FETAL *and* NEONATAL PHYSIOLOGY

FIFTH EDITION

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VOLUME 1

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1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

FETAL AND NEONATAL PHYSIOLOGY, FIFTH EDITION ISBN: 978-0-323-35214-7

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Library of Congress Cataloging-in-Publication Data

Names: Polin, Richard A. (Richard Alan), 1945- editor. | Abman, Steven H., editor. | Rowitch, David H., editor. | Benitz, William E., editor. | Fox, William W., 1940- editor.
Title: Fetal and neonatal physiology / [edited by] Richard A. Polin, Steven H. Abman, David H. Rowitch, William E. Benitz, William W. Fox.
Description: Fifth edition. | Philadelphia, PA : Elsevier, [2017] | Includes bibliographical references and index.
Identifiers: LCCN 2016015238 | ISBN 9780323352147 (hardcover)
Subjects: | MESH: Fetus–physiology | Infant, Newborn–physiology | Maternal-Fetal Exchange–physiology
Classification: LCC RG610 | NLM WQ 210.5 | DDC 612.6/47–dc23
LC record available at https://lccn.loc.gov/2016015238

Executive Content Strategist: Kate Dimock
Senior Content Development Specialist: Marybeth Thiel
Publishing Services Manager: Catherine Jackson
Senior Project Manager: Doug Turner
Designer: Maggie Reid

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1

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Helene Polin, Carolyn Abman, Risa Sorkin, and Andrea Benitz

Our children –
Allison Polin Steinbrenner, Mitchell Polin, Jessica Moseley, and Gregory Polin
Ryan Abman, Lauren Abman, Mark Abman, and Megan Abman
Sophie Rowitch
Lindsey Benitz, Maija Benitz, and Annika Benitz Chaloff

And our grandchildren –
Lindsey Steinbrenner, Eli Steinbrenner, Willa Polin, Jasper Polin, Casey Moseley,
Smith Moseley, and Winslow Broderick

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Preface

It has been nearly 6 years since the fourth edition of *Fetal and Neonatal Physiology* was published. During that interval, thousands of publications have focused on various aspects of the physiology of the fetus and the neonate. More than any prior edition of this textbook, the fifth edition has been substantially updated and revised. Nearly one third of the chapters have been written by new authors, and 16 chapters appear in this book for the first time. In addition, the book now concludes with a section on the pathophysiology of a variety of neonatal diseases that has 23 new or completely updated chapters. With this edition, we have made changes in the editors. Dr. William (Bill) Fox has become an emeritus editor, and we have added Drs. William (Bill) Benitz and David Rowitch. Bill Fox was one of the two original editors of *Fetal and Neonatal Physiology,* and his knowledge and enthusiasm will greatly be missed. David Rowitch and Bill Benitz bring their own skill sets to the textbook and have helped to make this edition truly outstanding. The challenge faced in the preparation of this edition was to provide an exhaustive update without allowing the size (and cost) of the textbook to escalate. To further this end, we have eliminated clinical information that can easily be found in one of the many standard textbooks on neonatology, without sacrificing our goal of making the book a comprehensive reference text.

As with any large textbook, there are a number of individuals we need to acknowledge. First, we wish to thank the hundreds of authors who contributed chapters to this latest edition. We realize that the "academic" benefits of being a chapter author are limited and that writing a new chapter or updating an existing one requires an enormous amount of time and effort. Only through the generosity of these contributors and their desire to educate has this edition been able to go forward. We wish to thank Marybeth Thiel at Elsevier, who has challenged us to make this edition ever better, for her tremendous help in the development and organization of the book.

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1 Basic Genetic Principles

Fred Levine

PRIMARY STRUCTURE OF NUCLEIC ACID

The two kinds of nucleic acid—deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)—are composed of recurring monomeric units called *nucleotides.* Each nucleotide has three components (Figure 1-1, *A*): (1) a phosphate group linked to (2) a five-carbon atom cyclic sugar group, which, in turn, is joined to (3) a purine or a pyrimidine base. DNA and RNA are distinguished by their base components and the makeup of their sugar-phosphate backbones. DNA consists of four deoxyribonucleotides that differ in their base components. The four bases are the purine derivatives adenine (A) and guanine (G) and the pyrimidine derivatives cytosine (C) and thymine (T) (see Figure 1-1, *B* and *C*). Similarly, four different ribonucleotides are the major components of RNA; they contain the purine bases adenine and guanine and the pyrimidine bases cytosine and uracil (U). Thus the major difference in base composition between RNA and DNA is that RNA contains uracil, whereas DNA contains thymine. The other difference between RNA and DNA is in their sugar-phosphate backbones: RNA contains ribose, and DNA contains 2-deoxyribose. Deoxyribose confers resistance to hydrolysis, which is important in conferring onto DNA a high degree of chemical stability, allowing it to remain intact for thousands of years, making possible the sequencing of the genomes of long-extinct species. In both DNA and RNA, the nucleotides are joined together by phosphodiester bonds linking the phosphate group of one nucleotide to a hydroxyl group on the sugar of the adjacent nucleotide. The purine and pyrimidine bases of the nucleotide constitute distinctive side chains and are not present in the backbone structure of nucleic acids.

By analyzing x-ray diffraction patterns of purified DNA and by building models, James Watson and Francis Crick, working in Cambridge in 1953, deduced that native DNA consists of two antiparallel chains in a structure that can be conceptualized as resembling a spiral staircase, the *double helix* (Figure 1-2, *A*). The two strands are held together by hydrogen bonds between pairs of bases on the opposing strands, similar to the steps on the staircase (see Figure 1-2, *B*). The bonding is specific: A always pairs with T, and C always pairs with G. As Watson and Crick noted, a pair of purines would be rather large to fit inside a double helix (which has a thickness of 2 nm), and a pair of pyrimidines would be too far apart to form stable hydrogen bonds with each other. The base pairs AT and GC, however, proved to be similar not only in size but also in shape. Overall, the discovery of the structure of DNA was one of the most important events in biology because it not only provided an explanation for how genetic information is carried but also indicated how this information is propagated. As a consequence of the hydrogen bonding between the two DNA strands, also known as *base pairing* or *hybridization,* a DNA molecule can replicate precisely by separation of the two chains followed by synthesis of two new complementary strands.

In contrast with the regular structure of DNA, most RNA molecules are single stranded. Base pairing, however, occurs between regions of an RNA strand with complementary sequences, with AU pairs instead of AT, giving RNA molecules a complex secondary structure that is poorly understood in most cases but plays an important role in cellular metabolism (e.g., as has been described for transfer RNA [tRNA]).

GENOMIC ORGANIZATION

CHROMOSOMES

Each cell in a human being contains an enormous amount of DNA. With the completion of the human genome sequence, we know now that the genome contains 3.23483×10^9 base pairs per haploid genome. Obviously, the cell must be able to organize such a large amount of DNA in a compact manner. This organization is accomplished by packaging the DNA into large macromolecular complexes called *chromosomes.* A major distinguishing feature of eukaryotic organisms, such as humans, versus prokaryotic organisms, such as bacteria, is the presence of a nucleus, the main function of which is to contain the chromosomes. In contrast to a long-prevailing notion that the DNA within the nucleus floated freely—sometimes characterized as a "noodle soup"—it has become clear that it is highly ordered, with specific sequences from distant regions being brought into proximity to regulate gene expression.

Of the 46 chromosomes in the human nucleus, 44 are *autosomes;* the remaining two are the *sex chromosomes*—those involved in sex determination. In the human karyotype (an organized microphotographic array of all of the chromosomes), the autosomes can be seen to exist in homologous pairs, numbered from 1 to 22 in order of decreasing size, with one member of each pair inherited from one parent. The two sex chromosomes are designated *X* and *Y*. Normally, females have two X chromosomes, whereas males have one X and one Y. A small segment of the Y chromosome includes the gene for the testis-determining factor (TDF) responsible for male development. The lack of this factor results in female development, so it is the presence or absence of the Y chromosome that actually determines gender. The TDF gene encodes a sequence-specific DNA-binding protein named *SRY* ("sex-determining region Y").

Each chromosome consists of one long DNA molecule complexed with large amounts of two types of protein, called *histone* and *nonhistone chromosomal proteins,* which serve to condense the DNA into an orderly, compact structure and play a key role in regulating gene expression. The five principal histone types—H1, H2a, H2b, H3, and H4—interact specifically with one another and with DNA to form structures called *nucleosomes.* Each nucleosome consists of a disk-shaped histone core plus a segment of DNA that winds around the core. The core contains two copies of H2a, H2b, H3, and H4, and the DNA wrapped

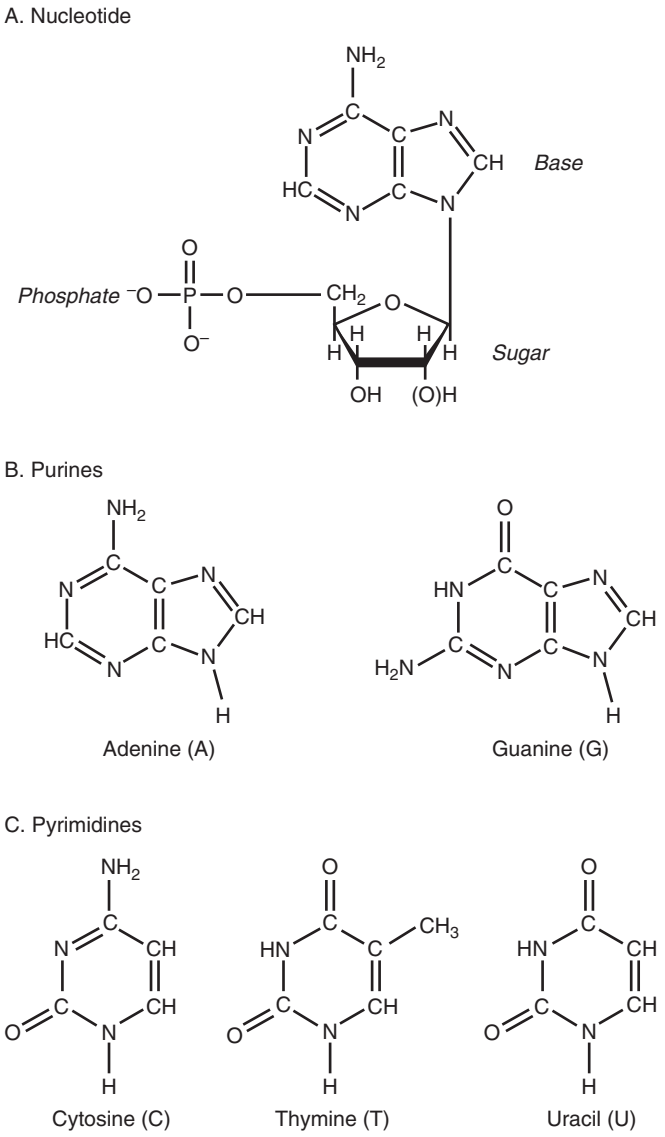

Figure 1-1 Nucleotide structure. A, General structure of nucleotides, consisting of a purine or pyrimidine base (in this case, adenine), a five-carbon atom sugar, and a phosphate group. The oxygen in *parentheses* in the sugar is present in the ribose of RNA but is absent in the deoxyribose of DNA. The plane of the sugar is perpendicular to that of the other subunits. **B,** Structures of the two purines in DNA and RNA. **C,** Structures of the three pyrimidines in nucleic acids. Cytosine is found in both DNA and RNA, thymine is unique to DNA, and uracil is unique to RNA.

around the histone core is approximately 140 base pairs in length. H1 binds to the DNA just next to the nucleosomes.

The complex of DNA and histones, called *chromatin,* forms coils to produce a fiber with a larger diameter. Histone-depleted metaphase chromosomes have been shown to consist of a nonhistone protein scaffold that has the shape characteristic of a metaphase chromosome surrounded by a halo of DNA. In this model of chromosome organization, the nucleoprotein fibers form radially oriented loops that converge onto the central scaffolding. Most chromatin fibers undergo a transition between dispersed and condensed configurations during the cell cycle. Before cell division, most chromatin is in the condensed form, with limited but functionally important transcriptional activity. Between cell divisions, the bulk of the chromatin in most cells is in a less-condensed, but still highly organized, configuration within the nucleus. Distant regions from the same chromosome, and even from different chromosomes, are brought into proximity with one another as an important aspect of gene regulation. Increasingly, it has been recognized that noncoding RNA molecules play an important role in organizing the structure of chromosomes and in gene regulation. For example, Xist, a noncoding RNA molecule, is a central regulator of X chromosome inactivation. It coats the inactivated X chromosome, which is structurally condensed, with most, but not all genes being transcriptionally inactive.

GENE STRUCTURE

All hereditary information is transmitted from parent to offspring through the inheritance of genes, which are defined as the DNA sequences necessary to produce a functional protein or RNA sequence. Approximately 20,000 genes are present on human chromosomes, although this number is subject to continuing revision, even with the completion of the human genome sequence. A surprising finding on completion of the genome sequence was that the number of genes in human beings is not substantially greater than that in lower organisms. Thus, evidently, it is not the number of genes that accounts for increased complexity, but rather the manner in which they are regulated.

Genes in eukaryotic cells are divided into regions called *expressed sequences (exons)* and *intervening sequences (introns),* of which only the exon sequences are present in mature messenger RNA (mRNA) and code for proteins (Figure 1-3, *top*). Some introns play a role in the control of gene expression, but in other cases, the introns may not have a function. However, they can greatly expand the size of genes. For example, the dystrophin gene, involved in Duchenne muscular dystrophy, is approximately 2300 kilobase (kB) pairs of DNA in length and includes 79 exons accounting for only 14 kB; the remainder consists of introns, some more than 100 kB long. By contrast, the α-globin gene, 835 base pairs long, includes two introns that total 261 base pairs. Overall, exons account for 1.5% and introns account for about 26% of the human genome.

In addition to the exons and introns, most of the eukaryotic genome consists of long DNA stretches that are not part of protein coding genes. For many years, it was thought that these long DNA stretches had no function—hence the term *junk DNA*. This is clearly an oversimplification, because it is clear that some noncoding sequences have roles in DNA replication, chromosome pairing, and recombination. An entirely new class of genetic element was revealed by large scale RNA sequencing projects, demonstrating the existence of genetic elements that code for multiple classes of noncoding RNAs. These are distinct from the transfer and ribosomal RNAs that are noncoding but have clear functions in protein synthesis. Other forms of noncoding RNAs include both micro-RNAs involved in gene regulation at a posttranscriptional level and long noncoding RNAs, some of which have functions (such as Xist mentioned earlier) but many of which are of unknown significance. Many sequences seem to have no obvious function. Recently, some researchers have taken the position that as much as 40% of the genome has a function, primarily involving regulating the expression of genes. However, much of the genome—in fact more than half—consists of various kinds of repeat sequences.

Interspersed throughout the noncoding DNA stretches are many repeated sequences, which are either clustered together or evenly distributed throughout the genome. These sequences can be short and consist only of 5 to 10 nucleotides, or they can be as long as 5000 to 6000 nucleotides. Whether these repeated sequences have important functions or are mere players in an intragenomic evolutionary battle for self-preservation and expansion remains largely a mystery. Some of the longer repeats, such

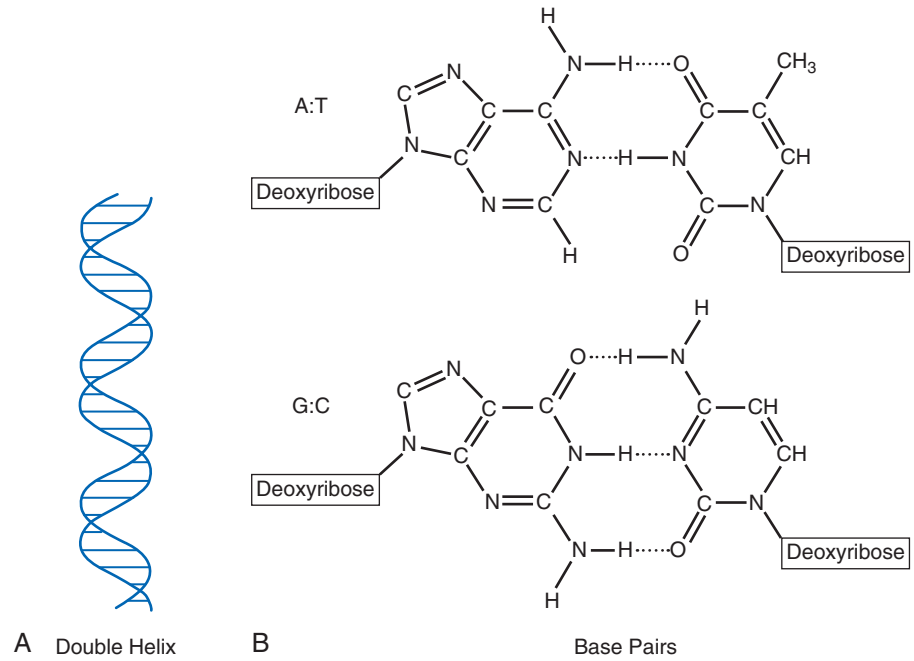

Figure 1-2 DNA structure and base pairing. A, Schematic representation of the double-helical DNA molecule. **B,** Base pairing of purines and pyrimidines in DNA. Hydrogen bonding between the pairs is indicated by *dotted lines.* The AT and GC base pairs are identical in size and nearly identical in shape. Note that the GC base pair has an additional hydrogen bond and therefore is held together more strongly.

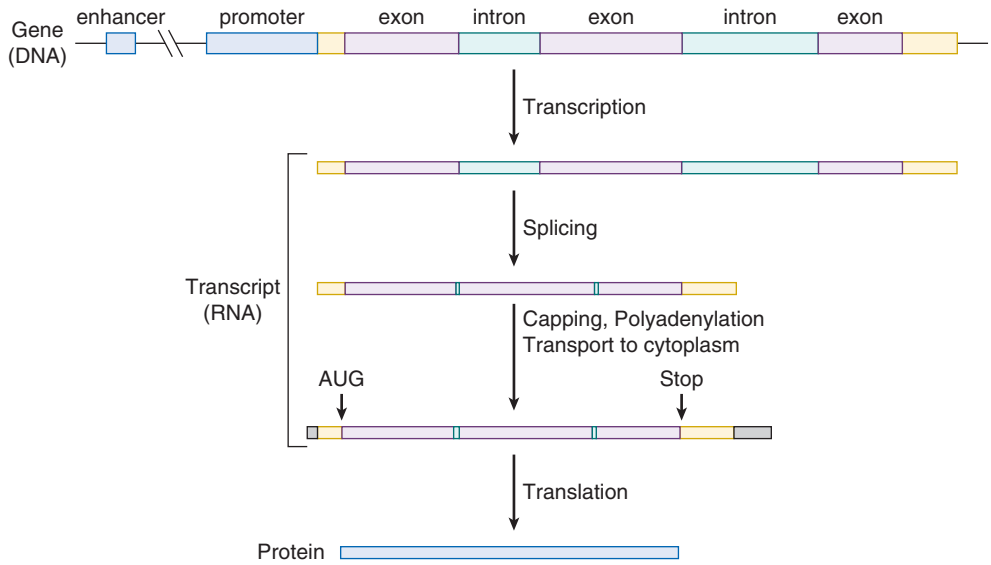

Figure 1-3 Gene structure *(top)* and the flow of genetic information from DNA to protein. *Tan boxes* indicate the regions of exons that do not encode amino acid sequences; *gray boxes* indicate posttranscriptional modifications. AUG is a codon that specifies the amino acid methionine and is also used to specify the first amino acid of a protein.

as the so-called *Alu sequences* or long interspersed nuclear elements (LINEs), have features similar to those of viruses and can move from place to place in the genome. Other repeats are short, consisting of a stretch of two bases, such as CACACA. It has been known for some time that short repeats exhibit extreme variability from individual to individual. This variability can be used to generate a type of fingerprint to uniquely identify any given individual. Such genetic "fingerprinting" is a powerful tool for gene-mapping studies and in forensic applications, such as paternity testing and criminal investigations. More recently, longer repeats and large regions of chromosomes have been found to exhibit copy number variation. This large-scale variation will almost certainly be found to contribute to genetically determined individual variation in human populations.

