Crook, A.D.; Aoyama, T.; Wang, X.Y.; Waller, M.; Kamisugi, Y.; Cuming, A.C.; et al. CLAVATA was a genetic novelty for the morphological innovation of 3D growth in land plants. *Curr. Biol.* **2018**, *28*, 2365–2376.e5. https://doi.org/10.1016/j.cub.2018.05.068.
7. Laux, T.; Mayer, K.F.; Berger, J.; Jurgens, G. The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. *Development* **1996**, *122*, 87–96. https://doi.org/10.1242/dev.122.1.87.
8. Schoof, H.; Lenhard, M.; Haecker, A.; Mayer KF, X.; Jürgens, G.; Laux, T. The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **2000**, *100*, 635–644. https://doi.org/10.1016/S0092-8674(00)80700-X.
9. Wang, X.; Wang, X.; Ren, J.; Ma, Y.; Yin, J. Characterization of tomato transcription factor WUSCHEL and functional study in Arabidopsis. *J. Integr. Agric.* **2012**, *11*, 1257–1265. https://doi.org/10.1016/S2095-3119(12)60122-1.
10. Xu, C.; Liberatore, K.L.; MacAlister, C.A.; Huang, Z.; Chu, Y.H.; Jiang, K.; Brooks, C.; Ogawa-Ohnishi, M.; Xiong, G.; Pauly, M.; et al. A cascade of arabinosyltransferases controls shoot meristem size in tomato. *Nat. Genetic*. **2015**, *47*, 784-792. https://doi.org/10.1038/ng.3309.
11. Nardmann, J.; Werr, W. The shoot stem cell niche in angiosperms: Expression patterns of WUS orthologues in rice and maize imply major modifications in the course of mono-and dicot evolution. *Mol. Biol. Evol.* **2006**, *23*, 2492–2504. https://doi.org/10.1093/molbev/msl125.

12. Bommert, P.; Lunde, C.; Nardmann, J.; Vollbrecht, E.; Running, M.; Jackson, D.; Hake, S.; Werr, W. Thick tassel dwarf1 encodes a putative maize ortholog of the Arabidopsis CLAVATA1 leucine-rich repeat receptor-like kinase. *Development* **2005**, *132*, 1235–1245. https://doi.org/10.1242/dev.01671.
13. Taguchi-Shiobara, F.; Yuan, Z.; Hake, S.; Jackson, D. The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. *Genes Dev.* **2001**, *15*, 2755–2766. https://doi.org/10.1101/gad.208501.
14. Je, B.; Gruel, J.; Lee, Y.K.; Bommert, P.; Arevalo, E.D.; Eveland, A.L.; Wu, Q.; Goldshmidt, A.; Meeley, R.; Bartlett, M.; et al. Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. *Nat. Genet.* **2016**, *48*, 785–791. https://doi.org/10.1038/ng.3567.
15. Tanaka, W.; Ohmori, Y.; Ushijima, T.; Matsusaka, H.; Matsushita, T.; Kumamaru, T.; Kawano, S.; Hirano, H.Y. Axillary meristem formation in rice requires the WUSCHEL ortholog *TILLERS ABSENT1*. *Plant Cell* **2015**, *27*, 1173–1184. https://doi.org/10.1105/tpc.15.00074.
16. Suzaki, T.; Sato, M.; Ashikari, M.; Miyoshi, M.; Nagato, Y.; Hirano, H.Y. The gene FLORAL ORGAN NUMBER1 regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to Arabidopsis CLAVATA1. *Development* **2004**, *131*, 5649–5657. https://doi.org/10.1242/dev.01441.
17. Chu, H.; Qian, Q.; Liang, W.; Yin, C.; Tan, H.; Yao, X.; Yuan, Z.; Yang, J.; Huang, H.; Luo, D.; et al. The *FLORAL ORGAN NUMBER4* Gene encoding a putative ortholog of Arabidopsis CLAVATA3 regulates apical meristem size in rice. *Plant Physiol.* **2006**, *142*, 1039–1052. https://doi.org/10.1104/pp.106.086736.
