codon anywhere in the coding sequence, whereas activations require precise substitutions at residues that can somehow lead to an increase in the activity of the encoded protein. Importantly, the specificity of oncogene mutations provides diagnostic opportunities, as tests that identify mutations at defined positions are easier to design than tests aimed at detecting random changes in a gene.

DNA AMPLIFICATION

The second mechanism for activation of oncogenes is DNA sequence amplification, leading to overexpression of the gene product. This increase in DNA copy number may cause cytologically recognizable chromosome alterations referred to as *homogeneous staining regions* (HSRs) if integrated within chromosomes, or *double minutes* (dmins) if extrachromosomal. The recognition of DNA amplification is accomplished through various cytogenetic techniques such as comparative genomic hybridization (CGH) or fluorescence in situ hybridization (FISH), which allow the visualization of chromosomal aberrations using fluorescent dyes. In addition, noncytogenetic, microarray-based approaches are now available for identifying changes in copy number at high resolution. Newer short-tag–based sequencing approaches have been used to evaluate amplifications. When paired with next-generation sequencing, this approach offers the highest degree of resolution and quantification available. With both microarray and sequencing technologies, the entire genome can be surveyed for gains and losses of DNA sequences, thus pinpointing chromosomal regions likely to contain genes important in the development or progression of cancer.

Numerous genes have been reported to be amplified in cancer. Several of these genes, including *NMYC* and *LMYC*, were identified through their presence within the amplified DNA sequences of a tumor and had homology to known oncogenes. Because the region amplified often includes hundreds of thousands of base pairs, multiple oncogenes may be amplified in a single amplicon in some cancers (particularly in sarcomas). Indeed, *MDM2*, *GLI*, *CDK4*, and *SAS* at chromosomal location 12q13-15 have been shown to be simultaneously amplified in several types of sarcomas and other tumors. Amplification of a cellular gene is often a predictor of poor prognosis; for example, *ERBB2*/*HER2* and *NMYC* are often amplified in aggressive breast cancers and neuroblastoma, respectively.

CHROMOSOMAL REARRANGEMENT

Chromosomal alterations provide important clues to the genetic changes in cancer. The chromosomal alterations in human solid tumors such as carcinomas are heterogeneous and complex and occur as a result of the frequent chromosomal instability (CIN) observed in these tumors (see below). In contrast, the chromosome alterations in myeloid and lymphoid tumors are often simple translocations, i.e., reciprocal transfers of chromosome arms from one chromosome to another. Consequently, many detailed and informative chromosome analyses have been performed on hematopoietic cancers. The breakpoints of recurring chromosome abnormalities usually occur at the site of cellular oncogenes. Table 101e-2 lists representative examples of recurring chromosome alterations in malignancy and the associated gene(s) rearranged or deregulated by the chromosomal rearrangement. Translocations are particularly common in lymphoid tumors, probably because these cell types have the capability to rearrange their DNA to generate antigen receptors. Indeed, antigen receptor genes are commonly involved in the translocations, implying that an imperfect regulation of receptor gene rearrangement may be involved in the pathogenesis. An interesting example is Burkitt's lymphoma, a B cell tumor characterized by a reciprocal translocation between chromosomes 8 and 14. Molecular analysis of Burkitt's lymphomas demonstrated that the breakpoints occurred within or near the *MYC* locus on chromosome 8 and within the immunoglobulin heavy chain locus on chromosome 14, resulting in the transcriptional activation of *MYC*. Enhancer activation by translocation, although not universal, appears to play an important role in malignant progression. In addition to transcription factors and signal transduction molecules, translocation may result in the overexpression of cell cycle regulatory proteins or proteins such as cyclins and of proteins that regulate cell death.

TABLE 101e-2 REPRESENTATIVE ONCOGENES AT CHROMOSOMAL TRANSLOCATIONS

Gene (Chromosome)	Translocation	Malignancy
ABL (9q34.1)–*BCR* (22q11)	(9;22)(q34;q11)	Chronic myeloid leukemia
ATF1 (12q13)–*EWS* (22q12)	(12;22)(q13;q12)	Malignant melanoma of soft parts
BCL1 (11q13.3)–*IgH* (14q32)	(11;14)(q13;q32)	Mantle cell lymphoma
BCL2 (18q21.3)–*IgH* (14q32)	(14;18)(q32;q21)	Follicular lymphoma
FLI1 (11q24)–*EWS* (22q12)	(11;22)(q24;q12)	Ewing's sarcoma
LCK (1p34)–*TCRB* (7q35)	(1;7)(p34;q35)	T cell acute lymphocytic leukemia
MYC (8q24)–*IgH* (14q32)	(8;14)(q24;q32)	Burkitt's lymphoma, B cell acute lymphocytic leukemia
PAX3 (2q35)–*FKHR/ALV* (13q14)	(2;13)(q35;q14)	Alveolar rhabdomyosarcoma
PAX7 (1p36)–*KHR*/ALV(13q14)	(1;13)(p36;q14)	Alveolar rhabdomyosarcoma
REL (2p13)–*NRG* (2p11.2-14)	Inv(2(p13;p11.2-14)	Non-Hodgkin's lymphoma
RET (10q11.2)–*PKAR1A* (17q23)	(10;17)(q11.2;q23)	Thyroid carcinoma
TAL1(1p32)–*TCTA* (3p21)	(1;3)(p34;p21)	Acute T cell leukemia
TRK (1q23-1q24)–*TPM3* (1q31)	Inv1(q23;q31)	Colon carcinoma
WT1 (11p13)–*EWS* (22q12)	(11;22)(p13;q12)	Desmoplastic small round cell tumor

Source: From R Hesketh: *The Oncogene and Tumour Suppressor Gene Facts Book*, 2nd ed. San Diego, Academic Press, 1997; with permission.

The first reproducible chromosome abnormality detected in human malignancy was the Philadelphia chromosome detected in CML. This cytogenetic abnormality is generated by reciprocal translocation involving the *ABL* oncogene on chromosome 9, encoding a tyrosine kinase, being placed in proximity to the *BCR* (breakpoint cluster region) gene on chromosome 22. Figure 101e-3 illustrates the generation of the translocation and its protein product. The consequence of expression of the *BCR-ABL* gene product is the activation of signal transduction pathways leading to cell growth independent of normal external signals. Imatinib (marketed as Gleevec), a drug that specifically blocks the activity of Abl tyrosine kinase, has shown remarkable efficacy with little toxicity in patients with CML. It is hoped that knowledge of genetic alterations in other cancers will likewise lead to mechanism-based design and development of a new generation of chemotherapeutic agents.

CHROMOSOMAL INSTABILITY IN SOLID TUMORS

Solid tumors are generally highly aneuploid, containing an abnormal number of chromosomes; these chromosomes also exhibit structural alterations such as translocations, deletions, and amplifications. These abnormalities are collectively referred to as chromosomal instability (CIN). Normal cells possess several cell cycle checkpoints, essentially quality-control requirements that have to be met before subsequent events are allowed to take place. The mitotic checkpoint, which ensures proper chromosome attachment to the mitotic spindle before allowing the sister chromatids to separate, is altered in certain cancers. The molecular basis of CIN remains unclear, although a number of mitotic checkpoint genes are found mutated or abnormally expressed in various tumors. The exact effects of these changes on the mitotic checkpoint are unknown, and both weakening and overactivation of the checkpoint have been proposed. The identification of the cause of CIN