HOW GENES FUNCTION

FLOW OF GENETIC INFORMATION

TRANSCRIPTION

Because DNA stores genetic information in the nucleus of eukaryotic cells, whereas protein synthesis occurs in the

cytoplasm, a mechanism by which the information is carried to the cytoplasm is essential. The first step in gene expression is the production of an RNA molecule from the DNA template. This RNA acts as a molecular messenger, carrying the genetic information out of the nucleus. The synthesis of mRNA is called *transcription* because the genetic information in DNA is transcribed without being changed into a new language. During the process of transcription, the two DNA strands separate, and one functions as a template for the synthesis of single-stranded RNA molecules by the action of enzymes called *RNA polymerases.* The initial RNA transcripts are quite long because they include both intron and exon sequences from the gene (see Figure 1-3). The intron sequences are cut out by specific enzymes, and the remaining exons are spliced together. To form the mature mRNAs that leave the nucleus, a methylated guanine nucleotide called a *cap* is added to the beginning; a string of 200 to 250 adenine bases usually is added to the end (see Figure 1-3). The cap is important for ribosomal binding in the initiation of protein synthesis, whereas the polyadenosine stretch at the end of the mRNA plays a role in the stability of the mRNA.

In addition to mRNA, other major classes of RNA are transcribed from DNA: ribosomal RNA (rRNA), tRNA, and microRNAs (miRNAs). In contrast with mRNA, these classes of RNA do not code for proteins. Ribosomal RNAs and tRNAs are required for protein synthesis. The recently discovered miRNAs play complex and as yet poorly understood roles in posttranscriptional regulation of gene expression. A subclass of small RNA molecules, termed *small interfering RNAs (siRNAs),* have homology to specific mRNAs in the cell. Binding of a target mRNA by siRNA results in its being degraded, with a consequent decrease in the production of the encoded protein. This phenomenon is widely used by researchers to shut down the expression of target genes. Ultimately, the hope is that this approach can be used to target genes that may be involved in diseases such as cancer. However, as with other forms of gene therapy, the major problem remains efficient delivery to specific target cells in the body.

Unlike prokaryotic cells, in which a single RNA polymerase makes all types of RNA, eukaryotic cells have three different RNA polymerases that transcribe different classes of RNA. The precursors to 18S and 28S rRNA are made by RNA polymerase I; the precursors to mRNA are made by RNA polymerase II; and 5S rRNA and tRNA are synthesized by RNA polymerase III. RNA polymerase I functions in a specialized region within the nucleus called the *nucleolus.*

TRANSLATION AND THE GENETIC CODE

The production of protein from an mRNA template is called *translation* because the genetic information that is stored in DNA as a sequence of nucleotides is translated into a sequence of amino acids. The method of storing genetic information is called the *genetic code.* Each member of the code, called a *codon,* consists of three adjacent bases. Each codon specifies a particular amino acid. Thus the linear nature of the codons in a DNA sequence specifies the sequence of amino acids in a protein. Because each of the three sites in a codon can be one of four possible nucleotides, a total of 4^3, or 64, different codons can be generated. Three of these 64 possible codons, UAA, UAG, and UGA, are called nonsense or termination codons because they do not code for amino acids, but rather serve to mark the end of a protein (i.e., a "stop signal"). The remaining 61 codons specify one of the 20 amino acids. The genetic code is said to be degenerate because each amino acid is specified by more than codon. A consequence of degeneracy in the genetic code is that some mutations do not result in a change in the amino acid sequence.

The three types of rRNA—28S, 18S, and 5S—associate with more than 50 proteins to form the ribosomes, which are the cytoplasmic sites of translation. The tRNAs are small molecules, approximately 80 nucleotides long, whose function is to position the correct amino acid for incorporation into the polypeptide. Before an amino acid can be incorporated into a polypeptide chain, it is first coupled to an appropriate tRNA by an aminoacyl-tRNA synthetase, which is specific for each amino acid–tRNA combination. A three-nucleotide region of each tRNA, designated the *anticodon,* includes a base sequence complementary to the appropriate mRNA codon and therefore hybridizes to it. In this way, each amino acid is brought into proper position and is added sequentially to the growing polypeptide chain by peptidyl transferase, an enzyme that is an integral part of the ribosome. Initiation of transcription almost always occurs at an AUG codon, which codes for methionine (see Figure 1-3). In many proteins, the initiation methionine is removed posttranslationally. Translation is terminated when a ribosome encounters a nonsense codon (see Figure 1-3). In the presence of the appropriate factors, the polypeptide chain is released from the last tRNA, and the ribosome disengages from the mRNA to start the cycle of protein synthesis over again.

REGULATION OF GENE EXPRESSION

The ability to control the production of proteins is central to the development and functioning of every organism. Although this control occurs at every stage of protein production, the most important level of control occurs at the level of mRNA production (i.e., transcriptional control). Transcriptional regulation is accomplished via the action of proteins that act on DNA, either by modifying it (e.g., cytosine methylation) or by binding to specific DNA sequences to activate or repress transcription from a gene.

In higher eukaryotes, such as humans, two major types of DNA sequences regulate gene expression: *promoter*s and *enhancers.* Promoters are located immediately adjacent to the start site of transcription, whereas enhancers can be located at large distances from the transcribed regions of the gene (see Figure 1-3). Several types of regulatory sequences have been identified in promoters that are important in transcriptional initiation by RNA polymerase II, including the *TATA box,* so called because it consists of a run of T and A base pairs. The TATA box is located approximately 30 bases before the transcription start site and functions as the binding site for a large, multisubunit complex of transcription factors (including RNA polymerase). Specific sequence elements that form part of promoters and enhancers are required for binding the approximately 1400 sequence-specific proteins that bind to DNA and modulate the rate of transcription up or down.

Transcription is regulated by interactions among proteins bound to enhancer and promoter sequences. Such proteins can have either stimulatory or inhibitory functions. For example, the receptors for steroid hormones such as the glucocorticoids, estrogen, and androgens have been isolated and shown to bind to specific sequences near steroid-responsive genes, such as vitellogenin and lactalbumin. Many transcription factors are expressed in a tissue-specific manner, contributing to the differences in gene expression between different cell types. In almost all cases, transcription factors are not absolutely specific for a particular cell type. Rather, it is combinatorial interactions between transcription factors that are expressed in some but not all cell types that lead to the formation of a particular protein complex at an enhancer and/or promoter site, leading to highly precise gene activation in a particular cell type.

EPIGENETICS

In addition to the classic transcription factors that bind to specific sequence elements in genes, gene expression is controlled by enzymes that modify DNA-bound proteins and even DNA itself. The major mechanism by which DNA is modified is by methylation of cytosine residues adjacent to guanosine. Methylation of these CpG dinucleotides by DNA methylases leads to

transcriptional inactivation, while demethylation by demethylases alters the conformation of chromatin, leading to transcriptional activation. Histone proteins are extensively modified by many enzymes, including acetylases, kinases, and methylases. The pattern of histone modification, particularly on lysines, controls to a great degree whether a particular region of chromatin will be transcriptionally active or inactive and is termed the *histone code*.

Modifications of chromatin proteins and DNA can be inherited through multiple cell divisions. Such heritable alterations in the pattern of gene expression are called *epigenetic,* as they do not involve changes to the DNA sequence itself and so are not classical mutations (i.e., a heritable phenotypic change with a change in genotype).

The importance of epigenetics is exemplified by genetic diseases that affect this process. For example, mutations in MeCP2, a protein that binds to methylated DNA to repress the expression of associated genes, cause Rett syndrome, an X-linked neurodegenerative disease. Rubinstein-Taybi syndrome is caused by mutations in the CBP gene, encoding CREB-binding protein, which acts to acetylate the histone proteins that are major components of chromatin. Environmental influences on the epigenetic control of gene expression are clearly important. For example, maternal folate intake affects the pattern of DNA methylation and consequently gene expression in infants. Note that DNA methylases are folate-dependent enzymes.

In some cases, epigenetic mechanisms operate in ways that appear to violate mendelian laws of inheritance. For example, while most epigenetic DNA modifications are eliminated during germ cell formation or at fertilization, some modifications persist and so are inherited transgenerationally. The extent to which this leads to altered patterns of gene expression with consequent phenotypic effects in humans is a matter of controversy.

POSTTRANSCRIPTIONAL REGULATION

In addition to transcriptional control, posttranscriptional mechanisms play important roles in controlling the level of gene product. Regulation takes place at virtually every level, including alternative splicing, transport of RNA from the nucleus to the cytoplasm, persistence of mRNA in the cytoplasm, translational efficiency, and regulation of the rate of protein degradation. Individual mRNA species differ widely with respect to metabolic stability. The half-lives of some mRNAs span several hours, or even days, whereas those of others are extremely short. The rate of turnover of some mRNAs can vary dramatically in response to changes in the cell cycle and in response to treatment with certain hormones. Protein binding and mRNA structural features also have been shown to influence susceptibility to decay. The significance of posttranscriptional influences on protein levels has given rise to the field of *proteomics,* in which techniques for measuring protein levels on a global and high-throughput scale are used to define the *proteome* (i.e., the total complement of proteins) within a cell.

STEM CELLS AND DEVELOPMENT

Development, in general terms, is the process by which a single fertilized egg becomes a complete organism. Central to development is the process of *differentiation,* whereby cells acquire different properties to carry out specific functions in separate tissues. To a large extent, differentiation is reflected in the production of tissue-specific proteins, which, of course, is the result of specific gene expression. A fundamental question in development is how a group of genetically identical cells comes to express sets of genes in a tissue-specific manner. Much progress in this area has come from the striking similarities between the process of development in organisms such as *Drosophila* and *Caenorhabditis elegans* and in humans. Important principles of development, such as the role of morphogen gradients (i.e., gradual changes in the concentration of molecules that influence cell fates), were first described in lower organisms but have proved to be relevant in humans as well.

In addition to knowledge gained from lower organisms, a revolution in the understanding of human development and human genetic disease has come from advances in the ability to manipulate mice genetically. This was originally done by introducing genetic material into mice by microinjection of fertilized eggs. The injected eggs give rise to mature animals that integrate the injected genes into their genome; such transgenic animals will pass the introduced genetic material through the germline to their offspring. In many cases, the introduced genes are expressed, allowing the effect of overexpression or ectopic expression of a gene product.

Inactivation of specific genes in mammals can be done by genetically manipulating embryonic stem (ES) cells derived from the blastocyst (Figure 1-4). A *stem cell,* by definition, is any cell that can both self-renew (i.e., replicate) and also give rise to more differentiated progeny. ES cells usually are derived from blastocysts, although ES cell lines have been derived even from eight-cell embryos. They can be grown in culture while retaining the ability to differentiate into all somatic tissues and the germline of mature mice. Thus a single cell grown in culture can be used to create a living mouse. Because ES cells can be grown in culture and genetically manipulated, mutations can be created in DNA using recombinant techniques. When the resulting DNA is incorporated into the DNA of ES cells by homologous recombination (Figure 1-5), they can be used to create mutant mice. To do that, the genetically manipulated ES cells are injected into blastocysts, giving rise to chimeric adult mice (Figure 1-4). Some of these mice contain the genetically manipulated cells in their germline and will produce genetically altered, nonchimeric offspring. Many mammals have been cloned using techniques such as somatic cell nuclear transfer, where nuclei from somatic cells are transferred into unfertilized oocytes. The original example was Dolly the sheep, but many other examples now exist.

Stem cell biology has been revolutionized by the isolation of embryonic stem cells from many mammals, including humans. These cells are being used to produce many different cell types in vitro for the study of human diseases. For example, dopaminergic neurons can be produced from human ES cells to study Parkinson's disease. In 2012, the Nobel Prize in Medicine was awarded for the discovery that virtually any cell type in the adult can be converted into a pluripotent stem cell, using gene transfer to express particular transcription factors that reprogram the pattern of gene expression to a pluripotent state. These cells, called *induced pluripotent cells (iPSCs),* are now the most common method used to study cellular differentiation in vitro, with the hope that they may become a source of cells for transplantation to treat diseases such as type 1 diabetes in the future.

An enormous advantage of being able to create adult mammals from stem cells is the ability to genetically alter the stem cells to study the function of genes and their role in disease. For example, a common investigational technique is to knock out genes in a particular tissue through modification of the target gene by flanking it with 34 base pair DNA sequences called *lox sites* that are the substrates for a recombinase enzyme derived from the bacteriophage P1 Cre recombinase. In mice, when Cre ("cyclization recombination") is expressed from a tissue-specific promoter element, the affected mouse will demonstrate deletion of the target gene only in cells expressing the tissue-specific promoter. Forms of the Cre recombinase that are activated by the drug tamoxifen have been developed, allowing for temporally controlled genetic alterations in adult animals. Recently, additional methods of making targeted genetic alteration in cells have been developed. These are all based on cooption of systems used by lower organisms to promote or protect from infection by recognizing specific DNA sequences in foreign organisms. Examples are the transcription activator–like effectors from *Xanthomonas*

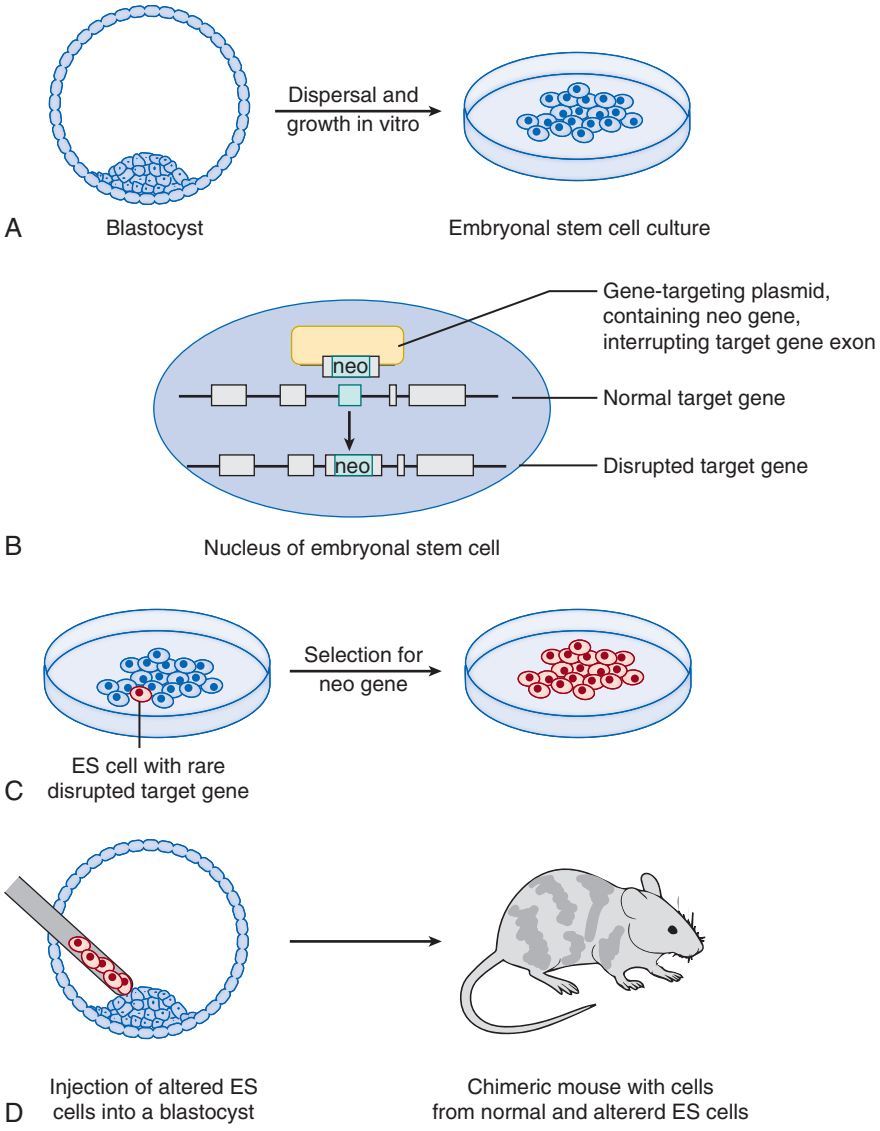

Figure 1-4 Genetic alteration of mice by homologous recombination.

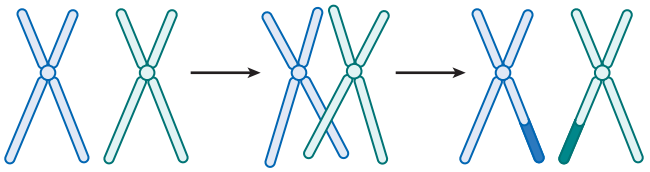

Figure 1-5 Recombination. In this simplified view of recombination, the two members of a homologous pair of chromosomes line up during the first meiotic prophase. Segments of the two chromosomes "cross over," and breakage and rejoining of the DNA strands occur.

bacteria and, more recently, the CRISPR/CAS system that is widely used by bacteria to protect against infection by bacteriophages.

CELL DIVISION AND RECOMBINATION

CELL CYCLE AND MITOSIS

The *cell cycle* is the process by which the cell divides to form two daughter cells. In *mitotic division,* the normal complement of 46 chromosomes is maintained through a process of DNA replication and subsequent separation of the chromosomes. Additionally, the cytoplasm of the eukaryotic cell cleaves into two approximately equal halves. Mitotic division takes up only a small part of the cell cycle. A complete cell cycle consists of four phases: G_1 (growth or gap 1), S (synthesis), G_2 (growth/gap 2), and M (mitosis). The cycle of each type of cell varies considerably in total duration, from minutes in some cells to weeks or months in others. The G_1 phase begins immediately after a cell division. It is followed by the S phase, during which DNA replication occurs, as described earlier. Immediately after the S phase, the cell is tetraploid; there are 92 chromosomes divided into 46 pairs of sister chromatids. Cells then pass into the premitotic G_2 phase, which ends with the onset of mitosis or actual cell division. The G_1, S, and G_2 phases are called *interphase* because despite continued growth and synthesis of macromolecules, such as DNA, RNA, and proteins, cell division takes place only during mitosis. DNA and the histone components of the chromatin are synthesized only during the S phase, whereas RNA, the cytoplasmic proteins, and organelles are synthesized continuously during the entire interphase. The cell finally divides in the M phase, during which the synthesis of RNA and protein is greatly reduced.

During interphase, chromosomes are not visible by light microscopy because chromatin is dispersed throughout the nucleoplasm. The beginning of mitosis is signaled by the appearance of chromosomes as thin threads inside the nucleus. Mitosis is divided somewhat arbitrarily into four phases: prophase, metaphase, anaphase, and telophase. During *prophase,* the nuclear envelope begins to break up, and each chromosome can be seen to consist of two identical or sister chromatids held together at specific regions called *centromeres.* Another structure that is important for proper segregation of chromosomes is the centriole, an organelle just outside the nuclear membrane. Each cell normally has a pair of centrioles, arranged opposite one another, but they are duplicated early in the S phase. During mitotic prophase, the two pairs of centrioles separate and migrate to define the poles of the cell. During *metaphase,* the chromosomes move to the equatorial plane of the cell and become attached to the spindle fiber apparatus, which is a structure consisting of microtubules of protein that radiate from the centrioles at either pole and extend from pole to pole. Each chromatid has a dense granule near its centromere called a *kinetochore* to which the spindle fibers attach. Because of the kinetochores and attached spindle fibers, the two sister chromatids are pulled toward opposite poles. During *anaphase,* the centromeres divide, and the two chromatids of each pair, now free of each other, move toward their respective poles by the contraction of spindle fibers. In *telophase,* the chromosomes and spindle fibers disperse and disappear, and new nuclear envelopes are assembled to surround the two sets of daughter chromosomes. Simultaneously, separation and segregation of the cell cytoplasm occurs, a process called *cytokinesis,* which results in the formation of a complete membrane around the cell and constitutes the end of the process of forming a new cell.

