Animal development is orchestrated by spatio-temporal gene expression programmes that drive precise lineage commitment, proliferation and migration events at the single-cell level, collectively leading to large-scale morphological change and functional specification in the whole organism. Efforts over decades have uncovered two ‘seemingly contradictory’ mechanisms in gene regulation governing these intricate processes: (i) stochasticity at individual gene regulatory steps in single cells and (ii) highly coordinated gene expression dynamics in the embryo. Here we discuss how these two layers of regulation arise from the molecular and the systems level, and how they might interplay to determine cell fate and to control the complex body plan. 

We also review recent technological advancements that enable quantitative analysis of gene regulation dynamics at single-cell, single-molecule resolution. These approaches outline next-generation experiments to decipher general principles bridging gaps between molecular dynamics in single cells and robust gene regulations in the embryo.

1. Introduction

The development of a multicellular organism from a zygote takes place following a well-defined genetic blueprint. In the past decades, extensive studies based on a combination of biochemical, cell biological, genetic and genomic approaches have systemically characterized genetic players controlling development [1–3]. Collectively, these studies have depicted a static gene regulatory network (GRN) that governs diverse cellular behaviours such as cell proliferation, cell fate determination and morphological movements during animal development [4–7]. However, these conventional cell population-based endpoint assays were unable to reveal the spatio-temporal dynamics or the three-dimensional (3D) architecture of molecular systems operating in single live cells that eventually give rise to precise gene regulation during embryogenesis. Recent development of a set of advanced imaging tools for single-cell, single-molecule analysis have opened up exciting new opportunities to address these questions [8–11]. Here, we will first discuss the molecular origin of gene expression stochasticity and dynamics, and how these properties are harnessed at the systems level to control distinct cellular functions and developmental events. We will also review recent technological advances and pinpoint emerging directions for applying these new methods to decode gene regulation at different levels.

2. Stochasticity in gene regulation

Fundamental gene regulation steps such as transcription and translation are inherently stochastic processes. The stochasticity originates from intrinsic randomness of molecular dynamics and interactions in a living cell. The inherent stochasticity is largely averaged out by an increased number of mRNA or protein molecules within the reactant pool; nonetheless, under many circumstances it could propagate through the GRN and influence the functioning of genetic circuits, sometimes leading to the variability of cellular phenotypes during developmental processes.
2.1. Transcriptional and translational bursting

As the first step of gene regulation, transcription is shown to be a highly dynamic and stochastic event. The first evidence came from single-molecule fluorescent in situ hybridization (smFISH) experiments. smFISH allows quantification of mRNA molecules within a single cell, revealing extensive variation of mRNA copy numbers in individual cells both in culture and in tissues [12–14]. By using an MS2 or PP7 live-cell RNA labelling system, it is feasible to image transcription at single-molecule resolution and in real time [15,16]. These experiments uncover drastic characteristics of eukaryotic transcription whereby the production of new mRNA molecules from a gene occurs in a bursting manner (figure 1a) [19,20]. The bursting kinetics can be roughly described by two parameters—bursting size and frequency. The statistics of these two parameters are speculated to be regulated by different components of the transcription machinery such as transcription factor concentration, enhancer–promoter architecture, epigenetic environment, gene positioning and chromatin remodelling [21]. Most developmental regulators are thought to be transcribed in bursting kinetics [22]. Using the MS2 system, both Garcia et al. [23] and Lucas et al. [24] investigated the activation of the gap gene Hunchback by the gradient of the Bicoid protein in Drosophila embryo. They have both identified strongly induced bursting in the cells at the anterior pole but quite stochastic switching in those cells at the posterior pole [23,24]. In addition, Nanog, which safeguards embryonic stem (ES) cell ground state, shows drastic transcription bursting kinetics in mouse ES cells [25]. More interestingly, the frequency and duration of Nanog transcription bursts can be altered by switching cells from serum to 2i culture condition.