18. Liu, X.; Kim, Y.J.; Müller, R.; Yumul, R.E.; Liu, C.; Pan, Y.; Cao, X.; Goodrich, J.; Chen, X. *AGAMOUS* terminates floral stem cell maintenance in *Arabidopsis* by directly repressing WUSCHEL through recruitment of Polycomb Group proteins. *Plant Cell* **2011**, *23*, 3654–3670. https://doi.org/10.1105/tpc.111.091538.
19. Itkin, M.; Seybold, H.; Breitel, D.; Rogachev, I.; Meir, S.; Aharoni, A. TOMATO AGAMOUS-LIKE 1 is a component of the fruit ripening regulatory network. *Plant J.* **2009**, *60*, 1081–1095. https://doi.org/10.1111/j.1365-313X.2009.04064.x.
20. Schmidtiai', R.J.; Veit, B.; Mandel, M.A.; Mena, M.; Hakeyb, S.; Yanofsky', M.F. Identification and molecular characterization of ZAG1, the maize homolog of the Arabidopsis floral homeotic gene AGAMOUS. *Plant Cell* **1993**, *5*, 729–737. https://doi.org/10.1105/tpc.5.7.729.
21. Suzaki, T.; Toriba, T.; Fujimoto, M.; Tsutsumi, N.; Kitano, H.; Hirano, H.Y. (2008.). Conservation and diversification of meristem maintenance mechanism in *Oryza sativa*: Function of the *FLORAL ORGAN NUMBER2* gene. *Plant Cell Physiol.* **2006**, *47*, 1591–1602. https://doi.org/10.1093/pcp/pcl025.
22. Yamaguchi, T.; Lee, D.Y.; Miyao, A.; Hirochika, H.; An, G.; Hirano, H.-Y. Functional diversification of the two C-class MADS box genes OSMADS3 and OSMADS58 in *Oryza sativa*. *Plant Cell* **2006**, *18*, 15–28. https://doi.org/10.1105/tpc.105.037200.
23. Rodriguez, K.; Perales, M.; Snipes, S.; Yadav, R.K.; Diaz-Mendoza, M.; Reddy, G.V. DNA-dependent homodimerization, subcellular partitioning, and protein destabilization control WUSCHEL levels and spatial patterning. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E6307–E6315. https://doi.org/10.1073/pnas.160767311.
24. Yadav, R.K.; Perales, M.; Gruel, J.; Girke, T.; Jönsson, H.; Reddy, G.V. WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex. *Genes Dev.* **2011**, *25*, 2025–2030. https://doi.org/10.1101/gad.17258511.
25. Zuo, J.; Niu, Q.W.; Frugis, G.; Chua, N.H. The WUSCHEL gene promotes vegetative-to-embryonic transition in Arabidopsis. *Plant J.* **2002**, *30*, 349–359. https://doi.org/10.1046/j.1365-313X.2002.01289.x.
26. Gallois, J.L.; Nora, F.R.; Mizukami, Y. & Sablowski, R. WUSCHEL induces shoot stem cell activity and developmental plasticity in the root meristem. *Genes Dev.* **2004**, 18, 375-380. https://doi.org/10.1101/gad.291204.
27. Xu, Y.Y.; Wang, X.M.; Li, J.; Li, J.H.; Wu, J.S.; Walker, J.C.; Xu, Z.H.; Chong, K. Activation of the WUS gene induces ectopic initiation of floral meristems on mature stem surface in *Arabidopsis thaliana*. *Plant Mol. Bio.* **2005**, 57, 773–784. https://doi.org/10.1007/s11103-005-0952-9.
28. Zhou, Y.; Yan, A.; Han, H.; Li, T.; Geng, Y.; Liu, X.; Meyerowitz, E.M. HAIRY MERISTEM with WUSCHEL confines CLAVATA3 expression to the outer apical meristem layers. *Science* **2018**, *361*, 502–506. https://doi.org/10.1126/science.aar8638.
29. Kim, H.-J.; Wu, C.-Y.; Yu, H.-M.; Sheen, J.; Lee, H. Dual CLAVATA3 peptides in Arabidopsis shoot stem cell signaling. *J. Plant Biol.* **2017**, *60*, 506–512. https://doi.org/10.1007/s12374-017-0083-2.
30. Mitchum, M.G.; Wang, X.; Davis, E.L. Diverse and conserved roles of CLE peptides. *Curr. Op. Plant Biol.* **2008**, 11, 75–81. https://doi.org/10.1016/j.pbi.2007.10.010.
