GENE REGULATION DYNAMICS

Noisy transcription under the spotlight

Real-time quantification of transcription initiation by nascent mRNA labelling reveals noisy transcriptional dynamics and a high level of inter-cell variability in transcription of environmental response genes.

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A long-standing question in biology is how noisy regulation at the cellular level impacts tissue and organismal responses. Plants offer the opportunity to address this, as the lack of cell motility allows the tracking of single-cell responses in intact tissues through time. However, our ability to quantify plant gene expression at cellular resolution has been limited by the methodology available. In this issue of *Nature Plants*, two independent works[1,2] apply an mRNA-labelling technique for real-time monitoring of transcription initiation dynamics at the cellular level in plants, revealing the single-cell basis of tissue-level responses to the environment (Fig. 1).

Both studies apply a previously developed method[3–7] to fluorescently label RNAs, based on bacteriophage proteins (PCP and MCP) that bind to specific RNA stem loops (PP7 or MS2, respectively). To assay transcription, a promoter of interest is used to drive expression of repeats of the RNA stem loops (Fig. 1a). The bacteriophage protein is fused to a fluorescent protein and constitutively expressed. In the absence of transcription from the promoter of interest, the bacteriophage protein–GFP molecules remain diffuse throughout the cell. When RNA polymerase II (RNAP) initiates transcription, the stem loops of the nascent transcripts are bound by the bacteriophage protein–GFP, and a bright spot, detectable by confocal microscopy, appears in the nucleus at the position of the allele being transcribed (Fig. 1c). The higher the number of RNAPs that have initiated transcription at the allele, the brighter the spot is. By tracking the spot brightness over time, the rate of transcription initiation can be dynamically measured at the individual alleles of a gene, for many cells of a tissue simultaneously (Fig. 1c,d).

Having carefully validated their methods against existing technologies, Alamos et al. and Hani et al. apply these systems to assay transcriptional dynamics for heat shock and phosphate-responsive genes, respectively. Hani et al. used microfluidics and time-lapse spinning disk confocal imaging to quantify transcriptional activity of *SPX1::MS2*, a reporter for the *SPX1* phosphate responsive gene, whilst precisely controlling the timing of phosphate application to seedlings. Upon phosphate addition, the average brightness of *SPX1::MS2* transcription spots in roots