Protein translation from mRNAs also occurs in a bursting fashion. Several independent studies have recently demonstrated imaging of this fundamental biological process at the single-mRNA level in mammalian cells (figure 1b) [17,26–29]. Specifically, they labelled the mRNA transcript by using an MS2 or PP7 system and in parallel engineered the transcript to express a set of ‘SunTag’ [30] or ‘spaghetti-monster’ epitopes [31] that recruit multiple copies of fluorescent protein to monitor the production of the nascent protein. An interesting consensus from these studies is that different mRNA molecules within the same cell are translated at heterogeneous rates, suggesting that, at the single mRNA level, translation is a stochastic process.

2.2. Molecular dynamics underlying stochasticity

The stochasticity of gene expression originates from intrinsic randomness of molecular dynamics in living cells. Boiled down to the bottom, biochemical reactions involved in gene regulation, such as transcription, translation, epigenetic regulation and protein degradation, are all driven by a complex cascade of dynamic molecular interactions at the single-molecule level that usually involve multistep complex assembly and extensive interactions between protein...
and nucleic acids. Traditional experimental approaches for studying gene regulation mostly rely on measuring average mRNA or protein concentrations from a mixed cell population at a given time point, thereby lacking the ability to probe these dynamic events in living cells with high spatio-temporal resolution [32,33]. Rapid development of live-cell labelling chemistry and fast high-resolution imaging platforms provide unique opportunities to elucidate the physical reality of biochemical reactions in living cells [34–36]. Gebhardt et al. developed reflected light-sheet microscopy and firstly achieved for fine-tuning the rates of TF complex assembly at subnuclear environments and serve as an important mechan-

-isms to generate and shape gene expression dynamics and stochasticity. With the rapid development of next-generation multimodal imaging modalities and new labelling strategies, it is important to further investigate how the three-dimensional genome organization specifically influences gene activity, and what gene products and mechanisms underlie the formation of these functional compartments in the nucleus.

3. Coupling stochasticity and dynamics in gene expression

The stochastic effects from fundamental steps of gene regulation will impinge and add onto the dynamic pattern of gene expression, resulting in temporal variation with stochastic fluctuations. The stochastic fluctuation, termed ‘gene noise’, occurs universally in microbial, single-cell eukaryotes and multicellular organisms that have developmental processes.

3.1. Gene expression noise originates from bursting

How does bursting contribute to gene expression noise? Pedraza & Paulsson presented a theoretical framework of the general quantitative relationship between bursting and gene expression noise [44]. Their analysis suggests that the random signals generated in one gene regulatory step have a determin-

-istic effect on the following steps. Mathematically, the stochasticity originated from each regulatory step propagates through the GRN and is dampened by a coarse-grained time-averaging factor. This analysis together with a number of following studies pinpoint three important properties of dynamic and stochastic gene expression [14,21,33]: (i) gene expression noise originates from intrinsic stochasticity of regulatory steps such as transcriptional and translational bursts; (ii) noise can propagate through the GRN and spread to connected regulatory steps; and (iii) noise is averaged out after each signal amplification step by increasingly higher copy numbers of reactants, such as mRNA and protein molecules.

3.2. Gene regulatory network structure modulates gene expression noise

Gene expression noise is regulated by GRN structure (figure 2). For example, the negative feedback loop can potently dampen gene expression noise, which might be necessary for precise
This was first demonstrated by a synthetic auto-inhibitory reporter [45], but was later shown to exist in a variety of naturally evolved systems. One example is a developmental patterning gene *snail* in *Drosophila* embryo, which negatively autoregulates its own promoter and thereby maintains stable gene expression upon induction [56]. On the contrary, positive autoregulation amplifies noise [57,58]. Besides feedback loops by transcription control, microRNA-mediated incoherent feedforward loop has the capability of effectively reducing noise in parallel with fine-tuning protein levels [59]. Interestingly, theoretical analysis predicted that the noise buffering function depends on the inhibitory strength of microRNA and a moderate strength has optimal noise-reducing ability, yet these predictions remain to be validated experimentally.

Gene expression noise could also be beneficial under certain circumstances and thus does not always have to be suppressed. A recent study on NF-κB dynamics uncovered that the intrinsic noise within the GRN enhances the robustness of NF-κB oscillation in response to periodic tumour necrosis factor (TNF) signals. Specifically, the frequency variations of the NF-κB oscillation poise the cell population to respond to a broad range of dynamic stimuli, leading to efficient gene expression in dynamically changing environments [60].