Progress through the cell cycle is controlled by a complex set of steps, many involving phosphorylation mediated by interacting kinases and phosphatases. Proteins called *cyclins,* so called because their expression is limited to specific stages of the cell cycle, control the initiation of the kinase-phosphatase cascade. That cascade, in turn, ultimately controls the ability of the cell to progress through the major cell cycle control points called *checkpoints,* at the G_1-S and G_2-M boundaries. Many of the proteins involved in cell cycle control are, not surprisingly, involved in the loss of cell cycle control that is the hallmark of carcinogenesis. Therefore they are classified as oncogenes or tumor suppressor genes, depending on whether their normal role is to promote or inhibit proliferation. In addition to the proteins that are directly involved in cell cycle control, proteins that are important in DNA repair also have been found to be important in carcinogenesis. Most prominently, the *TP53* tumor suppressor gene, encoding a protein that seems to be the central monitor of genomic damage, is also the most commonly mutated gene in human cancer.

MEIOSIS

Meiosis is the process by which germline cells form gametes. In contrast with mitosis, in which a single cell division and an exact duplication of the genetic material in the parent cell occur, meiosis involves two separate cell divisions from a diploid parent cell and a random reassortment and reduction of genetic material so that each of the four daughter cells has a haploid DNA content (i.e., 23 chromosomes). In this way, meiosis yields four haploid gametes, the sperm and the egg cells, which support sexual reproduction and a new generation of diploid organisms.

The first meiotic division, as with mitosis, is separated into four stages: prophase, metaphase, anaphase, and telophase. Before meiosis begins, the chromosomes in the cell are replicated to produce two pairs of sister chromatids, and each pair of sister chromatids remains together throughout the first meiotic division. In metaphase, the spindle fibers attach to chromosomes, and the paired chromosomes align themselves in the equatorial plane of the cell. In anaphase, the paired homologous chromosomes separate and move toward their respective poles. The daughter chromatids, however, remain attached to their centromeres. In telophase, the chromosomes arrive at the poles. One of the sets of chromosomes forms the first polar body, which eventually is lost. Because the number of chromosomes in each daughter cell is reduced by half, the first mitotic division is called the *reduction division.* This polar body can be removed from the unfertilized egg and the purified DNA amplified by polymerase chain reaction (PCR) techniques (described further on) and used for preimplantation genetic diagnosis.

The second meiotic division is completed after fertilization and occurs without DNA replication. A second polar body, containing a complete set of chromosomes, is extruded, leaving the egg with a single remaining set (i.e., it is haploid). The second polar body is also useful for preimplantation genetic diagnosis, particularly when recombination has occurred during the first meiotic division.

RECOMBINATION

During prophase of the first meiotic division, homologous pairs of chromosomes are held together by a protein-containing framework called a *synaptonemal complex,* which extends along the entire length of the paired chromosomes. Recombination between chromatids of the homologous chromosomes occurs at this stage, resulting in the exchange of DNA between the original parental chromosomes (see Figure 1-5). In males, the X and Y chromosomes are associated only at the tips of their short arms during meiotic prophase. This short associated region is called the *pseudoautosomal region* because recombination between the X and Y chromosomes occurs there (therefore it behaves as an autosome in terms of mendelian inheritance). This region probably plays an important role in sex chromosome pairing and segregation, as well as in male fertility. Recombination, in conjunction with mutation (see later), is important for generating genetic diversity through the exchange of DNA between different chromosomes, and it plays a critical role in gene mapping studies.

MUTATION AND GENETIC HETEROGENEITY

Mutation is defined broadly as any change in the sequence of DNA. Because most of the human genome does not consist of genes, most mutations are of no apparent functional consequence and thus are termed *silent.* Only mutations that affect the expression or function of a gene or its product are phenotypically apparent. Of these, many are not clinically relevant but instead contribute to normal population heterogeneity. Variations in hair and eye color, for example, originally arose through mutation. Thus the term *mutation* may be defined differently at the molecular genetic, biologic, and clinical levels. Along with recombination, mutation is a central element in producing the population diversity that is the substrate for evolution. The mutation rate in humans has been measured at about 1×10^{-10} mutations per nucleotide site per replication. Genomic sequencing has found about 75 new mutations in every person. The mutation rate and the types of mutations that occur, however, can vary dramatically among different loci. The consequence of this rate of mutation is that variations in the human genome occur, on average, approximately once per 1000 base pairs. Hence each genome differs from others at a few million sites. Although mutations occur in both germline and somatic (nongermline) cells, only mutations affecting the germline are inherited. Somatic cell mutations also are of major medical importance, particularly in the development of cancer.

SINGLE-GENE MUTATIONS

Mutations can range from those affecting only a single base pair to major alterations in chromosome structure. Mutations that affect only one nucleotide are called *point mutations* and involve the substitution of one nucleotide for another (Figure 1-6, *A*). Accordingly, such mutations also are referred to as *single-nucleotide polymorphisms (SNPs)*. When a point mutation occurs in a part of the gene that codes for a protein and alters the protein by changing the codon of which it is a part, it is called a *missense mutation.* Because the genetic code is degenerate, it is possible to have a point mutation that does not change the amino acid that is encoded. This is called a *silent mutation.* Insertion or deletion of a nucleotide in the protein-coding portion of a gene is called a *frameshift mutation* because it changes the entire reading frame of the gene at every codon distal to the site of the mutation. *Nonsense mutations* are those point mutations that result in the formation of one of the three codons (UAA, UAG, UGA) that do not code for an amino acid and so produce truncated proteins, which usually have little or no activity. Point mutations occurring near the boundaries between introns and exons can cause improper splicing of mRNA precursors, resulting in RNA instability or the production of truncated proteins, or both.

Regulation of gene expression also can be affected by mutations occurring in control elements, such as promoters and enhancers. Although the effect of such mutations usually is the production of less protein, such as occurs in some forms of thalassemia, some mutations also result in the increased production of a gene product, as in hereditary persistence of fetal hemoglobin.

An interesting mutational mechanism involves the expansion of triplet repeat sequences, caused by an increase in the number of copies of CCG or AGC repeats in or near a gene. These disorders include myotonic dystrophy, fragile X syndrome, and Huntington disease. The repeat number tends to increase with succeeding generations, and as the repeat number increases, so does the severity of the disease, giving rise to the phenomenon of *anticipation.* First described in myotonic dystrophy, anticipation refers to an increase in disease severity within succeeding generations of an affected family.

CHROMOSOMAL MUTATIONS

Mutations involving large alterations in chromosome structure are visible microscopically by karyotypic analysis (see Figure 1-6, *B*). These include deletions, duplications, inversions, and translocations from one chromosome to another. Because chromosomal aberrations usually result in the disruption of multiple genes, they often have profound clinical consequences. *Terminal chromosomal deletions* result from a single chromosomal break with subsequent loss of the piece of chromosome without a centromere. *Duplications* occur when a segment of a chromosome is repeated, either from inappropriate recombination or as a result of meiosis involving chromosomes with inversions or translocations. Most other chromosomal rearrangements, such as interstitial deletions, require multiple break-and-reunion events, so they usually are less common. Some common genetic diseases, however, such as Duchenne muscular dystrophy, result primarily from small interstitial deletions—demonstrating that chromosomal regions vary greatly in their propensity to undergo different types of mutational events.

Inversions result from two chromosomal breaks followed by reversal of the broken piece of chromosome and subsequent rejoining to form an intact but rearranged chromosome. An inversion by itself should not have any clinical consequences unless one of the breakpoints affects gene expression. In that circumstance, significant effects will then be seen in subsequent generations. When chromosomes with inversions (either

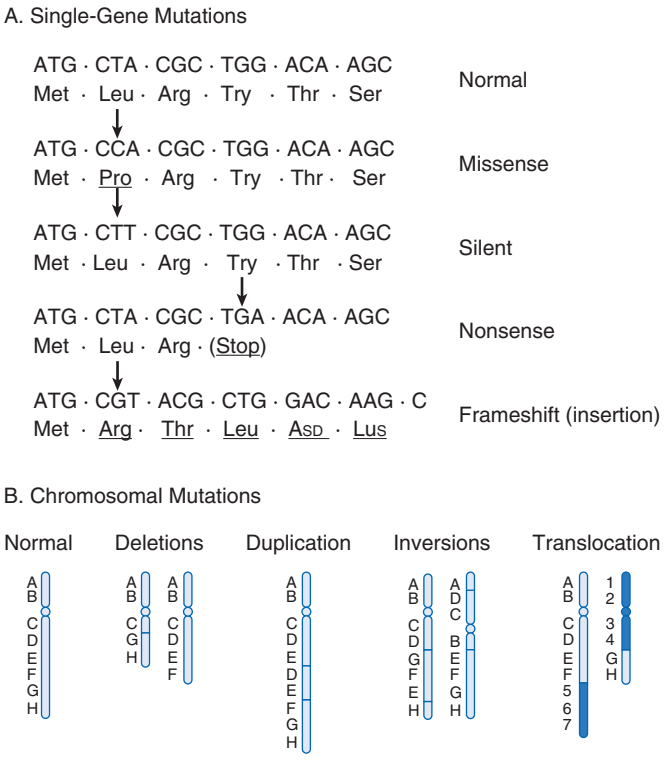

Figure 1-6 Mutation. A, Single-gene mutations. A prototypical normal gene sequence is shown on the first line, with the corresponding amino acid sequence. Examples of four types of common mutations also are shown. The substituted or inserted nucleotides are indicated by *arrows,* and the affected amino acids are *underlined.* **B,** Chromosomal mutations. A prototype normal chromosome is shown, with genes A through H. Examples of gross chromosomal mutations are shown to the *right,* and their effects on gene content and arrangement are indicated. In the translocation example, the two chromosomes are not members of a homologous pair.

pericentric, in which the inverted region includes the centromere, or *paracentric,* in which the centromere is not involved) go through meiosis and recombination with normal homologues, gametes may be formed that contain duplications and deletions of parts of the involved chromosomes. *Translocations* result from the exchange of genetic material between two nonhomologous chromosomes. Similar to inversions, they do not cause any clinical disease unless the breakpoints occur in a gene. Persons in whom a translocation is present but who have a normal amount of genetic material are called *balanced translocation carriers.* Like inversions, however, translocations can have severe effects in offspring, resulting from the consequent duplication or deficiency syndromes (or both).

GENETIC DISORDERS

Broadly, genetic disorders can be classified into three large categories: those caused by changes in a single gene, those involving a large genetic region or an entire chromosome, and those that are due to the cumulative effect of multiple genes (i.e., multifactorial). This section discusses these categories of disease, as well as factors that contribute to disease heterogeneity.

SINGLE-GENE DISORDERS

Single gene disorders are those in which the phenotype is due overwhelmingly to the effect of mutation of a single gene, with

little contribution from other genes. Environmental influences also tend to be less in these disorders than in polygenic diseases. Because they are caused by a single-gene defect, they display the simple patterns of inheritance dictated by mendelian laws. Single-gene disorders can be classified as being autosomal dominant (AD), autosomal recessive (AR), or X-linked. X-linked disorders result from mutations on the X chromosome. In contrast, AD and AR disorders are the result of mutations on the autosomes. It has been estimated that approximately 1% of people in the general population have a monogenic disorder.

AUTOSOMAL DOMINANT DISORDERS

AD disorders are those in which a patient manifests clinical symptoms when only a single copy of the mutant gene is present (i.e., the patient is heterozygous for the mutation). Inheritance of AD disorders follows several general principles (Figure 1-7, *A*):

- Each affected person has an affected parent.
- Affected persons, on average, have equal numbers of affected and unaffected children.
- Normal children of affected parents have only unaffected children.
- Males and females are affected in equal proportions.
- Each sex is equally likely to transmit the disorder to male and female children.
- Vertical transmission of the disorder occurs through successive generations.

These general rules of AD inheritance are based on the assumption, not always valid, that no new mutations occur. In fact, in some disorders the incidence of new mutations is quite high. For example, up to 50% of the cases of neurofibromatosis result from new mutations.

Dominant mutations occur in two settings: (1) a 50% reduction in the level of functional protein leads to a clinical phenotype—a phenomenon known as *haploinsufficiency,* or (2) a mutation leads to a gain of function that causes disease. Three classes of proteins are frequently involved: (1) proteins that regulate complex metabolic pathways, such as membrane receptors and rate-limiting enzymes in pathways under feedback control; (2) structural proteins; and (3) proteins with alterations that cause a dominant negative function—that is, in which the mutant protein interferes with the function of the protein expressed from the normal allele. Examples of AD disorders are familial hypercholesterolemia, which is caused by mutations in the low-density lipoprotein receptor; osteogenesis imperfecta, caused by mutations in some members of the collagen gene family; and Huntington disease, caused by a triplet repeat expansion in the Huntington gene.

A characteristic of many AD disorders is *incomplete penetrance,* whereby not all persons carrying the relevant gene(s) exhibit a specific trait. A particular gene defect can therefore manifest with widely variable severity. For example, tuberous sclerosis, one of the neurocutaneous disorders, can be clinically silent. Some persons are diagnosed with this disorder only when they have multiple affected children. At that point, careful examination may reveal subtle evidence of tuberous sclerosis, such as a minor abnormality on a computed tomography scan of the head. Similar observations have been made for many different dominant diseases. Incomplete penetrance is a manifestation of the interaction of other gene products with the product of the disease gene. Increasingly, this phenomenon is being recognized as a step in the continuum between simple completely penetrant monogenic disorders and so-called *complex disorders* in which no single-gene mutation is sufficient to cause disease.

The phenomenon of *germline mosaicism* is a complicating factor in incomplete penetrance. Germline mosaicism occurs when a mutation is present in some of the germ cells but not in most other cells. The affected person is completely healthy but is at risk for having multiple affected children. Germline mosaicism is fairly common in Duchenne muscular dystrophy and occurs in other disorders as well.

AUTOSOMAL RECESSIVE DISORDERS

AR disorders are those that are clinically apparent only when the patient is homozygous for the disease (i.e., both copies of the gene are mutant). The following general principles of inheritance are recognized for AR disorders (see Figure 1-7, *B*):

- The parents of affected children are clinically normal (i.e., carriers).
- Assuming that the carrier frequency in the population is low, only siblings are affected, and vertical transmission does not occur; the pattern therefore tends to appear horizontal.
- Consanguinity can be a factor. This can occur in outbred populations by mating between family members, or in inbred populations (e.g., the Amish) where the entire population is descended from a small number of ancestors.
- Males and females are affected in equal proportions.
- When both parents are heterozygous carriers of the mutation, 25% of their children are affected, 50% are carriers, and 25% are normal.

Every person is a carrier of AR mutations. Fortunately, the carrier frequency for most of these mutations is so low that the likelihood that carriers will have affected children is low.

Recessive mutations frequently involve enzymes, as opposed to regulatory and structural proteins. This is because 50% of the normal level of enzyme activity usually is sufficient for normal function. Complete enzyme deficiency produces an accumulation of one or more metabolites preceding the enzymatic block, such as the buildup of phenylalanine in phenylketonuria, and a deficiency of metabolites distal to the block. Either, or both, of these abnormalities may be responsible for the disease phenotype. Although many recessive disorders involve enzymes, two of the most common disorders with AR inheritance are cystic fibrosis, resulting from a mutation in a chloride channel, and

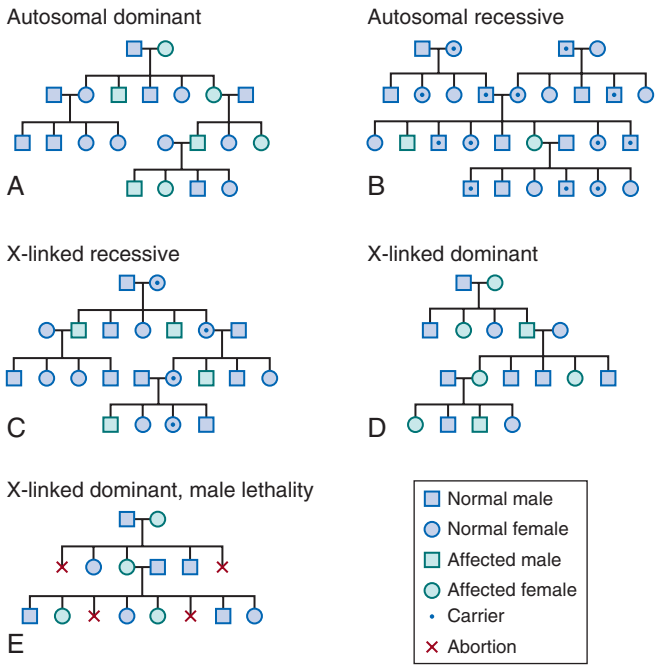

Figure 1-7 A-E, Pedigrees for disorders exhibiting the various mendelian modes of inheritance. These are idealized pedigrees, assuming full penetrance and no new mutations.

sickle cell anemia, resulting from a mutation in the β-globin gene.

It is important to be aware that the terms *dominant* and *recessive* refer to clinical phenotypes only. At the gene level, "dominance" and "recessiveness" do not exist. Persons heterozygous for a recessive disorder may be clinically normal, but the reduced level of functional or immunoreactive protein can be detected analytically and may lead to other biochemical abnormalities that have no obvious effect on the person's health. For example, short chain acyl-coenzyme A dehydrogenase deficiency, a disorder of short chain fatty acid metabolism, is detected by newborn screening but appears to have no clinical consequences. Patients homozygous for dominant mutations usually are more severely affected than are heterozygous patients. This is true in familial hypercholesterolemia. In many cases, the homozygous condition results in embryonic lethality, and so it is never seen clinically. Huntington disease stands out as an exception in that homozygous patients are not clinically different from heterozygous patients, presumably because the gain of function effect of the triplet repeat mutation is not dose responsive.

X-LINKED DISORDERS

X-linked disorders are complicated by the fact that females have two copies, with one being inactivated in every cell, and males have only one. Thus, for these disorders, clinical risk and disease severity will differ for males and for females.

The following rules of inheritance apply for X-linked recessive disorders (see Figure 1-7, *C*):

- In contrast with the vertical pattern of inheritance seen in pedigrees for AD disorders and the horizontal pattern for AR disorders, the inheritance pattern tends to be oblique, because patients have unaffected parents but affected cousins and uncles.
- Male-to-male transmission of the disorder does not occur, because fathers transmit X chromosomes only to daughters.
- Male children of carrier women have a 50% chance of being affected.
- All female children of affected men are heterozygous carriers but can be clinically affected to a significant degree, depending on the pattern of X inactivation.
- Unaffected men do not transmit the disease to any children.

Examples of X-linked recessive disorders are hemophilia, color blindness, and Lesch-Nyhan syndrome (hypoxanthine-guanine phosphoribosyltransferase [HPRT] deficiency).

Although X-linked recessive disorders generally are observed primarily in male patients, X-linked dominant disorders are approximately twice as frequent in females as in males and are characterized by transmission of the disorder from affected men to all daughters, but to no sons (see Figure 1-7, *D*). Relatively few X-linked dominant disorders have been described, but one example is hypophosphatemic (vitamin D resistant) rickets, in which males and females are equally affected even though females carry a normal and an abnormal gene. Several X-linked dominant disorders demonstrate embryonic lethality in hemizygous males (and presumably in homozygous females). In these disorders, affected mothers transmit the trait to one half of their daughters, but to no sons (see Figure 1-7, *E*). A high incidence of spontaneous abortions has been recognized, and the male-to-female ratio in children is significantly less than predicted. Disorders that appear to have this mode of inheritance include focal dermal hypoplasia, incontinentia pigmenti, and orofaciodigital syndrome type I.