31. Perales, M.; Rodriguez, K.; Snipes, S.; Yadav, R.K.; Diaz-Mendoza, M.; Reddy, G.V. Threshold-dependent transcriptional discrimination underlies stem cell homeostasis. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E6298–E6306. https://doi.org/10.1073/pnas.1607669113.
32. Daum, G.; Medzihradszky, A.; Suzaki, T.; Lohmann, J.U. A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 14619–14624. https://doi.org/10.1073/pnas.1406446111.
33. Brand, U.; Fletcher, J.C.; Hobe, M.; Meyerowitz, E.M.; Simon, R. Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity. *Science* **2000**, *289*, 617–619. https://doi.org/10.1126/science.289.5479.617.
34. Reddy, G.V.; Meyerowitz, E.M. Stem-cell homeostasis and growth dynamics can be uncoupled in the Arabidopsis shoot apex. *Science* **2005**, *310*, 663–667. https://doi.org/10.1126/science.111626.
35. Plong, A.; Rodriguez, K.; Alber, M.; Chen, W.; Reddy, G.V. CLAVATA3 mediated simultaneous control of transcriptional and post-translational processes provides robustness to the WUSCHEL gradient. *Nat. Commun.* **2021**, *12*, 1–13. https://doi.org/10.1038/s41467-021-26586-0.

36. Chongloi, G.L.; Prakash, S.; Vijayaraghavan, U. Regulation of meristem maintenance and organ identity during rice reproductive development. *J. Exp. Bot.* **2019**, 70, 1719–1736. https://doi.org/10.1093/jxb/erz046.
37. DeYoung, B.J.; Bickle, K.L.; Schrage, K.J.; Muskett, P.; Patel, K.; Clark, S.E. The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis. *Plant J.* **2006**, *45*, 1–16. https://doi.org/10.1111/j.1365-313X.2005.02592.x.
38. Nimchuk, Z.L.; Zhou, Y.; Tarr, P.T.; Peterson, B.A.; Meyerowitz, E.M. Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases. *Development* **2015**, *142*, 1043–1049. https://doi.org/10.1242/dev.119677.
39. Durbak, A.R.; Tax, F.E. CLAVATA signaling pathway receptors of arabidopsis regulate cell proliferation in fruit organ formation as well as in meristems. *Genetics* **2011**, *189*, 177–194. https://doi.org/10.1534/genetics.111.130930.
40. Cao, X.; He, Z.; Guo, L.; Liu, X. Epigenetic mechanisms are critical for the regulation of *WUSCHEL* expression in floral meristems. *Plant Physiol.* **2015**, *168*, 1189–1196. https://doi.org/10.1104/pp.15.00230.
41. Sun, B.; Zhou, Y.; Cai, J.; Shang, E.; Yamaguchi, N.; Xiao, J.; Looi, L.S.; Wee, W.Y.; Gao, X.; Wagner, D.; et al. Integration of transcriptional repression and polycomb-mediated silencing of WUSCHEL in floral meristems. *Plant Cell* **2019**, *31*, 1488–1505. https://doi.org/10.1105/tpc.18.00450.
42. Deyhle, F.; Sarkar, A.K.; Tucker, E.J.; Laux, T. WUSCHEL regulates cell differentiation during anther development. *Dev. Biol.* **2007**, *302*, 154–159. https://doi.org/10.1016/j.ydbio.2006.09.013.
43. Yang, Y.; Sun, M.; Yuan, C.; Han, Y.; Zheng, T.; Cheng, T.; Wang, J.; Zhang, Q. Interactions between WUSCHEL-and CYC2-like transcription factors in regulating the development of reproductive organs in *Chrysanthemum morifolium*. *Int. J. Mol. Sci.* **2019**, *20*, 1276. https://doi.org/10.3390/ijms20061276.
44. Barton, M.K. Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. *Dev. Biol.* **2010**, *341*, 95–113. https://doi.org/10.1016/j.ydbio.2009.11.029.
45. Prunet, N.; Morel, P.; Negrutiu, I.; Trehin, C. Time to stop: Flower meristem termination. *Plant Physiol.* **2009**, *150*, 1764–1772. https://doi.org/10.1104/pp.109.141812.
