Genes Classical And Genes Developmental: The Different Uses Of The Gene In Evolutionary Synthesis

Scott F. Gilbert
Swarthmore College, sgilber1@swarthmore.edu
Genes Classical and Genes Developmental

The Different Use of Genes in Evolutionary Syntheses

SCOTT F. GILBERT

ABSTRACT

Dobzhansky (1964) stated that "Nothing in biology makes sense except in the light of evolution," and the function of the gene is no exception. The use of genes in population genetics and developmental genetics differs significantly. This is reflected in the roles that genes are postulated to play in evolution. In the Modern Synthesis of population genetics and evolution, genes become manifest by differences in alleles that are active in conferring differential reproductive success in adult individuals. The gene is thought to act as a particulate, atomic unit. In current syntheses of evolution and developmental genetics, important genes are manifest by their similarities across distantly related phyla, and they are active in the construction of embryos. These developmental genes are thought to act in a context-dependent network. In the population genetics model of evolution, mutations in genes provide insights into the mechanisms for natural selection and microevolution. Different individuals will be selected and their genes will be represented in higher proportions in the next generation. For the developmental geneticist, mutations in the genes provide insights into the mechanisms of phylogeny and macroevolution. Different modes of regulation may enable the production of new types of structures or the modification of existing ones. The importance of developmental approaches to the role of genes is exemplified by the discovery and subsequent analysis of the developmental gene MyoD.

The concept of the gene has had its own radiation once it entered into the territory of developmental biology. As Morange has shown (1996 and this volume), the concept of the developmental gene was a major insight, and it changed the way development was discussed. It also changed the ways the gene was discussed with regard to evolution. Developmental biology and evolutionary biology are converging on
a new synthesis for macroevolution, and this synthesis is very different from the Modern Synthesis of population genetics and evolutionary biology that accounted for microevolutionary processes (see Carroll 1997; Gerhart and Kirschner 1997; Gilbert 1997; Gilbert, Opitz, and Raff 1996; Hall 1992, 1996; Raff 1996). Moreover, the concept of the gene is very different between the two types of synthesis.

GENES IN THE MODERN SYNTHESIS AND IN DEVELOPMENTAL SYNTHESSES

There are several differences that distinguish the gene of population genetics from the gene of developmental genetics. The main difference concerns the levels of events being explained. The gene of the Modern Synthesis of the 1940s was an abstraction. It was not sequenced, its structure was unknown (and generally thought to be protein), and the mechanisms accounting for genetic change (mutation, recombination) were unexplained. Moreover, given the abstract, mathematical, nature of the gene, none of this mattered. The gene could be anything that had the properties of transmittal with infrequent change. Alleles of \( A \) and \( a \) did not even need to be DNA. The genes of the Developmental Synthesis (to use a convenient shorthand for these new syntheses) are specific sequences of DNA containing not only protein-encoding regions, but regulatory sequences such as promoters, enhancers, silencers, insulators, introns, 5' untranslated regions, and 3' untranslated regions (see Gilbert, 1997). Thus, for a population geneticist, the problem of \( Drosophila \) sex determination was solved as early as 1905 (Stevens 1905; Wilson 1905) when it was discovered that the females have two X chromosomes (XX) while males have but one (XY or XO). This information was necessary and sufficient for modeling populations. However, for a developmental geneticist, this is but the starting point. The mechanisms of sex determination involve the binding of specific proteins to specific bases of DNA and RNA, and they can differ widely between phyla. What is mechanism to the population geneticist is correlation to the developmental geneticist.

A second difference concerns the tension between constancy and divergence. The genes of the Modern Synthesis are manifest by the differences they cause (Dobzhansky 1937, 19-49; Goldschmidt 1952;
see Dietrich, Gifford, and Schwartz, this volume). These differences
could be selected. The genes of the Developmental Synthesis are
manifest by their similarities. That the Pax6 genes (encoding a par-
ticular transcription factor) are expressed in photoreceptive cells
throughout the kingdom indicates that they may have an important
role in photoreceptor development and evolution (Quiring et al.
1994). More importantly, there are conserved pathways of conserved
genes. For example, the BMP4-chordin pathway (by which chordin
blocks the epidermal induction of the ectoderm and permits the
ectoderm to develop neurons) is critical for neural specification in
both arthropods and vertebrates (see De Robertis and Sasai 1996).
PCR allows these genes to be discovered through their similarities.