An important feature of X-linked disorders is the wide range of clinical expression in heterozygous females. The incomplete penetrance observed with AD disorders probably results from interactions among different genes, whereas the variability in X-linked disorders is affected by the process of *X-inactivation* or *lyonization* (after its discoverer, Mary Lyon). Because females carry two copies of the X chromosome and males carry only one, a mechanism called *dosage compensation* has evolved to equalize the amount of gene product that is produced from genes on the X chromosome. This could be accomplished in several ways. For example, X-linked genes in females could be transcribed at half the rate of those genes in males, as occurs in insects. The mechanism that operates in humans, however, involves the random inactivation of one of the X chromosomes in every cell of the body. Therefore only one X chromosome is active in each cell. This observation has major implications for X-linked diseases. Because the process of inactivation is random, on average, half of the cells inactivate the X chromosome carrying the normal gene and half inactivate the abnormal X chromosome. Unfortunately, in some cases, significant deviation from an equal ratio may be seen. Female patients in whom X chromosome inactivation has occurred in a high percentage of the body's normal cells may demonstrate significant symptoms. This may be one reason for the significant percentage of female carriers of the fragile X syndrome who exhibit some degree of mental retardation.

It also is important to distinguish between actual X-linked disorders and sex-influenced disorders. The latter category consists of disorders encoded by autosomal genes that are differentially expressed in the two sexes.

CHROMOSOMAL DISORDERS

Chromosomal disorders fall into two general categories: those involving an incorrect chromosome number, called *aneuploidy,* and those that result from large chromosomal mutations, as described earlier. Aneuploidy is the result of nondisjunction during meiosis, in which both members of a homologous pair of chromosomes move to the same daughter cell. As a result of nondisjunction, the fertilized egg receives either one or three copies of the chromosome instead of the usual two. Because they involve numerous genes, with disturbance in the normal genomic balance, most disorders affecting chromosome number are embryonic lethal, particularly if the defect is loss of a chromosome. Disorders that are not lethal usually result in sterility, because they prevent meiosis from proceeding normally. The best-known and most common chromosomal disorder is Down syndrome, which generally results from trisomy of chromosome 21 but also can be due to a duplication or translocation of a specific region of chromosome 21. Trisomies of chromosome 13 or 18 also occur but are much less common in live born infants than is Down syndrome. Turner syndrome occurs in women who receive only a single X chromosome, whereas Klinefelter syndrome occurs in men who receive two X chromosomes in addition to the Y chromosome.

Deletions that are too small to be visible using the cytogenetic techniques that were standard before the advent of molecular diagnostics are called *microdeletions,* and the resulting disorder is termed a *microdeletion syndrome* or *contiguous gene syndrome.* Microdeletions can be detected using large arrays of cloned genetic markers covering the entire genome. For some applications, the technique of *fluorescence in situ hybridization (FISH)* is still used. In the FISH technique, a cloned DNA probe is labeled with a fluorescent molecule and is then hybridized to a standard chromosome preparation on a microscope slide. The presence of two normal chromosomes can be visualized by the appearance of two fluorescent dots, whereas a heterozygous microdeletion appears as a single dot. It is likely that advanced DNA sequencing technologies will supplant all previously used techniques for diagnosing genetic diseases, including microdeletions.

Examples of microdeletion syndromes are DiGeorge syndrome, characterized by T cell immunodeficiency and cardiac

anomalies and due to a microdeletion of chromosome 22, and Prader-Willi syndrome, characterized by mental retardation, infantile hypotonia, and a compulsive eating disorder, and frequently due to a microdeletion of chromosome 15. A clinically unrelated disorder, Angelman syndrome, characterized by severe mental retardation, seizures, and a movement disorder, can also be due to a microdeletion in the same region of chromosome 15 as that affected in Prader-Willi syndrome. However, in Prader-Willi syndrome, the deletion is always on the chromosome inherited from the father, whereas in Angelman syndrome, the deletion is always on the maternally inherited chromosome. Both Prader-Willi and Angelman syndromes can arise from *uniparental disomy,* which means that both chromosomal homologues are derived from one parent, with no contribution from the other. For example, in approximately 15% of patients with Prader-Willi syndrome, both copies of chromosome 15 are maternally derived, whereas in Angelman syndrome, both copies can be inherited from the father.

Parent-of-origin effects on the occurrence of a genetic disease are a reflection of the phenomenon of *imprinting.* Imprinting refers to a process of transcriptional inactivation of a region of a chromosome derived from only one parent. The mechanism of this transcriptional inactivation involves methylation of cytosine residues during development. The reason for the existence of imprinting is not known, but it is clear that proper imprinting is necessary for normal development.

MITOCHONDRIAL DISORDERS

Mitochondria, cytoplasmic organelles whose major function is to serve as the sites of oxidative phosphorylation and energy production for the cell, also contain their own genetic material in the form of a small circular piece of DNA. The structure of the mitochondrial genome is similar in this way to that of bacterial genomes—a finding contributing to the theory that mitochondria originally developed from bacteria that established a symbiotic relationship within eukaryotic cells.

Many different clinical entities are due to mutations in genes that affect mitochondrial function. The great majority of those are due to mutations in nuclear genes that encode proteins important for mitochondrial function. However, mitochondria contain their own DNA, with 37 genes, of which 13 encode proteins important for oxidative metabolism and the rest encode transfer RNAs or ribosomal RNAs. Mitochondrial diseases frequently affect organs that are highly dependent on energy production and use, such as the central nervous system, muscle, and pancreatic beta cells. Examples of mitochondrial diseases are myoclonic epilepsy with ragged red fibers (MERRF) syndrome; mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome; and Kearns-Sayre syndrome, which has heart block and retinal disease as prominent components.

Many mutations in the mitochondrial DNA have been described, comprising both point mutations and deletions. The inheritance of mitochondrial DNA is unique because only maternal mitochondria are transmitted to the zygote. Therefore males and females can be equally affected by disorders due to defects in mitochondrial DNA; however, the mutation can be passed only through the maternal lineage. This is called *maternal* or *cytoplasmic inheritance* because the mitochondria are located in the cytoplasm. The biology of mitochondrial disease is also complicated by the phenomenon of heteroplasmy, in which more than one genotype exists within a single individual. This occurs with mitochondrial DNA to a much greater extent than with nuclear DNA because each mitochondrion contains multiple copies of mitochondrial DNA and each cell contains multiple mitochondria. Heteroplasmy greatly complicates efforts to diagnose and make prognostic judgments about mitochondrial disease resulting from mutations in mitochondrial DNA because each patient is literally unique in the frequency of mutated mitochondrial DNA in different organs.

MULTIFACTORIAL DISORDERS

Multifactorial disorders, which are by far the most common form of genetic disease, do not show clear-cut mendelian patterns of inheritance but tend to run in families. These disorders include common chronic diseases of adults, such as atherosclerosis, hypertension, diabetes, peptic ulcers, and schizophrenia, as well as birth defects, including cleft lip and palate, spina bifida, and congenital heart disease. Multifactorial disorders are thought to result from the interaction of multiple genes with environmental factors, leading to the observed familial clustering. The polygenic component of these disorders consists of a series of genes interacting in a cumulative manner. A particular combination of genetic and environmental factors pushes vulnerable persons past a threshold at which they are at risk for the disease. There has been a tremendous effort to define the genes that contribute to particular polygenic traits, with the hope that those genes would be good therapeutic targets. Those efforts have led to the identification of many genes, but in no case has any single gene contributed a large fraction of the genetic contribution to the propensity to develop disease. Efforts are now focused on large scale DNA sequencing.

HETEROGENEITY IN GENETIC DISORDERS

As discussed earlier, genetic disorders, particularly those that are dominant or polygenic, are quite heterogeneous as a result of the complex interactions between multiple genetic loci and environmental factors. For example, persons who are carriers for α_1-antitrypsin deficiency have a predisposition to develop emphysema, particularly if they are smokers, while nonsmoking carriers may never manifest any evidence of disease due to their carrier status. With respect to polygenic disease, the epidemic of type 2 diabetes is due almost entirely to the environmental factor of high food availability in the face of a genetic makeup that was likely to have been selected for under conditions of limited food availability.

An important example of gene-environment interaction is the observation that variation in single genes may produce enormous differences in the response to drugs. These pharmacogenetic differences exhibit all three mendelian modes of inheritance. The most common is glucose-6-phosphate dehydrogenase deficiency, inherited as an X-linked recessive disorder, which may induce hemolytic anemia in response to various drugs. Without exposure to these drugs, such patients otherwise appear normal. Genetic differences in drug metabolism are increasingly recognized as important in determining pharmacokinetics. This finding has spurred interest of pharmaceutical companies in the burgeoning field of pharmacogenomics, which is directed at understanding the genetic contribution to pharmacology and that has already become into routine clinical use in some settings.

In addition to the interactions between genetic and nongenetic components in both single-gene and multifactorial disorders, other factors serve to increase the heterogeneity of genetic disorders. As stated earlier, dominant disorders often are characterized by varying severity and incomplete penetrance. It is likely that specific disease loci interact with the genetic background of the individual patient. Some combinations of genes at other loci may minimize the pathologic consequences of the mutation, whereas other combinations may accentuate them. In addition, disease heterogeneity results from multiple mutant alleles for a single locus. For example, Duchenne muscular dystrophy is caused by mutations in the dystrophin gene that usually lead to complete absence of the protein. The less severe Becker muscular dystrophy results from mutations at the same locus that lead to shortened dystrophin molecules. In addition, mutations that

lead to partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity cause gout, whereas mutations that abolish HPRT activity lead to the severe neurologic manifestations of Lesch-Nyhan syndrome. These examples of allelic variation and disease are akin to the normal allelic variation that gives rise to the rich diversity of life.

An additional reason for heterogeneity in genetic disease is that mutations in different genes can sometimes have quite similar clinical manifestations. Certain forms of hemophilia, for example, are caused by mutations in either the gene for factor VIII, responsible for classic hemophilia, or the gene for factor IX, the cause of Christmas disease. Both of these genes are on the X chromosome, and both conditions are inherited as X-linked recessive disorders. Additional bleeding disorders result from mutations in other genes. Other diseases caused by mutations in multiple gene loci have different modes of inheritance in different families. For example, spastic paraplegia, Charcot-Marie-Tooth disease, and retinitis pigmentosa all have AD, AR, and X-linked recessive inheritance forms. The precise molecular diagnosis of such disorders is being revolutionized by the incorporation of next generation DNA sequencing into routine clinical testing.

GENETIC DIAGNOSIS

Perhaps one of the most significant scientific achievements in human history is the complete sequencing of the human genome. Because the haploid human genome constitutes about 3 billion base pairs, this was truly an enormous effort. The initial sequence, reported in 2003, cost a few billion dollars, but in the years since, the cost has dropped to the low thousands. Exome sequencing, which examines only the regions of the genome that encode proteins—about 1% of the total genome, or about 30 million base pairs—is now available in CLIA-certified laboratories as a clinical test. Widespread applicability of exome sequencing to the diagnosis of genetic diseases in children is limited primarily by the difficulty of interpreting the results of the test. Determining which sequence variants are disease-causing as opposed to being normal variants or even false positives due to sequencing errors is a difficult problem, but one that will eventually be solved. Having data from the parents and siblings can be invaluable in interpreting genome sequence data, but as the number of genome sequences from the general population increases, this will become less necessary, as distinguishing benign from disease-causing mutations will be less problematic. Clearly, it is inevitable that DNA sequencing, whether of the exome or even of the whole genome, will become the standard of care in the diagnosis of genetic disease.

GENE AND CELL THERAPY

Genome sequencing has revolutionized the diagnosis of genetic disease. However, the available therapies for most genetic diseases are inadequate. Treatments such as enzyme replacement for storage diseases, (e.g., Gaucher disease) or dietary restriction for disorders of amino acid metabolism have serious limitations, such as high cost and low efficacy. Additionally, protein replacement is applicable to only a few diseases in which delivery into the circulation is efficacious; this approach is problematic when it is necessary to deliver a missing component directly to cells, particularly when tissue specificity is necessary. The ultimate treatment for many inherited disorders is to use DNA itself as a pharmacological agent. The promise of gene therapy led to great hope and hype in the early 1990s, but that promise went unfulfilled, primarily due to limitations in the efficiency with which genes could be transferred into cells and tissues. However, there has been steady progress in the development of sophisticated gene transfer methods, and gene therapy is now a reality.

It is important to distinguish between gene therapy affecting the germline and therapy affecting only somatic tissues. Germline gene therapy would allow transmission of genetic modifications to future generations. Even if such modifications were limited to treating specific diseases, serious moral and ethical questions would arise. This situation is further complicated because it would theoretically be possible to alter traits to produce more "desirable" children. Because somatic cell therapy affects only the person treated, the ethical considerations are greatly simplified. All current gene therapy protocols in humans are limited to somatic cell experiments.

Although inherited mutations exist in every cell in the body, most genes are expressed in specific tissues, and, in most cases, gene therapy needs to be directed only toward the proper tissue. Even when a gene is expressed in all tissues, only one tissue may need treatment to ameliorate the disease phenotype. The effectiveness of this approach has already been proven for a number of inherited metabolic diseases, such as maple syrup urine disease, in which liver transplantation is effective despite the fact that the enzyme is expressed in other tissues as well. For some diseases, such as hemophilia, any cell that can secrete a protein into the circulation is a potential target for gene therapy, even if it is not the normal site of protein production.

The most desirable methods of gene therapy allow for gene transfer directly into the patient (i.e., in vivo gene therapy) (Figure 1-8). For some tissues, such as the central nervous system, this is likely to be the major mode of gene transfer. For organs, such as the bone marrow, cells can be removed easily, manipulated in vitro, and reintroduced into the patient (i.e., ex vivo gene therapy) (see Figure 1-8).

In the ideal gene therapy system, the normal gene would be introduced into cells in vivo and replace the defective gene through site-specific recombination. This would result in proper positioning of the gene relative to the various transcriptional regulatory elements. Unfortunately, efficient techniques for site-specific integration are not yet available, and all current methods of gene transfer result in predominantly random integration. Because of this limitation, gene *replacement* therapy is not yet possible. However, developments with gene targeting using genetic systems adapted from lower organisms raise the prospect that true gene correction may be possible. The most recent of those systems is based on the Cas9 RNA-guided DNA nuclease from *Streptococcus pyogenes*. Transfer into a mammalian cell of a gene encoding Cas9, along with an RNA oligonucleotide homologous to any genomic sequence called an sgRNA, leads to cleavage of the targeted genomic sequence, which then can become a substrate for homology direct repair, resulting in correction of the genetic defect.

Although gene correction is a long-term goal, gene *augmentation* therapy, in which the normal gene supplements the defective gene from a distal site, is a reality, with substantial success in clinical trials for a number of monogenic diseases. Although simpler, this method suffers from the risk of insertional mutagenesis, in which the transducing vector affects the expression of genes in the region in which it integrates. This phenomenon led to the development of leukemia in a number of patients enrolled in a gene therapy trial for severe combined immunodeficiency in which bone marrow was removed and transduced with a retroviral vector expressing the adenosine deaminase gene. Advances in vector design are reducing those risks.

To increase the efficiency of gene transfer, most gene therapy studies are using replication-defective genetic transducing vectors derived from viruses. Numerous viruses have been used, including adenovirus, adeno-associated virus, murine retroviruses, and even lentiviruses, such as HIV. Viral vectors can infect various cell types from several species at efficiencies often approaching 100%.

Although the initial thrust of research in gene therapy was to correct single-gene defects, work in the field has shifted toward

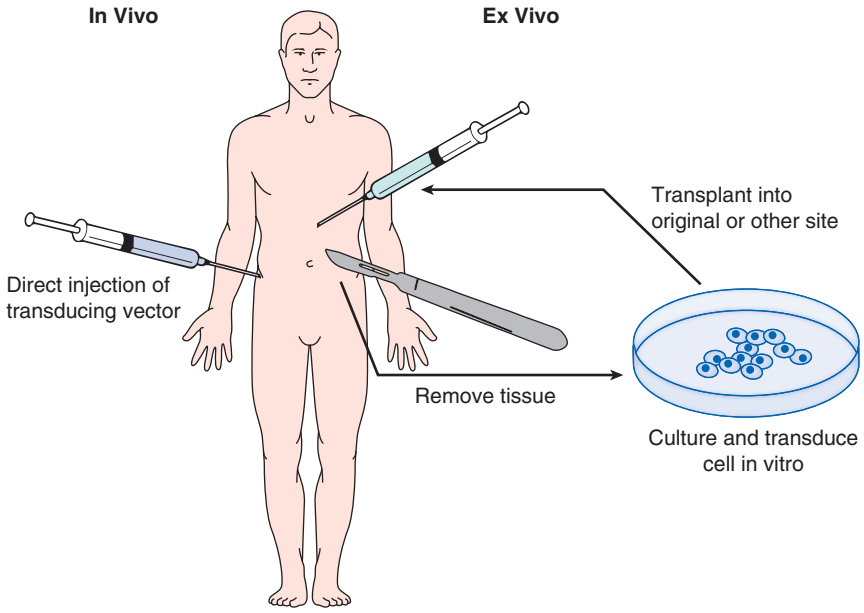

Figure 1-8 Models for human gene therapy. In vivo gene therapy involves direct introduction of a transducing vector into the patient. Ex vivo gene therapy involves removal of tissue and transduction in vitro.

applying gene transfer technology to more common polygenic diseases, such as cancer and coronary artery disease. Viral vectors are being used to deliver cytotoxic genes to cancer cells. In some cases, the vectors are designed specifically to target the cancer cells—a so-called magic bullet. Other experiments are using gene transfer techniques to enhance angiogenesis in ischemic cardiac tissue. The appeal of using gene therapy for common polygenic diseases is that these techniques can be applied to many more patients than would be the case if gene therapy were limited to the much rarer monogenic disorders.

STEM CELLS

The term *stem cells* is commonly used to refer to embryonic stem cells, derived from the inner cell mass of the blastocyst. These cells are totipotent, having the ability to differentiate into every cell of the body, leading to the formation of a whole organism. However, there are other types of stem cells. Adult stem cells reside within specific tissue compartments and have a restricted ability to differentiate—usually into cells of that tissue type. The prototypical example is the hematopoietic stem cell, residing within the bone marrow. Neural stem cells within specific brain regions are also well described, as are intestinal and skin stem cells.

An amazing breakthrough in science came with the demonstration that essentially any adult cell type can be converted into a pluripotent stem cell, with the ability to differentiate into essentially all cell types of the body under the influence of specific combinations of transcription factors. Such cells, termed *induced pluripotent stem cells (iPSCs),* have the potential for the personalized production of any cell type, eliminating the problem of organ rejection.

Two basic strategies are being pursued with regard to the therapeutic use of stem cells. In one, stem cells are transplanted into a site of damage to facilitate repair. In that application, the stem cells themselves do not replace the function of the damaged tissue, but rather produce factors that promote repair of endogenous tissue. The second, more ambitious, approach requires that the stem cells replace malfunctioning cells or cells that have been destroyed. An example of the former strategy is injection of stem cells into damaged myocardium. There is some evidence that improvements in cardiac function occur in patients, even though the injected stem cells do not persist in large numbers. An example of the second strategy is replacement of pancreatic beta cells in patients with type 1 diabetes, in whom the pancreatic beta cells are destroyed. A requirement for cell replacement applications of stem cells is that the stem cells be induced to differentiate into the target cell type. This is a challenging goal and must be based on a detailed understanding of the fundamental developmental mechanisms that lead to the formation of that cell type in the fetus.

Although stem cells have the potential to differentiate into any cell type in the body, major problems must be overcome. One problem is achieving the ability to direct stem cells to differentiate into all of the desired target cell types. Another is to find ways to stimulate the cells that are formed to become functionally incorporated into the target organ. For instance, dopaminergic neurons derived from stem cells must integrate properly into the substantia nigra so that dopamine release is appropriately regulated. Finally, transplanted cells will be rejected by host immune responses unless they are genetically identical to the host's. The use of iPSCs may eliminate that problem, as they would be derived directly from the patient's own cells.

