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BRANCH OVERVIEW AND SUMMARY

The last presentation to the Council by the Developmental Biology, Genetics and Teratology (DBGT) Branch four years ago coincided with the publication of a series of papers announcing the identification of the gene, *Sonic hedgehog*, which has been shown to play an important role in directing a number of developmental processes. Since that time, the advances in the field of developmental biology have been truly remarkable and have continued to move this area of investigation from a description of phenomenology to a discipline with a better understanding of the genetic and molecular mechanisms underlying developmental processes. Our staff takes great pride in the knowledge that DBGT and NICHD have supported many of the important advances that have been shaping the field of developmental biology.

The focus of this Branch is primarily on the basic studies of mechanisms regulating development. The motivation for understanding embryonic development includes both intellectual curiosity and the desire to predict and prevent human birth defects. In order to address the issues related to human birth defects, the field of developmental biology is elucidating the mechanisms that control normal development and examining how perturbations of these processes, both genetic and environmental, cause developmental abnormalities. While great progress has been made in preventing deaths resulting from either low-birth-weight, prematurity, respiratory distress syndrome or SIDS, birth defects remain the leading cause of deaths in infants under one year of age. Families of babies born with severe, non-fatal, birth defects are burdened financially by expensive special medical treatment, education, rehabilitation, and other supportive services to care for childhood and adult disabilities. The basic and translational research supported by DBGT will increase our understanding of normal and aberrant development and, ultimately, will provide the foundation for preventing, ameliorating, or treating these inborn errors of morphogenesis.

This Branch supports research and training in the following areas: clinical genetics, basic developmental genetics, early embryonic development, developmental neurobiology, limb development, chondrogenesis, myogenesis, teratology, and developmental and reproductive immunology (see figures 1-4, 7 and 8, and the table at the end of the report). Support for research is primarily through standard mechanisms: R01s, R29s, R37s, R55s, P01s and, most recently R03s. Currently, DBGT does not support Centers. Through these mechanisms, DBGT supports many of the foremost investigators in the field of developmental biology including three Nobel Prize laureates, three Presidential New Investigator awardees, and an ever increasing number of Howard Hughes Medical Institute investigators.

The research supported by these grants uses a variety of animal models from *Drosophila, C. elegans* and other invertebrates to zebrafish, *Xenopus*, chick, mice, non-human primates and humans. The use of animal models is very important, whether one seeks to understand the control of gene expression, the specification of cell fates, cell pattern formation, the ontogeny of immunity or the effect of a drug or other exogenous agent. An important breakthrough was the demonstration that genes known to control specific developmental events in invertebrate models (such as *Drosophila* and *C. elegans*) also regulate similar events during the development

of vertebrates, including humans. Consequently, lessons learned from *Drosophila* have direct applicability to vertebrate development. For example, the role of *hedgehog* and *fringe* in *Drosophila* wing development has led to the identification of homologous genes, *Sonic hedgehog* and *radical fringe,* in vertebrate limb development. This knowledge of commonalities between species allows us to perform experiments in animal models with genes that cause human birth defects so that the role of the gene can be determined. Similarly, genes identified in animals provide insight into the causes of human defects. The use of animal models also allows us to look at very early stages of development in order to identify critical periods in the formation of structures when an insult or aberration in the developmental process can lead to a birth defect.

The most effective way to minimize human morbidity and mortality is to understand more fully how and when developmental processes take place. In order to understand abnormal development, it is essential to have a firm grasp on mechanisms controlling normal development. Over the past few years, major breakthroughs have greatly increased our understanding of the molecular and genetic controls of embryonic development. In particular, it is now clearer how certain extracellular signaling proteins control critical developmental events and how perturbation of these events can lead to the formation of birth defects.

Biochemical and molecular biological techniques are being used to examine the mechanisms that regulate pre-mRNA splicing during development. When a given pre-mRNA molecule is spliced differently, its mature mRNA functions differently. This differential function is an important means of producing tissue-specific gene expression. Accordingly, elucidating the mechanisms that regulate where a pre-mRNA is spliced will help to explain how cell- and tissue-specific properties are produced during development.

Other areas of rapid expansion of knowledge include early embryonic inductions, axial patterning, cell fate determination, patterning of the nervous system and the limb, and a better understanding the complex genetic and biochemical networks by which developmental genes are regulated. This information is critical to elucidating the genetic and molecular mechanisms that control normal embryonic development and will enable us to predict, diagnose and prevent birth defects.

While incredible and exciting advances have been made over the last four years since our last report, there have been some unexpected occurrences. As can be seen in figures 5 and 6, the relative proportion of grants and funds within DBGT's various program categories has remained relatively constant. However, between FY93 and FY96, there was an overall downward trend in the total number of grants supported by the Branch and the accompanying dollars (see numbers above bar graphs in figures 5 and 6). Figure 9 illustrates the budget trend for DBGT over the last ten years. In current dollars, there was a drop in FY94 but there appears to be a gradual trend towards improvement since that time. Even with this improvement, there is a widening gap between current and constant dollars. However, it is clear that the past fiscal year has shown some improvement with an enhanced payline and an overall increased budget at the NICHD. Consequently, with the continuing support for research in developmental biology shown by our Director, Deputy Director, and Center Director and the Institute's improving budget outlook, the future of developmental biology at the NICHD is encouraging.

SPECIAL INITIATIVES

The Rat Genome Project

The rat is an important animal model for several human conditions and for basic research in biology. The Rat Genome Project is funded by 13 NIH institutes, including the NICHD. The Project is identifying over 6,000 genetic markers, creating a dense genetic map, and anchoring the map to the chromosomes with fluorescence *in situ* hybridation. The project has produced PAC, YAC and BAC libraries of genomic rat DNA. Finally, it has produced cDNA libraries from adult organs including lung, liver, heart, brain, and muscle, and from whole embryos at day 8, 12 and 18. These maps and reagents are being made available to the scientific community. A better understanding of the rat genome will enable us to study many developmental defects in the rat. Additionally, the high degree of similarity between the rat, mouse, and human genome means that the maps of the rat genome will improve our general understanding of mammalian genetics and its role in diseases and birth defects.

Zebrafish Research

An important new model of vertebrate development has received increased attention and will be of tremendous importance in the future. While experimental analyses of early development have previously been done in *Xenopus*, and genetic analyses of early development have previously be done in the mouse, *Danio rerio* is a single species in which both experimental and genetic analyses of early development can be studied. There are also additional advantages of the zebrafish as a model of vertebrate development. The embryo is small, transparent, and contains a relatively small number of cells that are identifiable early in development and are easily accessible to marking, observation, and manipulation. The ability to "clone" fish permits rapid mutant screens to be performed in the F1 generation to identify recessive embryonic lethal mutations. Their small size and short generation time (3-4 months) makes it easy to propagate large populations and characterize many mutations. These attributes have allowed for the construction of detailed fate maps (indicating the ultimate localization of a cell after migration) and cell lineages, transplantation and experimental manipulation of individual cells, and characterization of specific cell migrations and differentiation in living vertebrate embryos. Using this model, it is now known that gene expression patterns define developmentally distinct domains in axial structures and the nervous system. These domains include tissues at the embryonic midline, along the antero-posterior axis of the developing nervous system that foreshadow structural and functional divisions in the central nervous system, and the dorso-ventral domains within early neurogenic tissue that foretell divisions into ventral and dorsal neural tube, neural crest, ectodermal placodes, and non-neurogenic ectoderm. A variety of mutations have been identified that alter expression patterns and axial morphogenesis in these embryos, and a linkage map of the zebrafish genome, including many of these mutations and molecular markers is being generated.

Computer-assisted Imaging of Embryonic Development

The DBGT has promoted the uses of computer-assisted imaging for the study of embryonic and fetal development. The DBGT Branch sponsors projects that develop computer hardware and software to study some aspects of embryonic development. Advanced computer-assisted imaging techniques are being used to construct digital models of existing collections of human embryos. These models show the progressive growth and movements of the embryo's internal structures, which can not be viewed in any other way. The most advanced instrumentation is being used to study the development of animal models and is providing a new understanding of the cellular, molecular and genetic events that control cell movement, cellular interactions, and the formation of organs. These new techniques are enabling us to detect and to elucidate the mechanisms that cause birth defects so that they can be prevented.

Developmental Immunology

Two new initiatives were developed to increase our basic knowledge and understanding of the developing immune system and its role in perinatal host defenses. They were designed to take advantage of recent advances in biochemistry, molecular biology, genetics and biotechnology. The first initiative was an FY97 program announcement with set-aside funds (PAS) entitled "Developmental and Genetic Defects of Immunity." The PAS focused on basic studies to identify the genes and elucidate the molecular and genetic mechanisms responsible for normal and abnormal development of the immune system. Special emphasis was placed on identifying and characterizing mutated genes, their proteins, signal transduction pathways and mechanisms that cause primary immunodeficiencies. Funding was provided jointly by the NICHD and the Jeffrey Modell Foundation, a nonprofit organization supporting research and education on primary immune deficiency. This PAS was reissued for FY98. The second initiative, also issued in FY97, was a Request for Applications (RFA) entitled "Ontogeny of Perinatal Host Defenses." The goal was to promote critically-needed basic studies on the development of acquired perinatal host defense mechanisms in humans. The RFA encouraged fundamental research on the cellular, molecular and genetic mechanisms responsible for ontogeny of acquired host defenses.

HIGHLIGHTS OF RESEARCH PROGRESS

This section describes some of the advances made by investigators supported by DBGT. It is not meant to be a comprehensive review of the field. Due to space limitations, it does not present all of the work supported by this Branch but, rather, only a few highlights. For clarity, when describing animal studies, references to genes are in *italics*. When the protein products coded for by these genes are discussed, they are not italicized or, if abbreviated, are printed in upper case.

DEVELOPMENTAL GENETICS - - Clinical Studies

Recently, investigators have identified the genes that are responsible for several birth defects by

two different techniques, either by genetic mapping of affected families, or by examining an affected individual for the presence of candidate genes. The genetic mapping method starts with extensive examinations of families that include affected individuals. It compares the inheritance pattern of the disease phenotype with the inheritance pattern of identified genetic markers. Once the segregating genes are identified, they are examined to determine how they cause the condition. The candidate gene approach starts with an animal mutation having a phenotype similar to a human condition. If the mutant gene is present in humans with the condition, it indicates that the human condition is caused by similar mechanism to that previously identified in the animal model.

Identification of Birth Defect Genes by Genetic Mapping

The main advantage of the genetic mapping method is that it does not require an understanding of the disease's underlying biological cause. Instead, it uses extensive gene mapping of families that includes a high proportion of individuals with the condition. This technique has been used recently to identify the genetic mutations responsible for Hirschprung's Disease (HSCR; aganglionic megacolon), a congenital disease that occurs in 1 of 5,000 live births.

HSCR results in the absence of intrinsic ganglion cells in the nerve plexuses of the distal digestive tract and, thus, in a colon that lacks innervation. The absence of innervation causes intestinal blockage that usually presents in the neonatal period, and requires surgical correction. To find the genes responsible for HSCR, investigators identified a large inbred population of 61 nuclear families consisting of over 3,700 individuals with a high incidence HSCR. Families within this population underwent genome-wide screens, segregation analyses, identity-by-decent analyses, and linkage disequilibrium mapping. These examinations identified two genes that play a significant role in HSCR, called HSCR1 and HSCR2. HSCR1 maps to human chromosome 10q11.1, and encodes the proto-oncogene, RET; it accounts for about 10% of HSCR. HSCR2 is located on 13q22, and is the endothelin-B receptor gene. HSCR patients have a G to T mis-sense mutation in exon 4 of the receptor gene that impairs the receptor's ability to respond to its ligand. Homozygotes for this mutation have a 74% chance of developing HSCR. These findings show how complex human birth defects are caused by mutations in several genes. Other studies are using this information to alter these genes in mice and study the effects on development.

Identification of Birth Defect Genes by Candidate Search

When the cellular and molecular nature of a condition is known, this information can be used to identify the genes responsible for human birth defects by narrowing the search to candidates in particular gene families or in particular genetic pathways. The candidate genes are often determined by studying normal animal development, and by examining the genetic basis for defects in animal development. For example, examinations of mouse limb development led to the discovery that human Hand-Foot-Genital Syndrome (HFG) was caused by a mutation in *Hoxa-13.*

Examinations of mouse showed that *Hoxa-13* was expressed in the right place and time to be involved in the formation of the hand and foot, and that targeted mutations of the Hox clusters 9-

13 led to limb malformations. Mapping the naturally occurring mouse mutant, hypodactyly (Hd), showed that Hd was a mutation in *Hoxa-13*. The observation that *Hoxa-13* controls the formation of the distal limb of the mouse made it a good candidate for the gene that caused the similar human condition, HFG. People with HFG have small, malplaced phalanges, metacarpals, and metatarsals. Females with HFG have uterine, urethral and ureteral defects that often lead to infertility. DNA samples from a single HFG family (38 individuals) were genotyped for a marker that is linked to the Hoxa cluster. One allele of the marker was always present in affected individuals and absent in unaffected individuals. The probable involvement of a member of the *Hoxa* cluster, and the data showing that *Hoxa-13* was responsible for hypodactyly in the mouse, led investigators to sequence the coding region of *Hoxa-13* in an affected individual. Comparison to these regions from normal individuals showed that HFG is caused by an A to G transition in the homeodomain. This mutation is predicted to produce a truncated protein without transcriptional activities. Interestingly, this mutation is different than that responsible for hypodactyly. The exact role of this mutation in the limb and reproductive organs is being examined in mice engineered to have the HFG mutation.

Both gene mapping and candidate search analyses provide valuable insights into the causes of congenital human defects. They will enable identification of individuals that are at-risk, and they should lead to a means to prevent or cure devastating birth defects. In addition, these studies show the intricate genetic interactions that may be responsible for complex human diseases. Studies must combine basic genetic research and population studies in order to elucidate the nature of complex human diseases.

Glycerol Kinase Deficiency (GKD) and Complex GKD

Glycerol kinase deficiency (GKD) is an X-linked disorder that is heterogeneous in nature. Clinically it is recognized in three forms: infantile, juvenile and adult. The infantile form is the most severe and has the most extensive genetic damage associated with it. This form is referred to as complex GKD since the defect in the gene for the enzyme, glycerol kinase, is frequently complexed with defects in one or both of its contiguous genes that are responsible for Duchennes muscular dystrophy and adrenal hypoplasia congenita (AHC). These three genes are in close proximity on the X chromosome (Xp21) and large deletions in this region can leave all three genes damaged. In the juvenile form, only the GK gene is affected and in the adult form the patient presents with pseudohyertriglyceridemia due to the elevation of free glycerol. Using a specific genomic scanning approach for the identification of transcribed genes, the genes for GK and *DAX1*, an adjacent gene responsible for adrenal hypoplasia congenita, were located and sequenced. The latter gene encodes a novel member of the nuclear hormone receptor superfamily. This gene is also associated with hypogonadotropic hypogonadism and is expressed in the adrenal glands, gonads, pituitary and hypothalamus. Further studies of these genes involved in complex GKD will provide important information on the fundamental relationships between phenotype and genotype in patients with contiguous gene syndromes.

