**Gene Regulation: Stable Noise**

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Transcriptional regulation is noisy, yet despite this variability, embryonic development reproducibly generates form and function. Recent work demonstrates that patterns of transcriptional activity in embryos are stably inherited through mitosis. These observations have implications for how accuracy arises in development.

Implicit in many common descriptions of developmental regulation are assumptions that transcriptional programmes are somehow hard-wired into the mechanisms of differentiation. The alternative picture of cells flip-flopping around between gene expression states seems hostile, at least at a first glance, to building form and function in an embryo. However, when studied at the single cell level, transcription is frequently observed to be an extremely noisy process, hardly suggestive of hard-wiring. A recent study, using direct imaging of transcription dynamics in live *Drosophila* embryos, reveals the transcriptional behaviour of a developmental gene is inherited through mitosis from a cell to its descendants. These observations raise questions about how stable transcriptional behaviour can be regulated and what roles it may play in development.

The primary approach to observe real-time transcription dynamics uses a live cell RNA detection system, based upon RNA stem loops from the genomes of RNA bacteriophages [1, 2], such as MS2. Sequences encoding stem loops are placed after the promoter of interest. The repeats incorporate into nascent RNA during transcription, and are directly visualized in living cells by co-expression of the stem-loop binding proteins (for MS2 loops this is MCP) fused to GFP. For strongly expressed genes, with multiple RNA polymerases transcribing a gene at the same time**,** the RNA appears at the transcription site as a fluorescent spot (Figure 1) corresponding to a local enrichment of MCP-GFP. The dynamics of RNA production are inferred from fluctuations in spot intensity.

Ferraro et al investigated the timescale of transcriptional persistence in the early embryo of *Drosophila.* The early embryo is syncytial, with the first 13 embryonic divisions occurring without separating membranes. Divisions are synchronous, removing confounding variation in cell cycle and developmental time. MS2 loop expression was driven using a truncated enhancer of the *snail* gene. Snail is expressed ventrally in the embryo in around 1000 mesodermal precursor nuclei, and is required for mesoderm specification and cell movements during gastrulation. A small proportion (10-20%) of nuclei showed transcription spots in mitotic cycle 13 of the embryo. After following these nuclei through mitosis into cycle 14, it was clear the progeny of nuclei with spots in cycle 13 were more likely to show spots

early in cycle 14 than the progeny of non-transcribing neighbour nuclei. This initial "memory" phase persisted around 15 minutes, before the remainder of the ventral population initiated strong transcription.

**Regulation**

What causes this persistence, through cell division, of transcriptional activity? An obvious source of transcriptional regulation in development is the spatial cues that pattern the embryo. In the *Drosophila* embryo, more than any other developmental system, these cues have the reputation of being near deterministic [3]. Cells exposed to a specific set of spatial cues will divide, and in the absence of any significant cell motility, their daughters will continue to be exposed to the same signals, and so respond like their mothers.

Differential access to spatial cues could occur as a result of different cell positions relative to global activators, or stochastic local fluctuations in these activators. However, neither possibility explains the persistent transcriptional behaviour. Firstly, the study only considered ventral cells, where expression is generally strong. The difference in time-of-onset of expression between cells from expressing mothers and cells from non-expressing mothers did not depend on their relative position to the prospective furrow of mesoderm invagination. This discounts cell position relative to global signals as a source of transcriptional constancy. In addition, the difference in expression between expressing and non-expressing daughters does not depend on their distance relative to each other, as would be expected if local cues drove persistent expression. So the persistent transcriptional behaviour of the descendants of *snail*-expressing mothers does not seem to result from differential signal distributions, at least not above the length scale of a single nucleus.