ONLINE GENETICS RESOURCES

Genetics Home Reference. A clearly and simply written guide to genetics, put together by the National Library of Medicine, with links to many other resources: http://ghr.nlm.nih.gov/ (Accessed April 2015)

Online Genetics Education Resources. A compendium of sites put together by the National Human Genome Research Institute of the NIH that offers information ranging from that directed at lay audiences through professional geneticists: http://www.genome.gov/10000464 (Accessed April 2015)

Gene Tests. A comprehensive resource for available genetic tests: http://www.genetests.org/ (Accessed April 2015)

Online Mendelian Inheritance in Man. A comprehensive catalog of clinically relevant human genes and genetic diseases: http://omim.org/ (Accessed April 2015)

2 Prenatal Diagnosis

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Currently, the prenatal diagnosis of a fetal genetic disorder or a chromosome abnormality requires invasive testing; all of the tests in use carry small but recognized risks of miscarriage (0.5% to 1%). Accordingly, an important aspect of prenatal care is screening to identify those women who face an increased risk of a pregnancy complicated by aneuploidy, genetic syndrome, or congenital malformation. Screening modalities include review of the clinical history for both the patient and her partner, evaluation of maternal serum markers or noninvasive prenatal screening results, and ultrasound examination in both the first and second trimesters. Ultimately, however, the definitive diagnosis of a genetic condition or chromosome abnormality in the fetus requires fetal nucleic acids obtained by chorionic villus sampling (CVS), amniocentesis, or percutaneous umbilical blood sampling (PUBS). Recently, noninvasive prenatal screening using cell-free fetal (cff) DNA in maternal plasma has shown high sensitivity and specificity for common aneuploidies (trisomies 21, 18, 13) in high-risk women.[1,2] Screening for sex chromosome abnormalities and rare microdeletion syndromes using cff DNA is also becoming clinically available, and data validating these uses are being published currently.

SCREENING

Because women with "positive screens" (risk greater than a predetermined cutoff), which indicates increased risk, often proceed to an invasive prenatal diagnostic test with an inherent risk of miscarriage, screening methods should strive for a high level of detection with the lowest screen-positive rate. Concepts such as *screen-positive rate* (the number of women with an increased risk among those undergoing testing identified on the screening test), *positive predictive value* (the chances of an abnormal result among the screen-positive group), and *detection rate* (number of abnormal fetuses identified from within the screened population) provide useful parameters to compare screening approaches. Additionally, knowledge of the gestational age at which screening can be performed is important and may influence pregnancy options.

PARENTAL CLINICAL HISTORY

PARENTAL AGE

A long-recognized increase in aneuploidy as women become older is a cornerstone of prenatal diagnosis. For women who are 35 years of age at delivery, the chance of having a newborn with Down syndrome (trisomy 21) is approximately 1 in 308 pregnancies. Because trisomy 21 is associated with increased risk of miscarriage and stillbirth, for a 35-year-old woman the chance that Down syndrome will be diagnosed is actually higher at amniocentesis (1 in 258) or CVS (1 in 175). Although maternal age was the first screening criterion for Down syndrome, it performs poorly when assessed at a population level. Approximately 15% of women have children at age 35 years or older (screen-positive rate), and the likelihood in this subgroup of women that a pregnancy will be complicated by Down syndrome (positive predictive value) is only 1% to 3%. Furthermore, the detection rate is only approximately 20%; less than one fourth of Down syndrome infants are born to women in this older maternal age subcategory. When evaluated by these screening parameters, the utility of maternal age over 35 years alone as an indication for an invasive prenatal diagnostic test has been challenged.[3] Genetic conditions associated with the father's age are more difficult to delineate but include an increased risk of dominant mutations as exemplified by achondroplasia.[4]

REPRODUCTIVE HISTORY

Assessment of the couple's reproductive history may also signal an increased genetic risk for the pregnancy. A history of repeat miscarriages (2 or more) is associated with an increased risk of parental balanced translocation (6.8%). Other reproductive outcomes, such as a previous malformed stillbirth along with a single miscarriage, are also associated with an increased risk of a parental balanced translocation (5.4%).[5] A history of three or more consecutive first-trimester abortions carries a 9.6% risk of a parental balanced translocation.[4] Similarly, repeated failure of in vitro fertilization cycles (for more than 10 cycles) attributable to poor implantation is associated with an increased risk of a parental balanced translocation of 2.5%.[6] By comparison, the overall rate of balanced translocations in newborns is 0.2%.[7] A balanced translocation increases the person's risk that offspring may inherit an unbalanced complement of chromosomes, with associated implications for mental and physical delays.

In addition to previous pregnancies, a diagnosis of infertility warrants closer examination of the identified etiologic disorder and the possible recommendation for prenatal diagnostic testing. Balanced translocations and sex chromosome aneuploidy occur in 14.3% and 6.5% of men with absent and low sperm counts, respectively.[8] In addition, with male factor infertility related to obstructive azoospermia, congenital bilateral absence of the vas deferens (CBAVD) is a common diagnosis. Of men with CBAVD, almost two thirds carry at least one mutation in the gene responsible for classic cystic fibrosis (CF) (i.e., the CF transmembrane receptor gene [*CFTR*]). Almost half (54.5%) of the men are double heterozygotes, possessing two mutations for classic CF, although most often the second mutation is a variant specifically associated with infertility and not classic CF.[9] Because men with CBAVD can father children through assisted reproduction using intracytoplasmic sperm injection, carrier screening of the female partner is critical in view of the relatively high carrier frequency—1 in 25 in the white population. Couples in which both members carry a *CFTR* mutation face a 25% risk of having a child with CF; this finding emphasizes the importance of delineating the specifics of male factor infertility.

Female factor infertility also may have an underlying genetic etiology with subsequent risk to the offspring. In particular, poor ovarian reserve and oligomenorrhea or amenorrhea may reflect a premutation of fragile X. Classically, 3% of cases of sporadic premature ovarian failure and 13% of cases of familial premature ovarian failure are associated with a premutation of fragile X.[10] Of significance for female factor infertility, earlier menopause in women with a premutation of fragile X heightens the possibility that these women will seek infertility evaluation and treatment with a diagnosis of poor ovarian reserve.[11] In view of an overall frequency of fragile X premutations in the general population of approximately 1 in 200 women, infertility centers offer screening for fragile X to women. For fragile X premutation carriers,

Table 2-1 Cystic Fibrosis Detection and Carrier Rates Before and After Testing

Racial or Ethnic Group	Detection Rate* (%)	Carrier Rate Before Testing	Approximate Carrier Risk After Negative Test Result†
Ashkenazi Jewish	94	1/24	1/380
Non-Hispanic white	88	1/25	1/200
Hispanic white	72	1/58	1/200
African American	64	1/61	1/170
Asian American	49	1/94	1/180

Modified from the American College of Medical Genetics. *Technical Standards and Guidelines for CFTR Mutation Testing,* 2006 edition. Available at: http://www.acmg.net/Pages/ACMG_Activities/stds-2002/cf.htm. Retrieved December 16, 2010.
*Detection rate data based on use of a 23-mutation panel.
†Bayesian statistics used to calculate approximate carrier risk after a negative test result.

the implications for the offspring reflect the degree of expansion of the fragile X site (as discussed next under family history screening).

FAMILY HISTORY

Finally, review of the clinical history for both parents includes an assessment of family history. Ethnicity and country of origin are now routinely ascertained at preconception and prenatal visits. Various diseases of an autosomal recessive nature occur with increased frequency among specific populations reflecting historical physical or cultural constraints to gene migration. For some disorders, disease distribution is widespread and warrants screening in essentially all individuals. CF is an example of such an autosomal disease. Current recommendations are to offer *CFTR* carrier screening to all women, ideally in the preconception period, with education regarding disease frequency and testing sensitivity within the patient's specific ethnicity (Table 2-1). In persons of Northern European heritage, the carrier frequency is 1 in 25, with screening detecting 88% of carriers. However, in populations in which CF is less common, such as Asians (carrier frequency of 1 in 94), screening detects only 49% of carriers. In any population, screening can reduce but not totally negate the presence of a *CFTR* carrier.

Several autosomal recessive disorders occur more frequently within specific populations, and screening is then specifically directed by the individual patient's race or ethnicity. For example, hemoglobinopathies are more common in people of African, Mediterranean, or Asian origin. The carrier state for sickle cell occurs in approximately 1 in 12 persons of African American ancestry, and hemoglobin electrophoresis is the preferred method of screening. In some populations, further assessment for a hemoglobinopathy is warranted in the presence of a low mean corpuscular volume. β-Thalassemia will be detected by hemoglobin electrophoresis, whereas a low mean corpuscular volume without iron deficiency and with a normal hemoglobin electrophoresis is suggestive of α-thalassemia. Further diagnosis of this carrier state would require molecular diagnostic testing based on the individual patient's country of origin. Among persons of Ashkenazi Jewish heritage, the American College of Obstetrics and Gynecology (ACOG) recommends carrier screening for Tay-Sachs disease, CF, Canavan disease, and familial dysautonomia.[12] For each of these disorders, the carrier frequency is sufficiently increased and the molecular diagnostic tests are sufficiently sensitive to meet the criteria for a prenatal screening test. In addition to these four ACOG-recommended screenings, there are expanded carrier screenings that can be considered in patients of Jewish ancestry.[13] As with all autosomal recessive disorders, any offspring would have a 25% chance of inheriting the disease in question if both parents are carriers.

For couples in which only one person is of Eastern European Ashkenazi Jewish heritage, the recommendation remains to offer screening but with the knowledge that in non–Ashkenazi Jewish populations the carrier frequency is lower and is typically not established. Furthermore, among non–Ashkenazi Jewish persons, the sensitivity of the molecular diagnostic tests for specific disease mutations is substantially less. For example, in screening for Tay-Sachs disease carriers in a non–Ashkenazi Jewish population, the recommendation is to use a functional assay with 98% detection, compared with molecular diagnostic tests, which detect only 50% of carriers. Persons of Ashkenazi Jewish heritage also may avail themselves of information regarding additional autosomal recessive diseases (Table 2-2). However, for couples in which only one member is of Ashkenazi Jewish heritage, the constraints of accurate screening in the non–Ashkenazi Jewish person remain; functional assays are available only for Tay-Sachs disease. For couples in which both members are carriers for these autosomal recessive disorders, prenatal diagnosis is possible with use of the same molecular diagnostic tests used for fetal cells obtained by either amniocentesis or CVS.[14]

Of relevance to prenatal diagnosis, a family history of intellectual disability of unknown etiology or significant developmental delay or autism represents a positive screen for fragile X syndrome. Fragile X syndrome is the most common inherited cause of intellectual disability. The specific characteristics result from expansion of the fragile X mental retardation (FMR-1) region on the X chromosome. In most cases, 40 or fewer CGG repeats are present within FMR-1, and the region remains stable when passed from either parent to their offspring. Of note, however, some persons have inherited expansions of this repeat region, either slight (41 to 60—intermediate range) or larger (61 to 200—premutation range).[15] Approximately 1 in 200 women (1 in 113 to 1 in 350) carry a premutation for fragile X syndrome. When this unstable CGG repeat region expands to greater than 200 repeats (full mutation), increased methylation impairs translation, resulting in lack of production of the fragile X mental retardation protein. The size of the maternal premutation allele directly influences whether further expansion occurs during meiosis (Table 2-3).[16] Sons who inherit a full mutation have characteristics of typical fragile X syndrome. In daughters who inherit the full mutation, features of the syndrome are unpredictable because of the normal random silencing of one X chromosome (Lyon hypothesis). As many as two thirds of daughters with a full fragile X mutation may have mild to moderate retardation. Although general population screening for the premutation carrier state in women is not currently advocated, given the relatively high frequency of premutation carriers (1 in 200) and implications for disability, judicious review of the family history for characteristics of fragile X syndrome is encouraged.[17]

The family history of both partners also can yield important information regarding adult-onset dominant disorders such as Marfan syndrome, polycystic kidney disease, myotonic dystrophy, and Huntington disease. Such dominant adult-onset disorders may be noted in one or more seemingly remote family members, with no perception of the significance for the current pregnancy. Non–disease-related death of affected persons before disease manifestation and later age at onset of symptoms can cloud the inheritance pattern in a family. Additionally, especially in women with myotonic dystrophy, the most common adult-onset muscular dystrophy, the occurrence of congenital myotonic dystrophy with symptoms more severe than those typical of the adult-onset disease should be addressed. Disorders of recessive inheritance require attention with testing of the individual at risk of the specific DNA mutation known to be segregating within the family. Lack of knowledge of the specific mutation

Table 2-2 Autosomal Diseases With Increased Frequency Among Persons of Ashkenazi Jewish Heritage

		Ashkenazi Jewish		Non–Ashkenazi Jewish	
Disease	**Description**	**Carrier Rate**	**Carrier Detection**	**Carrier Rate**	**Carrier Detection**
Tay-Sachs	Neurologic deterioration, death in early childhood; juvenile- and late-onset forms	1/30	98% by Hex A testing 94% by DNA	1/300	98% by Hex A testing 50% by DNA
Canavan	Neurologic deterioration; death during early childhood, with some survivors into teens	1/40	98% by DNA	Undetermined	60% by DNA
Cystic fibrosis	Chronic pulmonary disease, pancreatic insufficiency, variable survivorship	1/29	97% by DNA	Varies by ethnicity	Varies by ethnicity
Familial dysautonomia	Impairment of sensory and autonomic nervous systems	1/32	99% by DNA	Unknown	Unknown
Fanconi anemia group C	Pancytopenia; developmental delay and failure to thrive	1/89	99% by DNA	Unknown	Unknown
Niemann-Pick type A	Lysosomal storage disease with degenerative course similar to that in Tay-Sachs	1/90	95% by DNA	Unknown	Unique mutations, enzymatic levels poorly discriminate normal and carrier states
Mucolipidosis IV	Neurodegenerative disorder with marked developmental and growth retardation	1/127	95% by DNA	Unknown	Unknown
Bloom	Pre- and postnatal growth restriction, susceptibility to malignancies	1/100	95% by DNA	Unknown	Unknown
Gaucher	Type 1—variable severity secondary to deposition in spleen, liver, and bones; presentation from chronic illness to asymptomatic	1/15	95% by DNA	Unknown	70% by >30 mutations

Data from ACOG Committee on Genetics: ACOG committee opinion. Number 298, August 2004. Prenatal and preconceptional carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 104:425–428, 2004; and Preconception and Prenatal Genetic Screening Pocket Facts. March of Dimes, 2001.

Table 2-3 Maternal Premutation Allele Size and Risk of Expansion to Full Mutation

	Full Mutation Risk: % (No. of Fetuses Affected)			
Maternal Repeat Size*	**Nolin, 1996**	**Pesso, 2000**	**Toledano-Alhadef, 2001**	**Nolin et al, 2003**
55-59	13 (3/22)	0 (0/11)	0 (0/22)	4 (1/27)
60-69	21 (7/34)	12(1/8)	10 (2/20)	5 (6/113)
70-79	58 (59/102)	50 (1/2)	17 (1/6)	31 (28/90)
80-89	73 (78/107)	50 (1/2)	—	58 (81/140)
90-99	94 (83/88)	100 (1/1)	—	80 (89/111)
100-200	99 (177/179)	75 (3/4)	—	98 (194/197)

Data from Nolin SL, Brown WT, Glicksman A, et al: Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet* 72:454–464, 2003.
*With less than 200 repeats.

within a family does not prevent testing the person at risk but will limit the assurance of exclusion of the carrier state. For example, in a woman with a brother who has CF arising from the most common mutation, homozygosity for deltaF508, a negative result for the most common CF mutations (including delta508) changes her risk of being a carrier from 2 in 3 (unaffected sibling of a patient with an autosomal recessive disease) to 1 in 208 (background residual risk of any Northern European individual for undetected CF carrier status). By comparison, if her brother had not been tested, perhaps because he died before molecular diagnosis and neither parent was available, then her two-thirds empiric carrier risk could be reduced to only 1 in 15 (as a result of residual undetectable CF mutations in a Northern European person).

NONINVASIVE PRENATAL SCREENING

Noninvasive prenatal testing (NIPT) for fetal chromosome abnormalities using cell-free DNA (cf DNA) in the maternal circulation became clinically available in the United States in October 2011. This testing was initially offered only for trisomy 21, with testing for trisomy 13 and 18 added shortly after launch. Because this testing was initially validated in a population at high risk of fetal aneuploidy, professional recommendations and insurance coverage have restricted it to singleton pregnancies with a high-risk indication (maternal age at least 35 at delivery, ultrasound anomalies associated with an increased risk of aneuploidy, a prior pregnancy with aneuploidy, parent is a known carrier of a translocation involving chromosome 13 or 21, or high risk of aneuploidy on serum screen). NIPT utilizes the presence of cf DNA in the maternal circulation to predict the fetal karyotype. There are three different methodologies for this analysis—massively parallel shotgun sequencing, targeted massively parallel sequencing, and single-nucleotide polymorphism (SNP)–based approaches. A recent metaanalysis concluded that these techniques have a 99.0% (95% confidence interval [CI], 98.2 to 99.6) sensitivity for Down syndrome, 96.8% (95% CI, 94.5 to 98.4)

sensitivity for trisomy 18, and a 92.1% (95% CI, 85.9 to 96.7) sensitivity for trisomy 13, with a false-positive rate of <1% for each condition.[18] The reporting criteria, redraw rate, and performance data are each reported to vary slightly among the four different companies offering this testing clinically.[1,2,19-21] These detection rates are superior to second trimester maternal quad screening using maternal serum AFP (alpha-fetoprotein), hCG (human chorionic gonadotropin), uE3, and inhibin-A (80% detection rate for Down syndrome and 60% for trisomy 18 at a 5% false-positive rate) and first-trimester combined screening using maternal serum hCG, PAPP-A (pregnancy-associated plasma protein A), and fetal nuchal translucency (85% detection rate for Down syndrome and 90% for trisomy 18 with a 5% false-positive rate) (Table 2-4).[3]

Educating patients and providers regarding the limitations of NIPT will be critically important as NIPT is offered to a wider population. Although NIPT is superior to traditional maternal serum-based screening, it remains a screening test. Any patient with an abnormal NIPT result should be offered diagnostic testing for fetal karyotype and/or chromosomal microarray analysis (CMA). Implementation of NIPT has also revealed a number of potential incidental findings including maternal metastatic disease,[22] maternal mosaicism,[23] and placental mosaicism that can confound results.[24]

USE OF CELL-FREE FETAL DNA TO SCREEN FOR Rh DISEASE

Use of cff DNA testing for fetal Rh status has become commercially available and has improved care for the isoimmunized patient. Because the mother's genotype is Rh-negative, the presence of any Rh-positive DNA in her circulation denotes a fetus with the Rh-positive gene from the father and for whom the risks of isoimmunization exist.[25] The methodology is reproducible and sensitive such that cff DNA is used in the management of Rh disease. When a woman is isoimmunized to Rh antigen and produces antibodies, the Rh status of the fetus becomes an important piece of information in the management of this disease. An Rh-negative fetus would not require the extensive surveillance and testing indicated for the Rh-positive fetus with other risk factors.[26] Before the advent of cff DNA, fetal Rh status

Table 2-4 Detection Rates for Down Syndrome With First- and Second-Trimester Screening and Noninvasive Prenatal Screening

First- and Second-Trimester Maternal Serum Screening (MSS)	First-Trimester Screen Traditional	First-Trimester Screen With Instant Risk Assessment	Second-Trimester MSS	Second-Trimester AFP Only	Noninvasive Prenatal Testing
When is the test performed?	11-14 weeks	Blood sample: 9-14 weeks Ultrasound: 11-14 weeks	15-23 weeks	15-23 weeks	After 10 weeks
What is involved?	Blood test and ultrasound on the same day	Blood test and ultrasound; blood test at least 1 week before ultrasound	Blood test	Blood test	Blood test and ultrasound
To whom is the test available?	All patients	All patients	All patients	All patients	Patients with a risk factor*
What does it screen for?	Down syndrome Trisomy 18 Trisomy 13	Down syndrome Trisomy 18 Trisomy 13	Down syndrome Trisomy 18 Open spina bifida Smith-Lemli-Opitz syndrome (SLOS)	Open spina bifida	Down syndrome Trisomy 18 Trisomy 13
What is the detection rate?	Down syndrome: 85% Trisomy 18/13: 90%	Down syndrome: 85% Trisomy 18/13: 90%	Down syndrome: 80% Trisomy 18: 60% Open spina bifida: 80% SLOS: unknown	Open spina bifida: 80%	Down syndrome: ~99% Trisomy 18: 98-99% Trisomy 13: 79-92%
What is the screen-positive rate?	5%	5%	5%	2.5%	<1%
When are results available?	3-5 days after the ultrasound and blood test	Immediately after the ultrasound	3-5 days after the blood test	3-5 days after the blood test	2 weeks after the blood test
Diagnostic options after results	Chorionic villus sampling (CVS) or amniocentesis	CVS or amniocentesis	Amniocentesis	Amniocentesis	CVS or amniocentesis
Advantage	Results available early in pregnancy Early ultrasound can identify some birth defects	Results available same day as ultrasound Early ultrasound can identify some birth defects	Less expensive Screen for all conditions in one step	Follow-up screening for those who choose first-trimester screening	High detection rate with low screen-positive rate Early ultrasound can identify some birth defects.

