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REVIEW

# A complex systems approach to Arabidopsis root stem-cell niche developmental mechanisms: from molecules, to networks, to morphogenesis

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**Abstract** Recent reports have shown that the molecular mechanisms involved in root stem-cell niche development in *Arabidopsis thaliana* are complex and contain several feedback loops and non-additive interactions that need to be analyzed using computational and formal approaches. Complex systems cannot be understood in terms of the behavior of their isolated components, but they emerge as a consequence of largely non-linear interactions among their components. The study of complex systems has provided a useful approach for the exploration of system-level characteristics and behaviors of the molecular networks involved in cell differentiation and morphogenesis during development. We analyzed the complex molecular networks underlying stem-cell niche patterning in the *A. thaliana* root in terms of some of the key dynamic traits of complex systems: self-organization, modularity and structural properties. We use these analyses to integrate the available root stem-cell niche molecular mechanisms data and postulate novel hypotheses, missing components and interactions and explain apparent contradictions in the literature.

**Keywords** Root stem-cell niche · *Arabidopsis thaliana* · Gene regulatory networks · Complex systems · Self-organization · Modularity

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## Introduction

Located at the tip of the root, the root stem-cell niche (RSCN) sustains the development and growth of all below-ground tissues. Given its anatomical simplicity and accessibility, the *Arabidopsis thaliana* RSCN has become an excellent model system. The RSCN has been amenable to cellular and molecular genetic analyses unraveling a plethora of molecular regulatory mechanisms (MRMs) involved in maintaining its pattern and functionality. Partially due to the lack of data, until recently, most of the RSCN MRMs were understood as fragmented and isolated processes that were many times assumed to exhibit a linear relationship between genotype and phenotype. For example, currently, the identity and location of the RSCN is explained by the intersection of the expression patterns of a small set of transcription factors (Aida et al. 2004). However, recent findings reveal that a complex network composed of many interacting elements underlie RSCN patterning.

In her great introductory book to complexity, Mitchell (2009) described a complex system as "…a system that exhibits nontrivial emergent and self-organizing behaviors". Indeed, complex systems comprise feedback loops and other non-linear interactions that produce the emergence of often non-intuitive behaviors that without the use of theoretical approaches seem impenetrable and many times preclude clear interpretations of the experimental data. The RSCN regulatory network involves several components interacting in non-linear ways. This does not mean that actual approaches are not useful; instead, systematic and integrative approaches can complement detailed analyses of particular molecular components, improving our understanding of the system. Such integrative approaches become more relevant if we consider that complex networks have systemic key structural and dynamic properties, such as self-organization

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and modularity, which cannot be understood by characterizations of isolated components.

Theoretical and computational approaches are necessary to study complex systems, like biological systems, affording the verification, prediction, and deeper understanding of experimental data with a more integral and systemic view (Strogatz 2001; Kitano 2002). While we will not review the many different tools available (But see: Alvarez-Buylla et al. 2007; Ay and Arnosti 2011), we will focus on the conceptual integration of experimental and theoretical approaches while analyzing the MRMs of RSCN patterning. To do this, we will describe some key properties, namely self-organization, modularity, and some structural and dynamic network properties, to guide the integrative description of the RSCN MRMs, detect missing components and interactions and provide novel hypotheses and plausible explanations for apparently contradictory data. Importantly, apart from the functional modularity and auxin-transport self-organization properties (Azpeitia et al. 2010; Mironova et al. 2010; Leyser 2011), the properties that we will describe have not been explicitly tested in the RSCN. However, the available data suggest that a robust complex molecular network with certain structural and dynamic characteristics, which are typical of complex networks, underlies RSCN patterning. Moreover, some of these properties appear to be generic to previously characterized MRMs (Barabási and Oltvai 2004; Kitano 2007).

In this review, first, we briefly describe the RSCN and the current explanation of how the RSCN is maintained. We then analyze the structural and dynamic characteristics of the RSCN MRMs with regard to complex systems approaches with a particular focus on network theory. Our analysis enabled us to propose novel explanations and propose experimentally verifiable predictions. For example, this approach is useful for uncovering and understanding the specific mechanisms of cell patterning, regenerative capacity and the maintenance of stem cells (SC) in the system under study. Finally, we discuss the implications and future directions of the ideas presented here.

## The RSCN

All primary tissues of the root develop from the RSCN. The RSCN consists of a small group of cells with low division rates called the quiescent center (QC), which are surrounded by a cell layer composed of four different cell types of initial or SCs (Fig. 1; Dolan et al. 1993). The QC is necessary for SC maintenance because its ablation or malfunction produces premature SC differentiation, RSCN consumption, and, finally, if not reestablished, root determinacy (van den Berg et al. 1995; van den Berg et al. 1997; Xu et al. 2006; Sarkar et al. 2007).

Multiple MRMs are involved in RSCN maintenance, and the most important MRMs identified thus far include the following: (1) The regulatory interactions sustained among the GRAS family transcription factors *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*) and a few additional genes, (2) the interplay between the redundant transcription factors *PLETHORA1* (*PLT1*), *2*, *3, BBM*, and the auxin signaling pathway, (3) the *CLAVATA LIKE40* (*CLE40*) and *WUSCHEL RELATED HOMEOBOX5* (*WOX5*) MRM, (4) many hormonal signaling pathways in addition to the auxin signaling pathway and (5) epigenetic mechanisms (reviewed in Scheres 2007; Benková and Hejátko 2009; Shen and Xu 2009; Sablowski 2011).

