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Logic of gene regulatory networks

Stefan C. Materna and **Eric H. Davidson**

Abstract

Regulatory networks of transcription factors and signaling molecules lie at the heart of development. Their architecture implements logic functions whose execution propels cells from one regulatory state to the next, thus driving development forward. As an example of a subcircuit that translates transcriptional input into developmental output we consider a particularly simple case, the regulatory processes underlying pigment cell formation in sea urchin embryos. The regulatory events in this process can be represented as elementary logic functions.

Introduction

Gene regulatory networks (GRNs) underlie the events which unfold during development to produce an animal from a fertilized egg [1••,2]. Their active constituents are transcription factors that activate, or repress, downstream genes, and signaling molecules that facilitate regulatory interactions across cell boundaries. Transcription factors bind to the *cis*-regulatory modules of downstream genes. These genomic regions are the nuts and bolts of gene regulatory networks: they are the information processors which execute basic logic operations to yield new transcriptional outputs, according to their transcription factor inputs [3••]. For example, two transcription factors both may have to be bound for activation of a downstream gene to occur, which corresponds to a logical AND operation. Similarly, OR and NOT operations are implemented by *cis*-regulatory modules.

The expression pattern of any gene can be understood in terms of the structure/function properties of its *cis*-regulatory apparatus. However, a single gene cannot by itself cause development; rather, many regulatory genes need to act in concert to drive development forward. Each cell at each moment in time can be characterized by its regulatory state, which is generated by the set of regulatory genes that are active in it. GRNs explain how and why spatial regulatory states are set up as development proceeds. Despite their seeming complexity, GRNs have a recognizable, underlying structure. They consist of modular entities, or subcircuits, each of which comprises the interactions necessary to achieve a discrete developmental task, for example the activation of an inductive signal, the lock-down of a transcriptional state, or the expression of differentiation genes [4]. Identification and functional analysis of subcircuits illuminates the character of the underlying regulatory apparatus, and reveals the logic operations (and their implementation details) that have to be executed for development to occur.

As an elementary example we here review what has been learned about the subcircuit underlying the initial specification of pigment cells in the sea urchin embryo. Our focus is on

corresponding author: Eric H. Davidson, Address: California Institute of Technology, Division of Biology, m/c 156-29, 1200 E. California Blvd., Pasadena, CA 91125, USA, phone: 626 - 395 4937, fax: 626 - 793 3047, e-mail: davidson@caltech.edu

the logic functions encoded in the genomic sequence, execution of which produces the pigment cell regulatory state.

The specification of pigment cells in the sea urchin embryo

The vegetal half of the blastula stage sea urchin embryo will give rise to endodermal and mesodermal cell types. The skeletogenic lineage occupies the central part of the vegetal plate. It is surrounded by a ring of cells which are precursors of endoderm and mesoderm (Figure 1a). During 8th cleavage this tier is subdivided radially into two rings, the outer of which will become the endoderm and form the gut. The inner ring will develop into the non-skeletogenic mesoderm, and will give rise to the pigment cells [5,6].

Delta/Notch signaling induces the specification of the non-skeletogenic mesoderm. The Suppressor of Hairless (SuH) transcription factor is the canonical effector of Delta/Notch signaling. In all cells of the sea urchin embryo this factor initially forms a complex with the obligatory repressor Groucho [7,8]. This complex binds to SuH target sites in the relevant *cis*-regulatory module of the *glial cells missing* (*gcm*) gene, and represses its transcription [9•]. At 7th cleavage the skeletogenic cells at the center of the vegetal plate express the Delta ligand and present it on their surface. It is received through the Notch receptor by the adjacent ring of cells [10,11,12•], which are in consequence specified as mesoderm. When the Delta ligand is bound, the intracellular domain of Notch (N^{ic}) is cleaved from the receptor. It forms a nuclear complex with SuH, in which it displaces the Groucho repressor, changing the behavior of the transcription factor to that of an activator. The SuH sites that mediate both mesodermal activation and non-mesodermal repression of the *gcm* gene are located in a single *cis*-regulatory module [9•].

