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Current Opinion in Genetics & Development

Modeling transcriptional networks in Drosophila development at multiple scales

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Quantitative models of developmental processes can provide insights at multiple scales. Ultimately, models may be particularly informative for key questions about network level behavior during development such as how does the system respond to environmental perturbation, or operate reliably in different genetic backgrounds? The transcriptional networks that pattern the Drosophila embryo have been the subject of numerous quantitative experimental studies coupled to modeling frameworks in recent years. In this review, we describe three studies that consider these networks at different levels of molecular detail and therefore result in different types of insights. We also discuss other developmental transcriptional networks operating in Drosophila, with the goal of highlighting what additional insights they may provide.

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Current Opinion in Genetics & Development 2011, **21**:711–718

This review comes from a themed issue on
Genetics of system biology
Edited by Norbert Perrimon and Naama Barkai

Available online 1st September 2011

0959-437X

DOI 10.1016/j.gde.2011.07.005

Developmental transcriptional regulatory networks

Development is both robust, producing reliable outputs in the face of genetic variation and environmental perturbation within species, and plastic, producing new outputs when parameters of the developmental program are altered between species [1]. Quantitative approaches at multiple scales, from the molecular to the circuit and network, promise a route to understanding how developmental networks achieve robustness under some circumstances and plasticity under others [2]. Success in understanding these properties holds great promise for medicine, as it could pinpoint the origins of developmental defects and guide the design of new diagnostics and therapies. Success will also inform fundamental questions about evolution, as we seek to understand when altering the parameters of a developmental program leads to new phenotypes and when the phenotypic variation is simply suppressed.

Different developmental programs use conserved processes, such as cellular division, differentiation and migration, to produce organisms with unique morphologies, physiologies, and behaviors. To control these processes, developmental programs make use of gene regulatory networks that consist of multiple components: signaling pathways to detect and relay information, transcriptional networks to produce different sets of RNAs and proteins, and the effectors that execute the various processes involved in differentiation. Here we focus on the transcriptional component of these networks, or transcriptional regulatory networks (TRNs). Drosophila embryonic development has a been a favorite model for systems-level studies of TRNs, owing to a variety of technical advantages and a strong conceptual foundation provided by decades of traditional molecular genetic study. In this review, we discuss three studies of TRNs that pattern the Drosophila embryo to illustrate how different data types can inform biological questions at different scales of resolution and how they can be integrated into explanatory or predictive computational frameworks. We then discuss selected TRNs in Drosophila that operate during other stages of development, the features we believe make them also amenable to modeling, and the technical advances that will enable more quantitative experimentation on TRNs.

Early development in Drosophila

Patterning of the Drosophila embryo begins with maternally provided cues that are transformed into concentration gradients of transcription factors that control the expression of downstream target genes along both the anterior/posterior and dorsal/ventral axes [3,4]. The targets for these TRNs include both regulatory and structural proteins that collaborate to define the position and identity of larval segments and to control the differentiation of the germ layers [5].

These TRNs operate in a highly dynamic environment. Zygotic transcription begins 2 h after fertilization, when the embryo contains approximately 2000 nuclei. During the next forty minutes, three further rounds of nuclear division take place and the cells migrate to the periphery, leading to a syncytial blastoderm embryo with approximately 6000 nuclei arranged in a monolayer at the surface. At the end of an hour-long interphase, during which cell

