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Review

The new dysmorphology: Application of insights from basic developmental biology to the understanding of human birth defects

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ABSTRACT **Information obtained from studies of developmental and cellular processes in lower organisms is beginning to make significant contributions to the understanding of the pathogenesis of human birth defects, and it is now becoming possible to treat birth defects as inborn errors of development. Mutations in genes for transcription factors, receptors, cell adhesion molecules, intercellular junctions, molecules involved in signal transduction, growth factors, structural proteins, enzymes, and transporters have been identified in genetically caused human malformations and dysplasias. The identification of these mutations and the analysis of their developmental effects have been greatly facilitated by the existence of natural or engineered models in the mouse and even of related mutations in *Drosophila*, and in some instances a remarkable conservation of function in development has been observed, even between widely separated species.**

A remarkable picture was recently featured on the cover of a journal—the head of a fruitfly (*Drosophila*) with a well formed eye located on one of the antennae (1). This eye, as well as other ectopic eyes on wings and legs, had been produced by the targeted expression of the cDNA for the *Drosophila* eyeless (*ey*) gene in the imaginal disks that gave rise to the eye-bearing structures. Perhaps even more remarkable was the image of an ectopic eye produced with the targeted expression of a cDNA from the mouse *Pax6* gene, which, in its mutant form ($Pax6^{Sey}$), causes the disorder Small eye. *Pax6* and *ey* are genes for homologous transcription factors which have two DNA-binding domains, a paired domain (named after the *Drosophila* paired-rule genes) and a homeobox domain (after the *Drosophila* homeotic genes).

Contrast these images with the description of a human newborn with bilateral anophthalmia (absence of the eyes), choanal atresia (obstructed nasal passages), microcephaly, and numerous other anomalies of the brain (2). The child's mother had aniridia (absence of the iris), and the father had only cataracts and decreased visual acuity. Genetic analysis revealed that the father and mother each had a different nonsense mutation of the *PAX6* gene (also termed Aniridia) and that the child was a compound heterozygote. As the gene symbols indicate, the human *PAX6* and mouse *Pax6* genes are homologous and, therefore, the human *PAX6* and *Drosophila ey* are also homologous.

As might be expected, the pathology observed in the infant with the two *PAX6* mutations was quite similar to that found in mouse fetuses homozygous for the $Pax6^{Sey}$ mutation. However, flies homozygous for *ey* mutations also have reductions or complete absence of their compound eyes. Thus, it appears that the same evolutionarily conserved "master control gene" (3) is essential for development of the eye in species as divergent as *Drosophila* and *Homo sapiens* and, therefore, that "the genetic control mechanisms for development are much more universal than anticipated" (1).

The implications of the eyeless–Small eye–Aniridia story for understanding the pathogenesis of human birth defects are enormous. It may seem too good to be true to expect that the same gene will subserve very similar functions throughout evolution. Nevertheless, the findings do suggest that there is much to be learned about the genesis of human birth defects from the study of developmental mechanisms, not only in closely related species such as the mouse but also in quite distant species and phyla as well.

Consider now the following clinical case. Three children in a family had a syndrome of optic nerve colobomas (clefts) and other associated eye abnormalities, renal hypoplasia, and vesicoloureteral reflux (passage of urine from bladder to ureter) (4). Their father also had optic nerve colobomas and evidence of renal dysfunction. This family thus appeared to have an autosomal dominant syndrome affecting two quite distinct developmental systems—the eye and the urogenital (kidney and ureter) system. Therefore, based on the known expression pattern of *Pax2* in mice—in developing kidney, eye, and other parts of the central nervous system—and on the phenotype of the transgene-induced mutation *Krd* (kidney and retinal defects), in which *Pax2* was deleted (5), the *PAX2* gene in the affected individuals was analyzed. A single base change, which resulted in a presumed truncation of protein product and elimination of an octapeptide motif, was found (4).

This family is instructive for many reasons:

(*i*) It illustrates the perplexing nature of many human birth defect syndromes in which seemingly unrelated abnormalities are present.

(*ii*) The ability to correlate the known pattern of expression of a specific gene (*Pax2*) during early development of the mouse with the clinical picture of the affected humans made its human homolog (*PAX2*) a candidate for further examination.

(*iii*) Similarly, the existence of a phenotypically similar mutant mouse (*Kdr*) with a known mutation affecting the same gene strengthened its status as a candidate gene for the human disorder.

(*iv*) Finally, the discovery that the syndrome could be attributed to a mutation of *PAX2*, after having previously identified a human birth defect resulting from mutation in another related gene, *PAX6* (and another one, to be discussed below, in *PAX3*), indicates that it is now possible to think of a class of human disorders resulting from mutations in a family of related genes of developmental importance.

