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CURRENT PROBLEMS OF DEVELOPMENTAL BIOLOGY

A Model Study of the Role of Proteins CLV1, CLV2, CLV3, and WUS in Regulation of the Structure of the Shoot Apical Meristem

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Abstract—In order to elucidate the role of proteins CLV1, CLV2, CLV3, and WUS in the mechanism underlying the maintenance of compartmental structure (spatial arrangement of the zones of biosynthesis of marker proteins) of the shoot apical meristem, a model of such mechanism was developed. Computational experiments led to biologically plausible solutions only when synthesis of substance W in a space between the organizing center and meristem apex was limited by the mechanism based on interaction of CLV3 with membrane receptor CLV1/CLV2 and lower boundary of the zone of W synthesis was determined by isoline of the corresponding threshold level of substance Y concentration. The model of the "reaction-diffusion" type formalizing the role proteins CLV1/CLV2, CLV3, and WUS can describe the basis of the mechanism underlying regulation of the compartmental structure of the shoot apical meristem and positioning of the organizing center in a certain site of the cell ensemble of such meristem.

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Key words: morphogenesis, shoot apical meristem, model of reaction-diffusion type.

According to the current concept (Sharma et al., 2003), the central zone of the shoot apical meristem (AM) is a source of stem cells, while the peripheral zone and organizing center are zone of transient amplifying cells. In the shoot vegetative AM, the central zone is located in three to four upper meristem layers (Reddy and Meyerowitz, 2005) in a radius of two to four cells from the AM vertical axis (Clark, 1997). The organizing center is located directly under the central zone and is two to three cells thick in the meridianal direction. Gene *CLV3* is expressed in the central zone cells (Clark et al., 1995) and gene *WUS* is expressed in the organizing center cells (Laux et al., 1996). The zone where *WUS* expression is observed is surrounded by an area, where membrane complex CLV1/CLV2 is synthesized, which is a receptor for CLV3. CLV3 binds to complex CLV1/CLV2 and inhibits *WUS* expression (Sharma et al., 2003). Thus, regulation of the compartmental structure of the shoot AM is reduced to establishment of boundaries between the central zone and peripheral zone and between central zone and organizing center; in the latte case this is establishment of boundaries between the compartments of *CLV3* and *WUS* expression.

MATERALS AND METHODS

Taking into account the data on presumed role of proteins CLV1, CLV2, CLV3, and WUS in regulation of the compartmental structure of the shoot AM, we developed a mathematical model of such regulation on two-dimensional area consisting of cells. A program written in MATLAB7 was developed on the model basis, which was used for computational experiments with aim of search for a set of parameters providing solution qualitatively agreeable with the published data.

Model prerequisites. As was already mentioned, protein CLV3 is synthesized in stem cells of the central zone of the shoot AM and is spread over the meristem from them (Lenhard and Laux, 2003), which allows simulation of this spreading by diffusion. Protein complex CLV1/CLV2 is a membrane receptor for CLV3, which is localized in the corpus upper layers, while the organizing center (area of *WUS* expression) is located in the zone of complex CLV1/CLV2 localization. When diffusing protein CLV3 reaches the zone of CLV1/CLV2 localization, it binds to receptor CLV1/CLV2 and forms a protein complex triggering the cascade of signal transmission for inhibition of

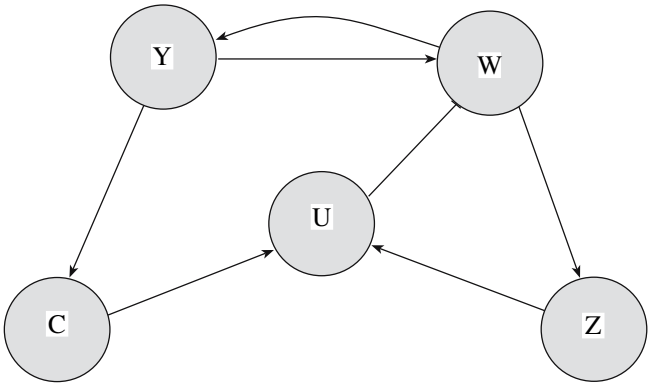


Fig.1. Schematic diagram of regulation of the concentrations of substances Y, C, Z, U, and W in the model (see text). (⟶) activation, (⟙) inhibition.

