Primer on Medical Genomics
Part XI: Visualizing Human Chromosomes

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In the past century, various methods to visualize human chromosomes were discovered. Chromosome analyses provide an overall view of the human genome that cannot be achieved with any other approach. The methods to visualize chromosomes include various techniques to produce bands along chromosomes, specialized procedures for specific disorders, and fluorescent-labeled DNA for targeted loci. Cytogenetic methods guide the study of the relationship between chromosome structure and gene function. They also aid in mapping locations of genes and identifying chromosome anomalies associated with medical disorders. The clinical diagnosis, prognosis, and response to treatment can be established for many malignant diseases. Cytogenetic methods provide an important diagnostic tool for clinical practice.

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AT = adenine-thymine; BrdU = 5-bromodeoxyuridine; FISH = fluorescence in situ hybridization; GC = guanine-cytosine; NOR = nucleolar organizing region; RBA = replication bands visualized with acridine orange stain; SCE = sister chromatid exchange

The term *chromosome* was coined by Waldeyer[1] in 1888 from the Greek words for colored body, *chroma* and *soma*. These cytological structures are the workbench on which genes are transcribed and DNA is replicated. During mitosis, the chromosomes divide and deliver appropriate copies of genetic material to somatic daughter cells. During meiosis, homologous chromosomes exchange material to provide opportunities for genetic recombination and form haploid gametes to ensure a constant chromosome number for the species. Cytogenetics, the study of structure and function of chromosomes, is important in medical practice today. This article describes various methods to visualize human chromosomes and discusses some applications of cytogenetic studies in clinical practice.

CHROMOSOME STRUCTURE

Chromosomes appear differently in interphase and metaphase stages of the cell cycle (Figure 1). In interphase, the morphology of individual chromosomes is not apparent because their chromatin is decondensed. As cells enter division, the chromatin condenses, and by metaphase, the morphology of individual chromosomes can be visualized. Each metaphase chromosome has 2 chromatids held together by the centromere (Figure 2). The centromere separates each chromosome into a short arm and long arm referred to as a p-arm and a q-arm, respectively.[2] In metacentric chromosomes, the centromere appears at or near the center of the chromosome. In submetacentric chromosomes, the centromere is nearer to one end of the chromosome. In acrocentric chromosomes, the centromere is nearly at the terminal end of the chromosome. Acrocentric chromosomes often have satellites on their p-arm (Figure 2). These satellites have stalks that contain genes that produce ribosomal RNA.

During cell division, the kinetochore forms within the centromeric region and is the site of spindle fiber microtubule attachment.[3] This structure can be visualized by using fluorescent-labeled monoclonal antibodies for the kinetochore (Figure 3).[4] The ends of the chromosomes are maintained by telomeres, which consist of TTAGGG sequence repeats. These structures can be visualized using fluorescent-labeled DNA probes specific for TTAGGG repeats (Figure 4).[5]

Using scanning electron microscopy, the chromatin of each chromosome has the appearance of a fibrouslike structure (Figure 5). The chromatin of each chromosome consists of a long continuous strand containing one double helix of DNA. The DNA is packed into each chromosome in a highly efficient manner to allow for transcription of single genes, gene regulation, replication, pairing of homologous chromosomes during meiosis, meiotic crossing over, and other functions.

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A glossary of terms frequently used in cytogenetic studies appears on page 75.