DEVELOPMENTAL GENETICS - - Basic Studies

Homeobox Genes in Development

The homeobox (Hox) genes encode DNA-binding proteins that play a significant role in regulating pattern formation of all animals from nematodes to humans. They are involved in controlling the formation of specific structures along the animal's anterior-posterior axis, in regulating the patterning of the limb, and in directing the formation of a variety of organs. Their significant role in the development of the fruit fly, *Drosophila melanogaster,* was first appreciated by Edward Lewis in the 1940s. In the 1980s, researchers demonstrated that Hox genes also controlled vertebrate embryogenesis by identifying vertebrate genes with sequence homology to the *Drosophila* Hox genes. This discovery lead to an entirely new understanding of the molecular and genetic control of vertebrate embryogenesis, and to the realization that the development of both vertebrates and invertebrates was controlled by the same genetic and molecular events. The realization that Hox genes control major aspects of vertebrate development has influenced virtually all subsequent research in the field.

On-going experiments are determining how Hox gene products control the regional development of the fruit fly. For example, the Hox gene, *Abdominal-B* (*Abd-B*) produces two protein products (I and II), that control the formation of different embryonic regions. The actual means of control is being examined in transgenic flies in which one of the protein products (II) is deleted from its normal territory and the other product (I) is expressed in that domain. When the expression of *Abd-B II* is prevented, specific structures are missing. If these structures are formed in flies in which *Abd-B I* is expressed in *Abd-B II*'s normal region, it will indicate that the differential distribution of their products determines which structures they form. The relation between the protein's structure and its function are also being examined by introducing genes with partial coding regions. These elegant experiments will provide important information about how gene products control the formation of specific embryonic structures.

One important vertebrate Hox gene, *goosecoid* was identified in 1991. It is homologous to the *Drosophila* genes *gooseberry* and *bicoid. Goosecoid* is enriched in Spemann' s Organizer, the region that controls pattern formation during gastrulation, and alters the fate of nearby cells. Accordingly, goosecoid protein was apparently the morphogen that had been sought since Hans Spemann first discovered the properties of the organizer in 1924. Researchers subsequently identified two important *goosecoid* targets, *chordin* and *Cerberus*. *Chordin* is homologous to the *Drosophila* gene, *short gastrulation* (*sog*). It interacts with Bone Morphogenic Proteins to regulate patterning. *Cerberus* is a novel gene that is involved in the formation of head structures, which has eluded all previous examinations.

Examination of Hox gene expression in zebrafish embryos has shown an unexpected spatial distribution. Several zebrafish Hox genes are not distributed in a co-linear pattern, as they are in *Drosophila* and mouse. Further comparisons of Hox gene distribution between zebrafish, *Drosophila* and mouse are expected to explain the significance of the Hox distribution pattern and to explain the phylogenetic origin of the pattern.

RNA Regulation by the 3' Untranslated Region

Many developmental events require that gene expression is confined to a specific embryonic region or to a specific developmental stage. One important way of obtaining spatial- and temporal-specific gene expression is to regulate the translation of the messenger RNA (mRNA). The RNA's 3' untranslated region (3' UTR) contains elements that control where and when the RNA is translated. For example, the temporal regulation of sperm and egg cells in the same *C. elegans* hermaphrodite is regulated by the 3' UTR of the *tra-2* RNA. Early in development, a repressor protein binds to the 3' UTR of *tra-2*, prevents translation of *tra-2* and thus causes the germ cells to make sperm. Later in development, when the repressor does not bind to the 3' UTR, *tra-2* is translated and causes the germ cells to make oocytes. The genes that produce the repressor proteins are currently being examined.

Translational control by the 3' UTR is particularly important during the earliest stages of development, which occur prior to embryonic gene transcription and therefore, rely on maternal mRNA. The role of 3' UTRs in regulating patterning and cell fate determination has been studied in several species. For example, the anterio-posterior patterning of the *Drosophila* embryo depends upon the localization of several maternal RNAs. Two of these RNAs, *oscar* and *bicoid,* become localized to opposite poles by control elements in their 3' UTRs. Normally, *bicoid* is localized to the anterior pole and *oscar* is localized to the posterior pole. When the 3' UTR of *oscar* is replaced with the 3' UTR of *bicoid*, *oscar* becomes localized to the anterior pole. This produces an embryo with two abdomens, a posterior abdomen, formed under the control of the endogenous, posterior *oscar* and an anterior abdomen, formed under the control of the ectopic anterior *oscar*. The anterior abdomen forms functional germ cells.

EARLY EMBRYO DEVELOPMENT

Control of Cell Fate by Localized Maternal RNA

During oogenesis, the mother synthesizes messenger RNAs and transports them into the oocyte to be used during embryonic development. Most of the mRNAs are distributed homogeneously. However, some are localized to a specific region of the oocyte. These localized mRNAs have a profound influence on the spatial organization of the embryo. As the oocyte divides into the first embryonic cells, the localized mRNAs become segregated to particular cells and cause them to follow a unique developmental pathway.

Early examinations of the ascidian provided the first evidence that a localized cytoplasmic substance could control a cell's fate. This embryo contains a yellow-colored substance called the yellow crescent that is specifically localized in the cells that will produce the muscles. Accordingly, the yellow crescent is also known as myoplasm. Since its initial discovery, the yellow crescent has served as an important model for studies of cytoplasmic localization. Recent DBGT-supported research has discovered two RNA components of the yellow crescent, Uro-1, and YC RNA. The identification of these RNAs culminates a 90-year search for the myoplasm's

active ingredients. Uro-1 is an mRNA that encodes for a protein tyrosine kinase and appears to be part of the signal transduction pathway that organizes the myoplasm. YC RNA is a non-coding RNA that is localized to the myoplasm, and is associated with the mRNA of proliferating cell nuclear antigen (PCNA), an mRNA that encodes a co-factor for DNA polymerase-delta and is required for DNA replication. The YC and PCNA RNAs are transcribed from opposite strands of the same DNA duplex, and have complementary 3' ends. Preliminary examinations of the spatial and temporal distribution of these RNAs indicate that they interact with one-another to regulate cell proliferation and to control myogenesis.

Other experiments are identifying the molecular components of *Drosophila* germ plasm, which controls the determination of the germ cells. The germ plasm consists of several localized proteins and RNAs. Recent studies show that the pattern-forming gene, *oskar*, plays a significant role in assembling and regulating these localized constituents.

Intracellular Transduction of Developmental Signals

DBGT has a long-standing interest in research that examines the genetic and molecular mechanisms that control cell fate determination. Because cell fate determination is mediated, in large part, by extracellular growth factors, this Branch has placed a heavy emphasis on research projects elucidating the mechanisms by which extracellular factors control cell fate. Many recent examinations show that growth factors regulate development by a complex series of interrelated chemical interactions. The complexity of the interactions is due to two main facts. Firstly, each growth factor exerts its influence through a long intracellular signal transduction pathway. Alterations in the pathway profoundly influence the factor's effect. Secondly, several unrelated growth factors interact extracellularly so that a factor's effect depends on the concentration of other substances in the local environment. Thus, a growth factor's pathways and interactions must be understood in order to elucidate the interactions that control embryonic development, to determine how developmental defects arise, and to devise means to correct these defects.

On-going research is examining the intracellular pathways of, and interactions between, members of the Fibroblast Growth Factor (FGF) superfamily, Transforming-Growth-Factor-β (TGF-β) superfamily, WNT and Hedgehog families. Some of the most interesting recent findings concern the extracellular interactions of the TGF-β family member, Bone Morphogenic Protein, and the intracellular and extracellular interactions of the WNT proteins.

Regulation of BMP Activity: Many studies had shown that several growth factors have opposing effects on developing systems. For example, activin, BMP-1, -2, and -4 cause early embryonic cells to develop ventral fates, whereas follistatin, chordin, nogin and nodal proteins cause them to develop dorsal fates. Recent DBGT-supported research has demonstrated a complex group of extracellular interactions between these opposing growth factors.

One of the first studies examined this issue by looking at the roles of the TGF-β family member, activin, and its antagonist, follistatin, in neural induction. In the intact embryo, the nervous system is induced by the underlying mesoderm. All previous work in this field (going back more

than 70 years) had indicated that the default state of ectoderm was epidermis and that inductive events direct some of the ectodermal cells into the neural lineage. Because activin is a potent mesoderm inducer, it was also expected to promote neural induction. In order to define these events at the molecular level, investigators produced dominant negative activin receptors, and identified and examined a naturally occurring antagonist of activin activity, follistatin. Surprisingly, blocking activin's activity caused the ectodermal cells to develop into components of the nervous system. These results indicated that the normal function of the activin receptor is to mediate the induction of the ectoderm into epidermis rather than into nervous system and that without this signal, nervous system would be formed. These unexpected findings and the hypotheses they engendered caused great excitement.

Other studies have shown that the formation of the dorso-ventral axis is controlled by extracellular interaction between antagonistic factors. Interestingly, homologous interacting factors perform the same functions in *Drosophila,* frog and mouse. These factors are sufficiently similar that the frog factor functions in the fly, and vice versa.

Vertebrate BMP-4 is homologous to *Drosophila* decapentaplegic (*dpp*). In both vertebrates and in *Drosophila*, these factors induce the formation of specific non-neural structures such as surface ectoderm, visceral endoderm, and blood. The action of these factors is antagonized by chordin and by *short gastrulation* (*sog*), which induce neural structures, in vertebrates and in *Drosophila*, respectively. BMP-4/dpp and chordin/sog are produced on opposite sides of the embryo and bind to each other extracellularly, preventing activation of the BMP receptor. Thus, the interactions between BMP-4/dpp and chordin/sog form a gradient along the embryonic axis that causes the formation of the different structures. Cells that receive no BMP-4 signal develop dorsal fates, including neural tissue and notochord; cells that receive moderate levels of BMP-4 develop intermediate fates, including somitic muscle and pronephric kidney; and, cells that receive high levels of BMP-4 develop ventral fates including blood.

Wnt Signal Transduction: The large Wnt family of secreted proteins (currently 16 members) has a role in many important developmental events, including proper axis formation, mesoderm induction, and formation of the nervous system and the limbs. The developmental importance of Wnt signaling has led to many studies to determine the genetic control of *Wnt* gene expression and the downstream signaling cascade of WNT proteins. Genetic and biochemical examinations of Wnt signaling in *Drosophila* and in *Xenopus*, supported mainly by DBGT, have identified the Wnt-receptor, frizzled, and have shown that activated frizzled causes β-catenin to accumulate in the cytoplasm. The cytoplasmic β-catenin binds to the Tcf-Lef transcription factor, translocates to the nucleus and activates pattern-forming genes such as *engrailed, goosecoid, siamois* and *Xnr-3*. These genes mediate the developmental events mentioned above.

These studies have also identified soluble WNT antagonists, called frizzled-related proteins (FRPs). FRPs bind to WNTs so that the frizzled receptor is not activated. This causes the cytoplasmic β-catenin to form a complex with the glycogen synthase-3 (GSK-3) and the adenomatous polyposis coli (APC) tumor-suppressor protein that leads to the degradation of β-catenin so that it cannot activate the aforementioned pattern-forming genes. Other studies have

shown that GSK-3 blocks WNT function. Obviously, improper localization of WNTs, FRPs and of GSK-3 lead to significant developmental defects. Thus, these studies have worked out the precise pathway by which WNTs activate a group of genes and leads to the formation of particular structures. Interestingly, mutations in APC that result in colorectal tumors prevent formation of the β-catenin complex causing inappropriate activation of Wnt-responsive genes. Continued examination of these important signaling molecules is expected to provide a significant breakthrough in our understanding of genes, growth factors, and pathways that regulate developmental events.

ORGANOGENESIS

The field of developmental biology has only recently reached the level of sophistication required to study the formation of organs. The DBGT Branch is supporting research that examines the development of several organs and organ systems, including the digestive, circulatory and nervous systems; the lungs; the pituitary; and the eyes.

Digestive System

Studies of *Drosophila* are examining the genetic pathways that control the formation of the posterior gut, and the kidney. These experiments are using recently discovered genes to examine all of the genetic and molecular events that lead to the formation of an organ, from the first step, when the primordium is patterned until the final form of the organ is achieved. For example, the gut forms by a series of steps and investigators are identifying genes that seem to control each of these steps. One of these genes, *arc,* is the only gene known so far that controls the process of convergent extension, a process of critical importance to many developmental events in most animals. Thus, these studies should provide significant genetic and molecular information about universal processes of organogenesis.

Another project is examining the function of homeobox-containing genes in the differentiation and morphogenesis of mammalian endodermal organs. It is studying the role of a mouse homolog to a *Xenopus* homeobox gene that is involved in endoderm formation. Homologous recombination in ES cells is being used to produce null mouse mutants for characterization. Genomic DNA maps and subclones are also being used to determine the mechanism of region-specific activation of this gene in the developing pancreas and duodenum.

Lungs

Recent research has shown how several growth factors are involved in lung morphogenesis. Specifically, BMP4, FGF10, SHH, and WNT-7b seem to be involved in particular aspects of the branching morphogenesis and the epithelial-mesenchymal interactions that are required for the formation of the lung. Each of these factors seems to be involved in a particular component of lung morphogenesis. These findings have lead to a new model of lung morphogenesis that proposes four stages in the formation of the lung, and that each of these stages is controlled by

one of these genes. On-going studies are using transgenic techniques to study precisely how the genes for these factors and for their receptors control their respective process and how that process contributes to the overall formation of the lung. These findings will provide valuable new information about the genetic control of the formation of all organs and structures whose formation involves branching morphogenesis and epithelial-mesenchymal interactions (e.g., the pancreas, the kidney, salivary glands, palate, tooth, etc.).

DEVELOPMENTAL NEUROBIOLOGY

The nervous system is formed shortly after gastrulation, when the cells of the dorsal ectoderm are induced by the underlying mesoderm. From this original ectodermal sheet, a highly stereotyped nervous system is created. As the neural tube is formed, pattern in the anteroposterior, dorsoventral, and mediolateral axes is generated. Neural progenitor cells and their offspring derive positional information from within these boundaries, neurons are generated, acquire specific cell fates, migrate to specific positions within the developing nervous system and send out processes. Specific patterns of connectivity are established with neighboring processes and with other targets. This series of events, which is recapitulated throughout phylogeny, results in the most complex and intricate structure. Supporting research that contributes to our understanding of how the nervous system develops under both normal and abnormal conditions is the major focus of the Developmental Neurobiology Program.