A third, related difference concerns what aspects of evolution
these genes attempt to explain. The gene of the Modern Synthesis is a
gene that could explain the mechanisms of natural and sexual selec-
tion (hence, the allelic genes are manifest by differences in adult
phenotypes; Dobzhansky 1937; Lewontin 1974). The gene of the
Developmental Synthesis attempts to explain phylogeny. This mac-
roevolutionary program harks back to what Bowler (1996) calls the
“first evolutionary biology” – the attempt to discover the origins of
the different phyla and classes. Thus, differential Hox gene expres-
sion is postulated to have brought about (a) the transformation from
fins to limbs in vertebrates (Shubin, Tabin, and Carrol 1997; Sordino,
van der Hoeven, and Duboule 1995) and (b) the transformation of
limbs into maxillipeds during crustacean development (Averof and
Patel, 1997). Developmental syntheses look at the possibilities and
constraints for the arrival of the fittest, while population genetics can
model their survival. Both approaches are obviously needed to un-
derstand evolution.

This leads to a fourth difference between the genes of the Modern
Synthesis and those of the Developmental Synthesis. In the Modern
Synthesis, evolutionary change was conceived to originate from al-
terations in the coding region that altered the performance of en-
zymes or structural proteins. For example, did the gene make a
functional or nonfunctional protein? Did the gene encode a slower or
faster variant of the enzyme? The genes that are important in the
Developmental Synthesis are not those necessarily encoding meta-
bulic enzymes or structural proteins. Rather, they are genes encoding
signal transduction components or gene expression proteins (such as transcription factors and splicing factors). Moreover, the important portions of these genes are not so much the protein encoding exons as they are the regulatory regions of these genes or the portion of the protein that binds to these regions.

A fifth difference involves when and where these genes are expressed. The genes of the Modern Synthesis are expressed in adults competing for reproductive advantage. The genes of the Developmental Synthesis are expressed during the construction organs within the embryo (see Raff 1992; Waddington 1953).

The sixth difference between the gene of the Modern Synthesis and the gene of the Developmental Synthesis concerns atomicity. The gene of the Modern Synthesis was independent from all other genes. It might be physically close to other genes on the chromosome, and this might cause the physically linked genes to be inherited together, but gene action was an individual phenomenon. The gene acted as an autonomous unit. The genes involved in the Developmental Synthesis are not autonomous actors. First, many of them are linked in physical aggregates, such as the Hox genes. Not only are these Hox genes, themselves, conserved throughout evolution, but their linkage is conserved between arthropods and vertebrates. The reason for this close linkage appears to be that these genes share regulatory elements in their promoters and enhancers (Duboule 1994; Morange, this volume). Therefore, the entire entity – consisting of many linked genes – is a developmentally functional unit. Another example of genes linked together in a developmental sequence are the mammalian globin genes. Here, they are each regulated by a Locus Control Region. Whereas the Hox genes are ordered in the genome according to their spatial expression patterns, the globin genes are present in the genome according to their temporal expression patterns (see Martin, Fiering, and Groudine 1996). Second, the developmental genes are linked together into networks of interacting genes and gene products (Gilbert, Opitz, and Raff 1996). For instance, the deletion of the muscle-forming MyoD gene in mice does not lead to marked deficiencies of muscle development, since the MyoD protein suppresses the activation of the muscle-forming Myf-5 gene. In the absence of MyoD, this suppression is lifted and myf-5 can be expressed and transforms the cells into muscle (Rudnicki et al. 1993).
Studies of the classical limb fields and the imaginal discs have dissected them into numerous pathways of reciprocal activation and suppression by genes and their protein products (see Gilbert 1997, for review). In development the gene is not an independent entity, but is part of a pathway.

This lack of autonomy has important consequences. First, "what" a gene does depends upon its context. In the liver, enolase is a glycolytic enzyme, while in the lens cell, it's a structural crystallin (Piatigorsky and Wistow 1991). The GSK-3β gene (He et al. 1995) can play a role in the Wnt signal transduction pathway for fly segmentation or frog neural axis formation, or it can help regulate glycolysis. Beta-catenin can hold cells together as part of the desmosome or it can be a developmentally critical transcription factor, depending on the cell in which it is expressed (Schneider et al. 1996).

