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Gene Regulation: Analog to Digital Conversion of Transcription Factor Gradients

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Transcription factor gradients trigger differential transcriptional responses based on concentration. But how, in some cases, do target genes maintain uniform transcription across portions of the gradient? Lessons from *Drosophila* demonstrate that organization of transcription into 'hubs' can lead to local increases in transcription factor concentration.

Several developmentally important transcription factors have been found to operate in gradients across tissues. It has been demonstrated, in many developing systems, that transcription factor gradients lead to differential regulation of target genes. Cells can thus, through the intracellular transcription factor concentrations, 'read' their position within the tissue and develop accordingly. At the molecular level, the readout of transcription factor amount operates through enhancers. Enhancers bear strong or weak (high or low affinity) binding sites for several transcription factors and their target genes can be differently expressed in cells that contain high or low transcription factor amounts [1,2]. However, the continuous information provided by transcription factor concentration gradients needs to

be transformed into discrete tissue domains where a given target gene is expressed with similar efficiency across cells. This would not be possible without additional control. In a study reported in a recent issue of *Current Biology*, Yamada and colleagues [3] found that uniform expression of a target gene responsive to a transcription factor gradient may be achieved by organization of transcription into local 'hubs' within the cell nucleus. The hubs increase local transcription factor concentration even in cells with very low levels of the factor. The authors showed in the *Drosophila* embryo that the hubs are organized by a so-called pioneer transcription factor that binds many enhancers. Local interactions of graded and pioneer transcription factors enable robust and homogenous activation of target genes across tissues patterned by transcription factor gradients.

This discovery comes at the front of a wave of renewed interest in the mechanistic understanding of transcription factor action during development. The cellular blastoderm stage of *Drosophila* embryogenesis, the system used by Yamada *et al.*, has been spearheading the genetic studies of developmental pattern formation for decades. The powerful genetic approaches have been increasingly complemented by functional genomic studies, and together they have made cellular blastoderm patterning probably the most understood developmental model system. However, both genetic and genomic analyses have their limitations. Despite being able to comprehensively chart the complex networks of transcription factor regulation in this system, we know relatively little about how these regulatory processes occur on the cell biological level, in the nucleus. This gap has led to a recent wholesale shift of the developmental patterning field towards application of high-resolution imaging approaches. With imaging one can 'see' when and where a transcription factor binds an enhancer and measure the dynamics, spatial distribution and noise of such interactions that are at the heart of all gene regulatory networks. Two technological enhancements and one conceptual breakthrough have led to this paradigm shift in the transcriptional field towards

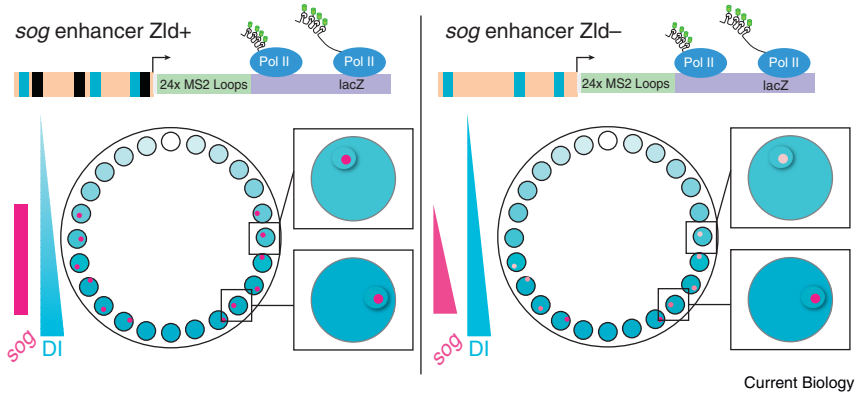

Figure 1. Local concentration of transcription factors into hubs impacts interpretation of graded positional information in the *Drosophila* embryo.
The activity of enhancers in promoting gene transcription can be visualized *in vivo* with reporters containing arrays of RNA loops bound by the MS2 RNA binding proteins fused to GFP. In the study by Yamada *et al.* [3], two versions of the *sog* enhancer with and without binding sites for the pioneer transcription factor Zelda (black boxes) were tested by imaging of the MS2 reporter in the cellular blastoderm stage of *Drosophila* embryos. With Zelda sites, graded Dorsal expression (cyan) along the dorsal ventral axis of the embryo is converted into a discrete lateral domain of *sog* reporter activity (magenta). Without Zelda sites, the *sog* reporter activity correlates with nuclear concentration of Dorsal. Colocalization analysis of Dorsal-containing nuclear hubs and reporter dots suggests that, in the presence of Zelda, Dorsal is concentrated in the hubs. This effectively flattens the gradient and converts the graded positional information into a discrete domain of transcriptional activation.

imaging. Firstly, advances in fluorescent probes and reporter biology enabled the detection of transcriptional events at the site of action in the nucleus (Figure 1), [4,5]. Secondly, advanced microscopy techniques such as lattice light sheet microscopy allowed visualization of the process of transcription and transcription factor dynamics in the nuclei of intact living organisms [6–8]. Finally, the concept of non-membrane-bound compartmentalization in cells, for example through disordered proteins undergoing liquid–liquid phase separation [9], has struck a cord with researchers looking for mechanisms that establish spatial organization within the nucleus [10–13]. The Yamada paper is a prime example of how these techniques and concepts come together and provide new insights into the extensively studied *Drosophila* blastoderm patterning model.