WUS expression in cells at the organizing center boundary (Rojo et al., 2002; Lenhard and Laux, 2003). Including of this mechanism in the model made it possible to study its role in the organizing center formation and positioning in the shoot AM.

It was already shown (Nikolaev et al., 2006) that positioning of *WUS* expression at a certain distance from the shoot AM apex can be ensured by a mechanism based on mutual activation of expression of *WUS* and a certain hypothetical gene *Y* by the products of their expression. Substance Y is synthesized in the first AM layer and diffuses over the shoot AM cell ensemble. An important role of the upper AM layer in the central zone formation was shown by Reinhardt et al. (2003) and, therefore, we proposed an activating role of Y in this model.

Since, on the one hand, we proposed that WUS activates *Y* expression in the cells remote from the organizing center and, on the other hand, there is no evidence that WUS is found beyond the organizing center boundaries, we assume that this protein in the synthesizing cells activates synthesis of a certain diffusing substance, direct activator of *Y* expression. In order to simplify the model, we identified protein WUS and WUS-dependent activator for *Y* as substance W.

Model. The two-dimensional cell array considered in the model represents a vertical section of the shoot AM: we will discuss here a variant, when the zone of cells is bounded by parabola - approximation of the shape of the AM vertical section by parabola was used, for example by Meicenheimer (1979). Let us assume that the diffusing substance Y is synthesized in the upper cell layer. This synthesis is regulated by substance W, which spreads from the zone of its synthesis. Let us assume also that diffusing substance C, which represents in our model CLV3, may be synthesized at Y concentrations above the threshold level. Synthesis of substance W is regulated according to the following principle. W may be synthesized in cells of the area, where Y concentration exceeds a certain threshold and this threshold does not exceed the threshold of C synthesis and, thus, the zone of permitted W synthesis spreads from the source of Y farther than the similar zone for C. Let us assume that everywhere when concentration of W exceeds a certain threshold, substance Z may be synthesized, which does not diffuse and represents the membrane complex CLV1/CLV2 in the model. Let us assume also, according to the above concept, that C and Z can form irreversibly complex U, which inhibits the expression of W.

The meaning of the presumed mechanism underlying the regulation of W synthesis is as follows. While Y stimulates synthesis of W, the latter produces a mechanism for inhibition of its synthesis by activating synthesis of Z. When W spreads against the gradient of its concentration, for example, by means of diffusion, Z will be synthesized in a certain zone, inside the zone W synthesis is located. If the zone of C synthesis does not coincide with that for W, it can be proposed that C diffuses from outside to "spot" Z and is "absorbed" as a result of complex U formation. At some ratios of the rates of C diffusion and "absorption" and rate of restoration of the level of Z inside "spot" Z, a C-free and, hence, U-free zone will be maintained. In this inner zone, synthesis of W will be permitted. Fig. 1 shows a scheme of "local" regulation of the dynamics of concentrations of substances Y, C, Z, U, and W.

Equations of the model. Let us consider a two-dimensional ensemble of n cells; cells i and j are separated by cell boundaries (cell walls and plasmalemmas) of area S_{ij}, across which substances Y, C, and W can be transferred. Cell boundaries are characterized by the coefficients of penetrability β_Y, β_C, and β_W, respectively. Reactions of synthesis of substances k, $k \in \{W, C, Y, Z\}$ with the rates of change of the corresponding concentrations p^k, $p^k \in \{y, c, w, z\}$, which depend on the presence of other substances.

$$\frac{dp^k}{dt} = \frac{1}{\tau_k} g(x^k),$$

$$\text{where } g(x^k) = \begin{cases} 0, x^k \longrightarrow -\infty \\ 1, x^k \longrightarrow +\infty, \end{cases}$$

$$x^k = \sum_j E_{kj} p^j + h_k, \quad k, j \in \{Y, C, W, Z\}.$$

Here, p^k is concentration of substance k; E_{kj} are coefficients of regulation > 0, if substance j stimulates synthesis of substance k and < 0 if it inhibits; τ_k are coefficients inverse to the maximum rate of expression, Parameters h_k determine threshold values of function $g(x^k)$. Thus, x^k is a dimensionless control variable of the function of dependence of the rate of synthesis of substance k from concentrations of other substances present in the cell. For description of the rates of

expression (synthesis) in this model, sigmoid function is used as $g(x)$:

$$g(x) = \frac{1}{2}\left(1 + \frac{x}{\sqrt{1+x^2}}\right).$$

If diffusion is described by Fick's law and the coefficient of diffusion inside the cells is much greater than that between the cells, them the concentrations of substances are always equal inside the cell. The amount of substance P in cell i will change within time Δt due to: (a) flows $J_{ij}(\tau)$ of substance P between cell i and cells j, $j \in \varepsilon(i)$, where $\varepsilon(i)$ is a set of cell i neighbors, (b) synthesis of a given substance in the cell at specific rate $g(x_i^P)$, and (c) its degradation as a result of the first order reaction with the coefficient of velocity d_P. In the case of diffusion, $J_{ij}(\tau) = \beta^P S_{ij}(\tau)(p_j(\tau) - p_i(\tau))$, where $p(\tau)$ is concentration of substance P. As a result, a balance equation for substance P can be written:

$$\Delta P_i = \sum_{j \in \varepsilon(i)} \int_{\Delta t} \beta^P S_{ij}(\tau)(p_j(\tau) - p_i(\tau))d\tau + v_P g_P(x_i^P(\tau))V_i(\tau) - d_P p_i(\tau)V_i(\tau).$$

Using the mean value theorem for integrals and passing to the limit $\Delta t \longrightarrow 0$, we will obtain:

$$\frac{1}{V_i}\frac{dP_i}{dt} = \sum_{j \in \varepsilon(i)} \frac{\beta^P S_{ij}(p_j - p_i)}{V_i} + v_P g_P(x_i^P) - d_P p_i.$$

We will derive an equation for concentration by the standard method from estimation of concentration $p = \frac{V}{P}$ according to the rules of differentiation:

$$\frac{dp}{dt} = \frac{V\frac{dP}{dt} - P\frac{dV}{dt}}{V^2} = \frac{1}{V}\frac{dP}{dt} - p\frac{1}{V}\frac{dV}{dt}.$$

If we substitute the first summand in the right part for expression $\frac{1}{V}\frac{dP}{dt}$, we will obtain:

$$\frac{dp_i}{dt} = \sum_{j \in \varepsilon(i)} \frac{\beta^P S_{ij}(p_j - p_i)}{V_i} + v_P g_P(x_i^P) - d_P p_i - p_i\frac{1}{V_i}\frac{dV_i}{dt}.$$

If the cells do not grow, then $\frac{dV_i}{dt} = 0$ and $S_{ij} = \text{const}$, $V_i = \text{const}$.

When applying these considerations to all above mentioned substances, we will obtain the model as a system of ordinary differential equations:

$$\frac{dy_i}{dt} = \sum_{j \in \varepsilon(i)} \frac{\beta^Y S_{ij}(y_j - y_i)}{V_i} + v_Y g_Y(x_i^Y) - d_Y y_i,$$

$$\frac{dc_i}{dt} = \sum_{j \in \varepsilon(i)} \frac{\beta^C S_{ij}(c_j - c_i)}{V_i} + v_C g_C(x_i^C) - d_C c_i - \alpha c_i z_i,$$

$$\frac{dw_i}{dt} = \sum_{j \in \varepsilon(i)} \frac{\beta^W S_{ij}(w_j - w_i)}{V_i} + v_W g_W(x_i^W) - d_W w_i,$$

$$\frac{dz_i}{dt} = v_Z g_Z(x_i^Z) - d_Z z_i - \alpha c_i z_i.$$

Here, v_k, $k \in \{Y, W, C, Z\}$ are maximal rates of synthesis of the corresponding substances. It is proposed that C(CLV3) can form irreversibly a complex with Z (dimeric receptor CLV2/CLV2), which triggers the mechanism of repression of W synthesis.

