Heterogeneity and its multiscale integration in plant morphogenesis

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Abstract:

Heterogeneity is observed at all levels in living organisms, but its role during the development of an individual is not well understood. Heterogeneity has either to be

limited to ensure robust development or can be an actor of the biological processes leading to reproducible development. Here we review the sources of heterogeneity in plants, stress the interplay between noise in elementary processes and regulated biological mechanisms, and highlight how heterogeneity is integrated at multiple scales during plant morphogenesis.

Introduction:

Heterogeneity$^{\gamma}$ ($^{\gamma}$=see definition in glossary in Box 1) is an inherent feature of all living organisms. It is observed at all organization levels and contributes to the function of higher-level structures: diverse molecules interact to form specialized sub-cellular structures that together build cells which, in multicellular organisms, can acquire different identities and form complex organs. Recently, another type of heterogeneity within specific structures, which could at first sight appear homogeneous, has gained attention. For instance, at the organ level, seemingly identical lateral root primordia can be formed by heterogeneous contributions of founder cells [1•]; at the tissue level, Arabidopsis leaf epidermal pavement cells are heterogeneous in size and shape [2]; and at the cellular level cortical microtubules (CMT) and cellulose synthase trajectories vary between the different sides of epidermal cells of etiolated hypocotyls [3,4].

With the expansion of quantitative approaches, the number of processes that now appear as involving heterogeneity is rapidly increasing. This raises two major questions: how is heterogeneity generated, and what are its biological consequences? In this review, we discuss some recent insights gained from reports of heterogeneity at different scales and its integration$^{\gamma}$ between different functional levels within a plant.

Subcellular processes are sources of heterogeneity.

Gene expression is by nature a highly stochastic$^{\gamma}$ process [5]. At the whole plant level, gene expression shows noise$^{\gamma}$ levels that are under genetic control, but the origin (intrinsic$^{\gamma}$ or extrinsic noise$^{\gamma}$) could not be identified [6]. At the individual cell level, gene expression fluctuates over time in leaf cells, mostly as a consequence of extrinsic noise [7•] (Figure 1), as reported for prokaryotes and other eukaryotes [8]. At the system level, additional levels of noise may arise from the gene regulatory network (GRN) topology. For instance, the noise in the expression of a gene coding for a transcription factor affects the expression of its downstream targets and when TFs target TF genes, noise propagates within the GRN [9]. One way to reduce this propagation relies on redundant regulations by multiple TFs that provide robustness to the transcriptional output of a gene [10,11].

Noise in gene expression can be used to generate heterogeneity in plants. For instance, a link between noise and plasticity in gene expression has been observed in Arabidopsis [12]. Noisiness of gene expression is used to drive differentiation during sepal development [13••]. Expression of the ATML1 TF in epidermal sepal cells shows a high level of noise. When ATML1 level exceeds a threshold in receptive cells in the G2 phase, it triggers endoreduplication and hence giant cell formation. This generates a loose pattern within the epidermis where the average proportion of giant cells, but not their position, is determined. This resembles the formation of retinal mosaics in Drosophila [14] or the selection of odorant receptors in mammals [15]. Relying on noise in gene expression to control cell fate when a precise pattern is not absolutely required may be more cost efficient than complex deterministic networks.

Stochasticity can also drive heterogeneity in other cellular components such as the cell wall. At the molecular level, while overall occurrence of the different monomers in lignin polymers is genetically and developmentally controlled, their precise polymerization pattern in the cell wall appears stochastic, leading to a high diversity of structures [16,17]. At a larger scale, cell walls are also heterogeneous, as a result of biologically regulated processes. In the epidermis of dark-grown Arabidopsis hypocotyls, specific loosening of the longitudinal anticlinal cell walls triggers anisotropic cell expansion. It is only in the latter step that CMT arrays and associated cellulose deposition switch to a preferentially transverse orientation to consolidate anisotropic growth [18]. The formation of lobes in Arabidopsis leaf epidermal pavement cells involves heterogeneity not only along but also across the cell wall [19••]. In both cases, spatial heterogeneity in the mechanical properties of the cell wall was attributed to heterogeneous distribution of pectins with different chemical properties, which suggests that pectins offer a more versatile way of tuning cell wall mechanical properties than other components such as cellulose microfibrils. These examples illustrate how chemical heterogeneity leads to mechanical heterogeneity, which in turn drives growth anisotropy.

