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EXECUTIVE SUMMARY

The field of developmental biology has grown tremendously over the period covered by this report. Enormous progress has been made in elucidating the cellular, molecular, and genetic events that drive embryonic development. This growth is reflected in the budget of the Developmental Biology, Genetics, and Teratology (DBGT) Branch, which has risen from approximately $60 million, to more than $90 million, following several years of near stagnancy (See Figures 5 and 7). The end result of extensive interactions with scientific panels and of recommendations from the scientific community via the World Wide Web was the publication, in February 2001, of *Developmental Biology: Understanding Normal and Abnormal Development*, which represents a blueprint for future planning in this area of research. The increase in budget and grants supported by the Branch is due, in part, to several Requests for Applications (RFAs) that dealt specifically with issues described in the strategic plan, as well as the increased visibility afforded to the research of the Branch by this plan. The program staff increased from four to six, and the program areas were reorganized to more efficiently manage the increasing holdings of the Branch.

While the quest for a better understanding of the genetic and molecular mechanisms underlying developmental processes has always been of interest to the National Institute of Child Health and Human Development (NICHD), this interest is now the cornerstone for the strategic plan and the stimulus for the recent NICHD Birth Defects Initiative. 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 most effective way to minimize human morbidity and mortality is to more fully understand how and when developmental processes take place. To understand abnormal development, it is essential to have a firm grasp of the mechanisms that control normal development. Accordingly, a primary focus of the DBGT Branch is the basic studies of mechanisms regulating development.

Over the past few years, a number of major breakthroughs have greatly increased the understanding of the molecular and genetic controls of embryonic development. New technologies and methods with enhanced sensitivity have allowed investigators to push back the time frame for investigation, so that they can now explore the earliest events initiating the formation of organs. The discovery of new genes has also allowed for a better understanding of specific events, such as the formation of forelimbs and hindlimbs in their appropriate locations. It is also clearer now how certain signaling molecules control critical developmental events, such as gastrulation, determination of cell fate, and patterning events. With further advances in functional genomics and proteomics, it will soon be possible to elucidate how complex networks of signaling pathways interact, and to explain how the same signaling molecule can have distinct effects in different organ systems.

While the use of technological and methodological advances has been important, the incredible breakthroughs in understanding developmental processes have primarily been due to the use of animal models. Clearly, 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. However, an

important advance in thinking is evident in the demonstration of the conservation of genes, genetic networks, and developmental processes across the animal kingdom. Acknowledgement of this critical fact has validated the use of such diverse models as *Caenorhabditis elegans, Drosophila, Xenopus*, zebrafish, chick, and mouse to study human developmental processes. This knowledge of commonalities between species allows researchers 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 can provide insight into the causes of human defects. Animal models also allow researchers 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. NICHD participation in initiatives across the National Institutes of Health (NIH) to support the development of genomic tools and advance the use of model systems is a major component of the DBGT Branch research portfolio.

However, the fact remains that the knowledge gained from animal models must be translated into clinical applications. The use of multidisciplinary research programs that bring together basic and clinical investigators to enhance the translation of basic findings into clinical applications is the stimulus behind the NICHD Birth Defects Initiative, and a major goal of the Institute's strategic plan in developmental biology. In pursuit of this goal, the Institute will continue to investigate birth defects from both clinical and basic perspectives. Clinical studies are important to address the genetics, phenotyping, and characterization of human birth defects. Basic studies that use experimentally manipulable systems are essential to clarify the underlying mechanisms of development. In addition, there must be a two-way flow of information between scientists who have different research interests and endeavors to fill the gaps in knowledge about how perturbation of normal processes causes developmental anomalies. One of the hopes for the grants funded by the Birth Defects Initiative is that findings from clinical and genetic epidemiological studies will provide insights to basic scientists, while, at the same time, allowing for the translation of findings from basic studies to clinical studies and applications.

In the pursuit of knowledge to understand developmental processes and the causes of birth defects, the DBGT Branch supports research and training in the following areas: developmental genetics, early embryonic development, organogenesis, developmental neurobiology, factors in teratogenesis, and developmental and reproductive immunology (Figures 1 and 2, and Table 1a and 1b).

To explore these topics, the Branch supports a large number of trainees through T32 and F32 mechanisms, as well as a number of physician scientists on career development awards (see the *Training and Career Development* Section, and Figures 3, 4, 8, and 9). Support for research is primarily through standard mechanisms: R01, R03, R37, and P01 (Figures 3 and 4). Through these mechanisms, the DBGT Branch supports many of the foremost investigators in the field of developmental biology, including Nobel Prize laureates, Lasker Prize winners, Presidential New Investigator awardees, and Howard Hughes Medical Institute investigators. The staff takes great pride in the knowledge that the DBGT Branch and the NICHD support many of the stars who continue to shape the field of developmental biology. Increased visibility of Branch programs and the judicious use of specific initiatives have helped the influx of grants. Consequently, with the continuing support for research in this area shown by the leadership of the Institute, the future of developmental biology at the NICHD looks bright.

THE NICHD STRATEGIC PLAN IN DEVELOPMENTAL BIOLOGY

The research supported by the NICHD strives to ensure that every child is born healthy and wanted and grows up free from disease and disability. In pursuit of this goal, the NICHD supports studies focusing on mechanisms that regulate normal and abnormal development in order to understand the molecular basis of birth defects. Over the past few years, the NICHD staff has engaged the scientific community in developing a strategic plan in order to facilitate completion of the Institute's mission, with one of the emphasis areas being developmental biology. The end result of extensive interactions with scientific panels and of recommendations from the scientific community via the World Wide Web was the publication of *Developmental Biology: Understanding Normal and Abnormal Development*, which represents a blueprint for future planning in this area of research, in February 2001. This plan, as is true of most of the work supported by the DBGT Branch, emphasizes of the basic biological science necessary to understand early developmental processes through the time when many organ systems form. While the plan focuses on the use of model systems, it also takes advantage of the conserved nature of genes, genetic networks, and developmental pathways across the animal kingdom. This type of research will allow translation of results from these model organisms to human development, while potentially providing the basis for understanding the formation of birth defects. The plan also recognizes that clinical studies are important to address the genetics and phenotyping of human birth defects. In this plan, the Branch tries to foster interactions between basic and clinical investigators to enhance the translation of basic findings to clinical applications, through multidisciplinary research programs on birth defects.

The plan defines a series of scientific goals, including genetic studies of birth defects, basic mechanisms of normal and abnormal development, and the development of model systems. Recommendations for research technologies and resources highlight functional genomics and proteomics, imaging technology, developmental databases, and programs that study the developmental biology of birth defects. The plan also addresses training and education issues associated with increasing the research workforce and the diversity of its expertise. Some of the Branch's ongoing efforts to address topics outlined in the strategic plan are highlighted below, including the Birth Defects Initiative and Branch involvement in promoting the use of animal model systems.

BIRTH DEFECTS INITIATIVE

Clearly, a long-term and major goal of such an initiative is the prevention of birth defects. However, the DBGT Branch also believes that effective prevention measures and strategies can only be developed with a thorough and in-depth knowledge of the epidemiology, etiology, and pathogenesis of birth defects. This Initiative will capitalize on the revolutionary discoveries of the human genome project and the extraordinary advances in biochemistry, genetics, and molecular and developmental biology in order to identify the genes, environmental factors, gene/environmental interactions, and underlying mechanisms responsible for birth defects. With this Initiative, the DBGT Branch hopes to create a setting where basic scientists can work closely and synergistically with clinicians, to enhance opportunities for translating basic findings to clinical applications, and to provide a fertile environment for training the next generation of

investigators. The foundation for this Initiative came from recommendations made by several panels of experts at two workshops: one focusing on clinical and epidemiological research, and the other on basic research. Recommendations from these workshops led to the publication and funding of two RFAs.

The first of these RFAs was co-sponsored and supported by the NICHD, the National Institute of Dental and Craniofacial Research (NIDCR), the National Institute on Environmental Health Sciences (NIEHS), and the Environmental Protection Agency (EPA) and was funded in 2000. The purpose of the RFA was to encourage collaborative, interdisciplinary, and innovative genetic epidemiological studies. These studies will exploit and integrate the extraordinary advances in developmental genetics, functional genomics, and high throughput biotechnology with cutting-edge mathematical, methodological, and statistical tools to evaluate data and determine the etiology, prevalence, distribution, and genetic susceptibility of birth defects in various populations. A total of 11 grants were funded from the RFA, six of those by the NICHD. These projects cover a variety of conditions, including neural tube defects, orofacial clefts, and congenital heart defects. Through these studies the research community will learn more about the potential impact of gene/environment interactions, nutrition, and specific genes on the formation of birth defects. Investigations such as these will provide a foundation for developing hypotheses concerning molecular genetics and the developmental biology of structural birth defects.

The second RFA was co-sponsored by the NICHD and the NIEHS and was funded in July 2001. The purpose of the RFA was to develop synergistic program projects that integrate basic, translational, and clinical approaches to elucidate the molecular mechanisms and genetic bases of human malformations. Using relevant animal models and recent advances in structural, functional, and comparative genomics, proteomics, and biotechnology, these projects will dissect the complex genetics, developmental processes, and molecular mechanisms responsible for human structural birth defects. The Branch selected the program project mechanism because it is ideal for combining multiple clinical and basic component projects with a central theme, and for fostering interactions and collaborations between basic and clinical scientists. A total of five program projects were funded through this RFA, four by the NICHD. The projects cover structural birth defects, such as congenital heart malformations, lung hypoplasia, neural tube defects, and orofacial and skeletal anomalies. Each program project consists of at least three component projects, including basic studies and at least one clinical or translational project.

As a component of the NICHD Birth Defects Initiative, investigators funded by both RFAs will meet periodically to discuss the progress of their research, exchange ideas, share resources, and foster collaborations that are relevant to the research goals of the Initiative. This requirement will help to establish an interactive network of investigators who are interested in multidisciplinary approaches to enhancing the community's understanding of the epidemiology, etiology, pathogenesis, and developmental biology and genetics of structural birth defects.

An important new addition to the Birth Defects Initiative is the Branch's participation in the NICHD Global Network for Women's and Children's Health Research. The cooperative agreement, conducted with substantial involvement by and support from the DBGT Branch, is a collaborative effort between investigators at the Department of Pediatrics at the University of Iowa Medical College, the South American Birth Defects Registry (ECLAMC), and the Centrinho Clinic in Bauru, Brazil. This project will measure the impact of the interventional use of folic acid supplementation on cleft lip/palate, and on neural tube defects. It will also measure the impact of having a child born with clefting on subsequent maternal/infant/family health during the first two years of the baby's life.

RESEARCH INITIATIVES IN DEVELOPMENT USING ANIMAL MODELS

The developmental biology strategic plan relies quite heavily on the use of animal models to clarify the mechanistic causes of birth defects. The discovery of the highly conserved nature of developmental processes across species, from worms (*C. elegans)*, flies (*Drosophila)*, zebrafish (*Danio rerio)*, frogs (*Xenopus)*, avians, and mice, to humans, has been an important conceptual advance because it has provided credence for using these diverse models to study human developmental processes. Consequently, the knowledge provided by work with model systems will provide the basis for translational studies of human congenital defects.

Now that the human genome has been sequenced, the next stage is to identify the genes relevant to development and determine their function. The NICHD leads several efforts to determine the role of genes that control embryonic development and that lead to birth defects when mutated. These studies take advantage of several animal models, including the mouse, the amphibian, *Xenopus*, and the zebrafish.

The Power of Mouse Mutagenesis for Birth Defects Research

For many years, the NICHD has appreciated the fact that the laboratory mouse offers unique advantages for elucidating the causes of human birth defects. In fact, over 15 years ago, the DBGT Branch and the Reproductive Sciences Branch issued an RFA funding projects that revolutionized the study of mammalian developmental genetics. Collectively, these projects devised and/or perfected the major methodologies that are used today to manipulate the genes of the laboratory mouse, including homologous recombination, retrovirally mediated random and targeted gene insertion, gene knockouts, spatial and temporal conditional gene expression, and strategies for gene identification and cloning. These projects enabled a large amount of subsequent research and led to the revolution in biomedical research that has elucidated the genetic basis of many aspects of mammalian biology. Indeed, research supported by the Branch has produced many mutant mouse strains that serve as important models of specific human congenital diseases, and provide critical information about the genetic basis of many birth defects.

However, the biomedical research community has long advocated the need for more models of human birth defects to enable elucidation of their genetic bases. This need became clear through a series of NICHD and NIH meetings, including the ground-breaking meeting in 1998, on *Priority Setting for Mouse Genomics and Genetics Resources*, which led to the development of

the Trans-NIH Mouse Genomics and Genetics Initiative (see http://www.nih.gov/science/models/mouse for more information). The functional analysis of the mouse genome is a high priority for the NIH and was specifically mentioned in the President's fiscal year 2000 NIH budget request to congress. The NICHD plays a key leadership role in this important effort.

The DBGT Branch also helped to establish the Trans-NIH Initiative on Mouse Mutagenesis and Phenotyping for Developmental Defects, which led to the establishment of the Baylor College of Medicine facility for mutagenesis and phenotyping of developmental defects. This Initiative is producing point mutations throughout the genome to enable the examination of two categories of genes: those that control events so critical that mutations in them prevent birth; and those that control less-critical events that, when mutated, lead to viable, but abnormal offspring. The ingenious strategy being used involves inserting a "balancer chromosome cassette," which contain a visible, expressed, coat-color marker, into an identified region of a chromosome. These cassettes inhibit recombination and enable rapid genetic mapping. The coat-color marker enables estimates of loss due to embryonic lethal mutations and tells the investigators to perform prenatal examinations of that cross in order to reveal the time and cause of embryonic lethality, and to provide critical information about genes that cause these severe birth defects. The inserted cassettes also enable mice with lethal mutations to be propagated in large numbers instead of dying, which is critical for thorough examination. Finally, because these cassettes are inserted into identified regions of the genome, they provide an entry point for genetic mapping. These specially engineered mice enable the Baylor facility to identify and examine genes whose role in embryonic development is so critical that they cannot be observed by other mutagenesis and phenotyping strategies.

Phenotypic and genetic data from the initiative are available to the community via the project's Web site, at http://www.mouse-genome.bcm.tmc.edu/ENU/MutagenesisProj.asp. Additionally, the mutant strains are available to researchers via national distributors. Many of the mutants generated so far have defects that are similar to human birth defects, as well as to other conditions, like diabetes and infertility. Because the Baylor facility is producing an enormous number of new mutants, the Branch recently issued an RFA for projects that perform additional phenotypic and genetic analyses. These projects will make the mutants maximally useful for elucidating the genetic causes of birth defects.

The Amphibian, *Xenopus,* as a Model for Developmental Biology Research

Over the past few years, the NIH has asked a broad representation of investigators within the biomedical research community to recommend which non-mammalian models would help to elucidate the basis of human health and disease. Many of these discussions highlighted the importance of the amphibian, *Xenopus*. Because recommendations identified *Xenopus* as a premier model for the study of cellular and developmental biology, the NICHD established the Trans-NIH *Xenopus* Working Group, composed of representatives from 11 Institutes.

For the past few decades, *Xenopus laevis* has been the predominant amphibian in the study of developmental processes because it possesses many unique advantages, including the production of many large embryos and rapid, external development. The embryos also recover well from experimental manipulations, and their cells and identified regions can be excised and cultured in

a simple salt solution. The combination of these advantages and the ease of using this model organism with many molecular techniques have enabled the use of *X. laevis* to elucidate the mechanisms that control several important vertebrate developmental processes, including cell fate decisions, patterning the basic body plan, organogenesis, and signaling pathways in development.