46. Endrizzi, K.; Moussian, B.; Haecker, A.; Levin, J.Z.; Laux, T. The SHOOT MERISTEMLESS gene is required for maintenance of undifferentiated cells in Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristem genes WUSCHEL and ZWILLE. *Plant J.* **1996**, *10*, 967–979. https://doi.org/10.1046/j.1365-313X.1996.10060967.x.
47. Long, J.; Barton, M.K. Initiation of axillary and floral meristems in Arabidopsis. *Dev. Biol.* **2000**, *218*, 341–353. https://doi.org/10.1006/dbio.1999.9572.
48. Krizek, B.A.; Blakley, I.C.; Ho, Y.Y.; Freese, N.; Loraine, A.E. The Arabidopsis transcription factor AINTEGUMENTA orchestrates patterning genes and auxin signaling in the establishment of floral growth and form. *Plant J.* **2020**, *103*, 752–768. https://doi.org/10.1111/tpj.14769.
49. Rojo, E.; Sharma, V.K.; Kovaleva, V.; Raikhel, N.V.; Fletcher, J.C. CLV3 is localized to the extracellular space, where it activates the Arabidopsis CLAVATA stem cell signaling pathway. *Plant Cell* **2002**, 14, 969–977. https://doi.org/10.1105/tpc.002196.
50. Lohmann, J.U.; Hong, R.L.; Hobe, M.; Busch, M.A.; Parcy, F.; Simon, R.; Weigel, D. A molecular link between stem cell regulation and floral patterning in Arabidopsis. *Cell* **2001**, *105*, 793–803. https://doi.org/10.1016/S0092-8674(01)00384-1.
51. Sun, B.; Ito, T. Regulation of floral stem cell termination in Arabidopsis. *Front. Plant Sci.* **2015**, *6*, 17. https://doi.org/10.3389/fpls.2015.00017.
52. Lenhard, M.; Bohnert, A.; Jürgens, G.; Laux, T. Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. *Cell* **2001**, *105*, 805–814. https://doi.org/10.1016/s0092-8674(01)00390-7.
53. Payne, T.; Johnson, S.D.; Koltunow, A.M.; KNUCKLES (KNU) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the Arabidopsis gynoecium. *Development* **2004**, *131*, 3737–3749. https://doi.org/10.1242/dev.01216.
54. Sun, B.; Xu, Y.; Ng, K.H.; Ito, T. A timing mechanism for stem cell maintenance and differentiation in the Arabidopsis floral meristem. *Genes Dev.* **2009**, *23*, 1791–1804. https://doi.org/10.1101/gad.1800409.
55. Guo, L.; Cao, X.; Liu, Y.; Li, J.; Li, Y.; Li, D.; Zhang, K.; Gao, C.; Dong, A.; Liu, X. A chromatin loop represses WUSCHEL expression in Arabidopsis. *Plant J.* **2018**, *94*, 1083–1097. https://doi.org/10.1111/tpj.13921.
56. Gross-Hardt, R.; Lenhard, M.; Laux, T. WUSCHEL signaling functions in interregional communication Arabidopsis ovule development. *Genes Dev.* **2002**, *16*, 1129–1138. https://doi.org/10.1101/gad.225202.
57. Heisler, M.G.; Ohno, C.; Das, P.; Sieber, P.; Reddy, G.V.; Long, J.A.; Meyerowitz, E.M. Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. *Curr. Biol.* **2005**, *15*, 1899–1911. https://doi.org/10.1016/j.cub.2005.09.052.
58. Landrein, B.; Vernoux, T. Auxin, Chief Architect of the Shoot Apex. In *Auxin and Its Role in Plant Development*; Zažímalová, E., Petrášek, J., Benková, E., Eds.; Springer-Verlag: Wien, Austria, **2014**; pp. 191–212. https://doi.org/10.1007/978-3-7091-1526-8_10.
59. Schaller, G.E.; Bishopp, A.; Kieber, J.J. The yin-yang of hormones: cytokinin and auxin interactions in plant development. *Plant Cell* **2015**, *27*, 44–63. https://doi.org/10.1105/tpc.114.133595.
60. Snipes, S.A.; Rodriguez, K.; DeVries, A.E.; Miyawaki, K.N.; Perales, M.; Xie, M.; Reddy, G.V. Cytokinin stabilizes WUSCHEL by acting on the protein domains required for nuclear enrichment and transcription. *PLoS Genet.* **2018**, *14*, e1007351. https://doi.org/10.1371/journal.pgen.1007351.