Inborn Error of Development

The term birth defects can be defined in many ways, but for the purpose of this review I shall restrict the definition to alterations of structure or form (dysplasias) and malformations that are not mechanical or physical in origin. It has been estimated that there are >1750 inherited human disorders with altered morphogenesis, of which >1000 are multiple defect syndromes (6). In addition, there are a very large number of multiple malforma-

Abbreviations: WSI, Waardenburg syndrome type I; HMG, high mobility group; bHLH, basic helix–loop–helix; G protein, guanine nucleotide-binding protein.

tion syndromes that result from chromosomal aneuploidy.

It is now becoming possible to treat birth defects as "inborn errors of development" or "morphogenesis" in obvious analogy with and as an extension of the classical inborn errors of metabolism (7, 8). In fact, some birth defects are caused by enzyme deficiencies, which clearly fall within the category of such metabolic errors. Although it is still too early to group birth defects according to sequential developmental pathways, as is done with the metabolic disorders resulting from enzyme deficiencies and as can be done with several groups of mutations in *Drosophila*, they can be grouped by the functional or structural similarities of the mutant genes. Such a classification of several human birth defects based on this approach is presented (Table 1). This table illustrates how our understanding of the pathogenesis of human birth defects has been furthered by knowledge of molecular and developmental mechanisms derived from the study of lower organisms, *Drosophila* in particular, and of basic mechanisms of cell biology. Much has been learned in just the past few years; in fact, many of the entries in Table 1 are based on information published while this review was being written.

As was pointed out in the introductory examples of disorders resulting from mutations in *PAX2* and *PAX6*, relevant genetic models for human defects may exist in the mouse, and the models for the conditions listed in Table 1 are summarized in Table 2. These mouse mutants, whether derived from spontaneous or engineered mutations, have aided in the identification, analysis, and/or validation of the human mutations. The ability to use the mouse mutants in this manner has been greatly facilitated by the existence of regions of conserved synteny between the human and mouse genome, which makes it possible to correlate mouse and human genes and mutant phenotypes based on the known homologies between the chromosomal regions in which they are located (47).

In the following sections, I shall review the progress that has been made in using information that has been obtained from the study of development and cellular processes in lower organisms to understand the pathogenesis of human birth defects. In doing so, I shall restrict my attention to conditions that are caused by mutations in systems of transcriptional regulation, signal transduction, and cellular adhesion and communication. Many birth defects are also caused by abnormalities of enzymes, transporters, and structural proteins, but they are not discussed because of lack of space. Furthermore, again for the sake of space, clinical phenotypes will not be elaborated in the text but will be briefly summarized in the tables.

Transcription Factors

The two examples of disorders resulting from mutations in *PAX* genes serve as an introduction to the consideration of human disorders caused by defects in transcription factors, which are presumed to have important roles in developmental processes. Such factors are recognized by the existence of specific nucleotide sequences or motifs in their genes and, therefore, of specific amino acid sequences in the corresponding proteins. Five motifs are represented among the human birth defects listed in Table 1.

***PAX* Genes.** The mammalian *PAX* genes contain a paired box motif coding for a 128-amino acid domain homologous to that found in the paired segmentation gene and other *Drosophila* developmental genes. In addition, the *PAX* genes also contain a conserved octapeptide sequence and/or all or part of a paired-specific homeodomain (see below for discussion of homeodomains) (11, 48). In the mouse, and presumably in humans as well, the *Pax* genes (with the exception of *Pax1*) are expressed in embryogenesis along the entire anterior–posterior axis in a tissue-specific manner, including the central nervous system (48).

Three human disorders attributable to mutations of *PAX* genes are now known. The human and mouse *PAX2* and *PAX6* disorders have already been discussed. Of note in the human cases is that whereas possession of a single *PAX6* mutation generally causes only aniridia (2), compound heterozygosity for two mutant alleles results in anophthalmia and severe craniofacial abnormalities. These findings have been interpreted as indicating a dosage relationship between gene expression and the severity of the abnormalities (2).

The third set of *PAX* mutations affect *PAX3* and result in dominantly inherited Waardenburg syndrome type I (WSI) in humans and Splotch in mice. The identification of the WSI gene as a *PAX3* mutation was facilitated by the prior delineation of mutations in the presumed homologous mouse gene *Pax3* (the mutant gene is designated $Pax3^{Sp}$) (49). However, unlike humans with WSI, Splotch heterozygotes do not have hearing loss. Analysis of *PAX3* in WSI has revealed both point mutations and deletions that interfere with the binding of the protein to DNA, and these mutations are thought to involve loss rather than gain of function (9).

