Summary

Morphogen gradients provide concentration-dependent positional information along polarity axes. Although the dynamics of the establishment of these gradients is well described, precision and noise in the downstream activation processes remain elusive. A simple paradigm to address these questions is the Bicoid morphogen gradient that elicits a rapid step-like transcriptional response in young fruit fly embryos. Focusing on the expression of the major Bicoid target, hunchback (hb), at the onset of zygotic transcription, we used the MS2-MCP approach which combines fluorescent labeling of nascent mRNA with live imaging at high spatial and temporal resolution. Removing 36 putative Zelda binding sites unexpectedly present in the original MS2 reporter, we show that the 750 bp of the hb promoter are sufficient to recapitulate endogenous expression at the onset of zygotic transcription. After each mitosis, in the anterior, expression is turned on to rapidly reach a plateau with all nuclei expressing the reporter. Consistent with a Bicoid dose-dependent activation process, the time period required to reach the plateau increases with the distance to the anterior pole. Despite the challenge imposed by frequent mitoses and high nuclei-to-nuclei variability in transcription kinetics, it only takes 3 minutes at each interphase for the MS2 reporter loci to distinguish subtle differences in Bicoid concentration and establish a steadily positioned and steep (Hill coefficient ~ 7) expression boundary. Modeling based on the cooperativity between the 6 known Bicoid binding sites in the hb promoter region, assuming rate limiting concentrations of the Bicoid transcription factor at the boundary, is able to capture the observed dynamics of pattern establishment but not the steepness of the boundary. This suggests that a simple model based only on the cooperative binding of Bicoid is not sufficient to describe the spatiotemporal dynamics of early hb expression.
early development, is semiautomatic, and requires minimal user intervention. It also includes a tool to combine data from multiple movies and visualize several features of the intensity traces and the expression pattern.

Carmina Angelica Perez-Romero, Huy Tran, Mathieu Coppey, Aleksandra M Walczak, Cécile Fradin, Nathalie Dostatni (2018 Oct 17)
**Live Imaging of mRNA Transcription in Drosophila Embryos.**  

**Summary**

Live imaging has been used in recent years for the understanding of dynamic processes in biology, such as embryo development. This was made possible by a combination of advancements in microscopy, leading to improved signal-to-noise ratios and better spatial and temporal resolutions, and by the development of new fluorescence markers, allowing for the quantification of protein expression and transcriptional dynamics in vivo. Here we describe a general protocol, which can be used in standard confocal microscopes to image early Drosophila melanogaster embryos, in order to learn about the transcriptional dynamics of a fluorescently labeled RNA.

Huy Tran, Jonathan Desponds, Carmina Angelica Perez Romero, Mathieu Coppey, Cecile Fradin, Nathalie Dostatni, Aleksandra M Walczak (2018 Oct 12)
**Precision in a rush: Trade-offs between reproducibility and steepness of the hunchback expression pattern.**  
*PLoS computational biology* : e1006513 : [DOI : 10.1371/journal.pcbi.1006513](https://doi.org/10.1371/journal.pcbi.1006513)

**Summary**

Fly development amazes us by the precision and reproducibility of gene expression, especially since the initial expression patterns are established during very short nuclear cycles. Recent live imaging of hunchback promoter dynamics shows a stable steep binary expression pattern established within the three minute interphase of nuclear cycle 11. Considering expression models of different complexity, we explore the trade-off between the ability of a regulatory system to produce a steep boundary and minimize expression variability between different nuclei. We show how a limited readout time imposed by short developmental cycles affects the gene’s ability to read positional information along the embryo’s anterior posterior axis and express reliably. Comparing our theoretical results to real-time monitoring of the hunchback transcription dynamics in live flies, we discuss possible regulatory strategies, suggesting an important role for additional binding sites, gradients or non-equilibrium binding and modified transcription factor search strategies.

Marie Clémot, Anahi Molla-Herman, Juliette Mathieu, Jean-René Huynh, Nathalie Dostatni (2018)
The replicative histone chaperone CAF1 is essential for the maintenance of identity and genome integrity in adult stem cells.

Development (Cambridge, England) : DOI : dev161190

Summary

Chromatin packaging and modifications are important to define the identity of stem cells. How chromatin properties are retained over multiple cycles of stem cell replication, while generating differentiating progeny at the same time, remains a challenging question. The chromatin assembly factor CAF1 is a conserved histone chaperone, which assembles histones H3 and H4 onto newly synthesized DNA during replication and repair. Here, we have investigated the role of CAF1 in the maintenance of germline stem cells (GSCs) in ovaries. We depleted P180, the large subunit of CAF1, in germ cells and found that it was required in GSCs to maintain their identity. In the absence of P180, GSCs still harbor stem cell properties but concomitantly express markers of differentiation. In addition, P180-depleted germ cells exhibit elevated levels of DNA damage and de-repression of the transposable I element. These DNA damages activate p53- and Chk2-dependent checkpoints pathways, leading to cell death and female sterility. Altogether, our work demonstrates that chromatin dynamics mediated by CAF1 play an important role in both the regulation of stem cell identity and genome integrity.

Year of publication 2016


PLoS computational biology : e1005256 : DOI : 10.1371/journal.pcbi.1005256

Summary

The simultaneous expression of the hunchback gene in the numerous nuclei of the developing fly embryo gives us a unique opportunity to study how transcription is regulated in living organisms. A recently developed MS2-MCP technique for imaging nascent messenger RNA in living Drosophila embryos allows us to quantify the dynamics of the developmental transcription process. The initial measurement of the morphogens by the hunchback promoter takes place during very short cell cycles, not only giving each nucleus little time for a precise readout, but also resulting in short time traces of transcription. Additionally, the relationship between the measured signal and the promoter state depends on the molecular design of the reporting probe. We develop an analysis approach based on tailor made autocorrelation functions that overcomes the short trace problems and quantifies the dynamics of transcription initiation. Based on live imaging data, we identify signatures of bursty transcription initiation from the hunchback promoter. We show that the precision of the expression of the hunchback gene to measure its position along the anterior-posterior
axis is low both at the boundary and in the anterior even at cycle 13, suggesting additional post-transcriptional averaging mechanisms to provide the precision observed in fixed embryos.

