Synthesizing Embryology And Human-Genetics: Paradigms Regained

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The only way to get from genotype to phenotype is through development, so nearly all mutations can be seen as affecting developmental processes. Whether a gene encodes a protein needed for limb morphogenesis or for the color of the iris, the effect is developmental. Indeed, a century ago, genetics and embryology were considered to be the same thing. However, within the ranks of fin de siècle physiological embryologists, a problem arose as to which compartment of the zygote—the nucleus or the cytoplasm—contained the hereditary-developmental potentials. Scientists such as T. Boveri and E. B. Wilson claimed that the nucleus contained the potentials, while other embryologists, such as T. H. Morgan, claimed that these inherited potentials lay within the cytoplasm (for details, see Gilbert 1979). This conflict formed the basis for the emergence of genetics out of embryology.

Study of the X chromosome led from embryology to genetics. The correlation between nuclear karyotype and organismal phenotype strongly suggested that the number of X chromosomes determined sex—i.e., the nucleus contained the potentials (Stevens 1905a, 1905b; Wilson 1905a, 1905b). Morgan’s research, first on parthenogenetic hickory aphids and later on Drosophila, began as an attempt to disprove that hypothesis. Instead, he reluctantly confirmed it. In doing so, he and his laboratory established the gene theory and set genetics on a course that diverged from embryology. By 1926, Morgan claimed that in the past a lot of confusion had resulted “from confusing the problems of genetics with those of development” (Morgan 1926, p. 27).

Genetics and development went their separate ways, evolving their own techniques, rules of evidence, favorite organisms, journals, vocabulary, and paradigmatic experiments. Genetics textbooks stopped discussing embryology, and embryology texts ceased discussing genes. However, in the 1930s, certain scientists—among them R. B. Goldschmidt, E. E. Just, J. Huxley, B. McClintock, B. Ephrussi, S. Gluecksohn-Schoenheimer, and C. H. Waddington—still believed that the two fields could be reconciled. Each proposed a different synthesis of embryology and genetics.

Salome Gluecksohn-Schoenheimer (now Gluecksohn-Waelsch) brought a combination of Morgan’s genetics and Spemann’s embryology to the study of mammalian development. Gluecksohn-Schoenheimer had been trained in experimental embryology in Hans Spemann’s laboratory but found that she could not perform the traditional types of experimental analyses on mouse embryos: “It is not possible yet to use transplantation, isolation, or vital staining methods on mammalian embryos as they have been used on amphibian embryos. . . . For the present, however, the experimenter is not able ‘to alter the course of events at a chosen point in a chosen manner and draw conclusions on their relations from the resulting changes’” (Gluecksohn-Schoenheimer 1938, p. 573). To study mouse development, Gluecksohn-Schoenheimer traced the mutant phenotypes back to their embryological causes “by studying the details of the results of the ‘experiment’ carried out by the gene” (Gluecksohn-Schoenheimer 1938, p. 573). This approach to development certainly differed from that of experimental embryology and would be carried out by a new type of scientist, the “developmental geneticist.”
Gluecksohn-Schoenheimer looked at the T-locus of the mouse and related the abnormalities to deficiencies of the mesodermal induction system: "The effects of the two alleles T and t on notochord and mesoderm might suggest that the two alleles act on two different structures. However, if considered from the embryological point of view, the notochord and mesoderm of the mouse have the same origin, namely in the tissue of the wall of the primitive gut" (Gluecksohn-Schoenheimer 1940, p. 399). Other mutations, such as kinky, fused, and short-tail, were also seen as encoding substances involved in organogenesis. Gluecksohn-Schoenheimer hoped that the genetic analysis of these murine mutants would shed light on the inducer molecules themselves and would lead to insights into the control of gene activity during mammalian development.