Pax3 is expressed during early neurogenesis in the dorsal neuroepithelium, in several regions of the developing brain, in various neural crest derivatives, and in the limb mesenchyme (49). Abnormal emigration or migration of neural crest-derived cells has been implicated in the pathogenesis of the defects in Splotch, and it has been suggested that there may be a disturbance of neural cell adhesion molecule (N-CAM) processing (49).

A missense mutation of Asn-47 in *PAX3* has been detected in the Klein–Waardenburg syndrome (WSIII), just 3 amino acids away from a mutation causing WSI (10). Another mutation of Asn-47 has been found in the craniofacial–deafness–hand syndrome, and it has been postulated that these mutations are acting as dominant negatives (11). The differences in phenotypes among these conditions make it clear that allelic differences can result in what may be considered distinct clinical syndromes.

Zinc Finger Domains. The zinc fingers, common domains found in many transcription regulatory proteins, consist of sequences of amino acids containing pairs of cysteine and histidine residues that bind a Zn(II) ion and form finger-like DNA-binding structures (50). Tandem repeats of the zinc finger motif are found in many regulatory proteins, and mutations in *GLI3*, a member of the family of genes related to the oncogene *GLI* and to the *Drosophila* gene Krüppel, have been identified in patients with the autosomal dominant Greig cephalopolysyndactyly syndrome (12). Support for the conclusion that *GLI3* is truly the responsible gene was derived from identification of a deletion in the homologous *Gli3* gene in mice with the phenotypically similar disorder Extra-toes (caused by the mutant gene $Gli3^{Xt}$) (39). Mice heterozygous for $Gli3^{Xt}$ also have polydactyly and an enlarged interfrontal bone analogous to the broad nasal root and forehead in the human condition. *Gli3* is highly expressed in the brain, limb bud mesoderm and, later, interdigital mesenchyme, and the head mesenchyme of developing embryos (39). It has been speculated that deficiency of *GLI3* causes a decrease in programmed cell death, leading to an increase in mesodermal condensation in the limb buds, causing polydactyly, and to decreased cell death in the interdigital mesenchyme, causing syndactyly (39).

Mutations of a zinc finger gene have also been identified in the Wilms tumor suppressor gene *WT1* in people with the Denys–Drash syndrome (13). Expression of *WT1* is high in the developing fetal gonads, metanephric blastema, and glomeruli (51). In view of the fact that deletions of *WT1* result in Wilms tumor alone (or, when the deletion extends into *PAX6*, of aniridia as well), but not the Denys–Drash syndrome, it has been speculated that the particular mutations causing this syndrome may act as dominant negatives (52).

High Mobility Group (HMG) Boxes. The presumed master control gene for mammalian testis development is *Sry* (sex-

Table 1. Classes of mutations causing human birth defects (dysplasias and congenital malformations)

Gene	Disorder	Ref.
Transcription factor(s)		
PAX		
PAX2	Optic colobomas, renal dysplasia, vesiculoureteral reflux	4
PAX3	WSI [deafness, dystopia canthorum (wide-spaced eyes), pigment abnormalities]	9
	WSIII [WSI with camptodactyly (finger contractures), proximal insertion of thumbs, limb malformations]	10
	Craniofacial–deafness–hand syndrome	11
PAX6/AN2	Aniridia (heterozygotes); anophthalmia (homozygotes)	2
Zinc fingers		
GLI3/GCPS	Greig cephalopolysyndactyly (postaxial polydactyly of hands, preaxial polydactyly of feet, macrocephaly, broad nasal root, prominent forehead)	12
WT1	Denys–Drash syndrome (Wilms tumor, male pseudo- or true hermaphroditism, renal insufficiency)	13
HMG domain		
SOX9	Campomelic dysplasia (male sex reversal with failure of testicular development, congenital bowing and angulation of long bones, missing ribs, cleft palate, small thorax)	14, 15
bHLH leucine zipper		
WS2	WSII (WSI without facial dysmorphology)	16
POU domain		
POU3F4/DNF3	X-linked mixed deafness (stapes fixation, abnormal dilatation of internal acoustic canal)	17
Msx homeodomain		
MSX2	Craniosynostosis (fusion of bones of skull), Boston type (with short first metatarsals)	18
Receptor/signal transduction		
Growth factor receptors		
FGFR1	Pfeiffer syndrome (craniosynostosis, large thumbs and great toes, interphalangeal ankylosis)	19
FGFR2	Crouzon syndrome (craniosynostosis)	20
	Jackson–Weiss syndrome (craniosynostosis, broad great toes with medial deviation, tarsal–metatarsal coalescence)	21
	Pfeiffer syndrome	22
	Apert syndrome (craniosynostosis with severe syndactyly)	23
FGFR3	Achondroplasia (chondrodysplasia with proximal shortening of long bones, relative macrocephaly, exaggerated lumbar lordosis in heterozygotes)	24
	Thanatophoric dwarfism [micromelia (small limbs), relative macrocephaly with frontal bossing, small thorax, neonatal lethality]	25
Hormone receptor		
PTH-PTHrP receptor	Metaphyseal chondrodysplasia, Jansen type (short limbed dwarfism with hypercalcemia and hypophosphatemia)	26
Other receptors, cell adhesion molecules, and gap junctions		
RET/HSCR1	Hirschsprung disease type 1 (aganglionic megacolon)	27, 28
Endothelin B receptor/*HSCR2*	Hirschsprung disease type 2 (aganglionic megacolon)	29
KAL	Kallmann syndrome (anosmia, hypogonadotropic hypogonadism)	30, 31
Connexin43	Complex cardiac malformations with visceroatrial heterotaxias	32
G proteins		
FGD1	Aarskog–Scott syndrome (disproportionate short stature, shortening of distal extremities, vertebral defects, facial defects, urogenital anomalies)	33
LIS1/MDCR	Lissencephaly, component of Miller–Dieker syndrome	34
GNAS1	Albright hereditary osteodystrophy: pseudohypoparathyroidism (mental retardation, short stature, short metacarpals, tissue calcifications, hypocalcemia) and pseudopseudohypoparathyroidism (same phenotype with normocalcemia)	35
Growth factor		
IGF2	Beckwith–Wiedemann syndrome (somatic and visceral overgrowth, neonatal hypoglycemia, embryonal tumors including Wilms)	36, 37