Molecular genetic approaches have suggested that the identity and location of the RSCN depends on the intersection of the SHR, SCR and PLT protein domains (Aida et al. 2004) and the negative regulation of the *WOX5* QC identity marker by CLE40 (Stahl et al. 2009). Because the PLT transcriptional and protein domains depend on the auxin concentration (Aida et al. 2004; Zhou et al. 2010), the maximum auxin concentration coincides with the QC location (Brunoud et al. 2012), and auxin signaling, transport and metabolism modifications alter the RSCN (Ding and Friml 2010), auxin is assumed to have a fundamental role in RSCN specification. Finally, epigenetic mechanisms modulate the expression location and level of, at least, the *SCR* and *PLT* genes (Shen and Xu 2009). We recently published a model that demonstrated that the concerted action of at least the first three MRMs mentioned above, and not the isolated activity of any such MRMs, is necessary to understand how the RSCN is specified and maintained (Azpeitia et al. 2010). Importantly, our results suggested that the characterized RSCN regulatory network is incomplete because the model was not capable of reproducing important processes observed in the RSCN such as its robustness. We believe that a complex systems perspective such as the one used here may be used to propose the missing components and interactions necessary for the production of the RSCN observed systemic behaviors and aid in the achievement of a better understanding of the properties of the MRMs underlying the RSCN.

## Complex system approaches to RSCN patterning

We now use a complex systems-based approach to analyze the integrated action of the above mentioned MRMs during RSCN patterning and study some systems-level traits and behaviors of the integrated network. We also discuss whether such a systematic and integrative approach reveals novel predictions to be tested experimentally or innovative approaches towards understanding how the cellular patterns and organization of the RSCN emerge.

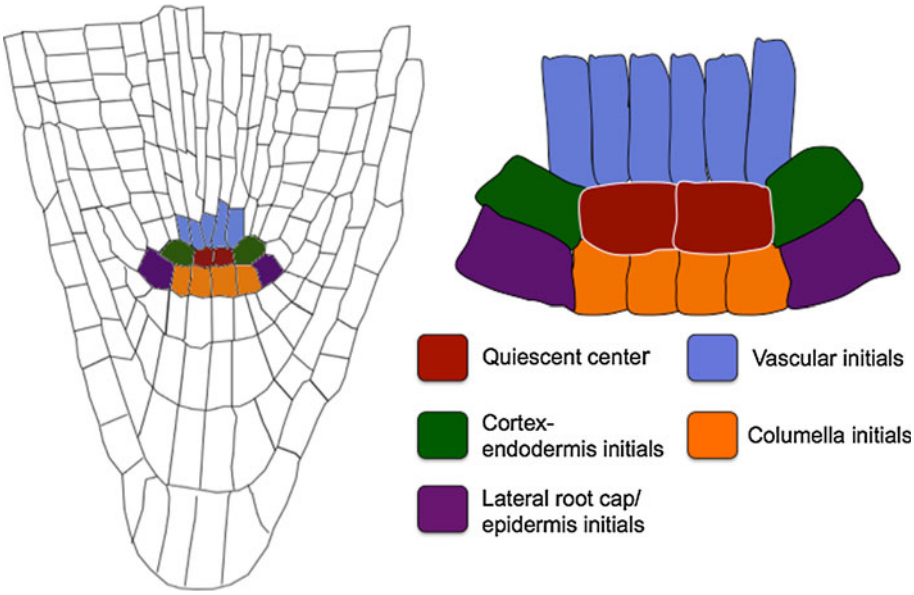


**Fig. 1** *Arabidopsis thaliana* root meristen and zoom to the root stem-cell niche (RSCN). The RSCN is located at the tip of the root meristem, here colored. The different colors stand for the different initial or stem cell types that compose the RSCN and two quiescent cells revealed in a longitudinal section

## Structural network-based study of the RSCN MRM

A network is composed of components called nodes that are connected through edges. In molecular regulatory networks, the nodes usually represent genes, proteins or molecules (e.g., hormones), while the edges represent regulatory interactions (reviewed in Barabási and Oltvai 2004; Albert 2007).

The most basic structural features of networks are their degree (also called connectivity) and degree distribution. The connectivity or degree $k$ refers to the number of direct links that one node has with the other nodes of the network, while the degree distribution $P(k)$ refers to the probability that a randomly selected node has a specific degree $k$. Many biological networks follow a power law degree distribution (Babu et al. 2004) or a similar long-tail distribution. A power law degree distribution means that $P(k) \approx Ak^{-\lambda}$, where $A$ is a normalization constant and $\lambda$ is the degree exponent. Networks with a power law degree distribution are also known as scale-free networks. As observed with the degree distribution, in scale-free networks, there are many low degree nodes, while a few of the nodes, known as hubs, have high degrees (Barabási and Oltvai 2004; Albert 2007). Because of their high connectivity, hubs have been proposed as important nodes for network functionality, connecting nodes that can participate in different processes and bringing together the network as a whole (Barabási and Oltvai 2004).

Although we lack a large enough network structure or architecture for RSCN patterning to allow for statistical analyses of the degree distribution, more than one of the genes involved in RSCN maintenance are probably hubs. For example, *SHR* and *SCR* regulate hundreds of genes (Sozzani et al. 2010), a fact that is reflected by the many processes in which they are involved apart from RSCN maintenance such as root regeneration (Xu et al. 2006; Sena et al. 2009), lateral root development (Lucas et al. 2011), cell cycle (Sozzani et al. 2010), root radial patterning (Helariutta et al. 2000), middle cortex formation (Cui and Benfey 2009), vascular development (Carlsbecker et al. 2010; Cui et al. 2011) and stress response (Cui et al. 2012).