Even though *Xenopus* has been an extremely important model organism, it has lagged behind other models in the development and use of genomic tools critical for the study of modern developmental biology. Consequently, at the NICHD meeting, *Genetic and Genomic Needs for Xenopus Research*, held in 2000, the community recommended two categories of research tools: 1) genomic tools for *X. laevis;* and 2) development of tools for *X. laevis'* genetically amenable relative, *X. tropicalis* (see http://www.nih.gov/science/models/xenopus/ for more information). Together, these tools will create the research infrastructure needed for investigators to use these models to elucidate the mechanisms that control embryonic patterning and organogenesis.

The first recommendation is important because it will enhance gene discovery and characterization in the widely used *X. laevis* model. To date, this recommendation has led to the production of genetic and genomic tools for *X. laevis*, including the construction of 50 cDNA libraries and more than 240,000 *Xenopus* Expressed Sequence Tags (ESTs) that will be helpful in the identification of new genes and their functions.

The development of *X. tropicalis* as a genetic model is the important second recommendation of this initiative. Despite *X. laevis'* advantages for cellular and molecular studies, it has not been used for genetic studies because it is pseudotetraploid and has a long generation time (one to two years). However, the related diploid species, *X. tropicalis*, maintains the advantages of *X. laevis*, but has a much shorter generation time (three to five months) and performs successfully in preliminary genetic studies. Thus, *X. tropicalis* seems to offer enormous potential for genetic and genomic studies of embryonic development.

Although *X. tropicalis* has many apparent advantages over *X. laevis*, the research community needs proof that the organism fulfills all of the requirements of a genetic model in order to draw a critical mass of investigators to use it. To address this need, the DBGT Branch issued an RFA in 2001, to optimize the parameters of mutagenizing and phenotyping *X. tropicalis.* The RFA established new projects that use three standard methodologies to produce and characterize mutations with defects in developmental processes: deletion mutations, random insertional mutations, and point mutations. Collectively, these projects will enable large-scale mutagenesis, phenotyping, and gene identification and characterization in this important model for developmental biology research. Because developmental processes are conserved across vertebrate animals, these resources will benefit all aspects of developmental biology research. Other recent DBGT Branch initiatives to create these resources and to establish a genetic *Xenopus* system will lead to major new advances, which will benefit *Xenopus* research, research in other model systems, and research into the causes of human birth defects.

Zebrafish Research

The use of zebrafish as a model of vertebrate development and disease has grown over the past few years, primarily because of the organism's value in both experimental and genetic analyses. Unlike *Xenopus*, which allows experimental analyses of early development, or the mouse, which allows genetic analyses of early development, *D. rerio* is a single species in which both experimental and genetic analyses of early development can be studied. 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. *D. rerio's* short generation time (three to four months) and small size make it easy to propagate in large populations and to characterize many mutations. These attributes have allowed researchers to construct detailed fate maps and cell lineages, transplant and experimentally manipulate individual cells, and characterize specific cell migrations and differentiation in living vertebrate embryos.

Acting on recommendations from the zebrafish research community, the NIH established the Trans-NIH Zebrafish Coordinating Committee (TZCC) to generate genetic and genomic resources needed to promote the use of the zebrafish system for genetic studies of vertebrate embryogenesis and disease. Co-chaired by the NICHD and the National Institute of Digestive Disorders and Kidney Diseases (NIDDK), the TZCC now comprises 18 Institutes and Centers (See the NIH Zebrafish Web site, at http://www.nih.gov/science/models/zebrafish/index.html for more information).

Since its inception in 1997, the TZCC has released a number of initiatives. The first of these (DK-98-006) funded six projects to generate and provide genomics tools. As a result of the individual and collaborative efforts of the investigators, many important resources and contributions have been made to the zebrafish research community, including:

- More than 225,000 ESTs assembled in 18,000 gene clusters;
- Radiation hybrid (RH) maps;
- Two cross-referenced genetic maps with a distance between markers of less than one centimorgan;
- Generation of over 60 gamma-ray-induced deficiencies that have been characterized and mapped; and
- A comparative genomics project to elucidate syntenic relationships between zebrafish and mammalian genomes.

Although these projects are coming to a close, several of the participating labs have begun to map the ends of BAC clones to the RH map to create a physical scaffold for the genomic sequence, which is being generated by the Sanger Center in Great Britain. The genomics data and tools developed through these projects are even more valuable now that the Sanger Center's genomic sequencing effort is underway.

In an effort to be responsive to the needs of the zebrafish research community, the TZCC sponsored a meeting in spring 1999, titled *Genomic and Genetic Tools for the Zebrafish*. This meeting reviewed the current state of zebrafish research and solicited recommendations for the future needs of the research community. The resulting recommendations covered a number of

important areas, including genomics, sequencing, mutagenesis, and bioinformatics issues. To address these needs, the NICHD and TZCC published an RFA entitled *Mutagenesis Screens/Phenotyping Tools for Zebrafish* (HD-00-004), to exploit the power of mutagenesis screening in zebrafish in order to detect and characterize genes, pathways, and phenotypes of interest in development, behavior, organ formation, and disease processes. The participating Institutes and Centers made 14 awards. In 2001, the TZCC also issued two Program Announcements (PAs) in response to the research community's needs, *Development of Zebrafish Mutagenesis and Screening Tools* (http://grants.nih.gov/grants/guide/pa-files/PA-01-070.html), and *The Zebrafish as an Animal Model for Development and Disease Research* (http://grants.nih.gov/grants/guide/pa-files/PA-01-095.html).

MAMMALIAN GENE COLLECTION (MGC)

Funded by 17 NIH Institutes and Centers, including the NICHD, the MGC is a trans-NIH initiative that supports the production of cDNA libraries, clones, and sequences. The original goal of the MGC was to provide a complete set of full-length sequences and cDNA clones of all expressed genes for both the human and the mouse. Over the past year, the MGC has produced over 80 cDNA libraries enriched for full-length cDNAs derived from human tissue and cell lines, as well as mouse tissue. This trans-NIH initiative has been tremendously successful, and all the resources generated by the MGC are publicly accessible to the biomedical research community. The MGC is moving toward the completion of a full-length cDNA collection for both the human and the mouse. Other model organisms, including non-mammalian models, will be considered for analysis by the MGC in the future. Of particular interest to the NICHD is obtaining the full-length cDNA sequences for two very important animal models relevant to the developmental biology community, *Xenopus* and zebrafish.

HIGHLIGHTS OF RESEARCH PROGRESS

The majority of the investigator-initiated grants supported by the DBGT Branch primarily focus on basic mechanisms regulating development. Most use animal models, but others have a human focus. This section describes some of the advances made by investigators supported by the Branch; due to space limitations, it does not present all of the work supported by the Branch but, rather, provides only a few highlights. For clarity, when describing animal studies, references to genes are in *italics*, whereas the protein products coded by these genes are **not** italicized. When mentioned, references to human genes and proteins follow the same convention, but are in capital letters. General references to a family of factors are indicated by the abbreviated name, in capital letters, regardless of species (such as, members of the BMP family).

THE DEVELOPMENTAL GENETICS AND GENOMICS PROGRAM

This Program supports investigations into the role of genetic determinants in development, and into the manner in which mutant genetic processes are involved in causing developmental defects. Congenital defects result from a variety of genetic anomalies, including: single gene defects, chromosomal defects, or multi-factorial conditions (classified as a combination of genetic factors with or without environmental influences). Research funding is provided by the Branch for: studying basic genetic regulatory mechanisms in animal models; identifying genes that initiate and control the developmental processes; analyzing individual genes or genetic networks that are differentially expressed at characteristic developmental times; and defining basic regulatory mechanisms for gene expression. This research includes studies to: determine inheritance patterns of developmental anomalies; understand how genes interact with each other and the environment to cause birth defects; define functional aspects of nucleotide sequences; and identify DNA sequence aberrations that result in a wide variety of clinically observed developmental abnormalities.

The Developmental Genetics and Genomics Program is particularly interested in developing research programs that utilize functional and comparative genomics and proteomics for the analysis of gene activity and protein function, and that use imaging technology in the visualization of developmental processes. Research using these emerging technologies will enable scientists to visualize developmental processes, and to monitor cellular and molecular changes at a level of resolution previously not possible. Support for these areas is essential for the future of integrated research in basic and clinical developmental biology.

DNA Methylation, Gene Expression, and Genomic Imprinting

DNA methylation has a profound effect on gene expression and plays a key role in the acquisition of functional epigenetic modifications to DNA. Methylation serves as a "tag" that enables regulatory factors to distinguish methylated sequences from identical, but unmethylated ones. DNA methylation is an important regulator of genetic information in species ranging from bacteria, to humans. Mammals use the modification of DNA by methylation to prevent transcriptional initiation, and to ensure the silencing of genes on the inactive X chromosome, imprinted genes, and the DNA of parasites. Further evidence that DNA methylation is critical for development comes from mice that have a targeted disruption of the gene, *Dnmt-1*, which encodes a DNA-methylating enzyme. Mice with this mutation die at an early embryonic stage.

One of the interesting and unexplained features of DNA methylation is the complexity of the methylation patterns. For instance, until recently, no DNA methyltransferase mutations had been reported in humans. However, investigators studying the mechanism of X chromosome inactivation have reported the first example of a naturally occurring mutation in a mammalian DNA methyltransferase gene (*DNMT-3B*). These mutations occur in patients with a rare autosomal recessive disorder, called Immunodefiency, Centromeric Instability, and Facial anomalies or ICF syndrome, which is characterized by a variable immunodeficiency, mild facial anomalies, centromeric decondensation, and restricted hypomethylation patterns. Further analysis of mutant methyltransferases such as the *DNMT-3B* forms found in ICF syndrome may lead to insights into methylation complexities.

Other investigators are studying the molecular mechanisms involved genomic imprinting. Genomic imprinting is a gene regulatory process in mammals that distinguishes the parental origin of alleles; that is, it creates a differential expression of genes depending on whether those genes are inherited from the male or female parent. Expression differences between alleles of imprinted genes are thought to be the consequence of the inheritance of parent-specific methylation patterns. Disturbances in this process, which ensures the establishment, transmission, and perpetuation of imprints, can result in human congenital anomalies. Certain types of cancer and Beckwith-Weidermann, Angelman, and Prader-Willi syndromes demonstrate a direct association between genomic imprinting and inherited diseases. Using the mouse as a model system, investigators have also demonstrated that an oocyte-specific DNA methyltransferase variant, *Dnmt-1o*, is required to maintain methylation patterns at imprinted loci during a specific stage of cell division. Further manipulation of DNA methyltransferases is likely to illuminate additional aspects of the pattern dynamics of genomic methylation during mammalian development. The use of animal models will help researchers uncover the molecular mechanisms involved the process of epigenetic regulation of gene expression and learn how perturbations of these processes result in human congenital anomalies.

Growth Factor Receptors and Lymphedema

Primary lymphedema is a rare, autosomal dominant disorder that leads to disabling and disfiguring swelling of the extremities. When untreated, the condition tends to worsen with time. The genetic basis for this condition, an under-studied developmental disorder of the lymphatic system, was initially localized to distal Chromosome 5q, with the Vascular Endothelial Growth Factor Receptor-3 (*VEGFR-3*) identified as a candidate. Subsequent work has established that missense mutations in the tyrosine kinase domain of the VEGF receptor affect its biological activity. These studies will ultimately lead to a better understanding of the developmental biology of the lymphatic system and could provide a means of identifying high-risk individuals in lymphedema families, as well as in the normal population.

Patent Ductus Arterious (PDA) and Char Syndrome

Until recently, a genetic defect specific to PDA had not been identified in humans. Char syndrome is a heart-hand disorder with a phenotype that includes PDA, abnormal fifth digits on the hands, and facial dysmorphology. Char syndrome has an inheritance pattern consistent with an autosomal dominant mechanism. However, the phenotypic expression is variable, particularly with respect to the PDA. Among Mendelian disorders with cardiac defects, Char syndrome is unusual in having PDA as the predominant heart lesion, which suggests a role for its disease gene in sixth aortic arch development.

Studies using a positional cloning/candidacy gene approach have identified a Char syndrome disease gene, *TFAP-2B*, that encodes a transcription factor also known as AP-2β. The family of AP-2 transcription factors has important functions in retinoid-regulated morphogenesis, particularly in affecting structures derived from neural crest cells. These studies are directed toward understanding the biological role of the AP-2 family in the development of the ductus arteriosus. In addition, the exploration of the pathogenesis of PDA in Char syndrome provides a rare opportunity to understand a pathway leading to this common, yet poorly understood form of congenital heart disease.

Homeobox *(HOX)* and T-box Genes: Relevance to Congenital Anomalies

Pioneering work using the animal model, *Drosophila melanogaster*, some 40 years ago has paved the way to the realization of the conservation of genomic organization and expression patterns in all animals. In every organism, architectural patterning genes are part of a complex developmental program encoded in that animal's genome. The genes have to be expressed at the right time, in the right place, and they have to exert precise control over their downstream target genes. Molecular and genetic evidence gathered over the past 20 years indicates that different animals possess many common genetic systems for embryonic patterning.

One class of important genes involved in patterning the anterior-posterior axis of all bilateral animals, including humans, are called homeotic genes, all of which contain a distinctive sequence of DNA bases that have been termed the "homebox." These genes, called *Hom* genes in invertebrates and *Hox* genes in vertebrates, govern similar aspects of body design in all animal embryos. Vertebrates have four clusters of *Hox* genes: *Hoxa, Hoxb, Hoxc, and Hoxd.*

Investigators funded by the DBGT Branch and others have demonstrated that mutations in several *HOX* genes in humans are responsible for a variety of anomalies, ranging from limb defects, to hand-foot-genital syndromes, to urinary tract anomalies. These findings further demonstrate the importance of the use of animal models in the quest for understanding the etiology of human disorders. In the race for understanding the molecular basis of disease, simple model organisms, such as *Drosophila, C. elegans*, and others will continue to be an indispensable tool for providing answers relevant for human biology.

T-box genes are another important family of transcriptional regulators of early development that were recently identified as the genes responsible for several complex human developmental disorders. DBGT-funded investigators have been instrumental in the phylogenetic study of the T-box gene family, which now numbers nearly 20 genes. Members of this family have been identified in both vertebrates and invertebrates, where the genes play key roles in the regulation of embryonic development, and, particularly, in morphogenesis and the assignment of cell fate. It is now clear that the T-box genes play essential roles in early development, including specification of the mesoderm, as well as in heart and limb morphogenesis.

The recent knowledge of an association between inherited developmental disorders and T-box genes may soon provide a medical pay-off from this basic research. Genetic data indicate that the limb and cardiac malformations that characterize the autosomal dominant Holt-Oram syndrome can be produced by nonsense mutations, deletions, rearrangements, and missense mutations of the *TBX-5* gene. Another pleotropic disorder that affects limb, apocrine-gland, tooth, hair, and genital development, called ulnar-mammary syndrome, has been shown to be caused by mutations of the *TBX-3* gene. Genetic analysis of other human T-box genes has demonstrated potential associations with other human conditions, such as DiGeorge syndrome (*TBX-1*), Bardet-Biedl syndrome 1 (*TBX-10*), and Acromegaloid Facial Appearance syndrome (*TBX-15*). The importance of T-box genes in human developmental diseases, coupled with the finding that the T-box genes are evolutionarily conserved, aids in the scientific justification of the use of lower organisms in the study of normal and abnormal human development.

The Developmental Role of Cholesterol

Of the several hundred inborn errors of metabolism discovered and characterized in the last century, relatively few involved abnormal *de novo* synthesis of an essential small metabolite. Furthermore, most inborn errors of metabolism feature postnatal evolution of metabolic deficiencies or toxicities in children who are phenotypically normal at birth. In 1993, researchers made a surprising discovery that Smith-Lemli-Opitz syndrome, one of the best-known autosomal recessive malformation-mental retardation syndromes, was caused by a primary defect of cholesterol biosynthesis. The finding not only raised important questions about embryological links between abnormal sterol biosynthesis and congenital malformations, but also focused attention on the special implications of a disorder that involves an essential fetal metabolite that cannot be supplied in adequate amounts by the mother during gestation.