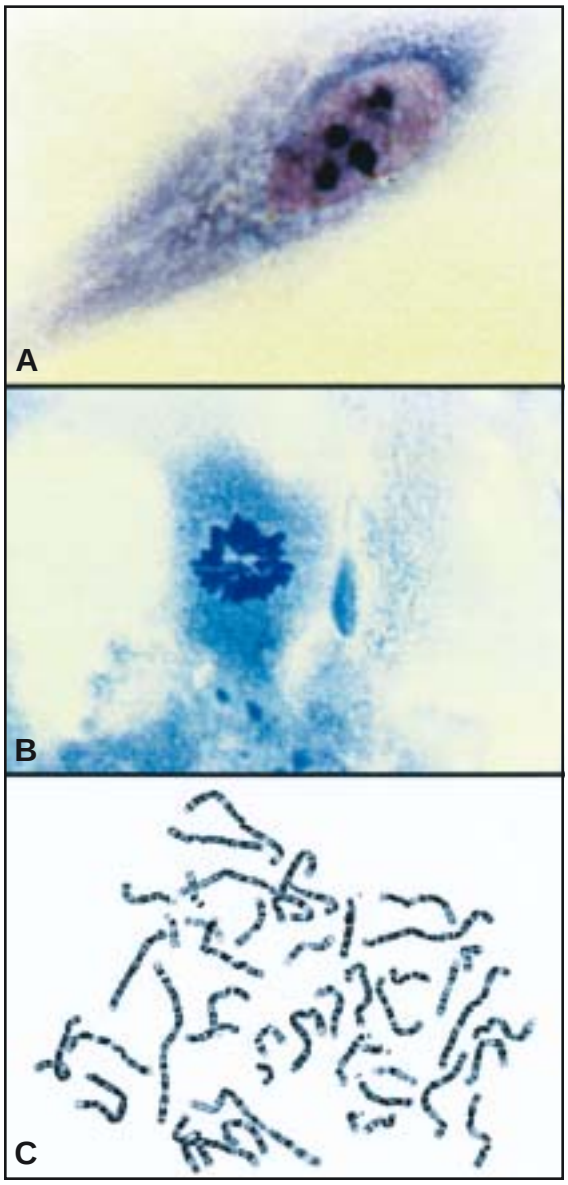

Figure 1. A, Fibroblast in interphase that contains 5 dark staining nucleoli within the nucleus. B, Chromosomes of a fibroblast aligned on a metaphase plate and viewed from a polar orientation. C, Metaphase chromosomes that have been treated with the spindle fiber inhibitor colcemid.

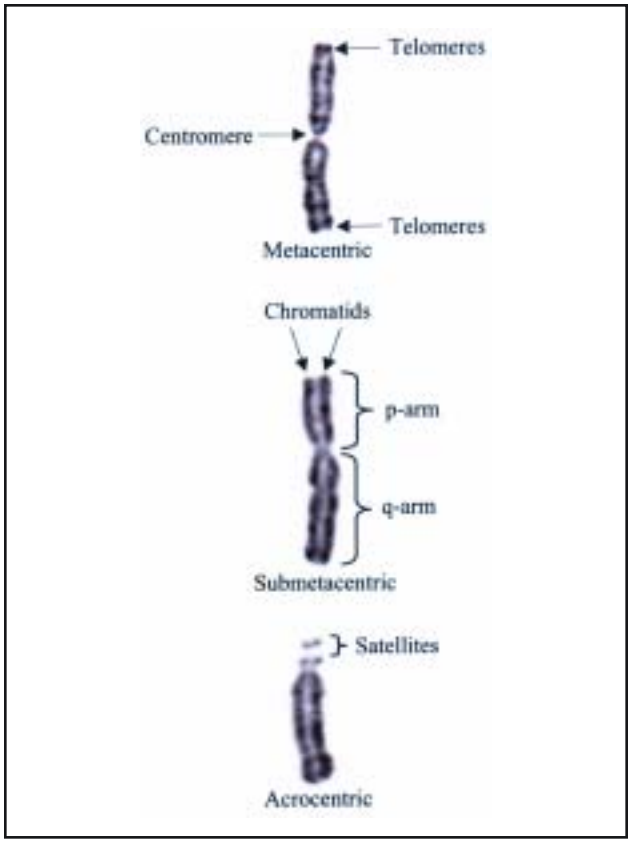

Figure 2. Representative human metaphase chromosomes. Top, Metacentric chromosome shows the location of the centromere and telomeres. Middle, Submetacentric chromosome shows 2 chromatids and the short arm (p-arm) and long arm (q-arm). Bottom, Acrocentric chromosome shows the satellite region.

CHROMOSOME ANOMALIES

Chromosome anomalies can be either constitutional or acquired. Constitutional anomalies cause birth defects, infertility, and other medical problems. Most acquired chromosome anomalies that develop after birth are associated with neoplastic processes.