61. Luo, L.; Zeng, J.; Wu, H.; Tian, Z.; Zhao, Z. A molecular framework for auxin-controlled homeostasis of shoot stem cells in Arabidopsis. *Mol. Plant* **2018**, *11*, 899–913. https://doi.org/10.1016/j.molp.2018.04.006.

62. Ma, Y.; Miotk, A.; Šutiković, Z.; Ermakova, O.; Wenzl, C.; Medzihradszky, A.; Gaillochet, C.; Forner, J.; Utan, G.; Brackmann, K.; et al. WUSCHEL acts as an auxin response rheostat to maintain apical stem cells in Arabidopsis. *Nat. Commun.* **2019**, *10*, 5093. https://doi.org/10.1038/s41467-019-13074-9.
63. Reinhardt, D.; Pesce, E.R.; Stieger, P.; Mandel, T.; Baltensperger, K.; Bennett, M.; Kuhlemeier, C. Regulation of phyllotaxis by polar auxin transport. *Nature* **2003**, *426*, 255–260. https://doi.org/10.1038/nature02081.
64. Leibfried, A.; To, J.P.C.; Busch, W.; Stehling, S.; Kehle, A.; Demar, M.; Kieber, J.J.; Lohmann, J.U. WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* **2005**, *438*, 1171–1175. https://doi.org/10.1038/nature04270.
65. Suzuki, T.; Miwa, K.; Ishikawa, K.; Yamada, H.; Aiba, H.; Mizuno, T. The arabidopsis sensor His-kinase, AHK4, can respond to cytokinins. *Plant Cell Physiol.* **2001**, *42*, 107–113. https://doi.org/10.1093/pcp/pce037.
66. Zhang, K.; Wang, R.; Zi, H.; Li, Y.; Cao, X.; Li, D.; Lin, D.; Tong, J.; Pan, Y.; Jiao, Y.; et al. AUXIN RESPONSE FACTOR3 regulates floral meristem determinacy by repressing cytokinin biosynthesis and signaling. *Plant Cell* **2018**, 30, 324–346. https://doi.org/10.1105/tpc.17.00705.
67. Zhao, Z.; Andersen, S.U.; Ljung, K.; Dolezal, K.; Miotk, A.; Schultheiss, S.J.; Lohmann, J.U. Hormonal control of the shoot stem-cell niche. *Nature* **2010**, 465:1089–1092.
68. Bao, S.; Hua, C.; Shen, L.; Yu, H. New insights into gibberellin signaling in regulating flowering in Arabidopsis. *J. Integr. Plant Biol.* **2020**, *62*, 118–131. https://doi.org/10.1111/jipb.12892.
69. Bolduc, N.; Hake, S. The Maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga20x1. *Plant Cell* **2009**, 21, 1647-1658. https://doi.org/10.1105/tpc.109.068221.
70. Yanai, O.; Shani, E.; Dolezal, K.; Tarkowski, P.; Sablowski, R.; Sandberg, G.; Samach, A.; Ori, N. Arabidopsis KNOXI proteins activate cytokinin biosynthesis. *Curr. Biol.* **2005**, 15, 1566–1571. https://doi.org/10.1016/j.cub.2005.07.060.
71. Chung, Y.; Zhu, Y.; Wu, M.F.; Simonini, S.; Kuhn, A.; Armenta-Medina, A.; Jin, R.; Østergaard, L.; Gillmor, C.S.; Wagner, D. Auxin Response Factors promote organogenesis by chromatin-mediated repression of the pluripotency gene SHOOTMERISTEMLESS. *Nat. Commu*. **2019**, *10*, 1–11. https://doi.org/10.1038/s41467-019-08861-3.
72. Jung, J.H.; Lee, S.; Yun, J.; Lee, M.; Park, C.M. The miR172 target TOE3 represses AGAMOUS expression during Arabidopsis floral patterning. *Plant Sci*. **2014**, *215*, 29–38. https://doi.org/10.1016/j.plantsci.2013.10.010
73. Kinoshita, A.; Vayssières, A.; Richter, R.; Sang, Q.; Roggen, A.; van Driel, A.D.; Richard, S.S.; Coupland, G. Regulation of shoot meristem shape by photoperiodic signaling and phytohormones during floral induction of Arabidopsis. *Elife* **2020**, *9*, e60661. https://doi.org/10.7554/eLife.60661.