In addition, DBGT-funded investigators studying the human condition, X-linked dominant chondrodysplasia punctatas, have discovered that mutations in a novel 3β-hydroxysterol dehydrogenase gene in the mouse, *Nsdhl*, were associated with X-linked male lethalities. These investigators subsequently demonstrated that mutations in the human *NSDHL* gene were responsible for most cases of CHILD syndrome (Congenital Hemidysplasia with Ichthyosis and Limb Defects).

These findings are yet another example of the importance of utilizing model organisms, in this case, the mouse. Such studies have provided insight into the elucidation of the developmental role of cholesterol and some of its intermediate metabolites. This research will further the knowledge of the role of sterols in various developmental processes and could eventually lead to the design of new therapeutic agents for skeletal and skin disorders.

From Slime Molds to Mice to Man – Genomic Sequencing and Functional Genomics

Perhaps the best way to find genes involved in developmental processes is to sequence the complete genome of a genetically tractable organism. The availability of complete genome sequences promises to accelerate research into the fundamental mechanisms of cell and developmental biology. One such organism is *Dictyostelium discoideum*, a simple multicellular organism that undergoes a small number of well-characterized events during its life. Such events are very similar to the developmental events that transform the multicellular vertebrate zygote into an embryo. *D. discoideum* has served as a model for vertebrate development for several decades; the current understanding of many developmental processes is derived from studies of this organism. Common processes are the basic intracellular and intercellular events that form the organs and organ systems during the development of all multicellular animals.

DBGT-funded investigators have nearly completed sequencing the *D. discoideum* genome. This information will allow researchers to learn the functions of vertebrate genes that control critical aspects of embryonic development. With the sequencing of the mouse and human genomes either completed or nearly completed, *D. discoideum* information will also serve as a valuable tool for the field of comparative genomics.

Even though the complete transcriptional map of the genomes and the entire genomic sequence of a variety of organisms, including human, may be known, the function of most of the genes will likely remain unclear. The problem of elucidating function for all of these genes in animals

has spawned a new era of research, called "functional genomics" or "new genomics." The goal of the functional genomics effort is to determine the biological function of a genome using strategies that will ultimately coalesce with the genetic maps, physical maps, DNA sequence, and gene transcription patterns. The unique value of functional genomics lies in the power of large data sets to provide a broad and less-biased view of biological systems. Such global studies are expected to reveal an organization within functional pathways and regulatory networks that is difficult to appreciate from analyses of small groups of gene products.

DBGT-funded investigators have essentially opened the door to the emerging field of functional genomics using a variety of model organisms. Studies underway utilizing *Dictyostelium* will further the understanding of diverse biological processes that are common to many eukaryotes, such as motility, cell differentiation, and morphogenesis. These investigations are exploring biological regulation from a genomic perspective by examining the function of several thousand genes in a simple developing organism.

The generation of mutations in the mouse, by either chemical mutagens or targeted gene disruptions, will also play an important role in unraveling the function of many developmentally relevant genes in this model organism. With the complete sequencing of the human genome on the horizon, new approaches are being developed for the elucidation of the functional content of the human genome. The utilization of model organisms will be paramount to this effort.

Bioinformatics

Gene expression patterns provide important insight into the molecular mechanisms of development, differentiation, and disease. The mouse serves as a pivotal animal model because it is closely related to humans, and because tissues from many different mouse strains and mutants are readily available for detailed expression analyses. The scientific community is generating an ever-increasing amount of expression data of an increasingly diverse and complex nature. This proliferation of scientific results raises questions of how to manage, integrate, and analyze these data. The DBGT-funded Gene Expression Database (GXD) is a publicly available, electronic tool developed to address the problems raised by these scientific results. The GXD is a community resource designed to provide integrated access to different types of expression data, and to place those data in the context of other biological information. The GXD has been integrated with the Mouse Genome Database to enable a combined analysis of genotype, expression, and phenotype data and has comprehensive links to other databases such as PubMed, OMIM, sequence databases, and databases from other species. The integrated system, called the Mouse Gene Expression Information Resource, will be an extremely valuable tool in studying and understanding the molecular basis of development, differentiation, and disease.

THE EARLY EMBRYONIC DEVELOPMENT PROGRAM

The Branch's Program in Early Embryonic Development supports research examining events that occur between the time of fertilization and gastrulation. These critical patterning events transform the fertilized egg cell into a multicellular organism with identifiable axes, and with primary germ layers. These mechanisms are so important for normal development that they are highly conserved across all animals. Additionally, the mechanisms that control these early events control many subsequent developmental events.

A complete understanding of these early events is required to elucidate the mechanisms that regulate all of embryonic development and, ultimately, to prevent and cure human birth defects. These early events include axis formation and patterning, germ layer specification, cell fate specification, cell-cell signaling, and cell migration. Studies supported by the DBGT Branch since its last report to the National Advisory Child Health and Human Development (NACHHD) Council have elucidated many of the cellular, molecular, and genetic mechanisms that control such critical events. Among the most exciting recent discoveries are those that provided a new understanding of the molecular and genetic mechanisms that form and pattern the embryonic axes. In the interest of spatial limitations, the following section describes only those discoveries related to embryonic axes.

Axis formation begins soon after fertilization, when a complex series of events initiates the formation of the dorsal-ventral axis. Continuing until the time of gastrulation, these events establish the axis and control the patterning of structures along the dorsal axis. A separate series of events, occurring mostly during gastrulation, establish the right-left axis. Alterations in these events lead to severe birth defects.

Development of the Dorsal-Ventral Axis

The first patterning event that occurs after fertilization initiates the formation of the dorsal-ventral body axis. The dorsal side of the embryo gives rise to the tissues that form the nervous system and axial musculature, whereas the ventral side gives rise to the digestive system. Formation of this axis is initiated by the cytoplasmic and nuclear localization of several transcription factors in the beta-catenin signal transduction pathway. Recent studies indicate that during the embryo's first cell cycle, the beta-catenin pathway component, Disheveled, moves along tracks of microtubules to the future dorsal side where it stabilizes beta-catenin. The activated beta-catenin leads to the formation of the dorsally located, embryonic organizing center shortly before gastrulation.

The embryo's dorsal organizing center is a source of many important signaling molecules that establish the dorsal-ventral axis and pattern structures along the dorsal axis. Previous studies supported by the Branch discovered many of the signaling molecules produced by the organizing center, including the important vertebrate homeobox gene, *goosecoid*, homologue of the *Drosophila* genes, *gooseberry* and *bicoid*. More recent research discovered the goosecoid target genes, *Chordin* and *Cerberus*.

In studies using *Xenopus*, it has been shown that the Chordin promotes the formation of dorsal structures by binding to the key ventral morphogen, bone morphogenic protein (BMP), with the

same affinity as the BMP receptor. Consequently, Chordin's extracellular complex with BMP prevents the ventral morphogen from activating its receptor, thereby preventing ventralization. Further biochemical studies showed that the secreted metalloprotease, Xolloid, cleaves the inactive Chordin/BMP complex and allows BMP to activate its receptor, which then leads to ventral development. Studies such as these show how Xolloid and Chordin regulate patterning along the dorsal-ventral axis. These findings also show that dorsal-ventral patterning is controlled by gradients of particular extracellular binding factors and proteases that regulate the precise amounts of morphogens available in each region of the embryo.

Cerberus is the protein responsible for patterning structures along the dorsal axis and induces the formation of the head. Cerberus' head-inducing activity is localized to the anterior-most endoderm, which gives rise to the foregut and liver. The secreted form of cerberus is a multivalent antagonist that binds to Nodal, Wnt-8, and BMP-4 in the extracellular space. Further, the protein undergoes a proteolytic cleavage that releases its carboxy-terminal domain, which specifically binds to and inhibits Nodal, the latter process being required for trunk formation. Its action as a Nodal inhibitor explains how processed Cerberus is able to induce head formation without trunk formation by establishing a trunk-free region at the anterior end of the embryo where the head can form.

Development of the Right-Left Axis

Although the vertebrate body seems to be bilaterally symmetrical, it actually has considerable asymmetry about the right-left axis. For example, some organs are located on only one side of the body, such as the liver and spleen; some organs display lateral differences, such as the lungs; and some organs spiral in a characteristic direction, such as the aorta and the intestines. Complete or partial reversals of this pattern accompany several birth defects.

Only recently have the mechanisms that control the formation of the right-left axis come under investigation. These studies demonstrate that several signaling molecules become localized to either the right or the left side of the embryo prior to lateralization. For example, at very early stages of development, Activin Beta-B is localized to the embryo's right side, where it induces expression of an Activin receptor, cAct-RIIA. This receptor subsequently represses Sonic Hedgehog (*Shh*) expression on the embryo's right side; this action localizes Shh to the embryo's left side, where it induces localized Nodal expression.

Studies on events leading to right-left asymmetries have shown that the gene for the bicoid-related homeodomain transcription factor, *Pitx2*, is expressed on the embryo's left side shortly after the time of *Shh* and *Nodal* gene expression, and that *Pitx2* gene expression can be induced by *Shh* and *Nodal*. Additionally, *Pitx2* and its gene product continue to be expressed in organs that have asymmetric placement or have asymmetric orientation (e.g., heart and digestive system). Thus, *Pitx2* seems to play a critical role in transforming lateralized gene expression into the embryo's asymmetrical body plan. These processes and factors also display a remarkable degree of conservation across all the animals that researchers have examined so far. In fact, the human homologue of *Pitx2* gene, *RIEG*, has been identified as the gene responsible for Rieger syndrome, an autosomal dominant birth defect that results in craniofacial dysmorphologies and heart, limb, and pituitary defects.

The formation and patterning of the embryonic axes are major formative events of the early embryonic period. Recent studies supported by the DBGT Branch demonstrate many of the evolutionarily conserved molecular and genetic components that control these critical processes. Thus, elucidating these early events will produce the thorough mechanistic understanding of the processes that is necessary to prevent or cure devastating early defects that can prevent birth. Additionally, the molecular and genetic components that control these early processes are used in many subsequent developmental events. Thus, a complete understanding of these early processes and the components that regulate them will help to elucidate many subsequent developmental events.

THE ORGANOGENESIS PROGRAM

The spectacular advances in molecular techniques and genomics over the last decade have moved the study of organ formation beyond the more traditional studies of morphology and tissue interactions, to an understanding of the molecular genetic mechanisms driving the development of organs and organ systems. The ability to detect spatial and temporal patterns of gene expression and to test gene function by targeted disruption or misexpression of specific genes has enhanced the understanding of the roles of specific genes in events like specification of the primordium, inductive signaling, outgrowth, and patterning. An emergent theme from such studies is the conserved role of growth factors, signaling molecules, and signaling pathways across vastly different animal species and organs. This universality of molecular mechanisms is giving rise to a template for organ morphogenesis. As seen in this section, the DBGT Branch is supporting research examining the development of several organs and organ systems.

Digestive System Development

Using the wide variety of genetic techniques available in *Drosophila*, researchers are now studying the molecular basis of hindgut formation. An evolutionarily conserved "cassette" of genes, including the genes *caudal*, *brachyenteron*, *fork head*, and *wingless*, appears to be involved in gastrulation and gut formation. Subsequent elongation of the hindgut, which is similar to elongation of the archenteron and of the entire embryonic axis in vertebrates, is driven by mediolateral cell rearrangement under the control of the genes *drumstick*, *bowl*, and *lines*.

Other studies are pursuing the role of Shh produced by the gut endoderm. Shh induces *Bmp* gene expression in the adjacent splanchnic mesoderm. Members of the BMP family are thought to control aspects of mesodermal smooth muscle development in the gut, possibly through regulation of proteins, such as integrins and/or laminins, that are associated with cell-matrix interactions. Shh also activates expression of posteriorly expressed *Hox* genes in the mesoderm of the gut. In general, the *Hox* genes play a pivotal role in the molecular control of anterior-posterior pattern formation throughout the developing embryo.

Heart Development

Experiments using *Drosophila* and several vertebrate animal models have confirmed a role for secreted signaling molecules within the BMP subfamily of the TGFβ superfamily during heart development. *Bmp-2, -4,-5,-6,* and *-7* are all expressed during early heart development. Targeted deletions of *Bmp-2* and *Bmp-4* cause early lethality before cardiac cushion formation.

However, deletions of *Bmp-5*, *Bmp-6*, or *Bmp-7* do not affect heart development. These finding suggest that some BMPs potentially share redundant functions during heart development. Analysis of the *Bmp-6/Bmp-7* double mutant has confirmed this suspicion because the mutant mice display a marked delay in the formation of the outflow tract endocardial cushions. This delay can lead to defects in valve morphogenesis and chamber septation, which ultimately leads to death due to cardiac insufficiency.

Lung Development

DBGT-supported researchers are studying the embryonic mouse lung as a model system for branching morphogenesis, a fundamental aspect of the development of many vertebrate organs. The temporal and spatial patterns of gene expression for signaling proteins and their receptors suggest that the distal ends of buds act as signaling centers with factors secreted by the endoderm affecting the mesoderm, and vice versa. *Bmp-4*, *Shh*, and *Wnt-7b* expression are tightly localized to the distal endoderm of both terminal and lateral lung buds. *Patched* (which encodes a Shh receptor), *Wnt-2*, and *Fgf-10* expression are restricted to the distal mesoderm immediately surrounding the buds. In particular, researchers have hypothesized that the interplay between Bmp-4 and Fgf-10 signaling may coordinate both branching morphogenesis and proximal-distal differentiation. These findings, as well as similar findings from the study of chick limb outgrowth (see below) and chick feather bud development, suggest that BMPs and Fgf10 form a conserved regulatory network that patterns outgrowths.

Pituitary Gland Development

The central role of homeobox genes in development makes them excellent candidate genes for defects related to growth, as well as birth defects. Recent genetic evidence demonstrates that at least five homeobox genes play crucial roles in development of the pituitary gland, the site of synthesis and secretion of six hormones that coordinate a diverse range of body functions. The *bicoid*-related *Pitx* family is among those homeobox genes that researchers have characterized most recently. The importance of each member of the *Pitx* gene family is emerging through analysis of human patients and targeted mutations in mice. *PITX-2* mutations in humans cause Rieger syndrome-type 1, a condition that includes defects of varying severity in the anterior segment of the eye, cataracts, glaucoma, missing or misplaced teeth, umbilical abnormalities, cardiac defects, and occasional growth insufficiencies due to pituitary irregularities. In the mouse, an allelic series of *Pitx-2* mutations of varying severity has demonstrated a minimum dosage requirement for *Pitx-2* in multiple organs (eye, brain, mandible, heart, lungs, and limbs), as well as in the pituitary gland. Differing sensitivities of various organs to *Pitx-2* deficiency have led researchers to conclude that both laterality and organogenesis have a tissue-specific dosage-dependence on *Pitx-2*.

Kidney Development

Mouse BMP family members, including *Bmp-2* and *-7*, display dynamic expression patterns during emergence of the ureteric bud and subsequent morphogenesis of the kidney collecting ducts, as well as during induction of the surrounding mesenchyme to form nephrons. Studies investigating the roles of BMP family members during kidney development demonstrate that only null mutations in *Bmp-7* result in defects largely confined to the kidney. Specifically, in the metanephric mesenchyme, where only the *Bmp-7* gene is expressed, loss of BMP signaling results in increased apoptosis, which leads to a massive depletion of metanephric mesenchyme.

Further studies indicate that Bmp-7, in conjunction with Fgf-2, is necessary for promotion and maintenance of metanephric mesenchyme. These findings have allowed the development of a more comprehensive model of growth factor signaling between the ureter, nephrogenic mesenchyme, and surrounding stromal precursor cells.

