

A dynamic model for stem cell homeostasis and patterning in *Arabidopsis* meristems

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Abstract

Plants maintain stem cells in their meristems as a source for new undifferentiated cells throughout their life. Meristems are small groups of cells that provide the microenvironment that allows stem cells to prosper. Homeostasis of a stem cell domain within a growing meristem is achieved by signalling between stem cells and surrounding cells. We have here simulated the origin and maintenance of a defined stem cell domain at the tip of *Arabidopsis* shoot meristems, based on the assumption that meristems are self-organizing systems. The model comprises two coupled feedback regulated genetic systems that control stem cell behaviour. Using a minimal set of spatial parameters, the mathematical model allows to predict the generation, shape and size of the stem cell domain, and the underlying organizing centre. We use the model to explore the parameter space that allows stem cell maintenance, and to simulate the consequences of mutations, gene misexpression and cell ablations.

Introduction

Growth of the aerial parts of higher plants relies on a life-long supply with cells by the shoot apical meristem (SAM). The SAM contains a small population of non-differentiating stem cells in the central zone at the meristem tip [1]. After cell divisions in the stem cell domain (SCD), daughter cells are shifted towards the surrounding peripheral zone, where organ primordia are initiated and cells can enter a differentiation pathway. The architectural makeup of flower primordia, which gives rise to the plant's reproductive organs, resembles that of the SAM with the main difference that stem cell activity is switched off in flowers after generation of a species-specific number of organs. It is evident that land plants such as trees, which can grow in size and produce new organs for hundreds of years, must have developed robust regulatory systems that enable them to maintain active stem cell populations also under changing or adverse environmental conditions. Disturbing stem cell regulation can arrest the growth of a plant's shoot tip, or may result in gross tissue overproliferation and failure to reproduce. More subtle alterations in stem cell proliferation can affect overall size of a seed-producing inflorescence structures, such as a maize cob, the size of a fruit, or the number of petals in a horticultural flower. We are only just beginning to understand how the fate of the stem cell population is regulated in higher plants.

Maintenance of the undifferentiating stem cell population depends on signals from cells of the organizing centre or OC, which reside underneath the SCD in a deeper region of the meristem. Several gene products have been identified that enable these adjacent cell groups to communicate with each other. The stem cells of *Arabidopsis thaliana* secrete the CLAVATA3 (CLV3) peptide, consisting of 12 amino acids [2,3,4]. CLV3 was shown to interact with the LRR-receptor kinase CLAVATA1 (CLV1) that is expressed in and surrounding the OC [5,6]. A second receptor system composed of the LRR-protein CLAVATA2 (CLV2) and the membrane associated kinase CORYNE (CRN) is more widely

expressed in the meristem and vasculature, and also contributes to signal perception [7,8]. CLV3 dependent activation of the two receptor systems represses the expression of WUSCHEL (WUS), a homeodomain transcription factor that is normally produced from OC cells, and which is required for the maintenance of stem cells [9,10]. WUS itself acts non-cellautonomously to promote stem cell fate at the meristem tip. The WUS protein does not seem to move, and it could control the expression of other genes that generate a diffusible signal which ultimately promotes stem cell identity [11]. Searches for target genes showed that several *ARABIDOPSIS RESPONSE REGULATOR (ARR)* genes, which are negative regulators of cytokinin signalling, are repressed by *WUS*, thus involving cytokinin in meristem maintenance [12]. However, *WUS* induces stem cell fate only at the meristem tip, and not in the (*WUS* expressing) OC cells or other surrounding cells, indicating that a spatially restricted cofactor, or a competent cellular state is required to respond to *WUS* activity [13].

Because stem cells signal back to the OC via CLV3 and its receptors to restrict *WUS* expression, a feedback circuitry is established that maintains a stable stem cell population. Support for this model of stem cell homeostasis comes from a number of experimental observations: 1) loss-of-function mutants of *WUS* cannot maintain stem cells [10]; 2) loss-of-function mutants of *CLV3* (or *CLV1*, *CLV2* or *CRN*) allow for less restricted *WUS* expression and production of excessive stem cells [3,4,8,14]; 3) constitutive high level expression of *CLV3* represses *WUS*, causing stem cell loss [4]; 4) when *WUS* expression is uncoupled from repression by *CLV3*, e.g. when controlled from a heterologous promoter, the stem cell domain expands [15,16]; 5) the *CLV3/WUS* circuitry is capable of self-organization. This was revealed by laser ablation experiments in tomato, showing that after elimination of both SCD and OC, new domains of *WUS* expression are generated at peripheral sites that then initiate new SCDs, which support further growth of the SAM [17].

However, all previous studies performed on various mutants or constitutive misexpression lines did not allow studying the immediate consequences of system perturbations. Cell ablation experiments are further complicated by wounding effects, and ectopic cell divisions in the SAM which are required for regeneration. Analyzing the dynamics of the *CLV3/WUS* circuitry at a shorter timescale required rapid and transient perturbations of gene expression. In a first study of this type, *CLV3* expression was silenced by Dexamethason-induced expression of a foldback *CLV3* RNA [18]. Live imaging of the SAM before and after *CLV3* silencing showed that expression of a *CLV3:GFP* transgene, acting as a reporter for stem cell identity, extended into cells adjacent to the central zone within 24 hours after induction. Importantly, this re-specification of peripheral cells to stem cell identity was not preceded by cell divisions. In a similarly designed experiment, induction of high level *CLV3* expression downregulated both *WUS* expression, and the stem cell marker *CLV3*, within 3 hours [14]. Together, these experiments showed that the *CLV3/WUS* circuitry is acting throughout development, and that the output, stem cell number, can be continuously readjusted in response to changing amounts of the signalling components. In line with this, fluctuations of central zone size were observed, indicating continuous activity of the circuitry [18].

However, the *CLV3/WUS* circuitry was also found to be surprisingly robust and to tolerate changes in *CLV3* expression levels over a tenfold range [14], indicating that stem cells do not directly communicate their number via the amount of released *CLV3* signal. Furthermore, while strong *CLV3* signalling rapidly repressed *WUS* expression, a slowly acting compensation mechanism appeared to upregulate *WUS* with time. The components of this compensatory circuitry are unknown, but may be found among the gene set that controls *WUS* expression. *SPLAYED* (*SYD*) encodes a *SNF2*-type chromatin-remodelling ATPase that is required for *WUS* transcription [19]. *BARD1*, carrying BRCT and RING domains, interacts

with and antagonizes SYD to restrict *WUS* expression to the OC [20]. *HANABA TARANU* (*HAN*), coding for a GATA-transcription factor, represses *WUS* postembryonically from the developing vasculature [21]. The interplay between these components is not understood, and they may act exclusively to establish a discrete *WUS* expression domain when meristems are generated. During development, a second feedback mechanism could operate via the cytokinin signalling pathway. *WUS* represses the expression of several *ARRs* in the meristem, which restrict cytokinin signalling [12]. In turn, continuous activation of *ARRs* arrests meristem activity and *WUS* expression, suggesting that *WUS* and *ARRs* mutually repress each other.

We have generated a computational model of stem cell fate regulation by the *CLV3/WUS* circuitry in the shoot apical meristem. Our model incorporates two feedback regulatory systems that merge upon *WUS* regulation. The driving force for modelling was to better understand the forces that shape the *CLV3* and *WUS* expression domains, while making the minimal number of necessary assumptions about the factors to be involved. We used the model to study the effects of targeted system perturbations, and to explore the parameter space that allows for stem cell homeostasis under fluctuating conditions.