74. Wang, H.; Caruso, L.V.; Downie, A.B.; Perry, S.E. The embryo MADS domain protein AGAMOUS-Like 15 directly regulated expression of a gene encoding an enzymes involved in gibberellin metabolism. *Plant Cell* **2004**, *166*, 1206–1219. https://doi.org/10.1105/tpc.021261.
75. Li, Q.F.; Wang, C.; Jiang, L.; Li, S.; Sun, S.S.M.; He, J.X. An interaction between BZR1 and DELLAs mediates direct signaling crosstalk between brassinosteroids and gibberellins in Arabidopsis. *Sci. Signal.* **2012**, *5*, ra72. https://doi.org/10.1126/scisignal.2002908
76. Browse, J. Jasmonate passes puster: A receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* **2009**, *60*, 183–205. https://doi.org/10.1146/annurev.arplant.043008.092007
77. Fernández-Calvo, P.; Chini, A.; Fernández-Barbero, G.; Chico, J.M.; Gimenez-Ibanez, S.; Geerinck, J.; Eeckhout, D.; Schweizerd, F.; Godoy, M.; Franco-Zorrillae, J.M.; et al. The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell* **2011**, *23*, 701–715. https://doi.org/10.1105/tpc.110.080788
78. Wang, H.; Li, Y.; Pan, J.; Lou, D.; Hu, Y.; Yu, D. The bHLH transcription factors MYC2, MYC3, and MYC4 are required for jasmonate-mediated inhibition of flowering in Arabidopsis. *Mol. Plant* **2017**, *10*, 1461–1464. https://doi.org/10.1016/j.molp.2017.08.007
79. Wu, K.; Zhang, L.; Zhou, C.; Yu, C.W.; Chaikam, V. HDA6 is required for jasmonate response, senescence and flowering in Arabidopsis. *J Exp. Bot.* **2008**, *59*, 225–234. https://doi.org/10.1093/jxb/erm300.
80. Zhou, C.; Zhang, L.; Duan, J.; Miki, B.; Wu, K. HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. *Plant Cell* **2005**, *17*, 1196–1204. https://doi.org/10.1105/tpc.104.028514.
81. Finkelstein, R.; Reeves, W.; Ariizumi, T.; Steber, C. Molecular Aspects of Seed Dormancy. *Annu. Rev. Plant. Biol.* **2008**, *59*, 387–415. https://doi.org/10.1146/annurev.arplant.59.032607.092740.
82. Wang, Y.; Li, L.; Ye, T.; Lu, Y.; Chen, X.; Wu, Y. The inhibitory effect of ABA on floral transition is mediated by ABI5 in Arabidopsis. *J. Exp. Bot.* **2013**, *64*, 675–684. https://doi.org/10.1093/jxb/ers361
83. Riboni, M.; Robustelli Test, A.; Galbiati, M.; Tonelli, C.; Conti, L. ABA-dependent control of GIGANTEA signaling enables drought escape via up-regulation of FLOWERING LOCUS T in *Arabidopsis thaliana*. *J. Exp. Bot.* **2016**, *67*, 6309–6632. https://doi.org/10.1093/jxb/erw384.
84. Schmidt, R.; Schippers, J.H.M. ROS-mediated redox signaling during cell differentiation in plants. *Biochim. Biophys. Acta-Gen. Subj.* **2015**, *1850*, 1497–1508. https://doi.org/10.1016/j.bbagen.2014.12.020.
85. He, L.; He, T.; Farrar, S.; Ji, L.; Liu, T.; Ma, X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell. Phy. Biochem.* **2017**, *44*, 532–553. https://doi.org/10.1159/000485089.

86. Zeng, J.; Dong, Z.; Wu, H.; Tian, Z.; Zhao, Z. Redox regulation of plant stem cell fate. *EMBO J.* **2017**, *36*, 2844–2855. https://doi.org/10.15252/embj.201695955.