THE LIMB DEVELOPMENT PROGRAM AND RELATED TOPICS

Developmentally generated limb anomalies constitute a spectrum of birth defects that pose long-term suffering and morbidity for many affected individuals. Significant advances in molecular biology and elucidation of the genetic networks involved in limb development have led to a greater understanding of both normal limb development and skeletal dysplasias.

In vertebrates, limb initiation occurs when inductive signals emanating from somites and intermediate mesoderm (IM) move into the lateral plate mesoderm (LPM). Although the paired limbs of different vertebrates vary with respect to which somite level they arise from, their position is constant with respect to the underlying axial *Hox* gene expression pattern. Ultimately, signals pass from the mesoderm to the surface ectoderm in the prospective limb areas. The overlying ectoderm forms a structure called the apical ectodermal ridge (AER) along the distal margin of the developing limb bud. Proximal-distal outgrowth and differentiation of the limb occurs under the influence of reciprocal interactions between the AER and the limb bud mesenchyme. Ultimately, development of the three limb axes is interrelated and coordinated. In particular, the central importance of morphogenetic signaling factors, such as WNTs, FGFs, BMPs, and Shh, has been clearly established. The DBGT Branch funds research at the molecular, biochemical, and morphological levels to address limb outgrowth and formation of the limb axes, cartilage and bone differentiation, as well as somitogenesis and myogenesis.

Limb Initiation and AER Induction

Recent animal studies have expanded and refined the research community's understanding of the role FGF and WNT family signaling molecules play in a complex series of cross-talk mechanisms initiating limb development. Numerous observations have led to a working molecular model for limb initiation and AER induction. The *Wnt-2b* gene (IM in the somites, and LPM at the forelimb level) and *Wnt-8c* (in the LPM at the hindlimb level) activate and restrict *Fgf-10* expression the prospective limb regions. A third Wnt gene, *Wnt-3a*, acting through Fgf-10 production, mediates *Fgf-8* expression in the overlying ectoderm, which triggers the induction of the AER and subsequent limb outgrowth. All three *Wnt* genes mediate their effects on *Fgf*s via β-catenin-dependent pathways. While other Fgfs are expressed in the AER, only Fgf-8 has been shown to be necessary for normal limb development. To complete the loop, Fgf-8 signals back to the nascent limb bud to maintain expression of *Fgf-10*. In addition, conditional knockouts of *Fgf-8* in the AER cause a substantial reduction in limb bud size, a delay in *Shh* expression, misregulation of *Fgf-4* expression, and hypoplasia or aplasia of specific skeletal elements.

Another series of studies supported by the Branch has shown that during normal limb outgrowth, BMPs expressed in the AER, as well as in the mesenchyme, play an important role. Not only do these BMPs maintain the asymmetrical distribution of the AER across the distal limb bud, but

they also cause the ultimate regression of the AER, thus limiting normal limb outgrowth. Experimental inhibition of BMP expression early in limb development causes the AER to expand anteriorly, and to adopt a more posterior morphology. Early stages of *Fgf-4* expression are now seen anteriorly as well. Experimental inhibition of BMPs later in limb development is accompanied by persistence of the AER, and excessive soft tissue growth occurs distally. In sum, the AER is negatively regulated by BMPs.

Reciprocal Interactions between the AER and Zone of Polarizing Activity (ZPA)

Outgrowth and patterning of the vertebrate limb occur through reciprocal interactions between two organizing centers: the distal AER, and the posterior ZPA. The key signaling molecules involved are the FGFs of the AER and Shh produced by the ZPA. Initial work suggesting that an Fgf-4/Shh positive feedback loop maintained the two signaling centers and provided a simple molecular mechanism for coordinating their activities. However, more recent studies suggest that the combined activities of two or more AER-*Fgfs* function in a positive feedback loop with *Shh* to control limb development. This work indicates that inactivation of *Fgf-4* in the AER does not effect *Shh* expression, while other work suggests that none of the other known AER-*Fgfs* alone are required to maintain *Shh* expression. Rather, the combined activities of two or more of the AER-*Fgfs* are necessary. Close examination of the $Shh^{-/-}$ mutant mouse suggests that a positive feedback loop exists between *Shh* and *Fgf-4*, *-9*, and *-17* because *Shh* is necessary to maintain their expression. However, AER expression of *Fgf-8* is not dependent upon *Shh* expression.

Manifestation of the Limb Pre-Pattern

Expression of *Shh* in the ZPA localized to the posterior margin of the limb bud is a key factor in establishing both anterior-posterior limb polarity, as well as in maintaining the AER and subsequent outgrowth of the limb. Targeted disruption of the *Shh* gene has provided a definitive opportunity to study the role of *Shh* in limb development, without the caveats associated with microsurgery or chemical disruption. Analysis of $Shh^{-/-}$ mutant mice and their defective limbs has led investigators to propose that a pre-pattern exists in the limb field for the three axes of the emerging limb bud, as well as for the specific limb skeletal elements. With regard to the patterning of limb elements, the upper limb is completely specified, including its anterior-posterior polarity, in the absence of *Shh* function. The lower limb and digits also develop in the absence of *Shh*, but they are incomplete and lack normal anterior-posterior polarity. These results suggest *Shh* input is necessary at, or just distal to the level of the upper limb (at the elbow/knee joint). The role of *Shh* in normal limb development would then be to stabilize and expand the limb pre-pattern by specifying those elements not found in $Shh^{-/-}$ limbs. *Shh* is known to stabilize and amplify asymmetric gene expression, including genes of the *Hoxd* family.

Hox Genes and Limb Development

From their expression profiles, as many as 20 of the 39 *Hox* genes may be involved in limb development. Ample evidence has demonstrated that the *Hox* genes do not function in isolation, but rather as members of a highly integrated network. Defining the roles of individual *Hox* genes and defining the genetic interactions between *Hox* genes can only be accomplished through the laborious process of generating loss-of-function mutations in each gene, and then in multiple genes, in mice. Building upon these findings, more recent studies are examining the downstream events regulated by the *Hox* genes. For example, mice homozygous for the

Hoxa-13 mutation show severe autopod malformations (i.e., abnormalities of the hands and feet). *Hoxa-13* mutants lack the normal mesenchymal condensations that form the digits. Recent studies have demonstrated that this defect is due to a disruption of the ephrin ligand/ephrin receptor system that mediates mesenchymal cell adhesion, sorting, and boundary formation leading to the cartilage condensations forming the digits. Therefore, *Hoxa-13* plays an important role in regulating genes whose products are required for mesenchymal cell adhesion.

Specification of Forelimb versus Hindlimb

While many of the genetic systems that control limb development operate in both the forelimb and hindlimb, there are clearly distinct morphological differences between the limbs. DBGT-supported researchers are making advances in understanding the genetic factors that determine these differences. Two T-box family transcription factors have been identified based on their expression in either the forelimb (*Tbx-5*), or hindlimb (*Tbx-4*). A member of the *Pitx* family of genes, *Pitx-1* has also been found to play a role in the specification of the hindlimb identity. Ectopic misexpression of *Pitx-1* in the forelimb causes ectopic *Tbx-4* expression, as well as misexpression of *Hoxc-10* and *Hoxc-11*, genes that are normally expressed in the hindlimb. Morphological changes characteristic of the hindlimb are observed in response to this ectopic gene expression; in addition, the underlying muscle pattern of the forelimb develops as that of the hindlimb.

Hox Genes and Patterning of Somitic Mesoderm

Members of the *Hox* gene family are expressed in an anterior-posterior sequence along the embryonic axis. Investigators have postulated that the unique combination of *Hox* gene expression at any given axial level is responsible for establishing global positional information. Somites of vertebrates, serially homologous paraxial mesodermal condensations that form along the anterior-posterior axis, contribute cells to the limb musculature. Laterally, mesoderm segregates into the LPM (or body wall), some of which gives rise to the limb skeleton and connective tissue of the limb. Recent experiments suggest that independent *Hox* codes are established in the paraxial mesoderm and the LPM. Migrating somitic cells in the dorsal compartment retain both their original *Hox* genes expression, and their morphological identity. However, cells that migrate laterally adopt *Hox* gene expression characteristic of the lateral plate. This finding has implications for both the development and evolution of the paired appendages of vertebrates.

Somite Patterning and Differentiation

The paraxial mesoderm that forms the somites gives rise to multiple tissues, including skeletal muscles, cartilage, bones, and dermis. To clarify the role of Shh produced by the notochord and floor plate of the neural tube in somitic myogenesis, researchers have characterized the expression of the myogenic determination genes, *Myf-5* and *MyoD*, in $Shh^{-/-}$ mice. These studies have demonstrated that Shh has multiple functions in the somite, including induction of myogenesis via activation of *Myf-5* and *MyoD* in the epaxial dermomyotome. Unexpectedly, Shh is not required for muscle cell induction at other sites of myogenesis, not even in those somitic cells that give rise to the limb muscles. Further studies have shown that *Myf-5* is a direct target of long-range Shh signaling through positive regulation by Gli transcription factors.

Vertebrates have three *Gli* genes, and their roles in somite formation have been investigated in avian embryos. All three genes are activated at the onset of somite formation. However, the *Gli* genes are differentially controlled. *Gli-2* and *Gli-3* are controlled by WNT signaling from the surface ectoderm overlying the somites, while *Gli-1* activation is regulated by Shh. These findings indicate that the Shh and WNT signaling pathways converge to control *Gli* gene activation in the somite. Gli transcription factors are responsible for activation and repression of a variety of target genes downstream in these signaling pathways, including *Myf-5*, as mentioned above.

Molecular Defects in the Skeletal Dysplasias

The skeletal dysplasias are a heterogeneous group of over 200 disorders. A multifaceted research approach that combines basic genetics, biochemistry, developmental biology, and clinical and pathological analyses has led to dramatic insights into the specific gene defects giving rise to skeletal dysplasias. Defects in collagen molecules, growth factors or their receptors, and *Hox* genes, among others, have all been identified as underlying causes of these birth defects. Investigators now propose using a new molecular classification for these disorders based on the specific pathogenetic mechanism involved, including abnormalities of structural proteins, inborn errors of cartilage metabolism, defects in local regulators of cartilage growth, systemic defects that secondarily affect cartilage, transcription factor defects, tumor suppressor genes defects, and defects in signal protein inactivators. The research emphasis has shifted toward understanding the clinical-molecular correlations and genetically mapping the defective genes. These efforts are providing a better understanding of the function of these gene products in normal and aberrant development, and are improving genetic counseling and expanding diagnostic opportunities.

THE DEVELOPMENTAL NEUROBIOLOGY PROGRAM

Formation of the nervous system in vertebrates begins shortly after gastrulation, when the cells of the dorsal ectoderm are induced by the underlying mesoderm to become the neural plate that extends the length of the embryo. From this very simple neural plate, convergence and extension morphogenetic activities begin a process that ultimately results in a highly stereotyped, complexly organized and integrated nervous system. During neurulation, the neural tube is formed, and the embryonic central nervous system (CNS) is patterned along its anteroposterior, dorsoventral, and mediolateral axes. Neural progenitor cells and their offspring derive positional information from within these boundaries; neurons and glia are generated, acquire specific cell fates, migrate to specific positions within the developing nervous system, and send out processes to specific targets. Once specific patterns of connectivity are generated, synapses form, and hormonal and trophic factors influence the survival, differentiation, and selective elimination of these connections. This series of events, which is recapitulated throughout phylogeny, results in the most complex, beautiful, and intricate structure.

Understanding the mechanisms that control these events is not merely academic. With the discovery that many neurological birth defects are caused by miscues during a number of these aforementioned events, developmental neurobiology has taken on added importance. The major focus of the Developmental Neurobiology Program is to support basic and clinical research that

contributes to the understanding of how the nervous system develops under both normal and abnormal conditions. Goals of this research are to understand how such a remarkable diversity of cell types is generated from a relatively undifferentiated neuroepithelium, and to identify the underlying mechanisms, which allow the multitude of newly identified signaling pathways to interact both temporally and spatially, in order to generate the complex adult nervous system.

While embryonic development of the CNS remains a complex and poorly understood process, the recent advances in genetics, genomics, and proteomics are becoming the most important weapons in the scientific arsenal for understanding the mechanisms that underlie CNS development. Many genes that have been identified in *Drosophila* and other invertebrates are known to have vertebrate homologues. Not only are the same genes conserved, but the event cascades in which they are involved and the timing of their activation and inactivation are also conserved, in large part, across species.

Patterning

In the developing vertebrate central nervous system, the regionalized expression of *Hox* genes helps to define distinct neural progenitor cell domains along the anterior-posterior and dorsal-ventral axes. Regionalization is achieved through the interactions of different signaling molecules acting along the two primary axes. The next step, then, is to understand how cells become committed to their specific fates, and how regulatory genes are specifically involved in these individual cascades following regionalization. This search has become one of the most rapidly evolving areas of developmental neuroscience. By exploiting genetics in *Drosophila*, zebrafish, and mouse models, investigators have made tremendous progress in identifying genes and defining the cascades that are likely to mediate neural patterning.

Anterior-Posterior Axis Patterning

Anterior-posterior patterning of the nervous system of vertebrates occurs soon after neural induction, when adjacent tissues produce signals that turn on regulatory genes in discrete domains of the neural plate. Along the anterior-posterior axis, signals divide this presumptive neural tissue into four major areas: forebrain, midbrain, hindbrain, and spinal cord. The working hypothesis is that neural tissues are initially defined anteriorally, and it is the actions and interactions of a number of signaling pathways that posteriorize these tissues in a progressive manner by encoding positional values along the anterior-posterior axis.

Development of the vertebrate hindbrain involves specification of the neural epithelium into lineage-restricted compartments called rhombomeres. Boundaries of *Hox* gene expression are observed along the anterior-posterior axis and have been shown to be involved in establishing the identities of different rhombomeres in the hindbrain, as well as in the spinal cord. While *Hox* genes control the identity of each rhombomere, at least in part, recent studies identified a family of receptor tyrosine kinases (Eph RTK) with restricted segmental expression during rhombomere formation. These receptors mediate repulsive cell-cell interactions in axonal guidance (See later section on axonal guidance for more information); a dominant-negative form of Eph receptor, sek-1, disrupts the establishment of sharp rhombomeres boundaries in zebrafish and *Xenopus.* These data suggest that Eph RTK play an important role in early CNS patterning.

During formation of the fly CNS, neuroblasts (NBs) form seven rows along the anterior-posterior axis. The genes activated in the neural ectoderm that enable a cell to become an NB are called the proneural genes. These genes encode the transcription factor complex, *achaete-scute*. This complex, in concert with the delta ligand and the notch receptor, act to ensure that not every ectodermal cell that expresses *achaete-scute* becomes a neuron by inhibiting NB formation in adjacent cells. Thus, the balance of proneural genes and *delta-notch* activity results in the formation of a single NB from each proneural cluster. Segment polarity genes, including *gooseberry*, act upstream of the proneural genes of the *achaete-scute* complex to pattern the neuroectoderm along the anterior-posterior axis.

Dorsal-Ventral Axis Patterning

Dorsal-ventral patterning occurs when mesodermal structures beneath the neural tube secrete diffusible factors that result in the specialization of cells in the ventral half of the neural tube. This cell fate determination occurs during and following neural tube closure and involves the action of two opposing signaling pathways: Shh ventrally from the notochord, and BMP dorsally from the boundary of neural and non-neural ectoderm and later from the roof plate. Shh may work by repressing the expression of genes encoding dorsal neural tube transcription factors, which would otherwise be expressed throughout the neural tube. In zebrafish BMP mutants, neural crest, dorsal sensory neurons, and interneurons display aberrant phenotypes ranging from complete loss, to dramatic expansion. Current efforts are underway to determine whether dorsal cells are specified within different levels of BMP activity during early gastrulation, thus explaining the range of phenotypes.

