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Dev Biol. Author manuscript; available in PMC 2017 April 15.

Published in final edited form as:

Dev Biol. 2016 April 15; 412(2 Suppl): S7–S19. doi:10.1016/j.ydbio.2016.01.020.

ERIC DAVIDSON: STEPS TO A GENE REGULATORY NETWORK FOR DEVELOPMENT

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Abstract

Eric Harris Davidson was a unique and creative intellectual force who grappled with the diversity of developmental processes used by animal embryos and wrestled them into an intelligible set of principles, then spent his life translating these process elements into molecularly definable terms through the architecture of gene regulatory networks. He took speculative risks in his theoretical writing but ran a highly organized, rigorous experimental program that yielded an unprecedentedly full characterization of a developing organism. His writings created logical order and a framework for mechanism from the complex phenomena at the heart of advanced multicellular organism development. This is a reminiscence of intellectual currents in his work as observed by the author through the last 30–35 years of Davidson's life.

Introduction

Eric H. Davidson's career had an uncommonly unified trajectory over a half-century span and more. His late works from 2009–2015 emphasized a general theory of gene regulatory networks that drive developmental processes (Davidson, 2009, 2010; Erwin and Davidson, 2009; Peter and Davidson, 2009b, 2011a, 2015; Peter et al., 2012), but harked straight back to two potent theoretical papers that he wrote with Roy J. Britten about gene control in networks in 1969 and 1971 (Britten and Davidson, 1969, 1971). His experimental work for 50 years stressed using the ensemble of all the transcribed and regulatory components of a system to explain development, not just a "minimal" set of "important" components, and in this sense he was a father of Systems Biology[1]. This emphasis held from his breakout book Gene Activity in Early Development (1st edition)(Davidson, 1968) to the comprehensive catalogues of sea urchin regulatory genes that his group published in the 2000's (Howard-Ashby et al., 2006a, b; Materna et al., 2006) and the exceptional comprehensiveness of the Boolean model that he developed with Isabelle Peter and Emmanuel Faure in 2012 (Peter et al., 2012). In his final written commentary he emphasized repeatedly the need for models to be complete in terms of the components they included, and also completely validated by experimental evidence for linkages between the components, in order to provide causality.

[1]That is, the ensemble of expressed protein-coding genes, their promoters, intronic regions, and extended cis-regulatory sequences across the genome were interesting to him from the earliest years. In the 1960's he speculated that the main specific regulators in gene networks would be noncoding RNAs, possibly similar to lncRNAs that are now being defined. Curiously, though, by the time that actual miRNAs and lncRNAs were discovered and characterized by others in the second half of his career, he argued that they played little role in the developmental systems of his greatest interest.

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He contrasted the explanatory value of models fully rooted in genomic sequence, which show how the genome controls development, against models based on more limited analysis of regulatory pathways mediating parts of a process, e.g. controlling signaling “off the DNA” (Davidson, 2016). By 2015, the completion of the trajectory of his work could be deeply inspiring but also somewhat intimidating.

By the last years, one could be forgiven for imagining that his career leaped directly from the late 1960’s, as a systems biology theorist and pioneer, to the magisterial repleteness of the 2010’s with nothing in between. However, this retrospective view would misstate the record of how Davidson, his group, and his intellectual collaborators progressively developed these causal networks. It would telescope into nothing the risk-taking exploration, the swift responses to the findings of others, the key role of teaching in shaping emergent hypotheses, and the exciting experimental progress partly based on a series of technological advances, which filled at least three decades and kept revealing new features of developmental biology along the way. In reality, Eric Davidson led a highly effective experimental group that discovered major properties of his own system, and the group’s research both repeatedly corrected and provided increasingly firm foundations for his theoretical extrapolations to generality. The path from his theory of the late 1960’s to his theory of the 2010’s involved a sequence of strategic research moves in different directions as well as dynamically changing system-level insights based on the new discoveries by colleagues inside and outside his group. As a colleague and friend since the early 1980’s I was in a position to observe many of these moves and to see them eventually lead to the mature, well-validated models that emerged.

Here I offer one person’s vantage on a key segment of Eric Davidson’s complex and multifaceted career, anchored primarily by my own memories. A fuller, documented story of Eric’s career at Caltech awaits telling by Jane Rigg, who helped to build the lab from Eric’s first arrival there, ran it with him for decades afterwards, and shared in a vast range of Eric’s enterprises in science, institution building, and writing till the last year of his life.

Some comments on background

Eric had already focused on the central importance of tissue-specific differential gene expression from his days as a student with Alfred Mirsky, but this was systematized into a theory when he met Roy J. Britten. Eric’s deep engagement with Roy Britten was extremely important to his career, as described by a number of the other contributors in this issue, and Roy brought a great influence on him in several ways. One was the intellectual glamor of physics, which Roy embodied. The lure of doing biology that could meet the lofty standards of physicists was further reinforced by Eric’s interactions with his immediate neighbor at Caltech, Max Delbrück. (It was always a point of pride with Eric that he accepted Delbrück’s challenge to take an intensive tutorial in advanced math with Delbrück’s research fellow. Eric enjoyed formulating and troubleshooting differential equations himself ever afterwards.) The emphases on logic, quantitative precision and big-picture conceptual orientation, all intrinsic to physics, were values Eric prized to the end of his life, and he also savored the honored physics tradition of bare-knuckle, direct intellectual argument. Another thing that the collaboration with Roy provided was a distinctive experimental path unlike

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others in developmental biology. It was a heady thing to be able to measure the behavior of whole ensembles of nucleic acids and the structural features of whole genomes simply by using biophysical measurements of hybridization kinetics. Many (including multiple members of my own graduate school class at MIT) were impressed by this, and articles by Roy and Eric's joint groups were a regular feature in issues of the journal *Cell* when it was launched in the 1970's (Davidson et al., 1975; Galau et al., 1976; Galau et al., 1977; Hough et al., 1975). The excitement of this approach for Roy and Eric themselves must have been heightened by their prediction that repeat sequences, the elements that were most prominently distinguished by this experimental method, included the regulatory sequences that control differential gene expression. They hoped that they were driving to the heart of gene regulation as well as characterizing genomic and transcript structures. But by the early 1980's when I first encountered Eric, characterizing repeat sequence expression and repeat sequence distribution in different genomes were regarded by many people as his main interest. This structural genomics focus had drifted some way from the larger theory about gene regulation.

Ironically, Eric had been drawn into this field from a much earlier immersion in classical embryology. This had started at least as far back as his undergraduate work with L. V. Heilbrunn at the University of Pennsylvania and was almost certainly primed by his high-school work during summers at the Marine Biological Laboratory in Woods Hole. By the late 1970's and early 1980's, Eric's group may have been doing experiments in the lab like biophysicists, but his mind was also filled with something else, which was already becoming increasingly rare for the field: namely, a vast furnishing of encyclopedic knowledge of classical observational embryology from the late 1800's and the early 1900's. The oddness of this combination was very evident when I first critiqued chapters of what became his 1986 edition of Gene Activity in Early Development (3rd edition) (Davidson, 1986). The meticulously reproduced glossy plates of hand-drawn interpretations of microscopy from the early 20th century by E. G. Conklin and E. B. Wilson were presented and discussed in Eric's book in intimate detail, interspersed with brand new gene expression reporter assay data, hybridization kinetic measurements, and theoretical primers on macromolecular synthesis and turnover kinetics and nucleic acid reassociation kinetics. Especially in the chapter on cytoplasmic localization and the origins of embryonic axes (Chapter VI), even the names of the organisms described were unfamiliar – very few of them have continued to be studied as "model systems" – and their modes of development seemed dizzyingly individualized. Use of body plan patterning features such as reversible extrusion of polar lobes in snails and budding segmentation in leeches made it obvious that these organisms had diverged in their developmental processes very far from patterns familiar from work in models such as mammals or flies.

