Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

The Engrailed Story

Antonio Garcia-Bellido

Centro de Biologia Molecular, Consejo Superior de Investigaciones Cientificas, Universidad Autonoma de Madrid, Madrid 28049 Spain

IN 1972, a paper on the developmental genetics of the mutation *engrailed* appeared in this journal. Pedro Santamaria, then a Ph.D. student in my laboratory, and I co-authored it. Being involved, I find it hard to comment on the paper itself, on its impact when it appeared and on its later relevance. I will, therefore, try to navigate along the strait between Scylla and Charybdis, avoiding my own views and considering what others may have thought of the findings.

The story begins at Caltech in 1968. Edward B. Lewis, in charge of the Carnegie Collection of Drosophila mutant stocks, knew their adult phenotypes intimately. One of the many conversations in which we, together with Alfred H. Sturtevant, used to indulge was about homeotic genes. Lewis pointed out to me that *engrailed* (*en*) mutants had, in addition to the reported duplicate sex-combs in the male forelegs, a posterior wing margin similar to the normal anterior one. I had made preparations of adult morphogenetic mutant flies from the Caltech collection (mutants were usually observed only under the dissecting microscope) for detailed microscopic examination. Ed was right; under the light microscope there appeared along the posterior margin a secondary pattern of chaetae, typical of the anterior margin. Moreover, the specific corrugation of veins (swellings of the veins, from either a dorsal or ventral aspect) corresponded to a replacement of the posterior venation pattern by the characteristic anterior one. The characteristic chaeta pattern of the legs, including the secondary sex-comb, also showed replacement of the posterior by the anterior half; *engrailed* was clearly a homeotic mutation. And a peculiar one at that; contrary to others, it affected several segments in a homologous way. But many things were happening at that time in the Caltech lab, and I put the *engrailed* problem aside. It was to receive full attention upon my return to Madrid in 1969.

To understand what became interesting in the study of *engrailed*, we have to go back in time for a perspective as to how development and the genes controlling it were conceived. When I arrived at Caltech in 1967, I was invited to give a series of talks in the lab on the state of the art in developmental genetics, in Zurich in particular. I remember talking about determination and transdetermination and prepatterns, morphogenetic fields and blastemata, all terms that appeared metaphysical to Ed, although not to Sturt. In fact, these terms reflected the notions about development at the time, carried out as a continuation of an experimental tradition coming from Roux and Spemann. Blastemata were made of cells, certainly, but the morphogenetic information was in the population of cells. Regeneration experiments repeatedly showed regulative properties that determine, in some mysterious way, what the cells would differentiate into at the last minute before entering into irreversible differentiation. Genes at that time were just alleles with phenotypes that could be modulated by temperature or by many genetic modifiers present in the genome. Mutations led to perturbation of the norm, but there was really nothing else to understand because, after all, evolution was a historically contingent event—the result of changes of alleles to generate fitter morphologies. Researchers described mutants, looking for changes in enzymes or their products that by some complex mechanisms would generate cascades of interactions leading to the abnormality, *i.e.*, "phenogenetic trees of action." Homeotic mutant transformations, for example, supposedly resulted from some abnormalities in the dynamics of growth. Still the central search was for nonautonomous effects of mutations, in transplants or in mosaics, because they could lead to the construction of metabolic pathways.

However, things were changing. "Transdetermination," *i.e.*, a sudden change in prospective differentiation upon regeneration and culture of fragments of imaginal discs, was akin to a homeotic mutant transformation. But it could be experimentally manipulated. It seemed to take place suddenly, in groups of cells, as was experimentally shown later (Gehring 1967). Thus, transdetermination may result from "assimilative induc-

tion" between cells. I had tried in Zurich in 1964–65 to provoke this induction in cell mixtures, after dissociation of imaginal discs. Ernst Hadorn had earlier shown that dissociated and reaggregated cells from the same type of disc (from two identical discs labeled with different mutant cuticular markers) did form mixed patterns upon metamorphosis. In 1964, in the Hadorn lab Rolf Nothiger had shown that cells from different discs sort out, with some exceptional mosaics of isolated cells of one disc trapped in territories of cells of the other disc. Thus, there were no indications of assimilative induction. I showed in a series of experiments that not only do cells of different disc types sort out, but so do cells from different regions within a disc, while if derived from the same region they could partially reconstruct patterns or differentiate unpatterned elements of the region of origin (Garcia-Bellido 1966, 1967). Clearly, the cells of mature imaginal discs of Drosophila already had great personality. They were, singly, determined to form adult pattern elements, and they must have surface labels as to the position in the primordium, which they used for pattern reconstruction in reaggregates. Cells were no longer merely bricks for construction.