Scale-free networks present the small-world propertiy. The small-world property refers to the shortest possible path to travel from a node to any other node using only directly linked network nodes (Watts and Strogatz 1998). In small-world networks, nodes are connected to each other through a short path. Importantly, the small-world property has been reported in biological networks (Wagner and Fell 2001). Most of the MRMs involved in RSCN maintenance were initially characterized as independent of each other; however, recent work has discovered some links among them, creating short communication paths. For example, the SHR/SCR and PLT/auxin MRM were first described as independent MRMs (Aida et al. 2004). However, Lucas et al. (2011) recently reported that *shr* single mutants have an excessive accumulation and synthesis of auxin during the first 6 days after germination and have a progressive reduction in the auxin transport facilitators *PINFORMED* (*PIN*) expression in the root tip, likely regulating *PIN* abundance at the posttranscriptional level or indirectly regulating their expression, as suggested by Levesque et al. (2006). Moreover, SHR and SCR up-regulate the expression of miR165a, miRNA166a and miR166b (collectively referred as miR165/6). miR165/6 can diffuse from its site of expression and negatively regulate the post transcriptional expression of the HD-ZIP III gene PHABULOSA

(*PHB*) (Carlsbecker et al. 2010; Miyashima et al. 2011). HD-ZIP III genes apparently act by antagonizing the *PLT* genes in the RSCN (Smith and Long 2010). Moreover, during embryogenesis, HD-ZIP III genes regulate auxin flow (Izhaki and Bowman 2007), a fact that needs to be tested in the case of the root. Importantly, the PLT/auxin MRM feeds back to the SHR/SCR MRM. An analysis of whole seedlings revealed that HD-ZIP III genes expression is induced by auxin (Zhou et al. 2007), and the inhibition of *PHB* and its redundant gene *PHAVOLUTA* in the basal pole during embryogenesis is necessary for *SCR* and *WOX5* expression and thus proper RSCN development (Grigg et al. 2009; Fig. 2).

The last example is not the only example of the communication of MRMs through short paths. Many hormones are important for the RSCN including auxin, cytokinins (CK), ethylene, brassinosteroids (BR), jasmonate and abscisic acid, all of which alter the RSCN pattern, functionality or development (Ortega-Martínez et al. 2007; Müller and Sheen 2008; Ding and Friml 2010; Zhang et al. 2010; Chen et al. 2011a; González-García et al. 2011). However, hormones do not act trough isolated pathways or MRMs; they instead regulate each other at the biosynthesis, signal transduction and transport levels. For example, ethylene, CK and auxin regulate the synthesis of each other (Nordström et al. 2004; Tsuchisaka and Theologis 2004; Stepanova et al. 2005; Swarup et al. 2007; Stepanova et al. 2008; Jones et al. 2010; Zhou et al. 2011), PIN auxin transporter expression is affected by CK, BR, ethylene and auxin itself (Blilou et al. 2005; Vieten et al. 2005; Ruzicka et al. 2007; Dello Ioio et al. 2008; Ruzicka et al. 2009; Zhang et al. 2011), and the effects of ethylene on cell elongation are dependent on the auxin signaling pathway (Swarup et al. 2007; Stepanova et al. 2007; Fig. 3). Indeed, hormonal cross-talk is important for root patterning (reviewed in Benková and Hejátko 2009). The evidence reviewed here suggests that the small-world property is present in the whole RSCN network and demonstrates that the SHR/SCR, the PLT/auxin MRMs, and the hormone signaling pathways, which were originally reported as independent MRMs, are interconnected through short and most likely multiple pathways.

Importantly, due to the presence of short communication paths, the small-world property proposes that modifications in one MRM can have unexpected effects in other MRMs (Watts and Strogatz 1998), while, at the same time, allowing for simpler and direct explanations of such effects. For example, the *PIN* genes and *WOX5* expression are affected in *scr* and *shr* single mutant backgrounds, even though neither *PIN* nor *WOX5* appear to be direct target genes of SHR or SCR (Levesque et al. 2006; Sarkar et al. 2007; Sozzani et al. 2010). Based on the fact that SHR directly up-regulates the expression of cytokinin oxidase 3 (CKX3; Cui et al. 2011), a CK catabolism enzyme, and that CK represses *PIN* expression (Ruzicka et al. 2009; Zhang et al. 2011), one possible explanation is that SHR indirectly regulates *PIN* expression through its down-regulation of CK synthesis. Other possible explanations are (1) that SHR and SCR regulate *PIN* expression through its effect on auxin, as *shr* mutants accumulate auxin and high concentrations of auxin reduce the PIN protein levels (Vieten et al. 2005; Lucas et al. 2011), or (2) through an effect of SHR and SCR on PHB (Carlsbecker et al. 2010) given that the

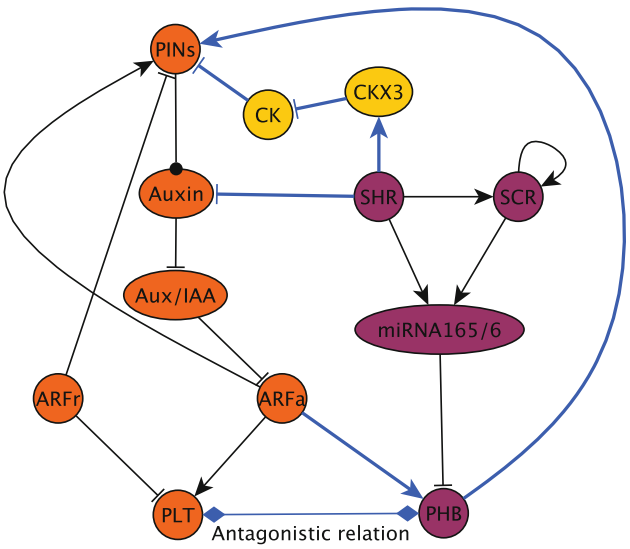


**Fig. 2** Shortest paths connecting the SHR/SCR (*purple*) and the PLT/Auxin (*orange*) modules as described in the main text. Simplified versions of these modules are depicted. *Blue* edges highlight the short paths that connect both modules. As observed, even when these were characterized as independent pathways or modules, they have multiple short communication paths. *Arrowheads* represent positive interactions, *T arrowheads* represent negative interactions, *doted arrowhead* auxin transport facilitation and *diamond arrowhead* antagonistic probably non-regulatory interactions. *ARFa* ARF activator, *ARFr* ARF repressor, *CK* cytokinin