Molecular Regulation of Neurogenesis

Embryonic development of the central nervous system (CNS) is a complex and poorly understood process. The CNS develops from a group of cells that is initially homogeneous in its developmental specification. With time, patterning of cells along the primary axes within the developing neuroepithelium creates regional differences that foreshadow the specialization which gives rise to the mature CNS. Understanding how cells become committed to their fates and how regulatory genes control CNS development is one of the most rapidly evolving areas of neurosciences. By exploiting our knowledge of genetics in *Drosophila* and mouse models, tremendous progress has been made in identifying genes that are likely to mediate aspects of neural patterning thereby generating cellular diversity in the CNS.

Anteroposterior (A/P) pattern: Genetics has become perhaps the most important tool for understanding the mechanisms underlying CNS patterning during development. Many genes that have been identified in *Drosophilia* have been shown to be very closely related to vertebrate genes. Recent studies confirm that those genes that play an important role in fly development, have vertebrate homologs that are also expressed during CNS development. In addition to *hox* genes, which have previously been shown to play a major role in A/P patterning of CNS rhombomeres, several segment polarity genes which act upstream of the proneural genes to pattern the neuroectoderm along the A/P axis have recently been identified. The *gooseberry* gene plays a fundamental role in specifying neuroblast (NB) identity in the neuroectoderm and is at the top of at least one gene regulatory hierarchy. The proneural genes *achaete* and *scute* are

downstream of *gooseberry* and are required for partial specification of NB fate, in addition to their well-known role in promoting neural precursor formation. Another gene, *huckbein*, is expressed in seven identified NBs and has been shown to play an important role in specifying cell fate in this lineage. Recent work has also shown that there are two activators (the secreted proteins Wingless and Hedgehog), and two repressors (the nuclear proteins, Gooseberry and Engrailed) of *huckebein*; in combination, these four genes sculpt the CNS pattern of *huckebein* expression along the A/P axis. While all of the individual genes that are involved in various aspects of neural development have not been identified, progress is being made in that direction. At the same time, researchers are actively placing these genes in their appropriate locations of their genetic networks. Completion of these activities will ultimately advance our understanding of how NB identity is established and patterning occurs.

Dorsoventral (D/V) Pattern: Families of genes have been implicated in D/V patterning of the neural tube, which is essential for the normal development of the brain and spinal cord. Expression of the proteins coded by some of these genes may be controlled by diffusible signals emanating from two midline structures (floorplate and notochord), which recent work suggests are critical in D/V patterning of the CNS. Sonic hedgehog and its homologues are well described examples of a gene and its product which has been shown to play an important role in the complex genetic network controlling the formation of the CNS of various species.

Of particular interest is our increased understanding of the role of transcription factors in controlling CNS development. One gene, *single-minded (sim)*, controls the formation of a group of neurons and glia that lie along the midline of the fly CNS. Several lines of evidence indicate that *sim* acts as a master regulator of CNS midline gene transcription and development. Genes that control D/V patterning activate *single-minded* transcription specifically in the midline precursor cells. *Single-minded* then activates transcription of a large group of genes that carry out the CNS midline lineage developmental program. Mouse homologues of *sim* have recently been identified. Preliminary studies of the mouse genes, *sim1* and *sim2* strongly suggest evolutionarily conserved functions. The SIM proteins contain a conserved protein sequence motif which is shared by several other proteins that have been shown to be predisposed to regulation by various environmental cues and suggests that SIM may also respond to specific environmental signals.

The Wnt family: Members of this large family have been implicated in all areas of embryogenesis including CNS development. The normal role of the signaling molecule encoded by the proto-oncogene, *Wnt*-1, is to regulate brain development. *Wnt*-$1^{-/-}$ mice are missing most of the midbrain and anterior hindbrain. It is unclear how the observed anatomical deletions are brought about. Currently, studies are being conducted to determine whether *Wnt*-1 is acting as a regional determinant, where loss results in cell fate changes. Alternatively, if it is acting as a trophic stimulus, cessation of mitosis and/or cell death may generate the observed phenotype.

Axonal Guidance, Pathfinding and Trophic Factors

For the CNS to form appropriate connections, neuronal precursors must migrate to their correct

location and extend processes into the extracellular environment. For the processes to reach their correct targets, factors in both the internal and external environments of the neurite are involved. Such guidance cues include extracellular and cell surface molecules, such as cell adhesion molecules and trophic factors, that can either inhibit or facilitate the progress of growth cones.

A wide variety of surface proteins, such as neural cell adhesion molecule (NCAM), L1, fasciclins, semaphorins and cadherins, appear to have either definable positive or negative influences on the fasciculation and guidance of neurites. For example, recent studies on NCAM have shown the presence of a large linear homopolymer of sialic acid (polysialic acid or PSA) that interacts with NCAM, thereby attenuating cell interactions. This unusual mode of regulation affects migration and cell-cell interactions during nervous system development in numerous model systems, including chick, rat, mouse and frog. One would expect that similar mechanisms are utilized during human development.

In *Drosophilia*, Fasciclin II has been shown to be a contact-mediated attractive guidance molecule of the immunoglobulin superfamily which is closely related to mammalian NCAM. Semaphorin II, a chemorepellent/inhibitor, is closely related to mammalian semaphorin III/collapsin and other secreted members of the semaphorin family. By exploiting the fly system, genetic analyses have been used to study the function of fasciclin II and semaphorin II, and to identify other proteins that interact with them during growth cone guidance and target recognition. Since molecules highly related to fasciclin II and semaphorin II exist in humans, understanding their function and interactions using the *Drosophila* model genetic system will have broad implications for our understanding of normal and abnormal human nervous system development.

Cell adhesion proteins have also been shown to be important in the organization of the cytoskeleton. Recently, it has been shown that neuroglian, the *Drosophila* homologue of the human L1-CAM, plays a role in the recruitment of the ankyrin membrane skeleton during development. Linkage of adhesion molecules to the cytoskeleton provides structural stability during mechanical stress. In addition, it may also communicate positional information to the cytoskeleton, causing its assembly at selected sites of the cell surface. Other proposed roles for CAM-cytoskeleton interactions include the regulation of cell migration and cell shape, the mediation of CAM-induced signaling events and the regulation of adhesive specificity and strength. Studies are currently under way to address these possibilities.

In other related arenas, investigators have examined how the biology of the growth cone itself is affected by inhibitory or excitatory guidance cues. Understanding the dynamics of microtubule rearrangements, actin localization and focal contacts during orientation away from a negative cue (e.g., chondroitin sulfate proteoglycans) or towards a positive cue (e.g., nerve growth factor) is critical for understanding how neurites function during development.

Neurotrophic factors appear to be essential for the proper development of the peripheral nervous system. Only recently has their importance been demonstrated in the CNS. At least in the developing isthmo-optic nucleus of the chick, the neurotrophin, NT-3, appears to support developing neurons. Neurotrophins can be transported from the terminals to the cell body as well

as from the cell body to the terminal. This result has significant implications for the concept of trophic signalling. The demonstration that intact neurotrophin can be transported to the terminals, released, and taken up by second order neurons, makes the classical neurotrophic hypothesis more complex in the CNS than was previously thought.

Neurotransmitters and Neuropeptides

In addition to classical neurotransmitters, many neurons also contain neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP), somatostatin, galanin, vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY). Neuropeptides, the broadest and largest class of chemical messengers in the nervous system, have been shown to act as classical neurotransmitters, neuromodulators and potentially as trophic factors in the nervous system. New studies are under way to identify the developmental mechanisms that control neuronal neurotransmitter and neuropeptide phenotype as well as examining other functions of these chemical messengers in the developing and adult nervous system.

In a recent series of studies, pituitary adenylate cyclase activating polypeptide (PACAP), a member of the VIP/secretin/glucagon family of neuropeptides has been shown to be the long sought noncholinergic regulator of sympathetic neuron function. PACAP has acute and long-term stimulatory effects on neuronal transmitter and bioactive peptide production. PACAP directly effects sympathetic neuronal membrane properties. The majority of sympathetic neurons express specific PACAP1 receptor mRNA and protein. PACAP mRNA is also found in sympathetic preganglionic projection neurons to the superior cervical ganglia. Most importantly, PACAP and PACAP1 receptors are expressed extremely early in neuronal development and in many neurogenic centers including the subventricular zone and also have important roles in neuronal survival and differentiation. Further studies of the role of this signalling mechanism are clearly indicated and should prove exciting.

Neural Tube Defects

Neural tube defects (NTDs) are the most frequent and severe developmental anomalies of the CNS. They include anencephaly, spina bifida and congenital hydrocephalus. The underlying causes of NTDs are poorly understood and only recently have animal models become readily available for researchers to study the etiology and pathogenesis of these abnormalities.

Mouse Models: Mouse models are particularly important for the study of NTDs as knowledge of the genetics of the mouse is far more advanced than that of other vertebrates. These genetic models are proving to be powerful tools in deciphering the mechanisms underlying both abnormal and normal neurodevelopment. Furthermore, homologous genes are frequently in conserved pathways in the mouse and human genomes, and, in conjunction with experimental analysis of mice and studies of human NTDs, provide strong evidence for similarities between neural tube closure in mice and man. Mouse mutations perturb normal development and allow us to understand what underlies defects and to define deficits at critical times. Several mouse models are available and are proving useful for our understanding of the mechanisms underlying NTDs.

These models also point out that the actual defect may not be confined to the neural tube but may also be due to defects in surrounding tissues.

For example, the *curly tail (ct)* mutation exhibits a defect in the proliferation of cells along the axis of the embryo in the developing endoderm. The disparity of growth between the neural tube and the gut results in a delay in the closure of the posterior neuropore as a result of abnormal mechanical forces. This mutation is primarily expressed in the lumbo-sacral region. While it has been convincingly demonstrated that diet supplementation with folate can reduce the incidence of some (but not all) human NTDs, this is not the case for the *curly tail* mutation. Consequently, researchers are now using *curly tail* as a genetic model of folate-resistant NTD. However, recent studies have shown that inositol, a water-soluble vitamin that plays a vital role in the inositol/lipid cycle, is capable of significantly reducing the incidence of spinal NTDs in *curly tail* mice. These results suggest the possible efficacy of combined treatment with folate and inositol in overcoming the majority of human NTDs. Other studies with these mice have identified modifier genes that may be important in understanding the issue of penetrance in genetically determined NTDs or elucidating genetic susceptibility for these defects.

Epidemiology: Epidemiological studies can also shed light on causes of NTDs. Recent studies have allowed us to begin to look at the hereditary basis of NTDs, by searching for genes that predispose humans to spina bifida and other NTDs. Preliminary work has been performed assesing the genetic contribution of affected candidates using detailed phenotypic descriptions, newly developed statistical techniques, and rapid genetic marker genotyping for a thorough genomic screen. Studies on individual traits are complemented by those examining the effects of environmental conditions, in order to attempt to tease out the complex relationships between genetic susceptibility and environment.

LIMB DEVELOPMENT AND RELATED TOPICS

Developmentally generated limb anomalies constitute a spectrum of birth defects that pose long-term suffering and morbidity for many affected individuals. In many cases the etiology has been unknown, and only recently has progress been made in understanding mechanisms underlying some of these conditions. Advances in molecular biology and genetic networks are also enabling investigators to push the frontiers of our understanding to much earlier stages than were previously possible. Molecular, biochemical and morphological studies addressing pattern formation, limb outgrowth and cartilage differentiation during limb morphogenesis in both normal and mutant animal models are of particular relevance in elucidating the causes of birth defects involving the appendicular skeleton.

Pattern Formation and the Specification of Limb Axes

The specialized activities of such regions of the developing limb as the posterior zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER) in directing the patterning and outgrowth of the developing limb have been known for many years. But these investigations of

axis specification and pattern formation essentially have been studies of phenomenology. However, by reinvestigating and analyzing "classic" experimental embryology investigations with modern molecular techniques, and by translating knowledge gained in *Drosophila* to vertebrate systems, the field of limb development has moved significantly within the last few years so that now there is a better understanding of the genetic mechanisms underpinning the developmental processes of the limb.

Sonic Hedgehog (Shh) and the ZPA: When the ZPA is transplanted from its normal position in the posterior portion of the limb bud to the anterior portion of the limb, it causes duplication of limb structures. Just what molecule is responsible for "polarizing" the limb has been controversial for many years with retinoic acid being a prime candidate. It is now known that *Shh* is expressed in the ZPA and that ectopic expression of this gene leads to limb duplications. These studies have shown that the protein encoded by the *Shh* gene is the important patterning factor produced in the ZPA. *Shh* is also expressed in other signaling centers, such as the notochord and the floorplate of the neural tube. This fact highlights the commonalities in the mechanisms leading to the formation of different tissues and organ systems.

Shh initiates the expression of secondary signaling molecules, including BMP-2 in the mesoderm, and FGF-4 in the ectoderm. Based on genetic evidence from *Drosophila* and its homologous molecules, it is likely that BMP-2 acts downstream of *Shh* to mediate patterning. *Shh* is also known to activate members of the *hoxd* gene cluster, which are transcription factors involved in regulating the pattern of chondrogenic rudiments in the limb. The mesoderm requires ectodermally derived factors, such as FGF-4, to activate target gene expression in response to SHH protein. The expression of *Shh* and *fgf-4* is coordinately regulated by a positive feedback loop operating between the posterior mesoderm and the overlying ectoderm. This positive feedback loop is important in coordinating the signals for anterior-posterior and proximal-distal patterning as the limb bud grows.

SHH protein appears to induce the expression of *patched* whose gene product acts as a receptor for SHH protein. Unlike the induction of BMP-2 and *Hoxd* genes by *Shh*, the induction of *patched* does not require FGF as a co-inducer. This would suggest that *patched* is a more direct target than FGF. Since *patched* is not expressed in the AER, the induction of FGF in this structure may depend on other mesodermal factors, although a direct effect via a non *patched*-dependent pathway is possible.

Dorsoventral (D/V) Patterning and the Origin of the AER: A complex genetic network is involved in establishing the D/V axis and the formation of the AER at the junction of the dorsal and ventral limb ectoderm. *Wnt-7a* is expressed in the dorsal ectoderm and it, in turn, induces expression of *Lmx-1* in the underlying dorsal mesoderm, resulting in dorsal-specific patterning. The promotion of ventral fate is mediated by expression of *Engrailed-1* (*En-1*). Again taking information from *Drosophila* and the establishment of marginal cells at the wing D/V border by the gene, *fringe*, it has recently been shown that the vertebrate homologue of this gene, *Radical fringe (R-fng)*, is restricted to the dorsal ectoderm by repression of its expression by *En-1* in the ventral ectoderm. The interaction of *R-fng*-expressing and non-expressing cells results in the

formation of the AER at their interface. Insight into the role of these genes has been obtained from studies of the chick limbless mutation, which lacks an AER. In this mutant, *En-1* is not expressed ventrally while *Wnt-7a* is expressed throughout the ectoderm and *Lmx-1* is expressed in the entire limb mesenchyme, resulting in a bidorsal limb bud. Since *En-1* suppresses *R-fng* expression, the absence of *En-1* in limbless mutants results in *R-fng* being expressed throughout the limb ectoderm, instead of being confined to dorsal ectoderm. In the absence of a juncture between *R-fng*-expressing and non-expressing cells, an AER does not form.

