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Molecular mechanisms of robustness in plants

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Robustness, the ability of organisms to buffer phenotypes against perturbations, has drawn renewed interest among developmental biologists and geneticists. A growing body of research supports an important role of robustness in the genotype to phenotype translation, with far-reaching implications for evolutionary processes and disease susceptibility. Similar to animals and fungi, plant robustness is a function of genetic network architecture. Most perturbations are buffered; however, perturbation of network hubs destabilizes many traits. Here, we review recent advances in identifying molecular robustness mechanisms in plants that have been enabled by a combination of classical genetics and population genetics with genome-scale data.

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Introduction

Phenotypic robustness is a measure of an organism's ability to buffer phenotype against genetic and environmental perturbations during development [2,3,4••] (Box 1). Robustness is commonly attributed to features of the underlying genetic networks, such as connectivity, redundancy, feedback, and oscillators, as well as to non-genetic mechanisms [4••,6••,7]. Targeted perturbation of these features decreases phenotypic robustness and releases cryptic genetic or epigenetic variation. The release of accumulated variation has been invoked as an important factor in evolutionary processes [9] and in disease susceptibility in humans [5].

Robustness is a quantitative trait. Traditionally, robustness of individuals has been measured as the degree of symmetry in morphological features [15]. Another robustness measure is the degree of accuracy with which a genotype produces a phenotype across many isogenic siblings. Robustness thus measured is trait-specific and may not be predictive of robustness in other traits [15]. Like any quantitative trait, robustness shows a distribution among genetically divergent individuals of a species and can be mapped to distinct genetic loci [16–18]. Non-genetic mechanisms also affect robustness, as mutation penetrance can vary among isogenic individuals [4••,6••,7]. Plants are excellent models to probe the molecular underpinnings of robustness. Because of their sessile life-style and continuous development, plants have likely optimized molecular mechanisms that buffer phenotype in the face of ever-changing environmental conditions. Here, we review some advances in identifying molecular mechanisms that contribute to robustness in plants and discuss future directions and challenges.

'Master regulators of robustness' affect connectivity of genetic networks.

One of the best characterized 'master regulators of robustness' is the molecular chaperone HSP90 [6••,18–27] (Box 1). HSP90 assists the folding of key developmental proteins, a function that is of even greater importance under stresses that compromise protein folding [29]. HSP90 inhibition decreases robustness in plants, flies, yeast, and fish and releases previously cryptic genetic and epigenetic variation [18,21–24,27] (Figures 1a,b and 2a,b). In worms, low HSP90 levels correlate with high mutation penetrance [6••]. HSP90's capacity to buffer many developmental phenotypes has been attributed to its high connectivity in genetic networks [31]. Perturbing HSP90 function impairs its numerous substrates, which is thought to reduce network connectivity and lead to decreased robustness and release of variation. In the progeny of genetically divergent *Arabidopsis thaliana* strains, every tested quantitative trait is affected by at least one HSP90-dependent polymorphism; most traits are affected by several [18,24].

The circadian regulator *ELF4* is another gene that reduces robustness when perturbed [33]. Circadian clocks are endogenous oscillators with remarkably robust periods, which persist in the absence of light cues and under increased temperature [14]. The robustness of plant clocks is thought to arise from multiple interconnected feedback loops [14]. In reporter assays, *elf4* mutants show highly variable periods before turning arrhythmic (without periods) [33]. It is unclear whether the initial, variable periods translate into increased variation of developmental traits or released cryptic variation; both seem likely given the importance of the circadian clock in orchestrating growth and development. In fact, HSP90's effect on robustness may arise in part from disrupted clock function: ZTL, a circadian regulator, is chaperoned by HSP90 [34].

Box 1 The term phenotypic robustness is often conflated with other terms, some of which have slightly different meanings or denote entirely different phenomena. In the following, we attempt to clarify our view and usage of these terms:

Developmental stability — is equivalent to robustness as defined here, describes 'the ability of organisms to withstand genetic and environmental during development, so as to produce a predetermined phenotype' [1].

Canalization — describes the notion that genetic systems evolve to a robust optimum through stabilizing selection. This robust optimum is thought to arise through elimination of deleterious alleles and reduction of additive genetic effects. Canalization pertains to populations with most individuals clustering around an optimal phenotype [3,5].