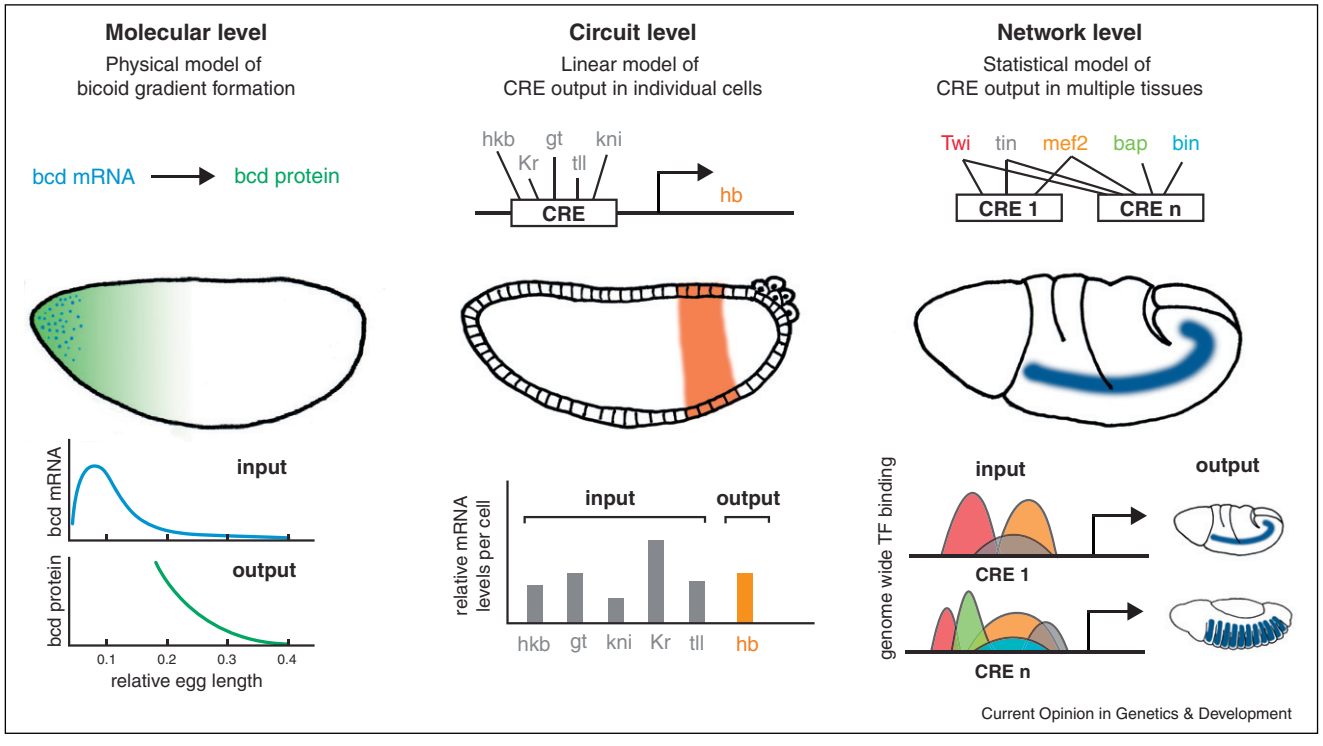
membranes invaginate to form the cellular blastoderm, the basic body plan is established and the embryo begins gastrulation [6]. During the next several hours of development, the gene expression patterns laid down before gastrulation are used to specify segmental identity, the three germ layers, and cell types within these tissues [7]. This patterning continues even as the cells in the embryo undergo complex movements to create the larval form [8].

Modeling of TRNs in the Drosophila embryo has been facilitated by a long history of genetic and molecular biology experiments. A majority of the key TFs involved in both anterior/posterior and dorsal/ventral axis specification were identified in pioneering genetic screens [9,10]. These TFs are also used in many other TRNs active at other stages and have been extensively characterized by decades of experimental work; in many cases, we know their DNA binding preferences [11], the *cis*-regulatory elements where they act [12], their spatial and temporal expression patterns, the effect of their disruption, and their roles in different TRNs. More recently, genomic analyses, such as ChIP-chip and ChIP-seq, have measured the *in vivo* binding of many TFs and sites of chromatin modification during several stages of embryonic development [13–16], facilitating the identification of *cis*-regulatory elements, elaboration of TRN topology and refinement of TF DNA binding preferences. Furthermore, microarrays and RNA-seq experiments have been used to measure the output of TRNs: the abundance and dynamics of mRNA transcripts in embryos at multiple stages [5,14,17••,18]. Spatial and temporal expression patterns have also been measured systematically at low-resolution for many genes across several developmental stages [19], and at high-resolution for fewer genes during cellularization of the blastoderm [20].