Chromosome anomalies are classified as numeric or structural. Numeric anomalies are further subclassified into polyploid or aneuploid (Figure 6). Ploidy refers to the number of haploid sets of chromosomes, in which a haploid set in humans is 23 chromosomes. This is the expected number of chromosomes in a gamete, whereas the diploid number, 46 chromosomes, is expected in somatic cells. Triploidy refers to 69 chromosomes, and tetraploidy refers to 92 chromosomes. Triploidy and tetraploidy have been observed in molar pregnancies and spontaneously aborted fetuses. Polyploidy is also associated with neoplastic disorders, although the set of chromosomes is not always complete.

Aneuploidy refers to chromosome complements that involve irregular multiples of the haploid chromosome number (Figure 6). The addition of a single chromosome is called *trisomy*, and the loss of a single chromosome is called *monosomy*. Thus, any cell that has trisomy 13 is characterized by 47 chromosomes, including 3 copies of chromosome 13. A cell that has monosomy 7 contains 45 chromosomes and is lacking one copy of chromosome 7. Aneuploid anomalies usually occur as a consequence of

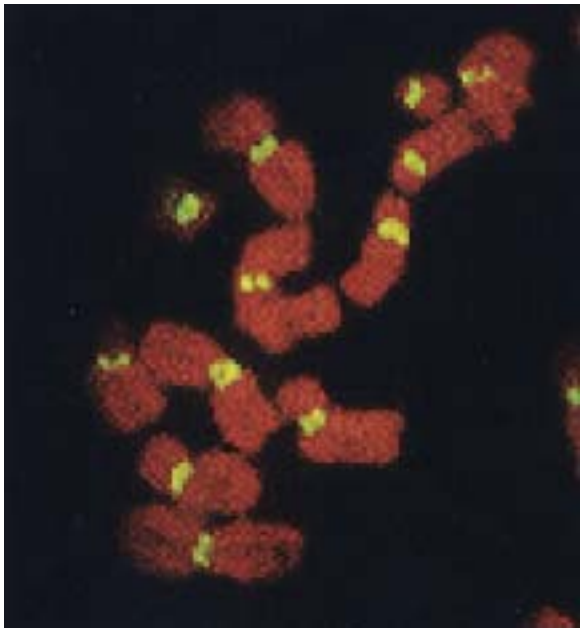

Figure 3. Bright staining structures at the centromeres are the sites of spindle fiber attachment called *kinetochores*. Fluorescent-labeled monoclonal antibodies were used for the kinetochores.

mitotic malfunction, such as chromosome nondisjunction. Gains of only a few specific autosomes (most commonly chromosome 21) or sex chromosomes are compatible with life. The only viable congenital monosomy is the loss of a sex chromosome, resulting in 44 autosomes and 1 X chromosome. Numeric abnormalities generally originate spontaneously; thus, the recurrence risk is usually minimal. Acquired additions and losses of chromosomes are common among clonal neoplastic disorders.

Figure 4. Yellow signals at the ends of each chromosome are fluorescent-labeled DNA probes for repetitive TTAGGG telomere sequences.

Anomalies of chromosome structure are classified as translocations, deletions, inversions, duplications, or isochromosomes (Figure 6). Structural anomalies can be present in either balanced or unbalanced forms. Reciprocal translocations involve the interchange of parts of different chromosomes. Inversions produce a reversal in the direction of an interstitial part of a chromosome and are either pericentric or paracentric. Pericentric inversions involve both the short arm and the long arm of a chromosome, whereas paracentric inversions occur in only 1 arm of a chromosome. Deletions involve loss of part of a chromosome and are either terminal or interstitial. Duplications produce 2 or more copies of a particular DNA segment on the same chromosome. Structural abnormalities may either arise spontaneously or be inherited. For de novo structural anomalies, the risk of recurrence is minimal. For most inherited structural anomalies, the recurrence risk ranges from 5% to 50% depending on the type of anomaly and the chromosomes involved.

EARLY MILESTONES IN THE PREBANDING ERA

Before 1970, methods to visualize chromosomes used nucleoprotein stains like Giemsa to produce uniformly stained chromosomes. This method is called *nonbanding*. The method of preparing karyotypes was first introduced for humans on the basis of nonbanded preparations. A karyotype is the organized arrangement of chromosomes from a single metaphase (Figure 7). The human karyotype contains 22 pairs of chromosomes called *autosomes* and 1 pair of sex chromosomes. Females have 2 X chromosomes, and males have 1 X and 1 Y chromosome.