Heterogeneity is also observed in the cell membrane system at multiple scales. Within the plasma membrane, the importance of polar distribution of proteins for patterning processes and physiology has been well demonstrated [20,21]. At the scale of the entire membrane system, rare phospholipids, the phosphatidylinositol-phosphates (PIPs), are heterogeneously distributed, with the amount of phosphatidylinositol 4-phosphate (PI4P) increasing from the Golgi apparatus to the endosomal compartments to reach a maximum at the plasma membrane [22-24]. The local

accumulation of this anionic lipid in the inner layer of the plasma membrane provides negative membrane surface charges, which establish a specific electrostatic identity and direct the plasma membrane localization of proteins such as PINOID or BRI1 KINASE INHIBITOR1 involved in hormone signaling [23,25••]. In animal cells, interaction between cationic residues of membrane protein and PIPs promotes the formation of nanodomains within the membrane [26,27], a mechanism also occurring in plants as the localization of the REMORIN proteins into nanodomains requires PI4P [28••]. This example illustrates the interaction between stochastic physical mechanisms and regulation by biological processes in the generation of heterogeneity at the cellular level.

Cell growth and division are heterogeneous processes

Heterogeneity in cellular patterns progressively appears during the formation of most organs: for instance, in both the developing embryo or in the lateral root primordium, growth and division patterns are initially stereotypical but become later more variable while preserving a stereotypical organ shape and size [1,29,30]. This suggests that fundamental cellular processes such as division and growth generate heterogeneity in the cellular patterns during development. In the shoot apical meristem (SAM), in which cell size is rather uniform, cell division timing and cell growth are coordinated at the individual cell level by a size-dependent accumulation of cyclin-dependent kinase activity that controls cell cycle progression [31,32••]. Cell division can be described according to a complex rule intermediate between critical size and critical size increment models [33••]. In addition, precision in the orientation of the division plane is controlled by a particular CMT structure, the preprophase band [34••]. Despite these regulatory systems, cell size just after division is variable due to unequal division [32••].

Cell division is an important source of heterogeneity, not only because daughter cells can have unequal sizes but also because of the unequal partitioning of molecules that may increase noise in biological processes such as gene expression [35]. Following an asymmetrical division, the smallest daughter cell grows at a faster rate than the largest one, thus partially compensating for the original difference in size [33]. A similar observation was made at a larger scale in the sepal, in which smaller epidermal cell lineages grow faster to catch up with larger cell lineages resulting in a homogenization of cell size [36•]. However, at later stages, differences in clone sizes are further amplified by growth. This indicates that mechanisms that integrate cell growth and cell division are acting at the multicellular or organ levels and that they are subjected to developmental regulations. However, heterogeneity in cellular processes can paradoxically contribute to robustness$^{\gamma}$ in development. Indeed, in developing sepals, the variability in cell growth is spatio-temporally smoothed out and this variability is required for the production of organs with reproducible size and shape [37••].

Mechanical stress as a contributor to cell integration

At any scale, heterogeneous growth generates heterogeneous mechanical stresses. At a small scale, in the SAM, mechanical stress can feedback on growth by enhancing heterogeneity between neighboring cells [38]. In developing sepals, mechanical stress generated by the fast growing trichomes leads to a mechanical shielding by the neighboring cells, thus buffering growth heterogeneity and reinforcing organ shape robustness [39•]. At the organ scale, mechanical stress provides a shape sensing mechanism contributing to the growth arrest at the sepal tip [40]. In addition to feeding back on cell growth, maximal tensile stress affects the orientation of division planes [41] or cell polarity [42], thus pointing to a possible coordination of different cellular

processes by mechanical signals and to the existence of multiple morphogenetic loops operating in parallel.