A second consequence of the context dependency of genes is that a gene's effects can differ when it is placed in a pathway containing different alleles of the other genes in that pathway. This constitutes the background effect well known to developmental biologists, immunologists, and clinical geneticists (Wolf 1995, 1997). For instance, in the formation of the limb, a gene deficiency in one individual may cause an absent limb; in a different individual the same genetic mutation may cause an absent thumb (Freire-Maia 1975). Certain histocompatibility alleles predispose mice to some disease, but only in particular strains. This has practical consequences for agriculture and pharmaceutical manufacture. The rationale for cloning both transgenic sheep and cattle is that the transgene does not function the same way when sexual reproduction places it in different backgrounds (Meade 1997; Schnieke et al. 1997).

The differences between the developmental and population approaches to evolution (and their different views of genes) were appreciated as early as 1953 by Conrad Hal Waddington. He claimed that in addition to "normative selection" (the elimination of less favorable phenotypes by natural selection), there must also be "stabilizing selection" within the embryo. At the same meeting where Waddington presented this view, J. B. S. Haldane (1953) concluded, "The current instar of evolutionary theory may be defined by such books as those of Huxley, Simpson, Dobzhansky, Mayr, and Stebbins. We are certainly not ready for a new molt, but signs of new organs are perhaps visible." He pointed to "a broader synthesis in the fu-
ture." This is what we are embarking upon now. The data of developmental genetics is complementing that of population genetics to provide a broader evolutionary synthesis that can explain macroevolutionary, as well as microevolutionary, phenomena.

**THE DISCOVERY OF A DEVELOPMENTAL REGULATORY GENE: MYOD**

The discovery of developmental genes has been accomplished by methods far removed from the ways that "classical" genes have been discovered. Classical genes were identified by looking for differences in individuals within populations; developmental genes have often been identified by looking at differences in the expression of these genes in a normal embryo. A muscle cell should be expressing a set of genes that differs from that of a fibroblast.

Michel Morange (this volume) has focused on one set of developmental genes – the homeotic (Hom-C/Hox) genes. As Morange and others have correctly noted, this work originally fell within the research programs of classical genetics, especially that of pseudoallelism. However, just as *Drosophila* is a very derived insect, so was research on the homeotic genes a very special case of developmental genetics. Most research in developmental genetics sought the causes of cell commitment and differentiation. This meant that one looked to see why a given cell became a muscle cell and not a fat cell, a skin cell and not a neuron, a blood cell and not a lymphocyte. The research on *Drosophila* maternal effect, segmentation, and homeotic genes was not in this category. It sought the means by which parasegments, segments, and compartments were specified. Each of these parasegments had nerves, blood cells, integument, etc., but arranged differently. The homeotic genes did not concern cell differentiation; rather, they regulated segment identity. So *Drosophila* research was studying a higher plane of development than most developmental geneticists, who were studying cell differentiation\(^1\) (see Emmons 1996).

I would like to trace the history that led to the identification of the first developmental gene involved in cell differentiation, the *MyoD* gene of vertebrates. This gene encodes a transcription factor protein that binds to a specific region of DNA, and the activation of this gene in any particular cell will transform that cell into a muscle precursor.

---

\(^1\) Emmons (1996)
The beginnings of this history are rooted in the search to find the eukaryotic equivalent of the operon. Sol Spiegelman and others had convinced biologists that differentiation was nothing but changes in protein synthesis, and the operon gave the first testable model concerning how different cells made different proteins (see Gilbert 1996). Britten and Davidson’s (1969) developmental operon hypothesis became one of the most quoted papers in all biology, and it predicted regulatory elements (sensors) in the DNA and diffusible regulators that would bind to them. This model would explain not only differential protein synthesis but also coordinated protein synthesis.² The program to find the eukaryotic operon can be divided into two main fronts – first, the search for the eukaryotic promoter, and second, the search for eukaryotic regulatory proteins. Both branches would be remarkably successful, and their first success was the discovery of MyoD – the “master regulatory gene” of muscle development.