Most striking was what was missing in this synthesis. François Jacob and Jacques Monod, and virtually all precedents for gene regulation from microbial molecular biology and genetics, were barely noted; Jacob and Monod were not even listed in the bibliography. Now, by the 1970's, most regulatory biologists in my own molecular biology orbit (at Harvard, MIT, University of California San Francisco, and the Salk Institute) had been massively influenced by Jacob and Monod's work, by models of bacterial operon regulation, and by the precedents for elegant λ phage regulation of lytic vs. lysogenic growth by a mini

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network of mutually antagonistic activator/repressor proteins (Jacob and Monod, 1961; Jacob et al., 2005; Maniatis et al., 1974; Monod et al., 1963; Ptashne, 1967; Ptashne et al., 1980). How could these be skimmed over so lightly in a book about differential gene regulation as the foundation for development? It was not just this particular work of Eric's that failed to draw upon Jacob and Monod. Interestingly, one of the most controversial predictions in the 1969 Britten and Davidson paper was that regulatory RNAs rather than regulatory proteins might be responsible for complex gene regulation (Britten and Davidson, 1969). Yet this was presented without regard for the clear evidence already in hand at the time that gene regulatory molecules were proteins in these bacterial systems. Why? Asked about this many years later, Eric often explained that for him in the 1960's, the evident differences between bacterial gene regulation and complex eukaryotic gene regulation in development completely dwarfed the similarities. Hybridization kinetic analyses of bacterial and multicellular eukaryotic genomes had already showed these to have vastly different kinds of sequence organization, with a severe paucity of repeat sequences in the bacterial genomes compared to the multicellular eukaryotes. If these were regulatory sites, then bacteria were missing this kind of regulation. Also, Eric's view of development was that this irreversible, hierarchical process of increasing complexity that he was interested in was so different from the reversible, physiological nutrient responses of bacteria that there was no reason to posit the same kinds of molecular mechanisms. In this way, Eric and Roy were indeed charting their own course. But were they actually solving developmental mechanisms?

A reorientation: cell type specificity and the significance of fate mapping

By the early 1980's when I reached Caltech, change was in the air. This was not yet evident in the publications that came out at the time, but various members of the lab were creating enabling technologies that would bring the lab back to developmental process. The advent of nucleic acid cloning in the mid 1970's had made a difference to the terms in which one could study embryo development. With a way to study genes individually within a developmental context, there was finally a choice about whether to study specific sequences or not. Now that one could get hold of different, unique mRNAs in cDNA form, one could monitor differential gene expression directly. Much of the research in the lab was still focused on characterizing complex sequence ensembles in genomes and bulk populations of RNA, but some projects began taking a different path. By using genes newly cloned in the lab or by collaborators, Eric's lab began to look systematically at how cell type specific gene expression patterns appeared.

In view of what followed, note that at this key juncture the most dynamic aspects of Eric's science did not require completeness or comprehensiveness at all. Instead, the goal was to find clear examples, individual examples, and to find out what the rules were that governed their behavior. The examples were there: genes with various patterns of elegantly localized, highly reproducible expression that shifted canonically with the progress of development. The excitement these results caused was witnessed in the *in situ* hybridization picture that Eric chose for the cover of the 1986 edition of his book: a section through a sea urchin embryo at the gastrula stage, with golden hybridization signals for one gene solely lighting up a row of recently specified mesenchyme cells (Davidson, 1986). The gauntlet had been

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thrown down: could the mechanism responsible for this localized expression be demonstrated?

Two elements were needed to address this question: genomic clones of the specifically expressed genes from which regulatory sequences could be obtained, and a gene transfer system to enable cis-regulatory activity to be tested. Cosmid cloning, reporter expression vectors, and new transfection methodologies being developed in the field as a whole made this possible. One crucial advance was made by Andrew McMahon as a postdoc in the group, who brought expertise from previous work on mammalian embryos to establish the microinjection-based gene transfer system for sea urchin eggs (McMahon et al., 1984). Other postdocs including Konstantinos (Costas) Flytzanis then rapidly exploited this gene transfer technique to locate genomic sequences capable of driving cell type specific regulatory activity for differentially regulated genes (Flytzanis et al., 1987). A few of these regulatory systems – those of cytoplasmic actin gene *CyIIIa* and *Endo16* especially – were then dissected by years of painstaking deletion and mutation analysis. Many of the regulatory sequence mutants showed not only failure to express in the right parts of the embryo, but also gain of ability to express in the wrong parts of the embryo. In fact, as shown by Roberta Franks in the lab, the mechanisms constraining ectopic expression could be more species specific than the positive functions enabling correct expression (Franks et al., 1988). Thus both negative regulation and positive regulation determined the normal boundaries of expression of these genes. Many members of the lab contributed to studies of these and other genes, following these precedents, over at least 10 years (Arnone et al., 1998; Calzone et al., 1988; Coffman et al., 1996, 1997; Hough-Evans et al., 1990; Kirchhamer and Davidson, 1996; Livi and Davidson, 2007; Ransick and Davidson, 2006, 2012; Wang et al., 1995; Yuh and Davidson, 1996; Zeller et al., 1995). The results from a few individual gene regulatory element analyses led much further, as noted below.

Cell lineage and cell transfer

Two other key recruits to the Davidson lab in the 1980’s had a transformational role. R. Andrew Cameron and Andrew Ransick, a little later, opened the door to experiments that would connect marker gene expression with the causal process of determining individual cell fates. Cameron brought a broad, expert knowledge of different kinds of invertebrate larval forms, and Ransick had been trained as a classic experimental embryologist. With their arrival, they brought to the group the ability to use microinjection, cell transfer, and elegant imaging approaches to track and manipulate cell fates. This was urgently needed because of an insight about sea urchin embryo development that had emerged quickly from descriptive analysis of the first handful of gene expression cases. These results showed that genes that would later be expressed as specific markers for particular tissues of the free-swimming larva were already expressed in confined patterns much earlier, in blastula stage embryos, long before any morphological differentiation. Gene expression patterns thus revealed an unexpected degree of early patterning, raising a question about when cell fate was actually decided. If one had a gene like the one encoding CyIIIa actin that was to be expressed exclusively in aboral ectoderm, at what point was this gene turned on, relative to the time that cells became committed to an aboral ectoderm fate?

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One way to see this was by cell lineage analysis, to determine when all the progeny of a given blastomere became the same cell type. The power of this approach was being dramatically illustrated by the complete embryonic cell lineage analysis of *Caenorhabditis elegans* by Sulston and coworkers at that time (Sulston et al., 1983) and the similar fate maps Nishida was producing for ascidian embryos [rev. in (Nishida, 1997)]. With a canonical fate map in hand, the conditionality or commitment of the cell fate determination could then be tested by cell transplantation experiments. With faculty colleague Scott Fraser, Cameron carried out detailed cell lineage mapping of the sea urchin embryo and set the biological framework for all the future work (Cameron et al., 1993; Cameron et al., 1989, 1990; Cameron et al., 1987). Ransick proved that endoderm fate could be specified conditionally in a signal dependent way by neighboring cells, using precise cell transfer and new molecular markers to build on a classical experiment of Hörstadius (Ransick and Davidson, 1993, 1995). The importance of intercellular signaling events in establishing the ultimate pattern of cell territories was then reinforced by a powerful and long-lasting collaboration that developed between Davidson and David R. McClay of Duke University. Integration of specific signaling events into the causal molecular chain leading to cell type specification provided a crucial turning point in Eric's research; it was fundamental to define the "process diagrams" that would become the basis for gene regulatory network models.