Induced mitotic recombination, first used to analyze development in genetic mosaics by Curt Stern and his group, had revealed the cell autonomous response of morphogenetic mutants in genetic mosaics to invariant morphogenetic fields ("prepatterns") (Stern 1954). It could also be used, as Hannah-Alava did, in the foreleg to trace clonal derivations of pattern elements. I used it with John Merriam at Caltech to describe the clonal parameters of wing disc development (Garcia-Bellido and Merriam 1971). I started with preparations I had made in Madrid of wings heterozygous for multiple wing hairs (*mwh*) irradiated as larvae and collected every 8 hr at puparium formation. The frequency of X-ray-induced mitotic recombination was reported to be low, and I expected only a few clones. But they would tell me how many cell divisions occur *in vivo* from mature larvae (the time I had made the cell dissociations) to the point of differentiation. It also had been reported that pupal discs undergo many cell divisions before differentiation, and this was contrary to the observation of a high degree of cell determination found in dissociated cells. To my surprise, adult wings of irradiated mature larvae contained hundreds of clones, each one cell in size. Larvae irradiated 8 hr earlier gave rise to wings with half the number of clones but twice the clone size, and the same trend was true for earlier irradiated larvae, with an average division time of 8 hr. Consequently, development could be described in terms of cells and clones, and in 1967, John and I became engaged in the study of the cell lineages of the wing. One of the first surprises was that clones could transgress neither the wing margin dorso-ventrally, nor the notum/wing boundary since very early in development (Garcia-Bellido 1968b). That meant the existence of determinative decision for cell territories. Chaetal mother cells separated clonally from trichome epidermal cells later, shortly before puparium formation. Thus, cells and cell decisions came to the foreground of developmental descriptions. The obvious next step was to combine cell markers with morphogenetic mutants, or lethals, in mosaics generated by mitotic recombination, at different times of development. I learned the required genetics from John and Ed and started a long project in Madrid of describing development in terms of cells and genes.

Well, not yet in terms of genes. I had come to Caltech to study the behavior of bithorax mutant cells in aggregates. My previous experience with other homeotic mutants, *aristapedia* and *Antennapedia*, was confusing (Garcia-Bellido 1968a). True, the homeotic leg cells of the antenna mixed well with leg and not with antenna cells, but the pattern limit of the mixing was more proximal in aggregates than in the mutant fly antenna. More specific limits of transformation were needed to exclude assimilative induction once and for all. This was provided by the two mutations, *bithorax* (*bx*) and *postbithorax* (*pbx*), with a net separation between wing homeotic tissue and haltere tissue along the middle of both the adult wing and the haltere. When the aggregates were made, the adult transformation boundary was respected; only *bx* homeotic cells will mix and reconstruct mosaic patterns with anterior wing cells, and the corresponding situation was true for *pbx* posterior haltere cells. The explanation for Ed was outright and categorical: "haltere posterior cells or haltere anterior cells cannot assimilate to become wing because in the mutant disc either the *bx* or the *pbx* cells are wildtype for the complementary region." This apparent tautology was all-revealing. Genes, *i.e.*, their wild-type alleles, were in charge of defining cell states, cell autonomously. For Ed it was clear because he had found gynandromorph mosaics of few *Ultrabithorax* (*Ubx*) cells differentiating mesothoracic chaetae in the metanotum (Lewis 1963). The work on *bithorax* aggregates was published much later (Garcia-Bellido and Lewis 1976), delayed by Ed's literary restraint.