Quantitative studies of early Drosophila development

Below, we discuss three recent examples of quantitative studies of TRNs operating in the Drosophila early embryo (Figure 1). These are not the only informative studies we could have chosen; there is an extensive literature on modeling the anterior/posterior and dorsal/ventral patterning networks operating in the blastoderm [21,22]. The three studies we chose interrogate TRNs at different scales and therefore provide a good illustration of how the goals of the analysis dictate the type of input

Figure 1



Three quantitative studies of TRNs in the embryo at different scales. Here we illustrate three examples of models of TRNs in the Drosophila embryo at the molecular, circuit and network levels. (A) To model the formation of the bicoid gradient, Little *et al.* [23••] measured the absolute levels of mRNA (input) and protein (output) in the early embryo. (B) To study TRNs at the circuit level, we modeled the control of the *hunchback* posterior stripe CRE by 5 regulators in the blastoderm [Wunderlich *et al.*, submitted]. We measured the relative mRNA expression levels of the 5 TF inputs and *hunchback* output at cellular resolution to create a model of the circuit's function. (C) Zinzen *et al.* studied the specification of mesoderm in the embryo using a network level approach [35••]. They measured the genome wide binding of 5 TFs and combined this with tissue level expression patterns driven by CREs to create a statistical model to predict CRE output based on the TF binding profiles.

data and the nature of the computational framework used in the study.

The use of morphogen gradients to dictate target gene expression in a concentration-dependent manner is a key concept in development. The anterior/posterior TRN begins with *bicoid*, a classic example of a morphogen gradient. The long-standing model for Bicoid gradient formation suggests that Bicoid protein diffuses from a point source of *bicoid* mRNA laid down by the mother in the egg and tethered to the anterior end of the embryo. Little *et al.* tested this mechanism by carefully measuring *bicoid* mRNA and protein distributions using fluorescent *in situ* hybridization (FISH), GFP tagged proteins, and sophisticated image processing software [23••]. Using a model of the synthesis, diffusion, and degradation of *bicoid* mRNA and protein, they showed that the actual distribution of mRNA, which is dispersed over the anterior 20% of the embryo, better explains the observed protein gradient than the previously assumed point source of mRNA. This finding has significant implications for how the gradient is constructed. Moreover, egg size is known to vary significantly both within and between Drosophila species [[24–28], Fowlkes *et al. PLoS Genetics*, in press], and this model of Bicoid gradient formation impacts our understanding of how the gradient will scale in embryos of different shapes and sizes.

Transcription factor binding sites are crucial for controlling expression of their target genes, but it is not known how they integrate information to produce specific gene expression patterns [22,29]. Changes in single sites can disrupt regulatory output, but it is currently difficult to predict which disruptions are likely to have an effect or what the effect will be. Part of the difficulty is that multiple configurations of sites are functional; evolutionary comparisons indicate that the positions and affinity of TF binding sites change quite rapidly over evolutionary time while gene expression output is conserved [30–32]. To understand how the arrangement of TF binding sites relates to their functional output, we analyzed the TRN controlling the zygotic expression of the gene *hunchback*, a transcription factor that is, partly, regulated by *bicoid* [Wunderlich *et al.*, submitted]. Using a quantitative *in situ* hybridization pipeline [20], we measured the relative mRNA levels controlled by a *hunchback cis*-regulatory element (CRE) and its five regulators at cellular resolution. This allowed us to model the relationship between TF mRNA concentrations (inputs) and mRNA expression directed by the *hunchback* CRE (output) in individual cells. We first measured both input levels and output levels in transgenic *D. melanogaster* lines that express a reporter under the control of the hunchback zygotic CRE from six different Drosophila species. We then measured the inputs and outputs in the endogenous settings of three Drosophilids [[20], Fowlkes *et al. PLoS Genetics*, in press]. Using these data, we fit a simple linear function connecting the inputs to the output of one CRE and used this function to predict expression for orthologous CREs, with and without a calculated value for the *cis*-regulatory contributions to output. We found that predicted TF binding site occupancy summed across the CRE is an effective measure of relative *cis*-regulatory function. This is surprising given that the calculation does not account for cooperative or mutually exclusive TF binding. This is likely because orthologous CREs have been selected for functional TF binding site arrangements, allowing a simple measure of overall site strength to capture functional differences between sequences. This result underscores the flexibility of CRE sequences with respect to TF binding strength and arrangement, which is known to vary between individuals and species [33,34].