A critical influence: the Woods Hole Embryology course

A catalyst for the next steps was Eric's service for two terms as Director or Co-Director of the Embryology Course at the Marine Biological Laboratory at Woods Hole, from 1988 through 1996. Eric felt that the faculty for this venerable course should be expanded to include multiple expert lecturers as well as a stable core consisting of leaders of three or four thematically integral modules of about two weeks each. In addition to labs and lectures, very importantly each lecture would be followed with an intensive question period afterwards in which other faculty present would take a very active role in the discussion[2]. This organization forced the directors of the course into close engagement with data and interpretations that were emerging in a wide range of developmental biology systems, and especially with each other's own outlooks. The period from 1992–1996 was especially dynamic because the course was then run by a triumvirate that had exceptional importance for the development of Eric's later work and thought: Michael Levine, then at the University of California San Diego, and David McClay, together with Eric. Work from both the Levine and McClay labs illustrated exciting principles involved in differential gene expression that became an inspiration and an experimental path forward to explain new aspects of sea urchin development. Eric was greatly impressed with Levine's demonstration of how discrete modular enhancers of the *eve* gene in Drosophila could each integrate effects of finite sets of different positive and negative inputs (Arnosti et al., 1996; Harding et al., 1989; Small et al., 1992), and this became a prototype for his views of information processing "by the genome". McClay's demonstration of very early, specifically localized activation of

[2]This was so stimulating that course faculty located in Southern California organized a Developmental Biology Colloquium with meetings for dinner and scientific talks to extend these interactions throughout the academic year, and the meetings continued from 1995–2001. Participants initially included Andy Cameron, Michael Levine (while he was at UC San Diego), James Posakony (UC San Diego), Joel Rothman (UC Santa Barbara), Richard Firtel (UC San Diego), Paul Sternberg (Caltech), Eric Davidson, and the author, later joined by Scott Fraser and Marianne Bronner-Fraser (Caltech).

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maternal signaling molecules in one region of the early sea urchin embryo provided the needed causal key – at the apex of a control hierarchy – to trigger regionally limited expression of specific genes, and thus to initiate a patterning cascade (Logan et al., 1999). These ideas were incorporated quickly as key elements of Eric's research priorities, and later served as key elements of his models of sea urchin development.

Another element of the Embryology course that left a vital legacy was the way each year's course would initiate. The first module of each course included a "zoo lab", in which the great diversity of marine life around Woods Hole itself was used for experimental and comparative embryology. During the years that Eric was involved in the directorate or the course, Andy Cameron ran the zoo lab and infused it with his own rich knowledge of marine invertebrate embryology. Because marine invertebrates cover a much wider phylogenetic span than terrestrial animals, the exposure to these organisms during the course in the late 1980's and early 1990's was an opportunity for participants to learn molecular principles of development in a context of animal evolution, in a way that has become vanishingly rare since then. In live developmental observation under microscope and microinjection needles, the course juxtaposed the differentiation of sea urchin pluteus larvae, *Xenopus* embryos, *Nereis* trochophore larvae, *Ciona* (ascidian) tadpole larvae, *Drosophila* embryos, occasionally *Ilyanassa* snail embryos, and the directly developing embryos of intelligent cephalochordates like *Loligo*, while also discussing models ranging from short germ band insects, leeches, and cnidarians to chick and zebrafish. Attempts in the course lab to use these embryos for comparative mapping of gene expression domains palpably confronted the whole group of course participants with homology and evolutionary issues.

How Embryos Work: a new classification of embryonic development modes

A watershed in Eric's understanding of developmental process in embryology as a whole can be mapped from the publication in 1989, 1990, and 1991 of a powerful sequence of review articles in *Development* (Davidson, 1989, 1990, 1991). With his renewed interest in "biology" as a tractable set of mechanisms, Eric looked back at the extraordinary heritage of classical embryology that he had mastered, now in a different light. From a perspective of many years later, he singled out the 6th chapter of the 1986 book, which considered a century's worth of evidence about specific localization of developmental determinants within the egg, as a turning point for his thinking. In the 1986 book he had been writing backwards across decades or centuries to address new discoveries to questions raised by earlier embryologists: e.g., "Thus the only real preformation is that of the genome itself" (Davidson, 1986, Chapter VI, p. 430). Now in 1989–91, he tackled the notoriously varied forms of embryonic development and wrestled out a core group of organizing principles from this "sea of phenomenology".

Looking at processes such as conditional vs. autonomous specification through the lens of potential transcription factor activation mechanisms, he resolved three major "types" of development (Davidson, 1991). The criteria he considered major were those that distinguished the "types" according to how spatially localized molecules could cause anisotropic biochemical activation of some transcription factor(s), thus initiating a distinct regulatory state in one part of the developing embryo. Signaling from one embryonic cell to

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its neighbor was now central to the process, and a key question was how different organisms positioned the sending and receiving cells appropriately. To explain how this process could implement cellular differentiation, Eric also invented the notion of the “smart histospecific gene” with a regulatory system that integrates timing, signaling, and cell type contextual regulatory inputs all at once (Davidson, 1990). He showed how the three major types of embryonic development made different uses of canonical cell cleavage planes, pre-setting of axes by molecules pre-localized in the egg, or cell migration as ways to bring about spatially correct transcription factor activation. He then considered how the phylogenetic distribution of these characters could hint at which modes of development were a conserved ancestral inheritance of bilaterians and which might instead be more recently derived.

The connection to molecular mechanism proposed here ran far ahead of the data at the time, but it was exceptionally exciting as an invitation. The promise was that a new kind of molecular embryology research would create a full “vertical linkage” (a phrase Eric used to describe his most admired outlook) from the most concrete molecular level through a definable cascade of consequences to the complete process of embryonic differentiation. It was an integrative analysis that could be read as a manifesto. In the Davidson lab itself, the conceptual re-investment in biology and deep analysis of specific cis-regulatory systems reaped a cornucopia of reward in the 1990’s. The group produced numerous analyses of cis-regulatory systems of cell type-specific genes, especially *CyIIIa* and *Endo16*; developed a highly influential theory of cis-regulatory elements as microprocessors; produced theories about the roles of cell lineage commitment timing in the evolution of complex organisms; began sea urchin genomics; and gained the first beachhead on solving the problem of the gene regulatory network.

Cis-regulatory elements as microprocessors

Two deeply studied cases of single gene cis-regulatory elements from Eric’s lab put his work into textbooks. Frank Calzone and then Chiou-hwa (Cathy) Yuh used the subsequences responsible for positive and negative regulatory function in CyIIIa and Endo16 to relate regulatory function of the DNA sequence to specific DNA-binding proteins, that they defined biochemically to bind to these sites (Calzone et al., 1988; Yuh et al., 1994). They then tested how these components determined the logic connecting the impacts of each protein-target site interaction into a full regulatory system for the gene.

The work on cis-regulatory system dissection was given strong impetus by the precedents from Levine’s work in Drosophila. However, there were two crucial differences from previous work that Eric built into these studies from the start, taking advantage of experimental features of the sea urchin system. In addition to characterizing the regions of the embryo where expression was obtained (spatial expression), he focused on dynamics of the gene expression driven by particular cis-regulatory elements, sensitively measuring the whole temporal profile of changes in level of transcript expression per embryo with development. These temporal activity profiles were often quite distinct, even between elements that helped to drive expression of the same gene in the same territories of the embryos (an early observation still relevant to current discussions about “shadow enhancers”). In addition, the work exploited quantitative gel shift technology based on dose-

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response titration with competitor oligonucleotides to track the changes in available transcription factor protein inputs, even before these factors themselves were identified. The ability to account for a protein with functional activity thus did not depend on prior genetic or other evidence for what factors “ought” to be important. These aspects of the approach were clearly related to the Britten-Davidson intellectual partnership, and also took practical advantage of the ability to culture millions of sea urchin embryos developing very synchronously from DNA-injected eggs after fertilization – a rarity among embryological systems.

The endoderm specific Endo16 regulatory system was dissected based on a saturation analysis of all the portions of the full regulatory region that were capable of being bound by sequence-specific nuclear proteins (Yuh et al., 1994). This study first demonstrated modularity in the clustering of the sites bound by different proteins, and then demonstrated that different modules served different regulatory functions (Yuh and Davidson, 1996). For example, different modules governed different temporal aspects of expression, different quantitative aspects of expression, and apparently exerted different negative regulatory functions to exclude expression in different inappropriate domains. Unlike the precedent of *Drosophila eve* stripe 2, the spatial repression functions for *Endo16* were found in different modules than the positive regulatory functions, showing that functional outputs of different modules must interact (Yuh and Davidson, 1996). The rules for combination of the different modules were not strictly additive, even when two modules were studied that could each be considered competent as minimal enhancers. In work done by C.-H. Yuh with computational analysis by James G. Moore, the combination of two modules of regulatory factor binding sites, A and B, did not simply drive a combination of the expression patterns of both, but sacrificed the distinctive timecourse pattern of one to amplify the pattern of the other: two individual enhancers had become a newly strengthened single entity, with the combination dependent on the presence of certain binding sites (Yuh et al., 1996). This was an early hint that a cis-regulatory system’s behavior could follow fairly complex logical rules.