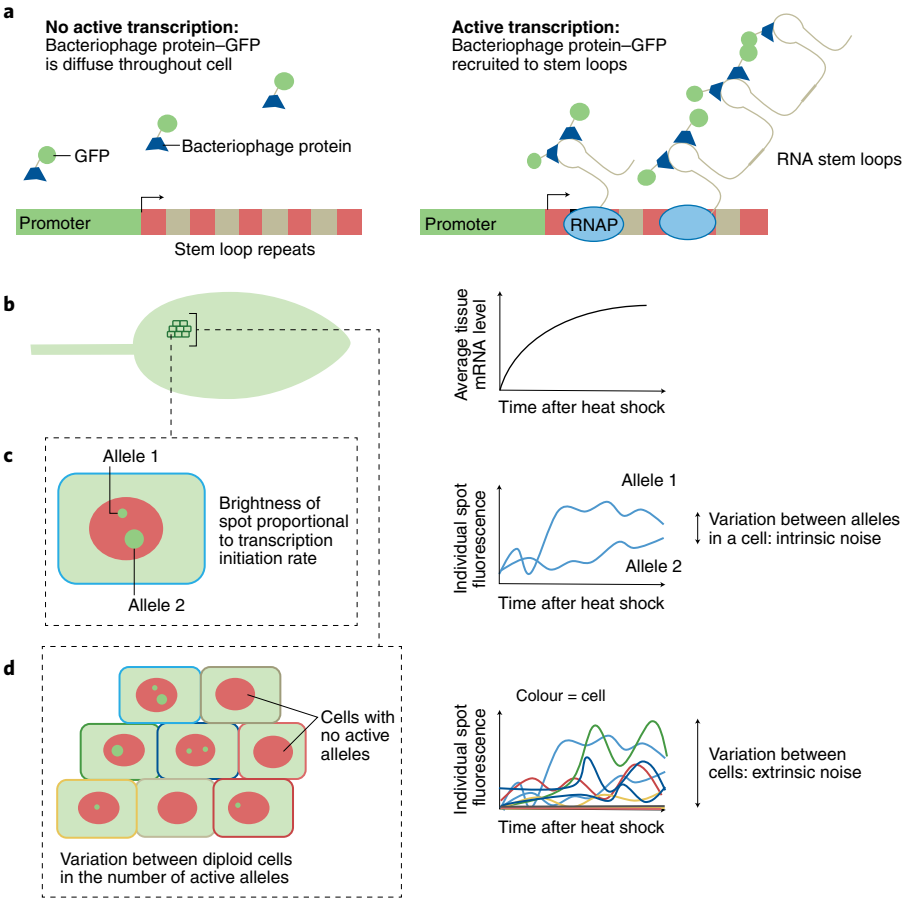

Fig. 1 | Single-cell basis of tissue environmental response revealed by RNA labelling. a, RNA labelling using PP7 and MS2 methods. In the presence of active transcription, the recruitment of the bacteriophage protein–GFP to the RNA stem loops results in a bright spot in the nucleus at the position of the active copy of the promoter reporter. Panels **b**–**d** illustrate the *HSP101::PP7* and *HsfA2::PP7* response to heat shock in leaf cells. The same principles apply to phosphate response in roots observed by Hani et al. **b**, Average tissue expression of *HSP101* in response to heat shock, as might be assessed by bulk RNA sequencing on pooled leaf tissue or low-volume RNA sequencing on a single leaf. **c**, PP7 RNA labelling allows the rate of transcription initiation at the promoter of interest to be assayed by tracking the brightness of nuclear spots over time using confocal microscopy. The two nuclear spots represent two alleles of the reporter construct. **d**, Quantification of spot fluorescence in multiple neighbouring cells. Colour of cell outline indicates corresponding traces on the right. Alamos et al. and Hani et al. found both variability in the rate of transcription initiation for alleles in the same cell (**c**) and variability between cells (**d**). Figure adapted with permission from ref. [1], Springer Nature Limited.

decreased rapidly. A significant difference could be detected within five minutes, faster than what was possible using quantitative reverse transcription–PCR. Strikingly, Hani et al. observed a high level of inter-cell variability in *SPX1* promoter activity, both in the absence of phosphate and in response to its resupply. Within a group of neighbouring root cells, some showed rapid reductions in nuclear spot intensity upon phosphate addition, whilst others continued to show high rates of transcription initiation. The cause of the inter-cell differences is unknown and will be an interesting question for future research.

In an analogous experiment, Alamos et al. quantified single-cell responses to heat shock in leaves (Fig. 1b–d). They used PP7 reporter lines for two heat-inducible genes (*HSP101* and *HsfA2*) and a constitutive promoter (EF-Tu) and tracked the brightness of individual nuclear spots as a heat treatment was applied. Similar to Hani et al., they found that reporter activity was highly variable between cells. For the heat-inducible and control promoters, a fraction of cells showed no detectable transcription throughout the experiment. Following the onset of the heat treatment, the fraction of cells that were actively transcribing from the heat-inducible promoters increased but remained constant for the constitutive gene. Amongst active cells, the average rate of transcription remained relatively constant. This change in the fraction of transcriptionally active cells is analogous to how *FLOWERING LOCUS C* (*FLC*) expression responds to prolonged cold treatments[8]. *FLC* is likely not needed for the function of cells in which it is expressed, but rather influences the expression of the mobile flowering signal, FT, such that the level of FT may provide an average read-out of the single-cell *FLC* expression. In contrast, in the case of the heat shock response, HSP101 (a chaperone) and HsfA2 (a transcription factor that induces heat shock proteins) most likely function cell-autonomously and thus would be needed by each cell to prevent protein misfolding. This makes the fractional response surprising and further work is required to understand how the absence of detectable transcription initiation over the time window of the experiments is related to cellular protein levels.

Both groups observed high levels of inter-cell heterogeneity in transcriptional initiation for the promoters tested and, for the first time in plants, observed evidence for transcriptional bursts. They attempted to understand the sources of the inter-cell variability. On one hand, there is 'intrinsic noise', which is reflected as differences in the expression of the alleles of a gene within a given cell, arising due to the stochastic nature of transcription initiation at each allele. On the other hand, 'extrinsic noise' refers to differences between cells and is caused by factors extrinsic to the alleles of a gene of interest, such as those due to inter-cell differences in transcription factor numbers or activation of signalling pathways. Both groups used the RNA-labelling techniques to measure these types of noise by comparing the level of variation in expression of the two alleles of a gene in the same cell (intrinsic noise) (Fig. 1c) with the level of variation between cells (extrinsic noise) (Fig. 1d). They each found that both sources of noise existed. Intrinsic noise was the major contributor to the variability in activity of heat shock promoters and in *SPX1* transcription under phosphate deprivation. Under phosphate addition, the *SPX1* alleles within a cell behaved similarly and inter-cell differences dominated.

The large contribution of intrinsic noise found in these studies contrasts with a previous study[9] in which, using fluorescent protein reporters for two constitutive promoters in *Arabidopsis*, the researchers found that extrinsic noise was the largest contributor. One hypothesis for this difference is that the contribution of extrinsic versus intrinsic noise differs between constitutive and environmentally responsive genes. Additionally, this difference between the studies reflects a general trend across model systems, where the estimated contribution of extrinsic versus intrinsic noise is often related to whether RNA or protein is measured *in situ* [5,10–13]. More work is needed in plants to understand the extent to which stochasticity at different stages along the central dogma contributes to inter-cell variability in protein levels and function. Another important question is how inter-cell variability in gene expression affects inter-plant variability. A recent RNA-sequencing study on individual seedlings found that approximately 9% of expressed *Arabidopsis* genes show high levels of inter-plant variability in expression[14]. The tools developed by Hani et al. and Alamos et al. will be vital for determining the cellular-level mechanisms underlying this inter-plant transcriptional variability. ❐

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Competing interests

The authors declare no competing interests.