Often a single TRN with a small number of TFs can specify several different cell types. Zinzen *et al.* used ChIP-chip binding data and tissue-level CRE activity data to investigate how a TRN specifies several different mesodermal cell types [35••]. They measured the genome-wide binding of five TFs involved in mesodermal specification and differentiation at several time points over ten hours of development, beginning before gastrulation. Though there are other TFs that also contribute to this process, the study was limited to the five TFs essential for mesodermal specification and differentiation. The goal of the study was to predict the expression patterns driven by candidate CREs identified by ChIP-chip. The strategy used was to make a statistical model that correlates ChIP-chip binding patterns with tissue-level expression patterns. They built this model by training on ChIP-chip data and previously measured expression patterns driven by ~300 CREs. The resulting statistical model was used to predict the expression patterns driven by 8008 candidate CREs, and a subset of these predictions was then tested with a high degree of success. This study shows that the binding patterns of a small number of TFs to CREs are sufficient to predict their spatio-temporal activity and emphasizes the capacity of different TF binding patterns to yield the same expression output. It also provides a way to predict the functional consequences of changes in TF binding, which is observed even over short evolutionary timescales [36]. This approach may also be effective for prediction at finer scales of resolution, by making use of binding data for more TFs and annotations of CRE activity at cellular resolution.

The examples above illustrate that a systems approach to investigating TRNs can address biological problems at multiple scales, from a physical model of gradient formation at the molecular level, to rules for CRE architecture at the binding site level, to a statistical model for predicting the tissue-level expression of new CREs. The three studies contend with an increasing number of