Results

Model components and basic assumptions

We propose a partial differential equation (PDE) model to follow the dynamics of gene regulation across the SAM (Fig1A). Conceptually, at the centre of the model lies the regulation of *WUS* via two separable feedback operated reaction-diffusion systems, a commonly used type of differential equation models for developmental processes in biology [22]. Instead of representing the entire meristem structure, we here restrict the spatial component to two dimensions using an artificial longitudinal section through the SAM (Fig1B). Cells within this meristem section are modelled as discrete entities. We neglected growth and cell divisions for two reasons: firstly, we are concentrating on meristem homeostasis, i.e. meristem size remains unaltered, and secondly, the gene regulation that is considered in this study is faster than the cell cycle. The cellular or tissue framework thus remains static. The regulative processes within cells are mapped to a set of PDEs constituting a gene regulative program, which is executed in each cell. The components and underlying assumptions of our model are summarized as follows (Fig1A):

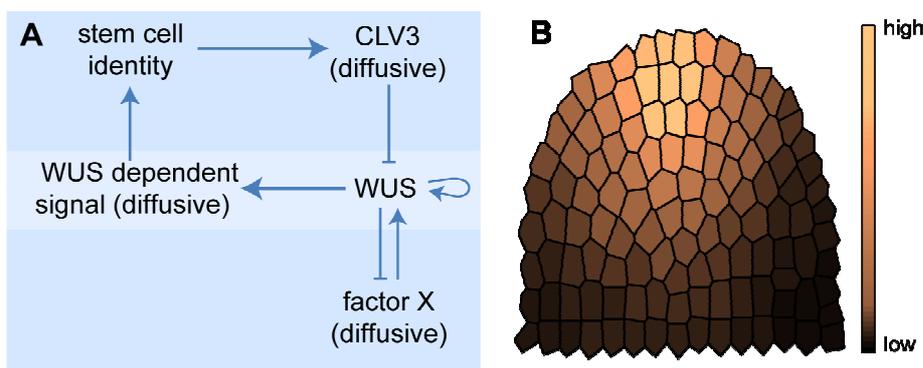


Figure 1: Modelling components and cellular framework.

(A) Components and their interactions regulating stem cell homeostasis in *Arabidopsis*.
(B) Two-dimensional meristem frame with assumed anchoring distribution indicated by colouring.

Stemness:

Cells of the meristem can acquire stem cell identity, reflected in their level of *stemness*, which is controlled by a *WUS*-dependent signal (*WUS-signal*, see below). We avoided an artificial and static cut-off concentration for *WUS-signal*, above which cells switch to the stem cell status, and instead established a dynamic but sigmoidal response to *WUS-signal*, that results in variable levels of *stemness* to represent a cell's state.

Experimental evidence from *WUS* misexpression shows that only outer cell layers acquire stem cell identity. The underlying factors responsible for this are not known. We are now not postulating another signal, but take this observation into account by allowing only cells in outer cell layers to acquire stem cell identity. Therefore, only cells in the outer layers of the meristem are competent to react to *WUS-signal* and we restricted *stemness* to the outer layers. Stem cells express the signalling molecule *CLV3*, proportional to their *stemness* level. The *stemness* levels are expressed by the model variable [*st*].

CLV3:

CLV3 freely diffuses to neighbouring cells. To avoid flooding the entire model with *CLV3*, the *CLV3* peptide is regarded to decay with time. We eliminated the need for receptor proteins or other signalling components because insufficient quantitative data are available to assess their contribution. Furthermore, *CLV*-signalling appears to be largely controlled by the amount of available *CLV3* peptide [14]. Thus, the local *CLV3* concentration is computed to directly restrict *WUS* expression. The *CLV3* levels are expressed by the model variable [*CLV3*].

WUS:

We assume that *WUS* protein is not mobile and therefore remains mostly in the cells where the *WUS* gene is expressed [11], except for weak leakage diffusion. While all cells of the

model meristem are in principal able to express *WUS*, we added a spatial parameter which makes cells that reside closer to the meristem tip more competent to activate *WUS* expression (Fig1B). This anchoring was found to be necessary in our model to ensure correct positioning of the two functional domains (SCD and OC) within the dome. Without the spatial component, immediately neighbouring SCD and OC are still formed, but at more random locations (Fig2). The requirement for a spatial component reflects the fact that our virtual meristem is not structured, i.e., all cells are intrinsically equal and carry no positional information. In plant meristems, such spatial information will be provided by signals within and between cell layers, or from the vasculature. We introduced a positive feedback loop for *WUS* via autoactivation. Although not experimentally proven, it is supported by the observation of a rapid upregulation of *WUS* expression in regenerating callus [23]. *WUS* promotes the expression of *WUS-signal*, which is mobile and can diffuse to neighbouring cells. The *WUS* levels are expressed by the model variable [*WUS*].

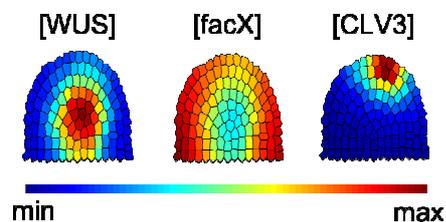


Figure 2: System without anchoring distribution.

Equilibrium state of a simulation where the anchoring distribution guiding *WUS* expression is exchanged by a constant *WUS* reaction rate. As a result of the missing positional information, SCD and OC keep their positions relative to each other, but the SCD is now formed at a random location in the outer meristem layers.

WUS-signal:

WUS-signal is generated by all *WUS* expressing cells, and the amount produced depends on the levels of *WUS* expression. Similar to *CLV3*, *WUS-signal* is mobile and degraded at a constant rate. Cells react to the amount of *WUS-signal* they receive with *stemness*. Only outer

cell layers of the meristem are competent to respond to the *WUS-signal*. The *WUS-signal* levels are expressed by the model variable $[WUS_{sig}]$.

Factor X:

To account for *CLV* independent regulation of *WUS* expression, we incorporated a *factor X* (*facX*). At the start of the simulation, *facX* is expressed homogeneously in all cells and is freely diffusing. *FacX* induces *WUS* expression, but is itself under negative feedback regulation by *WUS* [14]. This is implemented through active degradation or consumption of *facX* by *WUS*. The interactions between *WUS* and *facX* are thus based on an activator-substrate-like mechanism that will generate a discrete *WUS* domain. The *facX* levels are expressed by the model variable $[facX]$.

The described entities are compiled into a PDE representation of the intracellular gene regulative program given by Eqs. (0.1)-(0.5) (see Materials and Methods section). An overview of the interactions is given in Fig1A.

The resulting model depends on a set of parameters like kinetic constants; validation of our model therefore required first to identify a parameter setting that allow reproducing the two functional meristem domains, i.e. the *CLV3*-expressing SCD and the *WUS*-expressing OC, at approximately those locations which are experimentally observed in wildtype meristems (Fig3). The model parameters have been tuned by hand using a decomposition of the model (see Materials and Methods section).

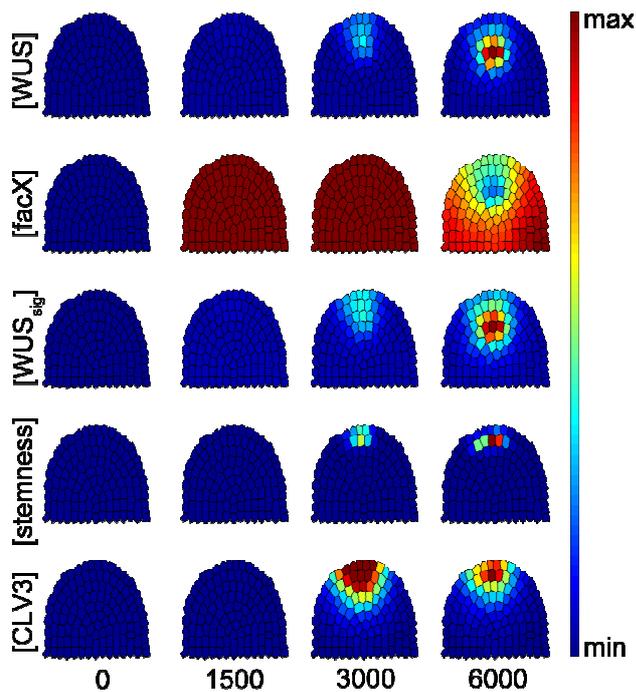


Figure 3: Time course simulation of a wild-type meristem.