The discovery of MyoD, unlike the discovery of the homeotic genes, was not a surprise. However, it did have its origins from a relatively unappreciated source – somatic cell genetics. Somatic cell genetics was one of Boris Ephrussi’s brainchildren, and it flourished during the late 1960s and early 1970s, declining precipitously by the 1980s (see Burian, Gayon, and Zallen 1991). Its technique was to fuse different types of cells together and look at the resulting state of differentiation. Most cell types (liver, neurons, melanocytes) lost their differentiated phenotypes when fused with other cells, so some intranuclear diffusible negative regulator was hypothesized for the disappearance of their specific differentiated functions (see Davidson, Ephrussi, and Yamamoto 1968). The nuclear constitutions of these hybrids were often unstable, and chromosome loss occurred. If particular lost chromosomes were correlated to the retention of a differentiation cell enzyme, then the genes for the negative regulatory protein (that would have repressed that “luxury” enzyme) could be postulated to reside on the absent chromosome. This was proposed to be the case for kidney-specific esterase (Klebe, Chen, and Ruddle 1970) and hepatic aminotransferase inducibility (Weiss and Chaplain 1971).

The exception to this rule was the skeletal muscle cell. First, myocytes retained their differentiated state in pure cell culture better than other cells. Second, proliferating myoblasts that had not yet made
contractile protein retained this commitment in culture (Konigsberg 1963). Third, several laboratories (Blau, Chiu, and Webster 1983; Ringertz, Krondal, and Coleman 1978; Wright 1981; see Pinney, de la Brousse, and Emerson 1990) found that when differentiating myoblasts were fused with other cells, not only was the muscle-specific phenotype retained, but the myoblasts could cause the nucleus of the other cell type to make muscle-specific proteins. There appeared, then, to be a positive regulator of muscle gene transcription. This agreed well with other experiments that showed coordinate transcriptional control of muscle gene expression (Devlin and Emerson 1978, 1979).

In 1984, Konieczny and Emerson showed that the mouse embryonic cell line C3H10T1/2 (generally called the T-one halfs) could be converted into stable proliferating myogenic, chondrogenic, or adipogenic cell lines following treatments that inhibited DNA methylation. They predicted that the high rate of myogenic phenotypes resulted from the activation of one or a very few regulatory loci. In 1986, Emerson’s laboratory and Weintraub’s laboratory both reported that the transfection of C3H10T1/2 cells with cDNA from either cultured muscle cells or from 5-azaC-treated C3H10T1/2 cells would transform the cells into myocytes (Konieczny, Baldwin, and Emerson 1986; Lassar, Paterson, and Weintraub 1986). The Weintraub group (Davis, Weintraub, and Lassar 1987; Weintraub et al. 1989) made cDNA copies of the mRNA, cloned them, and transfected the clones individually into the C3H10T1/2 cells. One of these clones, MyoD, was found to convert the C3HT101/2 cells solely into myoblasts, and at high frequency. Moreover, it converted freshly cultured endodermal gut cells, ectodermal neurons, and other cells as well, into skeletal muscle.

MyoD turned out to be a muscle-cell-specific transcription factor. It controls cell determination and differentiation by binding to regions of the DNA that precede several muscle-specific protein-encoding genes. It also binds to its own promoter to retain its own transcription, and it binds to the promoters or enhancers of other muscle-specific transcription factors to activate them, as well. (This MyoD-binding DNA sequence was discovered through a collaboration between Weintraub’s laboratory and David Baltimore’s group; Murre et al. 1989.)
This muscle differentiation research program was occurring at the same time as the *Drosophila* research program, yet along a fundamentally different line of approach. Charles Emerson is a well-known muscle developmental biologist; Hal Weintraub – until his recent death – was a major investigator of chromatin structure and transcription factors. Nowhere in this research program was a mutant used. This research program was strictly epigenetic. It was based on phenotype analysis – the appearance of muscle contractile proteins. When recombinant DNA became available, it was used to see if the cloned gene encoded a protein capable of changing the “phenotype” of the cell. Also, although other organisms were found to make *MyoD* as a muscle-specific transcription factor, that data did not play any major role in forming the research program or (at least initially) strengthening the program.

**DEVELOPMENTAL GENES AND THE REGULATORY NETWORK**

Homeotic genes and *MyoD* have been called master regulators. They are seen as being at the top of the developmental hierarchy, controlling the genes below them. Are they master regulators? Yes – the products of homeotic genes can convert a haltere segment into a wing or an antenna into a leg; *MyoD* can convert a neuron into a muscle if activated there. However, are they also “slaves,” genes that are themselves told what to do? Yes – homeotic genes such as *abd-A* are regulated to make the parapods in the abdominal segments of caterpillars (Carroll 1995; Carroll, Weatherbee, and Langeland 1995) and genes such as *Ubx* are regulated temporally to distinguish the third thoracic from the first abdominal segment (Castelli-Gair and Akam 1995). Some homeotic genes, such as the *Abd-B*-like *mab-5* in *C. elegans*, are regulated by the cell lineage in which the gene resides. In this last case, cell lineage plays a greater role than cell region in determining the gene’s expression (Harris et al. 1996; Salser and Kenyon 1996). Similarly, recent research suggests the paradoxical view that *MyoD* is both a master control gene and also one of the most tightly controlled genes in the genome.