The *gcm* gene serves two functions. First, it is required for activation of a variety of genes that are part of a pigment cell-specific gene battery, among them the *pks* (polyketide synthase) and *FvMo1*,*2*,*3* (Flavin Mono-oxygenase) genes which are part of the pigment synthesis pathway [13]. A second function is the repressive exclusion of *alx-1* gene expression (Sagar Damle and EHD, unpublished data). Alx-1 is a promoter of skeletogenic cell fate, and is a direct activator of skeletogenic differentiation genes. A wiring diagram of the pigment cell specification subcircuit is shown in Figure 1c. It is part of the GRN covering endomesoderm development, a complete version of which can be found at http://sugp.caltech.edu/endomes/.

A logic description of pigment cell formation

The pigment cell specification subcircuit is the transformation function that converts the Delta signal emitted by the cells of the skeletogenic lineage into developmental output, viz. the specification of pigment cells. At the same time it guarantees the exclusion of skeletogenic cell fate in mesodermal cells. These developmental tasks are summarized in the process diagram in Figure 1b.

The start of the pigment cell specification subcircuit is conditional and can be described with IF-THEN logic. Reception of the Delta signal by mesodermal cells kicks off the subsequent specification events. The Notch receptor, through which the Delta signal is received, is expressed widely in the early embryo [10,11]. However, the Delta ligand stays attached to the cells that express it, effectively limiting its range to its immediate neighbors [7]. In the sea urchin embryo, the cells that can physically receive this signal lie in the mesodermal ring that immediately surrounds the skeletogenic cells at the center of the vegetal plate (Figure 1a). If, and only if, the Delta signal is received by these cells will pigment cell specification be set in motion.

Downstream of Delta/Notch signaling lies the *gcm* gene. In the future mesoderm prior to 7th cleavage, as well as in all other embryonic territories, its default state is off, due in part to the SuH/Groucho repressor complex [8]. In the absence of signaling it represses transcription and, thus, executes a NOT operation (Table 1.1). Once the Delta signal is received, it is interpreted in the cis-regulatory region of the *gcm* gene: N^{ic} binds to SuH, displacing Groucho; only together can they activate *gcm* [9•]. Therefore, this obligate association is an AND operation (Table 1.2).

Subsequently, i.e., in the post gastrular period, *gcm* expression is controlled by a later acting module which includes an auto-activation function and depends on other inputs (A. Ransick and EHD, unpublished data). The choice of modules may be considered an exclusive OR operation, as discussed earlier [3••], since it is mediated by looping of the genome so that only a given *cis*-regulatory module can be in proximity to the basal transcription apparatus at one time (Table 1.3).

The first downstream function of Gcm is the direct activation of genes specific to pigment cells. These operations are dependent entirely on the binding of Gcm to the respective *cis*-regulatory regions. Thus, they can be considered a series of simple conditional operations (Table 1.4). The second function of Gcm is the inhibition of the *alx-1* gene, which may occur through direct binding of Gcm to the *alx-1 cis*-regulatory region. The result is a NOT operation (Table 1.5) and leads to the exclusion of skeletogenic fate in the mesodermal territory [14]. This kind of function is frequently employed in instances where adjacent cells, which are often the progeny of a recent cell division, adopt different cell fates. In fact, just as ectopic expression of *gcm* in skeletogenic cells results in repression of *alx-1*, the ectopic expression of *alx1* in mesodermal cells results in repression of *gcm.* In a similar fashion, endodermal cells are prevented from adopting mesodermal fate. There, the *foxA* gene is responsible for repressing *gcm* [15].