Hox Genes and Limb Patterning: The nested expression of the *Hoxa* and *Hoxd* clusters of genes along the proximal-distal and anterior-posterior axes, respectively, has suggested a role in limb patterning by virtue of the "Hox code" at different positions of overlapping gene expression. Uniform expression throughout the limb of a hox gene that normally was confined to a specific area resulted in homeotic-like changes that altered the pattern of bone rudiments within the limb. However, as knockouts of Hox genes in mice were developed and analyzed, the subtle changes or lack of phenotype suggested more of a role of Hox genes in controlling the localized recruitment and growth of prechondrogenic precursors initiating chondrogenic condensations. The role of Hox genes in patterning became more evident by combining mutations of paralogous Hox genes. For example, individual mutations of either *Hoxa-11* or *Hoxd-11* show only mild abnormalities in the formation of the radius and ulna. However, mice homozygous for both mutations have only vestiges of these bones. Thus, both genes are required for outgrowth of these long bones and suggest a significant role in the patterning of the limb along the proximal-distal axis.

The Role of Retinoids in Limb Patterning: Prior to the "discovery" of *Shh*, retinoic acid was thought to be a strong candidate for the limb morphogen, primarily because of its ability to produce limb duplications in a manner similar to that of the ZPA. Because ectopically applied retinoic acid on the anterior portion of the limb induces the expression of *Shh* prior to the formation of limb duplications, it is possible that retinoids are respecifying positional information. Consequently, they may play an important role at very early stages of development by exerting an influence on early flank mesenchyme and perhaps influencing the initial establishment of the ZPA and *Shh* expression. Antagonists of retinoic acid receptors, when applied to presumptive limb-forming regions, exhibit disruption of limb pattern without affecting cell viability and do not express *Shh*. Ectopic application of retinoic acid also induces *hoxb-8* expression, which is known to induce an ectopic polarizing region. These studies suggest a link between retinoid signaling and the initial establishment of polarizing activity.

Growth Factors and Their Receptors in Limb Development

Fibroblast Growth Factors (FGF): At least three forms of FGF are found in the developing limb: FGF-2,-4, and -8. Each is apparently able to support outgrowth of the limb which has been demonstrated by treatment of limb buds with FGF following removal of the AER. Without FGF, the growth of AER-free limb buds is truncated. FGF-4 is localized to the posterior AER and is involved in maintaining expression of *Shh*. FGF-8 is expressed early in limb development along an A/P stripe of ectoderm and later along the AER for its entire length. FGF-2 is more widely distributed throughout the limb being found in the limb ectoderm, the AER, and mesenchyme.

Interestingly, message for FGF-2 as well as its anti-sense message co-localize in undifferentiated mesenchyme but not in differentiated muscle or cartilage. This suggests a role for the anti-sense message in regulating the turnover of FGF-2 mRNA. Changes in the expression of FGF receptors (FGFR) mark distinct stages of chondrogenesis with proliferative mesenchyme, precartilage condensations and differentiating chondrocytes expressing FGFR-1, -2 and -3, respectively.

Bone Morphogenic Proteins (BMP): This group of proteins is related to the TGF-β family of signaling molecules. BMP-2, -4 and -7 are found in the limb. BMP-2 in particular is a secondary signaling center and has an expression domain overlapping that of *Shh*, which induces its expression. Two BMP receptors (BMPRs) are found in the limb bud, each with a different function. BMPR-1B prefigures future cartilage primordia, is necessary and sufficient to mediate mesenchymal cell condensation, and appears to be involved with the regulation of apoptosis. On the other hand, BMPR-1A is specifically expressed in prehypertrophic chondrocytes and appears to be involved in the regulation of chondrocyte maturation.

Insulin-like Growth Factor (IGF): In vitro studies have demonstrated a potential role for IGF-1 in stimulating the initial outgrowth of the limb. In these studies, IGF-1 can induce limb formation from prospective limb-forming regions before they have acquired the ability to form limbs. When grafted to ectopic sites, these buds give rise to nonpolarized, rudimentary limbs. Consequently, while IGF-1 may be involved in normal outgrowth, other signals are necessary to promote genes regulating patterning.

Other Molecules Potentially Involved in Limb Development

Dlx-5, a member of the *distal-less* family of homeobox-containing genes, is co-expressed with *Msx-2* in the AER, the anterior margin of the limb and a small region at the posterior margin. In addition, *Dlx-5* also is expressed at high levels in condensing mesenchyme at the onset of chondrogenesis. Using *in vitro* binding assays, it has been demonstrated that *Dlx-5* fusion proteins can bind to the cartilage-specific enhancer element in the first intron of type II collagen. This suggests that *Dlx-5* may be involved in regulating the expression of cartilage matrix protein genes at the onset of chondrogenesis. Expression of *Indian hedgehog* has also been associated with later stages of development in areas of chondrogenic expression. Recently, the T-box genes, *Tbx-4* and *-5*, have been shown to play a major role in the specification of forelimb and hidlimb.

Syndecan-3, a heparan sulfate, integral membrane proteoglycan, is present at several stages during the development of the limb and may mediate interactions with ECM components that control cell shape, adhesion, proliferation and differentiation. At early stages of development, expression of syndecan-3 in mesenchyme beneath the AER is dependent on the AER since this proteoglycan is absent or reduced if the AER is removed or missing, as in limbless and wingless mutants. It is possible that FGF binds to the heparan sulfate chains of syndecan so that the proteoglycan acts as a "presenter" of FGF to appropriate receptors. Syndecan is also expressed transiently at the onset of chondrogenesis when blocking with antibodies inhibits mesenchymal condensation and prevents accumulations of cartilage-specific molecules. Later, syndecan-3 is found in immature, proliferating chondrocytes in the growth plate but not in mature, hypertrophic chondrocytes.

Molecular Defects in the Chondrodysplasias

Birth defects affecting the skeleton are common but it is only recently that they have come to be understood at the molecular level. Through both positional cloning and candidate gene approaches, specific gene defects resulting in skeletal dysplasias have been identified. Defects occur in many cartilage molecules, such as the cartilage oligomeric matrix protein (e.g., pseudoachondroplasia, multiple epiphyseal dysplasia), cartilage-derived morphogenic protein-1 (e.g., Grebe Syndrome), type X collagen (e.g., Schmid-type metaphyseal dysplasia), and type II collagen (e.g., achondrogenesis, spondoepiphyseal dysplasia, Kniest dysplasia).

While it seems logical that conditions affecting the skeleton would be due to defects in genes specifically coding for cartilage structural proteins, this is not always the case. For example, a specific amino acid substitution in the transmembrane region of the FGF receptor 3 is responsible for most cases of achondroplasia while additional mutations in the extracellular region of the receptor results in thanatophoric dysplasia. Defects in genes thought to be involved with patterning also have been shown to cause limb anomalies, such as a partial deletion of the *Hoxa-13* being involved with hand-foot-genital syndrome (HFG). As described earlier, insight into this human condition, was obtained from studies of mutant mice with hypodactyly in which a normal gene product is critical for early branching in the formation of the mammalian digital arch. For many of the unmapped disorders, comparisons of animal studies with the linkage data for families with specific conditions represent essential steps in identifying the genetic defects underlying these conditions. This, in turn, will provide a better understanding of the function of gene products in normal and aberrant development as well as improving genetic counseling and expanding diagnostic opportunities.

Somite Formation

Sonic hedgehog protein is now known to be the signal emanating from the notochord and floor plate of the neural tube that influences the somites to subdivide into dermatome, scleratome and myotome which give rise to the dermis, vertebrae, and muscle, respectively. This signal suppresses the expression of *Pax-3* in the condensing epithelial somites and coincides with the onset of *Pax-1*. The *Pax-1*-expressing region expands and becomes the scleratome while the expression of *Pax3* is restricted to the dermomyotome. Subsequently, the myotome and muscle (as assessed by effects on expression of myoD, myf5, myogenin and actin) develops from the dermomyotome as a result of other signals from the notochord and ventral neural tube (but not floor plate). The notochord is required during somite formation and the initial induction of myoD. After this time, the ventral neural tube alone is sufficient to maintain expression of myoD. In contrast, the lateral plate mesoderm and the dorsal neural tube produce negative signals (perhaps dorsalin) repressing myogenic genes and localizing their expression to myotome. In the absence of the neural tube during these formative stages, somitic cells undergo apoptosis indicating that the neural tube is a source of trophic and proliferative signals.

TERATOLOGY

Birth defects constitute a major health problem in the United States. They are the leading cause of infant mortality, accounting for 1 in 5 infant deaths. It is estimated that more than 150,000 babies in the United States (about 4% of all live births) are born with significant structural birth defects each year. Our long-term goal is the prevention of birth defects. However, effective prevention measures and strategies can only be developed with a thorough and in-depth knowledge and understanding of the epidemiology, etiology and pathogenesis of birth defects. In the past teratology focused primarily on congenital gross morphologic malformations and their causes. Recently teratology has been dramatically transformed and expanded to include more subtle developmental anomalies, such as functional, biochemical, molecular, genetic, behavioral deficits or aberrations and intrauterine growth retardation. Unfortunately, tremendous gaps exist in our knowledge and understanding in these areas. The aim of the teratology program is to support studies to fill these gaps. In October 1997, a workshop was convened to develop a strategic plan to enhance clinical and epidemiological research on structural birth defects. Another workshop focusing on the molecular and genetic mechanisms of birth defects is planned for 1998. The recommendations of both workshops will serve as the bases for future initiatives to stimulate exciting new, emerging and innovative research into the next millennium.

Epidemiology

Since direct experimental studies are not conducted in humans, epidemiologic research, supported by *in vitro* and animal model studies, is essential for identifying potential teratogens and evaluating their teratogenicity. In the past, some teratogenic factors and environmental teratogens have been discovered by epidemiologic approaches. Using surveillance data, analytical and experimental methods, epidemiologists were able to incriminate thalidomide, isotretinoin (Accutane™) and etretinate as teratogens. Epidemiologic studies demonstrated the association between deficient folic acid intake by the mother during pregnancy and the subsequent development of neural tube defects in the offspring. Recent epidemiologic studies indicate environmental and genetic factors, as well as maternal obesity during pregnancy, are risk factors for neural tube defects in the neonate.

Case-control Studies: A case-control surveillance project study designed to identify the risks and safety of environmental exposures (primarily medications) in relation to birth defects provides essential data for epidemiologists and clinicians. Through systematic analyses of collected data or accrual of data, hypotheses on potentially teratogenic drugs or factors are generated and tested promptly and effectively. The project's unique infrastructure and data-collection system allows investigators to respond quickly on important issues. For example, they promptly dismissed the suggestion that Bendectin™ caused pyloric stenosis and refuted the teratogenicity of spermicides; but demonstrated a positive association between gastroschisis and the intake of decongestants. Currently the investigators are testing the hypothesis that ibuprofen and aspirin are risk factors for persistent pulmonary hypertension of the newborn.

Another team of investigators are developing new statistical methods for analyzing familial

aggregation of congenital malformations. By systematically analyzing various congenital malformations to identify more homogeneous phenotypic groups, followed by studies of familial aggregation and formal genetic analysis, the investigators expect to improve our understanding of the etiologic mechanisms underlying major congenital malformations. A population-based case-control epidemiologic study on clubfoot in the state of Washington is investigating the association between ligamentous laxity, intrauterine constraint, family history, and birth prevalence. The ultimate goal of the project is to identify risk factors and develop prevention strategies to reduce antenatal exposure to the causes of clubfoot. Interestingly, preliminary data suggest that maternal smoking during pregnancy increases the risk of clubfoot in the offspring.

Physical Factors

Hyperthermia is a well known teratogen in animals and a suspected teratogen in humans. Although data on humans are retrospective and from case-control studies, there is reasonable correlation between high maternal temperature during pregnancy and birth defects. Rat studies demonstrated that a variety of congenital CNS defects are induced by hyperthermia. Molecular studies indicate acute hyperthermia induces a heat shock response which alters transcription, translation, and synthesis of protective heat shock and antioxidant proteins. This results in accelerated hyperthermia-induced programmed cell death primarily in the developing CNS.

Clinical studies to date show prenatal ultrasound is safe in humans when properly used. However, to rule out any subtle and long-term delayed effects in humans, some experimental animal studies are being supported by the Branch. The findings indicated that high intensity ultrasound induces developmental defects as the result of elevated heat and acoustic cavitation in the fetus. Using pregnant mice as a model, ultrasound exposure to fetal gonads caused adverse changes. The exposed female offspring had decreased numbers of oocytes in the ovary while the male offspring had lower birthweights, reduced testicular weight and volume, or reduced daily sperm production. Exposing pregnant rats to high levels of ultrasound caused irreversible CNS cellular damage and biochemical changes in the embryos. The abnormalities were indistinguishable from those caused by hyperthermia; therefore, a thermal mechanism is implicated.

Retinoid Teratogenicity and Pharmacokinetics of Retinoids

Retinoids are naturally occurring or synthetic derivatives of vitamin A. Vitamin A (retinol) is essential for normal growth, development, reproduction, vision, and differentiation of epithelia. Retinoic acid (RA), a vitamin A derivative, plays a fundamental role in pattern formation within the developing limb and in the development of the CNS. However, deficient or excessive levels of vitamin A may be teratogenic. Some synthetic retinoids developed for treating dermatologic and oncologic diseases have also proven to be extremely teratogenic. The most commonly prescribed synthetic retinoid is isotretinoin (Accutane™) which is used for recalcitrant cystic acne. Administration of isotretinoin during embryonic development can cause either spontaneous abortion or severe malformations such as craniofacial, CNS, cardiovascular, respiratory, or thymic defects associated with the cardiac abnormalities. In a study comparing the teratogenic doses of various retinoids in pregnant mice, etretinate (Tegison™) was found to be most teratogenic.

Other studies have shown that *all-trans*-RA and its metabolites are also potent teratogens. These investigations demonstrate the importance of pharmacokinetics studies for evaluating the relative teratogenicity of retinoid drugs.