87. Wang, Y.; Shirakawa, M.; Ito, T. Dynamic changes in reactive oxygen species in the shoot apex contribute to stem cell death in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **2022**, *2022*, 3864. https://doi.org/10.3390/ijms23073864.
88. Berrios, L.; Rentsch, J.D. Linking reactive oxygen species (ROS) to abiotic and biotic feedbacks in plant microbiomes: The dose makes the poison. *Int. J. Mol. Sci.* **2022**, *23*, 4402. https://doi.org/10.3390/ijms23084402
89. Leijten, W.; Koes, R.; Roobeek, I.; Frugis, G. Translating flowering time from *Arabidopsis thaliana* to Brassicaceae and Asteraceae crop species. *Plants* **2018**, *7*, 111. https://doi.org/10.3390/plants7040111
90. Sheldon, C.C.; Rouse, D.T.; Finnegan, E.J.; Peacock, W.J.; Dennis, E.S. The molecular basis of vernalization: The central role of FLOWERING LOCUS C (FLC). *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3753–3758. https://doi.org/10.1073/pnas.97.7.3753.
91. Shindo, C.; Aranzana, M.J.; Lister, C.; Baxter, C.; Nicholls, C.; Nordborg, M.; Dean, C. Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. *Plant Physiol.* **2005**, *138*, 1163–1173. https://doi.org/10.1104/pp.105.061309.
92. Choi, K.; Kim, J.; Hwang, H.-J.; Kim, S.; Park, C.; Kim, S.Y.; Lee, I. The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in arabidopsis by recruiting chromatin modification factors. *Plant Cell* **2011**, *23*, 289–303. https://doi.org/10.1105/tpc.110.075911.
93. Kim, D.H.; Doyle, M.R.; Sung, S.; Amasino, R.M. Vernalization: Winter and the timing of flowering in plants. *Annu. Rev. Cell Dev. Biol.* **2009**, *25*, 277–299. https://doi.org/10.1146/annurev.cellbio.042308.113411.
94. Bastow, R.; Mylne, J.; Lister, C.; Lippman, Z.; Martienssen, R.; Dean, C. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **2004**, *427*, 164–167. https://doi.org/10.1038/nature02269.
95. Amasino, R.M.; Sung, S. Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature* **2004**, *427*, 159–164. https://doi.org/10.1038/nature02195.
96. Yang, H.; Howard, M.; Dean, C. Antagonistic roles for H3K36me3 and H3K27me3 in the cold-induced epigenetic switch at Arabidopsis FLC. *Curr. Biol.* **2014**, *24*, 1793–1797. https://doi.org/10.1016/j.cub.2014.06.047.
97. Blazquez, M.A.; Green, R.; Nilsson, O.; Sussman, M.R.; Weigel, D. Gibberelllins promote flowering *Arabidopsis* by activating the LEAFY promoter. *Plant Cell* **1998**, *10*, 791–800.
98. Liu, C.; Chen, H.; Er, H.L.; Soo, H.M.; Kumar, P. Direct interaction of AGL24 and SOC1 integrates flowering signals in *Arabidopsis*. *Development* **2008**, *135*, 1481–1491.
99. Sheldon, C.C.; Hills, M.J.; Lister, C.; Dean, C.; Dennis, E.S.; Peacock, W.J. Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2214–2219. https://doi.org/10.1073/pnas.0711453105.
100. Choi, J.; Hyun, Y.; Kang, M.J.; Yun, H.I.; Yun, J.Y.; Lister, C.; Dean, C.; Amasino, R.M.; Noh, B.; Noh, Y.S.; et al. Resetting and regulation of *FLOWERING LOCUS C* expression during Arabidopsis reproductive development. *Plant J.* **2009**, *57*, 918–931. https://doi.org/10.1111/j.1365-313X.2008.03776.x.
101. Angel, A.; Song, J.; Dean, C.; Howard, M.A Polycomb-based switch underlying quantitative epigenetic memory. *Nature* **2011**, *476*, 105–108. https://doi.org/10.1038/nature10241.
102. Yun, H.; Hyun, Y.; Kang, M.J.; Noh, Y.S.; Noh, B.; Choi, Y. Identification of regulators required for the reactivation of FLOWERING LOCUS C during Arabidopsis reproduction. *Planta* **2011**, *234*, 1237–1250. https://doi.org/10.1007/s00425-011-1484-y.