AFP, Alpha-fetoprotein.
*Risk factors for Down syndrome, trisomy 18, or trisomy 13 include maternal age ≥35 years at estimated due date, a positive first- or second-trimester screening test, abnormal ultrasound findings, or a prior affected pregnancy.

could be established only by determining the genotype of the fetal cells, typically obtained by amniocentesis.

Current serum screening and noninvasive prenatal screening options are summarized in Table 2-5.

PRENATAL ULTRASOUND EXAMINATION

Along with advances in biochemical screening in the 1990s, ultrasound screening for Down syndrome also gained popularity. For the more common aneuploidies, characteristic patterns of ultrasound findings emerged (Table 2-6). Even when isolated malformations exist, the risk of aneuploidy can range from a low percentage to greater than 50% (Table 2-7). Some of the increased risk associated with isolated ultrasonography-detected malformations is attributable to the possibility that further subtle dysmorphia or other minor malformations undetectable even by high-resolution ultrasound will be present in the newborn.

Ultrasound survey in the second trimester has become increasingly used for assessment of structural malformations. Nuchal fold thickness was the first marker introduced[27] and several others followed. "Soft markers" for aneuploidy are increased nuchal fold thickness, echogenic bowel, shortened femur, echogenic cardiac focus, choroid plexus cysts, and renal pelvis dilatation. These findings typically are transient and, if not associated with aneuploidy, have no functional significance in the fetus. The one exception is echogenic bowel, which may be associated with CF or fetal infection (typically cytomegalovirus infection). When ultrasound is used as a screening tool for aneuploidy, the screen-positive rate (based on the presence of any one of these subtle markers as a positive result) is relatively high (13.0%), with a detection rate for Down syndrome of approximately 50%.[28]

Different combinations of markers have been proposed under the term *genetic sonogram*. Genetic sonogram describes any mathematical formula based on second-trimester sonographic markers, which revises an a priori risk of Down syndrome.[27,29] The goals of the genetic sonogram are to reduce amniocentesis rates and to increase Down syndrome detection. The presence of a structural malformation or a soft marker increases the probability of Down syndrome. This increase can be expressed as a likelihood ratio, calculated as the ratio of the false-negative rate divided by specificity. Table 2-8 lists the published ratios for trisomy 21 using common second-trimester ultrasound markers.[29]

Studies of the genetic sonogram in the age of cf DNA are lacking. In cases where cf DNA results are positive, the genetic sonogram can be very helpful in determining the true risk. However, although cf DNA has an excellent detection rate, a diagnostic test is still recommended in cases where aneuploidy is suspected and the patient desires a definitive diagnosis.

FIRST-TRIMESTER SCREENING FOR ANEUPLOIDY

With improvements in ultrasound resolution, greater clarity of fetal structures in the first trimester emerged. Between 11 and 14 weeks of gestation, visualization of additional fluid collection at the nape of the fetal neck (nuchal translucency) is a sensitive indicator of aneuploidy in the fetus. In initial studies, 70% detection of Down syndrome was predicted, with only 5% of the population considered screen-positive (increased nuchal lucency >2 standard deviations for gestational age). The addition of maternal serum markers, primarily pregnancy-associated plasma protein-A and hCG, brought the screen-positive rate in line with 5%, with a detection rate of 80% for trisomy 21 in the fetus.[30] The benefit of screening using ultrasound and noninvasive prenatal testing is that they can be performed in the first trimester, allowing earlier diagnosis. The most recent improvements in the performance of nuchal translucency as a screening tool for trisomies have been based on improved statistical modeling of the data rather than changes in the measurement techniques.[31] Ductus venosus measurements and nasal bone assessments are two additional first-trimester ultrasound parameters that are used to decrease screen-positive rates without loss of detection.[32,33]

Although first-trimester screening with a combination of ultrasound examination and maternal serum screen yields high detection and low screen-positive rates, an alternative approach of

Table 2-5 Comparison of Prenatal Screening and Diagnostic Options

	Any Time After 10 Weeks	First Trimester			Second Trimester	
	Screening Test	Screening Test		Diagnostic Test	Screening Test	Diagnostic Test
Name	NIPT (noninvasive prenatal testing)	First-trimester screening—*traditional*	First-trimester screening with *instant risk assessment*	Chorionic villus sampling	Quad screen	Amniocentesis
Available to	Patients with a risk factor*	All patients	All patients	All patients	All patients	All patients
Timing	After 10 weeks	11 1/7 to 13 6/7 weeks	Blood: after 9 0/7 weeks Ultrasound: 11 1/7 to 13 6/7 weeks	10 to 13 6/7 weeks	15 to 22 6/7 weeks	After 15 weeks
Detection Rate	~99% for Down syndrome ~98%-99% for trisomy 18 ~79%-92% for trisomy 13	~85% for Down syndrome ~90% for trisomy 18 and trisomy 13	~85% for Down syndrome ~90% for trisomy 18 and trisomy 13	>99% accuracy (for chromosomal abnormalities)	80% for Down syndrome 60% for trisomy 18 80% for NTDs	>99% accuracy (for chromosomal abnormalities) 98%-99% for NTDs
Risk	Noninvasive	Noninvasive	Noninvasive	0.5% miscarriage risk	Noninvasive	0.2-0.3% miscarriage risk
Results	2 weeks	3-5 days	Immediate	10-14 days	1 week	10-14 days

NTD, Neural tube defects.

*Women 35 years or older, fetuses with ultrasonographic findings indicative of an increased risk of aneuploidy, women with a history of a trisomy-affected offspring, a parent carrying a balanced robertsonian translocation with an increased risk of trisomy 13 or trisomy 21, and women with a positive first-trimester or second-trimester screening test result. Source: ACOG committee opinion 640, 2015.

Table 2-6 Common Ultrasound Findings in Fetuses With Chromosome Abnormalities

Abnormality	Ultrasound Findings
Trisomy 21	Ventriculomegaly, brachycephaly
	Nuchal thickening
	Cardiac defect—AV canal
	Duodenal atresia, echogenic bowel
	Renal pyelectasis
	Shortened femur/humerus; clinodactyly involving fifth digit, sandalfoot
Trisomy 18	CNS—agenesis of corpus callosum, meningomyelocele, ventriculomegaly
	Cystic hygroma
	Cardiac anomalies
	Congenital diaphragmatic hernia
	Omphalocele
	Clenched hands with overlapping digits
	IUGR with polyhydramnios
Trisomy 13	CNS—holoprosencephaly, agenesis of corpus callosum, meningomyelocele, microcephaly
	Cleft lip/palate, midface hypoplasia, cyclopia, microophthalmia
	Nuchal thickening
	Cardiac anomalies
	Omphalocele, echogenic bowel
	Echogenic kidneys
	Radial aplasia, polydactyly
Turner	Cystic hygroma
	Cardiac defects (coarctation of the aorta)
	Horseshoe kidneys
	Hydrops
Triploidy	CNS—holoprosencephaly, agenesis of corpus callosum, meningomyelocele, Dandy-Walker malformation
	Hypertelorism, micrognathia
	Syndactyly involving third and fourth fingers
	Cardiac defects
	Omphalocele
	Early-onset IUGR affecting skeleton more than head
	Placental abnormalities—enlarged or small and calcified

Data from Benacerraf B, editor: *Ultrasound of fetal syndromes*, New York, 1998, Churchill Livingstone.
AV, Atrioventricular; *CNS,* central nervous system; *IUGR,* intrauterine growth restriction.

Table 2-7 Aneuploidy Risk Among Isolated Major Anomalies

Anomaly	Risk (%)*	Most Common
High-Risk Category		
Cystic hygroma	>50	45,X
Hydrops	>50	13, 21, 18, 45,X
Holoprosencephaly	50	13, 18, 18p–
Complete atrioventricular canal	40	21
Omphalocele	30	13, 18
Duodenal atresia	30	21
Bladder outlet obstruction	20	13, 18
Lower-Risk Category		
Hydrocephaly/ventriculomegaly	10	21, 13, 18, triploidy
Cardiac defects	10	21, 18, 13, 22–, 8, 9
Meningomyeloceles	7	18
Anencephaly	2	
Encephalocele	10	
Limb reduction	8	18
Clubfoot	6	47,XXY, 47,XXX, 18, 21
Facial clefts	1	13, 18, 22q–
Minimal-Risk Category		
Gastroschisis—must be differentiated from ruptured omphalocele		
Hydranencephaly		
Single umbilical artery		

Data from Nyberg D, Mahony B, Pretorius D, editors: *Diagnostic ultrasound of fetal anomalies: text and atlas*. St Louis, 1990, Mosby; and Sanders R, Hogge W, Spevak P, Wulfsberg E, editors: *Structural fetal abnormalities: the total picture*. St Louis, 2002, Mosby.
*Risk data are estimates, which are influenced by gestational age at detection and the resolution of ultrasound images in reported studies.

Table 2-8 Published Likelihood Ratios for Trisomy 21 Using Common Second-Trimester Ultrasound Markers

Marker	Smith-Bindman	Nuberg	Nyberg	Bromley	Agathokleous*
None	NA	0.4	0.36	0.22	0.37
Absent or hypoplastic nasal bone	NA	NA	NA	13.94	23.27
Nuchal fold	17	8.6	11	Infinite	23.27
Hyperechoic bowel	6.1	5.5	6.7	NA	11.44
Short humerus	7.5	2.5	5.1	5.8	4.81
Short femur	2.7	2.2	1.5	1.2	3.72
EIF	2.8	2	1.8	1.4	5.83
Pyelectasis	1.9	1.5	1.5	1.5	7.63

Data from Odibo AO, Ghidini A: Role of the second-trimester "genetic sonogram" for Down syndrome screen in the era of first-trimester screening and noninvasive prenatal testing. *Prenat Diagn* 34: 511–517, 2014.
EIF, Echogenic intracardiac focus; *NA,* not available.
*Pooled estimate.

so-called *integrated testing* has also been supported. Integrated testing consists of first-trimester ultrasound examination and measurement of serum markers in combination with select second-trimester serum markers. With integrated testing, the screen-positive rate is lower by a few percentage points and the detection rate is above 95%.[34] This methodology, however, delays screening results until the second trimester. A compromise between first-trimester screening alone and integrated testing is reached with *sequential screening*. Sequential approaches may be stepwise or contingent; both release high-risk screen results in the first trimester. Further screening is done in the second trimester for all (in the stepwise approach) or only a proportion of the women (in the contingent approach)[35,36] (see Table 2-5). Either sequential approach maintains a low screen-positive rate with (<5.0%) with a detection rate for Down syndrome greater than 90%. Currently, ACOG recommends offering women of all ages screening for aneuploidy in pregnancy, with initiation of screening in the first trimester optimal.[3]

The majority of fetuses with increased nuchal translucency but a normal karyotype proceed through gestation without complication. As the degree of first-trimester nuchal edema increases, however, the risk of other structural malformations detected on the second-trimester ultrasound or at birth also increases. Although a relatively low risk of 2.7% is present for fetuses with mild nuchal edema (nuchal translucency 3.0 mm), risk reaches 35.6% for those fetuses with markedly enlarged nuchal translucency (7.0 mm).[37] Although many of the identified abnormalities are cardiac malformations, other disorders such as congenital diaphragmatic hernia, skeletal dysplasias, fetal akinesis, and metabolic storage disease also are reported.[38] Persistence of markedly increased nuchal edema into the second trimester represents the greatest risk, with almost half (40.9%) of fetuses experiencing an adverse outcome such as structural malformation, hydrops, or in utero demise.[39] Conversely, for fetuses with mild increases (2.5 to 3.0 mm) in first-trimester nuchal fold, a majority continue through gestation without adverse outcome, and the risk of structural abnormalities or disability in the newborn period or early childhood is not significantly increased.[40]

A septated cystic hygroma in the first trimester is defined by extensive nuchal thickening extending along the entire length of the fetal back, where septations are clearly visible. This finding is seen in the first trimester and affects 1 in 300 pregnancies. A cystic hygroma is associated with aneuploidy 50% of the time.[41] In the 50% of cases without aneuploidy, there is a 1-in-2 risk of a major structural malformation, typically cardiac or skeletal. If complete prenatal evaluation reveals no evidence of additional abnormalities, the residual risk of an abnormal pediatric outcome ranges from 5% to 25%. Intrauterine demise occurs in 25% of fetuses with a cystic hygroma.[41]

DIAGNOSTIC TESTING

Although use of screening modalities for genetic disease and aneuploidy during pregnancy avoids the risk of miscarriage, none of the available tests provides a definitive answer. For diagnosis, fetal DNA needs to be studied; currently, appropriate samples can be obtained only through invasive testing: the placenta (CVS), amniotic fluid (amniocentesis), or fetal blood (percutaneous blood sampling [i.e., PUBS]) may be used. When patients are counseled regarding each of them, the miscarriage rate is frequently quoted, but other concerns such as procedure-related fetal morbidity and the likelihood of technical complications should also be considered.

CHORIONIC VILLUS SAMPLING

Available from 10 weeks of pregnancy onward, CVS currently affords the earliest diagnostic possibility. Trophoblast cells obtained from the placenta can be studied for specific genetic mutations, as well as chromosome analysis. CVS is performed under ultrasound guidance with the passage of a catheter into the placenta, either transcervically or through the maternal abdomen. Based on large multicenter collaborative studies using either transcervical or transabdominal CVS, a miscarriage rate ranging from 0.2% (in experienced centers) to 1.0% has been reported.[42] Concerns related to fetal morbidity include fetal infection (from maternal disease such as human immunodeficiency virus [HIV]), isoimmunization, and fetal damage. With regard to fetal injury, initial reports of higher rates of limb reduction defects among newborns after first-trimester CVS sampling have been extensively investigated. At this time, the risk of such fetal complications is considered rare: 5.2 to 5.7 per 10,000 after CVS compared with 4.8 to 5.97 per 10,000 without CVS.[43] Technical concerns also have surfaced with CVS. Sample procurement is technically different from amniocentesis, and the learning curve is longer. In addition, with multifetal pregnancies, it is possible to ensure sampling of each individual fetus, but this can be more problematic than with amniocentesis. Finally, although both CVS and amniocentesis will provide information concerning the chromosomal makeup of the fetus, CVS may also detect aneuploidy, which is confined to the placenta. Occurring in approximately 1% to 2% of CVS samples, confined placental mosaicism reflects a combination of karyotypically normal and abnormal cells. In two thirds of the cases, the abnormal cell line is confined to the placenta; thus further evaluation of the fetus by amniocentesis or PUBS is warranted. An additional outcome associated with confined placental mosaicism is fetal growth restriction, which is likely to be dependent on the specific chromosome abnormality.[44]

EARLY AMNIOCENTESIS (AT 9 TO 11 WEEKS OF GESTATION)

In order to provide diagnostic results in the first trimester, early amniocentesis has been investigated. From 10 to 18 weeks of gestation, however, the amniotic fluid volume is rapidly expanding such that the removal of 20 mL at 11 weeks represents as much as one third of the total volume. The same 20 mL removed at 18 weeks represents less than 10% of the total volume. An initial trial to assess the safety of early amniocentesis was halted owing to an increased miscarriage rate.[45] When compared with CVS, early removal of amniotic fluid carries a significantly higher relative risk of miscarriage of 1.92 (95% CI, 1.14 to 3.23).[46] Additionally, increased fetal morbidity was noted, with a higher rate of clubfoot, which was unrelated to whether or not rupture of membranes had occurred.[47] Technical concerns also arose, with increased rates of culture failure. Furthermore, efforts to decrease the amount of amniotic fluid removed were associated with an even greater chance of technical culture failure.

STANDARD AMNIOCENTESIS AT 15 WEEKS OR LATER

Removal of 15 to 20 mL of amniotic fluid in the second trimester is associated with a small risk of miscarriage. Collaborative studies in the 1990s noted a miscarriage rate of approximately 0.5% above the background rate. The definitive prospective randomized study by Tabor and colleagues in 1986 revealed a 1.0% risk of miscarriage above the background rate. This study was performed in low-risk women with both case and control groups of subjects of comparable gestational ages. In the amniocentesis and control groups, miscarriage rates between 16 and 24 weeks were 1.7% and 0.7%, respectively, with a relative risk of 1.72 (95% CI, 1.06 to 2.81).[48] More recently, analyses of data perhaps more reflective of the current practice of amniocentesis suggest that the loss rate may be less than that previously noted. Additionally, risk factors may be present that increase a particular woman's miscarriage rate after amniocentesis. When women with repeated miscarriages or bleeding in the current pregnancy are compared with women without these risk factors, increased

odds ratios of 3.03 and 2.4, respectively, have been reported.[49] If such predisposing risk factors are considered, the risk of miscarriage in the woman without significant history is only minimally and not significantly increased above the background rate.[50]

Fetal morbidity after amniocentesis also should be considered. In pregnant women who are Rh-negative, the combination of an Rh-positive fetus and an invasive diagnostic test carries a small risk of fetal-maternal hemorrhage. For this reason, Rho(D) immune globulin is given prophylactically to all Rh-negative women unless the partner is known to be Rh-negative or the woman declines it. For women with other red blood cell antigen incompatibilities with their partners, any invasive diagnostic test increases the risk of antibody formation and the effects of isoimmunization in the fetus. These risks also exist for CVS, although they are theoretically lower with amniocentesis, because amniocentesis can avoid the placenta. Similarly, infectious disease in the mother poses a theoretical risk. Transmission of hepatitis B in women who are chronic carriers does not appear to increase the risk of subsequent carriage in their infants. Likewise, although transmission of HIV through amniocentesis remains a theoretical possibility, amniocentesis in women with a low viral load is considered reasonable.

Fetal morbidity is also associated with rupture of membranes occurring after amniocentesis. In approximately 1% of procedures, the amniotic membranes do not promptly reseal, and amniotic fluid leakage occurs. In a majority of such instances (90%), the amniotic fluid will reaccumulate to normal levels, although the mean duration of the recovery period has been reported to be 3 weeks. Amniocentesis-related rupture of membranes with failure to reseal is associated with increased risk of intrauterine growth restriction and prematurity.[51] By contrast, spontaneous rupture of the membranes in the second trimester carries a poor prognosis, with only a limited chance of regaining normal fluid levels.