In 1923, Painter[6] indicated that human somatic cells have 48 chromosomes. Because it was difficult to make good chromosome preparations, 3 decades passed before the correct chromosome number was established. In 1956, Tjio and Levan[7] used a hypotonic solution to process fetal lung cells and show that humans have 46 chromosomes. During the next few years, the association of trisomy 21 with Down syndrome,[8] 45,X with Turner syndrome,[9] 47,XXY with Klinefelter syndrome,[10] and the basis of XXX syndrome[11] were established. In 1960, a small abnormal chromosome 22 was identified in neoplastic cells from patients with chronic myeloid leukemia.[12] Subsequently, this chromosome anomaly became known as the *Philadelphia chromosome*. In clinical practice, nonbanding methods have been primarily supplanted by banding techniques. Nevertheless, nonbanding is still useful in combination

with banding techniques for confirming certain subtle structural anomalies, for visualizing active centromeres, for scoring breaks and gaps, and for detecting fragile sites on chromosomes (Figure 8).

THE BANDING ERA

In the early 1970s, various banding techniques were discovered that allowed for accurate pairing and identification of each chromosome in the human karyotype.[13] With these new staining methods, it was possible to subdivide chromosomes into individual bands, each with a characteristic location, size, and staining intensity. Thus, more than 400 chromosome bands could be recognized in each haploid set of human chromosomes. The banding techniques allowed detection of subtle structural abnormalities and identification of specific chromosomes involved in aneuploid conditions. The bands also became important for precise mapping of genes to specific chromosomal loci. Several common methods to visualize banded chromosomes are discussed subsequently.

Q-banding

Caspersson et al[14] discovered one of the first chromosome banding techniques. This method involves staining chromosomes with a fluorochrome, such as quinacrine mustard or quinacrine dihydrochloride, and examining them with fluorescence microscopy. The Q-bands appear along each chromosome in alternating bright and dull bands with varying intensity (Figure 9).

Q-banding is produced by unequal distribution of adenine-thymine (AT) and guanine-cytosine (GC) pairs throughout each chromosome. The brighter Q-bands are thought to contain more AT nucleotides than the duller fluorescent bands, which are richer in GC nucleotides. Cytogeneticists believe that most functional genes lie within the dull fluorescing bands (euchromatin) rather than in the brightly fluorescing bands (heterochromatin).

Q-banding is useful for establishing the specific nature of almost any numeric or structural chromosome anomaly. The Y chromosome is easy to recognize by Q-banding because the distal band of the q-arm fluoresces brightly (Figure 9). The brightly fluorescing Yq band is highly polymorphic and is not present in about 2% of healthy males. Thus, this region probably does not contain clinically important genes.

G-banding

G-banding techniques were introduced in the early 1970s and are now the most common chromosome staining procedures. The most frequently used method to produce G-bands involves treating the chromosomes with trypsin and staining with a Giemsa solution.[15] This procedure pro-