Year of publication 2015


**Transcriptional Memory in the Drosophila Embryo.**

*Current biology : CB* : DOI : 10.1016/j.cub.2015.11.058

**Summary**

Transmission of active transcriptional states from mother to daughter cells has the potential to foster precision in the gene expression programs underlying development. Such transcriptional memory has been specifically proposed to promote rapid reactivation of complex gene expression profiles after successive mitoses in Drosophila development [1]. By monitoring transcription in living Drosophila embryos, we provide the first evidence for transcriptional memory in animal development. We specifically monitored the activities of stochastically expressed transgenes in order to distinguish active and inactive mother cells and the behaviors of their daughter nuclei after mitosis. Quantitative analyses reveal that there is a 4-fold higher probability for rapid reactivation after mitosis when the mother experienced transcription. Moreover, memory nuclei activate transcription twice as fast as neighboring inactive mothers, thus leading to augmented levels of gene expression. We propose that transcriptional memory is a mechanism of precision, which helps coordinate gene activity during embryogenesis.

Year of publication 2013

Tanguy Lucas, Teresa Ferraro, Baptiste Roelens, Jose De Las Heras Chanes, Aleksandra M Walczak, Mathieu Coppey, Nathalie Dostatni (2013 Jul 26)

**Live imaging of bicoid-dependent transcription in Drosophila embryos.**

*Current biology : CB* : 2135-9 : DOI : 10.1016/j.cub.2013.08.053

**Summary**

The early Drosophila embryo is an ideal model to understand the transcriptional regulation of well-defined patterns of gene expression in a developing organism. In this system, snapshots of transcription measurements obtained by RNA FISH on fixed samples cannot provide the temporal resolution needed to distinguish spatial heterogeneity from inherent noise. Here, we used the MS2-MCP system to visualize in living embryos nascent transcripts expressed from the canonical hunchback (hb) promoter under the control of Bicoid (Bcd). The hb-MS2 reporter is expressed as synchronously as endogenous hb in the anterior half of the embryo, but unlike hb it is also active in the posterior, though more heterogeneously and more
transiently than in the anterior. The length and intensity of active transcription periods in the anterior are strongly reduced in absence of Bcd, whereas posterior ones are mostly Bcd independent. This posterior noisy signal decreases progressively through nuclear divisions, so that the MS2 reporter expression mimics the known anterior hb pattern at cellularization. We propose that the establishment of the hb pattern relies on Bcd-dependent lengthening of transcriptional activity periods in the anterior and may require two distinct repression mechanisms in the posterior.

Year of publication 2010

Aude Porcher, Asmahan Abu-Arish, Sébastien Huart, Baptiste Roelens, Cécile Fradin, Nathalie Dostatni (2010 Jul 29)

The time to measure positional information: maternal hunchback is required for the synchrony of the Bicoid transcriptional response at the onset of zygotic transcription.


Summary

It is widely accepted that morphogenetic gradients determine cell identity by concentration-dependent activation of target genes. How precise is each step in the gene expression process that acts downstream of morphogens, however, remains unclear. The Bicoid morphogen is a transcription factor directly activating its target genes and provides thus a simple system to address this issue in a quantitative manner. Recent studies indicate that the Bicoid gradient is precisely established in Drosophila embryos after eight nuclear divisions (cycle 9) and that target protein expression is specified five divisions later (cycle 14), with a precision that corresponds to a relative difference of Bicoid concentration of 10%. To understand how such precision was achieved, we directly analyzed nascent transcripts of the hunchback target gene at their site of synthesis. Most anterior nuclei in cycle 11 interphasic embryos exhibit efficient biallelic transcription of hunchback and this synchronous expression is specified within a 10% difference of Bicoid concentration. The fast diffusion of Bcd-EGFP (7.7 mum(2)/s) that we captured by fluorescent correlation spectroscopy in the nucleus is consistent with this robust expression at cycle 11. However, given the interruption of transcription during mitosis, it remains too slow to be consistent with precise de novo reading of Bicoid concentration at each interphase, suggesting the existence of a memorization process that recalls this information from earlier cycles. The two anterior maternal morphogens, Bicoid and Hunchback, contribute differently to this early response: whereas Bicoid provides dose-dependent positional information along the axis, maternal Hunchback is required for the synchrony of the response and is therefore likely to be involved in this memorization process.

Aude Porcher, Nathalie Dostatni (2010 Mar 12)

The bicoid morphogen system.

Summary

Several fundamental concepts of developmental biology have emerged from studies on the early development of the Drosophila melanogaster embryo. In the late 1980s, studies on Bicoid provided the first solid experimental evidence for the existence of morphogenetic gradients and their implication in axial patterning. Bicoid has since stimulated further research, bringing together developmental and cell biologists, physicists and theoreticians to address fundamental biological questions. These include mechanistic aspects of transcriptional and translational control, molecular and functional aspects of evolution and, more recently with the development of quantitative approaches, the robustness of axial patterning in a systems biology view. However, recent studies provide data which lead to contradictory interpretations. Here, we discuss these recent observations, highlighting the data helping to understand how anterior patterning is achieved under the control of Bicoid and point to novel challenges for future studies.