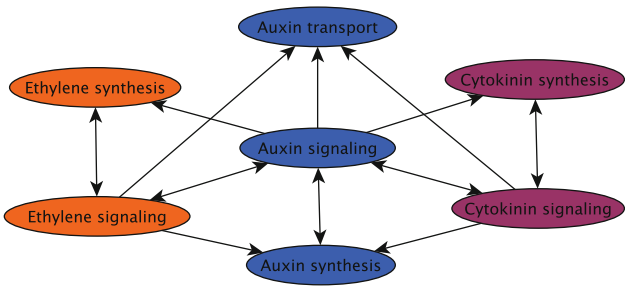


**Fig. 3** Cross-talk among auxin, cytokinin and ethylene pathways involved in RSCN patterning as described in the main text. As observed, these hormones pathways have multiple interactions or crosstalk connections at the signaling, synthesis and transport levels demonstrating that they are part of an integrated complex network with short communicating paths

HD-ZIP III genes regulate auxin flow (Izhaki and Bowman 2007; Fig. 2). SHR and SCR could modulate WOX5 through its regulation of the HD-ZIP III genes or through another indirect mechanism. For example, *CLE10* was recently reported as a putative SHR target gene (Cui et al. 2011). However, CLE10 has been described as a peptide involved in protoxylem vessel formation and not in RSCN maintenance (Kondo et al. 2011).

Interestingly, neither hub importance nor small-world properties are rules in biological networks. The deletion of the more connected genes does not necessarily lead to the most drastic phenotypes (e.g., Espinosa-Soto et al. 2004), and the perturbation of a MRM does not alter all other MRMs. Why does this happen in biological networks? High connectivity is not necessarily directly related to functionality in a network. Other measurements, such as betweenness (i.e., the number of shortest paths that pass through a node), may also determine the functionality of a node (Goh et al. 2002). In addition, positive feedback loops appear to make biological networks more robust against mutations in highly connected nodes (Espinosa-Soto et al. 2004). However, understanding how structure and function are related is a difficult task and entails different approaches. Theoretical biology has proposed other properties of biomolecular networks, such as modularity, which may help explain why neither hub importance nor small-world properties are rules in biological networks.

## The RSCN as a modular system

Recent research suggests that biological networks usually have modular organization. At a structural level, network modules are usually defined as a subset of network components that are more connected with each other than with other components of the network (Fig. 4; Wagner et al. 2007; Espinosa-Soto and Wagner 2010). Modular organization may reduce the pleiotropic effects of perturbations (such as mutants) in the network (von Dassow and Munro 1999; Wagner et al. 2007) because modules have a relatively autonomous behavior with respect to the rest of the network. Hence, such modularity may help explain why, in biological networks, mutations of highly interconnected nodes may not alter the phenotype, or they do not necessarily behave as expected for small-world networks.

As previously mentioned, some interactions occur between the SHR/SCR MRM and the PLT/auxin MRM. However, there are multiple interactions within PLT/auxin and SHR/SCR MRMs (Figs. 2, 5, 6). *PLT* genes expression patterns are altered by auxin addition, transport inhibition and signaling pathway mutants (Aida et al. 2004; Blilou et al. 2005; Galinha et al. 2007). The PLTs response to auxin is partially dependent on tyrosylprotein sulfotransferase (TPST). TPST controls the activity of the secreted

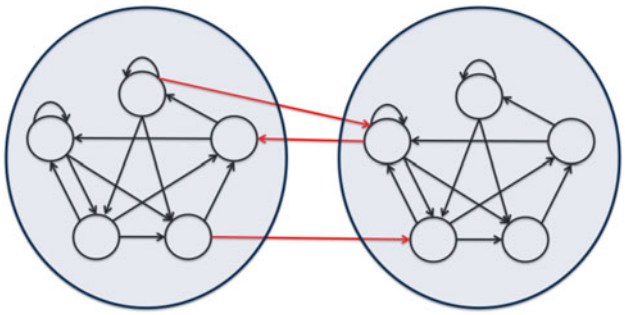

**Fig. 4** Structural modularity of gene regulatory networks where there are many more within-module interactions than between module interconnections. This property can reduce pleiotropic effects of mutants, due to the relative independence of different modules

peptide portion of the root meristem growth factors 1 (RGF1), 2 and 3 (herein RGFs) by Tyr sulfation (Matsuzaki et al. 2010). RGFs expression is auxin-independent, while TPST expression is auxin-dependent. Matsuzaki et al. (2010) proposed that RGFs probably stabilize PLT proteins based on the fact that wild-type seedlings treated with RGF1 expand PLT1 and 2 protein domains but not *PLT1* or *PLT2* transcriptional domains. However, other results demonstrated that *tpst* mutants reduce PLT at the transcriptional and protein levels, demonstrating that TPST can control PLT expression at both levels (Zhou et al. 2010). Interestingly, *PLT* genes control auxin transport, which is indispensable for the observed auxin graded concentration in the root, creating a loop in which PLT genes simultaneously control and are controlled by auxin distribution (Aida et al. 2004; Blilou et al. 2005; Galinha et al. 2007). The RopGEF7 gene is positively regulated by auxin and acts as a positive regulator of *PLT* expression. Interestingly, RopGEF7 also affects the auxin transport and response in the RSCN (Chen et al. 2011b). Moreover, as described below, there are multiple feedback loops within the auxin signaling pathway, greatly increasing the connectivity of the MRM (Fig. 5).