Cryptic genetic variation — is genetic variation that is phenotypically silent until revealed by environmental, genetic, or epigenetic perturbations [8].

Developmental noise — was used originally by Waddington to refer to differences among homologous replicated parts within a single individual and to describe the absence of developmental stability [2]. The term is currently often used as 'noise' to describe stochastic variation in traits such gene expression, caused by both intrinsic errors and extrinsic micro-environmental fluctuations [10,11]. Noise is thought to play an important role in fate determination and circadian clock function [12–14].

Fluctuating asymmetry — describes an organism's deviation from bilateral symmetry for the whole organism or particular morphological features such as fly wings or bristles. FA is an individual-based measure of robustness. Low FA is thought to correlate with high fitness [15].

Variable mutation penetrance — describes the phenomenon that certain mutations show different expressivity (i.e. severity of phenotypic effect) among isogenic individuals. We attribute these expressivity differences to differences in robustness among these individuals. Less robust individuals are expected to show higher mutation penetrance. Variable mutation penetrance among genetically divergent individuals arises from individual-specific genetic and non-genetic modifiers.

Phenotypic plasticity — is the ability of an organism to alter its physiology, morphology, and development in response to changes in its environment [2]. In our view, phenotypic plasticity describes changes in phenotype that are predetermined in existing genetic networks, rather than consequences of stochastic errors in development (that may be ultimately due to extrinsic micro-environmental differences rather intrinsic errors).

Epistasis — is the nonreciprocal interaction of nonallelic genes, in which one gene masks the effects of another. More recently also used to describe interactions of variants with a gene or regulatory region.

Pleiotropy — describes the phenomenon in which a single gene is responsible for several distinct and seemingly unrelated phenotypic effects.

Robustness master regulator — is used here to denote genes that strongly affect robustness. We use this term interchangeably with the terms network hub and fragile node. In yeast, genes that strongly affect robustness are network hubs [28]. Studies in plants [30••] and worms [32] have identified a small number of fragile nodes that affect the penetrance of mutants and natural variants in many other genes. Another frequently used term is 'capacitor', which refers to genes that keep genetic variation phenotypically silent when fully functional and release genetic variation when perturbed [22,23].

Of course, HSP90 and ELF4 are not the only robustness master regulators in plants. However, unlike in yeast, in which a systematic mutant analysis identified 300 robustness master regulators, all highly connected 'network hubs' [28], in plants a similar analysis has not been conducted; the sheer number of genes and the lack of high-throughput robustness assays have so far made such analysis unfeasible. In our hands, most tested plant mutants, some of them affecting key developmental genes, do not affect robustness of quantitative seedling traits.

Fine-tuning of gene expression stabilizes developmental traits.

The origins and consequences of gene expression noise have been extensively studied in single celled organisms [10–12], but less so in multicellular organisms [35], including plants [13]. In 2006, Hornstein and Shomron [36] hypothesized that microRNAs (miRNAs) may reduce gene expression noise and sharpen developmental transitions. In particular, feed-forward loops, in which a transcription factor regulates both a target and its miRNA with opposing effects on target protein levels, were predicted to buffer stochastic expression fluctuations [36]. As plant miRNAs tend to target key transcription factor and F-box genes, they modulate developmental transitions, variation in leaf morphogenesis, reproductive development, and root architecture [37•]. miRNAs have recently been shown to facilitate robustness. For example, *miRNA164* miRNAs control plant development by dampening transcript accumulation of their targets *CUC1* and *CUC2*, wherever expression of miRNAs and targets overlap. *miRNA164* miRNAs define boundaries for target mRNA accumulation in addition to reducing target expression levels [38].

In plants, small RNA-dependent regulation of gene expression is not limited to miRNAs — in fact, there are many plant-specific small interfering RNAs (siRNAs), some of which are mobile and facilitate robust pattern formation. Chitwood and co-authors [39••] demonstrated that a subset of trans-acting siRNAs (tasiRNAs), the low-abundant and conserved tasiR-ARFs, move intercellularly from the upper leaf side (adaxial), where they originate, to the lower leaf side (abaxial), generating a small RNA gradient that defines the expression boundaries of the abaxial determinant ARF3. tasiR-ARF biogenesis requires both miRNA activity (*miR390*) and siRNA pathway components, including the specialized