### Figure 2.

List of known GRNs with topology, dynamics, functions and examples. GRNs with defined topological structures are able to generate specific patterns of gene expression dynamics, including bistability, oscillation, adaptive response, noise regulation and diffusion in three-dimensional space, corresponding to different mechanisms for controlling cellular functions, cell fate decision and developmental patterning. References for examples: noise reduction [45]; toggle switch [46]; bistable switch and timing control [32]; damped oscillation [47,48]; robust oscillation [49]; adaptive response [50]; Turing model [51,52]; mutual inactivation [53].

| GRN topology | dynamics | function | example |
|--------------|----------|----------|---------|
|              |          | noise reduction | *snail* autoregulation in *Drosophila* embryo |
|              |          | toggle switch | PU.1-GATA1 in erythroid or myeloid differentiation |
| fast titration |          | bistable switch and timing control | RB/E2F network in control of cell cycle entry |
| delay        |          | damped oscillation | p53-Mdm2 signalling |
| activity     |          | limit cycle robust oscillation | CDK1-Plk1-APC signalling cascade |
| activity     |          | adaptive response | EGF-induced ERK activity |
| 3D diffusion |          | Turing model pattern formation of rings and stripes | Wnt-Dkk signalling nodal-lefty signalling |
| between cells |          | pattern formation mutual inactivation | Notch-Delta signalling |
3.3. Other factors that regulate gene expression noise

In parallel to GRN-mediated regulations, cells have evolved other mechanisms to control gene expression noise. For example, epigenetic modifications regulate noise through direct modulation of transcriptional bursting. Specifically, DNA methylation and histone deacetylation have been demonstrated to act as noise repressor, whereas histone acetylation does the opposite [61,62]. Interestingly, cellular compartmentalization was recently shown to function as a transcriptional noise filter through spatial partitioning of molecules in and out of the nucleus [63,64]. The nuclear retention of most transcripts is about twenty minutes, which is a similar time to scale transcription bursting or mRNA degradation and thus can efficiently average out the stochasticity of mRNA concentration by a factor of three to four [64]. The cost of this passive filtering is the loss of specificity in the spatial and temporal domains, yet it provides a general means of noise reduction for all the transcripts produced. Moreover, this interesting mechanism might have general implications in the evolutionary advantage of sophisticated cellular organizations in higher eukaryotes.

4. Gene expression dynamics at the systems level

Countless gene regulatory events take place within a single mammalian cell, driving dynamic expression of thousands of genes. The products of these genes functionally interact with each other in real time via feedback loops (figure 2), forming a number of interconnected circuits within the whole GRN network [3,65].

4.1. Gene regulatory network structure determines gene expression dynamics

From a physicist’s point of view, if we know the kinetic parameters describing each regulatory event and the topological structure of the GRN, we should be able to formulate a set of differential equations to quantitatively describe the expression dynamics of any gene within the network. The variable for each equation corresponds to the expression of a particular gene as the function of time. The solution for each equation describes the dynamic motion of the entire system. Depending on the parameter space, dynamic systems formulated by differential equations are able to generate diverse and interesting behaviours such as adaptive response, pulse, bistability, oscillation and chaos (figure 2) [49,50,66]. Meanwhile, the ‘one-to-one’ relationship between equations and solutions suggests that understanding GRN functionality requires precise measurement of gene expression over time.

4.2. Gene regulatory network shapes temporal dynamics

The relationship between gene expression dynamics and the GRN topological structure has been studied for over a decade by both ‘top-down’ network analysis and ‘bottom-up’ network engineering [3,65,67]. One emerging property in the GRN is the prevalence of bistability in the system (figure 2). Generally speaking, bistability means that the output of a system has two stable equilibrium states. Bistability is critical for biological systems, because it is required for generating digital and switch-like behaviours such as cell fate determination. Gardner et al. first built up a simple gene circuit comprising mutual transcriptional repression and showed that this genetic toggle switch can robustly generate bistability in gene expression [46]. Bistability also exists in natural systems. For example, the autoregulation of E2F transcriptional factor coupled with its ultrafast sequestration by the Rb protein generates bistability, dictating the switch of cell cycle between quiescence and proliferation [32].