Cellular and Molecular Mechanisms of Retinoid Teratogenicity

Retinoic Acid Receptors: Recently, studies on the cellular and molecular mechanisms of retinoid teratogenicity have been stimulated by the discovery of the nuclear retinoic acid receptors (RARs) and nuclear retinoid X receptors (RXRs). These receptors, belonging to the multigene family of steroid/thyroid hormone receptors, have significantly advanced our understanding of the molecular mechanism and function of RA. They are RA-inducible transcriptional regulatory factors which transduce the RA signal at the level of gene expression. Both the RARs and RXRs consist of several subtypes and are encoded by separate genes. Studies showed 9-*cis* RA is the ligand for RXRs while both all-*trans* RA and 9-*cis* RA are ligands for RARs. Each of the RAR subtypes and their respective isoforms display a distinctive spatial and temporal pattern of expression during development. This suggests specific and non-overlapping functions. Several supported laboratories are attempting to unravel the sequence of molecular events which mediate RA-induced dysmorphogenesis.

RAR-β Proteins in Dysmorphogenesis: A laboratory found that retinoid-induced embryonic dysmorphogenesis in mice occurs only when RAR-β2 mRNA and unbound retinoid levels remain elevated for at least 6-9 hours following retinoid treatment and results in a significant and prolonged elevation of RAR-β protein levels. These findings suggest the important role of RAR-β protein levels in embryonic dysmorphogenesis. In ongoing studies the investigators will compare the role of the 3 different isoforms of RAR-β (β1, β3, and β4) with that of RAR-β2. They will also conduct site-directed mutagenesis studies to examine the role of individual amino acid residues in RARβ compared with RARα and RARγ. This work will ultimately be useful in designing new retinoids for therapeutic treatments which have reduced teratogenic activity.

Transcriptional Regulation by cAMP and Retinoids: Previous studies suggest that the molecular mechanisms by which retinoids exert their teratogenic and physiologic roles are through binding to nuclear retinoid receptor proteins and altering expression of specific genes. Investigations have shown that the expression of a RA- response gene may be modified by the presence of the catalytic subunit of cAMP-dependent protein kinase (PKA). Phosphorylation by the catalytic subunit is a common mechanism by which protein structure and/or function may be modified. This research showed that RA receptors are phosphorylated by the catalytic subunit and this modification may contribute to the observed changes in gene expression. Recently, a cDNA has been cloned which encodes a novel transcription factor that can dramatically affect transcription of the RA-responsive reporter gene. A potential PKA phosphorylation site on this protein suggests the possible interaction between the RA and cAMP pathways. The goal of this research is to identify proteins involved in the expression of RA-responsive genes and to understand how these proteins may interact with the cAMP pathway.

Effects of Retinoic Acid on Hox Genes: Other investigations are using transgenic mouse models

to examine the effects of toxic doses of RA on the regulation of *Hoxa-1* and *Hoxa-2* gene expression during critical stages of fetal development. The correct spatiotemporal patterns of expression of these homeobox-containing genes are critical for normal craniofacial and cephalic development. Since the head regions are affected by teratogenic doses of RA, and a newly discovered retinoic acid response element (RARE) has been found at the 3' end of the Hox gene group, there is a strong possibility that RA exerts its toxic effects through directing the ectopic expression of these genes. It is planned first to identify putative regulatory regions of these genes and then, by site-directed mutagenesis, to test the functions of binding sites of regulatory factors in transgenic mice. These studies will be followed by an investigation of the role of the RARE in *Hoxa-1* and *Hoxa-2* expression using a knockout model. Finally, the *Hoxa-1* contribution to the phenotype seen in retinoid toxicity will be examined by giving *Hoxa-1* knockout mice toxic doses of RA and studying changes in Hox expression in RAR knockout mice.

DEVELOPMENTAL IMMUNOLOGY

The research projects supported in this area cover a broad range and involve several disciplines including basic, applied, and clinical studies on ontogeny of immunity, development of immune responsiveness, and reproductive immunology. The long-term goal is to translate the basic knowledge and understanding from these studies into new approaches for the effective diagnosis, treatment, and prevention of early infection and developmental disorders of immunity.

Ontogeny of Immunity

In a study using mice to define cellular interactions that guide T lymphocyte development, the investigators focused on characterizing recognition events involved in positive selection. They established that positive selection occurs on conventional peptide/MHC complexes, that peptides presented in the thymus are involved in shaping the T cell repertoire, and that recognition during positive selection is promiscuous rather than stringent in nature. Another study used transgenic mice to elucidate the control of B cell development and immunoglobulin expression. The findings will also provide new insights into immunological disorders with a B cell component. Although the immune system of the full-term human fetus is almost functionally mature, in general it is not as efficient as the adult immune system. At birth the neonatal B and T cells are generally naive and unprimed, so they have not undergone antigen-induced clonal expansion or maturation. This partially accounts for the human neonate's susceptibility to infection. Although neonatal B cells are mature and functional, they are unable to produce some specialized antibodies such as IgA and IgG2 against some types of encapsulated bacteria. The neonatal T cells have a diminished capacity to provide help for or actual suppression of immunoglobulin production by B cells, diminished generation and activity of cytotoxic T cells, and decreased capacity to activate macrophages. Recent studies showed that neonatal T cells also have a markedly diminished capacity to produce certain cytokines (e.g., IFN-γ and IL-4). Antigenic naivete is the principal mechanism for selective lymphokine deficiency in neonates. Current research is examining the mechanisms of cytokine regulation in order to devise strategies to enhance cytokine production and protective immunity in the human neonate.

Primary Immune Deficiencies

Hereditary angioneurotic edema (HANE), which affects approximately 1 in 50,000-100,000 people, is the clinical manifestation of the partial deficiency of C1 inhibitor (C1inh). C1inh is the sole inhibitor of the C1r and C1s components of the classical complement pathway and an important regulator of the activity of a number of other important plasma proteases. Therefore, it plays an important role in the regulation of the complement and contact activation cascades. Specific knowledge of C1inh function and of regulation of its gene can lead to improved therapies for HANE and other conditions in which C1inh may play a role. Studies to elucidate the function of C1inh and the regulation of its gene are in progress. They show that interferon-γ (IFN-γ) leads to enhanced synthesis and secretion of C1inh. This induction of C1inh synthesis is primarily regulated at the transcriptional level, and is controlled by elements in the 5' flanking region and first intron of the C1inh gene. The results indicate that mutated IFN-γ activated sequence 4 binds to signal transducer and activator of transcription-1α and is the primary element in the 5' flanking region responsible for IFN-γ induction of the C1inh gene.

Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by eczema, thrombocytopenia, platelets with reduced volume, recurrent bacterial and viral infections due to abnormal immune function, autoimmune diseases and increased risk of malignancies. The immunologic abnormalities observed in the classic phenotype of WAS are complex and involve both B and T cell function. The WAS gene was identified in 1994 by positional cloning and the protein product was designated WAS protein (WASP). The mutations are distributed throughout the WAS gene, affecting all exons except exons 5, 8 and 12. The most common defects found in mild cases of WAS are point mutations resulting in single amino acid substitutions located in exons 1, 2 and 3. In the past year, a major effort has been mounted to establish a genotype-phenotype correlation in WAS. Molecular studies indicate WASP plays a major role in cell signaling and in cytoskeleton formation. Nonfunctional or absent WASP interferes with the normal immune response and the production of platelets and the function of neutrophils. The investigators will continue to elucidate the function of the WASP and the genetic basis of WAS. The goal is to understand the key elements and mechanisms responsible for WAS so effective therapies and prevention strategies may be developed.

Reproductive Immunology

The projects investigate the immunobiology of the placenta and maternal/fetal immunologic interactions during pregnancy in humans and animal models. A group of basic studies focuses on the mechanisms of fetal/maternal tolerance. These studies are designed to identify the underlying immunologic and/or genetic mechanisms which protect the fetus from maternal rejection and allow successful completion of pregnancy. The findings show protection of the fetus from maternal immunologic rejection is multifactorial, possibly involving modulated or unique MHC/HLA expression, hormonal changes during pregnancy, unique expression of non-MHC cell surface molecules, and the specific function of cells and cytokines at the uteroplacental interface. Studies on expression of non-polymorphic HLA-G class I MHC molecules primarily on extravillous trophoblast cells strongly implicate a role for the HLA system in fetal tolerance.

Differential expression and/or repression of classical I molecules on fetal tissue seems to be required for the maintenance of pregnancy. Cell surface molecules, such as complement regulatory proteins, and various cytokines may also play a role in fetal survival. Recent evidence showed certain cytokines (e.g., TGF-β, TNF, and IFNγ) are important in establishing, maintaining, and regulating pregnancy. A fundamental understanding the basic mechanisms of fetal/maternal tolerance has important clinical and therapeutic implications for controlling transplacental transmission of infections, developing therapies for immunologic forms of infertility and recurrent spontaneous abortions, and correcting immunologic dysregulation that leads to unsuccessful pregnancy.

Maternal/Fetal/Neonatal Transfer of IgG: Studies are examining the molecular mechanisms involved in transplacental transfer of maternal IgG antibodies to the fetus; transport of colostral IgG across the intestinal epithelium in fetuses and neonates; and the effects of colostrum on stimulating intestinal maturation and host defense. Maternal IgG acquired both before and after birth contributes to the protection of rats and mice against perinatal infections. Receptors for the Fc portion (FcR) of IgG mediate the transfer of immunoglobulin across the placenta in fetal mice and rats. The FcR has a similar structure to class I MHC Ags, neonatal Fc receptor (FcRn), which mediates transport of IgG across the intestinal epithelium of suckling mice. The FcRn appears to be the only transporter of IgG from mother to young in the mouse. The onset of IgG synthesis in mice that received no colostral IgG lagged behind that in siblings with normal IgG transport, suggesting that maternal IgG stimulates antibody production in the neonate. FcRn is expressed predominantly in the small intestine of the neonate. However, a similar or identical FcRn is present in the fetal yolk sac of late gestational age rats and mice. Using knockout mice lacking functional FcRn, it was determined that FcRn is the only carrier of IgG from mother to young in the mouse. In humans, most materno-fetal IgG transport occurs after the 22nd week of pregnancy. The identity of the receptors that carry IgG across the human placenta is not known. However, IgG has been detected by immuno electron microscopy in vesicles of the human syncytiotrophoblast and endothelial cells of the placenta. These vesicles are presumed to mediate transcytosis of IgG in placenta. Recently a human FcRn (hFcRn) was found in syncytiotrophoblasts, but it was unclear if it was in the fetal capillary endothelium. Although the information strongly suggests that this receptor functions in IgG transport in humans, further studies are needed to confirm the function of hFcRn. In other studies, the IgG FcR from human fetal small intestine was identified, characterized, and cloned. The function and developmental regulation of this hFcR will be studied. Experiments will examine the receptor expression, signal transduction and molecular mechanisms responsible for transport of IgG across human fetal enterocytes. Moreover, the influence of different micro-environments and the effects of breast milk trophic factors (e.g., EGF, IGF-1, and TGF-β) on the ontogeny of human fetal small intestine and host defense will be studied.

Developmental Neurobiology

Important areas of research in developmental neurobiology focus on how the CNS forms and the characterization of critical, early developmental periods and their regulatory signals. While systematic morphological studies have described general aspects of mammalian neural tube formation, the cellular and molecular mechanisms associated with the complex pattern of temporal and spatial interactions giving rise to the adult nervous system remain to be defined. Cellular behavior patterns represent important aspects in the early shaping and, ultimately, the high levels of integration and function of the brain and spinal cord. These behavioral patterns include proliferation, developmentally regulated cell death, motility, changes in cell shape, cell-cell interactions and the interactions between cells and the extracellular matrix. Equally important are the mechanical and "inductive" influences exerted by the tissues surrounding the developing neural tube. Progress in recent years has included the development of antibodies specifically associated with neuronal development and the elucidation of a potential role for homeobox and other genes in neurulation. However, at the molecular genetic level, there is still a need to identify genes involved in early neural development and to place these genes in appropriate biochemical cascades to begin to understand how they exert their influences. In addition, there is a need to establish markers for critical periods of CNS formation and for regional specialization. Molecular signals associated with regulating or modulating phenotypic differentiation, such as growth factors, morphogens, extracellular matrix and cell surface molecules, still need to be identified. While much evidence for the trophic support of neuronal survival has been obtained *in vitro*, *in vivo* studies need to be continued in order to provide further evidence for their role. These studies will also open new avenues of research for potential therapeutic applications.

Zebrafish Genome Project

At the recommendation of the Director, NIH, a zebrafish genome project has recently been initiated by the NICHD, NIDDK and other NIH Institutes working through the Cross-NIH Zebrafish Coordinating Committee. The purpose is to facilitate the mapping and positional cloning of genes in the zebrafish. The specific objectives to be accomplished are: 1) the generation of a genetic map with a resolution of 0.3 cM or better; 2) the development of Expressed Sequence Tags (ESTs) both from existing libraries and also from new cDNA libraries from specific developmental time points and tissues generated under this RFA; 3) creation of a physical (radiation hybrid) map of the zebrafish genome; and 4) the implementation of mechanisms to insure accessibility and ease of use of zebrafish genomic information.

Birth Defects Research Initiative

Birth defects are the leading cause of infant mortality, accounting for 1 in 5 infant deaths. It is estimated that more than 150,000 babies in the United States (about 4% of all live births) are born with significant structural birth defects each year. The estimated lifetime cost of children born

each year with any of 17 major birth defects or cerebral palsy is more than $8 billion. Since many birth defects cannot be fully corrected, they become a major cause of childhood and adult disability. Our long-term goal is the prevention of birth defects. However, effective prevention measures and strategies can only be developed with a thorough and in-depth knowledge and understanding of the epidemiology, etiology, and pathogenesis of birth defects, which is currently lacking. By stimulating and expanding research support for birth defects, this proposed initiative promises to fill some of the gaps in our knowledge. This initiative will capitalize on the extraordinary advances in biochemistry, genetics, molecular and developmental biology to identify the genes, environmental factors, gene/environmental interactions, and underlying mechanisms responsible for birth defects. Ideally, by studying basic, translational, and clinical aspects of important human birth defects, this new initiative will create a setting where basic scientists can work closely and synergistically with clinicians, enhance opportunities for translating basic findings to clinical applications, and provide a fertile environment for training the next generation of investigators.

Computer-Assisted Imaging of Embryonic and Fetal Development

A major research direction for the future is the development and integration of projects that are using advanced computer-assisted imaging for education, for detection and treatment of developmental abnormalities, and for research into the causes of birth defects. DBGT plans to foster this area of research by supporting interdisciplinary research and training programs. These projects will produce a new generation of basic and clinical scientists who will have an improved understanding of embryonic and fetal development, better-trained obstetricians and perinatalogists, and more-informed and interested parents-to-be. They will develop new techniques to reduce the incidence and severity of birth defects, and they will enable an improved understanding of the cellular, molecular, and genetic causes of birth defects.