In both *Drosophila* and vertebrates, similar positional cues are thought to regulate dorsal-ventral patterning within the CNS. In the fruit fly, NBs form in three columns parallel to the ventral midline along the dorsal-ventral axis. Recent studies in *Drosophila* have shown that: *ventral nervous system defective* (*vnd*), an NK-2 type homeobox gene, is expressed in the ventral neuroblast column; *muscle segment homeobox* (*msh*) is expressed in the lateral column; *intermediate neuroblast defective* (*ind*), a novel homeodomain protein, is expressed in the intermediate column. These genes have vertebrate homologues (*Nkx-2.1/Nkx-2.2/Nkx-6.1*, *Msx*, and *Gsh-1/Gsh-2*, respectively) that play parallel roles during formation of the dorsal-ventral axis in vertebrate CNS development, which demonstrates regulatory and functional conservation. These columns ultimately give rise to motoneurons, interneurons, and sensory neurons. In *Drosophila*, it is the convergence of dorsalizing signal, Dorsal, decapentaplegic (Dpp), and EGF receptor (Egfr) signaling pathways that controls expression of the homeodomain proteins Vnd, Ind, and Msh, thus regulating dorsal-ventral expression domains. Studies are underway to determine if similar dorsalizing signaling pathways are involved in vertebrate patterning.

In addition, many of the genes in the *Drosophila* signaling cascade downstream of *hedgehog* (*Hh*) have been identified, and all have homologues in mammals. At the end of the pathway is a putative transcription factor, *cubitus interruptus* (*ci*). Hh induces *ci* protein and antagonizes the negative regulation of *ci* by the proteins, Patched and Protein kinase A. In mammals, the homologues of *ci* are the three *Gli* genes, *Gli-1*, *Gli-2*, and *Gli-3*. Current work aims to determine whether the three mouse Gli gene products have similar functions to the *Drosophila* ci protein, and whether they function in an analogous signaling pathway. An important role is suggested by the observations that humans with the dominant Greig cephalopolysyndactyly

syndrome have limb and craniofacial defects and spina bifida due to mutation in *GLI-3*, and mouse *Gli-3* mutants display similar abnormalities.

Because cells of the embryo must respond to cues in both dorsal-ventral and anterior-posterior axes, it is important to understand how this positional information is integrated at the level of a single gene in establishing the CNS plan in mammals. Studies have shown that *Pax-3* is an effector gene that functions in translating dorsal-ventral positional information into differentiation of discrete cell lineages within the neural tube and somites. The initial expression domain marks what will ultimately be the dorsal pole of the embryo. It may be *Pax-3* that acts to coordinate these opposing cues.

Molecular Regulation of Neurogenesis

The CNS develops from a group of cells that is initially homogeneous in its developmental specification. Current understanding is that the nervous system is established through progressive stages of regional development, in two major phases. In both the early and late phases, the interactions between neighboring tissues are sequential and are important in specifying regionally distinct structures. As discussed in previous sections, early stages include differentiation of the neuroectoderm, and the formation and segmental patterning of the neural tube. Later stages include determination of specific neuronal and glial phenotypes, and the increased specialization of CNS structures. Both the early and late phases of neural differentiation are controlled by a multitude of transcription factors that act as switching molecules by binding to DNA and activating or repressing gene expression, signaling factors, and growth factors. Researchers are learning that the same or similar pathways are used a number of times during the development of the CNS.

Determination of Laminar Neuronal Identity

In *Drosophila*, successive Ganglion Mother Cells (GMCs) are generated from a single NB, each of which has is own distinct fate. Recently it has been shown that there are four genes that control "birth-order" specification of GMC fate within all NB lineages. *Drosophila* NBs sequentially generate GMCs, whose neuronal progeny populate different layers of the CNS: early-born GMCs give rise to deep-layer neurons; middle-born GMCs produce middle-layer neurons; while late-born GMCs generate superficial neurons. Researchers also know that each layer expresses a characteristic transcription factor. What is exciting is that each NB goes through a temporal order of gene expression, with progeny maintaining the profile of transcription factors present at birth. These are the first genes known to regulate birth-order dependent "laminar" neuronal identity.

Axonal Guidance and Pathfinding

For the CNS to form appropriate connections, neuronal precursors must migrate to their correct location and extend processes into the extracellular environment. It is well established that axon growth is a highly directed process. During the process of axonal pathfinding, the axons are repeatedly confronted with choice points at which they must select their appropriate axonal pathways. Correct pathway selection requires the presence of spatially and temporally orchestrated cues at each choice point, and the responsiveness of individual neural growth cones to a specific set of cues only. Clearly, factors in both the micro- and intracellular environments must be involved. External guidance cues in the microenvironment can be short-range and

contact-mediated, long-range and diffusible, attractive, as well as repulsive. These cues include extracellular cell surface molecules, such as cell adhesion molecules, and trophic factors. Of special note is that research now shows that a single, individual factor inhibits or facilitates these processes, depending on the age of the growth cone and the environmental milieu.

Cell Adhesion Molecules (CAMs)

CAMs belong to the immunoglobulin (Ig) superfamily and play critical roles in neural development. They, along with other related surface proteins, such as L1, fasciclins, semaphorins, and cadherins, are known to have either definable positive or negative influences on the fasciculation, guidance, and stabilization of neurites. There has been much research activity into the mechanism by which growth is inhibited/repelled during neural development.

Some neural CAM variants have a large linear homopolymer of sialic acid (polysialic acid or PSA), which modulates interactions between neural CAM molecules, thereby attenuating cell interactions. The function of PSA stems from its ability to reduce cell interactions. Recent work on the regulation of PSA expression in synaptogenesis in the visual system of the chick has shown that the synapse eliminates PSA as part of its normal development, and that the loss of PSA from the site of axon-target interaction may serve to stabilize structures formed during synaptogenesis. In a different model that examines thalamocortical development in the neocortex of the rat, researchers have learned that membrane-bound axonal components are involved in laminar-specific branch formation, and that, in particular, PSA moieties contribute to the laminar specificity by inhibiting branch emergence in inappropriate layers. Scientists continue to examine this interesting role for PSA.

The Semaphorins are a family of secreted and transmembrane proteins, some of which function as repellents during axonal guidance. Of particular note is that the major family of Semaphorin receptors, called Plexins, were discovered in *Drosophila*; in particular, Plexin A was shown to play a pivotal role in axonal guidance. Current studies are aimed at understanding how these ligands and receptors function to control guidance.

Mutations Resulting in Guidance Errors

Recently a gene named *unplugged*, which functions in selecting motor axonal pathways in the embryo, was discovered in zebrafish. In *unplugged* mutants, motor axons exit the spinal cord in a common nerve path, but at a subsequent choice point, an identified subpopulation of motor axons specifically fails to select its appropriate axonal pathway. Analysis of chimeric embryos suggests that cells surrounding the neuron provide *unplugged* gene activity to which the migrating neuron responds.

In another series of studies, investigators learned that severe guidance errors occur in the tract of the postoptic commissure in the mouse forebrain of embryos mutant for the transcription factor *Pax-6*. This provides an opportunity to discover how early patterning genes, such as *Pax-6*, might provide positional information in the form of guidance cues later in development.

Receptor Tyrosine Kinases (RTKs)

RTKs, of which the Eph receptors are the largest known subfamily, and RTK ligands, the Ephrins, have been shown to play a critical role in the guidance of axons to their targets. Ligands in the Ephrins-B family are cell-surface anchored by a transmembrane domain and signal through their Eph receptors by direct cell-cell contact. This contact-mediated mechanism provides the potential for bi-directional signaling with a forward signal through the RTK, and a reverse signal through the ligand. This reverse signaling has been demonstrated biochemically and is postulated to play an important role in axonal pathfinding, as well as in related developmental processes, such as guidance of cell migration.

THE DEVELOPMENTAL IMMUNOLOGY PROGRAM

This Program includes basic, applied, and clinical studies in developmental genetics, ontogeny of immunity, primary immunodeficiencies, perinatal host defenses, and vaccine development. The long-term goal of the research is to translate the basic knowledge, insights, and understanding from these studies into new approaches and strategies for the effective diagnosis, treatment, and prevention of early infections and developmental disorders of immunity.

In recent years, molecular genetics and developmental biology have markedly enhanced the research community's fundamental understanding of the developing immune system. Considerable progress has been made in identifying and characterizing the genes and proteins that play a role in the structure, development, function, and interactions of the immune system. The pace has been greatly accelerated by the introduction of new and innovative technologies, methodologies, and high-throughput instrumentation developed for the Human Genome Project. The tools developed for genomic, proteomic, and bioinformatics research have contributed significantly to the basic understanding of developmental genetics and molecular immunobiology. These tools have unraveled complex gene networks, defined developmental processes and pathways, and elucidated gene-gene and gene-environment interactions involved in immunologic development.

Developmental Genetics

During early development in the thymus, T cells make sequential cell fate choices. These fate choices require initiation of new programs of gene expression. Once initiated, these programs must be faithfully propagated in a heritable manner, from parental cells, to their progeny. With the exception of the T-cell receptor, these changes in gene expression occur without a change in information encoded directly in the DNA sequence. Rather, these heritable programs of gene expression are imposed, at least in part, epigenetically through changes in chromatin structure and DNA methylation. These changes allow T cells to tune the threshold for expression of specific genes. A study showed that DNA methyltransferase 1, the maintenance enzyme for DNA cytosine methylation, is critical for the proper expression of certain genes that define fate and determine function of these early T cells in the thymus.

Ontogeny of Immunity

The thymus is important for producing self-restricted and self-tolerant thymocytic T cells. Within the thymus, T-cell precursors interact with thymic epithelial stromal cells and undergo a complex and well orchestrated process of positive selection. A recent study in mice showed that *Hoxa-3* and *Pax-1* transcription factors regulate the ability of fetal thymic epithelial cells to promote thymocyte development. It also showed that mutations in *Hoxa-3* and *Pax-1* result in the development of fewer thymic epithelial stromal cells and defects in their cellular differentiation. These mutations appear to act in a synergistic and dosage-dependent fashion to impair thymus development and the positive selection of thymocytes. Although it is recognized that positive selection and maturity of T cells require interaction with thymic stroma, the genes, molecular mechanisms, and transduction pathways involved are still poorly understood. Consequently, many studies are underway to elucidate the molecular events for the positive selection of T cells. Several studies are examining the biological properties of peptides that mediate positive selection of major histocompatibility complex, or MHC-restricted thymocytes. These studies aim to determine the biological properties of the soluble peptides, peptide/MHC complexes, and thymic peptides that initiate positive selection of CD4+ T cells. Moreover, the studies will measure the activation threshold of positive T-cell selection and compare the gene and protein expression profiles of thymocytes undergoing positive selection. These studies will provide important insights into the mechanisms and specificity of positive selection and T-cell survival.

Early in life, acquisition of humoral responsiveness is both antigen-specific and genetically controlled and occurs in a sequentially programmed manner. It also parallels the ontogeny of antibody repertoire diversity. The ability to rapidly generate B cells with maximum antibody diversity and specificity is extremely important for developing protective humoral immunity. Because newborns and infants are unable to generate a diverse antibody repertoire to specific antigens, they are more susceptible to certain infectious agents. A recent study demonstrated that development of B-cell immunocompetence is linked to sequence restriction and structure restriction of the antibody's third heavy chain complementarity-determining region (HCDR3). The HCDR3 region of B cells from human second trimester fetuses and neonates proved to be considerably shorter, more restricted in diversity, and lower in antigen-binding affinity, when compared to the same region in adult B cells. These findings indicate that the length of the HCDR3 region is crucial for antibody binding-site diversity, is genetically controlled temporally, and may explain why neonates are more susceptible to some infectious agents. Additionally, investigators have demonstrated that the process of histone acetylation regulates transcription of genes controlling late B-cell differentiation. These studies provide valuable information and insights into the molecular mechanisms that govern why neonatal B cells are less capable of responding to certain infectious agents. More importantly, they provide the basis and fundamental understanding for developing pharmacologic agents that will significantly enhance antibody diversity and humoral responsiveness in pre-term neonates.

The Branch also supports studies to define the cellular interactions that guide T-cell development. Initially, investigators characterized recognition events involved in positive selection; they then established that positive selection occurs on conventional peptide/MHC complexes, that peptides present in the thymus are involved in shaping the T-cell repertoire, and that recognition during positive selection is promiscuous rather than stringent in nature. These

studies showed that the immune system of the full-term human fetus is almost functionally mature, but it is not as efficient as the adult immune system. Clearly, there are functional differences between the adult and neonatal immune systems. At birth, neonatal B cells and T cells are generally naive and unprimed; they have also not undergone antigen-induced clonal expansion or maturation. This phenomenon 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, that protect against some types of encapsulated bacteria. In addition, neonatal T cells possess a diminished capacity to provide help for, or may actually suppress, Ig production by B cells, a diminished generation and activity of cytotoxic T cells, and a decreased capacity to activate macrophages.

Another current avenue of research focuses on identifying the cytokines that are important in neonatal host defenses, and studying the molecular mechanisms of cytokine regulation. These studies showed that T cells of newborn rats have a markedly diminished capacity to produce two cytokines, interferon-γ (IFN-γ) and interleukin-4 (IL-4), that are essential for intrapulmonary inflammatory responses. When newborn rats were administered bacterial lipopolysaccharide and gram-negative bacteria intratracheally, no intrapulmonary inflammatory response was observed. In contrast, adults treated similarly had an intensely florid inflammatory response. Intrapulmonary inflammation in newborn rats did not reach adult levels until after four weeks of age. It was found that IFN-γ and IL-4 levels correlated with, and were directly responsible for, the intensity of intrapulmonary inflammation. Studies continue to determine the underlying molecular mechanisms for the delayed cytokine response in the newborn rats. Information emanating from these animal studies will help elucidate the molecular pathogenesis of gram-negative respiratory infections in human infants. Furthermore, the information will help devise rational pharmacologic approaches and strategies for accelerating and increasing the production of cytokines and could lead to therapeutic modalities that enhance protective immunity for human neonates and infants.

Primary Immunodeficiencies (PIs)

Almost 100 genetic types of PI have already been described. Defective or missing PI genes may affect the normal development and function of T cells or B cells, phagocytes, natural killer cells, neutrophils, platelets, or complement components. Some mutated genes that cause PIs may be X-linked, so males would be clinically affected, whereas, females would be silent carriers. Although treatments are available for most PIs, the biggest research challenge is to develop more effective, practical, cost-effective therapies and screening methods for newborns.

Basic research studies are examining the molecular mechanisms of human B-cell PIs. Two projects are studying Bruton's agammaglobulinemia, or human X-linked agammaglobulinemia (XLA). In this X-linked B-cell PI, the gene encoding Bruton's tyrosine kinase (BTK) is mutated, normal B-cell development and activation are blocked, and B cells and plasma cells are virtually absent. Consequently, affected boys have a profound lack of antibodies and are highly susceptible to severe and chronic infections. B cells require BTK for receptor-induced, sustained calcium signals; these signals are critical for the transcriptional events that regulate normal B-cell development and activation. Therefore, investigators are trying to identify the key elements, molecular mechanisms, and pathways that regulate intracellular calcium levels in B cells. Studies are progressing to identify the phosphorylated protein ligand that binds to BTK to sustain

calcium signals. The long-term goal of this research is to develop therapies to correct the B-cell deficiencies in boys with XLA.

Another B-cell PI being studied is hyper-IgE (HIE) syndrome, a complex immunologic disorder characterized by extremely high levels of serum IgE and severe, recurrent infections and dermatitis. Investigators found that HIE patients have genetic defects in the cytokine IL-4 and its receptor signaling pathway. These defects interfere with normal signaling and cause certain B cells to selectively overproduce IgE.