On the other hand, SCR and SHR form a dimer, and they together directly up-regulate *MAGPIE* (*MGP*) expression and JACKDAW (*JKD*) postembryonic expression (Levesque et al. 2006; Welch et al. 2007; Cui et al. 2011). Mutations in *JKD* diminish *SCR* expression in the QC and cortex-endodermis initials (CEI), causing a misspecification of the QC and ectopic periclinal divisions of the CEI. The double mutant *jkd mgp* restores *SCR* expression in the QC and CEI, suggesting that MGP is a negative regulator of *SCR*. Yeast two-hybrid and transient assays have shown that SHR, SCR, JKD, and MGP can physically interact and modulate the expression of and transcriptional activity of each other (Welch et al. 2007; Ogasawara et al. 2011). SCR also acts as a negative regulator of *MGP* when it forms a dimer with like heterochromatin protein1 (LHP1) (Cui and

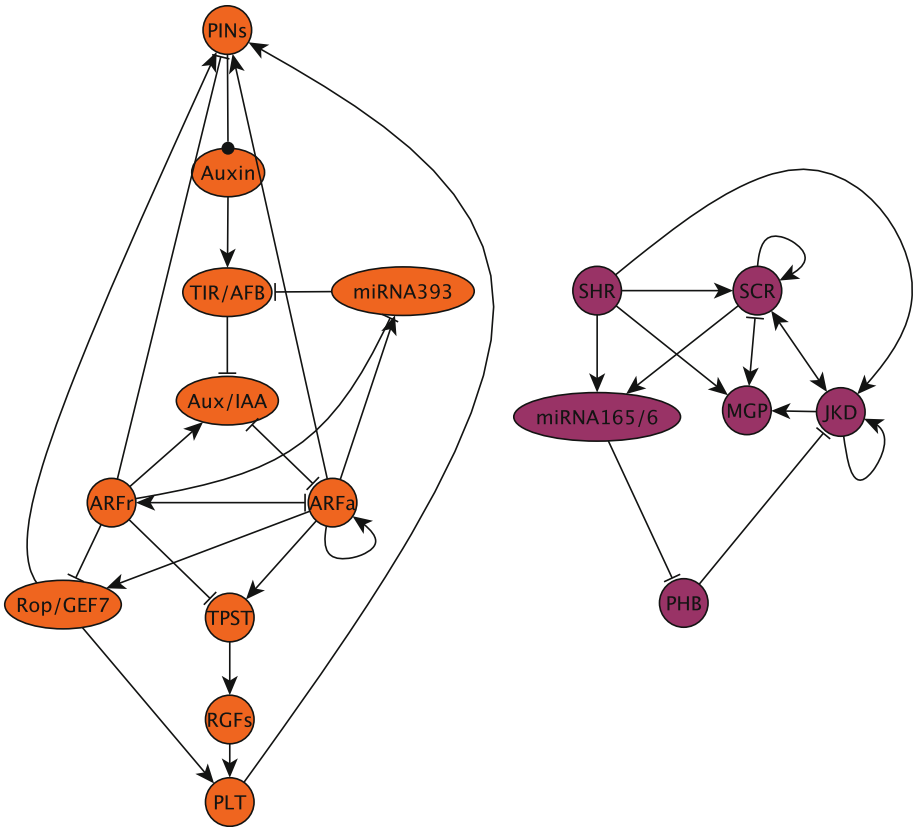


**Fig. 5** The PLT/Auxin (*orange*) and the SHR/SCR (*purple*) structural modules. The whole RSCN network is probably divided into several structural modules, based on their relative intra-module versus inter-module interactions: in these two modules there are many more within-module interactions than between-module connections (see also Fig. 2). *Arrowheads* as in Fig. 2

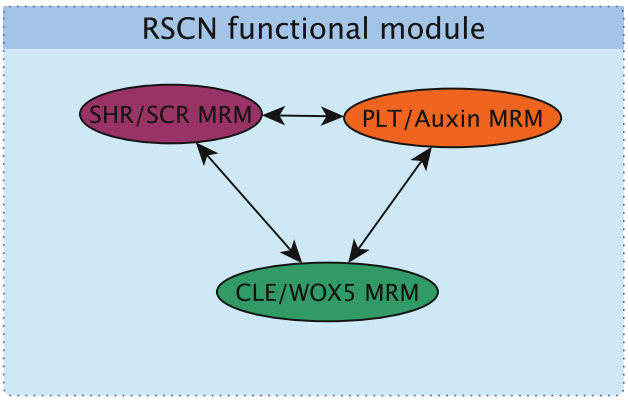


**Fig. 6** The root stem-cell niche functional module. As observed, the RSCN functional module is composed of several structural modules, indicating that structural and functional modules do not always coincide

Benfey 2009). LHP1 is a candidate protein for the histone trimethylation that is necessary for PcG repression activity (Exner et al. 2009). As mentioned above, SHR and SCR negatively regulate PHB through miR165/6. Recently, Miyashima et al. (2011) demonstrated that the restriction of *PHB* to the stele is necessary for maintaining *JKD* expression. Consistent with this result, the gain-of-function allele *phd-1d* has a similar ground tissue phenotype as the *jkd* single mutant with low *SCR* expression levels, which may explain why PHB repressed SCR expression in a previous study (Grigg et al. 2009; Fig. 5).

Until now, we have considered a structural definition of modularity. However, dynamic regulatory network models may be used to test whether a set of interacting genes or other molecular components constitute a functional module that is sufficient and necessary to recover an observed gene-state configuration and its spatial pattern. Importantly, structural and functional modules may not coincide (Ten Tusscher and Hogeweg 2011). For example, in Azpeitia et al. (2010), we aimed to analyze whether PLT/auxin, SHR/SCR, and CLE/WOX5 MRMs were sufficient to reproduce the stable gene configurations that characterize each cell type within the RSCN and their observed spatial distribution. We found that some important interactions are missing, but by adding a few predictions, our RSCN regulatory network model constitutes a functional module that incorporates the necessary and sufficient MRMs required to recover the genetic configurations that have been described for the main cell types of the RSCN and their observed qualitative spatial patterning. However, as mentioned above, each of the MRMs considered in the proposed RSCN network may constitute a different structural module (Fig. 6). Thus, dynamic analyses are fundamental to the understanding of how the MRM structure and function are related.