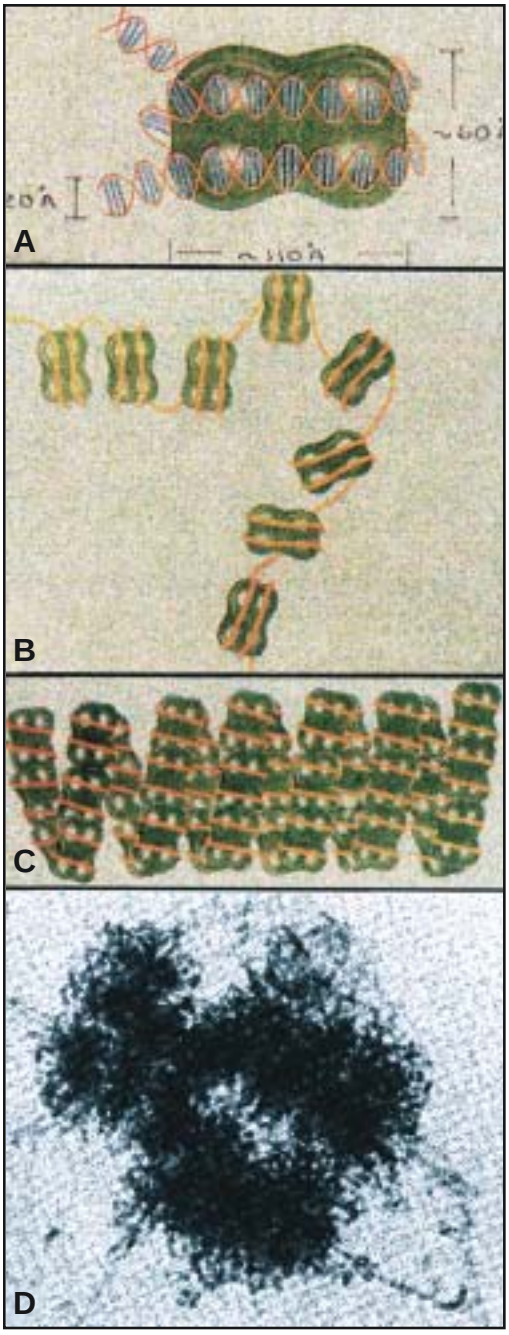

Figure 5. A, Within each chromosome, the DNA is wrapped around a set of 2 tetramers, each made up of 1 molecule each of H2a, H2b, H3, and H4 histones. Each histone tetramer complex and its associated DNA are called a *nucleosome*. Each nucleosome contains approximately 200 DNA base pairs that encircle the histone tetramers 1.5 times. B, Series of nucleosomes produces the appearance of "beads on a string," which traverses the entire length of a chromosome. C, Nucleosomes form a solenoid-like structure that is held together by H1 histones. D, Acrocentric chromosome viewed with scanning electron microscopy showing the fibrous-appearing arrangement of chromatin throughout the chromosome. D, From R. J. Green, Center for Disease Control, Atlanta, Ga.

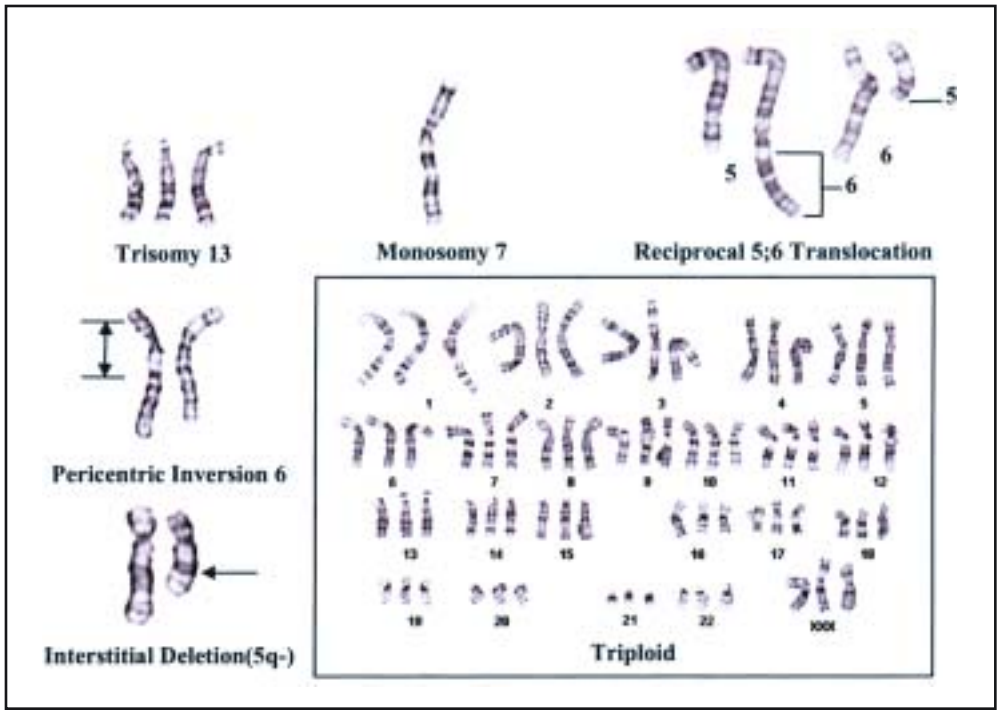

Figure 6. Chromosome anomalies.