PTH-PTHrP, parathyroid hormone–parathyroid hormone-related peptide.

determining region Y), the product of which contains a HMG box, an ≈80-amino acid DNA-binding motif (14). A subgroup of HMG box-containing genes coding for proteins with >60% homology to *SRY* has been termed the *SOX* (*SRY*-type HMG box) genes. One member of this group, *SOX9*, has been found to contain mutations in the presumed autosomal dominant syndrome of XY sex reversal and campomelic dysplasia (14, 15). Because of its chromosomal location in the mouse and the presence of similar skeletal abnormalities, although not sex reversal, it is conjectured that *Sox9* is the gene responsible for the mouse mutation Tail-short (*Ts*) (14, 41). Since the detected mutations appear to cause loss of function or haploinsufficiency, *SOX9* is thought to be active in a dosage-sensitive pathway (15). *SOX/Sox9* is expressed in sites of cartilage deposition and genital ridges and gonads during fetal life (14, 53).

Basic Helix–Loop–Helix (bHLH) Leucine Zippers. A form of Waardenburg syndrome without facial dysmorphology is known as Waardenburg syndrome type II (WSII). On the basis of its similarity to the recessive mouse mutation microphthalmia (*mi*) and the location of the loci for the

Table 2. Mouse models of known mutations causing human birth defects

Human gene	Mouse gene	Mouse disorder	Ref.
Transcription factors			
PAX			
PAX2	*Pax2/Kdr*	Kidney and retinal defects	4
PAX3	*Pax3^{Sp}* (Splotch)	White spotting, widely spaced eyes	38
PAX6	*Pax6^{Sey}* (Small eye)	Small eyes in heterozygotes; no eyes or nose in homozygotes	2
Zinc fingers			
GLI3	*Gli3^{Xt}* (Extra toes)	Extra digits on hind limbs; homozygotes have polydactyly all limbs, skeletal and central nervous system abnormalities	39
	Gli3^{add} (anterior digit pattern deformity); transgene induced	Elongated thumb, bent second digit	40
HMG domain			
SOX9	*?Ts* (Tail-short)	Short kinked tail, vertebral fusions, shortened skull, occasional short bent forelimbs, extra ribs	41
bHLH leucine zipper			
WS2	*mi* (microphthalmia)	Microphthalmia, deafness, pigmentary defects, osteopetrosis	42
Receptor/signal transduction			
RET (HSCR1)	*retk^{-}*	Aganglionic megacolon; renal agenesis in homozygotes	43
EDNRB	*s^{l}* (Piebald-lethal)	White spotting, aganglionic megacolon	44
Connexin43	*Gja1*	Enlarged conus of the heart with obstruction of right outflow tract (pulmonary stenosis)	45
Growth factor			
IGF2	Paternal disomy 7 (with *Igf2*)	Fetal overgrowth	46

two conditions in homologous chromosomal regions, the human homolog of *mi*, *MITF*, was analyzed and mutations were detected. *MITF/mi* was found to encode a bHLH leucine zipper protein, a putative transcription factor interacting with a potential melanocyte-specific promoter element (16).