Grading from blue (minimum) to red (maximum) illustrates the relative concentration of the indicated components. From left to right, the simulations were developed from close to zero concentrations for number of steps shown below, until the (stable) equilibrium state was reached.

Simulation of wildtype

Starting from almost zero concentrations of all considered components, the system was simulated until an equilibrium state was reached (Fig3); since we investigate system behaviour by means of numerical integration of the model equations, under ‘equilibrium state’ we understand a state where all derivatives are zero or reasonably close to zero. In the wild-type scenario, a given meristem showed *WUS* expression first at the meristem tip, triggered by a sufficiently high level of *facX*. *WUS* then increased, thereby repressing *facX* at the same location. The centre of the OC shifted downwards. Distribution of *WUS-signal* overlaps with that of *WUS*, but since *WUS-signal* is diffusible, it is located in a wider domain and extended always to the meristem tip. Together with an increase of *stemness* at the meristem tip, *CLV3* became expressed, pushing the OC downwards from its initial position.

This time course reproduced the dynamic changes in gene expression patterns that are observed during embryonic development of the shoot meristem.

Simulations of mutants and system perturbations

I. Reducing CLV3 expression

We next tested the consequences of reducing *CLV3* expression. When *CLV3* is downregulated during plant development, both the SCD and the OC expand laterally due to unrestricted *WUS* expression [4,16,18]. Furthermore, *WUS* expression is then no longer excluded from the meristem tip.

Our in silico analysis started from an equilibrated wild-type meristem, thus simulating a conditional knock-out of *CLV3*. When *CLV3* expression was stopped, the SCD rapidly enlarged due to recruitment of lateral cells. At the same time, the OC expanded and shifted towards the meristem tip (Fig4). A similar, but less pronounced effect was seen when *CLV3* was still expressed, but at reduced levels (see FigS1 in supporting information).

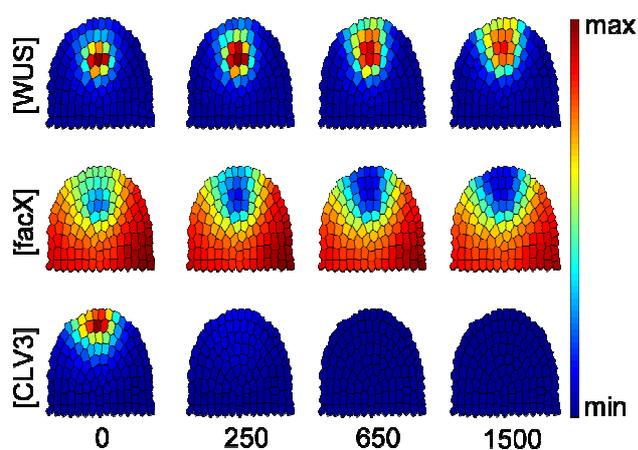


Figure 4: Time course simulation for a *clv3* loss-of-function mutant.

Only the concentrations of *WUS*, *facX* and *CLV3* are shown, starting from an equilibrated wild-type (0). At calculation time point 1, *CLV3* concentration is set to 0, and simulations proceed until equilibrium. Note that the *WUS* domain expands and shifts upwards.

In addition, we simulated the effects of reducing *CLV3* receptor activity, i.e. *CLV1* or *CLV2* and CRN constructs (see FigS2 in supporting information). These simulations showed behaviour comparable to reductions in *CLV3* expression and thereby supports the assumption that from a modelling perspective it is sufficient to model *CLV3* activity as a representative for *CLV* signalling.

II. Increasing CLV3 expression

Plants that continuously express *CLV3* fail to maintain a shoot meristem due to an early arrest of *WUS* expression and stem cell differentiation [4]. However, inducible overexpression during development was found to be compensated in some flower meristems, resulting in a recovery of *WUS* expression at later stages [14]. In our simulations, high level expression of *CLV3* in all cells caused a rapid shrinkage of the OC, downregulation of *WUS*, and a reduction in *stemness*, concomitant with a reduction in *CLV3* expression levels from the SCD (Fig5A). Overexpression of *CLV3* set at an intermediate level resulted in *WUS* repression and a rapid loss of *stemness*, which recovered with time (see Fig5B). A similar behaviour was observed in plant floral meristems in response to induced overexpression of *CLV3* [14]. Low level overexpression of *CLV3* allowed the system to reach a new stable equilibrium state, with a smaller OC and SCD (see FigS3 in supporting information).

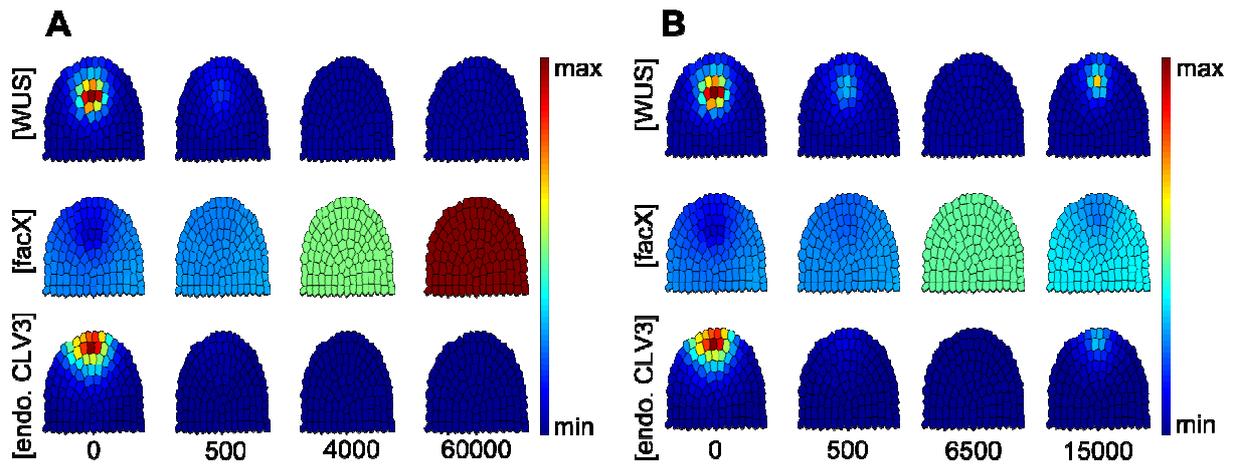


Figure 5: Response to *CLV3* overexpression in the entire meristem.

Time-evolution of *WUS*, *FacX* and *CLV3* concentrations upon strong (A) or intermediate (B) level overexpression of *CLV3* is shown, starting from equilibrated wild-type meristem (time-point 0) until the simulations reach a new equilibrium state. *endoCLV3* = *CLV3* expression from the endogenous promoter, taken as reporter for stem cell identity. Note that both a *WUS* expression domain and stem cells are reinitiated in (B), but not in (A).

We further tested the robustness of the system against perturbations by analyzing the response to altered endogenous *CLV3* expression in small, discrete steps: the effectiveness of *stemness*-dependent *CLV3* expression is tested in a range from 10% to 620% in 10% steps. Varying *CLV3* levels from 90% to 620% compared to wildtype affected the size of the SCD, while OC cell number remained constant (see FigS1B in supporting information). This indicates that OC and SCD sizes are not strictly coupled, which has been also noted experimentally when analyzing the sizes of OC and SCD in plants grown under diverse environmental conditions [24]. In the simulations, we varied *CLV3* expression in 10% steps in a range from 10% to 620%.

