

"A Genomic Regulatory Network for Development" (2002), by Eric H. Davidson, et al.

In 2002 Eric Davidson and his research team published "A Genomic Regulatory Network for Development" in *Science*. The authors present the first experimental verification and systemic description of a gene regulatory network. This publication represents the culmination of greater than thirty years of work on gene regulation that began in 1969 with "A Gene Regulatory Network for Development: A Theory" by Roy Britten and Davidson. The modeling of a large number of interactions in a gene network had not been achieved before. Furthermore, this model revealed behaviors of the gene networks that could only be observed at the levels of biological organization above that of the gene.

Britten and Davidson's 1969 publication presented a theoretical model of the process of gene regulation, in which five different types of genes interact to control the amount of products made from genes. This basic model of gene regulation suggested how development proceeds in a manner consistent across generations of organisms, yet maintains enough flexibility to evolve novel body plans. The model correctly predicted the functional existence of transcription factors, cis-regulatory elements, and RNA-polymerase before these molecules were experimentally discovered. "A Theory" represents the theoretical origins of the experimental conclusions reached by "A Genomic Regulatory Network for Development."

Davidson used the purple sea urchin, *Strongylocentrotus purpuratus*, as a model to investigate gene regulatory networks for several reasons. First, the sea urchin had a long history as a model for embryonic development. In 1877 Hermann Fol in Geneva, Switzerland discovered pronuclear fusion, the joining of the genetic materials of sperm and egg, in sea urchins. In 1907 Theodor Boveri in Würzburg, Germany showed that each chromosome carries distinct hereditary factors, and that all chromosomes must be present in urchin eggs for proper embryonic development. In 1939 Sven Horstadius in Stockholm, Sweden showed that cells of the early blastomere interact to direct cell fate within the embryo. Second, Davidson used the sea urchin because he had been working with urchins for over 30 years at the California Institute of Technology in Pasadena, California. He had investigated their gene expression and messenger RNA (mRNA), specification, transcription factors and gene regulation, and maternal inheritance. Third, through decades of research, Davidson's lab had compiled many gene libraries that they could use in a system-wide approach to investigating the genetic control of development.

"A Genomic Regulatory Network for Development" begins by outlining then current information about gene regulation, and it argues for investigating development at a systems level. Systems biology focuses on the interactions of multiple components in biological systems, as opposed to investigating single phenomenon or simple interactions. At the time scientists had described the phenotypic effects of single or small groups of genes, yet there was not a mechanistic description of a developmental process beginning with the genotype and ending with the phenotype. Davidson and his teams instead investigated a gene regulatory network that included all the genes operating in a single network, as well as their functional connections, as they control the specification of endomesoderm cells from earlier embryonic cells.

The purple sea urchin is a member of the group bilateria, animals in which early embryos develop three germ layers: ectoderm, mesoderm, and endoderm. The mesoderm and endoderm develop from a group of progenitor cells in early embryos called endomesoderm. The specification of endomesoderm cells occur approximately ten hours post fertilization, and "A Gene Regulatory Network for Development" describes the network through the first twenty-four hours of development.

The relatively homogenous group of cells of the early embryo provided the researchers a blank cellular slate with which to investigate the gene regulatory network directing the specification of the endomesoderm from surrounding cells. Davidson focused on the endomesoderm because it is one of the first tissues specified in the sea urchin embryo, and he proposed that a network operating early in development would capture features that are common across all species of bilateria.

The next section of “A Genomic Regulatory Network for Development” presents the basic physiology of sea urchin embryos during the first twenty-four hours of development. The publication uses fate maps to demonstrate how the endomesoderm is spatially distinct from other cell types (ectoderm progenitors) in the early embryo. These maps show that the endomesoderm originates from around two-dozen cells in the vegetal pole of the embryo approximately eight to ten hours post-fertilization. The tissues that derive from these cells are indicated in these maps, specifically several types of mesoderm, the gut endoderm, and for the study of body plan evolution, the skeletogenic mesenchyme. This section also mentions what developmental genes are expressed during the first-twenty four hours, notably Beta-catenin, Wnt8, and Delta, a member of the Notch signaling pathway. After twenty-four hours the sea urchin embryo is at the blastula stage, and while the cells of the embryo exhibit no physiological distinction, the regulatory state of these cells, defined by which genes are being expressed in each cell, has changed.