Severe combined immunodeficiency (SCID) is caused by a genetic defect in the development and/or function of both T cells and B cells. Different mutations can cause SCID; one common cause is adenosine deaminase (ADA) deficiency. ADA is an enzyme essential for catalyzing the deamination of the substrates adenosine and deoxyadenosine. Without ADA, excess substrates accumulate that are specifically toxic to developing lymphocytes. Research on the molecular mechanisms of lymphotoxicity has demonstrated that a phosphorylated metabolic derivative of the substrates inhibits lymphocyte development. Deficiency of Janus-associated kinase 3 (JAK3) causes at least 5 percent of human SCID cases. JAK3 is important for intracellular signaling and lymphocyte development and differentiation. In a study of nine patients, the severity of immunodeficiency varied depending on the type of the *JAK3* mutation. Recently, an investigator reported the first case of a CD45 SCID in a two-month-old boy. CD45 is a hematopoietic-specific transmembrane protein tyrosine phosphatase that functions to regulate the *Src* kinases required for T- and B-cell antigen-receptor signal transduction. Without CD45, signal transduction by lymphocyte antigen receptors is blocked, and T- and B-cell development is prevented.

Wiskott-Aldrich syndrome (WAS) is an X-linked, recessive disorder. Patients exhibit complex immunological and hematological abnormalities that affect the development and function of their B-, T-, platelet, and hematopoietic cells. The gene for WAS encodes a multi-domain WAS protein (WASP). WASP plays a major role in cell signaling and in cytoskeleton formation. Nonfunctional or absent WASP interferes with normal immune responses, production of platelets, and neutrophil functions. WAS lymphocytes and platelets undergo increased apoptosis. This phenomenon may explain the progressive lymphopenia and thrombocytopenia in WAS patients. Recently, an investigator found that WASP stimulates actin assembly through its C-terminus. He also identified a calcium integrin-binding protein (CIB) as a WASP N-terminus binding partner and found that WASP and CIB participate in platelet aggregation and chemotactic migration of leucocytes.

Hereditary angioedema (HAE) is an autosomal dominant condition that is caused by a partial deficiency of C1 inhibitor (C1INH). Because C1INH is the sole inhibitor of the C1r and C1s components of the classical complement pathway, it plays an important role in the regulation of the complement and contact activation cascades. Recent studies have shown that bradykinin is a major mediator of vascular permeability in C1INH deficiency. C1INH structure-function analysis studies have characterized the determinants of target protease specificity. These findings, combined with the analysis of the role of heparin in enhancement of C1INH function, may lead to the development of improved therapy for HAE.

THE REPRODUCTIVE IMMUNOLOGY PROGRAM

Most of the research projects in this Program investigate the immunobiology of the placenta and the mechanisms of maternal-fetal tolerance. The basic studies are designed to identify the underlying immunologic and/or genetic mechanisms that protect the fetus from maternal rejection. Fetal protection appears to be multi-factorial, 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 strongly implicate a role for the HLA system in fetal tolerance. Differential expression and/or repression of Class I MHC molecules on fetal tissue seems to be required for the maintenance of pregnancy. Other cell surface molecules, such as complement regulatory proteins and various cytokines (e.g., TGF-β, TNF, and IFN-γ) may be important in establishing, maintaining, and regulating pregnancy. Understanding the basic mechanisms of fetal-maternal tolerance also 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 dysregulations that lead to unsuccessful pregnancy.

Immunotherapy for Recurrent Spontaneous Abortion (RSA)

Recently, an important multi-center, randomized, double-blinded clinical trial demonstrated that immunization with paternal mononuclear blood cells does not improve pregnancy outcome in women with unexplained RSA. RSA was defined as three or more spontaneous abortions. In this trial, the pregnancy success rate was higher in the untreated controls than in patients receiving immunotherapy. Thus, there was no evidence of benefit from immunization with paternal mononuclear blood cells for the prevention of RSA. Furthermore, higher rates of pregnancy loss were observed among patients immunized with paternal cells than in those immunized with saline. This significant finding suggests that immunotherapy may actually increase the rate of clinically recognized pregnancy losses. The findings of this clinical trial clearly indicate that immunotherapy with paternal mononuclear cells is not efficacious, and should not be offered as a treatment for unexplained recurrent pregnancy loss.

Maternal-Fetal-Neonatal Transfer of IgG

Studies are examining the molecular mechanisms involved in the 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 neonates against perinatal infections. In humans, most maternal IgG is transplacentally transferred to the fetus after the 22nd week of pregnancy. Maternal IgG must pass through two cell layers of the placenta to enter the fetus. First, it must pass through the epithelial syncytiotrophoblast cell layer, which possess MHC Class I-like human Fc receptors (FcRh) that actively transport the maternal IgG across this cell layer. Maternal IgG must also pass through the fetal villous endothelial cell layer, where specialized endothelial cells possess FcγRIIb2 receptors that actively transport the maternal IgG into the fetus. In addition to its role in transporting maternal IgG to the fetus transplacentally, FcRh is also involved in maternal IgG transfer enterically via the fetal small intestine from amniotic fluid and pre-term colostrum. Interestingly, FcRh in human placenta and fetal enterocytes is identical in molecular structure

and function, even though it is found in two different organs during fetal development. Understanding the structure and function FcRh, as well as the molecular mechanisms of placenta and enteric IgG transfer may lead to useful clinical applications, such as pharmacologic receptor-blocking strategies to prevent detrimental maternal IgG antibodies and infectious agents from entering the fetus.

TRAINING AND CAREER DEVELOPMENT PROGRAMS

In addition to supporting research grants, the DBGT Branch supports numerous training and career development awards that provide training in the latest research methodologies. For example, the Branch supports a large number of institutional training grants (T32s) at prestigious and successful training programs at universities, medical schools, and research institutions across the country. Collectively, the Branch's T32s support about 150 graduate students and 75 postdoctoral fellows per year. This number represents about 40 percent and 25 percent, respectively, of all the graduate students and postdocs that the NICHD supports on T32s. The DBGT Branch also supports an additional 40 postdoctoral fellows, training them at the top developmental biology laboratories in the country, on individual fellowships (F32s). Finally, the Branch supports advanced training via career development awards (K series) for more than 20 researchers per year. These awards enable medical professionals, mostly MDs, to learn and perform basic and clinical research. The grants produce clinical researchers and medically trained basic researchers who can perform translational research. Collectively, these Training and Career Development Programs are helping to produce future generations of researchers to perform basic developmental biology research, as well as clinical research on the causes of birth defects.

FUTURE PLANS

MOUSE FUNCTIONAL GENOMICS

In collaboration with the NICHD Reproductive Sciences Branch, the DBGT Branch is initiating several new projects to perform in-depth analyses of mutants with developmental and fertility defects. These analyses include phenotyping and gene characterization. The research will make the mutants being generated by the mutagenesis facilities more useful to the community, and thereby help to reach the goal of producing more models of human birth defects.

XENOPUS

The most exciting development for *Xenopus* research is the U.S. Department of Energy's recent plan to sequence the genome of *X. tropicalis*. The plan calls for the production of a sequence with 4 – 5 X coverage by the end of 2003, and subsequent completion to about 8X coverage. The completion stages require BAC end sequencing and fingerprint maps. Accordingly, the

DBGT Branch is in the process of constructing *Xenopus* BACs with large inserts and arranging for them to be fingerprint-mapped and end-sequenced. These resources will help to identify new genes and to elucidate the organization of the genome and will play an important role in sequencing the genome.

ZEBRAFISH

With its initial genomics projects coming to a close and the sequencing of the zebrafish genome being undertaken by the Sanger Center, the TZCC is again meeting with the scientific community in spring 2002, to re-evaluate the needs of the community. At this meeting, members of the community will prioritize such projects as generating full-length cDNAs, systematic gene disruption projects, microarray development, and other resources crucial for the efficient use of this important model of development. In addition, the NICHD and other members of the TZCC will continue to assist in the support of the Zebrafish Resource Center, which is overseen by National Center for Research Resources. This Resource Center acts as a stock center for the maintenance and distribution of zebrafish mutants and provides state-of-the-art informational resources via the World Wide Web. Also being developed is a new zebrafish database, which will make sequencing data, atlases, and other information available to the community.

MOLECULAR GENETIC SCREENING FOR DISEASES IN NEWBORNS

A major new initiative will focus on the development of new and innovative approaches and strategies for the molecular genetic screening of diseases in newborns. It will adapt the technology, methodology, and automated high-throughput instrumentation used in genomics and proteomics to screen newborns for defective genes and abnormal proteins. Techniques to be evaluated for adaptation will include: microarrays for gene and protein analyses, and mass spectrometry for protein identification and analysis. The initiative's long-term goals are to greatly improve the accuracy, efficiency, and cost-effectiveness of newborn screening tests, so that infants at-risk for certain genetic, metabolic, and infectious diseases can be identified rapidly. This knowledge would enhance the opportunities for early diagnosis and treatment and would significantly reduce the morbidity, mortality, and associated disabilities of affected infants.