III. Altering *WUS* expression

Lowering *WUS* expression levels reduces the sizes of both SCD and OC to a similar extent, and will cause a loss of both domains when *WUS* is fully repressed (see FigS4 in supporting information). Ectopic *WUS* expression was tested by changing the effect of *CLV3* signalling on *WUS* activity from repressive to activating. In plants, this has been achieved by expressing *WUS* from the *CLV3* promoter [15], which caused the coalescence of OC and SCD at the

meristem tip together with lateral expansion of this joint domain. This cell behaviour is also observed in our simulations (Fig6).

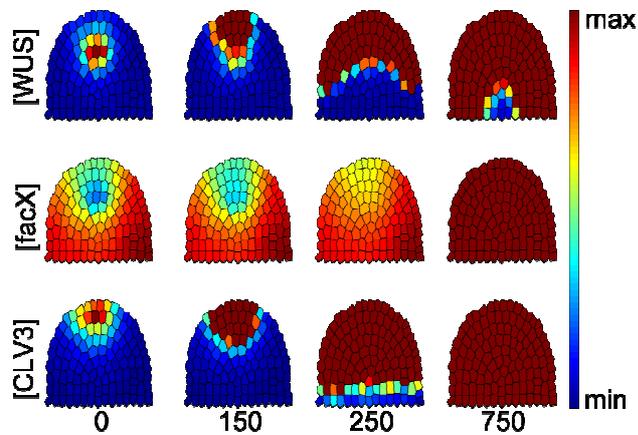


Figure 6: Simulation for *WUS* misexpression in the stem cell domain.

Starting from an equilibrated wild-type meristem, misexpression of *WUS* from the *CLV3* promoter (*CLV3*>>*WUS*) is simulated until a new equilibrium state is reached. Cells in the meristem now acquire mixed identities and express both the OC and SCD marker genes.

IV. Regeneration and de-novo generation of OC and SCD

After ablating SCD and OC from the meristem by pointing a laser beam at the meristem tip, *WUS* becomes expressed at the periphery, and the OC and SCD are regenerated with time [17], highlighting that cells at the periphery are capable of, but normally inhibited from the acquisition of OC identity. We simulated the laser ablation experiment starting from an equilibrated wildtype meristem, and eliminated all *WUS* or *CLV3* expressing cells from the meristem. We found that a new OC was generated which induced a SCD nearby (Fig7). This shows the self-generative capacity of our meristem model. During normal plant development, new meristems are generated during embryogenesis, flowering, and when axillary meristems are initiated. By simply altering *facX* expression levels in a given cellular framework, we could simulate the generation of a new OC and SCD, which coordinated increase in size when *facX* is further upregulated (see FigS5 in supporting information).

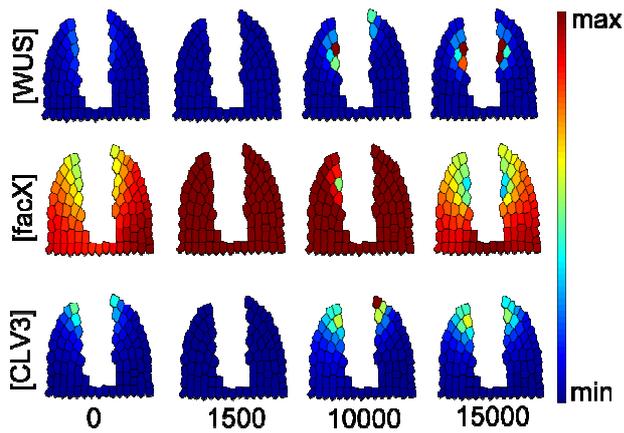


Figure 7: Simulation for a cell ablation scenario.

The central region of the meristem was eliminated by virtual cell ablation. Note that even in the absence of an SCD and underlying OC, both domains can be partially restored.

V. Role of $facX$ and WUS feedback regulation

In addition to the already described scenarios that are all inspired by previously conducted experiments, we analysed the role of $facX$ and the interaction of WUS with $facX$. Without feedback of WUS to $facX$, $facX$ could be exchanged for a constant expression of WUS . We tested this idea by eliminating the feedback term of WUS on $facX$ (see Eq. (0.2) in Materials and Methods). However, using an evolutionary algorithm to search the parameter space of the resulting model, we could not identify any parameter settings resulting in the desired system behaviour, with separate but adjacent SCD and OC. We therefore conclude that $facX$ and the feedback between WUS and $facX$ are vital for the model to initiate stable Turing patterning. Candidates to realize the role of $facX$ are genes and functions that control WUS expression. Because eliminating $facX$ destabilizes the OC, we predict that mutations in genes contributing to $facX$ function should result in either unrestricted WUS expression and meristem overproliferation (Fig8), or meristem arrest.

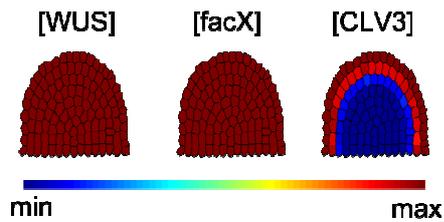


Figure 8: System neglecting feedback of *WUS* on *facX*.

Archetype system behaviour for simulations without feedback of *WUS* on *facX*: the meristem overproliferates, *WUS* is expressed in all meristem cells and all cells in the outer layers acquire stem cell identity indicated by their expression of the stem cell marker *CLV3*.

VI. Influence of anchoring factor distribution on system behaviour

We have introduced artificial positional information in form of an anchoring distribution (Fig1B) to stimulate SCD and OC positioning at the meristem tip. To test the possible influence of this anchoring distribution on the pattern formation capabilities of the model, we exchanged the distribution for a constant value. Although positioning of SCD and OC became more variable now, we were still able to identify parameter sets that result in spatially confined and adjacent SCD and OC (Fig2), indicating that the anchoring distribution has no significant influence on the qualitative behaviour of our model.

Discussion

Mathematical modelling is a tool that allows asking the most stringent questions concerning the dynamic behaviour of predicted gene regulatory networks; it also quickly uncovers the restrictions and shortcomings of assumed interaction maps, and thus provides guidance to direct future experiments. We had initially attempted to build a model for the SCD and OC, based solely on the interaction between two activator-inhibitor based systems (*CLV3/WUS*, and *WUS/facX*) which were linked via *WUS* as the common node. Conceptually, the underlying assumption was that SCD and OC could originate independently of each other, but that their maintenance and relative position are controlled by mutual feedback regulation. However, such a model failed to reproduce the domain arrangement observed in actual meristems for the model's parameter space which we explored using a stochastic parameter estimation technique, namely an evolutionary algorithm [25]. For the evolutionary algorithm, we used an objective function described previously [26], extended by a simple domain recognition procedure capable of identifying circular domains. This indicates that an essential component was missing from this model. The most common outcome which we achieved was not juxtaposition, but an overlap of the SCD with the OC. To improve the spatial separation of the two domains, we considered that cells within an actual meristem differ from each other by their position. Several misexpression experiments using *WUS* had previously shown that the cellular response to *WUS*-derived signals depends on a cell's relative position within the meristem, corresponding to its developmental trajectory. Only by adding spatial components to our model we were able to achieve a realistic sizing and arrangement of the two domains within the meristem; removal of this spatial component causes extensive spatial overlapping of the SCD and OC.