To describe the basic principles of how gene regulatory networks operate, “A Genomic Regulatory Network for Development” next discusses experimental studies of gene regulation. The vast majority of genes active in the early embryo code for transcription factors, a type of protein that binds to DNA in a sequence specific manner. When transcription factors bind to DNA the rate of transcription of the nearby gene can either increase or decrease. Britten and Davidson first proposed the existence of transcription factors as a regulatory mechanism in 1969, although they had originally termed these molecules ‘activator RNA’.

Transcription factors bind at sequence specific sites called cis-regulatory elements. Because a gene locus contains multiple cis-regulatory elements, the combinatorial input of many transcription factors binding around a gene is what controls the level of activity of that gene. In several papers prior to the publication of “A Genomic Regulatory Network for Development”, Chiou-Hwa Yuh and Davidson annotated the regulatory elements of *endo16*, a gene active during endoderm specification. This work describes the combinatorial interactions of regulatory regions and transcription factors, and how the timing, location, and amount of *endo16* expression changes throughout formation of the sea urchin gut. This examples illustrated that the inputs of transcription factors controls the transcription process of target genes, a central process of gene regulatory networks.

Having equipped the reader with the conceptual framework of gene regulation, “A Genomic Regulatory Network for Development” next discusses the methods used to discover what genes are active during endomesoderm specification, and how they link to each other in the network. In genetic studies, researchers often interrupt the normal functioning of a gene and record what happens. The authors interrupted a single gene coding for a transcription factor, they measured amounts of gene products from other genes, and they compiled a list of all the genes that are important in the endomesoderm specification network. Once the authors identified the major genes in the network, the specific interactions between genes were systematically explored by analyzing the regions around each gene for cis-regulatory elements. These elements represent the connections and functional nodes between otherwise unconnected genes. The quantity and location of these elements control gene regulation, and thus development.

The authors present the structure of the regulatory network as a wiring diagram. Britten and Davidson first published this kind of diagram to describe how genes interact in a network in their 1969 publication. These wiring diagrams became common features of Davidson’s papers about gene networks, and they illustrate how changes at a single node in the network can impact the transcription of multiple downstream genes. The interactive version of the diagram in this publication is located [here](#).

By diagramming the interactions between genes, the authors discovered several features of gene networks that theoretical models hadn't revealed. First, the majority of genes in the network code for transcription factors. This result supports the theory that an important act of early develop-

ment primarily is the construction of the regulatory states of cells, defined by the combination of transcription factors active in the nucleus. Second, the remainder of the genes that do not code for transcription factors are generally members of either the Wnt or Notch signaling pathways. These signaling pathways regulate transcription levels of target genes based on the signals from surrounding cells. The combination of intracellular network regulation with cell-cell signaling drives the spatial organization of the developing embryo. The authors state that this regulatory mechanism, measured by what classes of genes are active during early development, is a general fact of life and should direct development of all multicellular life.

The authors further discuss how expression of a single gene can impact the network as a whole. As an example they discuss *foxa*, a gene that codes for a transcription factor that, when bound to a cis-regulatory element, represses transcription of a target gene. Conversely, when expression of *foxa* is interrupted, the level of transcription of the genes increases. Repression is a basic regulatory function, but what is significant about the *foxa* gene is that it also inhibits itself. When upstream activating transcription factors bind near *foxa*, the gene is expressed. The corresponding *foxa* transcription factor binds near several other genes, inhibiting their expressions, while at the time shutting down its own expression. The *foxa* transcription factor degrades over time, leading to increased expression of target genes, including its own gene. This negative auto-regulation results in the oscillation of gene expression, and it has a major role in many biological phenomenon, such as driving the biological clocks of circadian rhythms, the segmentation clock of developing somites (somitogenesis), and the root clock of growing plants.