These results inspired creation of a vitally important new collaboration, when Eric and computational biologist Hamid Bolouri met and discovered a common cause. Together, Davidson and Bolouri pushed the dissection of the Endo16 cis-regulatory system to a summit of abstract regulatory logic. In the *Science* paper by Chiou-hwa Yuh, Bolouri and Davidson, functions of at least 12 qualitatively different molecular inputs were defined in spatial, kinetic, and interaction-dependence terms (Yuh et al., 1998). Even though some of the responsible factors were not yet identified, their behavior could be discerned in detail based on the effects of specific cis-regulatory mutations on reporter expression patterns and dynamics. Not only was cis-regulatory element function fundamentally combinatorial (Arnone and Davidson, 1997). The highly detailed picture that emerged was a dramatic demonstration that cis-regulatory systems could act as tiny computers.

An important historical point is that although the Bolouri-Davidson models included AND, OR, and NOT logic relating the inputs between different transcription factors, they were initially assumed throughout the 1990’s to be quantitatively modulated as well (Bolouri and Davidson, 2002a). Bolouri and Davidson collaborated on a canonical treatment of developmental gene regulation cascades based on differential equations that related

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transcription factor binding affinities and concentrations to outputs in dynamically changing systems (Bolouri and Davidson, 2003). Systems of differential equations with continuously variable inputs and outputs were a natural approach in view of the emphasis on timecourse measurements that had characterized the reporter assays for cis-regulatory element activity in the Davidson lab (Ben-Tabou de-Leon and Davidson, 2009). A continuously-varying input was also implicitly assumed to be important in the highly quantitative analysis of transcription factor expression dynamics across the first day and a half of embryonic development, surveys later expanded through the sea urchin genome project. Thus, when Peter, Faure and Davidson later came to use Boolean modeling to express the sea urchin endomesoderm gene regulatory network (Peter et al., 2012), it was a new conceptual step in a journey that had begun in a different direction.

Hamid Bolouri was a pivotal colleague in the creation of the first network models, and his influence in Eric's group and the group of researchers sharing general interests with Eric was extremely important. However, there was a long conceptual step between the dissection of an individual gene's cis-regulatory system and the creation of complex, hierarchical, multigene regulatory networks explaining an entire developmental process, and several technological advances and discoveries in the latter part of the 1990's were critical to make that transition.

Lee Hood and genomics

One person who made a great impact on Eric's thinking and work throughout the 1980's and 1990's was his long-term colleague, friend, and fellow visionary at Caltech, Leroy (Lee) Hood. Lee and Eric had become partners in many enterprises at Caltech when both were young faculty members in the 1970's. In the 1980's, when Lee became Chairman of the Biology Division, he and Eric became a team on a series of far-sighted institution-building projects that were aimed to strengthen development, molecular biology of the genome, and development of advanced technology that would later create the proteomic and genomic revolutions. Working together, they attracted funding for this vision from the Lucille P. Markey Charitable Trust in the 1980's, and then contributed these perspectives strongly to the establishment of the Beckman Institute at Caltech. However, these organization-building undertakings and involvement in Divisional leadership were very time-consuming, and thus a certain amount of energy was released for Eric's own research when Lee Hood stepped down as Chairman at the beginning of the 1990's. Even so, the visionary teamwork between the two continued. Even after Lee moved to Seattle, they collaborated strongly to help establish the first incarnation of the Stowers Institute for Medical Research in Kansas City, MO, in the mid-1990's. Both the Beckman Institute at Caltech and the Stowers Institute continue as vibrant and highly successful undertakings.

Lee's influence contributed in multiple ways, both technological and theoretical, to make possible Eric's assault on the gene regulatory network problem. Lee's team had already created technology for protein sequencing, but in the 1980's Lloyd Smith and other members of Lee's group were actively working to create the technology that became the DNA sequencing standard for the field (ABI dye-terminator capillary sequencing). The feasibility of the human genome project arose because of these kinds of advances. Lee was

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very expressive about the impact that high throughput sequencing could have, and this inspired Eric to look at genome structures in terms of the potential advantage that could be gained through this kind of information. He rapidly appreciated the ability to use noncoding sequence conservation between distinct species as a shortcut to identify candidate regulatory sequences around any gene of interest. Thus, Eric began to campaign for facilities to sequence corresponding BAC (bacterial artificial chromosome) clones from his own research animal, *Strongylocentrotus purpuratus*, and from the east coast sea urchin *Lytechinus variegatus* for comparison. Researchers with an interest in comparative genome bioinformatics, especially C. Titus Brown, were drawn to the lab as comparative sequencing became a reality (Brown et al., 2002). Andy Cameron acquired a new and lasting role as the head of the sea urchin genomics program, in Eric's lab and for a broadening community of sea urchin researchers (Cameron et al., 2000).

Lee's interest in high-throughput methods and robotics made it possible to dream of ways to expand the growing gene-by-gene characterization of the sea urchin embryo, to full comprehensiveness. A major hurdle at that time was basic gene discovery: how many genes were really involved in the early specification of embryonic territories, and what were the ones that were not yet discovered? In the 1990's this would not have been easy to overcome except for a major investment that also owed a great deal to Lee Hood. Lee had made contact with James (Jim) and Virginia Stowers, a generous and courageous philanthropic couple with an interest in applying new genomic technology to medical research. Eric became involved in the discussions with them and persuaded them of the value of solving molecular mechanisms underlying developmental processes. The original establishment of their Stowers Institute for Medical Research created an interim foundation somewhat like the Howard Hughes Medical Institute, to begin to realize the vision of Jim and Virginia Stowers even before the creation of the bricks and mortar research building that now houses the Insitute. During this initial period, the Stowers Institute made significant investments in research instrumentation to enable Eric to create a pilot demonstration project, including DNA sequencers and robotics for high-density clone arraying. This enabled Eric's group to take the principles that had been learned from the single-gene test cases and begin to define the complexity of the full system required for specifying the territories of the early sea urchin embryo.

Gene discovery and the network: a beginning of genomically-based systems developmental biology

In the mid 1990's, Eric recruited Jonathan Rast to the group under the aegis of the new Stowers Institute. Jonathan's background was in molecular comparative immunology and was rich in experience with screening gene libraries for rare clones based on differential expression and "fuzzy" structural criteria (Rast et al., 1997). Jon initiated the experimental systems biology of the sea urchin embryo by setting up a platform for high-throughput discovery of all the relevant genes. With others including Carolina Livi and Andy Ransick, he made high-density macroarrays of cDNA clones from sea urchin embryos at 20–40 hr of development and optimized probe hybridization conditions that would allow the same macroarray of clones to be screened iteratively for clones that met either structural or

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differential expression criteria. The group then screened these arrayed libraries to identify all those cloned genes that were expressed specifically in normal sea urchin embryo endomesoderm (Ransick et al., 2002; Rast et al., 2000; Rast et al., 2002). To do this, they exploited earlier results showing that all the events leading to endomesoderm specification were β-catenin dependent (Logan et al., 1999). This meant that a whole-embryo cDNA preparation that had been subtractively hybridized to remove sequences expressed in β-catenin-inhibited embryos would be automatically enriched for endomesoderm specific genes, and then could be used as a probe to identify all these endomesoderm genes among the individual clones in the macroarray. Individual macroarray clones flagged as endomesoderm-associated were then characterized by sequence. The genes identified as specifically expressed, with differential expression in spatial and temporal domains during the first 20–40 hours of development, served as a foundation for endomesoderm development at a system level.