Cell mosaics and cell aggregates revealed an undreamed of cell autonomy. These findings allowed the connection between cells, genes, and development, at least in my mind. And this was the title of the final seminar I gave in Caltech in 1968, before coming to Madrid.

One thing is left in the reflections about the background of the *engrailed* story. Once in Madrid, I gave a seminar in the Centro de Investigaciones Biologicas on my work at Caltech and assembled thereafter three Ph.D. students. One, Pedro Santamaria, was encouraged to work on the development of tergites (for comparison with the wing), Gines Morata to work on the clonal analysis of the *bithorax* mutants; and Pedro

Ripoll became engaged in the clonal analysis of lethals and genetic aneuploids in cells. I put myself to work on the dorso-ventral induction in wing vein formation. By so doing, I came to encounter very large wing clones, much larger than the earliest ones initiated in the embryo, but still normal-sized in tergites. How could that be? Early chromosome loss, as in gynandromorphs, but affecting the progeny of wing but not of tergite embryonic cells? I asked Gines to work it out. When he came up with the answer, along with Pedro Ripoll who had joined the enterprise, I could not believe it: it was too much cell autonomy, this time for the pace of growth. Clones were large because of a factor that could be mapped in the same chromosome arm of the *mwh* cell marker, and when removed by recombination led the cells to grow faster than their surrounding cells. The mosaic flies developed more slowly and had Minute chaetae, and this reminded me that C. Stern had used Minutes because on this background clones were large and thus could be easily detected under the dissecting microscope. The interesting thing here, however, is that the wing M^+ clones would respect not only the wing margin border, as normal clones do, but would also stop along a mysterious but constant line, running some cell diameters anterior to the fourth longitudinal vein of the wing. This is obviously the anterior-posterior clonal restriction boundary that separates an anterior from a posterior compartment, from the very beginning of development. The wing appeared then to be made of eight compartments consisting of growing cells that do not transgress the corresponding restriction lines after specific moments of development (Garcia-Bellido *et al.* 1973, 1976). Since early gynandromorph mosaics could have male/female boundaries running through the wing, compartments had to be polyclonal in origin.

This was the conceptual background that led to the analysis of *engrailed* (*en*), the real subject of this *Perspectives* essay. The 1972 paper (Garcia-Bellido and Santamaria 1972) contains two different sections. In the first, the clonal analysis of *engrailed* is subdivided into two parts, one describing how the mutant wing grows a "cell lineage", the other what *en* mutant cells do in "morphogenetic mosaics" within en^+ wings. The first part shows that the posterior part of the wing grows like the anterior one, with a very fuzzy clonal separation between anterior and posterior cells. In fact, cell aggregates of posterior regions of *engrailed* wing discs with whole wing disc cells showed mixing in both territories. The second part of the mosaic analysis revealed that mutant cells autonomously differentiate anterior structures in "homologous" posterior positions. In the second section, we showed the phenotype of double mutant combinations of *bx* and *pbx* with *en*, indicating that the duplicated posterior cells do not derive from anterior cells migrating to posterior positions. In the double *pbx en* mutants, the posterior haltere is transformed into an anterior wing. This observation demonstrated that the specification of the posterior part of the haltere is performed by the combined activity of pbx^+ and en^+ genes.

In the discussion section, in light of the notion of an invariant prepattern for segments and for posterior parts of segments to which mutant cells respond, it is pointed out that prepatterns become entities with no heuristic value in understanding morphogenesis. Thus, if any mutant transformation indicates that the corresponding alternative is its default condition or prepattern, *Contrabithorax* (*Cbx*) alleles, which show a wing to haltere transformation, would reveal the existence of an invariant haltere prepattern, and *Ubx* alleles that of a wing prepattern.