Technical issues arise with amniocentesis when the genetic diagnosis being pursued relies on molecular genetic studies. All amniotic fluid samples contain a small number of maternal cells, probably obtained as the needle passes through the maternal skin and uterus. When cultured amniotic fluid cells are evaluated, the chance of overgrowth by the maternal cells, with consequent assessment of the karyotype of the mother and not the fetus, is low. Nevertheless, when molecular diagnostic studies are initiated on direct, uncultured amniotic fluid, it is necessary to ensure that the fetal genome is being assessed. This can be accomplished either by (1) clearing the initial 1 or 2 mL from the needle before obtaining the amniotic fluid sample or (2) simultaneously evaluating maternal blood for additional polymorphic markers to ensure that the amniotic fluid sample represents a discretely different genome (maternal cell contamination study).

PERCUTANEOUS UMBILICAL BLOOD SAMPLING

PUBS is performed at 18 weeks or later in gestation by the removal of a blood sample from the umbilical cord under ultrasound guidance. The procedure is accompanied by an approximate 1% to 2% risk of pregnancy loss, which is higher with fetuses with other risk factors such as hydrops. Nevertheless, PUBS can provide rapid and often more extensive information than that obtained by CVS or amniocentesis. Karyotype analysis yields results within 48 hours, because the rapidly dividing peripheral blood cells do not require the extensive (often 2-week) period of culture needed for both CVS and amniocytes. Additionally, molecular genetic studies are possible on the extracted DNA, as is functional assessment of the bone marrow, immune system, and hepatic system. Fetal anemia can be diagnosed and treated by umbilical vessel transfusion in situations in which the hematologic suppression is expected to be transitory, such as parvovirus infection. Polymerase chain reaction (PCR) analysis for specific viruses such as parvovirus and cytomegalovirus can also be performed using fetal blood. Altered hepatic or bone marrow function may point to an underlying metabolic disease, guiding more specific molecular testing in the fetus.

NEW AVENUES FOR PRENATAL DIAGNOSIS

PREIMPLANTATION GENETIC DIAGNOSIS

After in vitro fertilization, directed assessment of a single cell from a blastocyst before transfer to the uterus—preimplantation genetic diagnosis (PGD)—can reveal single-gene disorders, as well as chromosome abnormalities. For persons with a balanced chromosome translocation, single blastomeres can be studied with fluorescence in situ hybridization (FISH)—a method to determine copy number for specific, predefined segments of DNA in nondividing cells. With probe combinations designed for the specific translocation carried by the individual, couples proceed through assisted reproduction and in vitro fertilization. On day 3 after fertilization, single cells are removed from each blastocyst and assessed within 12 to 24 hours with the translocation-specific FISH probes. On completion of the FISH analysis, the information obtained is used to guide selection of blastocysts to transfer to the woman. This methodology also allows determination of chromosome copy number and was initially applied as preimplantation genetic screening in women at increased risk related to older maternal age, previous aneuploidy, or repeat miscarriages. Development of the optimal panel of probes remains challenging because increases in the number of chromosomes studied are associated with higher technical error rates and the potential for eliminating from consideration for transfer a blastocyst that may indeed be chromosomally normal.[52] As currently performed, PGD is a screening method to decrease the chance that the pregnancy will be complicated by a chromosome abnormality, but it does not improve outcomes for women of advanced age or habitual miscarriage. Application of comparative genomic hybridization array technology may be helpful in this area.

Various factors can contribute to the approximate 10% error rate, including lack of possibility of retesting owing to use of a single cell, existence of mosaicism in the early blastocyst, and inherent technical difficulties of resolution with FISH probes. Approaches using a molecular approach to chromosome number (such as microarray or PCR methods) are likely to address this concern in the future.

Similarly, individual patients or couples at risk of a single-gene disorder that is inherited in either a recessive or dominant fashion may be candidates for PGD. As the technology continues to progress and the accuracy of PGD improves, current indications range from diagnosis of autosomal recessive disorders associated with childhood lethality to autosomal dominant disorders of adult onset. Areas of ethical controversy in this field are human leukocyte antigen matching for an affected sibling and family balancing.

CHROMOSOMAL MICROARRAY ANALYSIS

In the field of pediatric and adult genetics, chromosomal microarray analysis (CMA) has replaced karyotyping as a first-tier test to detect chromosome abnormalities in the setting of developmental disabilities or congenital anomalies.[53] CMA is able to detect genomic imbalances to within ~400 kb. In contrast, routine karyotyping is able to detect genomic imbalances in the 5- to 10-Mb range.[53] Additionally, SNP-based microarray testing has the capability to detect regions of homozygosity that may be indicative of uniparental disomy or parental consanguinity. In 2013, ACOG recommended CMA as a first-tier prenatal diagnostic test for fetuses with one or more major structural

abnormalities detected by ultrasound, replacing the need for fetal karyotyping. ACOG also stated that either fetal karyotyping or CMA can be performed when invasive testing is done in the setting of a normal ultrasound.[54]

The ACOG guideline was heavily influenced by a 2012 large-scale multicenter National Institute of Child Health and Human Development trial that compared prenatal CMA with traditional standard karyotyping.[55] The results indicated that CMA will detect a finding missed by standard karyotyping in 6% of fetuses with abnormal ultrasound findings and in 1.7% of fetuses with no ultrasound findings. However, CMA can also lead to counseling challenges because 3.4% of fetuses had a detected copy number variant (CNV) of unknown clinical significance. Just over half of these CNVs (1.8%) were classified as "likely benign" after investigation, with the other half (1.6%) classified as "likely pathogenic."[55] It is important to note that those CNVs that are classified as "likely pathogenic" may be complicated by reduced penetrance, variable expressivity, and delayed onset into adulthood, all of which make phenotyping difficult to predict and complicate counseling.

A recent metaanalysis indicated that when CMA is performed after detection of a structural abnormality by ultrasound, CMA will detect a significant finding missed by routine karyotyping in 10% (95% CI, 8% to 13%) of pregnancies with a variant of unknown significance detected in 2.1% (95% CI, 1.3% to 3.3%) of pregnancies.[56] As CNVs of unknown significance remain one of the greater interpretation challenges of CMA, some authors have advocated not to report them to patients.[57] However, there is no consensus on how to handle the reporting of these CNVs in the clinical setting. Regardless of the reporting strategy in place, pretest counseling and consent are critical components of this process and should include contracting with patients regarding the types of results that can be reported and their potential implications.

As CMA becomes used more widely in prenatal diagnosis, insurance coverage and economic impacts are important clinical considerations. CMA can be performed concurrently with standard karyotyping, in place of it, or as a reflex test if standard karyotyping is normal. Cost-benefit analysis favors ordering CMA alone.[58] However, utilizing rapid aneuploidy detection (interphase FISH or qualitative fluorescent PCR) for the most common aneuplodies followed by CMA if this testing is normal and standard karyotyping if this testing is abnormal was not included in this economic analysis and should be evaluated.

FUTURE DIRECTIONS

At this time, noninvasive prenatal screening using cf DNA is limited to the most common aneuploidies. However, it is not unreasonable to imagine that this technology will be expanded to include other conditions. As the technology of sequencing continues to improve and costs continue to decrease, sequencing the entire fetal genome through the maternal blood sample will be possible. This in turn would allow for noninvasive testing for single-gene disorders.

Exome sequencing, or sequencing of all the protein coding regions of the genome, is rapidly expanding into adult and pediatric practice. This technology will likely expand into prenatal diagnosis, but the challenge will be how to interpret large datasets and variants of uncertain significance and how to appropriately apply the information in clinical practice.[59]

SUMMARY

Prenatal genetic diagnosis is currently possible only by means of invasive procedures, with their inherent risk of miscarriage, fetal morbidity, and technical constraints. CVS, amniocentesis, and PUBS can all provide the fetal DNA needed for the assessment of a wide range of genetic and chromosome disorders. A good clinical history, which includes parental ages, reproductive history, and family history has value in screening for genetic and chromosome risks. During pregnancy, cf DNA, or maternal serum markers, and ultrasound examination are productive screening tools used primarily to assess Down syndrome risk. Ideally, such screening should be initiated in the first trimester. Most important, genomics is rapidly changing the field of prenatal screening and diagnosis, and further developments in the use of cf DNA will allow noninvasive prenatal genetic screening for a broad array of conditions from a maternal blood sample and decrease the need for invasive testing.

Complete reference list is available at www.ExpertConsult.com.

REFERENCES

1. Palomaki GE, Deciu C, Kloza EM, et al: DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 14:296–305, 2012.
2. Bianchi DW, Platt LD, Goldberg JD, et al: Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 119:890–901, 2012.
3. ACOG Practice Bulletin No. 77: Screening for fetal chromosomal abnormalities. *Obstet Gynecol* 109:217–227, 2007.
4. Kuhnert B, Nieschlag E: Reproductive functions of the ageing male. *Hum Reprod Update* 10:327–339, 2004.
5. Gadow EC, Lippold S, Otano L, et al: Chromosome rearrangements among couples with pregnancy losses and other adverse reproductive outcomes. *Am J Med Genet* 41:279–281, 1991.
6. Stern C, Pertile M, Norris H, et al: Chromosome translocations in couples with in-vitro fertilization implantation failure. *Hum Reprod* 14:2097–2101, 1999.
7. Hansteen IL, Varslot K, Steen-Johnsen J, Langård S: Cytogenetic screening of a new-born population. *Clin Genet* 21:309–314, 1982.
8. Nagvenkar P, Desai K, Hinduja I, Zaveri K: Chromosomal studies in infertile men with oligozoospermia and non-obstructive azoospermia. *Indian J Med Res* 122:34–42, 2005.
9. De Braekeleer M, Ferec C: Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. *Mol Hum Reprod* 2:669–677, 1996.
10. Conway GS, Payne NN, Webb J, et al: Fragile X premutation screening in women with premature ovarian failure. *Hum Reprod* 13:1184–1187, 1998.
11. Murray A, Ennis S, MacSwiney F, et al: Reproductive and menstrual history of females with fragile X expansions. *Eur J Hum Genet* 8:247–252, 2000.
12. ACOG Committee on Genetics: ACOG committee opinion. Number 298, August 2004. Prenatal and preconceptional carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 104:425–428, 2004.
13. Ferreira JC, Schreiber-Agus N, Carter SM, et al: Carrier testing for Ashkenazi Jewish disorders in the prenatal setting: navigating the genetic maze. *Am J Obstet Gynecol* 211:197–204, 2014.
14. ACOG Committee on Genetics: ACOG committee opinion. Number 318, October 2005. Screening for Tay-Sachs disease. *Obstet Gynecol* 106:893–894, 2005.
15. Hagerman PJ, Hagerman RJ: The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 74:805–816, 2004.
16. Nolin SL, Brown WT, Glicksman A, et al: Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet* 72:454–464, 2003.
17. Sherman S, Pletcher BA, Driscoll DA: Fragile X syndrome: diagnostic and carrier testing. *Genet Med* 7:584–587, 2005.
18. Gil MM, Akolekar R, Quezada MS, et al: Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 35:156–173, 2014.
19. Canick JA, Kloza EM, Lambert-Messerlian GM, et al: DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn* 32:730–734, 2012.
20. Nicolaides KH, Syngelaki A, del Mar Gil M, et al: Prenatal detection of fetal triploidy from cell-free DNA testing in maternal blood. *Fetal Diagn Ther* 35:201–217, 2014.
21. Nicolaides KH, Syngelaki A, Ashoor G, et al: Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 207:374, 2012.
22. Osborne CM, Hardisty E, Devers P, et al: Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. *Prenat Diagn* 33:609–611, 2013.
23. Wang Y, Chen Y, Tian F, et al: Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. *Clin Chem* 60:251–259, 2014.

24. Mao J, Wang T, Wang BJ, et al: Confined placental origin of the circulating cell free fetal DNA revealed by a discordant non-invasive prenatal test result in a trisomy 18 pregnancy. *Clin Chim Acta* 433:190–193, 2014.
25. Zhong XY, Holzgreve W, Hahn S: Risk free simultaneous prenatal identification of fetal Rhesus D status and sex by multiplex real-time PCR using cell free fetal DNA in maternal plasma. *Swiss Med Wkly* 131:70–74, 2001.
26. Daniels G, Finning K, Martin P, Soothill P: Fetal blood group genotyping from DNA from maternal plasma: an important advance in the management and prevention of haemolytic disease of the fetus and newborn. *Vox Sang* 87:225–232, 2004.
27. Benacerraf BR, Figoletto FD, Jr, Laboda LA: Sonographic diagnosis of Down syndrome in the second trimester. *Am J Obstet Gynecol* 153:49–52, 1985.
28. Weisz B, Pandya PP, David AL, et al: Ultrasound findings after screening for Down syndrome using the integrated test. *Obstet Gynecol* 109:1046–1052, 2007.
29. Odibo AO, Ghidini A: Role of the second-trimester 'genetic sonogram' for Down syndrome screen in the era of first-trimester screening and noninvasive prenatal testing. *Prenat Diagn* 34:511–517, 2014.
30. Nicolaides KH: Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 191:45–67, 2004.
31. Wright D, Kagan KO, Molina FS, et al: A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 31:376–383, 2008.
32. Gonce A, Borrell A, Martinez JM, Fortuny A: First-trimester screening for Down syndrome with ductus venosus Doppler studies in addition to nuchal translucency and serum markers. *Prenat Diagn* 25:901–905, 2005.
33. Nicolaides KH: First-trimester screening for chromosomal abnormalities. *Semin Perinatol* 29:190–194, 2005.
34. Wald NJ, Watt HC, Hackshaw AK: Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* 341:461–467, 1999.
35. Wright D, Bradbury I, Benn P, et al: Contingent screening for Down syndrome is an efficient alternative to non-disclosure sequential screening. *Prenat Diagn* 24:762–766, 2004.
36. Wald NJ, Rudnicka AR, Bestwick JP: Sequential and contingent prenatal screening for Down syndrome. *Prenat Diagn* 26:769–777, 2006.
37. Souka AP, Snijders RJ, Novakov A, et al: Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation. *Ultrasound Obstet Gynecol* 11:391–400, 1998.
38. Bahado-Singh RO, Wapner R, Thom E, et al: Elevated first-trimester nuchal translucency increases the risk of congenital heart defects. *Am J Obstet Gynecol* 192:1357–1361, 2005.
39. Souka AP, Krampl E, Bakalis S, et al: Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester. *Ultrasound Obstet Gynecol* 18:9–17, 2001.
40. Senat MV, De Keersmaecker B, Audibert F, et al: Pregnancy outcome in fetuses with increased nuchal translucency and normal karyotype. *Prenat Diagn* 22:345–349, 2002.
41. Malone FD, Ball RH, Nyberg DA, et al: FASTER Trial Research Consortium. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. *Obstet Gynecol* 106:288–294, 2005.
42. Alfirevic Z, Gosden CM, Neilson JP: Chorion villus sampling versus amniocentesis for prenatal diagnosis. *Cochrane Database Syst Rev* (2):CD000055, 2000.
43. Kuliev A, Jackson L, Froster U, et al: Chorionic villus sampling safety. Report of World Health Organization/EURO meeting in association with the Seventh International Conference on Early Prenatal Diagnosis of Genetic Diseases, Tel-Aviv, Israel, May 21, 1994. *Am J Obstet Gynecol* 174:807–811, 1996.
44. Wilkins-Haug L, Quade B, Morton CC: Confined placental mosaicism as a risk factor among newborns with fetal growth restriction. *Prenat Diagn* 26:428–432, 2006.
45. Sundberg K, Bang J, Smidt-Jensen S, et al: Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. *Lancet* 350:697–703, 1997.
46. Alfirevic Z: Early amniocentesis versus transabdominal chorion villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev*(2):CD000077, 2000.
47. Yoon G, Chernos J, Sibbald B, et al: Association between congenital foot anomalies and gestational age at amniocentesis. *Prenat Diagn* 21:1137–1141, 2001.
48. Tabor A, Philip J, Madsen M, et al: Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1:1287–1293, 1986.
49. Papantoniou NE, Daskalakis GJ, Tziotis JG, et al: Risk factors predisposing to fetal loss following a second trimester amniocentesis. *Br J Obstet Gynaecol* 108:1053–1056, 2001.
50. Antsaklis A, Papantoniou N, Xygakis A, et al: Genetic amniocentesis in women 20-34 years old: associated risks. *Prenat Diagn* 20:247–250, 2000.