The example of the pigment cell specification subcircuit gives a flavor of the regulatory processes that lie at the base of development. Linked through their *cis*-regulatory information processors, transcriptional regulators are joined to form the subcircuits that produce the phenomena we observe in development. Given transcription factors are often found to take part in different subcircuits at different locations or times during development [16••]. In these different contexts the factor may be used in the execution of different logic functions; the logic functions in which it participates are not ordained by the biochemical identity of the factor, but by the structure of each *cis*-regulatory module and the architecture of the subcircuit. This general principle is emerging from the many examples that are now understood at the same level as the rather simple pigment cell specification circuit [1••].

Outlook

The long term goal is to gain a complete understanding of the logic functions that are involved in the making of an animal. Indispensable to this process is detailed knowledge of temporal and spatial expression patterns of regulatory genes, as these data indicate the identity of the constituents of the various subcircuits. Due to the enormous complexity of metazoans, an exhaustive mapping is no simple task, but current efforts to gather this information for entire embryos, body parts or organs are bearing fruit [16••,17•]. A major challenge in many systems is to obtain large scale perturbation data, which can be technically demanding, not at least in mammals. But only by means of experimental perturbation results can descriptive expression catalogues and correlations be transformed into logic models that explain the spatial progression of regulatory states during development.

Expressing developmental logic operations using formal language opens up other interesting possibilities as to how GRN can be examined [3••]: Networks constructed from experimental

data thus become amenable to analytical methods which can be used to test their validity. This includes tests of whether a chosen network representation is the most parsimonious interpretation of the data and may help to identify gaps and predict unknown players, or indicate wrong or missing links [18]. Automatic assembly of entire GRNs from experimental data is still in its infancy and has mainly been applied to single cell organisms [19], but this is now changing. A good example is the Biotapestry program (http://www.biotapestry.org) which is used to generate the wiring diagrams for the sea urchin endomesodermal GRN, and many others [18]. This program goes significantly beyond the primary role as a visualization tool, and it assists researchers in finding a representation that is consistent with their experimental data. Thus, expressing developmental functions more formally may not only help in conceptualizing developmental logic functions, it may also serve as a tool to automatize the discovery process.

As more and more regulatory networks become well understood we will soon have an overview of regulatory architectures from a diverse group of biological systems and developmental subcircuits. Together they will provide a detailed comparative image of the logic functions that lie at the base of development, such that the form of development, e.g. stem cell development, embryonic development, or post-embryonic organogenesis, can be predictively understood in terms of abstract logic functions.

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References and recommended reading

1••. Davidson, EH. The regulatory genome. Academic Press; San Diego: 2006.

2. Extensive treatment of gene regulatory networks in developmental systems covering topics from *cis*-regulation, structure and function of GRNs, to the implications for evolution.

2. Stathopoulos A, Levine M. Genomic Regulatory Networks and Animal Development. Dev Cell 9:449–462. [PubMed: 16198288]

3••. Istrail S, Davidson EH. Logic functions of the genomic cis-regulatory code. Proc Nat Acad Sci USA 2005;102:4954–4959. [PubMed: 15788531]

5. This paper is an attempt to conceptualize the events at the promotor. It illustrates the repertoire of *cis*-regulatory logic operations, as an approach toward a functional interpretation of the genomic regulatory code.

4. Ben-Tabou de-LeonSDavidsonEHGene Regulation: Gene control network in development. Annu Rev Biophys Biomol Struct2007 in print

5. Ruffins SW, Ettensohn CA. A fate map of the vegetal plate of the sea urchin (Lytechinus variegatus) mesenchyme blastula. Development 1996;122:253–263. [PubMed: 8565837]

6. Davidson EH, Cameron RA, Ransick A. Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. Development 1998;125:3269–3290. [PubMed: 9693132]

7. Lai E. Notch signaling: control of cell communication and cell fate. Development 2004;131:965–973. [PubMed: 14973298]

8. Range RC, Venuti JM, McClay DR. LvGroucho and nuclear beta-catenin functionally compete for Tcf binding to influence activation of the endomesoderm gene regulatory network in the sea urchin embryo. Dev Biol 2005;279:252–267. [PubMed: 15708573]

9•. Ransick A, Davidson EH. cis-regulatory processing of Notch signaling input to the sea urchin *glial cells missing* gene during mesoderm specification. Dev Biol 2006;297:587–602. [PubMed: 16925988]

12. This paper is a detailed account of *gcm* regulation by SuH. It teases apart the activating and repressive functions of the SuH binding sites.