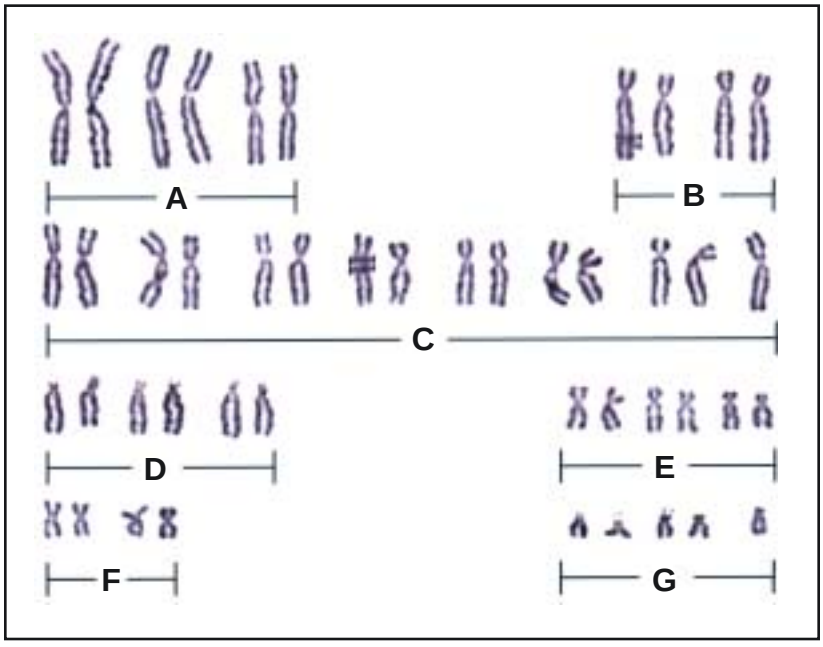

Figure 7. Karyotype preparation based on chromosome morphology from a nonbanded metaphase. Homologous chromosomes are paired and classified into 7 groups (A-G) on the basis of their size and centromere location. Although not all chromosomes can be accurately paired because the morphology of many chromosomes is similar, it is frequently possible to accurately pair homologous chromosomes 1, 2, 3, 9, 16, 17, and 18 and to identify Y in nonbanded preparations.

duces a series of alternating dark and light bands along each chromosome. The band pattern is similar for homologous chromosomes but is different for nonhomologues. Thus, G-band patterns can be used to pair and identify each of the human chromosomes accurately (Figure 10).

G-bands are distinct, permanent, and easy to photograph, and they can be visualized with an ordinary light microscope. The mechanism of G-banding is related to the basic structure of chromosomes. The exposure of chromosomes to trypsin most likely results in unequal extraction of DNA and proteins from the GC-rich regions. This alteration occurs differently along each chromosome and leads to the appearance of dark AT-rich bands and light GC-rich bands.

Fewer G-bands are observed in contracted short mid-metaphase chromosomes than among longer chromosomes in earlier cell division (Figure 11). This finding eventuates because as chromosomes become more contracted, small subbands appear to coalesce. The number of chromosome bands in a haploid set of mid-metaphase chromosomes is approximately 400. With high-resolution banding methods, chromosomes of early metaphase and late prophase have more than 850 bands per haploid set of chromosomes.[16] This allows for more precise localization of genes and detection of subtle chromosome anomalies.

C-banding

Pardue and Gall[17] first reported C-bands in 1970 when they discovered that the centromeric region of mouse chromosomes is rich in repetitive DNA sequences and stains dark with Giemsa. Arrighi et al[18,19] reported the same banding pattern for pericentromeric regions and the distal part of the Y q-arm of human chromosomes. Comings et al[20,21] suggested that the staining pattern may be a consequence of less DNA extraction in C-band regions than in non–C-band regions. Various methods can be used to produce C-bands. One common method involves treating the chromosomes with hydrochloric acid, barium hydroxide, and salt solutions at a high temperature and then staining the chromosomes with Giemsa (Figure 12, A). Another method involves staining chromosomes with the fluorescent dyes distamycin A and 4′,6-diamidino-2-phenylindole dihydrochloride (Figure 12, B).

Many chromosomes have regions that differ among individuals but have no pathological importance. These polymorphic regions can be visualized optimally with C-band methods and are most often seen on acrocentric chromosomes, the centromeric region of chromosomes 1, 9, and 16, and the distal portion of the Y chromosome. C-banding is also useful to show chromosomes with multiple centromeres, to study the origin of diploid molar pregnancies and true hermaphroditism, and to distinguish between donor and recipient cells in bone marrow transplantation.