These observations lead the authors to infer a stable transcription-dependent change in the nuclear state of cells, via nuclear retention of a key activator during mitosis or a change in the structure or protein composition and/or modification at the locus. Distinguishing between these possibilities seems non-trivial, and would ideally involve imaging individual regulatory factors in action (for example [4]) to address how their dynamic behaviour relates to transcription at a single locus. Following an analysis of the patterns of spot activation in reactivating cells, Ferraro et al favour a model where individual alleles suffer a transcription-dependent change in their template promoting reactivation post-mitosis. The cell cycle time at this embryonic stage is short (21 minutes at 25˚C[5]), giving little possibility for the gene to reprogram to a pre-transcriptional state before mitosis returns. The initial state could

comprise a repressive protein or modification, removed by a polymerase-coupled process, with no time for remedial action. Alternatively, the passage of polymerase may have recruited features with the potential to promote reactivation- or at least resist repression. Previous work showed that the coordination of *snail* expression required paused Pol II[6]. However, using different core promoters fused to the *sna* enhancer reveals the transcriptional persistence effect does not scale with the level of paused Pol II. In addition the presence of a TATA box in the core promoter was not required for inherited transcription. Reactivation may not be programmed in a specific manner. With the many dozens of proteins involved in a full transcription reaction, the possibilities that within a few minutes these, and the nascent RNA, will completely disperse, or that local transcription-triggered changes in nucleosome modification and organisation will have sufficient time to be reset, may be unrealistic. The slightest hint of a distinct configuration may be sufficient to nucleate the early reactivation of transcription as interphase begins.

**Function**

What value do variable transcription states inherited through mitosis have for development? Scattered or heterogeneous expression is a feature of self-organising differentiation, for example in microbial and stem cell differentiation. Here heterogeneity is proposed to act as a spontaneous source of cell diversity [7] and may also provide flexibility in cell response, with the more stochastic portfolio of gene expression allowing cells to perceive a greater range of stimuli than a coherent fully hard-wired program. "Useful" heterogeneity may also apply to pre-implantation mammalian development[8], which may be illustrative- the mammalian blastocyst has (unlike the fly) days to resolve any incoherence in pattern formation, and flexibility may be at a premium during the convolutions of implantation.

In contrast, the *Drosophila* embryo is the model of developmental precision, with extensive sub-division of body axes by multiple sharp expression boundaries in little more than 2 hours. Adding variance in gene expression would interfere with the accuracy required, and initially seems surprising given the degree to which the embryo has features to avoid such variance, such as the use of dominant maternal influences on polarity, a syncytium for spatial averaging of noise[9] and synchronous division cycles to further reduce cellular heterogeneity.

Ferraro et al estimate the early expressing nuclei synthesize about two fold more RNA than later expressing neighbours. Any resulting variability in gene expression may fall below the threshold for the embryo to have a problem. In addition, buffering may occur

transcriptionally [10], as the *snail* transgene used in this study lacked control elements present in the intact enhancer, and endogenous *snail* expression is more homogeneous[6]. Indeed, the endogenous gene may use "memory" processes simply for fast mitotic activation in all nuclei. More generally, in cycle 14, the embryo is still initially syncytial, so any expression differences may be further smoothed out through diffusion of RNA and protein[11], in addition to other post-transcriptional buffering processes[12-14]. The question emerges whether variable expression patterns are adaptive or simply neutral with regard to natural selection in otherwise precisely determined developmental processes.

It seems tenuous to propose expression heterogeneity might be important during gastrulation, although ventral furrow invagination initiates with a subpopulation of cells stochastically changing their shapes, before cell movements become more collective[15]. An emerging theme is that embryos require a degree of variable gene expression to counteract error during initial cell fate choices[16]. Although the rough positioning of differentiation might be established, fluctuations in levels of inducer, and other stochastic effects, such as the orientation of cell division, and variable cell cycle durations[17] will mean that some cells are in the wrong place for their allotted fate. Any incoherence in gene expression will allow partially committed cells to stay receptive to compensatory advice from their neighbours- although cells may have slipped into the wrong epigenetic valley, the additional variance allows exploration of paths leading to a meaningful solution. The availability of multiple RNA stem loop systems now means that the transcription dynamics of multiple genes can now be observed within single living cells[1]. The gene expression trajectories cells use to adapt to noisy influences on their differentiation can now be measured.

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**Figure 1**

**Imaging transcriptional fluctuations in living cells.**

Imaging transcriptional fluctuations using MS2 RNA stem loops. Sequences encoding MS2 loops are inserted into the transcription unit of the gene of interest and the MS2 loops are incorporated into the transcribed RNA. The newly synthesized RNA is detected as a fluorescent spot at the site of transcription, corresponding to the recruitment of MCP-GFP to MS2 loops present in the RNA. Spot intensity fluctuates over time, reflecting the changing transcript load at the gene. Image taken from reference [18].