The next section of “A Genomic Regulatory Network for Development” describes how expression levels of the genes in the network change over time. Proteins present in the unfertilized egg control the first four to seven cleavage stages. The embryo gains control of its development when it begins to express genes from its own DNA seven to twelve hours post fertilization. The activation of the endomesoderm network occurs at this time by the expression of two genes: *krox* and *krl*. The gene *krox* codes for a transcription factor that activates many of the genes in the endomesoderm network. The gene *krox* also regulates itself, locking in its own expression. This type of regulatory motif, the feed-forward loop, the authors suggest to be the primary reason why development proceeds in a forward direction, and why the cellular fate of differentiated eukaryotic cells cannot typically change.

Meanwhile, the *krl* gene codes for a transcription factor that represses *soxb1*, a gene expressed in the cells throughout the embryo. Thus another principle of early development is revealed; to activate a cascade of gene expression leading to cellular identity, such as the activation of the gene *krox*, the embryo must also repress all other gene networks that could potentially cause conflicting regulatory networks, such as the repression of gene *krl*. Hence, gene regulatory networks lock in specific developmental trajectory, while simultaneously repressing alternate outcomes.

Davidson concludes “A Genomic Regulatory Network for Development” by arguing for the value of systems biology. By combining genetic and molecular techniques with computational tools, Davidson argues that we can explain the complexities of bilaterian development with gene regulatory networks. However, he also notes that the biochemical and kinetic interactions between the molecules in the network are ignored in this paper. Thus, this publication presents but one component of an encompassing systems view. Nevertheless, this paper experimentally describes a complete gene regulatory network, changes in which may result in variation in body plan development. The authors suggest that by describing multiple regulatory networks and how network changes relate to variations in morphology, future researchers may be able to answer questions of the evolution of body plans.

After the publication of “A Genomic Regulatory Network for Development” in 2002, researchers described and compared gene regulatory networks across species. Davidson has described additional gene regulatory networks within the purple sea urchin, as well as within other species such as the sea star *Asterina miniata*. In 2003 the comparison of the endomesoderm networks of these two species shared many features, despite the difference in the larval forms for each species. In 2007 Veronica Hinman and Eric Davidson showed that one difference between these two embryos, the presence of an embryonic exoskeleton in the sea urchin but not in the sea star, is caused by a similar gene network involved in developing the adult exoskeleton in both species. Sea urchins,

but not sea stars, evolved to co-opt adult skeletogenic network into their endomesoderm networks, leading to the development of an exoskeleton in the sea urchin embryo.

The evolution of an embryonic exoskeleton in the sea urchin indicates how changes in the cis-regulatory elements of a specific gene can drive changes to morphology. From results such as these, researchers posed theories of hierarchical levels of gene networks, in which networks occur at different stages of development and evolve independently of each other. Furthermore, mutations at different hierarchical levels will impact organisms' phenotypes in different ways. For example, a mutation in a lower level of a network hierarchy may change color patterning of an insect wing. Conversely, an interruption in a higher level, such as in the endomesoderm network, may result in the lack of two germ layers. Some researchers argue that the level of a gene network defines the evolvability and robustness of the network. Biologists such as Günter Wagner in the US used the principles of gene regulatory networks to describe the emergence of variation observed in nature.

"A Genomic Regulatory Network for Development" details the genetic interactions that result in a specific developmental outcome, but this network is but one aspect in the early development of embryos from a single species. The authors argue that uncovering the structure and function of gene regulatory networks, as opposed to annotating evolutionary changes in genes themselves, should be a primary goal of the field of evolutionary developmental biology (evo-devo).

In 1969 Britten and Davidson proposed a simple model of gene regulation that allows for both developmental consistency within species, and the variation between organisms and species observed in nature. Thirty-three years later "A Genomic Regulatory Network for Development" presented the first experimental confirmation of a complete gene regulatory network for development, an achievement that confirmed many of the conclusions drawn in "A Theory." While many of the theoretical claims of "A Theory" were individually confirmed throughout the years leading up to this publication, "A Genomic Regulatory Network for Development" offered the first causal mechanistic view of development at the systems level.

Sources

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