RESULTS AND DISCUSSION

It follows from the above assumptions, underlying the considered model, that the set of geometrical forms of the zones C, Z, U, and W syntheses is determined by the mechanism of distribution (diffusion, in a given case) of substances Y, C, and W with the corresponding bound conditions and processes of absorption and/or degradation of the diffusing substances. The isolines of concentrations of these substances determine the entire set of forms of different zones at any given moment. Hence, it becomes clear that the geometry of stationary zone of W synthesis is markedly affected by that of the zones of Y and C syntheses. In particular, it was shown (Nikolaev et al., 2007) that if the boundary of the zone of C synthesis is convex with reference to the zone apex, the zones of W synthesis may reach the zone boundaries (Fig. 2a), but if it is concave, these zones may be located in the middle (Fig. 2b). In turn, the boundary of the zone of C synthesis is an isoline of Y concentration corresponding to a certain set of parame-

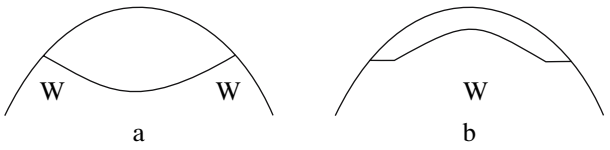
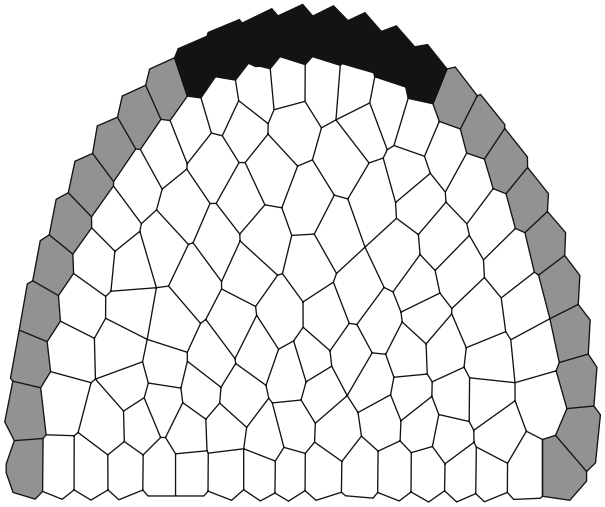
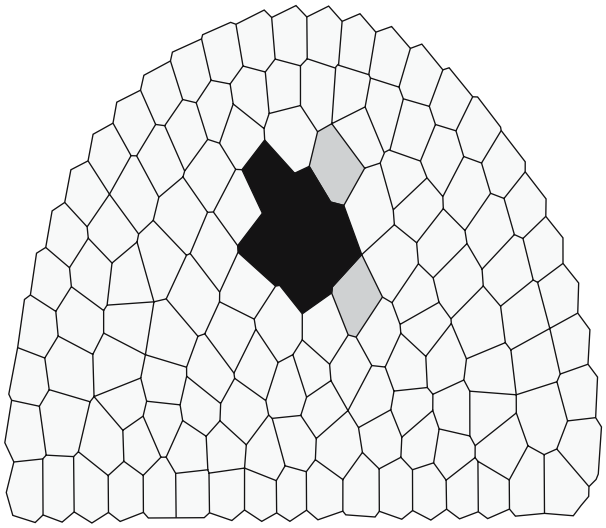


Fig. 2. Examples of location of the zone of W synthesis: (a) near the zone boundary in the case of convex boundary of the zone of C synthesis, (b) in the middle in the case of concave boundary of the zone of C synthesis.



Zone structure

Fig. 3. An example of cell distribution in the initial zone. Y (■) and C (■, ▩) synthesis in the cells is permitted.



Zone of W synthesis

Fig. 4. Stationary distribution of the level of W synthesis for one of model calculations of the zone cell structure.

ters or, in addition, it may be determined morphologically; it was shown, for example, that C synthesis is only possible in two upper cell layers of the meristem (Rojo et al., 2002). Hence, a morphological restraint was introduced for admissible zone of synthesis, which prohibits C synthesis outside the limits of several upper cell layers.

The model was calculated on hypothetical two-dimensional cell ensembles constructed according to the below described algorithm. One of such cell ensembles is presented in Fig. 3, where the area of permitted Y and C syntheses is indicated. Cell distribution in the zone was obtained using the following procedure: an initial zone with the shape of semicircle and radius R was set and a polar system of coordinates (r, φ) was introduced in this zone with a center at point with coordinates (0,0). The zone was then "populated" with random (with a certain mean density) points, cell centers, and this zone was then partitioned using *voronoi()* function from the library of the system MATLAB7 functions. Each cell of the obtained ensemble has its own unique index i. The concentrations of the considered substances are taken as constant inside one cell, but may differ in different cells. The concentrations of substances W, Z, C, and Y inside cell i are designates as w_i, z_i, c_i, and y_i, respectively.