Jönsson et al. had previously described a model for the *WUS/CLV3* interaction that concentrated on the generation of the *CLV3* domain (the SCD in our model) by a *WUS*

derived signal [27]. This model did not yet include the negative feedback regulation of *CLV3* signalling upon *WUS* expression, and the creation and maintenance of the *WUS* domain was not simulated. To confine the *CLV3* domain to the meristem tip, the authors proposed that an (unidentified) factor diffusing from the outermost meristem layer, the L1, together with the *WUS*-dependent signal, induced *CLV3* expression. They later used a reaction-diffusion model combined with two repressive signals, derived from the L1 and stem tissue, to activate *WUS* expression in a deeper meristem region [28]. Both models were successful at reproducing either the OC or the SCD, but did not incorporate the mutual interdependence between the factors that shape the two domains, and were less parsimonious with system components than the model we describe here.

A recently published model by Geier et al. [24] did not describe the spatial arrangement of domains, but addressed the observation that SCD and OC sizes vary strongly under changing environmental conditions. The model describes the SCD and OC as cell pools that are connected via differentiation rates and expand due to cell proliferation, which is regarded as an externally controlled parameter. Variation in the relative sizes of SCD and OC can be explained by assuming that a differentiation signal *X* is produced by OC or SCD, which can buffer the response of the cell pools against changes in proliferation rates. Although this model did not allow reproducing all mutant and overexpression experiments that we have simulated here, it combined modelling approaches with quantitative data, and highlighted the enormous developmental plasticity of the meristem.

We challenged our model by altering central system parameters to simulate mutant phenotypes and published transgenic experiments. In all experiments, our model proved to be robust against small-scale perturbations (see suppl. Information: sensitivity analysis). This stability probably results from the combination of two feedback operated system, whereby one of them, the *WUS/facX* system, acts as a buffer that dampens fluctuations in *WUS* levels.

Furthermore, the reaction rates that influence *WUS* expression in our simulations are one order of magnitude smaller than those controlling *CLV3* levels. Increased stability against signalling noise was uncovered in the analysis of coupled positive feedback systems if the two linked regulatory loops operated at different speeds [29,30]. Our modelling approach has revealed that combined feedback systems are sufficient to allow the generation and robust maintenance of two distinct cellular domains in a meristem, requiring only minimal assumptions about spatial restrictions of the system. The challenges ahead are now to extend the cell model into the third dimension, and incorporate cell divisions, but also to add other regulatory networks that control organ initiation and cell differentiation, approaching the goal of a virtual meristem.

Materials and Methods

Based on the gene interaction diagram shown in Fig1A, a system of coupled PDEs is set up that describes the temporal evolution of concentrations of the factors $[st]$, $[CLV3]$, $[WUS]$, $[facX]$ and $[WUS_{sig}]$ inside the cells of the SAM. The three-dimensional dome of cells constituting the SAM is represented in the model by a two-dimensional artificial longitudinal section (Fig1B). This section was generated by positioning cell centres and using a Voronoi decomposition in order to generate possible cell walls and thereby defining the cells. For the simulations we assume zero flux boundaries confining the simulated domain. The model equations are given in the following:

$$\frac{\partial [WUS]}{\partial t} = D_{WUS} \Delta [WUS] + w_{ko} \xi \rho_{anc} \frac{[WUS]^2 [facX]}{1 + ([CLV3] + [CLV3_{ext}])^3} - \mu_{WUS} [WUS] + \sigma_{WUS}, \quad (0.1)$$

$$\frac{\partial [facX]}{\partial t} = D_{facX} \Delta [facX] - w_{ko} \xi \rho_{anc} \frac{[WUS]^2 [facX]}{1 + ([CLV3] + [CLV3_{ext}])^3} + \frac{\sigma_{facX}}{1 + \frac{[facX]}{K_{facX}}}, \quad (0.2)$$

$$\frac{\partial [WUS_{sig}]}{\partial t} = D_{WUS_{sig}} \Delta [WUS_{sig}] + \rho_{WUS_{sig}} [WUS] - \mu_{WUS_{sig}} [WUS_{sig}], \quad (0.3)$$

$$\frac{\partial [st]}{\partial t} = D_{st} \Delta [st] + \mathbf{1}_{Id}(i) \rho_{st} \frac{\left(\frac{[WUS_{sig}]}{K_{st}} \right)^5}{1 + \left(\frac{[WUS_{sig}]}{K_{st}} \right)^5} - \mu_{st} [st], \quad (0.4)$$

$$\frac{\partial [CLV3]}{\partial t} = D_{CLV3} \Delta [CLV3] + c_{ko} \rho_{CLV3} [st] - \mu_{CLV3} [CLV3]. \quad (0.5)$$

The model depends on the following parameters: reaction rates ρ , basal expression rates σ , degradation rates μ , and kinetic constants K . While most parameters settings are similar for all cells of the considered simulated domain, there are two exceptions: (i) the reaction rate ρ_{anc} (Eqs. (0.1) and (0.2)) is given by a distribution with its maximum in the meristem tip (Fig1B). It is an artificial spatial component necessary for correct location of developing SCD and OC within the meristem. In addition ρ_{anc} is perturbed by a small uniformly random value $\xi \in [-0.025, 0.025]$ which is a random influence necessary for this subsystem to produce patterns. (ii) The reaction term guiding $[st]$ depends on the indicator function 1_{ld} . Since we assumed that only cells in the outer cell layers are competent to acquire stem cell identity, for competent cells i the indicator function returns a value $1_{ld}(i) = 1$ and $1_{ld}(i) = 0$ otherwise. While the former parameters refer to processes taking place within the cells, the model includes interactions between neighbouring cells as well. This interaction is modelled by diffusion terms $D\Delta$, where Δ is the Laplace operator in two dimensions and D is a diffusion rate. Note that although WUS and st are considered to be only locally active, their model equations (Eqs. (0.1) and (0.5)) contain diffusion terms since for these two factors we assume a weak leakage diffusion.

On top of the parameters we already described, the model contains a set of parameters that is used to accommodate the different modelled mutants and experiments. In this context, c_{ko} is used to simulate regulation of endogenous $CLV3$; c_{ko} modifies the reaction term guiding $CLV3$ expression where $c_{ko} = 1$ simulates a wildtype situation while $c_{ko} = 0$ represents a knockout and values $c_{ko} > 1$ represent overexpression of endogenous $CLV3$. Choosing c_{ko} values $c_{ko} \in [0, \infty)$, graded scenarios can be simulated. Using a comparable parameter $w_{ko} \in [0, 1]$, we are able to simulate graded WUS knockouts. Again a value of

$w_{ko} = 1$ represents a wildtype scenario while $w_{ko} = 0$ represents a *WUS* knockout. The parameter $[CLV3_{ext}]$ is used to simulate a *CLAVATA* background in all cells. In a wildtype scenario $[CLV3_{ext}]$ is set to 0 and with $[CLV3_{ext}] \in [0, \infty)$ a range of graded *CLV3* background strengths can be simulated. In addition, for simulations testing conditions with respect to *facX* under which OC and SCD are generated the already introduced parameter K_{facX} is varied.

Parameter Estimation

The model parameter setting for the wildtype simulations as well as for the simulations of mutants are given in Tab. 1 and 2. The model parameters have been tuned by hand using a type of hierarchical decomposition of the system: from a developmental perspective firstly the OC is formed which then induces the formation of a SCD. For the parameter tuning process we therefore divided the system into two parts. (i) Equations (0.1) and (0.2) which are responsible for the formation of the OC, and (ii) Eqs. (0.3) to (0.5) that constitute the SCD generating part of the system. During a three-stage process we started the tuning process of the model parameters by considering only the OC generating system, in order to identify parameters resulting in a single and spatially confined OC domain. For this subsystem, we started with parameter settings as documented previously (Koch and Meinhardt, 1994), and we were able to identify fitting parameters reasonably close to the initial settings. Using the resulting OC as input, we then tuned the parameters for the SCD generating part of the system. Here we ignored the feedback of *CLV3* on *WUS* and aimed on the identification of parameters which resulted in an SCD of appropriate size and an area under *CLV3* influence that extends the SCD but remains spatially confined as well. In the last step we considered the full system adjusting the parameters responsible for the feedback process between the two subsystems.