Table 1

Properties of *Drosophila* transcriptional regulatory networks

Name	Parts	Cell types	Precision	Dimensionality of tissue	Time scale	Repeating structures	Other notable features
eggshell patterning: the specification of dorsal appendage location [41••]	**TFs**: br, brk, pnt **Signaling**: DPP, EGFR	Dorsal, midline, anterior, roof, and floor cell types	Tissue consists of ~1000 cells; there are several cells per cell type.	2D	**Duration**: ~20 h **Stage**: Oogenesis stages 9-12	N/A	One of the best examples of the integration of signaling and transcriptional networks
cardiac specification: the specification of cardiac mesoderm from mesoderm [42•,43,44]	**TFs**: bin, Doc1, Doc2, Doc3, H15, Hand, mid, pnr, slp1, slp2, tin, tup, ush **Signaling**: DPP, Wg	N/A	Each cluster consists of tens of cells.	3D	**Duration**: ~2 h **Stage**: Embryonic stages 6–10	There are 10 paired clusters of cardiogenic cells.	Cardiac mesoderm specification happens during late gastrulation.
central nervous system patterning: starts with the creation of cell clusters in each hemisegment, continues to neuroblast formation, ends with neuroblast divisions and specification of ganglion mother cells (GMCs) [45,46,47••]	**TFs**: abdA, abdB, ase, cas, D, dm, dpn, Dr, eve, ftz, hb, ind, jumu, Kr, l(1)sc, lbe, nub, nvy, pdm2, pros, run, toy, Ubx, vnd, wor, and others **Signaling**: Notch, TK and others	30 types of neuroblasts that divide to form ganglion mother cells (GMCs), which make different kinds of neurons and glia	There are exactly 30 neuroblasts per hemisegment; each divides in a stereotyped way.	3D	*Neuroblast formation:* **Duration**: ~4 h **Stage**: Embryonic stages 8-11 *Whole CNS formation*: **Duration**: ~24 h	Each hemisegment has 30 neuroblasts that form in a reproducible pattern.	Neuroblasts divide asymmetrically, and GMCs move towards the dorsal side of the embryo after division. The pros TF is localized in a cell cycle specific way in the neuroblasts. Lineage and birth order are important for neuroblast specification.
larval muscle development: from cell differentiation in promuscular groups to fiber formation [48,49••]	**TFs**: ap, abd-A, Antp, crl, eve, Kr, lad, Mad, Mef2, nau, pan, pnt, scr, slou, tin, twi, Ubx **Signaling**: DPP, Notch, Ras, Wg	Founder cells (which can be divided into ~30 subtypes based on TF expression profiles), fusion competent myoblasts	Each fiber starts with a single founder cell that fuses with several fusion-competent myoblasts.	3D	**Duration**: ~11 h **Stage**: Embryonic stages 10-16	Each hemisegment has ~30 somatic muscle fibers, each of which originates from a single founder myoblast. 6 of the segments (A2-7) have a repeating muscle pattern.	Asymmetric divisions form progenitor cells. Hox genes specify segment identity.
cardiac morphogenesis: the transformation of cardiac mesoderm into the embryonic dorsal vessel [42,43]	**TFs**: abd-A, Abd-B, Antp, dl, eve, Mef2, pnr, tin, Ubx, and others **Signaling**: DPP, Hh, MAPK, Notch, RTK, Slit/Robo, Wg	3 major cell types: dorsal body wall muscles, cardial cells (also differentiated into ostium and non-ostium cells), pericardial cells	Each cluster of cardiogenic cells has ~6 pairs of cardioblasts, 4 pairs express tin, 2 pairs express svp, Doc1, Doc2, and Doc3.	3D	**Duration**: ~15 h **Stage**: Embryonic stages 10-17	There are 10 paired clusters of cells. Of these, the 3 most posterior sections form the embryonic/larval heart, which contains ostia (inflow tracts), and the 4 next most posterior form ostia in the adult heart.	Lineage is important for dorsal body wall muscle and pericardial cell specification; lateral inhibition also plays a role for dorsal wall body muscles.

Table 1 (*Continued*)

Name	Parts	Cell types	Precision	Dimensionality of tissue	Time scale	Repeating structures	Other notable features
bristle (macrochaete) formation: from the formation of proneural clusters to division of the sensory organ precursor cell (SOP) [50,51]	**TFs**: ac, ap, ase, B-H1, B-H2, chn, da, E(spl)-C, emc, h, iro-C genes (ara caup mirr), l(1)sc, pnr, pros, salm, salr, sc, ush **Signaling**: DPP, EGFR, Notch, Wg	Proneural clusters give rise to 2 macrochaete. One cell in each cluster becomes the SOP that divides twice to form 4 cell types.	Proneural clusters arise in steorotyped positions in the fly; each cluster contains 20-30 cells. There is exactly 1 SOP cell per cluster.	3D	**Duration**: ~2 days **Stage**: Third instar larva	Proneural clusters are used to make as many as 11 pairs of macrochaete.	Lateral inhibition helps ensure that there is only one sensory organ precursor cell per proneural cluster.
wing disc patterning: from the definition of the wing field to the establishment of the wing primordium [52]	**TFs**: ap, brk, dve, en, fj, hth, iro-C genes (ara, caup, mirr), nab, nub, rn, sd, tsh, vg,, many others identified **Signaling**: EGFR, DPP, Hh, Hippo, Notch, Wg	**Tissues**: notum, tegula, hinge (proximal, intermediate, distal), blade, margin	There is a population of cells in each tissue type, and there are roughly 75,000 cells total in the wing disc at the end of the third larval instar stage.	The 3D wing is patterned from a 2D imaginal disc.	**Duration**: ~2 days **Stage**: Third instar larva	N/A	Wing disc patterning is concurrent with a large number of cell divisions.
retina: creation of the ~800 ommatidia that make up the compound eye [53•,54,55]	**TFs**: dac, ey, eya, eyg, hth, Optix, so, toe, toy, tsh, and others **Signaling**: DPP, EGFR, Hh, Notch, Wg	There are 4 major cell types: lens secreting cone, photoreceptor, pigment and bristle cells.	Each ommatidium has exactly 20 cells: 4 lens cells, 8 photoreceptor cells, 6 pigment cells, and 2 bristle cells	There is a 2D field of ommatidia and a 3D ommatidium structure.	**Duration**: ~2 days **Stage**: Pupa	The retina is composed of ~800 ommatidia with three different subtypes, depending on photoreceptor type.	The ommatidia form as a wave, with the posterior ommatidia forming first and the anterior last, allowing ommatidia in various stages of development to be observed simultaneously.

