Engineering cellular symphonies out of transcriptional noise

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Development unfolds through a series of orchestrated spatial and temporal gene-expression patterns. Despite relying on the noisy process of transcription, expression patterns remain robust to myriad disturbances. To achieve the goal of building complex tissues from the bottom up, synthetic biology must learn how to buffer and harness transcriptional noise.

Eukaryotic cells navigate a chaotic environment, in which stochastic cellular processes converge to generate cellular phenotypes. Emerging from the chaos, precisely coordinated patterns of gene expression support complex cellular behaviours such as tissue homeostasis and developmental patterning. Following cell-fate transitions, cells retain stable identities over long periods of time despite internal and external disturbances. How do cell populations maintain stability while retaining sufficient plasticity to respond to external cues and insults? Endogenous mechanisms that buffer and harness transcriptional noise are emerging as regulatory systems that confer both stability and plasticity. Whereas current synthetic circuits are designed primarily around perturbing mean levels of gene expression, insights gained from studying transcription regulation suggest that harnessing transcriptional noise holds promise for engineering eukaryotic cells and tissues.

Tuning the hum of intrinsic noise

At the smallest regulatory scale, that of a single transcribed gene, the activity of RNA polymerases is highly variable, or 'bursty', giving rise to intrinsic 'noise' (that is, variance in gene expression) within single cells[1]. The mammalian transcription pre-initiation complex requires the assembly of over 100 components. Due to the diffusive nature of intranuclear reactions, each of these proteins may vary in concentration across space and time in a single nucleus, contributing to the intrinsic noise of gene expression. Furthermore, the formation of transcriptional condensates coincides with large transient increases in concentrations of transcription-machinery components[2,3]. Such spatial and temporal gradients in the availability of transcriptional machinery contribute to the intrinsic noise between genes in single cells. These gradients may also increase the variance in expression between alleles.

Given its nature, intrinsic noise of engineered gene circuits remains challenging to control. However, two viable control schemes suggest how intrinsic noise can be constrained by design choices to produce coordinated patterns of gene expression. Incorporating feedback loops between spatially separated circuit elements may alleviate some intrinsic noise. Alternatively, co-transcription of genes significantly reduces intrinsic noise. Because of the highly stochastic behaviour of transcription initiation, genes expressed from a single transcript display smaller variation in expression compared with independently transcribed genes[4]. Thus, co-transcriptional circuit designs may enable the coordinated expression of sets of genes required for multicellular patterning.

The rhythm of extrinsic noise

Extrinsic noise is defined as the correlated fluctuations that all genes in a cell undergo when compared with the entire cell population; it arises from fluctuations in cell resources and from divergence in transcriptional states. By understanding the sources of these fluctuations, we can design circuits that coordinate processes within this variance. The challenge of controlling extrinsic transcriptional variance is compounded by cell-cycle progression and its multiple correlated processes. Genome-wide, gene expression levels correlate with cell size and cell-cycle stage. Across the stages of the cell cycle, chromatin structure varies in packing density both locally and globally. At the largest length scales, polymer models suggest that the fractal complexity of packed chromatin drives non-linear changes in gene expression[5]. Changes in chromatin packing enhance cellular plasticity through increases in transcriptome heterogeneity. As the degree of chromatin packing varies globally with cell-cycle stage, temporally choreographed changes in chromatin density may provide an important mechanism by which the cell cycle potentiates cell-fate transitions, such as those necessary for development and regeneration. Treatments with small molecules that reduce the complexity of chromatin packing lead to decreased plasticity and better chemotherapeutic efficacy[5]. Conversely, higher variance in transcription rates correlates with greater plasticity in cellular reprogramming[6]. Synthetic sensors capable of

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identifying conditions that contribute to plasticity, such as high expression of topoisomerases, could provide feedback to entrain or amplify noise to limit or expand highly plastic cell populations.

In addition to genomic sources of noise, processes that locally modify chromatin and thus introduce additional intracellular and intercellular variability may promote cell-fate transitions. For example, DNA repair increases the intercellular variance in transcript numbers. By blocking actively transcribing polymerases, the DNA repair enzyme APEX1 increases the accumulation of DNA supercoils and bound polymerases. When repair is complete, release of the accumulated polymerases generates a large burst of transcription at recently repaired genes, resulting in a transcriptional 'tailwind'. When perturbing the global rate of DNA repair, the variance of resumed expression most significantly affects weakly transcribed genes (for example, those producing tens of transcripts)[7]. Thus, the resulting repair-mediated expression bursts may significantly affect key regulatory genes such as developmental transcription factors, which are expressed at the level of tens of transcripts.

Learning from developmental dynamics

A variety of natural cell consortia rely on constraining extrinsic and intrinsic noise to develop precise patterns. For example, proper segmentation of somites in the developing zebrafish spinal cord requires coordinated oscillating expression of the clock genes *her1* and *her7* in each cell and across the entire spinal cord. Disruptions of these oscillations result in morphological defects[8]. Within each cell, intrinsic noise must be mitigated, as normal spinal cord development requires highly correlated expression of *her1* and *her7*. This correlation is achieved through gene adjacency, which represents a common mechanism of gene co-expression in metazoans. Adjacency of genes may remove the spatial contribution to noise that induces variation in transcription bursting. Endogenously separated by only 12 kilobases, *her1* and *her7* display highly correlated transcription and expression. Whereas *her1* and *her7* expression from the same allele generates robust somite segmentation, their expression from different chromosomes results in reduced correlation and irregular somite boundaries[8]. Thus, designing circuits that coordinate the expression of multiple genes through spatial proximity may improve the precision of synthetic clocks and multicellular patterning.

Building genetically encoded synthesizers

The endogenous gene regulation mechanisms discussed above evoke methods to engineer synthetic circuits by composing regulatory elements that enhance or restrict responsiveness to intrinsic and extrinsic noise. Co-localized or co-transcribed genes provide more correlated expression at the expense of sensitivity to spatial gradients. By contrast, separating circuit elements buffers circuits from locally generated sources of intrinsic noise while simultaneously increasing variance between circuit elements. Inspired by the DNA repair-mediated 'tailwind' of transcription, synthetically introducing protein-binding sites to the DNA of actively transcribed genes may enable inducible, site-specific tuning of intrinsic noise. Cell transitions may be enhanced by timing these interventions to coincide with exit from mitosis, when cells are most vulnerable to cell-fate reset.

Extrinsic noise introduced through the phases of the cell cycle and extracellular cues induce large changes in cellular processes. Noise in these processes can be measured by synthetic reporters and translated into signals that drive cell-fate transitions. Spatiotemporal coordination of complex cell patterns such as the radial patterns that form during osteoblast regeneration will require circuits capable of rejecting extrinsic noise[9]. Alternatively, noise can be harnessed to stochastically trigger cell-fate-modifying circuits that generate distinct phenotypes within engineered cell subpopulations, mimicking the asymmetric differentiation of stem cells.

With these synthetic noise-responsive elements in hand, complex, coordinated multicellular behaviours can be engineered. Robustly coordinating synthetic oscillators will require combining intercellular feedback elements with noise-resistant elements that reject cell-cycle induced noise. Using a cell-cycle synchronized population, synthetic transcription programmes could be cyclically activated to induce spatial and temporal expression patterns. By coopting endogenous mechanisms of gene regulation, synthetic biology could construct cellular ensembles that are capable of coordinating robust, multicellular responses such as tissue regeneration and repair in mammals[10].

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Competing interests
The authors declare no competing interests.