Parameter	D_{WUS}	D_{facX}	$D_{WUS_{sig}}$	D_{st}	D_{CLV3}	ρ_{anc}	ρ_{st}	ρ_{CLV3}	$\rho_{WUS_{sig}}$
Value	0.002	0.02	0.02	0.002	0.02	<i>dist.</i>	0.5	0.6	0.03

Parameter	μ_{WUS}	$\mu_{WUS_{sig}}$	μ_{st}	μ_{CLV3}	σ_{WUS}	σ_{facX}	K_{facX}	K_{st}
Value	0.004	0.05	0.05	0.01	0.0002	0.004	0.2	1

Table 1: Summary of constant model parameters.

All parameters are dimensionless. ρ_{anc} follows a constant distribution shown in Fig1B.

<i>Parameter/Scenario</i>	$[CLV3_{ext}]$	$[WUS_{ext}]$	c_{ko}	w_{ko}
WT	0	0	1	1
Laser ablation	0	0	1	1
$CLV3 \uparrow$, medium	1	0	1	1
$CLV3 \uparrow$, strong	1.5	0	1	1
$CLV3 \downarrow$	0	0	0	1
$CLV3 \downarrow$, gradual	0	0	0.2	1
$CLV3 \gg WUS$	0	0	1	1

Table 2: Scenario dependent model parameters with their respective values.

All parameters are dimensionless. WT describes the wild type setting. $CLV3 \uparrow$: over-expression of $CLV3$ in all cells (stem cells and non-stem cells). $CLV3 \downarrow$: *clv3* loss of function mutant. $CLV3 \gg WUS$: Expression of WUS in the stem cell domain, controlled by the $CLV3$ promoter.

Simulation

For the numerical simulation of the PDEs, we assume zero flux conditions on the boundaries of the cell plane. In order to simulate the time evolution of the model, the model equations are numerically integrated. The equations have to be discretized with respect to time and space, here using a constant time step $\delta_t = 0.25$ and applying a finite difference scheme in cellular resolution to the space-dependent diffusion terms. Each cell is thereby represented by its centre and for the sake of simplicity, cell volumes are considered to show the gradients that would be assumed between the concentrations simulated for the cell centres. In addition, we assume free diffusion as the only means of communication between cells. Because the precise communication underlying *WUS* dependent signalling is still unknown, free diffusion represents a sort of ‘maximum entropy choice’. In terms of modelling complexity we benefit from this fact: with free diffusion, extracellular spaces, cell walls and membranes can be neglected during the simulation process. In addition, due to the Voronoi decomposition used to generate the considered section through the meristems, cell volumes and surfaces tend to even out. Since the model is not supposed to generate quantitative data, but rather to investigate qualitative behaviour, we neglect the influence of cell surfaces and volumes during simulations.

The diffusion terms in the considered system tend to be stiff and we therefore chose to use a variant of the second order implicit Crank-Nicolson integrator as presented previously by others [31] for these terms. To the only-time-dependent terms a faster explicit Adams-Bashford scheme [31] is applied instead. This implicit-explicit method is chosen in order to reach an appropriate trade-off between necessary computational effort and simulation accuracy.

The numerical simulations for the considered scenarios are done in a two-stage process. The first stage is used to equilibrate the system starting from the initial conditions. Here, under ‘equilibrium state’ we understand a system state in which all derivatives are reasonably close to zero. During the second stage the parameters are adapted in order to accommodate the considered scenarios. As initial condition for the first stage, the [WUS] level of all cells is homogeneously initialized with a starting concentration of 0.01. All other species are initialized with a value of 0. In the first stage, the system is simulated for 30000 time units. For the second stage we use the equilibrium concentrations obtained in the first stage as initial conditions, the parameters are adapted and in case of the laser ablation scenario the tissue topology is adapted. Afterwards the system is simulated for further 15000 time steps.

Modelling Background

To model biological systems there exists a range of different mathematical modelling approaches, e.g. stochastic molecular simulations, differential equation models, or discrete dynamic models. The available models vary with respect to possible level of detail where a gain in detail comes at the cost of additional computational effort. Since the focus of this study is to provide a model capable of reproducing the intricate dynamics underlying the maintenance of the SAM, we chose a differential equation model—an approach that provides the necessary level of detail and is commonly used to model the considered type of systems.

From a qualitative perspective, SAM maintenance is a question of developing and maintaining a patterning of a tissue with respect to distinct domains with specific gene expression profiles. A popular approach in developmental biology to capture pattern formation are reaction diffusion systems developed by Turing in the 1950s [22,32]. Relying on diffusion, these systems are capable of producing spatially heterogeneous patterns of

somewhat antagonistic reactions initiated by an initial small perturbation. Here we employ a similar mechanism: Eqs. (0.1)-(0.2) are a variant of the activator-substrate model [33] - a system that is known to produce circular domains that remain mobile and thereby allow an OC that is forming in the meristem tip to move down after initiation of the SCD. Eqs. (0.3)-(0.5) are derived guided by the law of mass action. Still, parameters like the Hill coefficients in Eq. (0.4) have been further tuned, e.g. large Hill coefficients are used in Eq. (0.4) in order to produce a sharper transition between cells showing a high level of *stemness* and neighbouring cells with low levels of *stemness*. In turn, such parameter choices reflect possible underlying biological reactions like the formation of homodimers or other forms of cooperativity.

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Supplementary Information for:

A dynamic model for stem cell homeostasis and patterning in *Arabidopsis* meristems

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Results from additional simulations

I. Reducing *CLV3* expression

Conditional loss-of-function of *CLV3* results in an expansion of the OC and the SCD. In addition, the OC is shifted towards the tip due to a loss of negative feedback by *CLV3*. When *CLV3* expression levels are only mildly reduced, a graded response was observed: SCD and OC enlarge, but the location of the OC is not affected due to active *CLV3* signalling. A typical time course of a simulation for a conditional reduction in *CLV3* expression is shown in FigS1A. Interestingly, OC size remained unaffected for a wide range of endogenous *CLV3* expression levels (FigS1B), indicating a partial uncoupling of OC and SCD sizes.

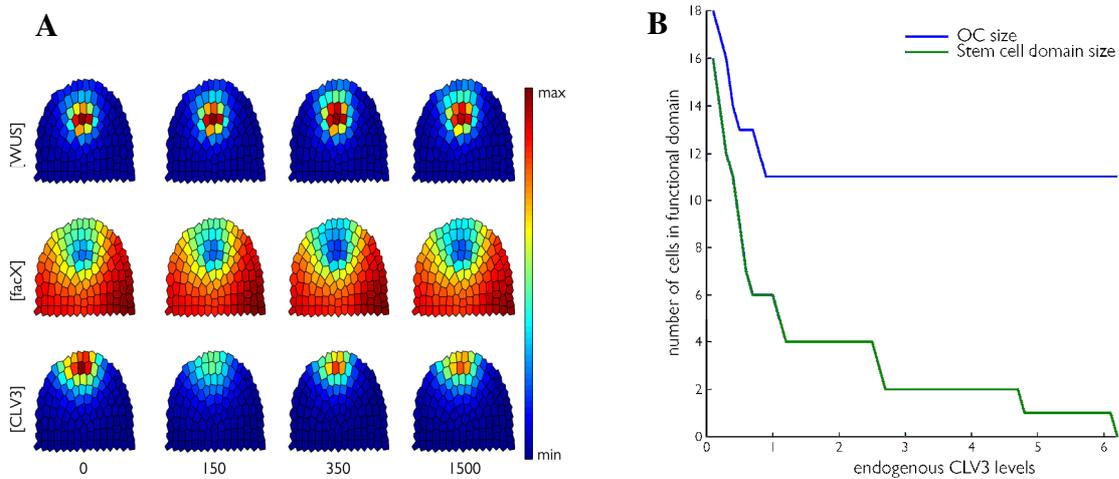


Figure S1: Effects of reduced *CLV3* levels on OC and SCD
(A) Time course simulation for a conditionally reduced *CLV3* expression.
(B) Impact of *CLV3* expression levels on the sizes of OC and SCD. To assess the number of cells in the respective domains, the concentrations were discretized using thresholds relative to the wild type concentrations: For *stemness*: $\delta_{st} = 0.21$, for *WUS*: $\delta_{WUS} = 0.31$.