Another dynamic feature in the GRN is oscillation, which is important for regulating periodic cellular processes such as cell cycle and circadian rhythm [49,68–70]. Although a two-node module, such as mutual repression with time delay, is able to generate damped oscillation, a three-node module is the minimum set-up for robust oscillation cycles (figure 2) [49]. Rust et al. probed the origin of circadian oscillation in cyanobacteria, and found that a small network comprising coupled positive and negative feedback loops maintains synchronized oscillation per day for several weeks [70]. Interestingly, several oscillation circuits can be coupled with each other to execute more complex regulations [71]. The GRNs governing mammalian circadian oscillation and the cell cycle could be even more intricate in terms of the number of genes and the degree of feedback/feedforward loops involved.

In higher eukaryotes, the integration of epigenetic regulations into the GRN provides more controls to gene expression dynamics. For example, Bintu et al. found that distinct types of epigenetic modifications, such as DNA methylation, histone deacetylation and histone methylation, have different effects in shaping gene expression dynamics [72]. Specifically, although all these modifications lead to transcriptional repression, they work at different time scales and thereby generate distinct temporal kinetics of epigenetic memory.

4.3. Gene regulatory network orchestrates spatio-temporal dynamics

The cell positional information has to be taken into account as a parameter for modelling gene expression during animal development, particularly for the case dealing with spatio-temporal distribution of morphogens. In this scenario, the dynamic evolution of a system can be formulated by a set of partial differential equations, which describe the variation of morphogens. The GRNs represented by partial differential equations are capable of generating molecular gradients within three-dimensional space (figure 2). For example, Cao et al. explored a synthetic circuit that forms self-organized core-ring patterns and showed that the ring width scales with the colony size, suggesting a self-controlled scaling mechanism dictated by the GRN structure [73]. In cultured mammalian cells, Sorre et al. monitored the expression dynamics of Smad4 protein as well as the transcriptional activity of Smad3 at the single-cell level to investigate the response of the GRN to different types of ligand stimuli [74]. Their results suggested that a TGF-β-mediated GRN responds to ligand stimuli in an adaptive mode. Specifically, high-frequency pulsed stimulations result in higher output than that from a mono-phase, sustained input, which serves as a mechanism for accelerating cell fate decision by morphogen gradients.
5. Shaping developmental processes by stochastic and dynamic gene expression

Animal development is a highly regulated spatio-temporal process in which cells undergo lineage commitment, proliferation and migration, giving rise to patterned tissues and organs. The amplification of cell number is accompanied by increasingly more complex organization and patterning of different cell lineages. Although the whole developmental process is regulated by numerous genes, a specific event may only heavily rely on a couple of regulators, consistent with a hierarchical topology of the developmental GRN [1,75].

5.1. Cell fate plasticity

The intrinsic stochasticity of gene regulatory events results in heterogeneous gene expression in single cells. This is not always deleterious and could be very useful for generating a repertoire of cells with plastic identities. The cell fate plasticity has been extensively studied by emerging single-cell transcriptional profiling technology (reviewed in [76–78]). By using a single-cell droplet-barcoding RNA sequencing approach, Klein et al. revealed that when the leukaemia inhibitory factor (LIF) was withdrawn from the culture medium, ES cells follow a ‘bet-hedging’ strategy that can prime pluripotent or differentiated states. This study exemplifies an avenue for studying similar types of questions.