10. Sherwood DR, McClay DR. LvNotch signaling mediates secondary mesenchyme specification in the sea urchin embryo. Development 1999;126:1703–1713. [PubMed: 10079232]

11. Walton KD, Croce JC, Glenn TD, Wu SY, McClay RD. Genomics and expression profiles of the Hedgehog and Notch signaling pathways in sea urchin development. Dev Biol 2006;300:153–164. [PubMed: 17067570]

12•. Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, et al. A Provisional Regulatory Gene Network for Specification of Endomesoderm in the Sea Urchin Embryo. Dev Biol 2002;246:162–190. [PubMed: 12027441]

16. This paper describes the experimental techniques that are used to unravel the architecture of the GRN underlying endomesodermal development in the sea urchin and presents the first version of this network.

13. Calestani C, Rast JP, Davidson EH. Isolation of pigment cell specific genes in the sea urchin embryo by differential macroarray screening. Development 2003;130:4587–4596. [PubMed: 12925586]

14. Oliveri P, Davidson EH. Development. Built to run, not fail. Science 2007;315:1510–1511. [PubMed: 17363653]

15. Oliveri P, Walton KD, Davidson EH, McClay DR. Repression of mesodermal fate by foxa, a key endoderm regulator of the sea urchin embryo. Development 2006;133:4173–4181. [PubMed: 17038513]

16••. Howard-Ashby M, Materna SC, Brown CT, Tu Q, Oliveri P, Cameron A, Davidson EH. High regulatory gene use in sea urchin embryogenesis: Implications for bilaterian development and evolution. Dev Biol 2006;300:27–34. [PubMed: 17101125]

21. This paper summarizes a large scale project to discover and gather exression profiles for all transcription factors in the sea urchin genome. The vast majority of transcription factors is transcribed by late gastrulation underlining the need to act combinatorially for proper regulation of downstream genes to occur.

17•. Satou Y, Satoh N. Cataloging transcription factor and major signaling molecule genes for functional genomic studies in Ciona intestinalis. Dev Biol Evo 2005;215:580–596.

23. This paper describes a project aimed at revealing the time and place where transcription factors are expressed during early development in sea squirt. This paper was the first step in establishing a GRN for early *Ciona* development.

18. Longabaugh WJR, Davidson EH, Bolouri H. Computational representation of developmental genetic regulatory networks. Dev Biol 2005;283:1–16. [PubMed: 15907831]

19. Geier F, Timmer J, Fleck C. Reconstructing gene-regulatory networks from time series, knock-out data, and prior knowledge. BMC Sys Biol 2007;1:11.