Of these differentially expressed genes, only a few dozen encoded transcription factors, and these became the core focal points for assembly of the gene regulatory network for development. With such a finite, tractable system of genes of interest, it was possible to apply recently available gene knockdown technologies (microinjection of morpholino antisense oligonucleotides) to perturb each regulatory gene individually and then assay the effect on expression of all the others. Each perturbation could be tested for quantitative effects, using highly quantitative RNA measurement methods harking back to Eric's emphasis on rigorous biophysics, and each perturbation could also be tested for qualitative changes in spatial expression patterns, in recognition of the importance of correct boundaries of expression for gene regulation already established. In the sea urchin embryo system, this strategy was extremely efficient and scientifically fruitful. Within a short time, an initial gene network model could be laid out, in which each gene's expression could be provisionally associated with the activity level of at least one hierarchically "upstream" regulator (Davidson et al., 2002a; Davidson et al., 2002b). It was a network because the regulators were themselves regulated, offering a first glimpse of causality at a system level (Davidson et al., 2003; Levine and Davidson, 2005).

Most important was that this network gave a proof in principle of an experimental strategy for solving complex networks (Oliveri and Davidson, 2004a). The organization of the network for display was carried out with major guidance from Hamid Bolouri, and then with Hamid's associate William Longabaugh, who devised the network display software program BioTapestry (Bolouri and Davidson, 2002b; Longabaugh et al., 2005). This presentation format became a signature of the kind of network analyses that emerged from the kind of strategy that Eric and his group had demonstrated (Oliveri and Davidson, 2004a, b; Oliveri et al., 2008a). The network could always be further revised: indirect regulatory effects could be separated from direct ones, determination could be refined as to whether two genes were actually simultaneously coexpressed in the same cell, etc. Signaling interactions could be defined as necessary or coincidental for action across a cell interface using cell transplantation and other perturbations. The next decade, Eric's lab spent a major effort clarifying, refining, correcting, and recasting details of the endomesoderm network and extending it to other embryonic domains and later times of development. But the experimental pathway had been established.

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The “96 well plate project”: toward the sea urchin genome project

As the earliest results began to emerge for the network, by 1997, it was anticipated that such a network could include many indirect linkages as well as direct linkages. One criterion for direct linkage would be whether the cis-regulatory elements of the target genes actually contained binding sites for the factors whose changes in activity affected the targets’ expression. Although large-scale DNA sequencing was still not widely available, Eric decided that the best way to determine this on a comprehensive scale was to isolate BAC clones containing all the genes of interest in the emerging gene network model, get complete sequence from each BAC including noncoding as well as coding regions, and and define the regions to test for transcription factor binding by patches of evolutionary sequence conservation between the *Strongylocentrotus* version and the *Lytechinus* versions of these genes. There was as yet no complete human or mouse genome, but Eric persuaded Elbert Branscomb, founding director of the DOE Joint Genome Institute (JGI), that it would be worth trying a demonstration project with sea urchin genes to give sequence information an immediate functional tie-in. The “96-well plate project” was to submit several score BACs for this analysis, and the group was galvanized to come up with a gene list and prepare the DNAs. This was the beginning of sea urchin genomics (Cameron et al., 2000).

The sea urchin genome

By the end of the 1990’s, the human genome project had prompted the creation of national centers for genomic sequencing, and as the first full draft human genome sequence was nearing completion, Eric lobbied strongly to expand the “96-well” BAC sequencing project to a full-fledged sea urchin genome project. With a change of leadership, JGI withdrew from the project, but Eric now established a strong alliance with Richard Gibbs, George Weinstock, and Erica Sodergren, then at the Baylor College of Medicine Human Genome Sequencing Center. They undertook the sea urchin genome project and worked closely with Eric via regular, lengthy conference calls throughout the entire sequencing and assembly process. This turned out to require significant research and development because of the extremely high polymorphism of natural sea urchin genomes as compared to humans or inbred mice (4% divergence between single copy sequences within the same population, even between the two alleles in a single individual). The high prevalence of mismatches at a given position caused problems for alignment of reads from conventional shotgun sequencing, and Eric repeatedly requested that the facility correct linkages and fill gaps by sequencing the discrete BACs that his group had provided.

In the end, although gaps remained, the sea urchin genome assembly yielded a virtually complete list of genes and most of their flanking sequences (Cameron and Davidson, 2007; Sea Urchin Genome Sequencing Consortium et al., 2006). The sea urchin genome became a field-wide resource, and Andy Cameron, working with C Titus Brown, Qiang Tu and others, created the infrastructure to curate it and make it accessible (Cameron, 2014; Cameron et al., 2004; Cameron et al., 2009; Tu et al., 2014; Tu et al., 2012). There were remarkable findings about immune cell genes from the completed sequence (Rast et al., 2006), but also a complete catalog of transcription factor genes. The expression patterns and dynamics of these were characterized in an industrial-scale undertaking by a team in the lab led by

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Meredith Howard and Stefan Materna (Howard-Ashby et al., 2006a, b; Materna et al., 2006; Tu et al., 2006). By 2006, the whole genomic parts list was in hand to make the network complete.

The double negative gate and network circuit logic

Even before it was complete, the network analysis shed brilliant illumination on the essential solvability of developmental process. Here, by working outward from one highly specialized cell type, a whole pattern of regulatory logic fell into place.

Cell biology of sea urchin development had pointed to one cell type as particularly favorable to reveal the mechanism of cell type specification. This was the skeletogenic micromere lineage, the precursors of primary mesenchyme cells that would all give rise to larval skeleton. Unlike most of the rest of the embryo, skeletogenic mesenchyme cells appeared to be specified autonomously: if removed from the embryo before differentiation, they proceeded to differentiate into skeleton anyway, giving rise to nothing else. They had therefore become committed at an early stage, probably as soon as the mitosis splitting them from other micromeres that would delay differentiation and only much later yield future adult sea urchin tissues. Several postdocs in the lab had studied the highly specialized set of genes expressed in these cells. Paola Oliveri had come to the lab as a postdoc with a highly divergent homeobox gene called *Pmar1* to study, which was already known to be expressed specifically in future skeletogenic cells, aiming to show what its role might be in these cells. Analyzing the exact role it played ended up having an outsize effect on the sea urchin gene network project and on Eric's conception of gene network theory itself (Oliveri et al., 2002). For the presumption was originally that if the Pmar1 transcription factor did anything, it would act as a positive regulator of skeletogenic cell identity. Thus, gain of Pmar1 function should cause more skeletogenic cells to be formed, and it did (Oliveri et al., 2003). Loss of Pmar1 function should cause a loss of skeletogenic cells, and it did. Swamping the effect of endogenous Pmar1 with an artificial obligate repressor form of Pmar1 should also cause a loss of skeletogenic cells… but it did not. Instead, the obligate repressor form of Pmar1 acted more like Pmar1 itself. What had always lurked as a theoretical possibility was suddenly a reality: the lineage-determining factor that caused the autonomous specification of skeletogenic cells was a repressor.

How could a repressor confer positive functional identity on a cell lineage? Only if it could repress the expression of another repressor (Oliveri et al., 2002). Suddenly, the gene network had to create cell type specific positive function by an algebraic function of negating a negative one. But there was more. In this light, the restricted anatomical expression of natural Pmar1 and the ability of ectopic Pmar1 to convert the whole embryo to skeleton forced the deduction that the positive regulator of skeletogenic gene expression was actually ubiquitous, and the unidentified repressor had to be everywhere in the embryo that Pmar1 was not. [This was later confirmed when Roger Revilla-i-Domingo identified the repressor as HesC (Revilla-i-Domingo et al., 2007).] In other words, to explain the localized activation of skeletogenic genes in one cell lineage at the vegetal tip of the embryo, one had to account for regulatory gene expression all over the rest of the embryo as well.

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Paola's discovery and its implications threw a sharp light onto the network not only as a molecular mechanism but also as a structure of logic, and logic deployed for spatial patterning. This was a double repression circuit which could be considered the algebraic equivalent of a positive driver, but one which was really more than that because of its use in space. The spatial extent of the action of the first repressor was narrow while the spatial extent of the second repressor was broad, and that pattern thus specified not only skeletogenic cells but also the fates of many non-skeletogenic cells through one network subcircuit, termed the "double negative gate".