Developmental Immunology: Ontogeny of Lymphocytic and Thymic Cytokines, Cytokine-receptors, and Signaling Molecules

The proposed initiative is an expansion of the existing program in basic research in human developmental immunology. A related, but more comprehensive RFA on "Ontogeny of Perinatal Host Defenses," was funded in FY97. This initiative, planned for FY99, however, will focus specifically on lymphocytic and thymic cytokines, cytokine-receptors, their signaling molecules, as well as the molecular mechanisms of action during human perinatal and infantile development. In recent years our basic knowledge and understanding of cytokines in adult humans and rodents have expanded dramatically. Unfortunately, this has not been true for cytokines in human fetuses, neonates and infants. The short-term objective of this new initiative is to rectify this disparity by supporting basic research studies on perinatal and infantile cytokines and their roles in immunologic development and disease. The long-term objective is to translate the new knowledge, approaches and insights from these basic studies into effective diagnostic, prophylactic, therapeutic and prevention strategies against perinatal and infantile infections.

DBGT PERSONNEL AND STAFF ACTIVITIES

STAFF

A. Tyl Hewitt, Ph.D., has been with the DBGT Branch since 1991 and was appointed as Branch Chief in August 1997. In addition to duties of Branch Chief, he is also responsible for the programs in limb development, chondrogenesis, myogenesis and related topics in developmental genetics. Prior to joining DBGT, he was on the faculty of the Wilmer Eye Institute, at the Johns Hopkins University School of Medicine. His dissertation research at Emory University centered on changes in cell surface properties during limb development.

Steven L. Klein, Ph.D., was trained as a developmental neurobiologist and a developmental biologist. Prior to joining the NICHD in 1991, he was on the faculty of the Department of Anatomy and Cell Biology of the University of Virginia, where he studied the cellular and molecular events involved in embryonic pattern formation, cell migration, cell interactions and cell fate determination in *Xenopus*. He now is responsible for the programs on Early Embryonic Development and Developmental Genetics.

Deborah B. Henken, Ph.D., joined the staff of the DBGT Branch as a Health Scientist Administrator in 1996. She is responsible for administering research in the area of developmental neurobiology and related topics in genetics. She comes to us after completing the NIH Grants Associate program and has the distinction of being the program's last graduate. Prior to this, she was an intramural scientist in the Laboratory of Experimental Neuropathology, NINDS, where she studied biological responses to virus infection in the nervous system. A graduate of Swarthmore College in Pennsylvania, she received her doctorate degree from Dalhousie University, Halifax, Nova Scotia, in the area of nervous system regeneration.

Allan Lock, D.V.M., is a Health Scientist Administrator responsible for directing research and training programs in developmental immunology, neonatal infections, reproductive immunology, teratology, and fetal tissue research. He also serves as the project officer for the NICHD Transgenic Mouse Development Facility; and the NICHD representative to the Hematology Subcommittee of the Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee and the NIH Chronic Fatigue Syndrome Committee. Dr. Lock came to the NICHD from the National Center for Research Resources in 1991.

Jean Shrier joined DBGT in 1991 as an Office Automation Assistant. Since 1994, she also has served as the Branch secretary and, in this additional capacity, has continued to provide excellent support to all members of the staff in coordinating administrative functions and managing our daily Branch activities.

PUBLICATIONS

Branch D.W., Ducat L., Fantel A., Low W.C., Zhou F.C., Dayton D.H., Gill T.J. The Human Fetal Tissue Working Group. Suitability of fetal tissues from spontaneous abortions and from ectopic pregnancies for transplantation. *JAMA* 1995;273:66-68.

Computer-Assisted Imaging of Embryonic and Fetal Development. 1996. Guest Editor: Steven L. Klein. Special Issue of *Computerized Medical Imaging and Graphics*, Vol 20, No 6. Pergamon Press, Great Britain.

LIAISON ACTIVITIES

Arthritis and Musculoskeletal Diseases Interagency Coordinating Committee

Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee

Skin Diseases Interagency Coordinating Committee

Trans-NIH Birth Defects Special Interest Group

Trans-NIH Group on Issues Related to Human Specimen Resources

Committee on NIH Interactions with Howard Hughes Medical Institute-Supported Scientists

NIH Rat Genome Coordinating Committee

NIH Coordinating Committee for the Zebrafish Genome Project

NIH Cystic Fibrosis Coordinating Committee

NIH Chronic Fatigue Syndrome (CFS) Coordinating Committee

NIH AREA Award Committee

NIH Neuroscience Reorganization Committee

NIH Joint Neuroscience Training Committee

NICHD Neuroscience Steering Committee

NICHD Large Grant Committee

NICHD Small Grant (R03) Committee

NICHD Training Committee

NICHD Program Planning and Program Organization Task Groups

Editorial Board of *Computerized Medical Imaging and Graphics*

"*News From the NICHD*" column for the Society for Developmental Biology Newsletter.

CONTRACTS

Developmental Studies Hybridoma Bank (www.uiowa.edu/~dshbwww/)

Human Embryo Magnetic Resonance Microscopy (embryo.mc.duke.edu)

Human Developmental Anatomy Center (Magneta.AFIP.MIL/embryo/)

The NICHD Transgenic Mouse Development Facility (NTMDF) (transgenics.bhs.uab.edu/page1.htm)

Distribution of Animal Models for Neural Tube Defects (www.jax.org/resources/documents/ntd/NTDhome.html)

Production of cDNA Libraries From Temporal and Spatial Embryonic Mouse Tissue (www.macconnell.com)

CONFERENCES AND WORKSHOPS

Applications of Anti-sense Technology in Development, March 8-9, 1994, Bethesda, MD.

Mammalian Developmental Mutants Workshop, May 10-11, 1994, Bethesda, MD

Workshop on Computer-Assisted Imaging of Embryonic and Fetal Development, June 23-24, 1994, Bethesda, MD.

Symposium on Anti-sense for Basic Research and Biotherapeutics, June 27, 1994, Las Croabas, Puerto Rico, at the Teratology Society Meeting.

Workshop on Molecular Mechanisms of Primary Immunodeficiencies, September 12-13, 1994, Bethesda, MD.

Symposium on Advances in Imaging Techniques for Teratology Research, June 26, 1995, Newport Beach, CA, at the Teratology Society Meeting.

Second Postdoctoral Fellows' Workshop, September 20-22, 1995, Bethesda, MD.

New Approaches for Assessing the Causes and Risks of Developmental Abnormalities from Chemical Exposures, December 11-12, 1995, Washington, D.C.

Chernobyl: Implications of a Decade (in conjunction with The International Congress of Human Genetics), August 24, 1996, Rio de Janeiro, Brazil.

Workshop on the Molecular Genetics of Ectodermal Dysplasias and Related Rare Genetic Disorders Affecting the Craniofacial Complex, November 14-15, 1996, Bethesda, MD.

Developmental Imaging Workshop, September 18-19, 1997, Bethesda, MD.

Workshop on Structural Birth Defects, October 8-9, 1997, Bethesda, MD.

Approaches to the Diagnosis and Treatment of Primary Immunodeficiency Disease (Interactive Satellite Telecast Symposium), October 31, 1997, Falls Church, VA, New Orleans, LA, and nation-wide.

REQUESTS FOR APPLICATIONS

AI-94-023 - The Mechanisms of Embryonic/Fetal Maternal Tolerance: This RFA was co-sponsored by the NICHD to invite applications for basic studies designed to identify the underlying immunologic and/or genetic mechanism (s) which protect the embryo and fetus from maternal rejection, and to elucidate the interactions of the fetal and maternal immune systems in successful pregnancy.

HD-97-002 - Ontogeny of Perinatal Host Defenses: This RFA was issued to invite innovative and hypothesis-driven basic research applications to address the large gaps in our basic knowledge and understanding of the developing human immune system and its role in perinatal host defenses.

PROGRAM ANNOUNCEMENTS

PAS-96-027 - Developmental and Genetic Defects of Immunity: This was a PA with set-aside funds which was jointly sponsored by the NICHD and the Jeffrey Modell Foundation. The purpose was to invite investigator-initiated basic research applications to identify the genes and elucidate the molecular and genetic mechanisms that are responsible for normal and defective development of the fetal, neonatal, infantile, and pediatric immune system.

PAS-97-072 - Developmental and Genetic Defects of Immunity (reissuance of PAS-96-027)

The following Program Announcements were initiated by NIAID and co-sponsored by NICHD:

PA-97-073 - Mucosal Immunity in Pathogenesis/Prevention of Human Disease

PA-97-081 - Basic and Clinical Research on Immune Tolerance

PA-97-099 - Genes and Mechanisms Underlying Primary Immunodeficiency

PA-97-101 - Basic Mechanisms of Vaccine Efficacy

DEVELOPMENTAL BIOLOGY, GENETICS & TERATOLOGY BRANCH
BY PROGRAM CATEGORY, FY 1997

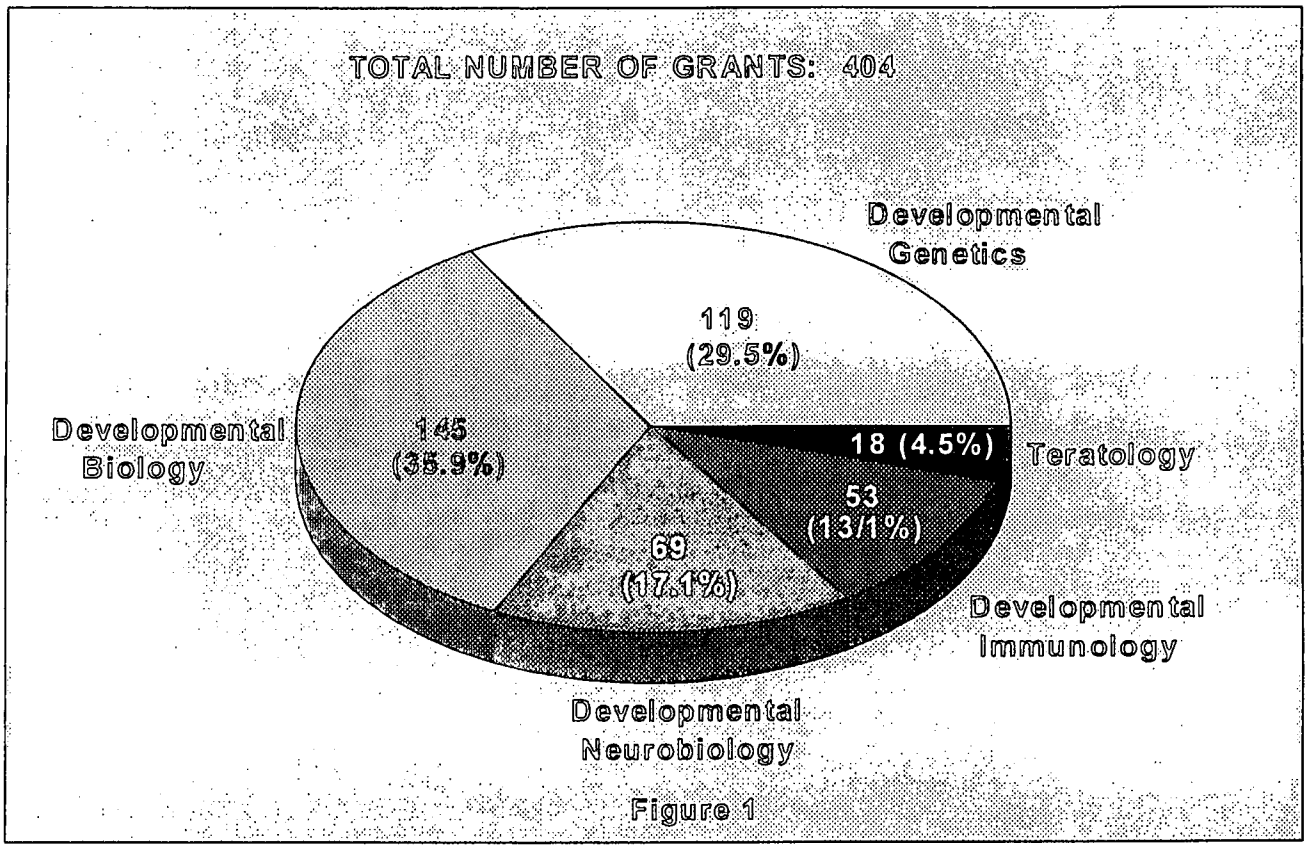

Figure 1

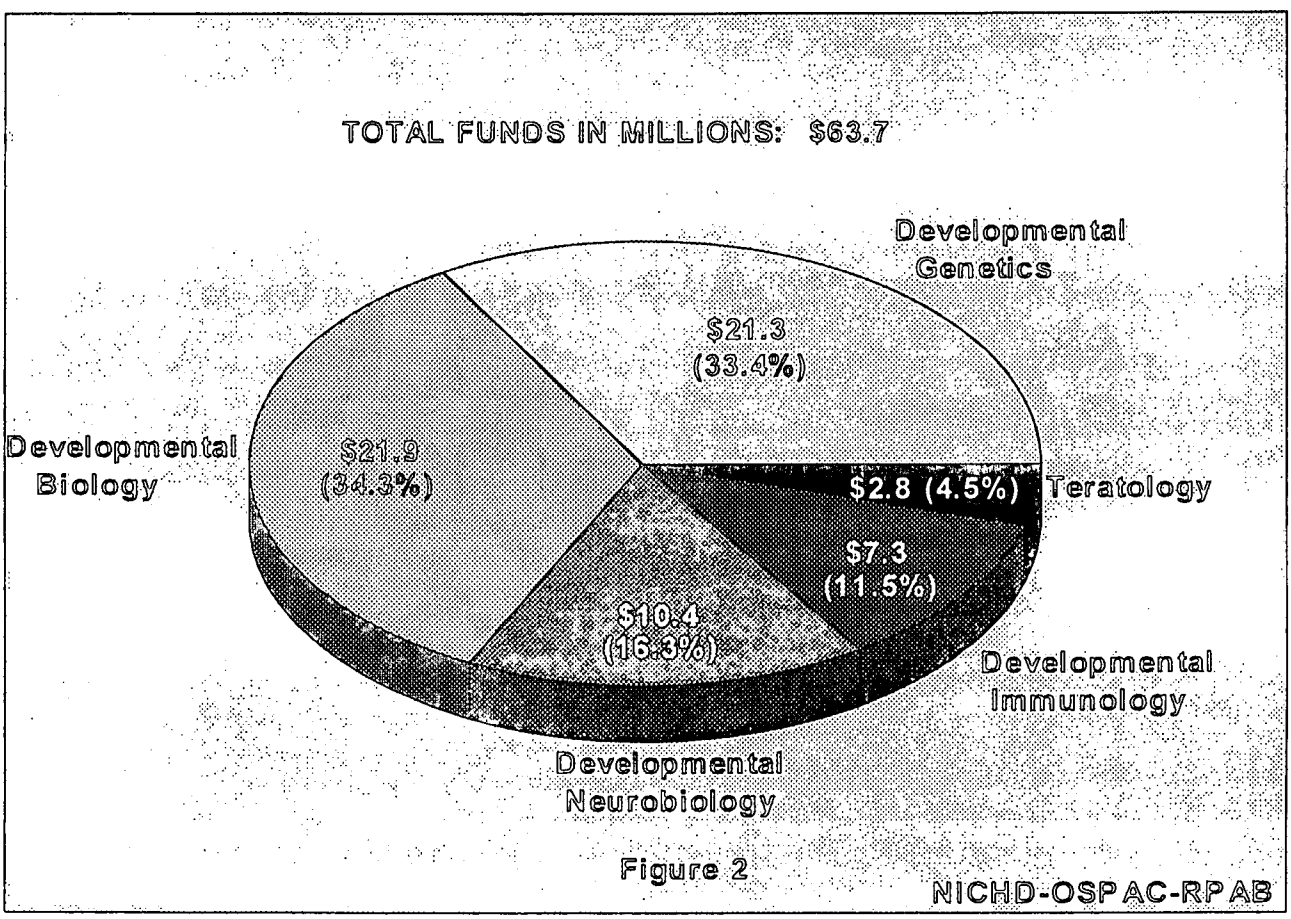

Figure 2

NICHD-OSPAC-RPAB

DEVELOPMENTAL BIOLOGY, GENETICS & TERATOLOGY BRANCH
DEVELOPMENTAL BIOLOGY SUBCATEGORIES, FY 1997

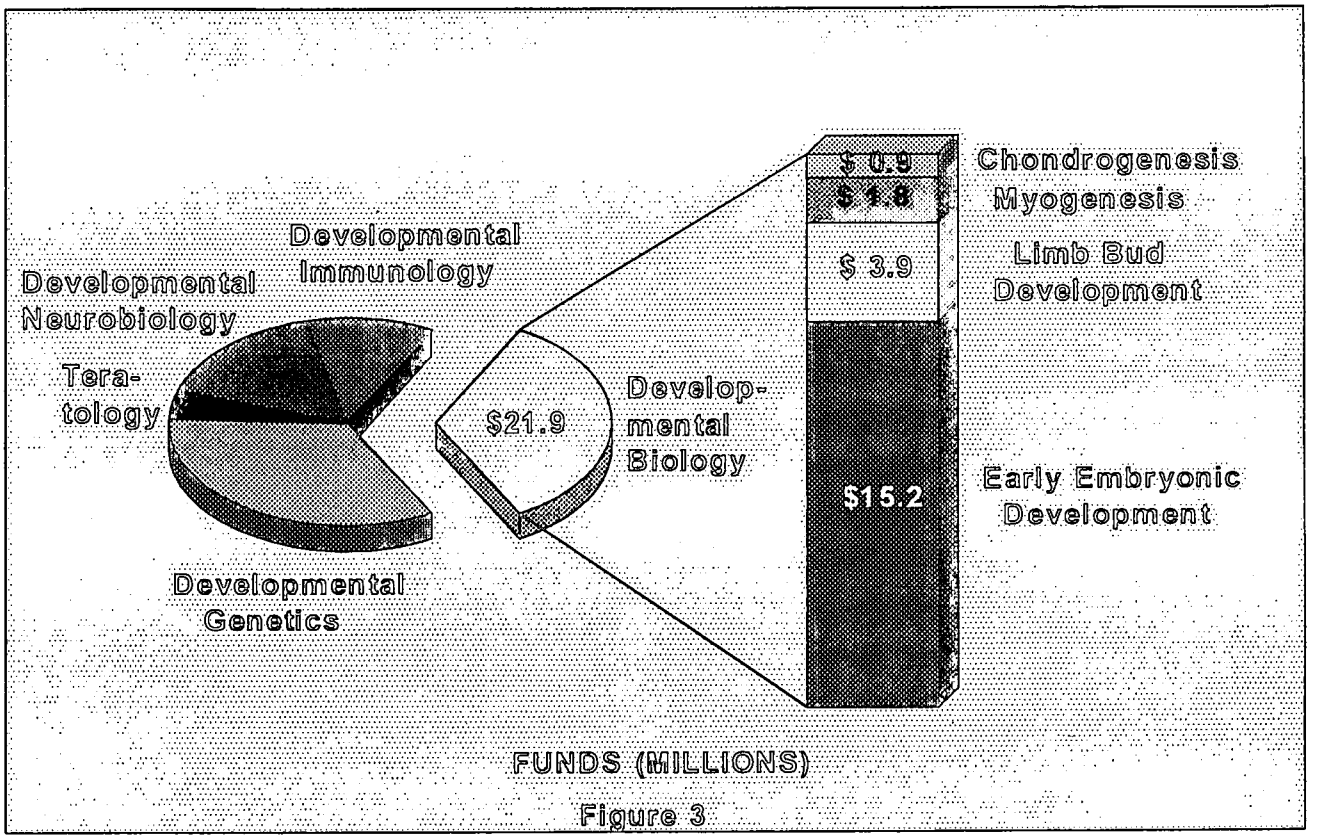

Figure 3

DEVELOPMENTAL IMMUNOLOGY CATEGORIES, FY 1997

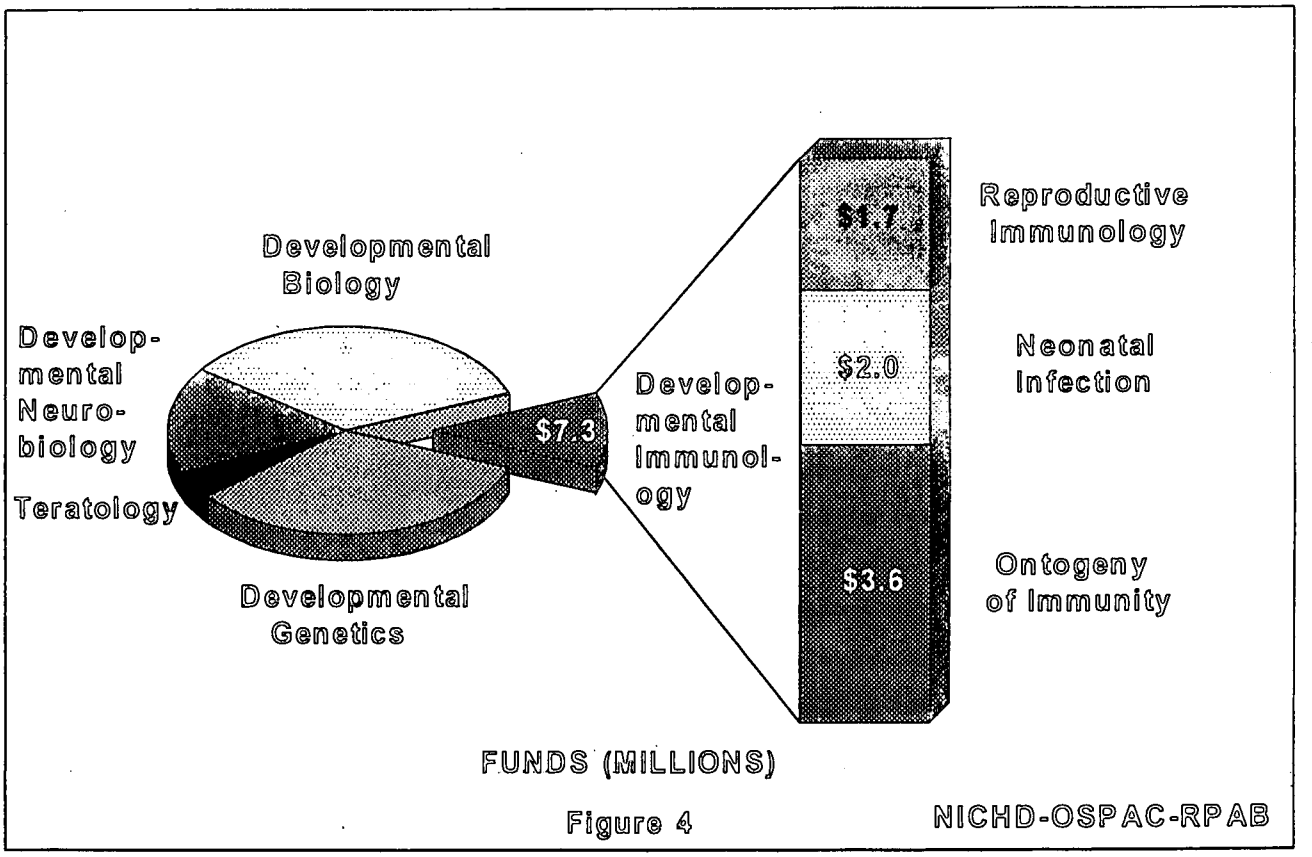

Figure 4

NICHD-OSPAC-RPAB

DEVELOPMENTAL BIOLOGY, GENETICS & TERATOLOGY GRANTS BY PROGRAM CATEGORY, FY 1993 - FY 1997

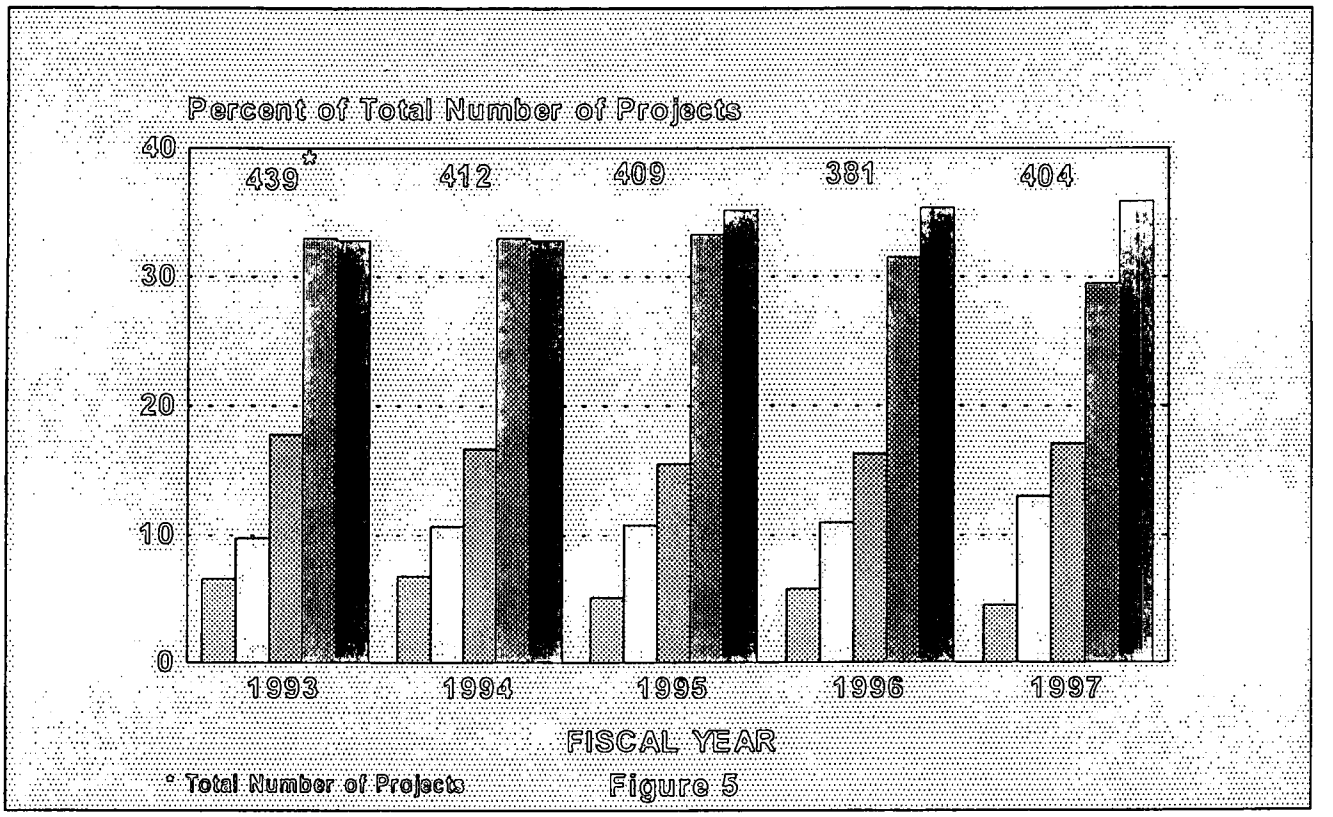

Figure 5

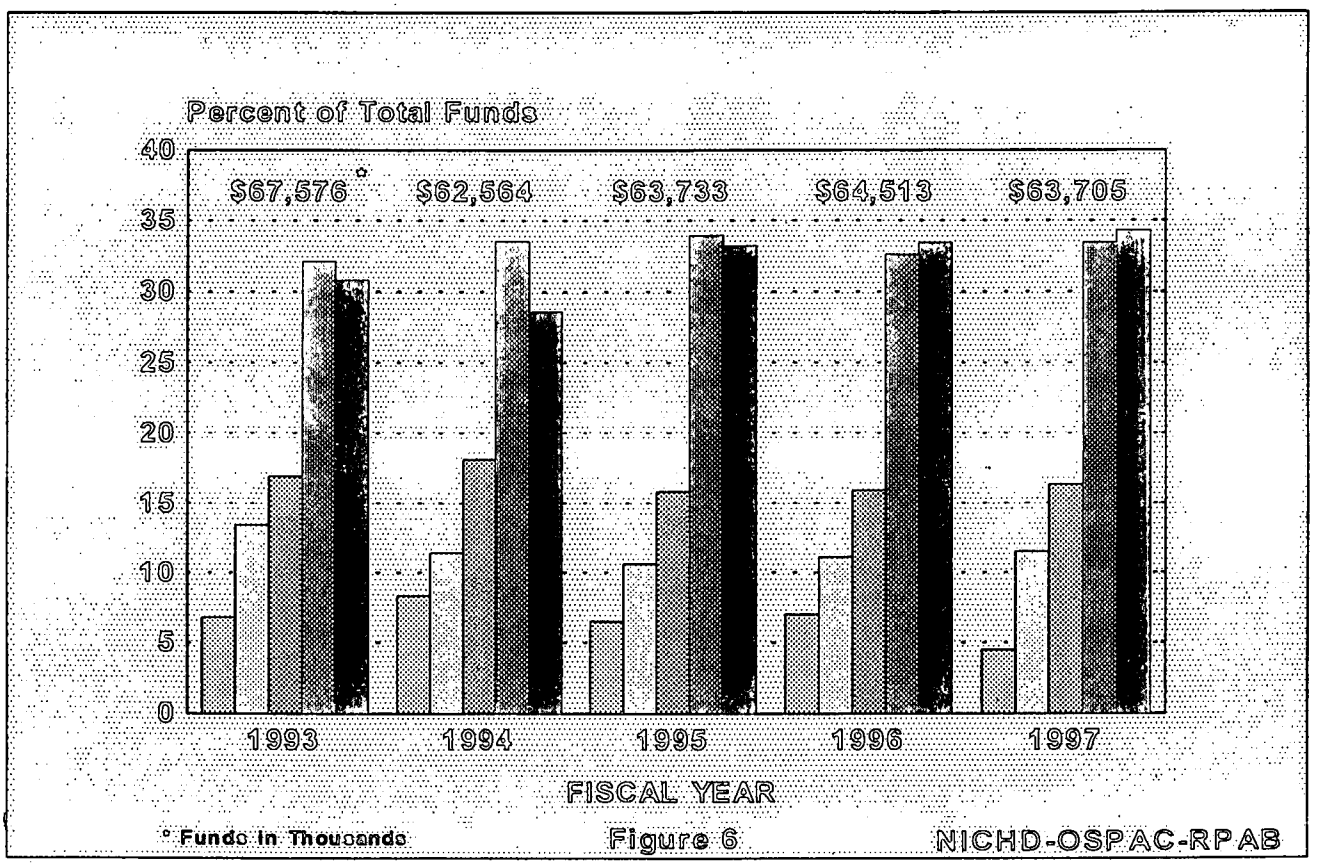

Figure 6

NICHD-OSPAC-RPAB

DEVELOPMENTAL BIOLOGY, GENETICS & TERATOLOGY BRANCH
TRAINING ACTIVITIES, FY 1993 - FY 1997

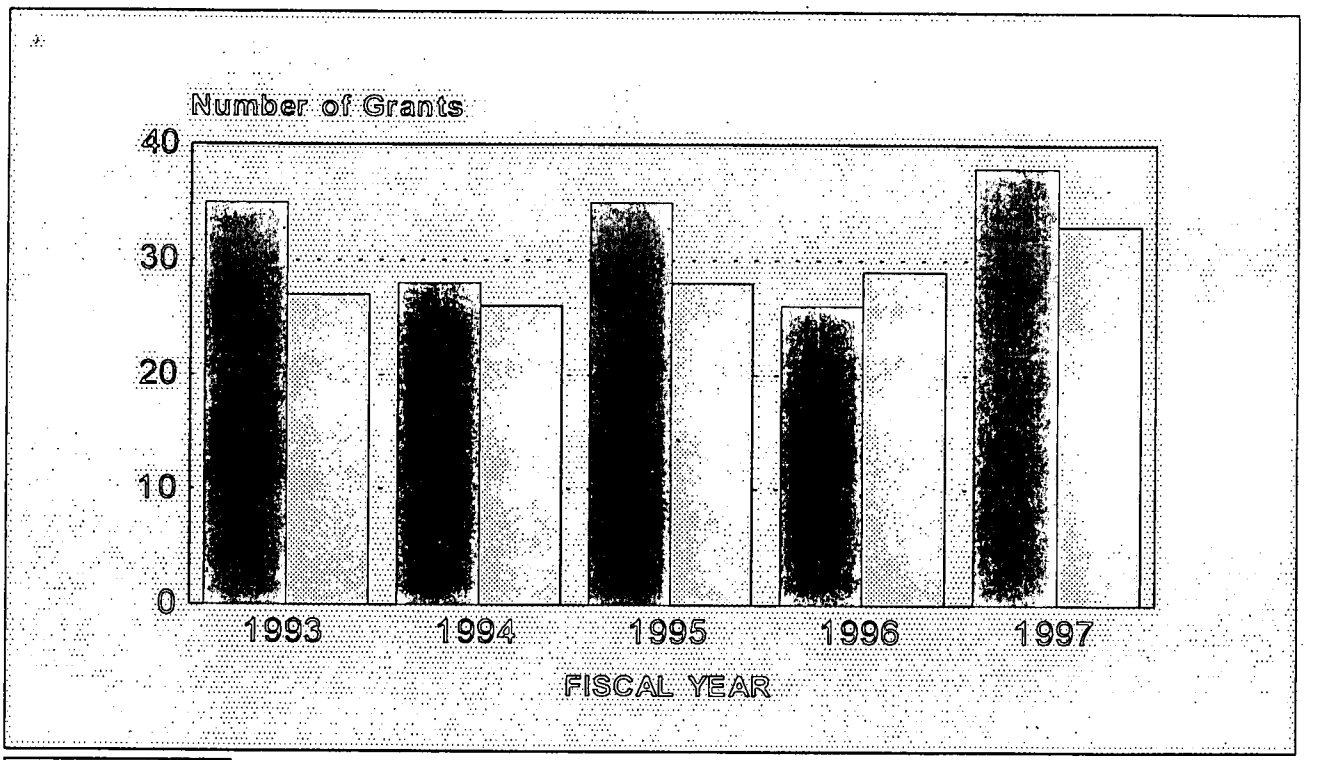

Figure 7

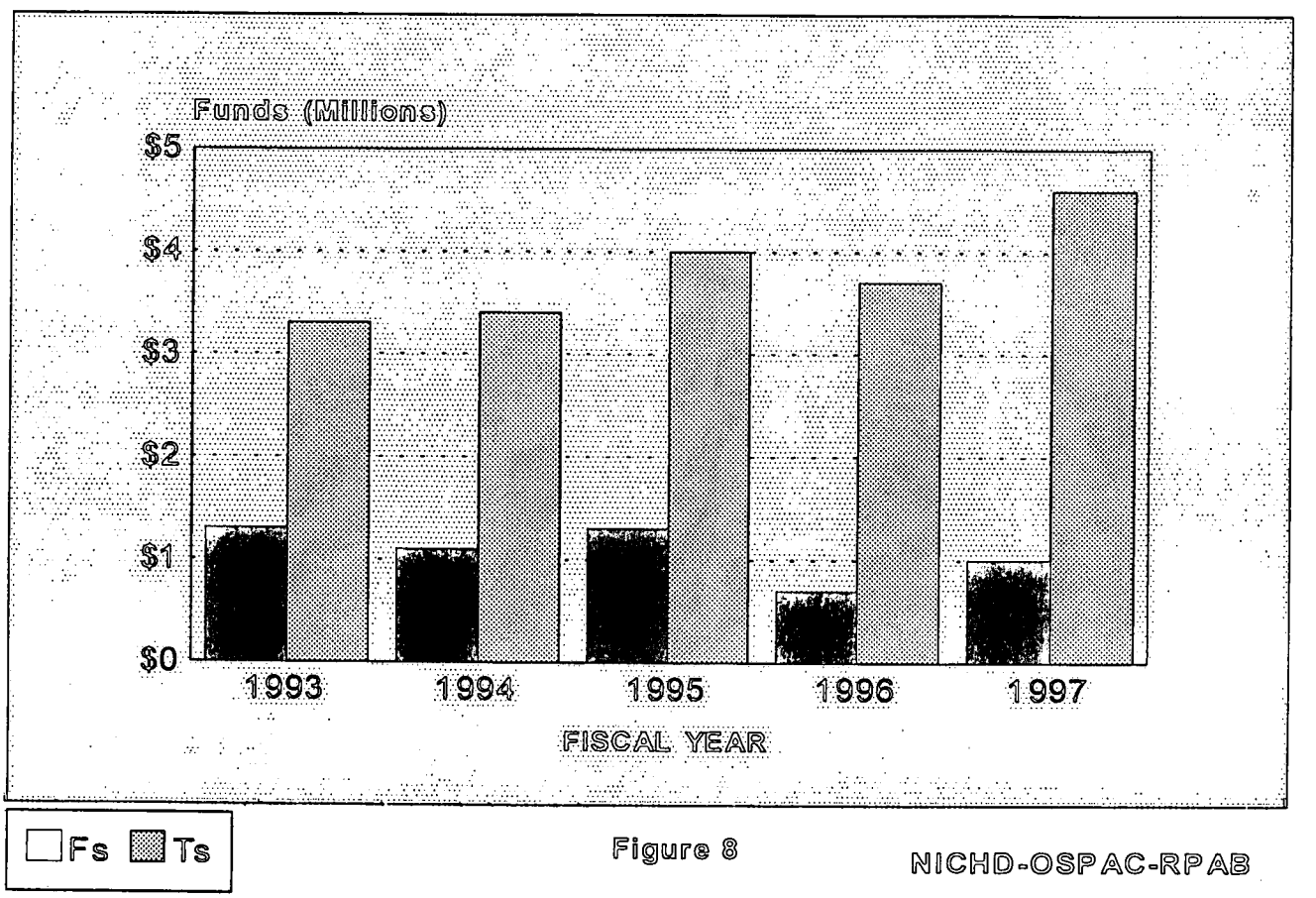

Figure 8

NICHD-OSPAC-RPAB

DEVELOPMENTAL BIOLOGY, GENETICS & TERATOLOGY BRANCH
FY 1987 - FY 1997
FUNDS IN CURRENT AND CONSTANT DOLLARS (1987=100)

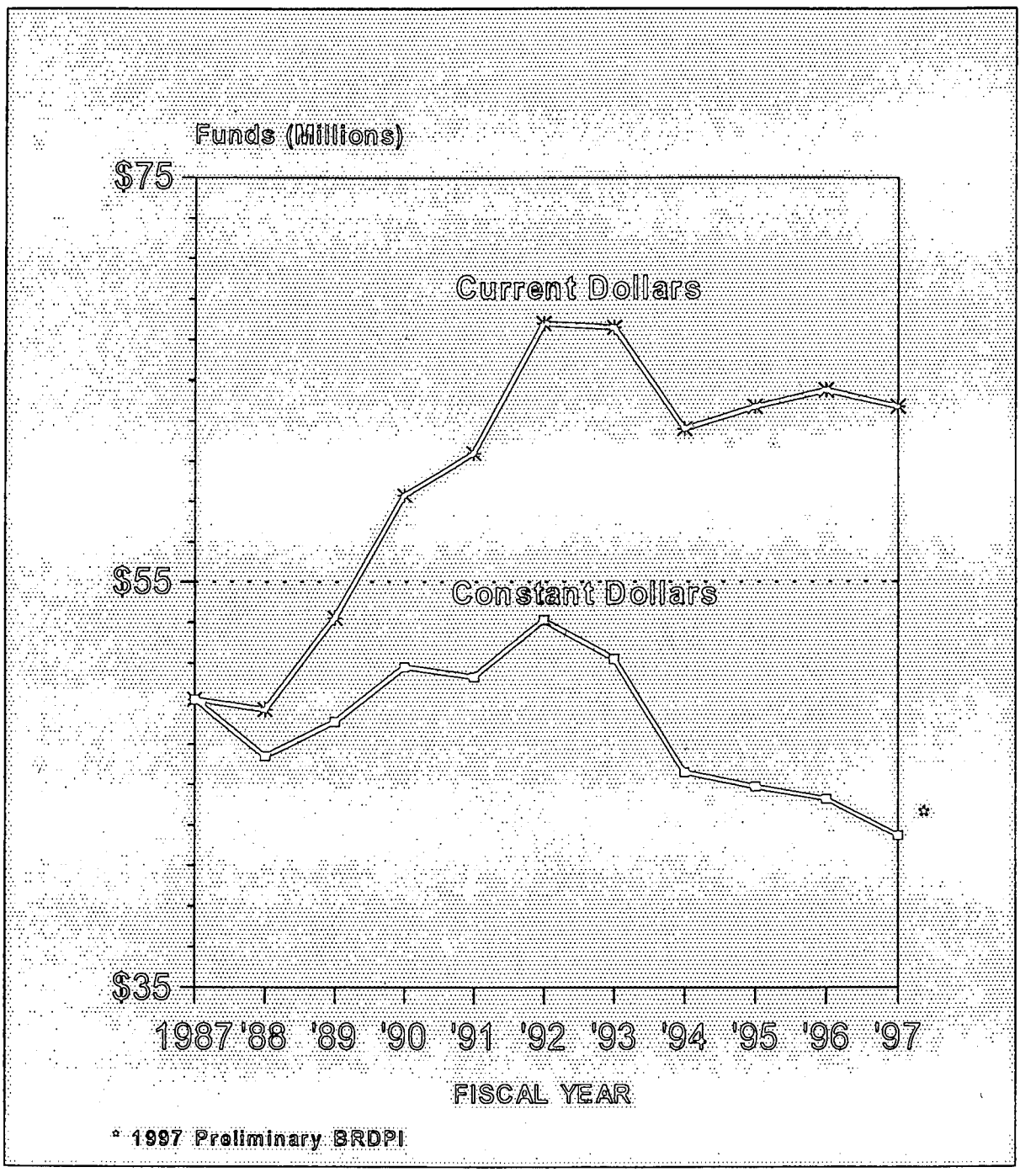

Figure 9 NICHD-OSPAC-RPAB

DEVELOPMENTAL BIOLOGY, GENETICS AND TERATOLOGY BRANCH
GRANTS AND CONTRACTS BY PROGRAM CATEGORY, FY 1997