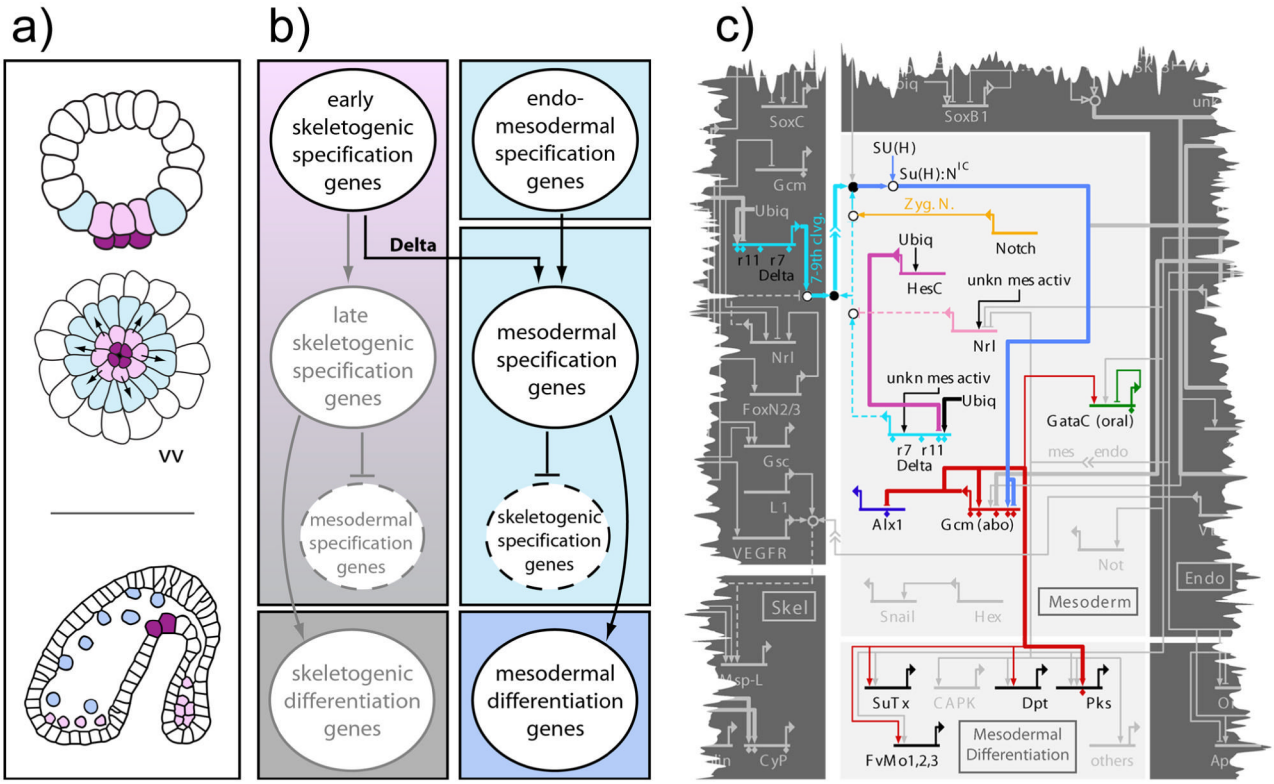

Figure 1.

Subcircuit underlying pigment cell specification in the sea urchin. a) Developmental scheme of the sea urchin embryo. At blastula stage (upper half) the cells of the skeletogenic lineage (pink) at the bottom of the vegetal plate are surrounded by a ring of presumptive mesoderm. The skeletogenic lineage is the source of the Delta signal (arrows) which induces mesoderm specification. Small micromeres (purple) are descendants of the skeletogenic cells; they contribute to the coelomic pouches. b) The process diagram of the mesodermal territory summarizes the regulatory events leading to pigment cell specification. Signaling from the skeletogenic lineage turns on the mesodermal program and leads to exclusion of skeletogenic fate. c) The mesodermal subnetwork details all identified linkages. It is part of the GRN of endomesodermal development (A current version of the endomesodermal GRN can be found at http://sugp.caltech.edu/endomeso). At the center of the pigment cell specification subcircuit lies the GCM gene which, once activated, locks the regulatory state through positive feedback, before turning on genes in the differentiation gene batterry and repressing the *alx-1* gene, a promoter of skeletogenic cell fate.

Table 1

Logic functions implemented by the pigment cell specification subcircuit.

Activation of GCM	
1) **IF** (Groucho **AND** SuH) **AND NOT** N^{ic}	⇒ **NOT** GCM
2) **IF** (N^{ic} **AND** SuH)	⇒ GCM
3) **IF** ((N^{ic} **AND** SuH) **OR** GCM)	⇒ GCM
Downstream functions of GCM	
4) **IF** (GCM)	⇒ pigment cell genes
5) **IF** (GCM)	⇒ **NOT** ALX-1

N^{ic}, nuclearized Notch intracellular domain; SuH, Suppressor of Hairless