In many of these processes, the dynamic reorientation of CMT upon stress is the main mechanism associated with the multiscale integration of mechanical signals into morphogenesis, although microtubule-independent stress responses have also been reported [42]. However, how mechanical stresses are translated into CMT dynamics is still unknown. Mechanical stress has been proposed to contribute to the accumulation of PIP in the boundary around organ primordia in the shoot apical meristem, which in turn may impact CMT and signaling, thus possibly forming a multiscale feedback between the organ, tissue and cellular levels [43•]. Mechanical stress could feed into morphogenesis by other pathways. Cell walls and plasma membrane may constitute both sensors and the source of signals. For instance, wall associated kinases and mechanosensitive ion channels are involved in the mechanotransduction pathway [44]. One emerging actor of the mechanotransduction pathway acting at the PM is DEFECTIVE KERNEL 1 (DEK1), a transmembrane protein exhibiting similarity to animal calpains, a class of Ca^{2+}-dependent cysteine proteases. The transmembrane domain of DEK1 is required for mechanosensitive Ca^{2+} influx, which in turn promotes the autocatalytic cleavage of DEK1, releasing the C-terminal cytosolic calpain-like domain [45••]. Because this domain is sufficient to complement embryo lethality of *dek1* mutants [46], it suggests that it may act as an integrator of mechanical signals, responding to Ca^{2+}. Mechanical stresses have also other effects on the PM, inducing dynamic reorientation of polarly distributed PM associated proteins, like for instance PIN-FORMED1 [47] which may involve Ca^{2+} modulation of the PINOID kinase [48-50]. Finally, mechanical signals contribute also

to robust gene expression patterns [51]. In summary, mechanical stress emerges as a signal patterning and coordinating growth at multiple scales.

Communication between cells organizes heterogeneity

Cell-to-cell communication is essential for multicellular organisms and can have opposite effects on cellular heterogeneity. Developmentally regulated symplastic cell-to-cell movement of informative molecules such as proteins, hormones or small RNAs through plasmodesmata contributes to the establishment and maintenance of heterogeneous cell identities or growth patterns [52-54]. One characteristic of such movement is that it can generate gradients of molecules that contribute to heterogeneity at the organ level. For instance, in the SAM, movement of the WUSCHEL protein out of the organizing centre provides cues for the spatial separation between domains of distinct cell fates [55,56•]. Movement of small RNAs produced from the epidermis on either side of the developing leaf establishes clear-cut expression patterns of their targets and hence position a robust developmental boundary in the leaf [57,58,59•]. Based on modeling, it was suggested that diffusing signals emanating from the SAM epidermis could provide the link between SAM geometry and stem cell niche homeostasis [60]. While these examples illustrate how cell-to-cell communication reinforce heterogeneity, intercellular movement of proteins can also coordinate growth between different cell layers in the leaf [61]. Because the topology of the mobile signal sources within the organ shapes the gradients, cell-to-cell communication may constitute a feedback loop between organ and tissue heterogeneity. Thus, short range mobile signals contribute to organize the heterogeneity at the organ/tissue scale by enabling the formation of distinct domains or by reducing heterogeneity.

Conclusion and perspectives

During the last years, research on heterogeneity in plants has widely expanded. However, while heterogeneity at the cellular level (mainly cell growth and cell division in relation with the associated mechanical stress) is starting to be characterised, heterogeneity at lower scales is far less studied. In particular, the level and roles of heterogeneity in gene expression as a result of noise in gene transcription and translation are still poorly characterised compared to what is known in other systems [8,62,63].