II. Simulating loss-of-function mutants in *CLVI*

The phenotype of *clv1* loss-of-function mutants is comparable to a downregulation of *CLV3* expression. Mathematically, a *clv1* loss-of-function mutant can be incorporated by extending Eqs. 1 and 2 (see Materials and Methods section in the main article) with a factor $c1_{ko}$ that modulates the response of *WUS* to *CLV3* expression. The corresponding equations are given here:

$$\frac{\partial [WUS]}{\partial t} = D_{WUS} \Delta [WUS] + w_{ko} \xi \rho_{anc} \frac{[WUS]^2 [facX]}{1 + (c1_{ko} [CLV3] + [CLV3_{ext}])^3} - \mu_{WUS} [WUS] + \sigma_{WUS}$$

$$\frac{\partial [facX]}{\partial t} = D_{facX} \Delta [facX] - w_{ko} \xi \rho_{anc} \frac{[WUS]^2 [facX]}{1 + (c1_{ko} [CLV3] + [CLV3_{ext}])^3} + \frac{\sigma_{facX}}{1 + \frac{[facX]}{K_{facX}}}$$

By setting $c1_{ko}$ to values $c1_{ko} \in [0, 1]$, different residual activity levels of *CLVI* can be simulated, where $c1_{ko} = 0$ represents a loss-of-function, and $c1_{ko} = 1$ represents the wild type scenario. Typical simulated time courses for graded *clv1* loss-of-function mutants are shown in FigS2.

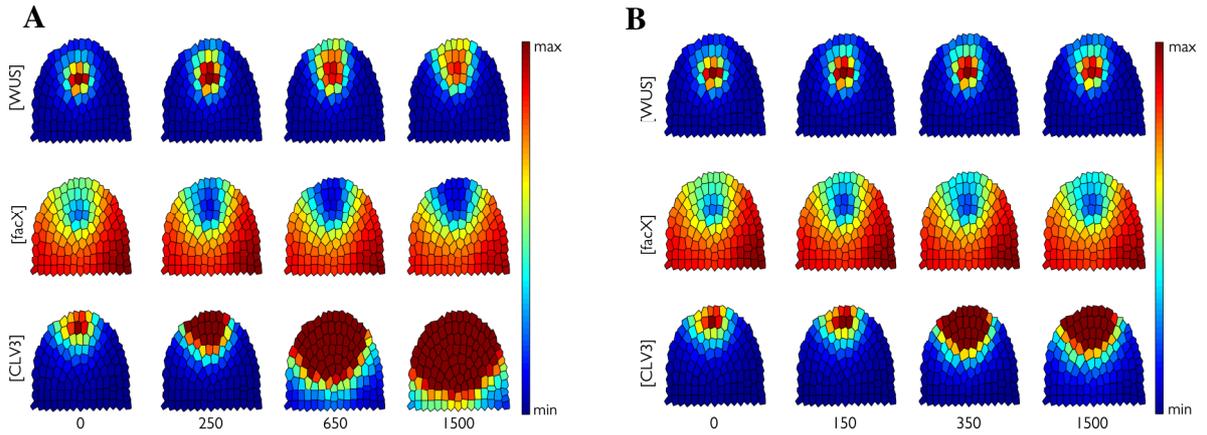


Figure S2: Time courses for graded *clv1* loss-of-function mutant simulations

(A) *clv1* loss-of-function scenario ($c1_{ko} = 0$), (B) a simulation where *CLVI* retains some activity ($c1_{ko} = 0.2$).

III. Gradual increase of exogenous *CLV3* expression levels

We reported simulations for meristems with strongly increased *CLV3* expression levels, resulting in meristem arrest, and intermediate overexpression levels that caused an only transient loss of the SCD, which recovered with time. By studying a range of *CLV3* overexpression levels, we uncovered that both domains are gradually reduced in size (FigS3A). However, the SCD (green line in FigS3B) responds more readily, indicating no strict interdependence of domain sizes.

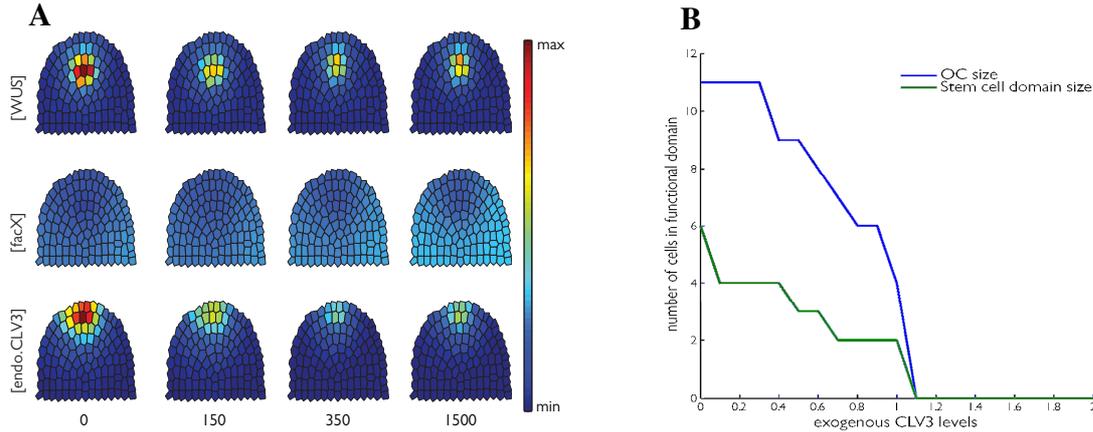


Figure S3: Gradual response to exogenous *CLV3* expression

(A) Time course simulation for a low level *CLV3* overexpression ($CLV3_{ext} = 0.7$). (B) Graded system response to different exogenous *CLV3* expression levels. The $CLV3_{ext}$ level is varied in $[0, 2]$. To assess the number of cells in the respective domains, the concentrations of *stemness* and *WUS* were discretized using thresholds relative to the wild type concentrations. For *stemness*: $\delta_{st} = 0.21$; for *WUS*: $\delta_{WUS} = 0.31$ is used.

IV. Altering *WUS* expression

We simulated the consequences of reducing *WUS* expression to different levels. Minor reductions of *WUS* levels cause a corresponding small reduction in OC and SCD size (FigS4A). A medium reduction causes a transient loss of SCD and OC until *facX* reaches a level that allows to re-initiate a new meristem (FigS4B). In case of a *WUS* knockout, the meristem is arrested without recovery (FigS4C).