The developmental genetic analysis of *engrailed* and the subsequent one of the *bithorax* complex (Morata and Garcia-Bellido 1976) were the bases for the notion of selector genes. Systemic transformations, like these homeotic ones, affecting individual cells, meant that the abstract specification of whole cell territories (as to segment or compartment) resided in developmental operations carried out by the individual cells. Moreover, the expression of *engrailed* (and of the *bithorax* genes) is limited to these territories. These two steps required, in turn, other genes in addition. The model was put forward in a Ciba Symposium in 1974 (Garcia-Bellido 1975) that "activator" genes delimit the realm of expression of "selector" genes, which in turn control "realizator" genes, in charge of performing the actual cell behavioral operations that end in final morphologies. Since several selector genes act in combination in the same cell, development results from a combinatorial or parallel processing of specific genetic functions acting in single cells (Garcia-Bellido 1975; Garcia-Bellido *et al.* 1976). Compartments within clonal restrictions were the realm of expression of these genes: *bithorax*, *postbithorax*, and *engrailed*.

The work on *engrailed* has since followed different paths, each paradigmatic. One path extends the notion of selector gene to other segmentation genes, like *bithorax*, *Antennapedia*, *proboscipedia*, and others. Their adult transformations and later the larval phenotypes of lethal null alleles of these genes (first done by Ed for the *bithorax* complex) revealed an underlying logic. Different segments require the function of different selector genes to specify alternatives to an evolutionary primitive segment, made of two parts, both corresponding to the anterior prothorax-mesothorax of the actual flies and by extension of insects (Garcia-Bellido 1977).

The 1972 observation that anterior wing cells and posterior *en* wing cells do not distinguish between each other in aggregates was confirmed by Gines and Peter A. Lawrence in clones, as posterior *en* M^+ clones invade the anterior compartments, but not vice versa (Morata and Lawrence 1975). This in turn indicates that *en* is not required in the anterior compartment.

This cell behavior [as well as the phenotype of *Cbx* mutants, corresponding to dominant gain of function alleles of *Ultrabithorax* (*bx* and *pbx*)], strongly suggested that these genes were real morphogenetic genes controlling developmental pathways (a term coined by Ed in contrast to metabolic pathways) in specific territories of the fly, while not active in others. These inferences could only be confirmed later, when it became possible to visualize gene expression patterns, after the advent of molecular techniques applied to the study of these genes.

The molecular analysis of *engrailed* was carried out by Thomas Kornberg and his colleagues (Poole *et al.* 1985) and extended by Patrick O'Farrell and his colleagues (Kuner *et al.* 1985). The *en* gene encodes a protein with a homeodomain (similar to that of *Ubx*), a transcription factor that binds to DNA and regulates the expression of other genes. The work of several laboratories analyzed the patterns of embryonic expression of early segmentation genes, corresponding to zygotic lethal mutations discovered by Eric Wieschaus and Christiane Nüsslein-Volhardt in 1978 (Nüsslein-Volhard and Wieschaus 1980). Three hierarchical classes of genes showed patterns that subdivided the syncytial egg and later the early blastoderm into shorter and shorter regions. Their expression depended on positive and negative controls by the earlier acting genes and, between them, finally specifying cell territories corresponding to embryonic segments. Among these genes were those controlling the limits of expression of *engrailed* and other segmental selector genes. They corresponded to the "activator" genes of the hierarchical model of selector genes. By making use of protein DNA crosslinking and cytological mapping of the *en* protein to salivary chromosomes, the search for the *en* down-stream "realizator" genes started later. It still continues, but among these genes are other selector genes (like *Ubx* and *en* itself) and genes encoding ligands (like *hedgehog*, *hh*) in cell-cell communication. The latter are possibly required to maintain the coherent expression of cell territories specified by *en*. But how these realizator genes control the characteristic behavior of compartment cells, how these systemic signals are converted into compartment-specific morphogenesis, is still unknown.

With the possibilities of manipulating the *en* gene in transgenic flies, it became possible for the first time to express it ectopically; if it is expressed in anterior compartment cells, it causes a transformation of an anterior into a posterior compartment (Zecca *et al.* 1995).