FIGURES AND TABLES

FIGURE 1: DBGT BRANCH PORTFOLIO BY PROGRAM CATEGORY—NUMBER OF PROJECTS, FISCAL YEAR 2001

Total Number of Projects: 440

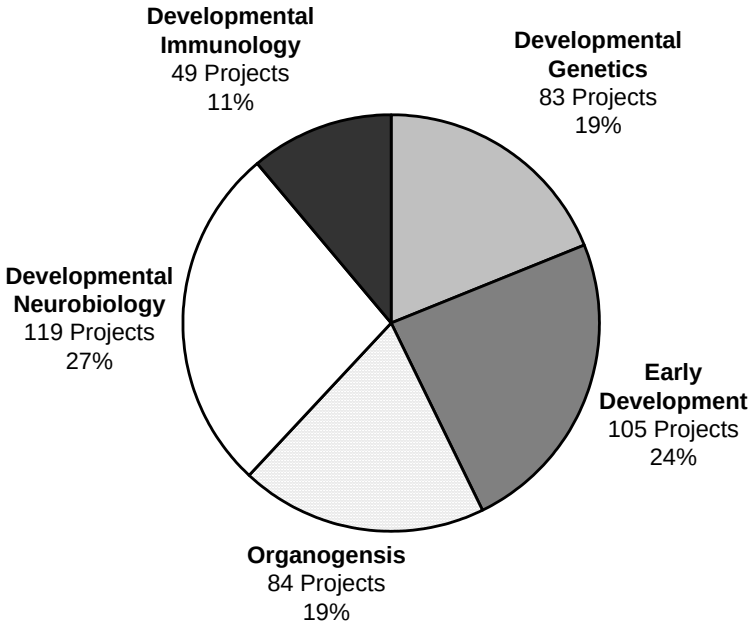


FIGURE 2: DBGT BRANCH PORTFOLIO BY PROGRAM CATEGORY—FUNDING, FISCAL YEAR 2001

Total Funds in Millions US$: $92.9

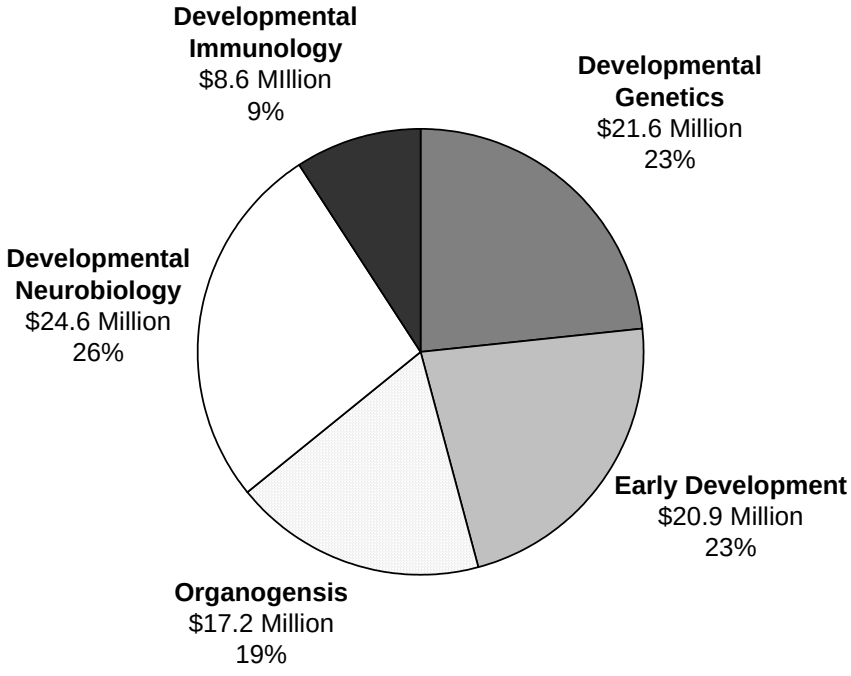


FIGURE 3: DBGT BRANCH PORTFOLIO BY SUPPORT MECHANISM, NUMBER OF PROJECTS, FISCAL YEAR 2001

Total Number of Projects: 440

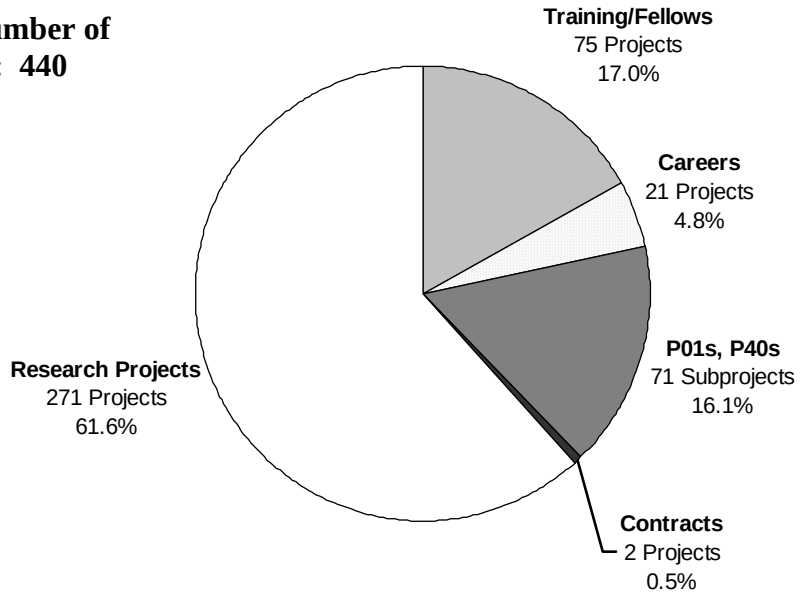


FIGURE 4: DBGT BRANCH PORTFOLIO BY SUPPORT MECHANISM, FUNDING, FISCAL YEAR 2001

Total Funds in Millions US$: $92.9

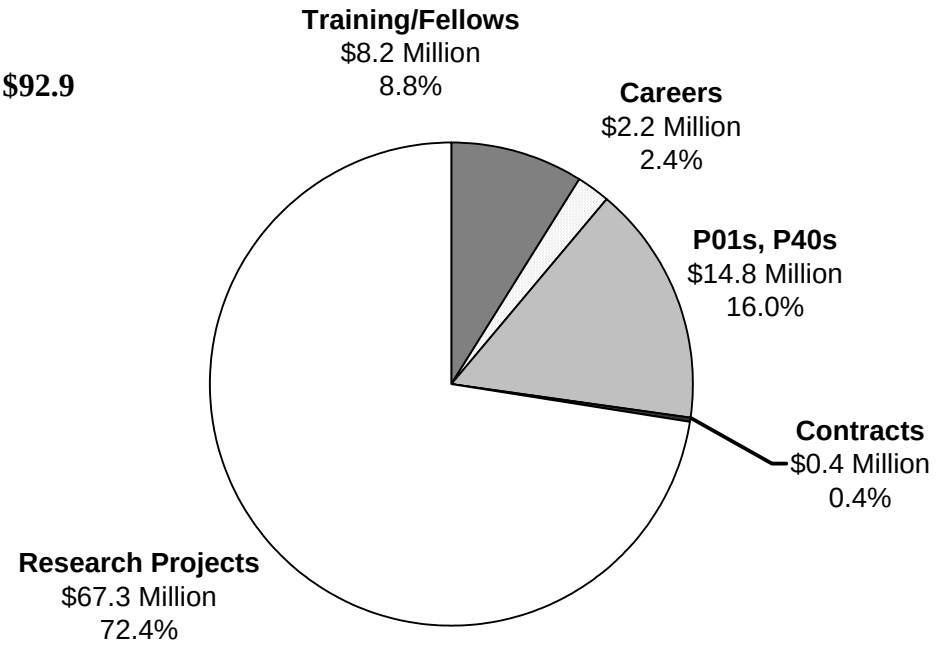


FIGURE 5: DBGT BRANCH FUNDS IN CURRENT AND CONSTANT DOLLARS, FISCAL YEAR 1990–FISCAL YEAR 2001

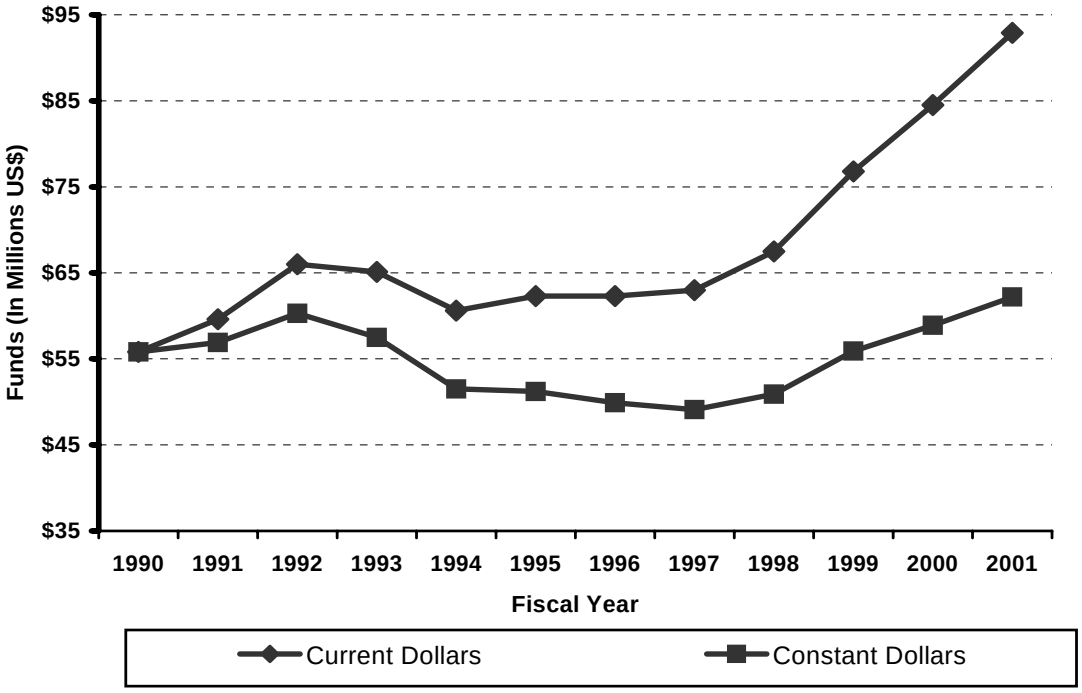
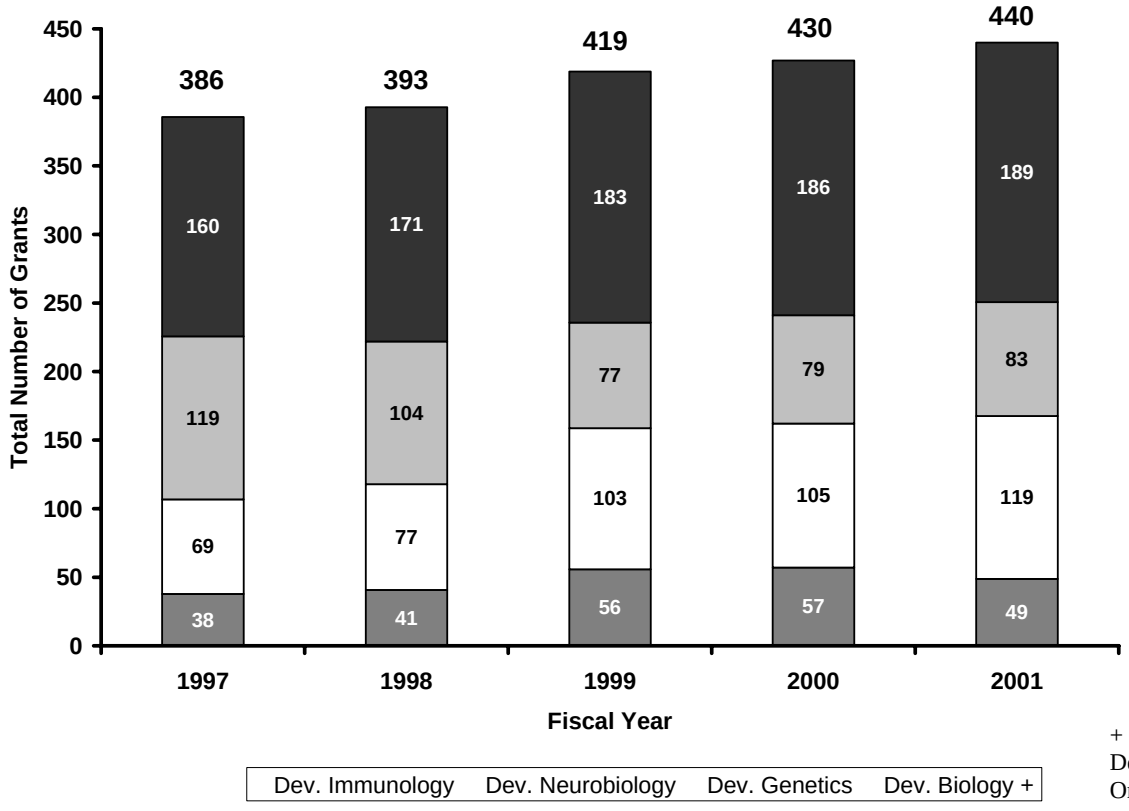
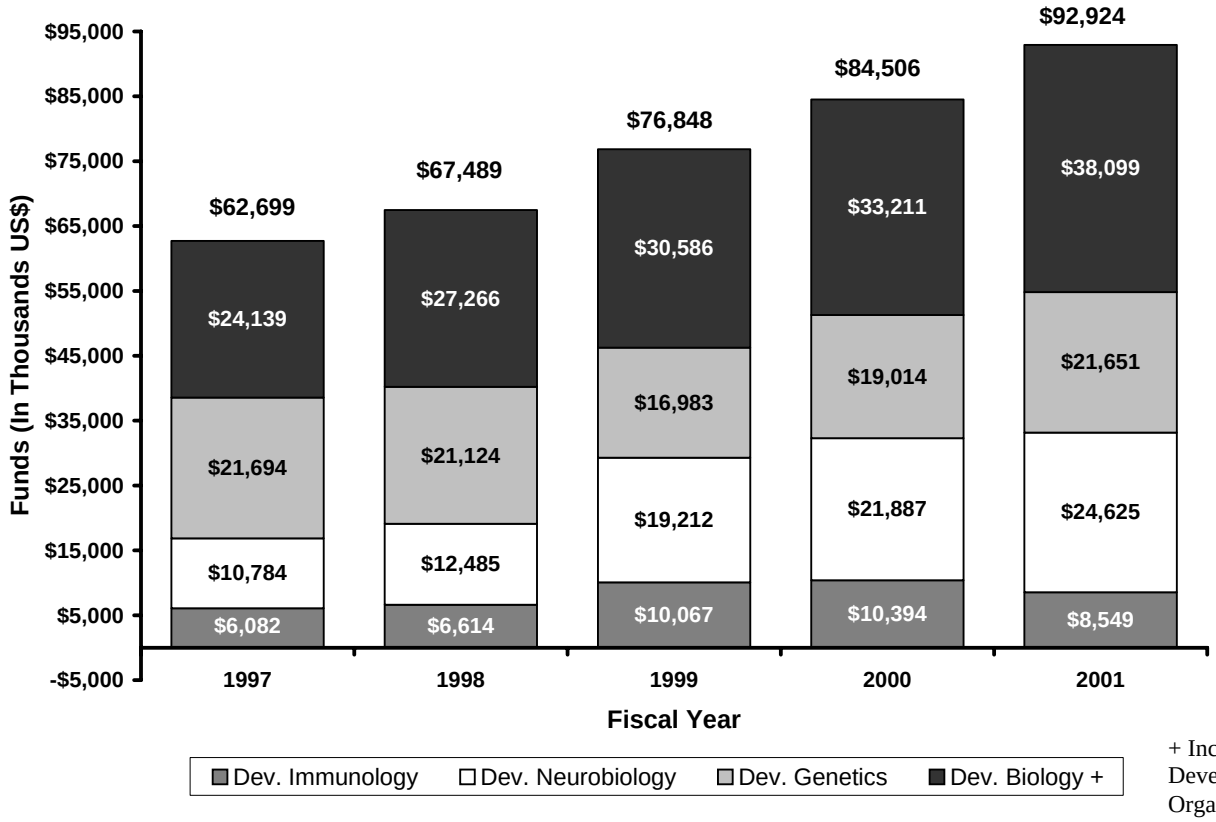


FIGURE 6: DBGT BRANCH PROJECTS BY PROGRAM CATEGORY—NUMBER OF PROJECTS, FISCAL YEAR 1997–FISCAL YEAR 2001



+ Includes Early Development and Organogenesis

FIGURE 7: DBGT BRANCH PROJECTS BY PROGRAM CATEGORY—FUNDING, FISCAL YEAR 1997–FISCAL YEAR 2001



+ Includes Early Development and Organogenesis

FIGURE 8: DBGT BRANCH TRAINING SUPPORT MECHANISMS—NUMBER OF PROJECTS, FISCAL YEAR 2001

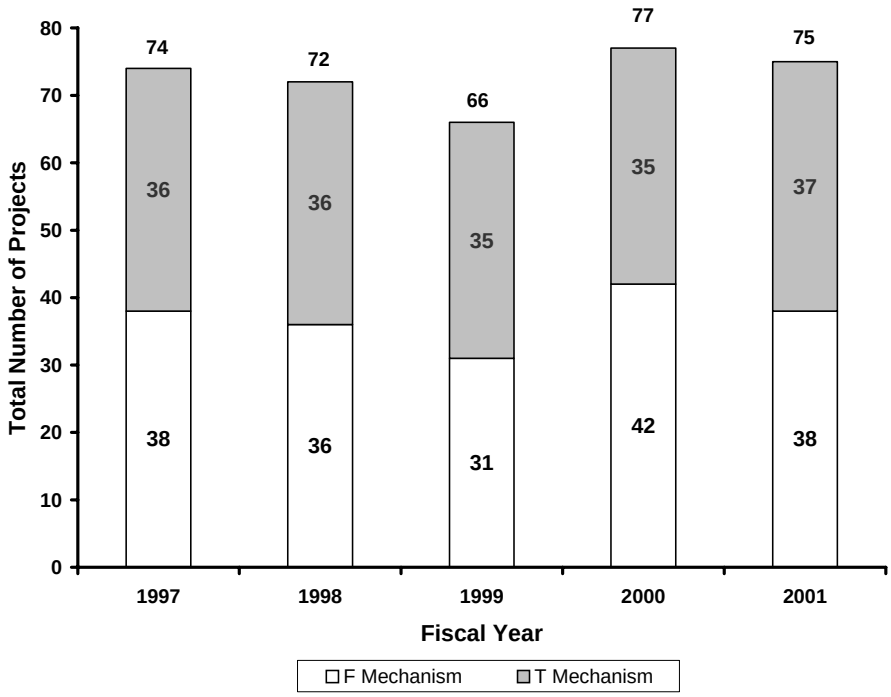


FIGURE 9: DBGT BRANCH TRAINING SUPPORT MECHANISMS—FUNDING, FISCAL YEAR 2001

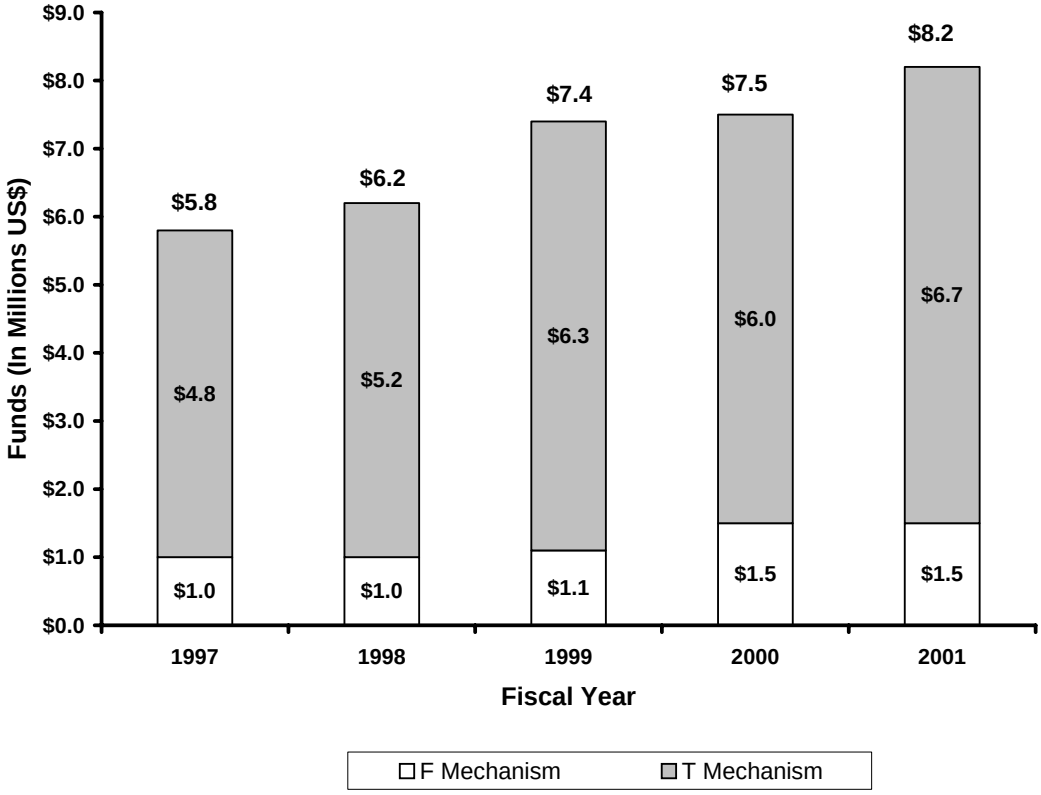


TABLE 1A: DBGT BRANCH GRANTS AND CONTRACTS BY PROGRAM CATEGORY—NUMBER OF PROJECTS, FISCAL YEAR 2001

Program Category	Total Projects		Research Projects (Incl. P01, P40)		Research Career Program Awards		National Research Service Awards		Research Contracts	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
TOTAL	440		342		21		75		2	
Early Development	105	23.9%	65	19.0%	4	19.0%	36	48.0%	-	-
Organogenesis	84	19.1%	71	20.8%	6	28.6%	6	8.0%	1	50.0%
Developmental Genetics	83	18.9%	72	21.0%	3	14.3%	8	10.7%	-	-
Developmental Neurobiology	119	27.0%	97	28.4%	5	23.8%	16	21.3%	1	50.0%
Developmental Immunology	49	11.1%	37	10.8%	3	14.3%	9	12.0%	-	-

Note: Subprojects of program projects are counted individually.