Yamada *et al.* focussed their analysis on the enhancer of the *short gastrulation* (*sog*) gene, which is bound by the Dorsal transcription factor [14]. Dorsal forms a nuclear concentration gradient across the blastoderm, patterning the dorsal ventral axis of the embryo [15]. *sog* is one of Dorsal's target genes and it is normally expressed in the central portion of the Dorsal gradient on the lateral side of the embryo. The *sog* enhancer contains Dorsal binding sites, but also binding sites for the ubiquitous transcription factor Zelda. Zelda is a well-established pioneer transcription factor required for zygotic genome activation in flies [16–18]. Yamada and colleagues used the *sog* enhancer to drive expression of a reporter that makes use of the aforementioned advance in reporter biology to visualize gene transcription as bright fluorescent dots in nuclei expressing *sog* (Figure 1). The appearance and disappearance of these dots was imaged live by confocal microscopy. This provided spatially resolved, quantitative data on target gene activation during the synchronous nuclear divisions — a characteristic feature of *Drosophila* pre-blastoderm development.

The authors used this system to ask an important question: how does the presence of Zelda binding sites in this enhancer influence *sog* transcription along the dorsal ventral axis? They engineered *sog* enhancers with and without Zelda binding sites upstream of the transcriptional reporter, introduced them to flies and imaged them in early embryos. To probe the spatial distribution of reporter activation along the dorsal ventral axis, they divided the *sog* expression domain into zones, according to their distance to the ventral source of the Dorsal gradient. This allowed them to

accurately quantify the effect of Zelda on enhancer-mediated transcription in ventral *versus* lateral regions of the embryo. The results revealed a very interesting phenomenon. While the wild-type enhancer was able to indiscriminately trigger *sog* reporter transcription in all Dorsal zones, a mutant enhancer with deleted Zelda binding sites failed to do so in the lateral regions of low Dorsal concentration. The effect was stronger during earlier nuclear divisions and was gradually ameliorated at later nuclear cycles. Although all nuclei eventually managed to express *sog* in the later nuclear cycles, they failed to do so earlier, indicating that the efficiency of the enhancer to trigger homogeneous *sog* transcription was compromised in the mutant enhancer. In other words, its response to the Dorsal gradient was concentration-dependent and mimicked the gradient of Dorsal morphogen itself. In contrast, the wild-type enhancer behaved in an 'all-or-nothing' fashion, where all lateral cells could trigger *sog* transcription, regardless of the exact concentration of Dorsal.

It was conceivable that although the total amount of Dorsal in nuclei is graded, locally, inside the nucleoplasm, Dorsal may concentrate around its target gene even in nuclei with very low levels of Dorsal. Since Zelda forms nuclear hubs [8,19,20], the authors asked if Zelda might help concentrate Dorsal close to the enhancer in the nuclei of lateral cells. They found that, indeed, Dorsal-containing hubs form in the vicinity of *sog* transcription foci (as well as in many other loci in the nuclei). If the enhancer contained Zelda binding sites, the local concentrations of Dorsal around the foci also remained high in lateral cells of the blastoderm. By contrast, for the enhancer without Zelda binding sites, the local concentrations of Dorsal around the foci decreased in lateral cells. This correlates with the delays in output of the *sog* transcriptional reporter revealed by live imaging and suggests that Zelda may act by creating an environment where Dorsal concentrates. Since Zelda is one of the most intrinsically disordered proteins encoded by the fly genome, it will be interesting to investigate whether Zelda phase separation plays a role in the formation of these hubs.

The conclusion of this study is strong and beautifully simple. If you have a gene regulated by a transcription factor that operates as a gradient and you want to prevent the gradient from influencing the gene's activation strength or homogeneity of transcription across cells, it is a good idea to add binding sites for a second, ubiquitous pioneer transcription factor to the gene's enhancer. This will help increase the local concentration of the specific transcription factor and thus ameliorate the effect of the concentration decrease along the gradient. Fundamentally, any morphogen gradient operates by converting the analog gradient information into the digital domain of target gene expression. The organization of transcription factors into nuclear hubs, able to exploit the physico-chemical properties of participating proteins to adjust local concentrations, may be a powerful mechanism to convert the continuous concentration information provided by graded transcription factors into discrete transcriptional outputs.

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