DNA sequences or antibodies raised against the homeodomain of *engrailed* have found it to be conserved throughout metazoan evolution. The gene *en* or its Drosophila paralog *inv* are expressed in insects and crustacea in the posterior half of embryonic segments, but also in the mesoderm and in certain types of neurons. This expression, related not to territorial domains but to histotypes and cell types, is also conserved. In fact, in the leech, arthropods, and vertebrates, *en*/*inv* orthologs are expressed in certain cephalic segments in specific neurons, as well as in somites (Wedeen and Weisblast 1991; Holland *et al.* 1997). In vertebrates, midbrain development requires *engrailed*, and how this relates to the insect functions is unclear. The problem of how the evolutionary functions of these selector genes arose and later diversified remains a central issue in metazoan evolution.

Mutations other than those in selector genes cause homeotic transformations in flies or in clones. They correspond to failures in function of genes involved in the activation of selectors or in the maintenance throughout the rest of development of the initial state, active or repressed. Their phenotypes (*e.g.*, *trithorax* or *Polycomb*) correspond to those of alleles of the selector genes. We now know that this maintenance, memory of gene activity, is because of changes in the chromatin organization of the activated selector gene, with positive regulatory feedback loops. These transformations are actual pathway substitutions. But a new type of gene has recently been found whose ectopic expression causes, at least in epidermal structures, the appearance of a complete eye, *i.e.*, a field of ommatidia with perfectly differentiated light receptor cells (Halder *et al.* 1995). In some mutants of the gene *eyeless* (*ey*) there is complete absence of eyes. This gene is also conserved in metazoans and expressed in primordia of primitive light receptor cells. Those ectopic eye territories result from recruitment of surrounding epidermal cells to differentiate into eyes, another case of assimilative induction. This peculiar behavior of *ey* has led to the coining of a new term, that of "master" genes, to be distinguished from "selectors" because they open developmental pathways, not merely substitute archetypic ones. But there are many cases of genes of the histotypic differentiation class that have similar functions, as *myo-D* for muscle cells (Olson 1990), the *achaete-scute* complex for neuronal specification (Campuzano and Modolell 1992), the *tinman* homologs related to the formation of the heart (Lilly *et al.* 1994), *vestigial* for the wing, and others that could be considered in principle also to be master genes.

engrailed plays a very important morphogenetic role, in addition to acting as a selector gene within compartments. Its function generates the transcription of a signal (*hh*) that releases a cascade of genetic effects on the cells on the other side of the compartment border. Those are mediated by a receptor that activates a nuclear gene, which in turn controls the transcription of another signal, in the wing the ligand *decapentaplegic* (*dpp*) (Zecca *et al.* 1995). The A/P boundary becomes then a reference for cell proliferation and for cell polarity, with symmetries at both sides of the clonal restriction border. In fact, the embryonic effects of *en* lethal alleles are changes in both the differentiation and

the polarity of epidermal segments. For that reason, it is included in the class of segmentation genes called "segment polarity" genes (Nüsslein-Volhard and Wieschaus 1980). Compartment borders and, in general, clonal restriction borders are associated with both the changes of polarity of cells and the references or signals for cell proliferation. Thus, *apterous* (*ap*), a recently discovered selector gene for the dorsal compartment of wing and haltere (Diaz-Benjumea and Cohen 1993), also starts a cascade of genetic effects on both sides of the dorso/ventral (D/V) border. Interestingly, *ap* is expressed in the wing primordium before the clonal restriction appears, and only later does its expression become restricted to the dorsal polyclone; mosaics of mutant *ap* cells close to the dorsal border become incorporated into the ventral polyclone (Blair *et al.* 1994). However, when these mosaics appear in central regions of the wing, they cause a D/V duplication growing perpendicular to the wing blade, formed by surrounding wild-type dorsal cells (Diaz-Benjumea and Cohen 1993). Thus, *ap* mutants fail to grow wings, possibly because the D/V border cannot be formed, and hence the signal for cell proliferation emanating from it fails to appear. Similarly, if the gene *engrailed* is ectopically expressed in clones in the anterior compartment of the wing, the associated signals generate a new border and cell proliferation ensues, giving rise to mirror-image duplicated A/P patterns (*e.g.*, veins) made by surrounding normal wing cells (Tabata *et al.* 1995; Zecca *et al.* 1995). Interestingly, the size of these duplications depends on the position of the clone (*en* or *ap*) within the compartment and not on the size of the clone; duplications are larger when the clones arise further away from the corresponding compartment border. These, and results with other types of mutations, indicate that growing compartments are internally heterogeneous, with discontinuities related to presumptive veins (also associated with clonal restrictions) and positional values between them (Garcia-Bellido and deCelis 1992). This is not the place to go into details, but it is through studies of these discontinuities that we eventually will connect selector function in actual morphogenesis with the species-specificity of sizes and shapes of organs.