Sorin Istrail, a computational biologist from Celera Genomics, had become acquainted with Eric through discussions about genome sequencing, and this vision of logic in a gene network subcircuit galvanized his interest. He and Eric began to work together to find out how many examples of such "double negative gates" there might be in the sea urchin embryo gene network. They also looked at other circuit motifs: for example, various kinds of feed-forward and feedback loops, mutual repression and expression-stabilizing circuits, among which, cases of intergenic positive feedback that Eric called "positive regulatory embrace" were especially prevalent. By 2005, Sorin and Eric developed a catalog of circuit types executing different logic functions in embryos and related them to their equivalents in formal logic and in standard electrical circuit designs (Istrail and Davidson, 2005; Istrail et al., 2007). Each subcircuit type appeared repeatedly in the network, because basic modes of spatial subdivision and regulatory state stabilization were needed repeatedly in embryo development. This translated the processes of embryogenesis catalogued in Eric's 1990 and 1991 reviews into a composite of modular network circuit elements that made the whole system intrinsically programmable (Ben-Tabou de-Leon and Davidson, 2009; Davidson and Levine, 2008; Istrail et al., 2007; Materna and Davidson, 2007; Oliveri and Davidson, 2007; Oliveri et al., 2008b).

A parallel strand: Evolution of development

The development of the first gene network model was intertwined with another major project of the Davidson lab: understanding the evolutionary relationships among the different variants of the developmental program within a clade. The comparative analysis inspired by the Embryology Course "zoo labs" in the late 1980's and early 1990's was brought explicitly into Eric's group when paleontology-trained Kevin Peterson, a student from the Embryology Course, joined the group in the early 1990's. Kevin's challenges that developmental control machinery should be related to the actual fossil record inspired Eric to make contact with Douglas (Doug) Erwin of the Smithsonian Institution Museum of Natural History to learn first-hand about the Burgess Shale fossils and the earliest fossil forms of echinoderms. In looking at these fossils, Eric's investment in studying skeletogenic cells and skeletogenic genes was also fortunate, because there is almost complete overlap between the genes expressed in the larval skeleton of the sea urchin and in the adult skeletal plates that are left to form the most visible part of the fossil record. The history of echinoderm evolution lay before Eric in these stones, "written" in terms of the deployment of the same genes he was studying in the lab.

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Eric then successfully lobbied sympathetic officials within NASA to create a small grant program to look at the molecular basis of evolution, proposing the rationale that some of these principles could inform the variations of life that might be encountered extraterrestrially. The grant program was kicked off with a series of workshops in which prospective PIs shared the opportunities for evolutionary insight that might emerge from their own systems, thus also learning from each other about the status of multiple interesting organisms (Davidson and Ruvkun, 1999). The funding released by this program enabled several postdocs with interests in evolution to be recruited to the lab, including Veronica Hinman as well as Peterson, and a subgroup interested in the evolutionary origins of deuterostome immunity (including Jonathan Rast and Zeev Pancer, following earlier postdoc L. Courtney Smith). Peterson and Davidson published theories about phylogeny and mechanistic origins of different modes of development (Peterson et al., 1997, 2000; Peterson and Davidson, 2000), and this drew interest from the paleontology community. At a 1999 conference with paleontologists in southwestern China, Eric was introduced to Jun-yuan Chen (Early Life Research Center, Kunming), the most knowledgeable explorer of the Doushantuo phosphorite deposits and an expert on their extraordinarily fine preservation of ancient fossils. Eric created new collaborations with Chen, and also with US paleontologists Erwin and David Bottjer (University of Southern California), in order to begin to apply his knowledge of diverse embryological forms to classification of the tiny, deeply pre-Cambrian fossils that emerged from imaging of these rocks (Chen et al., 2006; Chen et al., 2004; Chen et al., 2002; Chen et al., 2000).

As the pieces of the sea urchin endomesoderm gene network began to come into focus, therefore, the group was already poised to use them to test models of evolutionary change and conservation in developmental processes. Veronica Hinman tackled directly the question of how different the gene network emerging for sea urchin embryos might be from the gene network driving the corresponding endomesoderm structures in a very distantly related echinoderm, the starfish *Asteria miniata* (Hinman et al., 2003). This was an especially promising and straightforward comparison as the role of Pmar1 in sea urchin skeletogenic mesenchyme specification came into focus, because the starfish do not have a larval skeleton at all. She found that at least one gene that is skeletogenic-specific in the sea urchin (*Tbr*) is deployed entirely differently in the starfish, but that the circuitry controlling endoderm development was strikingly similar in starfish and sea urchin. A densely interconnected web of the same transcription factors was used, with only modest apparent changes in the “wiring”, in the core endoderm specification processes of both starfish and sea urchin (Hinman et al., 2003). This result made an extremely important point: evolutionary change of networks was modular, with some parts changing much more rapidly than others over the same evolutionary timespan (Davidson, 2001).

Eric and Doug Erwin began to elaborate this insight into a general theory, proposing how different parts of networks could be differentially exposed to or protected from evolutionary change (Davidson and Erwin, 2006, 2009; Erwin and Davidson, 2009). The buzzword that ultimately became memorable for readers was “kernel theory”, referring to the high conservation of densely cross-regulating gene circuits, but the “kernels” themselves were not actually the most important point. The central take-home message was that the developmental impact of evolutionary changes in any given gene’s expression depended on

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the topology of wiring of that gene into a specification network. Eric began to emphasize that these networks were intrinsically hierarchical, and that the evolutionary impact of change in a gene's activity pattern could be quite different depending on its level in the hierarchy. This implied that solving a given organism's gene regulatory network not only explained its own developmental process but also led to insights into the organism's evolutionary path. Furthermore, it opened the door to the possibility to test how evolutionary change in body plan could occur by targeted experimental rewiring of the organism's gene regulatory network, a strategy Eric dubbed Synthetic Experimental Evolution (Erwin and Davidson, 2009).

Culmination of the topological network: sea urchin as paradigm

By the time Eric wrote his 2006 book, *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution* (Davidson, 2006), his work had led to a revolution in understanding. The first endomesoderm gene network was published and was already being iteratively completed and corrected, as parts of the circuitry that seemed interesting came under closer examination. The sea urchin genome sequence was being completed as the book was written, providing material for extension of the network beyond the genes specific to the endomesoderm territory, to the whole embryo. The network topology had been shown to be extremely revealing about evolutionary processes. Dynamic modeling of parts of the network by differential equations, using synthesis and turnover values originally measured by Davidson and Britten in the 1970's, were yielding detailed insights about how fast the activation of a transcription factor gene could begin to affect expression of its target genes. Finally, analysis of the properties of the network had led to a theory of subcircuit modularity and logic.

I remember a conversation with Eric in 2007 or 2008, when he asked a remarkable question: what direction should he go next? Our discussion turned to a series of lectures that he had recently given at the Kavli Institute for Theoretical Physics at the University of California, Santa Barbara, where Boris Shraiman had invited him for an advanced quantitative biology course (QBIO, Feb 2007). As the audience included many physicists, Eric had decided to show less experimental data than usual but had instead inserted a number of text slides in which he stated what he felt were the emergent principles of the system of development he had been studying for the last 35 years. He had returned from those talks elated by the interaction with the physicists and the license this had given him to focus on abstract system principles. Unlike his earliest network speculations in 1969, this was now abstraction grounded in experimentally well-validated reality. We talked about the idea that he was opening a door to a kind of gene network meta-analysis that would shed light on principles operating far beyond the sea urchin embryo. There was indeed a sense that organized summing-up was now an important part of his mission.

By the time of the talk he gave to another broad audience, the Nobel Symposium on Systems Biology in June 2009, Eric had shifted completely to a presentation style centered on these statements of emergent principle. The principles included methodological ones: what is the proper way to solve a gene network to reveal causality? These lectures emphasized the crucial importance of basing proposed linkages on experimental perturbation tests, a

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necessary exhortation to audiences otherwise used to being assured that simple correlations were enough. But these lectures also included many emphases on the functions of the individual subcircuit elements that were found in the network, which could explain the component “jobs” that a network would serve, and the implications of network structure for evolvability. With a recently recruited postdoc in the lab, Isabelle S. Peter, Eric had begun actively to mine literature for the occurrence of similar network features in different biological systems: the heart specification networks of Drosophila vs. vertebrates; the pancreas specification network in mammals. These provided an expanding set of examples that Eric argued were functionally analogous to the subcircuits found in the sea urchin endomesoderm gene network. Isabelle and Eric began to write a series of integrative reviews that codified these points (Peter and Davidson, 2009a, b, 2010, 2011a).