5.2. Cell fate decision

Although noise generates cell fate plasticity within a cell population, a committed cell has one unique trajectory for gene expression and differentiation. Therefore, measuring expression dynamics of master cell fate regulators (such as TFs) would provide valuable information regarding how the GRN regulates cell fate decision. For example, combining network analysis and the measurement of E2F transcription dynamics at the single-cell level, Dong et al. uncovered that the network structure modulates E2F dynamics to generate bistability for coordinating two distinct functions—the control of the probability of cell-cycle entry by Myc and the control of cell-cycle length by G1 cyclin/CDKs (figure 2) [32]. This research highlights the fact that a naturally evolved system might have complex GRN structure to achieve multitask control of cellular processes. On the other hand, this study also addressed the question about how cell-cycle length is controlled, opening new opportunities for studying the relationship between cell cycle and lineage commitment.

One hallmark of cell differentiation is the increase of cell-cycle length, in particular the G1 phase [83,84]. For example, in the mouse nervous system, cell-cycle length in the ventricular zone increases from 8 h at the onset of neurogenesis to up to 18 h at the end [85]. Conversely, overexpression of G1 cyclin/CDKs or loss of CDK inhibitors, such as p27, shortens cell-cycle pace and impairs neurogenesis [86–88]. These findings suggest that the complex cell fate decision during differentiation is likely to be a synergistic effect from a GRN controlled by master cell-fate and cell-cycle regulators. As a result, measurement of the dynamics of lineage-specific TFs at different cell-cycle stages might provide key insight into understanding the cell fate decision process. Indeed, Kueh et al. measured the expression dynamics of PU.1 during myeloid differentiation and found that, in individual cells, the accumulation rate of this central regulator remains constant, while its final concentration varies according to the cell-cycle length [89]. Mathematical modelling revealed that the overall GRN comprising PU.1 and the cell-cycle network allows the system to switch between one undifferentiated state and two alternative differentiated states. This study exemplifies an avenue for studying similar types of questions.

5.3. Developmental patterning

GRNs that regulate morphogenesis have much more complex structures, which include not only regulators of cell division and fate but also components governing intercellular communication, such as Wnt, TGF-β superfamily, Notch, FGF and Hedgehog pathways [2,3,90]. The interconnected network topology are able to coordinate dynamic expression of a cohort of intracellular proteins, as well as some small secretory ligands that diffuse within three-dimensional space to control the patterning of different tissues. For example, during Drosophila embryo development, the Bicoid protein molecules are synthesized at the anterior pole and diffuse along the embryo axis, forming an exponentially decreased gradient [91]. This gradient then triggers compartmentalization for four target gap genes that establish the initial body segmentation. Although the Bicoid gradient was demonstrated to be essential, one important unsettled issue in this field is whether the Bicoid gradient alone is able to generate the pattern with such stunning precision, or whether it requires additional regulations, regarding the inevitable stochasticity in gene expression. Through mathematical modelling of the GRN, Manu et al. suggested that the cross-talk among gap genes is sufficient to reduce the patterning variance, though this prediction needs to be validated by more experiments [92,93].

Apart from morphogen gradient-dependent control, a few patterns such as rings or stripes can also be shaped within three-dimensional space by specific GRN structures. One well-studied example is the Turing model [94,95]. This model considers a reaction–diffusion mechanism in a simple GRN of two nodes—A and B. A diffuses slowly and produces B, whereas B diffuses rapidly but inhibits A (figure 2). Thereby, as the process initializes, the fast-diffusing B suppresses A at its surrounding regions, resulting in the spontaneous formation of rings and stripes along the diffusion axis. The Turing model-defined structure has been identified in many developmental GRNs. For example, during murine hair follicle formation, the Wnt ligand was shown to serve as a short-range
activator and the Dkk protein as a long-range inhibitor. The
dynamic interplay between this pair of genes thus fits the
Turing model and was indicated to determine the hair follicle
spacing [51]. Another example is in mesendoderm formation,
where a module comprising Nodal and its antagonist Lefty
was speculated to function in a similar way to define the
mesendoderm territory and to prevent its expansion into the
ectoderm [2,52].

Differing from the reaction–diffusion Turing mechanism,
the Notch-Delta GRN regulates multicellular patterning
via direct cell to cell contacts [53,96]. Specifically, the Delta
ligand trans-activates Notch in neighbouring cells, while
cis-inhibiting Notch in its own cells through different config-
urations of molecular interactions. Mathematical modelling
of the GRN suggested that the network is able to generate
mutual-inactivation dynamics between Notch and Delta in
the same cell, leading to an ultrasensitive switch between
mutually exclusive signal sending (high Delta/low Notch) and
receiving (low Delta/high Notch) states [53]. This
mutual inactivation can amplify small differences in ligand
concentration among neighbouring cells and facilitate cell
fate decision and pattern formation.