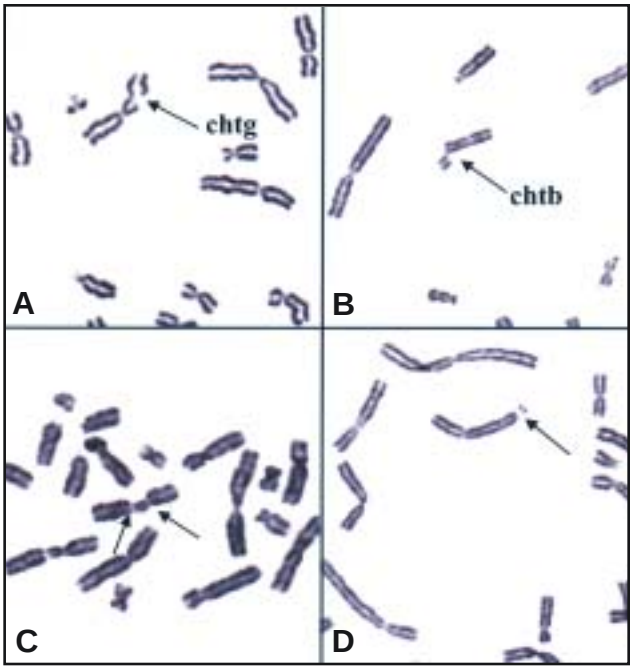

Figure 8. Nonbanded chromosomes showing a chromatid gap (chtg) (A), chromatid break (chtb) (B), dicentric chromosome (C), and fragile site (D).

Nucleolar Organizing Region–Banding

Nucleolar organizing region (NOR)–banding is a technique that stains NORs of chromosomes.[22] These regions are located in the satellite stalks of acrocentric chromosomes and house genes for ribosomal RNA. NOR-bands

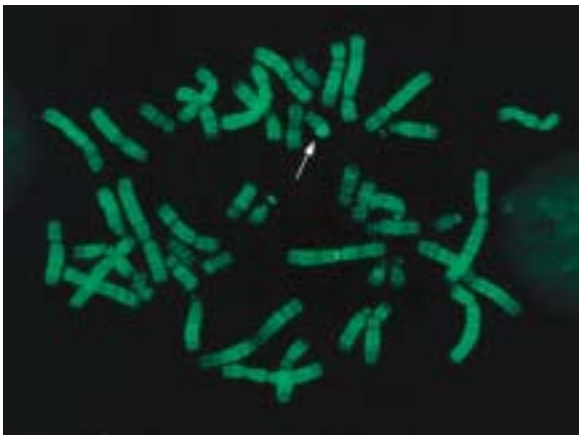

Figure 9. Q-banded metaphase from a healthy male with the Y chromosome (arrow). The characteristic banding pattern can be used to identify each chromosome accurately.