Once a dynamic model is developed, the model can be validated with experimental evidence. For example, our model is capable of reproducing most of the mutant phenotypes observed, but it is not as robust as expected from experimental observations and from comparison with other biomolecular networks (Azpeitia et al. 2010). This indicates that the uncovered regulatory module is incomplete. Additional components, interactions or complete modules may be necessary to recover the observed robustness and overall RSCN behavior. For example, epigenetic MRMs were not considered in our model and are fundamental to RSCN maintenance because *SCR* or *PLT* have an altered expression in mutants of the *TONSOKU* (*TSK*), *TEBICHI* (*TEB*), *FASCIATA1* (*FAS1*) and *FAS2*, NAP1-related protein1 (NRP1) and NRP2, Polycomb group (PcG), general control nonderepressible protein5 (GCN5) and Pickle (PKL) genes, which are all involved in epigenetic regulation (reviewed in Shen and Xu 2009). Moreover, all mutants of these genes have alterations in RSCN patterning, the expression of QC markers, and/or columella SC differentiation (Shen and Xu 2009). These epigenetic MRMs, which are part of structural modules that are different than the ones incorporated thus far, may be required to recover observed dynamic behaviors in the RSCN patterning and the regulatory networks involved within it. Thus, such epigenetic mechanisms should be an important part of the RSCN functional module even when they are likely a part of different structural modules. However, we believe that one of the most interesting and important properties of the dynamic analysis of complex networks is self-organization. This may also provide important biological insights into such networks, e.g., in terms of uncovering missing components or interactions.

## The RSCN as a self-organized system

Self-organization refers to the emergence of patterns or behaviors at the global system level as a consequence of non-linear interactions among system components without depending upon the action of a central controller (Seeley 2002). For example, the stable gene-state configurations (attractors) that characterize each cell type within a RSCN constitute a self-organized property of the complex regulatory module. These attractors emerge as a consequence of the concerted action of all of the molecular interactions considered in the particular network under consideration. At a different level of organization, the spatial cellular arrangement that characterizes the RSCN may also be considered as a self-organized pattern resulting from the coupled dynamics of intracellular networks in a multicellular spatial domain. In the case under consideration, the coupling of the dynamics of the intracellular networks occurs via the intercellular movement of some of the network components (Azpeitia et al. 2010).

The lack of a central controller means that self-organization arises from the local interactions of the system components and not from an individual component at any level of organization that works as a "guiding" unit. In this sense, it is relevant to uncover the structure and dynamics of intracellular biological networks and their coupling mechanisms among cells, rather than only concentrating on the role of so-called "key" genes. In contrast, we should understand, in terms of integrated regulatory networks, what makes a "key" gene key and why the mutation of such genes are sometimes sufficient to take the system from one multigenic and multicellular configuration to another one in contrast to mutations in other genes that are not "key".

In the root, there are many traits and behaviors that suggest the existence of self-organizing processes at the macro and micro levels. The regeneration process is perhaps one of the most obvious and best examples of self-organization in the RSCN. Normally, the RSCN develops from early embryonic stages (Dolan et al. 1993); however, when the root tip is excised or the QC ablated, a new RSCN is formed (Xu et al. 2006; Sena et al. 2009). The process of root and RSCN regeneration follows an ordered sequence of events. It begins with the formation of a new auxin maximum, which induces *PLT* expression and, later, SHR nuclear localization and *SCR* expression where the new RSCN will be relocated. *SHR*, *SCR*, *PLT* genes and the auxin maximum are all necessary for the RSCN regeneration process (Xu et al. 2006). After the root tip is excised, the RSCN is completely eliminated, and the root tip pattern is severely affected. However, even in the absence of the original pattern, RSCN regeneration proceeds (Sena et al. 2009). Moreover, callus regeneration from root, cotyledon, and petals, all of which have completely different morphologies, resemble the root regeneration process (Sugimoto et al. 2010). The fact that the RSCN can regenerate without a specific pre-established pattern and employ multiple molecular components strongly suggests that no single molecular component, module, external agent or pre-pattern directs the RSCN regeneration and patterning process. The process is instead due to the self-organizing capability of the molecular regulatory network involved, and it is likely that additional coupling constraints such as physical and chemical (e.g., hormone) fields are also involved.

The root auxin gradient is another excellent example of a self-organizing process involved in RSCN maintenance (Leyser 2011). Theoretical and experimental studies have suggested that the graded distribution of auxin along the root longitudinal axis depends on the polar localization of the PIN proteins (Blilou et al. 2005; Vieten et al. 2005; Grieneisen et al. 2007; Mironova et al. 2010) and on auxin metabolism (synthesis and degradation; Stepanova et al. 2008; Petersson et al. 2009). However, the polar localization of the PIN transporters is, in turn, regulated by the auxin-signaling pathway (Vieten et al. 2005; Sauer et al.

2006), which partly depends on the auxin cell concentration that at the same time, depends on the auxin gradient (Tiwari et al. 2001; Kepinski and Leyser 2005; Fig. 5). Hence, the auxin gradient is at the same time a cause and effect through its interdependences with the auxin signaling and transport mechanisms and not a process directed by any specific fixed force.

Self-organizing systems have a dissipative structure behavior, meaning that they are far from thermodynamic equilibrium. Interestingly, dissipative structures are maintained in steady states or attractors (Prigogine 1978), which can adapt and adjust to internal and external changes, allowing them to maintain coherent patterns or functions under a wide range of conditions and several types of perturbations (Heylighen 2001) that could include genetic loss or gain of function mutations. The latter behaviors are observed in the above examples of RSCN regeneration because it occurs in different types of RSCN lesions and different organs (Xu et al. 2006; Sena et al. 2009; Sugimoto et al. 2010). The auxin gradient is maintained even under some PIN and auxin signaling mutant backgrounds and during auxin addition or overproduction (Blilou et al. 2005; Vieten et al. 2005; Grieneisen et al. 2007). Finally, the overall multigenic RSCN configurations and cellular patterns are maintained in the presence of several mutations. However, without understanding how such apparently dispensable nodes are connected to the "key" nodes, we cannot understand the molecular regulatory basis of the overall behavior of the system.