However, very few investigators were able to follow this program. The analysis of mammal development proved extremely difficult, as mammals generally have few and small embryos per litter and have evolved the remarkable trick of developing within another organism. Gluecksohn-Waelsch, Hans Grünberg, and others isolated several mouse mutations that affected development, but these mutations did not tell us much about how mammalian genes acted or whether human developmental malformations occurred in ways similar to those of mice. At the Oak Ridge National Laboratory symposium on mammalian genetics and reproduction, Hans Grünberg reviewed murine developmental genetics and reflected that "for most of the mutants individually as for the problem of gene action in general, the retrograde analysis by means of morphological methods will not carry us much farther than it has done" (Grünberg 1960, p. 55). Grünberg would repeat this pessimistic appraisal in The Pathology of Development (Grünberg 1962, pp. 278–279) 2 years later. Meanwhile, at the Oak Ridge symposium, Salome Gluecksohn-Waelsch presented her studies, not on genetic variants of mouse morphology but on genetic variants of mouse hemoglobin. She pointed to studies on globin and other protein variants as having "set a pattern for future research in mammalian genetics, which promises to lead us further toward our understanding of the physiology of the gene" (Gluecksohn-Waelsch 1960, p. 97).

So, a second paradigm emerged. If it is not possible to study gene activity in actual mammalian embryos, study it in cultured cells and in developing adult cells, such as lymphocytes and erythrocytes. In the 1950s and 1960s, embryology had expanded to become developmental biology and had allowed such extrapolations to be made (Weiss 1959; Monroy and Moscona 1966). This program has proved extremely successful. The thalassemias and related disorders have told us much about gene regulation at the transcriptional, processing, translational, and posttranslational levels. X-chromosome inactivation has demonstrated a remarkable mammalian mechanism of gene dosage compensation. Mutations of human hormones and their receptors (e.g., ILGF-1, androgen receptor, etc.) have shown the developmental importance of these molecules. However, while these studies gave us enormously important information about gene expression, they told us little about the development of the mammalian embryo.

Recently, this situation has changed. The genetics of the mammalian embryo is again becoming the object of intense research. David Baltimore has noted: "We learned about five years ago how to add genes to a mouse, and we learned over the past two years how to subtract genes.... Together, these new approaches have compensated for the slow breeding and cost of mice, and promise to make the 1990s the Decade of the Mouse" (Baltimore 1991). We are at the point where we can analyze the effects of normal or altered genes responsible for the development of the mammalian phenotype. Indeed, we are on the verge of a Renaissance of mammalian developmental genetics. In this day of DNA hybridization and gene transfer, one can reasonably extrapolate from mice to humans. During the spring of 1991, four such extrapolations have shown some of the new ties forming between mouse development and human genetics. These experiments are paradigmatic of the new relationship being forged between development and genetics.

Transgenic Mice: Sex Determination

The first situation concerns the SRY (sex-determining region—Y chromosome) gene for human testes determination. Using the DNA from human XY females and XX males, the laboratories of David Page and Albert de la Chapelle (Vergnaud et al. 1986) found that the gene responsible for human male sex determination was located on a particular region of the short arm of the Y chromosome. Sinclair et al. (1990), using more refined DNA probes, further delimited this region to a 35-kb fragment of DNA. Within this region is an open reading frame capable of synthesizing a 223-amino-acid protein. The ability of frameshift mutations and point mutations within this open reading
sequence to cause XY individuals to develop as females further confirmed the notion that the SRY gene initiated testes determination. But more definitive proofs could only come from genetic manipulation in mouse embryos. Here, the mouse analogue (Sry) of the human SRY gene was inserted into the genome of XX mouse embryos, and it caused some of them to become males (Koopman et al. 1991). The extrapolation is that the human SRY gene similarly regulates male sex determination in humans.