The mouse mutations in *mi* are known to affect neural crest-derived melanocytes and retinal pigmented epithelium (54). Although several of the mouse *mi* mutations act as dominant negatives *in vitro* (42, 54), two human mutations in *MITF*, which are splice site abnormalities, are thought to act through haploinsufficiency (54).

POU Domains. Based on its chromosomal location and temporal and spatial expression during mouse embryogenesis, the gene *POU3F4* was considered to be a candidate gene for DFN3, a common form of X chromosome-linked mixed conductive and sensorineural deafness (17), and mutations in the POU-specific and POU homeodomains were found.

MSX Homeobox Family. In vertebrates, the gene *MSX2*, a member of the homeobox gene family defined by the *Drosophila* gene *msh*, is expressed in craniofacial structures, particularly in the region of the calvarial sutures, heart, limb buds, and otic and optic vesicles (18, 55). In mapping the location of the gene responsible for the Boston form of human autosomal dominant craniosynostosis (premature fusion of the bones of the skull), a missense mutation of a highly conserved amino acid in *MSX2* was detected (18).

***HOX* Genes.** Throughout this discussion of transcription factor mutations, reference has been made to homeobox or *Hox/Hox* genes. These genes contain a 183-bp region that encodes a 61-amino acid DNA-binding domain. This domain is present in several classes of *Drosophila* homeotic developmental control genes, genes that control the specification of different body segments. Of these, the genes in the homeotic complex, HOM-C, which is composed of the Antennepedia and Bithorax clusters, control the organization of the fly body along the anterior–posterior axis (56–58). In mice and humans there are four clusters of *HOX/Hox* genes, with each cluster containing different arrays of homologs of some or all of the *Drosophila* HOM-C genes (57). The homeologous genes in each of the different mammalian clusters are referred to as paralogs. The expression of the mouse *Hox* genes follows the same anterior–posterior orientation as that of their *Drosophila* homologs, with the most 3′ genes being expressed earlier and most anteriorly and being most responsive to retinoic acid (57).

Because of the central role of homeogenes in *Drosophila* and presumably mammalian development, it would be expected that mutations of *HOX/Hox* genes would show up among human birth defects or spontaneous mouse mutants (59). However, none has been detected as yet. The reasons for this are unclear, since it has been possible to create many types of abnormal mice by the deletion or insertion of *Hox* genes by homologous recombination or transgenesis, respectively (56, 57). One example of note is that a gain of function mutation of *Hoxa7*, a gene in the middle of the complex, results in severe craniofacial abnormalies and abnormalities of the axial skeleton interpreted as representing a posterior homeotic transformation (60). It is of interest that the abnormalities in the *Hoxa7* mutants are quite similar to those resulting from the teratogenic effects of retinoic acid (60). Overall, these experiments have shown that the *Hox* genes, like their *Drosophila* counterparts, are truly homeotic complexes. Loss-of-function mutations generally resulted in anterior homeotic transformations in the axial skeleton or neural crest (with specific structures taking on the identities of more anterior structures), and gain-of-function mutations caused posterior transformations (57).

Receptors and Signal Transduction

Although transcription factors may be central to the regulation of gene expression, developmental processes rely on the transfer of information along and within cells. With the delineation of receptors and signal transduction pathways likely to be important in morphogenesis has come the identification of several human birth defects attributable to mutations in these systems. Most striking in this regard has been the elucidation of the mutations responsible for several forms of chondrodysplasias—skeletal abnormalities attributable to defects in chondrogenesis.

Growth Factor Receptors. Achondroplasia, in the heterozygous form, is the most common form of dwarfism. When homozygous, it is a much more severe and lethal disorder. The gene for achondroplasia was identified by linkage mapping and found to encode the receptor for fibroblast growth factor 3 (FGFR3), a gene that is expressed in many tissues,with highest levels in the brain. Virtually all affected individuals have the same mutation in the transmembrane domain of this

receptor tyrosine kinase protein (24), and it has been postulated that the mutation is acting as a dominant negative, even though it causes much more severe effects in homozygotes.

Very similar in phenotype to homozygous achondroplasia is thanatophoric dwarfism, a sporadic lethal disorder. Analysis of *FGFR3* has revealed two characteristic amino acid substitutions in the kinase extracellular domains, each in a specific subgroup of the disorder (25). In addition, mutations in the intracellular domain have been found. It is clear, therefore, that the exact site and nature of the mutation can affect the phenotype and that different mutations in the same gene can result in different although related phenotypes. The limited number of different mutations detected in these conditions suggests that other mutations in the gene are likely to be lethal.