We have seen that the *en* embryonic compartment appears in groups of cells. This is so because they result from the subdivision of a continuous blastoderm. Further subdivision of the primordium is also polyclonal for the same reasons. Possibly, the coherence of the territory specified by selector genes results from signals between cells [like *hedgehog* (*hh*)] maintaining the selector activity in all the cells. How these later subdivisions appear, for example, that of *ap* generating the D/V symmetry, is still a mystery. Possibly, it reflects undetected heterogeneities of expression of other genes within the primordium, as happens for *en* and *bx* in the embryo. This may explain why we failed to assimilatively induce metathoracic cells to be converted into mesothoracic ones in gynandromorph mosaics of *Ubx* mutant cells in the embryonic metathorax (Miñana and Garcia-Bellido 1982). But that cells communicate with each other in genetic specification is evident, for example in the specification of territories with partial transformations owing to homeotic hypomorphic alleles. In these chimeric territories the expression of, *e.g.*, the Ubx protein occurs in contiguous patches not related by clonal origin (Botas *et al.* 1988). We have mentioned that the same applies to transdetermination events, which affect groups of cells. But the cell interactions at work in the transdetermination or in homeotic changes go beyond specifying cell type. The apposition of transdetermined (allotypic) territories to similar (autotypic) territories, owing to cell recognition, is associated with mirror-image copies of their patterns, or their integration into one single pattern, although both cell territories are of different clonal origin (Garcia-Bellido 1972). Thus, the phenomenon of assimilative induction eludes our understanding. Much more knowledge about active genomes defining cell behavior is obviously needed to explain development in terms of genes and cells.

LITERATURE CITED

Blair, S. S., D. L. Brower, J. B. Thomas and M. Zabortink, 1994 The role of apterous in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of Drosophila. Development **120:** 1805–1815.

Botas, J., C. V. Cabrera and A. Garcia-Bellido, 1988 The reinforcement extinction process of selector gene activity: a positive feed-back loop and cell-cell interactions in Ultrabithorax patterning. Roux's Arch. Dev. Biol. **197:** 424–434.

Campuzano, S., and J. Modolell, 1992 Patterning of the Drosophila nervous system: the achaete-scute gene complex. Trends Genet. **8:** 202–208.

Diaz-Benjumea, F., and S. M. Cohen, 1993 Interactions between dorsal and ventral cells in the imaginal disc directs wing development in Drosophila. Cell **75:** 741–752.

Garcia-Bellido, A., 1966 Pattern reconstruction by dissociated imaginal disc cells of *Drosophila melanogaster.* Dev. Biol. **14:** 278–306.

Garcia-Bellido, A., 1967 Histotypic reaggregation of dissociated imaginal disc cells of *Drosophila melanogaster* cultured in vivo. Wilhelm Roux's Arch. Entwicklungsmech. Org. **158:** 212–217.

Garcia-Bellido, A., 1968a Cell affinities in antennal homeotic mutants of *Drosophila melanogaster.* Genetics **59:** 487–499.

Garcia-Bellido, A., 1968b Cell lineage in the wing disc of *Drosophila melanogaster.* Genetics **60:** 181.

Garcia-Bellido, A., 1972 Pattern formation in imaginal disks, pp. 59–91 in *Results and Problems in Cell Differentiation*, Vol. 5, edited by H. Ursprung and R. Nöthiger. Springer-Verlag, Berlin.

Garcia-Bellido, A., 1975 Genetic control of wing disc development in *Drosophila*, pp. 161–182 in Ciba Found. Symp. 29, *Cell Patterning.*

Garcia-Bellido, A., 1977 Homeotic and atavic mutation in insects. Am. Zool. **17:** 613–629.