PROGRAM CATEGORY	TOTAL FUNDS		Research Projects (Incl. P01)		Research Career Program Awards		National Research Service Awards		Research Contracts	
	Funds	%	Funds	%	Funds	%	Funds	%	Funds	%
TOTAL	$63,704,788	100.0%	$53,247,981	100.0%	$2,637,333	100.0%	$5,645,996	100.0%	$2,173,478	100.0%
Developmental Biology	$21,866,924	34.3%	$16,652,419	31.3%	$820,099	31.1%	$4,019,811	71.2%	$374,595	17.2%
Developmental Genetics	$21,275,409	33.4%	$19,877,168	37.3%	$720,783	27.3%	$502,530	8.9%	$174,928	8.0%
Developmental Neurobiology	$10,394,528	16.3%	$9,603,301	18.0%	$246,403	9.3%	$457,726	8.1%	$87,098	4.0%
Teratology	$2,846,276	4.5%	$2,119,565	4.0%	$477,725	18.1%	$248,986	4.4%	$0	0.0%
Developmental Immunology	$7,321,651	11.5%	$4,995,528	9.4%	$372,323	14.1%	$416,943	7.4%	$1,536,857	70.7%
Numbers of Projects										
	Number	%	Number	%	Number	%	Number	%	Number	%
TOTAL	404	100.0%	261	100.0%	55	100.0%	71	100.0%	17	100.0%
Developmental Biology	145	35.9%	78	29.9%	21	38.2%	44	62.0%	2	11.8%
Developmental Genetics	119	29.5%	90	34.5%	18	32.7%	10	14.1%	1	5.9%
Developmental Neurobiology	69	17.1%	51	19.5%	7	12.7%	10	14.1%	1	5.9%
Teratology	18	4.5%	12	4.6%	3	5.5%	3	4.2%	0	0.0%
Developmental Immunology	53	13.1%	30	11.5%	6	10.9%	4	5.6%	13	76.5%

Notes: 1) The Scientific Evaluation Grants are excluded.
2) The Minority Biomedical Support Grants (S06) are included in the research projects.
3) Subprojects of Program Projects are counted individually.