An emerging conclusion from these studies is that heterogeneity is not only a biologically-generated process but can be a biological readout of noise in elementary reactions. Understanding how the biological context in turn affects the level of noise and what are the constraints it imposes on the translation of noise into a biological response are challenges for the future. These studies also underline the importance of the integration across different scales, with multiple mechanisms allowing either to exploit or on the contrary to buffer heterogeneity from one scale to the other. In this respect it is important to stress that such integration does not only occur from small to large scales but also conversely from organ to the tissue or cell level. Such an integrative view requires a systemic vision of heterogeneity in order to understand its contribution to morphogenesis. By allowing the objective assessment of noise and cellular heterogeneity together with the prediction of mechanical stresses and growth patterns, image analysis and computational modelling have been instrumental in many studies reported here. The recent advent of deep learning in image enhancement, restoration, segmentation and classification tasks [64] will strengthen and widen the importance of digital image analysis in quantitative cell biology. However, dealing with

heterogeneity also introduces new image analysis problems, in particular when it comes to identify principles of organization from noisy spatial image data. Methods based on image normalization and spatial statistics are emerging to address such problems [65,66]. Similarly, it can be anticipated that stochastic modeling approaches will be promoted in the coming years, as deterministic models have shown their limits when addressing noise and heterogeneity in various processes such as cell division [67]. or phyllotaxis [68].

An additional challenge for the future will be to shift towards multiscale models integrating the various dimensions of noise and heterogeneity to better decipher the processes involved in the building of robust organ shapes.

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Box 1: Glossary :

Heterogeneity is a property of a system that refers to its composite nature or to the variability of the elements that compose this system.

Integration refers to the processes/mechanisms whereby the individual characteristics and behaviors of cells are summed, leading to global growth at the tissue or organ scale.

Noise refers to the random variations in a biological process. For instance, gene expression level can fluctuate over time in a single cell (**intrinsic noise**), or vary between genetically identical cells growing in an homogenous environment (**extrinsic noise**). Noise can be measured by the coefficient of variation, the dimensionless ratio of the standard deviation over the mean.

Intrinsic noise is directly related to the stochasticity of the molecular interactions driving a biological process and occurs without variations in the number of molecules. It differentially affects biological processes of the same kind.

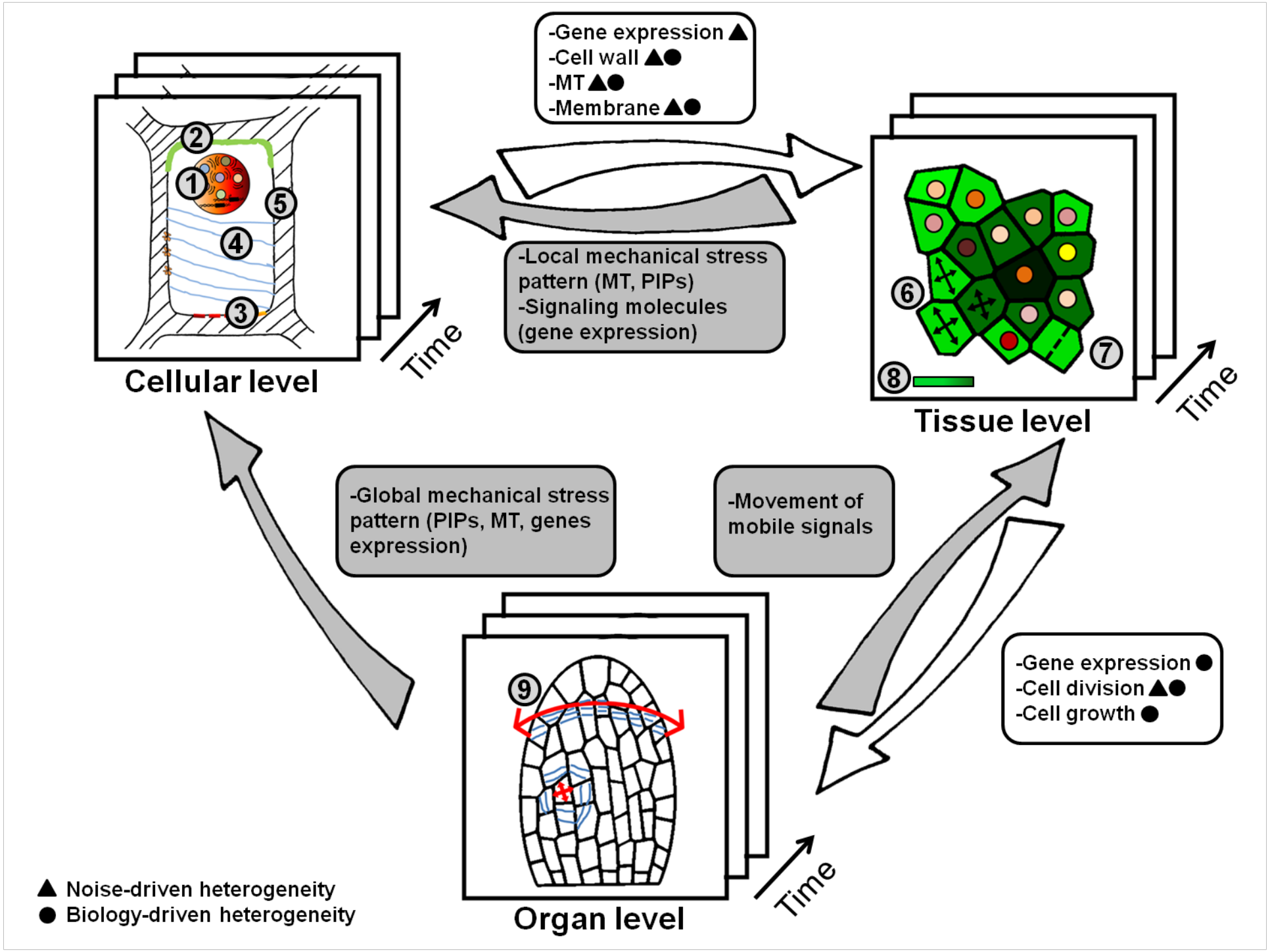
Extrinsic noise results from variations in the amount or activity of molecules that drive a biological process. Such variations can be observed between individual cells and affect similarly all the biological processes of the same kind occurring in a cell.