We summarize characteristics of other developmental TRNs amenable to quantitative modeling approaches. However, it is important to note that each of these TRNs is described by an extensive literature, and our summary is likely not comprehensive. We have listed references for each of them which can provide more detail.

components, from a single TF, to a handful of TFs controlling a single CRE, to a handful of TFs controlling many CREs. They also occur at increasingly later developmental time points, as the embryo itself becomes more complex. The computational frameworks needed to answer the questions that are posed in these studies require data of different breadths and resolutions. Notably, the data sets used in each study decrease in spatial and temporal resolution as they increase in the number of components, from single particle resolution at ~8 min intervals, to cellular resolution at ~10 min intervals, to tissue and embryo resolution data at ~2 h intervals; yet they are all successful in providing a satisfying answer to the questions they pose. These differences in data type emphasize that only the appropriate amount of detail should be included in an effective computational framework. Though not addressed directly in each study, the results also provide a computational framework that can be used to contextualize morphological or genetic variability within and between species.

Multiple developmental TRNs are amenable to quantitative analysis

Comparing insights from studies of different TRNs may shed light on how they are designed to accommodate different timescales, tissue types and output requirements. Many other TRNs have attractive features for systems-level studies, summarized in Table 1. The relevant players for these TRNs are largely known (Parts). Many of them give rise to a discrete number of morphologically distinct cell types, which may facilitate quantitating network output (Cell types). Some TRNs produce structures precisely, while the output of others is more variable (Precision). Many TRNs pattern relatively simple tissue structures, such as 2D sheets, making them relatively easy to image and analyze using image processing (Dimensionality of tissue). These different TRNs process inputs and produce outputs over a range of timescales, from hours, such as in early Drosophila development, to days, such as in eye formation (Time scale). Finally, many TRNs produce repeating structures, which can be useful for getting good statistical power out of a single sample (Repeating structures). Comparing across studies that interrogate at the same level of resolution may be particularly fruitful, as the modeling frameworks will probably be more similar than those employed at different levels [22].

Outlook

Often whole embryo measurement of the inputs and outputs of TRNs is sufficient to address questions at the tissue level, making genomic technologies such as ChIP-seq and RNA-seq informative. However, for studies at the molecular and circuit level, there is currently a trade-off between obtaining highly spatially and temporally resolved information for few components, which is achievable using imaging, and obtaining lower resolution data comprehensively using genomic technologies. To study the behavior of many TRNs, we do not require comprehensive information on every component in the cell – only information on a few tens of relevant regulators. Unfortunately, this is still beyond the reach of most imaging technologies, as only a handful of molecules can be labeled simultaneously in fixed tissue, and even fewer can labeled in live tissue [37–39]. An alternative solution is to increase the spatial and temporal specificity of biochemical techniques, such as ChIP-seq and RNA-seq, which could be achieved by lowering amount of material necessary and increasing the ability to purify specific cell types [40]. Together, the vast amount of information known about developmental TRNs and technical advances in quantitative experimentation make Drosophila an ideal choice to model TRN behavior, and address some of the most exciting questions about how development accomplishes the monumental task of creating an adult organism from a single cell.

Acknowledgements

We would like to thank Marc Kirshner, Tara Martin, Sean Megason, Becky Ward, Justin Kumar, Eileen Furlong, Robert Zinzen, Thomas Gregor, Thomas Klein, Richard Cripps, Alan Michelson, and Stas Shvartsman for useful feedback on the manuscript. ZBW is supported by a fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

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