Comprehensiveness validated: the Boolean Model

The advent of Isabelle Peter gave Eric a colleague within the lab who was in harmony with him on ways to think about abstract as well as concrete problems, and their collaboration led to the final large research milestone of his career. Isabelle had already led a subgroup within Eric’s lab to expand (and correct) the specification network for endoderm, showing it to be unexpectedly complex, with new insights about the way two signal response subcircuits could operate independently within the same cell to influence fate determination of future descendant cells (Peter and Davidson, 2010, 2011b). However, in her theoretical discussions with Eric, they struggled with the question of how to validate the network as a complete system explanation of the developmental process. Eric’s dynamic modeling of the network before had focused on continuous valued, differential equation-based models, but he was well aware that the key rate constants and absolute concentrations needed to make such models predictive were not known. If these parameters were estimated by fitting to the data, some values could always be found to satisfy the data well enough; but then to use such a model predictively would be tautological.

Eric considered an alternative way to think about modeling: could a Boolean model be sufficient? This had three attractions. First, increasingly Eric was seeing the specification process in terms of creating boundaries between cell types or territories, separations between mutually exclusive regulatory states. This would be intrinsically Boolean, not only in terms of the outputs of specification but also in terms of their underlying cause, the activity of mutually exclusive combinations of transcription factors. Second, the cell by cell domains of detectable “expression” vs. “nonexpression” had already been determined non-quantitatively for all the genes in the network. Third, Eric read increasing significance into the outcome of his earlier 2003 network dynamics work with Hamid Bolouri, which had shown that activation of a transcription factor gene might affect the factor’s target genes long before expression of the first transcription factor reached its maximum (Bolouri and Davidson, 2003). At that time, the predicted “step time” between gene activations was theoretical, based on assuming total accessibility of target regulatory sequences and simple systems based exclusively on positive regulation. However, now Eric wondered if this predicted system behavior could be a key to a radical simplification of modeling network activity: to convert the whole system to a Boolean model.

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Isabelle became excited about this idea and agreed to work with Eric to see whether they could code the activation requirements of all the known genes in the endomesoderm network into Boolean vector equations. This became a grueling effort lasting many months of concentrated work. Isabelle and Eric realized quickly that the model had to contain not only the best current data for the logic of transcriptional inputs at each gene's cis-regulatory system, but also detailed information about the location of each cell type relative to neighboring cells at each timepoint of development, since neighboring cells could be sources of inductive or inhibitory signaling molecules. They also had to define the step function for each re-computation in their Boolean model, and they made a choice to use a clock-based step which could include multiple synchronous changes at different parts of the embryo rather than the more usual asynchronous steps. As Isabelle and Eric developed rules and curated data quality to define these equations, they were joined by a programmer, Emmanuel Faure, who made it possible to build their model into a live simulation program (Faure et al., 2013).

The Boolean model they created (Peter et al., 2012) has been extensively discussed and is properly described by Isabelle elsewhere in this volume. However, in the context of this retrospective, it was important because it provided the very first opportunity to test whether the endomesoderm gene network model that Eric had been refining for ten years was actually complete and predictive. There was no circular logic inside it that would force it to appear predictive: the vector equations were built on interpretation of effects of a finite number of perturbations that had been tested, but with no guarantee that these relationships explained all expression features of all the genes. The step time function that was used, the same step time calculated theoretically by Bolouri and Davidson (Bolouri and Davidson, 2003), would not have worked if there had been chromatin barriers to remove, or extensive competition between activators and repressors on the genes that needed to be turned on. The number of genes included in the perturbation-based network was far smaller than the total number of genes expressed in developing endomesoderm, and they were selected for territory specificity: they did not even include the genes encoding transcriptional regulators that were expressed uniformly throughout the developing embryo. If any ubiquitous or misidentified factors played a rate limiting role anywhere in the endomesoderm, the model would have come up with an inconsistency from observed data. But in fact, when the Boolean model ran a complete predicted computation of the first day of endomesoderm development, the number of inconsistencies between model prediction and measured in vivo gene expression was extremely small. Further *in silico* tests of alternative step times showed that the Bolouri-Davidson theoretical step time actually reflected reality best. Thus, for the first time, there was not only a model that might account for observed biology, but actually a model that did account for observed biology (Peter et al., 2012). Eric's mission to explain development as a gene network had yielded something very close to complete.

Home stretch

In the wake of the Boolean model, Eric was ready to write another book, and he sensed that this would be his final book. At this point, his health was weakened due to progressive spinal stenosis, and walking more than a couple of steps with a walking stick was very difficult for him. Still, despite needing physical assistance, he was still extremely vibrant, and one could

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judge that his sense of urgency was not anything more than the relentless sense of mission that had characterized his whole life. Deciding that he could not write this book alone, he persuaded Isabelle to work with him on this enterprise too, assuming that it would take about a year with each of them writing different parts in parallel. In fact, Isabelle had never written a book before and was still responsible for day-to-day guidance of a key research team within Eric's lab, so this time line proved unrealistic. Eric's drafts laid out chapters 3, 7, and most of 1 before she was fully entrained in the writing. But for the next year, Eric and Isabelle went through every line together, decided what needed to be said on every point, and worked out every sentence side by side. It was an extremely intensive process. The book that finally emerged was heroic in scope, even more encyclopedic than Eric's previous books, yet still one that closed with the excitement of using gene networks in the future to see into the deep time of animal evolution (Peter and Davidson, 2015).

By the time the book was finished, Eric's physical condition had deteriorated significantly. Tragedy struck again during the correction of the proofs, as his long-time dear associate Jane Rigg became seriously ill. Jane had not worked on this last book at all, and now there was concern for her life. Eric could no longer walk or stand, and everything was becoming difficult for him. Still, the publication of the book was celebrated with a warm gathering of many Caltech community members who appreciated Eric, had worked with him, or simply admired him and wished to pay respect. It was a high point, muted only by Jane's absence.

As the spring proceeded, Eric worked intensively on additional papers and grant applications. He had new ideas; he would write a new theoretical article about measuring the total information complexity in a network so that different networks could be compared. Again, he persuaded Isabelle to join him on this. His health condition had now become alarming, but he continued to travel for seminars, to research new mechanical devices that might help his mobility and mitigate his now-unremitting discomfort, and to plan international conference trips and teaching stints. By the summer he was quite sick most days. In mid-August his symptoms improved, and he was elated to feel better. It seemed that there would be a future after all. He spent August 31 working on the new manuscript with Isabelle throughout the afternoon. At 2 am that night, as he was being helped into bed, Eric suffered a heart attack and was pronounced dead by paramedics an hour later.

Completeness

In the gene network, Eric and his colleagues had approached something close to completion, but this was a goal, not a method. In fact, it would matter whether networks could be established to be complete: for at the time of his death, the theoretical metric of network properties that Eric was working on would only be valid if used to make comparisons between two complete networks. A provisional network with only a few links filled in yet could not reliably show what kinds of circuit elements it contained, since some links could be missing or mis-assigned. One could not even be certain what the position of a given gene was in a regulatory network hierarchy, for evaluating its potential evolutionary impact, unless the other linkages in the network were extensively known. Thus, Eric at the end of his career had become interested in idealized network property characterizations that should only be applied to fully analyzed networks at the asymptote of completeness.