**Mutant Mice Produced through Chimeras with Genetically Altered ES Cells: The Hox 1.5-deficient Mouse**

A second interaction between embryology and human genetics concerns the ability to manufacture mouse mutants of developmentally interesting genes. The *hox* genes of mice and humans are closely related to the homeotic genes of *Drosophila* that encode transcription factors that specify the anterior-posterior body axis. Moreover, the mammalian *hox* genes are found in the same order, on their respective chromosomes, as are their *Drosophila* counterparts, and this gene order on the chromosomes likewise reflects the order of anterior boundaries of gene expression along the anterior-posterior axis (Harding et al. 1985; Duboule and Dolle 1989; Graham et al. 1989). Since the *Drosophila* homeotic genes specify which organs will form in a particular segment, is it possible that *hox* genes also specify anterior-posterior organ formation, despite the enormous divergence, in developmental programs, between mammals and insects? (Mammals form their segment-specific structures by reciprocal inductive interactions, while insects form theirs by evaginations of ectodermal imaginal disks.)

Chisaka and Capechi (1991) were able to mutate a cloned *hox-1.5* gene by inserting a neomycin-resistance gene into the region encoding the protein’s DNA-binding site. When this gene was added to mouse inner-cell-mass blastomeres (ES cells), they became incorporated into their genome and, by homologous recombination, could replace one of the alleles. These *hox-1.5*/*hox-1.5* heterozygous blastomeres could be selected by neomycin resistance and could be injected into a normal embryo. The resulting chimeric mouse was then bred to a wild-type mouse to create a heterozygous line which was then interbred to generate mice that were homozygous wild type, homozygous *hox-1.5* mutants, and heterozygotes. The mice that died within 12 h proved to be *hox-1.5* mutant homozygotes. The syndrome that resulted in death included the absence of parathyroids and thyroids, dysmorphogenesis of the heart and major blood vessels, and abnormalities of cervical cartilage. Here is a condition very similar to that of DiGeorge syndrome. The aberrant cell type(s) is not known, but this will be a project for classical embryological transplantation procedures.

**Isolation of the Human Regulatory Gene by the Homologous Murine Gene: The Formin Genes**

Clinicians know that certain abnormalities come in pairs or triads. If a kidney is missing, one should also look for limb and ear deformities. Many of these syndromes are known to be inherited in a Mendelian fashion, and are assumed to be caused by a single gene pair. How do multiple developmental anomalies arise from the malfunction of a single gene pair? One possibility is that the gene product is necessary for the development of a structure that influences several other tissues. (This would formally be the case with sex determination, which is a “syndrome” involving gonads, cartilage, bone growth, etc.) Another possibility is that the gene product is essential for the function of a structure that itself forms portions of several organs. (This would be the case, for instance, if the *hox-1.5* gene specified the positional information of neural crest cells that form facial cartilage and the mesenchyme of the parathyroids and thyroids.) A third possibility is that the gene product is necessary for the morphogenesis of several different organs. Mass et al. (1991) have shown that the murine limb-deformity (*ld*) gene is homologous to the human limb-deformity (*LD*) gene and that it is expressed in kidney and limb buds, which is where the major malformations caused in the human syndrome arise.

In this case, the research was first done on the mouse. Fortuitously, an insertion mutant was detected in experiments involved with transgene-induced tumorigenesis. This transgene insertion caused an *ld*-like phenotype that segregated with naturally occurring alleles at this locus and was expressed concurrently with the appearance of the mutant phenotype. Three of the five mutant-*ld* strains of mice contain disrupted transcripts at this locus. The naturally occurring mRNA from this “formin gene” region could then be used to isolate the human homologue from a genomic DNA library. The exons and 5’-flanking regions of these genes are extremely well conserved. By cloned DNA derived from a human formin exon,
the expression pattern of the human gene could be determined, and the chromosomal location of the human gene was found by in situ hybridization. Although there are several renal aplasia–limb-deformity syndromes, none has a phenotype identical to that of the murine condition. However, we now know the chromosomal location and expression pattern of a gene that may be critical in human development. The use of transgenic mice as models for inherited human developmental anomalies is just beginning.

