primers are added to the DNA solution. The temperature of the solution is increased to dissociate the individual DNA strands and is then reduced to permit annealing of the primers to their complementary template target sites. A thermostable DNA polymerase is included in the reaction to synthesize new DNA in the 5′ to 3′ direction from the primer annealing sites. The temperature is then increased to dissociate duplex structures, after which it is reduced, enabling another cycle of DNA synthesis to occur. Several temperature cycles (usually up to 40) are used to amplify progressively the concentration of the sequence of interest, which can be identified as a PCR product by agarose gel electrophoresis with a fluorescent dye. The product can be isolated and sequenced to identify the suggested mutation.

If the gene is large and the site of the mutation is unknown (especially if it is a point mutation), other methods can be used to identify the likely mutated site in the exonic sequence. A common approach involves scanning the gene sequence for mutations that alter the structural conformation of short complexes between parent DNA and PCR products, leading to a shift in mobility on a nondenaturing agarose gel (i.e., single-strand conformational polymorphism). A single-base substitution or deletion can change the conformation of the complex compared with wild-type complexes and yield a shift in mobility. Sequencing this comparatively small region of the gene facilitates precise identification of the mutation.

When the gene thought to cause the disease phenotype is unknown, when its likely position on the genome has not been identified, or when only limited mapping information is available, a candidate gene approach can be used to identify the mutated gene. In this strategy, potential candidate genes are identified on the basis of analogy to animal models or by analysis of known genes that map to the region of the genome for which limited information is available. The candidate gene is then analyzed for potential mutations. Regardless of the approach used, mutations identified in candidate genes should always be correlated with functional changes in the gene product because some mutations may be functionally silent, representing a polymorphism without phenotypic consequences. Functional changes in the gene product can be evaluated through the use of cell culture systems to assess protein function by expressing the mutant protein through transiently transfecting the cells with a vector that carries the cDNA coding for the gene of interest and incorporating the mutation of interest. Alternatively, unique animal models can be developed in which the mutant gene is incorporated in the male pronucleus of oocytes taken from a superovulating, impregnated female. This union produces an animal that overexpresses the mutant gene; it produces a transgenic animal, an animal with more than the usual number of copies of a given gene, or an animal in which the gene of interest is disrupted and the gene product is not synthesized (i.e., a gene *knockout* animal or an animal with one half [heterozygote] or none [homozygote] of the usual number of a given gene).

Molecular Diagnostics

The power of molecular techniques extends beyond their use in defining the precise molecular basis of an inherited disease. By exploiting the exquisite sensitivity of PCR to amplify rare nucleic acid sequences, it is possible to diagnose rapidly a range of infectious diseases for which unique sequences are available. In particular, infections caused by fastidious or slow-growing organisms can be rapidly diagnosed, similar to the case for *Mycobacterium tuberculosis*. The presence of genes conferring resistance to specific antibiotics in microorganisms can also be verified by PCR techniques. Sequencing of the entire genome of organisms such as *Escherichia coli, M. tuberculosis*, and *Treponema pallidum* offers unparalleled opportunities to monitor the epidemiologic structures of infections, follow the course of acquired mutations, tailor antibiotic therapies, and develop unique gene-based therapies (discussed later) for infectious agents for which conventional antibiotic therapies are ineffective or marginally effective.

The application of molecular methods to human genetics has revolutionized the field. Through the use of approaches that incorporate linkage analysis and PCR, point mutations can be precisely localized and characterized. At the other end of the spectrum of genetic changes that underlie disease, chromosomal translocations, deletions, or duplications can be identified by conventional cytogenetic methods. Large deletions that can incorporate many kilobase pairs and many genes can be visualized with fluorescence in situ hybridization (FISH), a technique in which a segment of cloned DNA is labeled with a fluorescent tag and hybridized to chromosomal DNA. With the deletion of the segment of interest from the genome, the chromosomal DNA fails to fluoresce in the corresponding chromosomal location.

Advances in molecular medicine have elucidated the mechanisms of carcinogenesis and revolutionized the diagnosis and treatment of neoplastic diseases. According to current views, a neoplasm arises from the clonal proliferation of a single cell that is transformed from a regulated, quiescent state into an unregulated growth phase. DNA damage accumulates in the parental tumor cell as a result of exogenous factors (e.g., radiation exposure) or heritable determinants. In early phases of carcinogenesis, certain genomic changes may impart intrinsic genetic instability that increases the likelihood of additional damage. One class of genes that becomes activated during carcinogenesis is oncogenes, which are primordial genes that normally exist in the mammalian genome in an inactive (proto-oncogene) state but, when activated, promote unregulated cell proliferation through specific intracellular signaling pathways.

Molecular methods based on the acquisition of specific tumor markers and unique DNA sequences that result from oncogenetic markers of larger chromosomal abnormalities (i.e., translocations or deletions that promote oncogenesis) are broadly applied to the diagnosis of malignancies. These methods can be used to establish the presence of specific tumor markers and oncogenes in biopsy specimens, to monitor the presence or persistence of circulating malignant cells after completion of a course of chemotherapy, and to identify the development of genetic resistance to specific chemotherapeutic agents. Through the use of conventional linkage analysis and candidate gene approaches, future studies will be able to identify individuals with a heritable predisposition to malignant transformation. Many of these topics are discussed in later chapters.

The advent of *gene chip* technologies or expression arrays has revolutionized molecular diagnostics and has begun to clarify the pathobiologic structures of complex diseases. These methods involve labeling the cDNA generated from the entire pool of