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But completeness was not a working criterion to guide the steps toward solving a new network. The great legacy to the rest of the developmental gene network field was instead the method Eric's group actually used. The problem was defined at a whole system level but was taken apart piece by piece, step by step, with every individual implied link submitted to direct test. The lab carried out not only trans-perturbations to sketch out network relationships but also cis-regulatory analyses on an industrial scale, to determine whether an apparent regulator-target relationship could actually be validated biochemically. An astonishing number of individual genes in the network were thus submitted to cis-regulatory analysis, one at a time, locating regulatory element candidate sequences in BACs large enough to contain whole regulatory systems of multiple elements as well as the regulated genes themselves. Eric's lab had developed an early bar-coding method for multiplex parallel enhancer assays *in vivo* (Nam and Davidson, 2012; Nam et al., 2010), and this could be used to test the relevance of a whole set of candidate elements by gain and loss of function in reporter assays. Then putative transcription factor binding sites could be located in the individual functional elements and mutated individually to test for effects of loss of the predicted input's effect, to prove where the functional influences were direct. This was neither high-throughput mapping of correlations nor a worm's eye view of miscellaneous unconnected facts. Rather, the framework provided by the network model allowed this infinitude of detailed results to be kept in context all the time and transmuted into biological significance, sometimes to see a small correction propagate to a structural shift in the network architecture itself.

Retrospectively, Eric achieved something like completeness in the sea urchin genome model, and arguably in his life as well. He had started out as a tough, uncompromising, confrontationally brave outsider who bulled his way into the upper reaches of science, infuriating some while charming others with his talent and swashbuckling zest for life. He used his physical strength, athleticism, and energy throughout his earlier life as a kind of shield, but he could be undiplomatic to the point of injuring his own causes. He could be extremely warm and generous but always insisted on doing things his own way. In this sense, his disability in the last years of his life paradoxically gave him a chance to gain in stature. This is what he did. As more and more was taken from him, as vanity was progressively laid aside, his persistent creativity in work was fueled increasingly by basic courage and selfless love for science. At the same time, he grew deeper in perspective, in empathy, and in the capacity for gratitude. He became truly admirable. His passing is a great loss.

Acknowledgments

Two members of the Davidson lab in the 1980's and early 1990's who have died since then were close friends and very helpful colleagues to me in those early years. They were founding-generation Davidson lab postdoc Barbara Hough-Evans and computational analyst (and Eric's football teammate) James Moore. Conversations with them during those years gave me considerable insight into research in the group and the currents of intellectual excitement at the time, and I owe them great debts for material included in this reminiscence. My own group's research program was one that benefited from some of the institutions that Eric helped to create, and the intellectual exchange that was an integral part of each of these institutions was in a way the most lasting benefit of all. Great thanks are also due to Andy Cameron for correcting my recollections in a draft of this article. I am grateful for support by an Albert Billings Ruddock Professorship of Biology at Caltech and grants to my lab from NIH.

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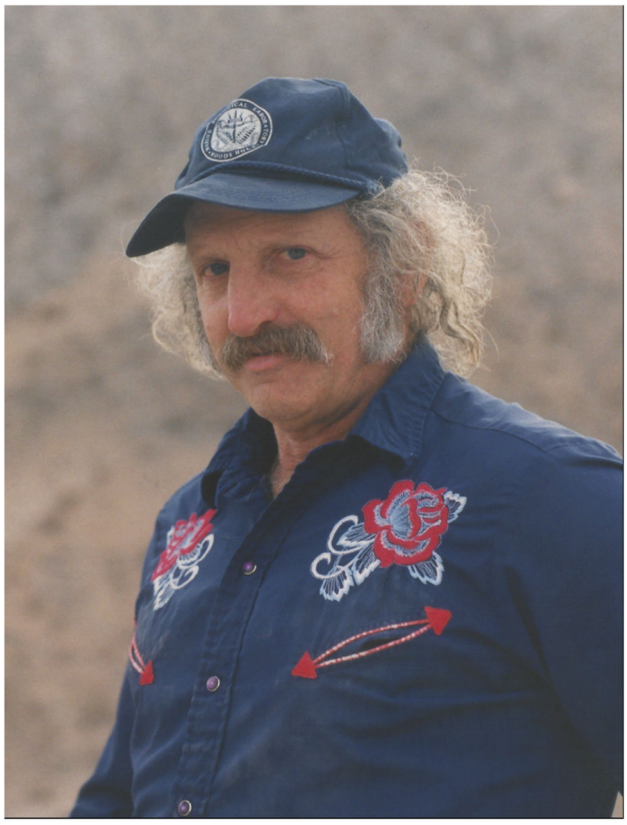

Fig. 1.
Eric Davidson on fossil hunting trip in California desert, November 1994

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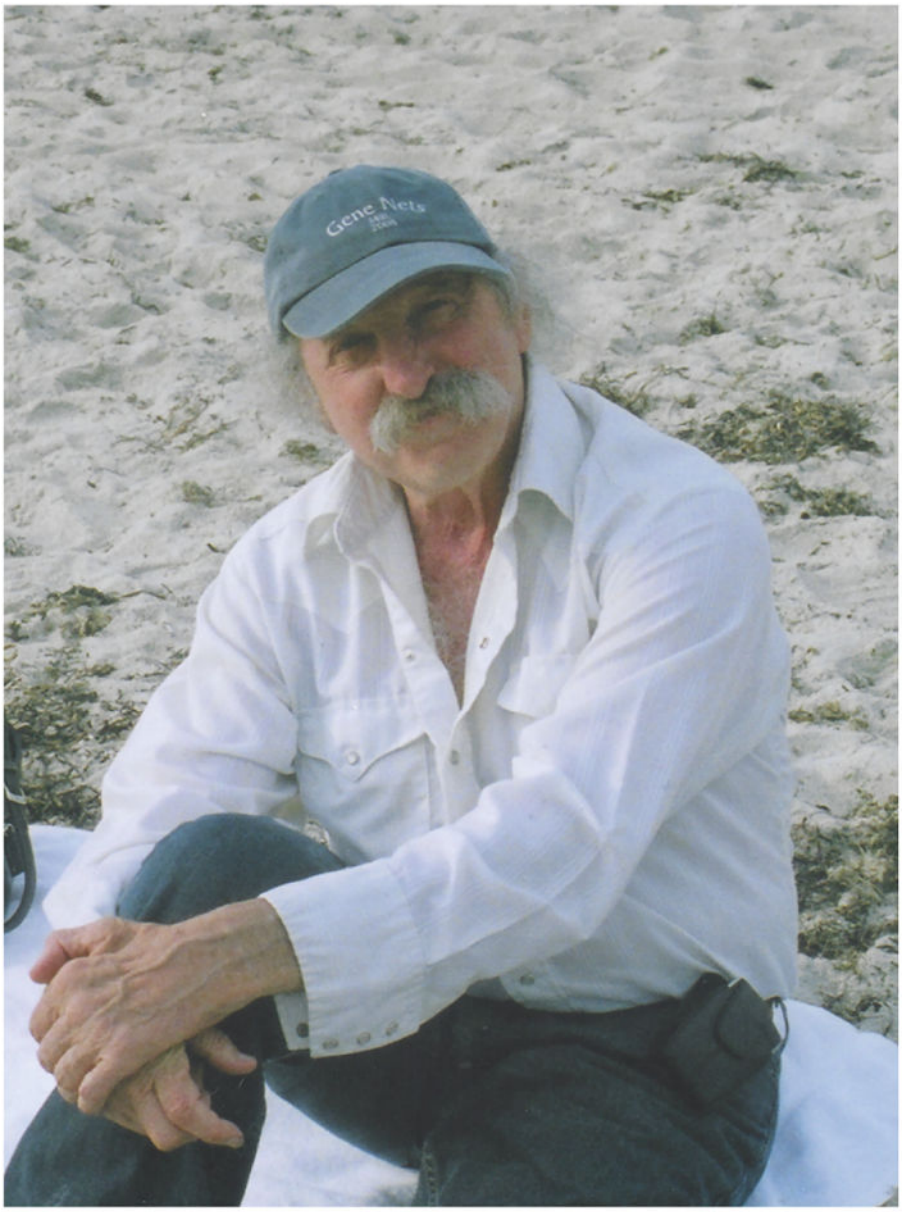

Fig. 2.
Eric Davidson on Nobska Beach, Woods Hole, MA, August 2009

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Fig. 3.
Eric Davidson near his home in Kinneloa Canyon, Pasadena, CA, February 2015