Robustness is an inherent property of a system that provides invariable output in response to input variations or heterogeneity.

Stochasticity refers to a random biological process that can not be accurately predicted as it is governed by probabilistic laws. Stochasticity is observed in chemical reactions involving multiple partners present at low numbers leading to infrequent interactions.

Figure 1: Heterogeneity and its integration over multiple scales in plant morphogenesis.

Heterogeneity is found at all levels of the organism, from the cellular to the organ level. At each level, the heterogeneity can be spatial and/or temporal. At the cellular level, gene expression fluctuates over time or can vary from cell-to-cell (1); plasma membrane proteins are polarly distributed (2); distinct phosphatidylinositol-phosphates (PIPs) are found in the membrane system (3) and microtubules (MT) orientation (4) and cell wall composition and structure (5) are variable. At the tissue level, neighboring cells have distinct growth rates and directions (6); cell division is unequal (7) and the concentration of mobile signals varies between cells (8). At the organ level, main directions of mechanical stress vary within the organ (9). This heterogeneity originates either from noise (triangles) or from biologically regulated process (discs). Heterogeneity at a low level impacts the functioning of the higher level (white arrows): for instance, noise-driven heterogeneity between different cells can impact tissue formation. Conversely, the higher level feeds back on the heterogeneity at the lower level (grey arrows): for instance local mechanical stress pattern generated at the tissue level by growth heterogeneity feeds back at the cellular level by impacting MT dynamics.


-Gene expression ▲
-Cell wall ▲●
-MT ▲●
-Membrane ▲●
-Local mechanical stress pattern (MT, PIPs)
-Signaling molecules (gene expression)
Cellular level
Time
Tissue level
Time
-Global mechanical stress pattern (PIPs, MT, genes expression)
-Movement of mobile signals
-Gene expression ●
-Cell division ▲●
-Cell growth ●
Organ level
Time
▲ Noise-driven heterogeneity
● Biology-driven heterogeneity