MOLECULAR CYTOGENETICS

Molecular cytogenetics provides a link between chromosome and molecular analysis and overcomes some of the limitations of standard cytogenetics. Deletions smaller than several million base pairs are not routinely detectable by standard G-banding techniques, and chromosomal abnormalities with indistinct or novel banding patterns can be difficult or impossible to interpret. To carry out cytogenetic analysis, cells must be dividing, which is not always possible to obtain (e.g., in autopsy or tumor material that has already been fixed). Finally, growth selection or bias may occasionally cause the results of cytogenetic studies to be misleading because cells that proliferate *in vitro* may not be representative of the original population, as is often the case with tumor specimens.

Fluorescence *in situ* hybridization (FISH) is a combined cytogenetic-molecular technique that solves many of the aforementioned problems. FISH permits determination of the number and location of specific DNA sequences in human cells. FISH can be performed on metaphase chromosomes, as with G-banding, but can also be performed on cells not actively progressing through mitosis. FISH performed on nondividing cells is referred to as interphase or nuclear FISH (Fig. 83e-2). The FISH procedure relies on the complementarity between the two strands of the DNA double helix and uses a molecular probe, which can be a pool of sequences across an entire chromosome, a DNA sequence for a repetitive part of the genome (e.g., centromeres or telomeres), or a specific DNA sequence found only once in the genome (e.g., a disease-associated gene). The choice of probes for FISH studies is important and will vary with the information needed for the diagnosis of a particular disorder. The most common type of probes are locus-specific probes, which are used to determine if a critical gene or region is absent (indicating a deletion), or present in the normal number of copies, or if an additional copy of the region is present. FISH on metaphase chromosomes will give the additional information of the location of the additional copy, which is necessary information to determine whether a structural rearrangement, such as a translocation, is present. FISH can also be performed with probes that bind to repeated sequences, such as DNA found in centromeres or telomeres, or with probes that bind to an entire chromosome ("painting" probes), to determine the chromosome composition of an abnormal chromosome. Interphase FISH studies can also help to identify structural alterations when probes are used that map to both sides of a translocation breakpoint. Each side of the breakpoint is labeled in a different color, and when no translocation is present the two probes appear to be overlapping. When a translocation is present, the two probes appear separate from one another. These set of probes, called "break-apart" probes, are commonly used to detect recurrent translocations in cancer cells.

ARRAY-BASED METHODOLOGIES (CYTOGENOMICS)

Array-based methods were introduced into the clinical lab beginning in 2003 and quickly revolutionized the field of cytogenetics. These techniques used arrays (collections of DNA segments from the entire genome) which could be interrogated with respect to copy number. With standard cytogenetics, the missing or extra pieces of DNA have to be big enough to see in the microscope on banded chromosomes (usually larger than 5 Mb). FISH requires a preselection of an informative molecular probe prior to analysis. In contrast, array-based techniques permit analysis of many regions of the genome in a single analysis, with greatly increased resolution over standard cytogenetics. Array-based techniques allow for scanning of the genome for small deletions or duplications quickly and accurately. The resolution of the

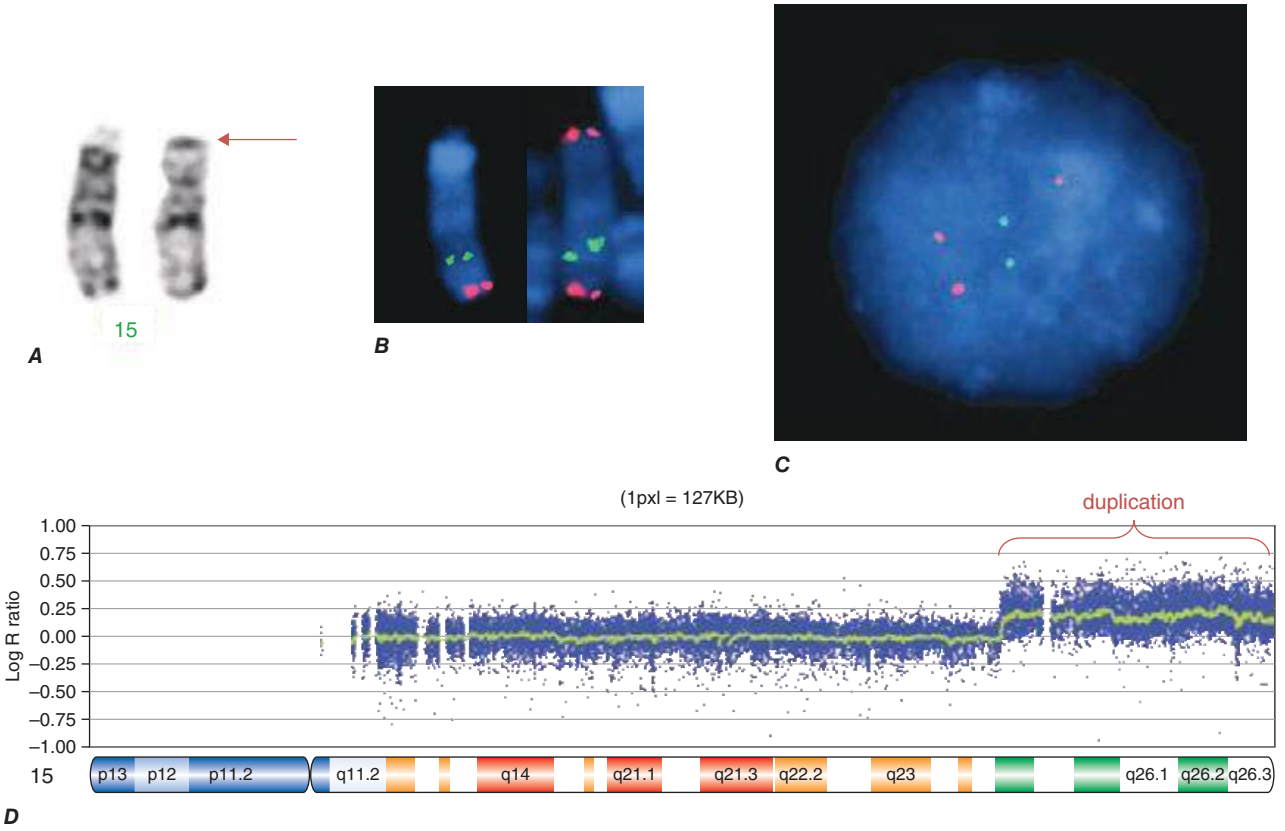

FIGURE 83e-2 G-banding, fluorescence in situ hybridization (FISH), and single nucleotide polymorphism (SNP) array demonstrate an abnormal chromosome 15. ***A.*** G-banding shows an abnormal chromosome 15, with unrecognizable material in place of the p arm in the chromosome on the right (*top arrow*). ***B.*** Metaphase FISH (only chromosome 15s are shown) using a probe from the 15q telomere region (*red*) and a control probe that maps outside of the duplicated region (*green*). ***C.*** Interphase FISH demonstrates three copies of the 15q tel probe in *red*, and two copies of the 15q control probe (*green*). ***D.*** Genome-wide SNP array demonstrates the increased copy number for a portion of 15q. Note that the G-banding alone indicates the abnormal chromosome 15, but the origin of the extra material can only be demonstrated by FISH or array. The FISH analysis requires additional information about possible genetic causes to select the correct probe. The array can exactly identify the origin of the extra material, but by itself would not provide positional information.