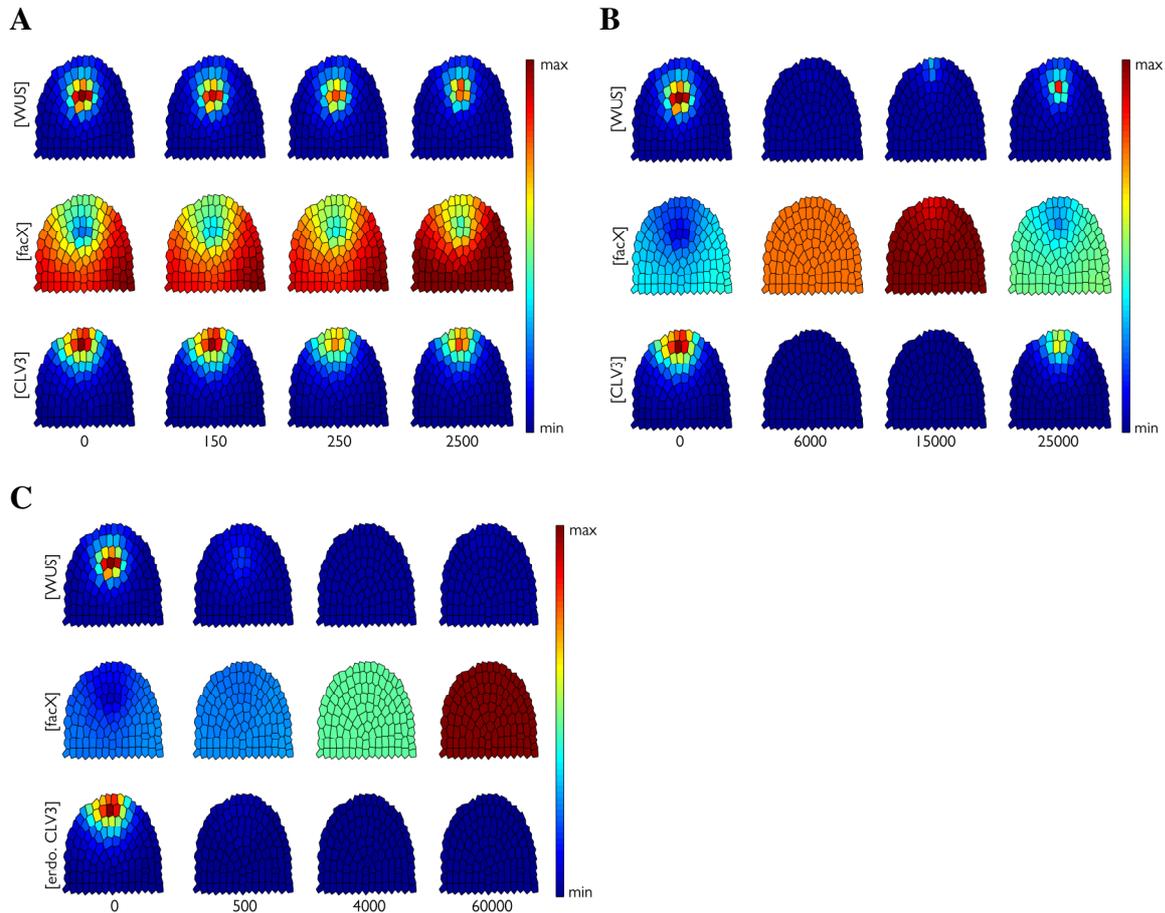


Figure S4: Time courses for graded *WUS* knockout mutant simulations

(A) A simulation for slightly reduced *WUS* expression level ($w_{ko} = 0.6$), (B) a simulation for with intermediate *WUS* expression ($w_{ko} = 0.4$), (C) a simulation for a *WUS* loss-of-function scenario ($w_{ko} = 0$).

V. Regeneration and *de-novo* generation of OC and SCD

New meristems can be formed during embryogenesis or flowering. Our model allows simulating the *de novo* generation of meristems by varying *facX* regulation. Figure S5 shows the system response to different levels of the kinetic constant K_{facX} and the resulting sizes of OC and SCD. K_{facX} is varied over a range from 0.1 to 1, while $K_{facX} = 0.4$ is used for wild type simulations.

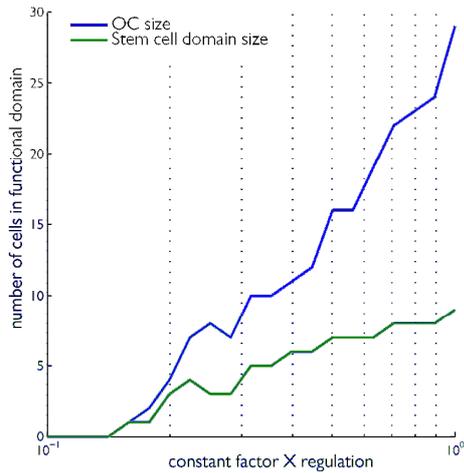


Figure S5: Consequences of *facX* expression levels for the sizes of OC and SCD

To assess the number of cells in the respective domains, concentrations were discretized using thresholds relative to the wild type concentrations. For *stemness*: $\delta_{st} = 0.21$, for *WUS*: $\delta_{WUS} = 0.31$ is used.

VI. Sensitivity analysis for model parameters

We investigated the sensitivity of the proposed model with respect to perturbations of single parameters of the wild type parameter setting. The parameters have been perturbed using the following changes in absolute values: $\{-50\%, -25\%, -10\%, 25\%, 50\%, 100\%, 150\%, 200\%\}$.

The corresponding simulations document a considerable robustness with respect to these perturbations: apart from varying the five parameters $(\rho_a, \mu_a, \rho_{WUS_{sig}}, \mu_{WUS_{sig}}, K_{st})$, the simulated system response was qualitatively unchanged; only slight variations in domain size and degree of separation between OC and stem cell domain occurred.

ρ_a Variation

For a perturbation in ρ_a of -50% , OC and stem cell domain are no longer spatially separated but both are located in the tip. For variations of $+150\%$ or $+200\%$, the functional domains are enlarged but separated, still during simulations small numerical instabilities occurred indicating that ρ_a is reaching level that becomes intractable by the current simulation setup. For the remaining perturbations the system remained qualitatively stable.

μ_a Variation

For a perturbations of $+150\%$ and $+200\%$ the formed domains are no longer spatially separated and located in the meristem tip. For the remaining perturbations the system remained qualitatively stable.

$\rho_{WUS_{sig}}$ Variation

With larger $\rho_{WUS_{sig}}$ levels domain sizes start to shrink and for perturbations of $+100\%$ to $+200\%$ simulations show small numerical instabilities. For the remaining perturbations the system remained qualitatively stable.

$\mu_{WUS_{sig}}$ Variation

For a perturbation of -50% domains are no longer spatially separated and both are located in the tip. This simulation shows small numerical instabilities as well. For perturbation levels of $+150\%$ and $+200\%$ simulations again no longer show spatial separation between functional domains, this time the simulations have been numerically stable. For the remaining perturbations the system remained qualitatively stable.

K_{st} Variation

Only for a perturbation of -50% , the system shows small numerical instability, but for all tested perturbation levels the system remains qualitatively stable.

Numerical Instabilities

As reported above, in 7 simulations of the sensitivity analysis instabilities occurred. In these cases, the simulations showed regular high-frequency oscillations with respect to time. There are two different possible explanations for the occurrence of these oscillations: (i) they could represent regular system behaviour which is rather improbable due to the fact that the oscillations are of high frequency and their exact patterns change slightly when varying the size of the time step used during integration. (ii) The used IMEX scheme for numerical integration employs a variant of the Crank-Nicolson integration scheme for the stiff diffusion terms. This modification was introduced to show better damping properties of the scheme with respect to false high-frequency oscillations [1]. Nevertheless, the observed oscillations could result from comparable false oscillations.

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