Organ Culture and Antisense Oligodeoxynucleotides (a-ODNs): The Genetic Control of Cell-Cell Interactions

Since the time of R. G. Harrison, organ culture techniques have been used to study development, but these techniques have been of limited use for genetic analysis. This is changing because of the blending of organ culture with the recombinant-DNA technology of a-ODNs. When added to cells, the a-ODNs bind to a complementary sequence of DNA or RNA and prevent the transcription, processing, or translation of that particular complementary sequence. It has recently been found that cells in tissue and organ culture can incorporate these a-ODNs from the medium. Thus, the a-ODNs allow one to make a physiological analogue of a specific mutation. This technique has recently been used to study the epithelial/mesenchymal transformation from cardiac endothelial cells. This transition is essential for the formation of the atrial and ventricular septa, the defects of which constitute a majority of congenital cardiac malformations. Potts et al. (1991) have shown that when an a-ODN specific for growth factor TGF-β3 is added to the medium in which portions of the chick heart tissue have been cultured, the normal transformation that occurs in the culture is inhibited. Recent studies by Sariola et al. (1991) show that a-ODN to the nerve growth-factor receptor inhibitors nephron formation and subsequent ureter bud branching during rat kidney morphogenesis in culture. Here, the a-ODNs were added directly onto the organ anlage as they developed in culture.

Recombinant-DNA technology has revitalized old embryological questions, has strengthened the ties linking embryology and genetics, and has made possible new studies of gene expression during mammalian embryogenesis. But it must be remembered that development is more than the realization of inherited genetic potentials. There is no one-to-one mapping of genotype onto phenotype. (If there were, the concordance rates of identical twins would always be 100%.) Genes are always presented in an environmental context, and there are numerous reasons for the ability of a genotype to generate a variety of possible phenotypes. The first reason concerns the statistical probability of certain developmental phenomena. X-chromosome inactivation is one of these phenomena in which chance plays a definite role. Two XX human embryos with identical genotypes may develop different phenotypes because of the random inactivation, in a particular organ, of one X chromosome versus the other. The placement of blood vessels in capillary beds is another example.

The second reason concerns developmental plasticity. There are several examples of this in invertebrates, but developmental plasticity is most characteristic of mammalian development. Mammals have evolved genetic systems that can respond even to environmental factors that do not exist in nature. The memory B cells of genetically identical organisms differ when the organisms have been exposed to different sets of antigens. Similarly, studies of songbirds and rats (Nordeen and Nordeen 1988; Alvarez-Buylla et al. 1990; Black et al. 1990) show that neuronal connections change in response to learning. Here, different synapses are formed by different experience. Development is more than just genetics. An individual with a multiple-personality disorder shows a wide spectrum of behaviors despite having a single genotype. In this age of the Human Genome Project and the enormous successes of the genetic approach to development, this is important to remember.

We thereby return to a second paradigm—the plasticity of the genome. This plasticity was first suggested by T. H. Morgan (Morgan 1934) and was elaborated on by L. C. Dunn when he related embryology to genetics (Dunn 1965, pp. 190–191). This view that “the genes are changing in some way during development” has been confirmed in lymphocyte differentiation and in the remarkable studies showing that methylation and the expression patterns of certain genes depend on their being packaged in the nuclei of the egg and the sperm. Moreover, research on morphogenically important genes in Drosophila and mammals has made the “one gene–one peptide hypothesis” anachronistic; as shown in the formins, differential RNA processing can make several different proteins from the same gene. As Pontecorvo stated in his reflections on the T-locus, “this is probably only a foretaste of what we are likely to find when we pass from the
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analysis of simple genes, like those providing the information for such a minor matter as the mere synthesis of an enzyme . . . to the genetic organization necessary to carry the information for morphogenetic processes” (Pontecorvo 1958, p. 64).

The past year has seen a revitalization of those studies seeking the genetic basis of mammalian development. A few years ago, it was feared that molecular biology might overwhelm and destroy embryology. While certain fears remain, it is possible that molecular biology may create a renaissance in the study of the mammalian embryo: paradigms regained.

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