REFERENCES

1. Palomaki GE, Deciu C, Kloza EM, et al: DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 14:296–305, 2012.
2. Bianchi DW, Platt LD, Goldberg JD, et al: Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 119:890–901, 2012.
3. ACOG Practice Bulletin No. 77: Screening for fetal chromosomal abnormalities. *Obstet Gynecol* 109:217–227, 2007.
4. Kuhnert B, Nieschlag E: Reproductive functions of the ageing male. *Hum Reprod Update* 10:327–339, 2004.
5. Gadow EC, Lippold S, Otano L, et al: Chromosome rearrangements among couples with pregnancy losses and other adverse reproductive outcomes. *Am J Med Genet* 41:279–281, 1991.
6. Stern C, Pertile M, Norris H, et al: Chromosome translocations in couples with in-vitro fertilization implantation failure. *Hum Reprod* 14:2097–2101, 1999.
7. Hansteen IL, Varslot K, Steen-Johnsen J, Langård S: Cytogenetic screening of a new-born population. *Clin Genet* 21:309–314, 1982.
8. Nagvenkar P, Desai K, Hinduja I, Zaveri K: Chromosomal studies in infertile men with oligozoospermia and non-obstructive azoospermia. *Indian J Med Res* 122:34–42, 2005.
9. De Braekeleer M, Ferec C: Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. *Mol Hum Reprod* 2:669–677, 1996.
10. Conway GS, Payne NN, Webb J, et al: Fragile X premutation screening in women with premature ovarian failure. *Hum Reprod* 13:1184–1187, 1998.
11. Murray A, Ennis S, MacSwiney F, et al: Reproductive and menstrual history of females with fragile X expansions. *Eur J Hum Genet* 8:247–252, 2000.
12. ACOG Committee on Genetics: ACOG committee opinion. Number 298, August 2004. Prenatal and preconceptional carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 104:425–428, 2004.
13. Ferreira JC, Schreiber-Agus N, Carter SM, et al: Carrier testing for Ashkenazi Jewish disorders in the prenatal setting: navigating the genetic maze. *Am J Obstet Gynecol* 211:197–204, 2014.
14. ACOG Committee on Genetics: ACOG committee opinion. Number 318, October 2005. Screening for Tay-Sachs disease. *Obstet Gynecol* 106:893–894, 2005.
15. Hagerman PJ, Hagerman RJ: The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 74:805–816, 2004.
16. Nolin SL, Brown WT, Glicksman A, et al: Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet* 72:454–464, 2003.
17. Sherman S, Pletcher BA, Driscoll DA: Fragile X syndrome: diagnostic and carrier testing. *Genet Med* 7:584–587, 2005.
18. Gil MM, Akolekar R, Quezada MS, et al: Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 35:156–173, 2014.
19. Canick JA, Kloza EM, Lambert-Messerlian GM, et al: DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn* 32:730–734, 2012.
20. Nicolaides KH, Syngelaki A, del Mar Gil M, et al: Prenatal detection of fetal triploidy from cell-free DNA testing in maternal blood. *Fetal Diagn Ther* 35:201–217, 2014.
21. Nicolaides KH, Syngelaki A, Ashoor G, et al: Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 207:374, 2012.
22. Osborne CM, Hardisty E, Devers P, et al: Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. *Prenat Diagn* 33:609–611, 2013.
23. Wang Y, Chen Y, Tian F, et al: Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. *Clin Chem* 60:251–259, 2014.
24. Mao J, Wang T, Wang BJ, et al: Confined placental origin of the circulating cell free fetal DNA revealed by a discordant non-invasive prenatal test result in a trisomy 18 pregnancy. *Clin Chim Acta* 433:190–193, 2014.
25. Zhong XY, Holzgreve W, Hahn S: Risk free simultaneous prenatal identification of fetal Rhesus D status and sex by multiplex real-time PCR using cell free fetal DNA in maternal plasma. *Swiss Med Wkly* 131:70–74, 2001.
26. Daniels G, Finning K, Martin P, Soothill P: Fetal blood group genotyping from DNA from maternal plasma: an important advance in the management and prevention of haemolytic disease of the fetus and newborn. *Vox Sang* 87:225–232, 2004.
27. Benacerraf BR, Figoletto FD, Jr, Laboda LA: Sonographic diagnosis of Down syndrome in the second trimester. *Am J Obstet Gynecol* 153:49–52, 1985.
28. Weisz B, Pandya PP, David AL, et al: Ultrasound findings after screening for Down syndrome using the integrated test. *Obstet Gynecol* 109:1046–1052, 2007.
29. Odibo AO, Ghidini A: Role of the second-trimester 'genetic sonogram' for Down syndrome screen in the era of first-trimester screening and noninvasive prenatal testing. *Prenat Diagn* 34:511–517, 2014.
30. Nicolaides KH: Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 191:45–67, 2004.
31. Wright D, Kagan KO, Molina FS, et al: A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 31:376–383, 2008.
32. Gonce A, Borrell A, Martinez JM, Fortuny A: First-trimester screening for Down syndrome with ductus venosus Doppler studies in addition to nuchal translucency and serum markers. *Prenat Diagn* 25:901–905, 2005.
33. Nicolaides KH: First-trimester screening for chromosomal abnormalities. *Semin Perinatol* 29:190–194, 2005.
34. Wald NJ, Watt HC, Hackshaw AK: Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* 341:461–467, 1999.
35. Wright D, Bradbury I, Benn P, et al: Contingent screening for Down syndrome is an efficient alternative to non-disclosure sequential screening. *Prenat Diagn* 24:762–766, 2004.
36. Wald NJ, Rudnicka AR, Bestwick JP: Sequential and contingent prenatal screening for Down syndrome. *Prenat Diagn* 26:769–777, 2006.
37. Souka AP, Snijders RJ, Novakov A, et al: Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation. *Ultrasound Obstet Gynecol* 11:391–400, 1998.
38. Bahado-Singh RO, Wapner R, Thom E, et al: Elevated first-trimester nuchal translucency increases the risk of congenital heart defects. *Am J Obstet Gynecol* 192:1357–1361, 2005.
39. Souka AP, Krampl E, Bakalis S, et al: Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester. *Ultrasound Obstet Gynecol* 18:9–17, 2001.
40. Senat MV, De Keersmaecker B, Audibert F, et al: Pregnancy outcome in fetuses with increased nuchal translucency and normal karyotype. *Prenat Diagn* 22:345–349, 2002.
41. Malone FD, Ball RH, Nyberg DA, et al: FASTER Trial Research Consortium. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. *Obstet Gynecol* 106:288–294, 2005.
42. Alfirevic Z, Gosden CM, Neilson JP: Chorion villus sampling versus amniocentesis for prenatal diagnosis. *Cochrane Database Syst Rev* (2):CD000055, 2000.
43. Kuliev A, Jackson L, Froster U, et al: Chorionic villus sampling safety. Report of World Health Organization/EURO meeting in association with the Seventh International Conference on Early Prenatal Diagnosis of Genetic Diseases, Tel-Aviv, Israel, May 21, 1994. *Am J Obstet Gynecol* 174:807–811, 1996.
44. Wilkins-Haug L, Quade B, Morton CC: Confined placental mosaicism as a risk factor among newborns with fetal growth restriction. *Prenat Diagn* 26:428–432, 2006.
45. Sundberg K, Bang J, Smidt-Jensen S, et al: Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. *Lancet* 350:697–703, 1997.
46. Alfirevic Z: Early amniocentesis versus transabdominal chorion villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev*(2):CD000077, 2000.
47. Yoon G, Chernos J, Sibbald B, et al: Association between congenital foot anomalies and gestational age at amniocentesis. *Prenat Diagn* 21:1137–1141, 2001.
48. Tabor A, Philip J, Madsen M, et al: Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1:1287–1293, 1986.
49. Papantoniou NE, Daskalakis GJ, Tziotis JG, et al: Risk factors predisposing to fetal loss following a second trimester amniocentesis. *Br J Obstet Gynaecol* 108:1053–1056, 2001.
50. Antsaklis A, Papantoniou N, Xygakis A, et al: Genetic amniocentesis in women 20-34 years old: associated risks. *Prenat Diagn* 20:247–250, 2000.
51. Borgida AF, Mills AA, Feldman DM, et al: Outcome of pregnancies complicated by ruptured membranes after genetic amniocentesis. *Am J Obstet Gynecol* 183:937–939, 2000.
52. Munne S: Preimplantation genetic diagnosis and human implantation—a review. *Placenta* 24(Suppl B):S70–S76, 2003.
53. Miller DT, Adam MP, Aradhya S, et al: Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individual with developmental disabilities or congenital anomalies. *Am J Hum Genet* 86:749–764, 2010.
54. ACOG Committee on Genetics: ACOG committee opinion. Number 581, December 2013. The use of chromosomal microarray analysis in prenatal diagnosis. *Obstet Gynecol* 122:1374–1377, 2013.
55. Wapner RJ, Martin CL, Levy B, et al: Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 367:2175–2184, 2012.
56. Hillman SC, McMullan DJ, Hall G, et al: Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 41:610–620, 2013.
57. Brady PD, Delle Chiaie B, Christenhusz G, et al: A prospective study of the clinical utility of prenatal chromosomal microarray analysis in fetuses with ultrasound abnormalities and an exploration of a framework for reporting unclassified variants and risk factors. *Genet Med* 16:469–476, 2014.
58. Harper LM, Sutton AL, Longman RE, Odibo AO: An economic analysis of prenatal cytogenetic technologies for sonographically detected fetal anomalies. *Am J Med Genet A* 164A:1192–1197, 2014.
59. Dugoff L: Application of genomic technology in prenatal diagnosis. *N Engl J Med* 367:2249–2251, 2012.

3 Basic Embryology

David L. Bolender | Stanley Kaplan

The human embryo begins as a single large cell, approximately 0.1 mm in diameter, just visible to the unaided eye. During the 266 days of gestation after fertilization, this cell increases in size, weight, and surface area in a rapid and markedly nonlinear fashion. From newly fertilized egg to newborn, length increases by a factor of 5000, surface area by a factor of 61 million, and weight by a factor of nearly 6 billion.[1] During this process the fertilized egg divides and differentiates into more than 200 different morphologically recognizable cell types. Orchestration of the increase in size and specialization in cellular function is a complex process about which much remains unknown. It has been argued, however, that the principles of development have been established and that details are missing only at the molecular level.[2] This claim is undoubtedly an overstatement; nevertheless, during the last decade or so, understanding of the molecular control of development has increased substantially. That human embryonic development occurs normally in most pregnancies is a tribute to the design of the control mechanisms that are operating. This chapter presents a brief description of the growth and differentiation of the human embryo, along with a limited discussion of certain factors that play a part in control of these activities.

GAMETES AND THEIR MATURATION

The human egg and sperm are two highly specialized cells that share little in common with the other cells of the adult body. They are different in both form and function. Like other cells, however, they must achieve a degree of maturity before they can perform their function (i.e., combining to form the zygote). The steps and the chronology leading to this maturation are quite different in the male and in the female, and such differences reflect the diverse pathways of the two sexes beginning early in human development.[3]

ORIGIN OF THE GAMETES

The human egg and sperm are derived from large, round primordial germ cells that can be identified in the wall of the yolk sac as early as 24 days after fertilization.[4] As the yolk sac begins to

be incorporated into the embryo, the germ cells migrate along the dorsal mesentery of the hindgut to the gonadal ridges, which they reach by the end of the fourth or early fifth week (Figure 3-1). This migration has been observed in vitro in pieces of hindgut, mesentery, and gonadal ridges of mouse embryos.[5] It is facilitated in humans by a striking ameboid shape (which persists even after the cells have reached the gonad[6]) and pseudopodia typical of those found in ameboid cells. The pseudopodia disappear after the migration is complete.[5,7] In humans these cells contain glycogen stores that diminish over time and disappear when the cells have reached their destination in the gonad, suggesting that the glycogen may be the energy source for their journey.[8]

ORGANIZATION OF THE GONAD

The coelomic epithelium covering the medial aspect of the gonadal ridges undergoes proliferation at about week 7 of gestation. As the epithelial cells multiply, they grow into the underlying mesenchyme in a series of fingerlike cords of cells called *primitive sex cords.* The primordial germ cells associate with these cords. If the embryo is to become a male, these cords continue to be prominent and eventually develop into the seminiferous tubules and rete testis. The early male gonad can also be recognized by the separation of the cords from their parent epithelial covering by a fibrous connective tissue layer, the tunica albuginea, which forms just under the epithelium. If the gonad is to become an ovary, the primitive sex cords remain rudimentary. The origin of the follicular cells of the ovary remains unclear, but likely candidates are cells from the coelomic epithelium and the mesonephros. The follicular cells associate with the primordial germ cells to form primordial ovarian follicles.[8]

DEVELOPMENT OF THE FEMALE GAMETE (SEE CHAPTER 150)

If the gonad develops into an ovary, the primordial germ cells become oogonia, and mitotic division continues.[9] Mitotic division of these cells has been observed in humans up to the seventh fetal month[10] but ceases sometime shortly before birth. No oogonia form after the birth of the infant after a normal full-term pregnancy.

In both males and females the germ cells form a syncytium while dividing.[11,12] These intercellular connections permit communication and facilitate the high degree of synchrony that has been observed during both mitotic division and meiotic division.[13-15]

By the eighth or ninth week after fertilization, some oogonia enter prophase of meiosis I and become primary oocytes.[9-16] Meiosis begins first deep to the surface of the human ovary and then expands toward the surface. Thus, at an appropriate fetal stage, oogonia are found superficially, oocytes deep to the surface, and small follicles at the inner part of the ovarian cortex.[9] It has been suggested that a diffusible meiosis-activating substance is secreted by rete cells (derived from the mesonephros), which lie in the center of the ovary, and good experimental evidence is available to support this hypothesis.[17-20]

The oocyte goes through the leptotene, zygotene, and pachytene stages of meiosis I, and it then stops at the diplotene stage.

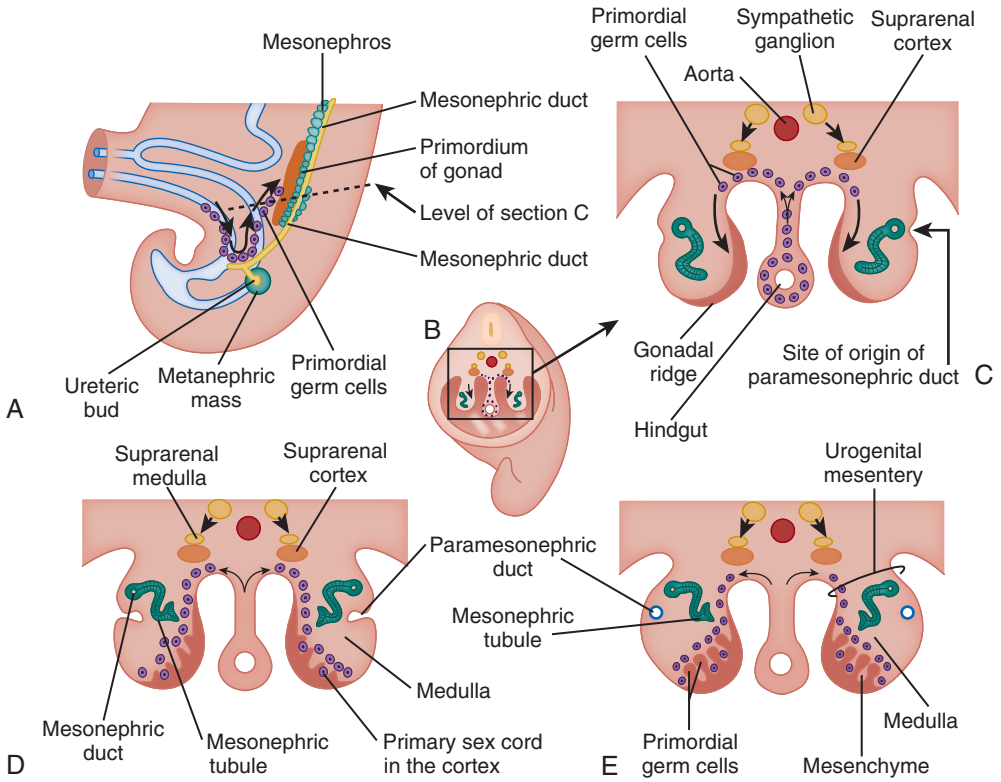

Figure 3-1 **A,** Sketch of a 5-week-old embryo, illustrating the migration of primordial germ cells from the yolk sac. **B,** Three-dimensional sketch of the caudal region of a 5-week-old embryo, showing the location and extent of the gonadal ridges on the medial aspect of the urogenital ridges. **C,** Transverse section showing the primordium of the suprarenal (adrenal) glands, the gonadal ridges, and the migration of primordial germ cells into the developing gonads. **D,** Transverse section through a 6-week-old embryo, showing the primary sex cords and the developing paramesonephric ducts. **E,** Similar section at a later stage showing the indifferent gonads and the mesonephric and paramesonephric ducts. (From Moore KL, Persaud TVN: *The developing human: clinically oriented embryology,* ed 5, Philadelphia, 1993, WB Saunders, p 281.)

At this point the oocyte becomes surrounded by a single, incomplete layer of flat follicular cells[9]; this unit is called a *primordial follicle.* The follicle's large central nucleus is known as the *germinal vesicle.* A crescent-shaped assembly of cellular organelles containing mitochondria, endoplasmic reticulum, the Golgi complex, lysosomes, and annulate lamellae (stacked parallel membrane arrays with pores) remains clustered adjacent to the nucleus.[15,21] Once it has been incorporated into a primordial follicle, the oocyte enters a long period of quiescence, beginning before birth in humans and ending in either atresia or ovulation.

Once sexual maturity is attained, a small number of oocytes begin the process of folliculogenesis, or follicle maturation, during each menstrual cycle.[22] The oocyte grows and eventually becomes one of the largest cells in the human body.[23] The organelles disperse throughout the cytoplasm, and the germinal vesicle (nucleus) enlarges. It increases its complement of nuclear pores, facilitating transport of molecules between nucleoplasm and cytoplasm. The follicular cells resume mitosis and increase markedly in size, changing in shape from squamous to cuboidal, and the follicle becomes surrounded by a basement membrane. Those follicles containing an oocyte surrounded by a single layer of cuboidal follicular cells are known as *unilaminar primary follicles,* to distinguish them from cells of earlier or later stages.[3]

During further growth of the primary follicle, a thick, acellular coat, the *zona pellucida,* begins to form between the oocyte and the follicular cells. Mitotic activity increases the number of follicular cell layers, and the follicle is now called a *multilaminar primary follicle.* The expanding follicle compresses the surrounding ovarian stoma, which organizes into a compact layer adjacent to the basement membrane of the follicle. This layer of stromal cells is called the *theca interna,* and its cells have the capacity to produce androgens when stimulated by luteinizing hormone activity (Figure 3-2). The theca interna is vascularized, but the epithelial layers of follicular cells remain avascular.

The zona pellucida is important in the process of fertilization because it contains sperm receptors, takes part in induction of the acrosome reaction, and becomes a block to polyspermy. It may also act after fertilization as a smooth, slippery envelope to contain the sticky ball of cells of the morula-stage embryo; these cells are free to adhere to the uterine endothelium when the zona breaks down, just before implantation.

The zona pellucida is made up of three separate filamentous glycoproteins, zona pellucida glycoprotein 1 (ZP1) ZP1 through ZP4, which differ in molecular weight and isoelectric point and account for virtually all protein in the zona pellucida. ZP1 crosslinks these filaments, resulting in a three-dimensional matrix that is permeable to large macromolecules. ZP3 serves as a species-specific sperm receptor and also induces the acrosome reaction in sperm on contact. At or shortly after fertilization, these two characteristics are lost, reducing the likelihood of polyspermy.[24-26] The ZP3 gene has been cloned; it is expressed only in oocytes, and then only during the growth phase of oogenesis.[27] ZP1, ZP2, and ZP3 are located on chromosomes 19, 7, and 5, respectively, while ZP4 is located on chromosomes 11, 16, 7, and 1.[27a] The interesting story of these zona pellucida proteins has been the subject of a popularized account[29] and several reviews.[28,30,31] Radiolabeling studies in mice indicate that all three glycoproteins are synthesized by the oocyte itself, rather than by the follicular cells.[32] Furthermore, immunofluorescence studies show that zona pellucida antigens are present within human oocytes but not in follicular cells.[33] Studies in species other than the mouse, however, suggest that the granulosa cells that surround the oocyte also may play a role in the synthesis of zona pellucida components.[30]

Numerous cytoplasmic projections of the follicular cells penetrate the zona pellucida to contact the cell membrane of the oocyte. In humans these filopodial extensions of the follicular cell may actually lie deeply buried in the oocyte, in straight invaginations or pits.[34] These pits are lined by the oocyte cell membrane; however, no cytoplasmic continuity exists between the two cell types. Animal studies have demonstrated the presence of gap junctions along the association of these two cell membranes, permitting transfer of small molecules (molecular weight of approximately 1000) between them.[22]

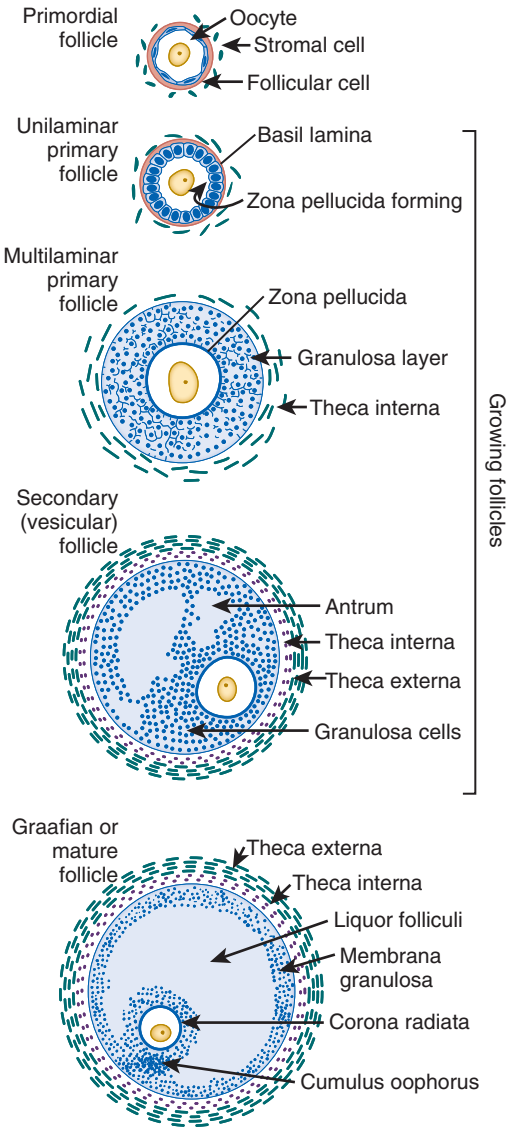

Figure 3-2 Schematic drawing of development of ovarian follicles, starting with the primordial follicle and ending with mature follicles. (From Junqueira LC, Carneiro J, Kelly RO: *Basic histology,* ed 7, Norwalk, 1992, Appleton & Lange.)

As the primary follicle enlarges, the follicular cells begin to produce follicular fluid, which collects within the intercellular spaces between follicular cells. These spaces coalesce to form a large fluid-filled cavity called the *antrum,* which is characteristic of the secondary (vesicular) follicle. The antrum expands, and the oocyte becomes located on one side of the follicle, where it is embedded within a mound of follicular cells known as the *cumulus oophorus.* The layers of follicular cells immediately surrounding the oocyte are termed the *corona radiata.* Because of its increased size, the follicle further compresses the surrounding ovarian stroma. A looser, less organized layer of flattened stromal cells encircles the follicle superficial to the theca interna.