The developmental expression of *FGFR3* has been studied in mice. In bone, it has been found to be high in the cartilage rudiments of developing bone and later, during endochondral ossification, only in resting cartilage (61). This pattern is distinct from that of *Fgfr1* and *Fgfr2*, which, later in development, are expressed predominantly in osteoblasts and hypertrophic cartilage and in perichondrium and periosteum, respectively. From this it has been inferred that the three different FGFRs must perform different functions during the latter stages of bone development (61), a conclusion to be considered critically in view of the following group of disorders.

Following the discovery of the mutations in achondroplasia, several other syndromes with skeletal abnormalities were examined, and several were found to be caused by mutations in *FGFR1* and *FGFR2* (Table 1) (62). Of note are the facts that identical mutations were found in cases of Crouzon and Pfeiffer syndrome and that Pfeiffer syndrome can result from mutations in either *FGFR1* or *FRGF2*. Furthermore, it is noteworthy that bony abnormalities, particularly of the digits, are present in several of the disorders caused by *FGFR1* and *FGFR2* mutations. It is well known that FGF4, one of the nine FGFs that may interact with one or more of the four FGFRs, is intimately involved in limb morphogenesis (63, 64) and that several FGFs can influence limb development (65). Less is known about the roles played by the different receptors, but the human findings should provide valuable insights into their functions. Furthermore, in view of the roles attributed to Sonic hedgehog (*Shh*), *Wnt7*, and Bone morphogenetic proteins 2 and 4 (*Bmp2* and *Bmp4*) in limb development (63, 64), it is likely that mutations in these and related genes will also soon be implicated in birth defects with abnormal limb development.

Hormone Receptors. Hormone receptor defects have long been recognzied as the cause of abnormal sexual differentiation, as, for example, testicular feminization resulting from mutation of the androgen receptor in XY individuals (66). In this condition, the anomalous sexual development is directly attributable to lack of androgen effect during all stages of development.

Mutation of the gene encoding the parathyroid hormone–parathyroid hormone-related peptide receptor, a member of the class of guanine nucleotide-binding protein (G protein)-coupled receptors, has been shown to be the cause of a form of short-limbed dwarfism, the Jansen-type metaphyseal chondrodysplasia, which is associated with hypercalcemia and hypophosphatemia (26). The mutation causes a dominant gain of function with constitutive activity of the receptor, which is believed to result in abnormal formation of enchondral bone.

Other Receptors, Cell Adhesion Molecules, and Gap Junctions. Hirschsprung disease, or aganglionic megacolon, is associated with congenital absence of the neural crest-derived myenteric and submucosal plexuses of the distal colon. Mutations in the genes for two different cell surface receptors have been identified in familial Hirschsprung disease (HSCR). In HSCR1, point mutations have been found in the *RET* oncogene, a receptor tyrosine kinase with cadherin motifs that may be involved in cell–cell interactions (27, 28). In the mouse, a targeted mutation in mouse *ret* (termed ret^{-}) causes, when homozygous, total intestinal agangliosis, as well as renal agenesis (43).

A missense mutation of the endothelin B receptor gene, which codes for a G-protein-coupled receptor, has been identified in persons with HSCR2. Persons heterozygous and homozygous for this mutation have a 21% and 74% risk, respectively, of developing Hirschsprung disease (29). Again, there is a mouse model for this condition, the piebald-lethal (s^{l}) mouse, with either natural or targeted mutations in the endothelin B receptor gene resulting in white spotting and aganglionic megacolon (44). Similar results were obtained with mice having targeted mutations of the gene for the ligand endothelin 3 (44). These findings implicate interaction of endothelin 3 with the endothelin B receptor in development of both enteric neurons and melanocytes.

Both of the genes just discussed code for products required for the proper development and migration of cells that take up a final location at sites distant from their place of origin. The same also applies to the gene *KAL*, responsible for X-linked Kallmann syndrome. The cardinal features of this syndrome are anosmia and hypogonadotropic hypogonadism, both stemming from the failure of neuronal migration (67). However, unilateral renal aplasia and other defects that may correlate with the sites of *KAL* expression observed in the developing chicken also occur (68). The product of the Kallmann syndrome gene is a protein with a cysteine-rich ("4-disulfide core") domain characteristic of several protease inhibitors and neurophysins and with a fibronectin type III repeat found in various neural cell adhesion and migration guidance molecules (30, 67).

Mutations of the gap junction protein gene, connexin 43, which interfere with intercellular communication, result in complex cardiac anomalies with abnormalities of location of organs (including asplenia or polysplenia) and with pulmonary atresia or stenosis (32). In mice, engineered mutations of the same gene (*Gja1*) also cause obstruction of the pulmonary artery outflow tract (45).

G Proteins. Abnormalities of signal transduction pathways involving G proteins or their relatives have been identified in three human genetic disorders. One is faciogenital dysplasia or the Aarskog–Scott syndrome. The gene responsible for this disorder has been found to be homologous to Rho/Rac guanine nucleotide exchange factors, which are involved in signal transduction and regulation of development (33).