Hormone signaling pathways are probably better examples of self-organizing, dissipative, and adaptable systems, i.e., they return to a basal state or attractor after perturbation. Such attractors are usually the inactive state of the pathway when the hormone is absent or present at low concentrations. Such systems also modulate and stabilize (adapt) their response when their inputs, usually hormone concentrations, change. There are multiple ways in which hormone pathways can reach this adaptable behavior (e.g., Dreher and Callis 2007).

As previously mentioned, one of the best-studied pathways during RSCN patterning is the auxin pathway. Auxin signaling can be modulated by adjusting the auxin concentration. As stated above, the auxin concentration depends on its transport, which is a self-organizing process, but it is also dependent on auxin metabolism. Auxin metabolism is regulated by multiple signals. Interestingly, auxin signaling also regulates some of these signals. For example, auxin inhibits CK synthesis (Nordström et al. 2004) and promotes ethylene synthesis (Rahman et al. 2001), while both CK and ethylene promote auxin synthesis (Stepanova et al. 2008; Jones et al. 2010; Zhou et al. 2011; Fig. 3). The internal components of the auxin pathway are also regulated to maintain a coherent adaptive response. Receptors of the transport inhibitor response1/auxin signaling f-box protein1–5 (TIR1/AFB) detect auxin. TIR/AFB are components of the SKP1/Cullin/F-box protein $(SCF)^{TIR1/AFB}$ ubiquitin ligase complex (Mockaitis and Estelle 2008) and are negatively regulated post-transcriptionally by miR393. The $SCF^{TIR1/AFB}$ complex promotes Aux/IAA degradation in the presence of auxin (Tiwari et al. 2001; Kepinski and Leyser 2005). Interestingly, Aux/IAAs act as an auxin co-receptor because TIR/AFB cannot readily bind auxin in the absence of Aux/IAA proteins (Calderón Villalobos et al. 2012). The Aux/IAA proteins form heterodimers with the Auxin Response Factor (ARF) proteins that mediate auxin transcriptional regulation (Tiwari et al. 2001). Some ARFs act as transcriptional activators (ARFa), while others act as transcriptional repressors (ARFr; Guilfoyle and Hagen 2007), and all compete for the regulation of the same target genes (Ulmasov et al. 1999); thus, the ARFa/ARFr ratio also modulates the auxin signaling response (Vernoux et al. 2011). Through its transcriptional activity, ARFa generates many feedback loops inducing the induction of *Aux/IAA* expression, and probably miR393 and some ARF family members (Wang et al. 2005; Paponov et al. 2008; Parry et al. 2009; Chen et al. 2011c; Fig. 3).

Importantly, the auxin signaling pathway can differentially modulate its response, depending on the Aux/IAA, ARF and TIR/AFB members involved in each specific process, because the members of these gene families have partially redundant functions, but also have specific characteristics that are involved in different responses (Hardtke et al. 2004; Parry et al. 2009; Calderón Villalobos et al. 2012; Rademacher et al. 2012). For example, the members of the TIR/AFB family have different strengths of interaction with Aux/IAAs, and different Aux/IAA-TIR/AFB complexes have different sensitivities to the auxin concentration (Parry et al. 2009; Calderón Villalobos et al. 2012). Thus, auxin signaling can adapt to several conditions, modulating the auxin concentration, adjusting the expression level and activity of the different receptors and proteins involved in the pathway through many feedback loops, adjusting the ARFa/ARFr ratio and selecting specific members of the Aux/IAA, ARF and TIR/AFB gene families that are involved in each response and condition.

Explicitly considering the self-organizing properties of a complex dynamic system could help resolve some apparently conflicting points regarding the MRMs involved in RSCN patterning. For example, some reports have shown that auxin promotes the expression of *WOX5* (Gonzali et al. 2005; Sarkar et al. 2007; Sena et al. 2009; Sugimoto et al. 2010). In contrast, a recent manuscript by Ding and Friml (2010) demonstrated that elevated auxin concentrations in the root tip lead to the consumption of the RSCN and WOX5 expression inhibition. It is likely that some of these apparent contradictions can be explained by invoking the different

responses and roles of the members of the ARF, Aux/IAA and TIR1/AFB gene families. However, another complementary hypothesis that may help reconcile such apparently contradictory results is that different responses to auxin signaling are explained by the self-organizing and self-regulating properties of the pathway. For example, self-organizing mechanisms generally involve, as has been uncovered for biological networks, many regulatory motifs such as feedback loops, which provide several properties to self-organizing processes. Positive feedback loops amplify a received signal or stimulus, allowing switch-like behavior, bistability, and hysteresis (Kitano 2004; Mitrophanov and Groisman 2008). On the other hand, negative feedback loops play an important role by dampening fluctuations, providing stability and limiting the fluctuating range of the components (Kitano 2004; Becskei and Serrano 2000). As mentioned above, positive and negative feedback loops are present in the auxin signaling pathway self-organizing process. The expression of ARF16, which is a putative ARFr, and ARF19, which is an ARFa, is promoted by auxin, thus creating a negative and a positive feedback loop, respectively (Wang et al. 2005; Paponov et al. 2008). The presence of these feedback loops could generate different auxin responses under different conditions, which could help explain why, under some circumstances, auxin inhibits *WOX5* expression, while in other conditions auxin promotes *WOX5* expression. For example, ARFa and ARFr compete for the same target genes (Ulmasov et al. 1999), and the expression patterns of ARF16 and ARF19 in the root tip are similar (Rademacher et al. 2011). Thus, if ARF16 expression is promoted to a greater extent by auxin than ARF19 expression, we expect that auxin addition would repress *WOX5* expression as observed by Ding and Friml (2010). However, if ARF19 is induced by auxin to a greater extent than ARF16, we could expect an induction of *WOX5* under auxin addition conditions as observed by the research of Gonzali et al. (2005) and Sugimoto et al. (2010). Hence, we may observe auxin inductive and repressive responses over *WOX5* under different conditions, and this would not imply contradictory experimental observations.