Taking into account the influence of geometry on the above discussed solutions, we assume that C synthesis is permitted only in the cells, for which the coordinates of center satisfy the condition:

$$r_C \le r \le R,$$

and Y synthesis in the cells for which the coordinates of center satisfy the condition:

$$r_Y \le r \le R, \quad \frac{\pi}{2} - \delta \le \varphi \le \frac{\pi}{2} + \delta.$$

As a result of the model investigation, we found the parameters that allowed us to obtain stationary distribution of concentrations and rate of synthesis of substance W, which are in qualitative correspondence with the experimentally obtained results. The parameters' values are presented below and the stationary solution for the rate of W synthesis in Fig. 4.

Parameters value

number of cells	D_W	d_W	τ_W	h_W	T_{WY}	T_{WU}	d_Z	α	τ_Z	h_Z	R	r_Y	T_{ZW}	D_C	d_C	τ_C	h_C	T_{CY}	D_Y	d_Y	τ_Y	h_Y	T_{YW}	r_C	δ
128	5	1	1	–88.9379	1500	–11900	1	1	1	10	100	85	0	50	1	1	–88.937	1500	50	0.2	1	–0.1	1000	70	$\frac{\pi}{6}$

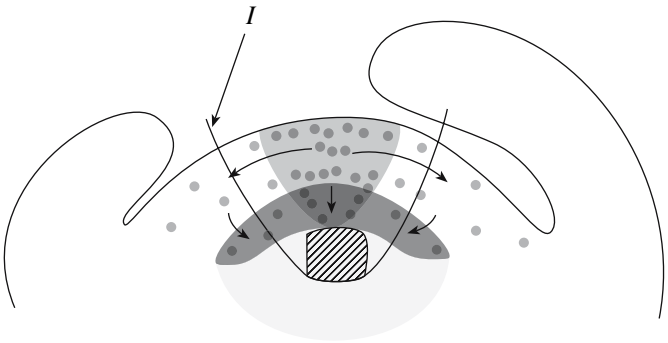


Fig. 5. Schematic diagram of the mechanism of regulation of gene *WUS* expression (after Lenhard and Laux, 2003, with modifications). I, isoline of the threshold concentration of substance Y, below which synthesis of W is absent.

( ) synthesis of protein CLV3, ( ) presence of CLV3, ( ) distribution of CLV3, ( ) presence of complex CLV1/CLV2, ( ) complex of CLV3 and CLV1/CLV2, ( ) expression of gene *WUS*.

Note that computational experiments led to biologically plausible solution only when W synthesis in a space between the organizing center (zone of real W synthesis) and meristem apex (zone of potential W synthesis) was restricted according the mechanism described above during formulation of CCW model. In all cases, the lower boundary of the zone of W synthesis was determined by an isoline of the corresponding threshold level of Y concentration. This detail has not been considered in the publications dedicated to the above considered mechanism but the authors confine themselves to the discussion of mutual regulation based on the interaction of protein CLV3 with receptor CLV1/CLV2 and depict the zone of CLV1/CLV2 synthesis around the organizing center, although Lenhard and Laux (2003) presented the situation (Fig. 5) which we obtained in our model studies as an illustration of the mechanism of regulation of *WUS* expression.

According to the postulated distributed regulation, the interaction between the organizing center and zone of Y synthesis in the first (outer) layer of the shoot AM is a basis of the organizing center positioning and simultaneous regulation of Y synthesis intensity, the latter affects, in turn, the size of the zone of certain threshold Y values. The fact such mechanism "works" allows us to propose the following biological interpretation: substance Y determines the central zone, a niche of stem cells, rather than protein WUS.

The role of diffusing morphogens in formation of the compartmental tissue structure is still an object of studies (Ashe and Briscoe, 2006). The model of the reaction-diffusion type considered in this paper and formalizing the role of proteins CLV3, CLV1/CLV2, and WUS may describe the basis of the mechanism underlying the regulation of compartmental structure of the shoot AM and organizing center positioning in a certain area the shoot AM cell ensemble. Note also that high coefficients of the sensitivity of regulation required for the appearance o compartmental boundaries suggest the existence of additional mechanisms underlying the formation of compartmental boundaries, such as direct juxtacrine interactions.

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