The second disorder in this group is the Miller–Dieker syndrome, with lissencephaly, a brain malformation characterized by a smooth cerebral surface and abnormal neuronal migration. The gene presumed to be responsible for this disorder, *LIS1*, which is altered in patients with the syndrome, has been found to encode a protein with significant homology to the β subunits of heterotrimeric G proteins (34). This protein is assumed to have an important role in a signal transduction pathway crucial for brain development, and 50% activity (haploinsufficiency) appears to be insufficient for normal function.

Albright hereditary osteodystrophy, with pseudohypoparathyroidism or pseudopseudohypoparathyroidism (same phenotype with normocalcemia), is believed to be caused by mutations in the gene for the G_s-protein α subunit that activates adenyl cyclase in response to parathormone. These mutations result in reduced levels of the mRNA for the α subunit or, on occasion, may have a dominant negative effect (35). By contrast, although not a hereditary disease *per se*, the McCune–Albright syndrome (polyostotic fibrous dysplasia, cafe-au-lait spots, and multiple endocrinopathies) is characterized by the reverse of what occurs in Albright hereditary osteodystrophy—by activating mutations of the G_s-protein α subunit (69). These mutations are presumed to occur somatically early in embryogenesis and to result in mosaicism for mutant and normal

cells in many tissues, with, among other consequences, increased osteoblast proliferation and abnormal differentiation.

Growth Factors

At the present time, only the Beckwith–Wiedemann syndrome has been attributed to genetically altered growth factor production. Three types of genetic etiologies implicating overexpression of the gene for insulin-like growth factor 2 (*IGF2*) have been proposed. These are all based on the fact that only the allele derived from the father is expressed, with the maternal allele being imprinted and, as a result, inactive. The three etiologies are a duplication of *IGF2* on the paternally derived chromosome, uniparental disomy (both copies of the chromosome or region being derived from the same parent) for all or part of paternal chromosome 11 on which *IGF2* is located, and a breakdown in the imprinting of the normally inactive maternal allele (36, 37). Each of these mechanisms would result in twice normal expression of *IGF2* and, presumably, in overgrowth and other aspects of the syndrome. This inference is supported by the observation that the regions of highest *IGF2* expression during human embryogenesis correlate well with the sites of overgrowth and tumor formation (70). It is also strengthened by the facts that imprinting of the maternally derived allele of *Igf2* in the mouse has been recognized and that paternal disomy for mouse chromosome 7 (homologous to human 11p) results in fetal overgrowth (46).

Uniparental disomy has been proposed as a mechanism for several other human birth defects, such as the Prader–Willi and Angelman syndromes. In these conditions, for which the specific imprinted genes involved have not been identified (although *SNRPN* is a candidate for the Prader–Willi gene), the disomy is for the chromosomes from the parent transmitting the inactivated allele (71). This results essentially in a null situation insofar as expression of the responsible gene or genes is concerned, even though the structural genes are still present. The same situation is also produced when a mutation in or a deletion of the responsible gene(s) occurs on the active chromosome (72).

Aneuploidy and Contiguous Gene Syndromes

The previous discussion has been entirely concerned with birth defects that can be attributed to mutations of single genes. Chromosomal aneuploidy, whether monosomies/deletions or trisomies/duplications, is also a major cause of multiple malformation syndromes. These conditions differ from many of the single gene abnormalities in two fundamental ways: many genes are likely to be involved in the pathogenesis of the phenotype and the problem is one of increased or decreased dosage of normal, not mutant, genes. In the monosomies/deletions, multiple simultaneous haploinsufficiencies are created. As has been commented on several times earlier, many loci appear to be quite dosage sensitive and haploinsufficiency is sufficient to produce developmental abnormalities (73, 74). It would appear that the same should also be true for conditions of increased gene dosage, although it is often more difficult to visualize the mechanisms that might be involved (73). However, understanding of these disorders will increase as the genes in the regions of imbalance are identified and their functions are delineated.