### Missing links in the RSCN network

The analyses of structural and dynamic MRM properties also allow for the detection of missing links and non–intuitive behaviors in the MRM that may guide future experimental studies that may otherwise be obviated. For example, Miyashima et al. (2011) suggested that miR165/6 acts as a morphogen. Theoretical research has demonstrated that the morphogen graded distribution alone is not capable of generating such highly precise patterns as the ones observed in the root vasculature. Theoretical analyses of complex systems have demonstrated that feedback loops have a critical role in the generation of robust and precise patterns (e.g., Jaeger et al. 2008). HD-ZIP III genes form a negative feedback loop with ZPR proteins in the shoot (Kim et al. 2008). It will be interesting to investigate if HD-ZIP III genes also create this or similar feedback loops in the root as suggested by theoretical studies and the observed patterns (Jaeger et al. 2008).

CLE40/WOX5 MRM is fundamental for RSCN maintenance (Sarkar et al. 2007; Stahl et al. 2009). However, until now, little information has been gathered regarding this MRM in the RSCN. Stahl et al. (2009) reported that CLE40 reduces *WOX5* expression. A similar MRM acts in the shoot stem cell niche, where the expression of WUS, a WOX5 homolog, is repressed by CLV3 (Sablowski 2011). The WOX5/CLE40 and WUS/CLV3 MRMs are likely similar in structure and dynamic behavior. This is supported by the fact that WOX5 can be substituted by WUS, that CLV3 can be partially substituted by CLE40 in the shoot, and that *CLV3* and *CLE40* over expression and peptide addition produce similar root phenotypes (Hobe et al. 2003; Fiers et al. 2005; Sarkar et al. 2007). Moreover, the protein phosphatases POLTERGEITS (POL) and PLL1 act downstream of the WOX5/CLE40 and WUS/CLV3 MRMs. In both cases, the CLV pathway inhibits POL and PLL1 activity, which is necessary for *WOX5* and *WUS* expression in the root and shoot, respectively (Song et al. 2008; Gagne et al. 2010). However, unlike the WOX5/CLE40 MRM, the WUS/CLV3 MRM has been thoroughly studied, revealing the presence of many feedback loops (Gordon et al. 2009; Chickarmane et al. 2012). The similarity between the WOX5/CLE40 and the WUS/CLV3 MRMs and the key importance of feedback loops in network behavior and emerging pattern robustness may guide researchers to search for feedback motifs that are similar to the ones that have been uncovered in the shoot in the WOX5/CLE40 MRM. Our own studies suggest a few yet uncovered feedback loops in the root MRMs that should be experimentally documented: a negative feedback loop between CLE40 and WOX5 and a positive self-regulation of WOX5, which likely occurs via the auxin signaling pathway (Azpeitia et al. 2010, and unpublished data).

## Conclusions

Complex systems approaches are becoming fundamental to the understanding of regulatory systems that result from non-linear interactions among multiple components that act in concert during a particular cell differentiation or morphogenetic process, rather than by being directed by single and isolated genes or any type of central controller. In this review, we have used structural and dynamic properties of

complex networks, like modularity and self-organization, to integrate and provide novel explanations for the plethora of molecular genetic involved in the RSCN of *A. thaliana*. Due to space limitations, we only focused on a few structural and dynamic properties of complex networks. However, there are other properties, such as robustness, which are pervasive among complex systems. Robustness refers to the ability of systems to retain their functionality despite perturbations. For the interested reader, excellent reviews have focused on this aspect (Kitano 2007; Whitacre 2012) and other important properties of complex systems (Mitchell 2009).

These properties constitute useful conceptual frameworks for analysis at the systemic level, and importantly, they yield complementary information to achieve a better and more profound mechanistic understanding of complex systems such as the regulatory networks involved in the patterning of SC niches and other biological structures. For example, structural analyses allowed for a global picture of the involved network topology, which may be useful for uncovering missing components or interactions and identifying novel behaviors or roles for particular nodes. Modularity at the structural level also helps us understand why relying on only the simplest structural traits, such as degree and degree distribution, may be misleading with regards to the role of particular nodes in overall network dynamics. However, importantly, structural modularity does not necessarily coincide with functional modularity; hence, when trying to uncover the structure–function relationship, additional dynamic approaches are required to verify the necessity and sufficiency of the components that are being considered in the overall system behavior or dynamics. Hence, structural and dynamic analyses complement each other, which also allows for the prediction of novel components and interactions and the evaluation of the functional role of characterized components or the proposal of innovative systemic explanations. As we observed, all of these properties are interconnected and together provide a powerful vision for the study of complex systems.

In this review, we have shown that enough molecular genetic information has been uncovered for the MRMs involved in RSCN patterning to allow for an integrative approach that explores the structural and dynamic properties characteristic of complex systems. This approach has allowed us to postulate a novel, non-intuitive hypothesis, which may guide future experimental studies, and provide explanations for apparently contradictory experimental evidence. Therefore, this type of analysis may guide research and then feedback to further the understanding of RSCN patterning. A dynamic interplay among theoretical, experimental and comparative analyses will greatly contribute to the further understanding of the complex nature of the regulatory systems involved in cell differentiation and morphogenesis during plant and animal development.

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