6. Advances in imaging technology for
probing dynamic and stochastic gene
expression

6.1. Imaging molecular dynamics in gene regulation

Modelling and analysing different GRNs underly ing develop-
mental control are limited by the precision of constructed
transfer functions which describe the input–output relation-
ship of fundamental gene regulatory steps, such as
transcription and translation. However, great challenges exist
for increasing precision, because every step dictates a complex
cascade of dynamic molecular interactions. For example, a
single transcription step includes the binding of TFs onto
enhancers or promoters, the assembly of pre-initiation com-
plex, elongation and termination in parallel with chromatin
remodelling events such as nucleosome remodelling and
epigentic modifications [97]. Moreover, components in this
machinery work with high-order cooperativities, bringing in
more complexity for determining the physical nature of these
biochemical reactions [98]. Conventional approaches such as
biochemistry and structural biology provide little information
about in vivo kinetics and stochasticity, and thus cannot resolve
this layer of regulation, whereas imaging provides a unique
opportunity by directly observing these dynamic processes
in real time. Recent improvements in chemical dyes and
fast high-resolution imaging platforms have allowed the
direct labelling of single molecules and the tracking of their
binding, dissociation and diffusion dynamics in live cells
[11,34,99,100]. For example, we can directly image the binding
of a TF or other DNA-binding protein at its genomic
cis-regulatory elements and calculate its resident time [8,101].

Moreover, improved aberration-corrected multi-focus micro-
scopy (MFM) can generate an instant stack of images from
nine focal planes, opening an avenue for high-resolution
three-dimensional imaging of single-molecule dynamics
in real time [36]. Importantly, the newly developed state-
of-the-art lattice light sheet scope enables the painting of a
three-dimensional molecular interaction density map of TF
within a single cell (figure 3a) [18,35]. These emerging
techniques for non-invasive high-resolution imaging thereby
provide us with effective tools for accurately measuring
spatio-temporal dynamics of molecular systems in live cells
at the single-molecule level.

6.2. Imaging gene expression heterogeneity within cell
population

Several imaging techniques have been developed recently for
measuring gene expression heterogeneity within a hetero-
genous cell population or a tissue. One promising approach
is smFISH, which can quantitatively determine the number of
RNA molecules in individual cells. This approach has been con-
tinuously optimized with probe barcoding and sequential
hybridization to enable multiplexed quantitative profiling of
hundreds of genes at the single-cell level (figure 3b) [13,102].
It has been applied for analysing embryonic stem cell pool
as well as three-dimensional mouse hippocampus, revealing
novel cell identities within different regions of the tissue
[62,104]. Recently, this technique was improved for cell lineage
tracing. The principle is to create barcoded recording sequences
that can be integrated into the genome and altered by CRISPR/
Cas9-targeting mutagenesis during cell division. Finally, the
lineage information will be read out from altered recording
sequences through multiplexed RNA smFISH [105]. However,
the smFISH experiment can only be performed on fixed
samples, limiting its ability for resolving the information of
gene expression at the temporal scale.