TABLE 1B: DBGT BRANCH GRANTS AND CONTRACTS BY PROGRAM CATEGORY—FUNDING, FISCAL YEAR 2001

Program Category	Total Funds		Research Projects (Incl. P01, P40)		Research Career Program Awards		National Research Service Awards		Research Contracts	
	Funds	Percent	Funds	Percent	Funds	Percent	Funds	Percent	Funds	Percent
TOTAL	$92,923,642		$82,078,284		$2,240,447		$8,207,740		$397,171	
Early Development	$ 20,912,777	22.5%	$ 15,310,715	18.7%	$ 390,491	17.4%	$ 5,211,571	63.5%	-	-
Organogenesis	$ 17,202,874	18.5%	$ 15,645,677	19.0%	$ 624,325	27.9%	$ 912,872	11.1%	$ 20,000	5.0%
Developmental Genetics	$ 21,616,216	23.3%	$ 20,857,110	25.4%	$ 343,186	15.3%	$ 415,920	5.1%	-	-
Developmental Neurobiology	$ 24,612,633	26.5%	$ 22,589,794	27.5%	$ 551,412	24.6%	$ 1,094,256	13.3%	$ 377,171	95.0%
Developmental Immunology	$ 8,579,142	9.2%	$ 7,674,988	9.4%	$ 331,033	14.8%	$ 573,121	7.0%	-	-

Note: Subprojects of program projects are counted individually.

APPENDIX A: DBGT BRANCH PERSONNEL

A. Tyl Hewitt, PhD, joined the staff of the DBGT Branch as a Health Scientist Administrator (HSA) in 1991. He was appointed as Branch Chief in August 1997. Prior to joining the DBGT Branch, he was on the faculty of the Wilmer Eye Institute at The Johns Hopkins University School of Medicine. His postdoctoral training in connective tissue biochemistry at the National Institute of Dental Research followed completion of his dissertation research at Emory University, which focused on changes in cell surface properties during limb development.

Deborah B. Henken, PhD, joined the staff of the Branch in 1996. She is responsible for administering research in the area of developmental neurobiology and related topics in genetics. She came to the NICHD after completing the NIH Grants Associate Program and has the distinction of being the Program's last graduate. Prior to this position, she was an intramural scientist in the Laboratory of Experimental Neuropathology at the National Institute on Neurological Disorders and Stroke, 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 and plasticity.

Steven L. Klein, PhD, was trained as a developmental neurobiologist and a developmental biologist. Prior to joining the DBGT Branch, he was on the faculty of the Department of Anatomy and Cell Biology of the University of Virginia School of Medicine, where he studied the cellular and molecular events involved in embryonic pattern formation, cell migration, cell interactions, and cell fate determination. He is now responsible for the Branch's Early Embryonic Development Program. Additionally, he leads the trans-NIH Initiatives on Mouse Mutagenesis and Phenotyping for Developmental Defects and on *Xenopus* Genetics and Genomics. Finally, he is the chair of the NICHD's Training Policy Committee, which helps to oversee the Institute's Training and Career Development Programs.

Lorette C. Javois, PhD, joined the staff of the DBGT Branch in 2000. She is responsible for administering the program on organogenesis, including the areas of limb development, somitogenesis, chondrogenesis, and myogenesis. She received her PhD in biology from Purdue University, where she studied pattern formation using the developing vertebrate limb as a model system. She competed her postdoctoral training at the Developmental Biology Center at the University of California, Irvine, where she continued to study aspects of pattern formation using the coelenterate, hydra. She spent 14 years on the biology faculty at The Catholic University of America in Washington, D.C., conducting research in the area of cellular and molecular pattern formation and teaching developmental biology and anatomy.

Allan Lock, DVM, MA, is a board-certified veterinary pathologist trained in infectious diseases and immunology. He began at the NIH in 1976, as a diagnostic and experimental pathologist at the Laboratory of Comparative Pathology in the Division of Research Services, and later became the chief of the Laboratory. In 1989, he became a program director of the Division of Comparative Medicine in the National Center for Research Resources. In 1991, Dr. Lock joined

the DBGT Branch to direct research and training programs in birth defects, teratology, developmental immunology, congenital infections, reproductive immunology, and fetal tissue research. He also served as the project officer for the NICHD Transgenic Mouse Development Facility from 1990-2001.

Michael E. Whalin, PhD, joined the staff of the DBGT Branch as an HSA in 1998. Before joining the Branch, he was an intramural scientist in the Office of the Scientific Director, Section on Growth Factors at the NICHD, where he studied the mechanism of action of nerve growth factor. Dr. Whalin is a graduate of the University of South Alabama, where he received his doctorate in Basic Medical Sciences/Pharmacology. He was a postdoctoral fellow at the FIDIA-Georgetown Institute for the Neurosciences prior to joining the NICHD. He is now responsible for the Programs on Developmental Genetics and Genomics.

Marlene Sheriff Taulton joined the staff of the DBGT Branch as its secretary in 1998. She currently serves in the capacity of program assistant for the Birth Defects Program. In this capacity, she provides outstanding support to the staff of the Branch, as well as to other members of the Center for Research for Mothers and Children in coordinating administrative functions and conference planning in addition to managing daily Branch activities.

APPENDIX B: DBGT BRANCH ACTIVITIES, 1998–2002

CONFERENCES AND WORKSHOPS

- *Priority Setting for Mouse Genomics and Genetics Resources* (Functional Genomics Portion), Bethesda, MD, March 19-21, 1998
- *NICHD Symposium: Genomics in Birth Defects Research*, San Diego, CA, at the Teratology Society Meeting, June 23, 1998
- *Second NICHD Workshop on Structural Birth Defects*, Bethesda, MD, July 20-21, 1998
- *Third Postdoctoral Fellows' Workshop*, Bethesda, MD, September 2-4, 1998
- *NIH Birth Defects SIG Meeting: Limb Development and Congenital Limb Defects*, Bethesda, MD, January 29, 1999
- *NIH Non-Mammalian Models Meeting* (Session on *Xenopus).* Bethesda, MD,
- February 16-17, 1999
- *External Zebrafish Advisory Panel/Zebrafish Genomics Initiative Grantees' Meeting*, Bethesda, MD, March 16, 1999
- *Course on Immune Response to Vaccines and Infection*, San Francisco, CA, presented at the Pediatric Academic Societies' Meeting, May 1, 1999
- *Workshop on Genomic and Genetic Tools for the Zebrafish*, Bethesda, MD, May 10-11, 1999
- NIH Predoctoral Neuroscience Training Meeting, Bethesda, MD, June 10-11, 1999
- Developing Scientists for the Year 2000 and Beyond: A Conference for NICHD-Supported Under Represented Minority Scientists, Bethesda, MD, July 29-30, 1999
- NIH Birth Defects SIG Mini-Symposium: Skeletal Biology and Disorders, Bethesda, MD, at the NIH Research Festival, October 6, 1999
- External Zebrafish Advisory Panel/Zebrafish Genomics Initiative Grantees' Meeting, Bethesda, MD, December 14, 1999
- Identifying the Genetic and Genomic Needs for Xenopus Research, Bethesda, MD, March 2-3, 2000
- Meeting on Advances in Primary Immunodeficiency Diseases: Risk of Cancer, Washington, DC, March 18-19, 2000
- Symposium on Gene-based Understanding of X-linked Primary Immunodeficiency Disorders, Boston, MA, at the Pediatric Academic Societies' Meeting, May 15, 2000
- Symposium on Genomics, Proteomics, Bioinformatics, and Developmental Toxicology in the 21st Century, Palm Beach, FL, at Teratology Society Meeting, June 29-30, 2000
- NIH Clinical Center Grand Rounds: Advances in the Understanding and Treatment of Human Severe Combined Immunodeficiency Diseases, Bethesda, MD, September 21, 2000
- Workshop on the Developing Immune System: Frontiers of Knowledge, Arlington, VA, September 20-21, 2000
- NIH Birth Defects SIG Meeting: Molecular Mechanisms of Alcohol Teratogenesis, Bethesda, MD, January 18, 2001
- External Zebrafish Advisory Panel/Trans-NIH Zebrafish Project Meeting, Rockville, MD, February 20, 2001
- First Structural Birth Defects Investigators' Meeting, Bethesda, MD, July 10-11, 2001
- NIH Predoctoral Neuroscience Training Meeting, Bethesda, MD, July 12-13, 2001

- NIH Meeting on Gene Therapy for Primary Immunodeficiency Diseases, Rockville, MD, August 29-30, 2001
- NIH Primary Immunodeficiency Diseases Advisory Meeting, Bethesda, MD,
- September 6, 2001
- NIH Roundtable: Primary Immunodeficiency Diseases: Diagnosis and Treatment, Bethesda, MD, October 26, 2001
- Fourth Postdoctoral Fellows' Workshop, Potomac, MD, December 5-7, 2001
- Workshop on Genomic and Genetic Tools for the Zebrafish, Bethesda, MD,
- April 1-2, 2002

LIAISON AND COMMITTEE ACTIVITIES

- Arthritis and Musculoskeletal Diseases Interagency Coordinating Committee
- Association for Women in Science-Bethesda Chapter
- Brain Molecular Anatomy Project
- CSR Biology of Development and Aging (BDA) IRG Steering Committee
- CSR BDA Working Group
- CSR Bone Biology Discussion/Application Referral Group
- CSR Neuroscience Reorganization
- Joint Neuroscience Training Program
- Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee
- NICHD/Highland Elementary Adopt-a School program
- NICHD HIV/AIDS
- NICHD Minority/Disability Supplement Review Committee
- NICHD Neuroscience Steering Committee
- NICHD Program Organization Task Group
- NICHD R03 Second Level Review Committee
- NICHD Training Policy Committee
- NICHD WEB Site Committee
- NICHD Workplace Improvement and Diversity Advisory Committee (WIDAC)
- NIH Child Care Committee
- NIH Shannon Review Committee
- Project Officers/Program Officials Forum (POPOF)
- Staff Training in Extramural Programs Committee (STEP)
- Training Advisory Committee (TAC)
- Trans-NIH Birth Defects Special Interest Group
- Trans-NIH Non-Mammalian Model Organisms for Biomedical Research Committee
- Sub-committee on *C. elegans*
- Sub-committee on *Drosophila*
- Sub-committee on *D. discoideum*
- Trans-NIH Single Nucleotide Polymorphism (SNPs) Initiative Committee
- Trans-NIH Group on Issues Related to Human Specimen Resources
- Trans-NIH Committee on Genetics in the Developing World

- Trans-NIH Committee on the Haplotype Map
- Trans-NIH Mammalian Gene Collection Initiative
- Inter-Institute Coordinating Committee
- Subcommittee on cDNA Technology Development
- Trans-NIH Mouse Genetics and Genomics Coordinating Committee
- Trans-NIH Xenopus Working Group
- Trans-NIH Zebrafish Coordinating Committee

APPENDIX C: DBGT BRANCH SOLICITATIONS, 1998–2002

REQUESTS FOR APPLICATIONS (RFAS)

NICHD-Sponsored

- HD-99-002 - Genetic Susceptibility & Variability of Human Malformations
- HD-99-007 - Mouse Mutagenesis and Phenotyping: Developmental Defects
- HD-99-008 - Developmental Mechanisms of Human Malformations
- HD-00-004 - Mutagenesis Screens/Phenotyping Tools for Zebrafish
- HD-01-008 - Developing the Potential of *Xenopus tropicalis* as a Genetic Model
- HD-01-020 - Mouse Phenotyping: Developmental and Fertility Defects

Co-sponsored by the NICHD

- DK-98-006 - Genomic Resources for the Zebrafish
- MH-00-002 - Gene Expression Profiling in the Nervous System

PROGRAM ANNOUNCEMENTS (PAS)

NICHD-Sponsored

- PA-98-074 - Zebrafish as an Animal Model for Development and Disease Research
- PA-01-070 - Development of Zebrafish Mutagenesis and Screening Tools [This PA was to continue efforts outlines above in the RFA Mutagenesis Screens/Phenotyping Tools for Zebrafish (HD-00-004)]
- PA-01-095 - Zebrafish as an Animal Model for Development and Disease Research [Reissuance of PA-98-074]

Co-sponsored by NICHD

- PAR-97-073 - Jointly Sponsored NIH Predoctoral Training Program in the Neurosciences
- PA-98-050 - Neurosciences Technology Development
- PAS-98-040 - Opportunities in AIDS Research: Human Immunology
- PA-98-105 - Research on Skeletal Growth and Development
- PAR-00-037- Jointly Sponsored NIH Predoctoral Training Program in the Neurosciences [Reissuance of PAR-97-073]
- PA-02-073 – Innovation Grants for Research in Human Immunology

CONTRACTS AND INTERAGENCY AGREEMENTS

- The NICHD Transgenic Mouse Development Facility (NTMDF)
- Distribution of Animal Models for Neural Tube Defects
- Gene Discovery in the Developing Nervous System
- *Drosophila* Stock Center at Indiana University

APPENDIX D: PUBLICATIONS BY DBGT PERSONNEL, 1998–2002

(DBGT staff names appear in bold.)

Henken, D.B. (1998). Zebrafish Research: Activities at the NIH. *The Zebrafish Science Monitor,* (5): 3-4.

Report of the NIH meeting for Priority Setting for Mouse Genomics and Genetics Resources [Functional Genomics Section]. (1998). http://www.nih.gov/about/director/reports/mgenome.htm.

NIH Non-Mammalian Models Meeting Report [Section on *Xenopus*]. (1999). http://www.nih.gov/science/models/nmm/.

S.G. Voit & **D.B. Henken.** (1999). Program Promotes Neuroscience Careers. *The NIH Record,* (51): 10.

When the Body's Defenses are Missing: PRIMARY IMMUNODEFICIENCY. (1999). DHHS, NICHD (NIH Publication No. 99-4149), Washington, DC, U.S. Government Printing Office.

Klein, S.L. (1999). The Need for Computers in the Study of Embryonic and Fetal Development. In: *Imaging and the Internet in the Study of Embryonic and Fetal Development, Special Issue of Computerized Medical Imaging and Graphics*. **S.L. Klein** (Ed). Elsevier Scientific, Ltd; Great Britain; 23(1-2).

Klein, S.L. (Ed). (1999). *Imaging and the Internet in the Study of Embryonic and Fetal Development, Special Issue of Computerized Medical Imaging and Graphics*. Elsevier Science, Ltd; Great Britain; 23(1).

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Henken, D., Rennert, O., **Whalin, M., Hewitt, A.T., Lock, A.** (2000). Summary of Second Workshop on Structural Birth Defects. *Developmental Biology,* 216: 423-425. Erratum. (2000). *Developmental Biology* 219: 163.

Clifton, S.W., S.L. Johnson, **S.L. Klein,** B. Strausberg, D. Pape, and the Washington University Genome Sequencing Center EST Team. (2000). *Generating Xenopus expressed sequence tags (ESTs) at Washington University Genome Sequencing Center*. The Eight International *Xenopus* Conference, Estes Park, Colorado.

Henken, D.B. (2000). *Neuroscience Research Support at the NICHD*. (pamphlet)

Scientific Frontiers in Developmental Toxicology and Risk Assessment. (2000). National Research Council, National Academy of Sciences; Washington, DC; National Academy Press.

From Cells to Selves: Strategic Plan 2000 [Section on Developmental Biology]. (2000).

Henken, D.B. (2001). Zebrafish Research: An Update on Extramural Activities at the NIH. *The Zebrafish Science Monitor*.

Developmental Biology: Understanding Normal and Abnormal Development. (2001). National Institute of Child Health and Human Development.