A subset of syndromes produced by small chromosomal deletions has been distinguished and referred to as the contiguous gene or microdeletion syndromes (75). These include the Prader–Willi, Angelman, Miller–Dieker, Smith–Magenis, DiGeorge (including Shprintzen), Langer–Giedion, Williams, and Beckwith–Wiedemann syndromes, and the syndrome of Wilms tumor, aniridia, genitourinary tract malformations, gonadoblastoma, and mental retardation (WAGR). Mention has already been made of *WT1* and *LIS1* as specific genes known to be present within the deletions causing the WAGR and Miller–Dieker syndromes, respectively. However, in light of the earlier discussion, it is worth nothing that a transcription factor gene has been suggested as a candidate for defects found in the DiGeorge syndrome. This is *TUPLE1*, a gene encoding a protein with repeated WD40 domains found in the β-transducin/enhancer of split family of proteins (76). In addition, a gene encoding a gelsolin-like protein homologous to the *Drosophila* flightless-I gene has been found within the microdeletion that causes the Smith–Magenis syndrome (77), and the deletion casuing Williams syndrome has been found to contain the elastin gene (78). Since abnormalities of elastic fiber architecture may be involved in the vascular and connective tissue pathology of the latter condition, it is assumed that haploinsufficiency for the elastin gene plays a significant role in the pathogenesis of the syndrome. A zinc finger gene, *ZNF141*, has been mapped to the deletion of chromosome 4p that causes Wolf–Hirschhorn syndrome, a condition not generally included with the microdeletion disorders (79).

Genes in Search of Human Diseases

In the earlier discussion of *HOX* genes, mention was made of mutations introduced in mice that resemble, to a greater or lesser extent, abnormalities found in humans. With the greater number of mice now being generated either by the insertion of transgenes or by gene interruption (by either homologous recombination or inadvertent insertion of a transgene), similar findings have been made with a large number and variety of other types of genes. In addition, not all mouse mutations affecting transcription factors are induced by genetic engineering techniques. For example, the mutation Kreisler (*kr*), which causes segmentation abnormalities of the hindbrain and defective development of the inner ear with deafness and a circling behavior, results from abnormality of a basic domain leucine zipper protein related to the *Maf* subfamily of transcription factors (80). Loss of this protein is thought to cause loss of the fifth rhombomere of the hindbrain. Similarly, the mutations at the T-locus (Brachyury), which affect mesoderm formation and notochord differentiation, are in the gene for what appears to be a unique transcription factor with a nearly palindromic DNA-binding site (81).

In addition to the specific examples just given, a large number of other genes have been shown to play important roles in development of many other organs and tissues, including the brain (82), ear (83), limb (64, 84, 85), and heart (86). Furthermore, although all of the examples provided are based on experiments with mice and often refer back to *Drosophila* genes, work in other species such as *Xenopus* and zebrafish is also highly relevant (87–89).

Teratogens

Reference has already been made to the fact that mutations in *Hoxa7* produce abnormalities similar to those resulting from the teratogenic effects of retinoic acid. It is also known that retinoic acid regulates *HOXB1* expression (90), and introduction of a mutation into the retinoic X receptor α gene causes heart and eye defects (91). These observations are, of course, germane to understanding the craniofacial, cardiac, thymic, and central nervous system abnormalities found in human infants exposed to isotretinoin (13-*cis*-retinoic acid) *in utero* (92). Limitation of space does not allow for further exploration of the obvious importance of an appreciation of basic developmental mechanisms for an understanding of the mechanisms of teratogenesis.

Conclusions

What has basic developmental biology taught us about human birth defects? I have used a large number of human disorders of morphogenesis and development as the starting point for an examination of this question, and several things should be apparent.

(*i*) The general functions of many of the human genes identified as causing developmental abnormalities are already known

from studies of lower organisms ranging from flies to mice.

(*ii*) In some instances, it may be possible to relate the specific functions of a human gene to functions demonstrated for homologous genes in lower organisms.

(*iii*) Many classes of genes believed, on the basis of studies of lower organisms, to play a role in development have been shown to do so in humans as well. These include not only genes for transcription factors, growth factors, receptors, and signal transduction pathways, but also for structural proteins.

(*iv*) In addition to mutations affecting the types of genes conventionally thought to be important in development and differentiation, mutations affecting enzymes and transporters can also give rise to birth defects not unlike those caused by mutations in the other types of genes.

(*v*) The techniques of transgenesis and homologous recombination have made possible the construction of animal models for many human disorders.

(*vi*) The prior identification and analysis of mutant mice, coupled with the known homologies between the human and mouse genomes, have made it possible to implicate or corroborate candidate genes for phenotypically similar human disorders.

(*vii*) The detailed analysis of gene expression patterns in the mouse, particularly during fetal development, has aided the interpretation of the effects of mutations of these genes in both mice and humans.

Although the preceding analysis has essentially been a retrospective one, starting with human disorders and working back to basic biology, the likelihood of a prospective relationship between basic developmental biology and human birth defects can also be envisioned. Elucidation of developmental mechanisms and identification of the genes controlling them will guide us in where and how to look when investigating abnormalities of human development. The patterns of malformations observed will then no longer hold the mystery that they now do. Rather, they will serve as signposts to direct our attention to particular developmental pathways and, ultimately, to specific genes.

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