6.3. Imaging gene expression dynamics at the single-
cell level

At the systems level, monitoring gene expression dynamics
over time at single-cell resolution is critical to understanding
the functionality of the GRN. One common strategy to achieve this goal is via long-term imaging
of cultured live cells with integrated fluorescence biosensors
[106]. Recent technological advances from several aspects
have greatly expanded the application of this approach, in par-
ticular for mammalian cells. The first advance comes from the
design and generation of biosensors. Depending on the layer of
dynamics chosen for monitoring, a cassette of fluorescence
protein coding sequence can be placed downstream from pro-
moters for detecting transcriptional dynamics, or directly fused
to the protein of interest for capturing protein concentration
dynamics [107]. In other cases, fluorescence resonance energy
transfer (FRET) biosensors with phosphorylation-responsive
elements have been used for probing the activity of protein
kinases [108]. A remarkable improvement was made by
Regot et al., who engineered the responsive domain of kinase
substrate to convert phosphorylation into localization changes,
providing a general approach for rapidly generating reporters
for protein kinase activities [109]. Moreover, the development
of revolutionary CRISPR/Cas9 genome editing tools have
enabled efficient construction of reporter cell lines with
knock-in alleles that can faithfully reflect gene regulation
within the native chromatin [110,111]. The second advance
comes from the improvement of imaging platforms. Although
the wide-field epi-illumination scope is still sufficient for
recording intensity-based dynamic signals, more advanced
platforms such as the wide-pinhole confocal microscope, light-sheet microscope, two-photon microscope and adaptive optics are available for imaging more challenging samples and can generate images with increased resolution and volumes [106,112]. One of the breakthroughs in live imaging during the past decade is the development of state-of-the-art light-sheet microscopy platforms for imaging the developmental process of whole embryos at high spatio-temporal resolution for long periods of time (figure 3c) [10,100,113].

One emerging challenge accompanying the advancements in microscopy is that the 'big data' acquired by these high-resolution, fast-imaging platforms require convenient and efficient computational tools for data storage, management and imaging analysis. Many open-source or commercial software programs are currently available to address specific imaging analysis steps such as cell segmentation, tracking, signal quantification and clustering [103,114,115]; however, none of these programs can provide a complete set of solutions. The automation of certain steps, in particular cell tracking, remains an intimidating challenge because of the difficulties in tracking fast-moving cells or identifying cells with dramatic morphological change during cell division. A bright future in the field will be to integrate automated imaging platforms, programmable microfluidic-guided cell sorting and endpoint single-cell genomic sequencing techniques to probe gene regulation at the single-cell level with both temporal dynamics and whole-genome coverage.

7. Concluding remarks

Over the past decades, extensive genetic and biochemical studies have mapped out complex pathways and interactions that connect individual regulatory elements to a hierarchical GRN. However, a central remaining question is how the GRN operates in living systems, eventually giving rise to the precisely ordered execution of developmental programmes. To address this problem, we need to understand dynamic gene regulation at both molecular and systems levels. On the one hand, dissecting molecular dynamics at the single-molecule level will uncover the biophysical principles governing fundamental gene regulatory processes. Specifically, this may lead to the delineation of exact roles of each gene regulatory layers, including site-specific TFs, epigenetic regulators, housekeeping transcription machinery and the three-dimensional chromatin architecture. On the other hand, measuring gene expression dynamics at the single-cell level will reveal the information processing framework underlying the GRN. This may not only delineate the control logic underlying different core GRN structures governing cell fate determination and tissue morphogenesis, but also

![Figure 3. Imaging technology for probing dynamic and stochastic gene expression. (a) Imaging of the 3D Sox2 cluster by using a lattice light sheet scope. Left panel, photograph of an assembled lattice light sheet scope; right panel, three-dimensional density map of a Sox2 cluster in a single ES cell nucleus. (b) Composite four-colour FISH data from three rounds of hybridizations on multiple yeast cells. Genes are encoded by multiple rounds of hybridization using different probe sets. The boxed regions are magnified in the bottom right corner of each image. Spots co-localizing between hybridizations are detected and have their barcodes extracted. Spots without co-localization are attributed to non-specific binding. Scale bar, 5 μm. (c) Three-dimensional raw image projection (up) of zebrafish embryos (6 h post fertilization) expressing fluorescent markers labelling all membranes and segmentation results of sliced embryo (bottom). Scale bar, 50 μm. Panel (a) is modified from reference [18,35], (b) is adapted from [102] and (c) is modified from [103], with permission.](http://rsob.royalsocietypublishing.org)
might uncover the overall organizing principles that coordinate the transition through different developmental stages. Finally, mathematical modelling will integrate the knowledge gathered from different perspectives to reconstruct a quantitative and comprehensive view of how gene regulation orchestrates the spatio-temporal choreography of development.

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