So it appears that these master control genes are themselves under masterful regulation. There can be no top of the hierarchy in a life...
cycle. The hierarchy has become a network of interactions. *MyoD* is such a powerful protein that the cell must control it at all ectopic times and places so that it is not expressed in the wrong cell or at the wrong time. If even a small amount of *MyoD* is made, that cell will become muscle. So *MyoD* is regulated at transcription, RNA processing, and by at least two post-translational regulators\(^4\) (see Gilbert 1997). Governors govern the governor. Regulators must be regulated by factors that are themselves both regulated and regulators. Moreover, *MyoD* regulation works within a field – the limb field or the somite field – because the regulators are soluble proteins coming from outside the cell: BMP-4, Wnt-4, FGFs (Kopan, Nye, and Weintraub 1994; Li et al. 1992; Vaidya et al. 1989). The basic state of *MyoD* and the *Hox* genes is to be inhibited. Like so much in developmental biology, activation consists of inhibiting the inhibitor; suppression is the inhibition of the inhibitor of the inhibitor.

So these developmental genes have to be both regulators and regulatees. The things they regulate and the things that regulate them are part of a pathway. In the end, it is not the conservation of the gene that is important, but the conservation of these developmental pathways that include them. The inheritance is not of a gene but of a regulated network of genes and the binding regions for their products. The genes encoding GSK-3\(\beta\) and \(\beta\)-catenin are particularly instructive cases. They can be considered structural genes or developmental genes depending upon which tissue is being considered. This is to be expected from our knowledge of evolution. As Jacob (1977) noted, nature should use what it has before inventing something new. Proteins have multiple sites. The fact that a gene can be used for different purposes within the body should not be troubling except by those people trying to name the gene. The interesting questions of evolutionary biology will involve how these pathways were modified to bring about the formation of new cell types and new body plans during the development of life on earth.

**CODA**

The genes of the Modern Synthesis and those of the Developmental Synthesis are quite different. They were invoked to explain different aspects of evolution, and they emphasize different aspects of genetic
The discovery of the first developmental gene associated with cell commitment and differentiation, MyoD, was accomplished in a manner very distinct from the methods used to identify classical genes. Furthermore, the analysis of the MyoD and homeotic genes has given rise to a principle of developmental biology – that the major regulatory genes are themselves highly regulated in a complex network. It would not be surprising if different “alleles” of regulatory genes were to be found between closely related species and that morphological changes may involve changes in the dissociation constants between ligands and their receptors or between components of the chromatin around the promoter. While the molecular bases of macroevolution may reside in the changes in these networks of gene regulation, both the classical and developmental “natures” of the gene are required to account for evolutionary processes.

NOTES

1. The aptness of Christiane Nüsslein-Volhard’s address is worthy of noting: The Friedrich-Miescher Laboratorium on Spemannstraße. It combines the institutional authority of DNA (the lab named for the discoverer of DNA is part of the Max Planck Institut) and the epigenesis of Spemann. The perfect place for the molecular biology of development.

2. E. B. Lewis (1963) also used the operon model, but he used it very differently than developmental biologists. He saw it as a mechanism for sequential gene activation, not for the differentiation of particular cell types.

3. It would have been extremely difficult to discover MyoD by mutational analysis. There is overlapping redundancy in the myogenic bHLH transcription factors and Myf-5 can compensate for the absence of MyoD (Rudnicki et al. 1993; Wang et al. 1996). Mutants were eventually constructed to test the binding site of the MyoD protein (Murre et al. 1989; Davis et al. 1990).

4. The wisdom of the control genes appears to be Sophrosyne. This Apollonian principle of Greek ethics is characterized by the disciplined self-restraint of great power, or as Helen North (1966) defined it, “the harmonious product of intense passion under perfect control.”

REFERENCES