Garcia-Bellido, A., and J. F. DeCelis, 1992 Developmental genetics of the venation pattern of Drosophila. Annu. Rev. Genet. **26:** 277–304.

Garcia-Bellido, A., and E. B. Lewis, 1976 Autonomous cellular differentiation of homeotic bithorax mutants of *Drosophila melanogaster.* Dev. Biol. **48:** 400–410.

Garcia-Bellido, A., and J. Merriam, 1971 Parameters of the wing

imaginal disc development of *Drosophila melanogaster.* Dev. Biol. **24:** 61–87.
Garcia-Bellido, A., and P. Santamaria, 1972 Developmental analysis of the wing disc in the mutant engrailed of *Drosophila melanogaster.* Genetics **72:** 87–101.
Garcia-Bellido, A., P. Ripoll and G. Morata, 1973 Developmental compartmentalization of the wing disc of Drosophila. Nat. New Biol. **245:** 251–253.
Garcia-Bellido, A., P. Ripoll and G. Morata, 1976 Developmental compartmentalization in the dorsal mesothoracic disc of *Drosophila melanogaster.* Dev. Biol. **48:** 132–147.
Gehring, W., 1967 Clonal analysis of determination dynamics in cultures of imaginal disks in *Drosophila melanogaster.* Dev. Biol. **16:** 438–456.
Halder, G., P. Callaerts and W. J. Gehring, 1995 Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. Science **267:** 1788–1792.
Holland, L. Z., M. Kene, N. A. Williams and N. D. Holland, 1997 Sequence and embryonic expression of the amphioxus engrailed gene (AmphiEn): the metameric pattern of transcription resembles that of its segment-polarity homolog in Drosophila. Development **124:** 1723–1732.
Kuner, J. M., M. Nakanishi, A. Zehra, B. Drees, E. Gustavson *et al.*, 1985 Molecular cloning of *engrailed*: a gene involved in the development of pattern in Drosophila melanogaster. Cell **42:** 309–316.
Lewis, E. B., 1963 Genes and developmental pathways. Am. Zool. **3:** 33–56.
Lilly, B., S. Galewsky, A. B. Firulli, R. A. Schulz and E. N. Olson, 1994 D-MEF2: A MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during Drosophila embryogenesis. Proc. Natl. Acad. Sci. USA **91:** 5662–5666.
Miñana, F. J., and A. Garcia-Bellido, 1982 Preblastoderm mosaics of mutants of the bithorax-complex. Wilhelm Roux's Arch. Dev. Biol. **191:** 331–334.
Morata, G., and A. Garcia-Bellido, 1976 Developmental analysis of some mutants of the bithorax system of Drosophila. Wilhelm Roux's Arch. Dev. Biol. **180:** 125–143.
Morata, G., and P. A. Lawrence, 1975 Control of compartment development by the engrailed gene in Drosophila. Nature **255:** 614–617.
Nüsslein-Volhard, C., and E. Wieschaus, 1980 Mutations affecting segment number and polarity in Drosophila. Nature **287:** 795–801.
Olson, E. N., 1990 MyoD family: a paradigm for development? Genes Dev. **4:** 1454–1461.
Poole, S. J., L. M. Kauvar, B. Drees and T. B. Kornberg, 1985 The engrailed locus of Drosophila: structural analysis of an embryonic transcripts. Cell **40:** 37–43.
Stern, C., 1954 Two or three bristles. Am. Sci. **42:** 213–247.
Tabata, T., C. Schwartz, E. Gustavson, Z. Ali and T. B. Kornberg, 1995 Creating a Drosophila wing de novo, the role of engrailed and the compartment hypothesis. Development **121:** 3359–3369.
Wedeen, C. J., and D. A. Weisblast, 1991 Segmental expression of an engrailed-class gene during early development and neurogenesis in an annelid. Development **113:** 805–814.
Zecca, M., K. Basler and G. Struhl, 1995 Sequential organizing activities of engrailed, hedgehog and decapentaplegic in the Drosophila wing. Development **121:** 2265–2278.