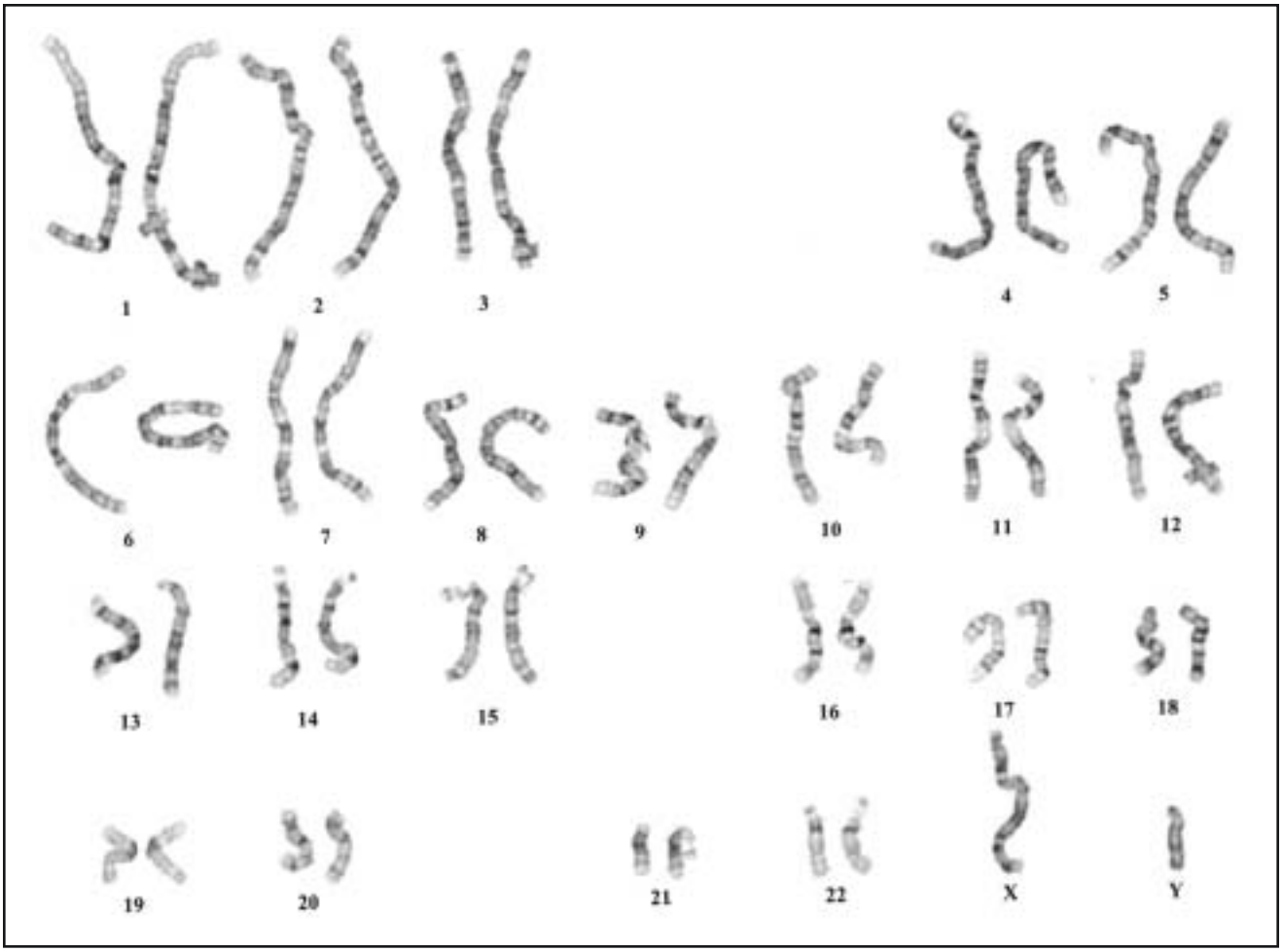

Figure 10. Representative G-banded karyotype. G-banding is widely used in clinical practice because it produces distinct permanent bands that allow identification of all human chromosomes and accurate characterization of numeric and structural anomalies.

may represent structural nonhistone proteins that are specifically linked to NOR and bind to ammoniacal silver. Goodpasture et al[23,24] developed a simple silver nitrate staining technique that is now used widely.

Not all satellite stalks stain NOR-positive. The clinical importance of NOR-negative staining in a seemingly structurally normal acrocentric chromosome is unknown. NOR-banding is useful in clinical practice to study certain chromosome polymorphisms, such as double satellites (Figure 13). This method is also helpful to identify satellite stalks that are occasionally seen on nonacrocentric chromosomes.

Replication Bands

Viable cells in humans require one functional X chromosome. Any additional X chromosomes become inactivated in early embryogenesis and replicate late in subsequent cell divisions. In interphase cells, late-replicating X chromosomes can be observed as condensed chromatin structures attached to the nuclear membrane (Figure 14). In metaphase cells, late-replicating X chromosomes can be identified by replication bands visualized with acridine orange stain (RBA) (Figure 15).[25,26] To produce RBAs, 5-bromodeoxyuridine (BrdU) is added to a cell culture for 3 to 12 hours before the cells are harvested for chromosome studies. Then, BrdU is incorporated into DNA in place of thymidine during replication. This procedure creates a bandlike pattern along each chromosome because the dark G-bands replicate later than the light G-bands.[27] Since late-replicating chromosome bands contain mostly inactivated genes, RBA can be useful in clinical practice to study the activation status of chromosome regions on structurally abnormal X chromosomes in congenital disorders.

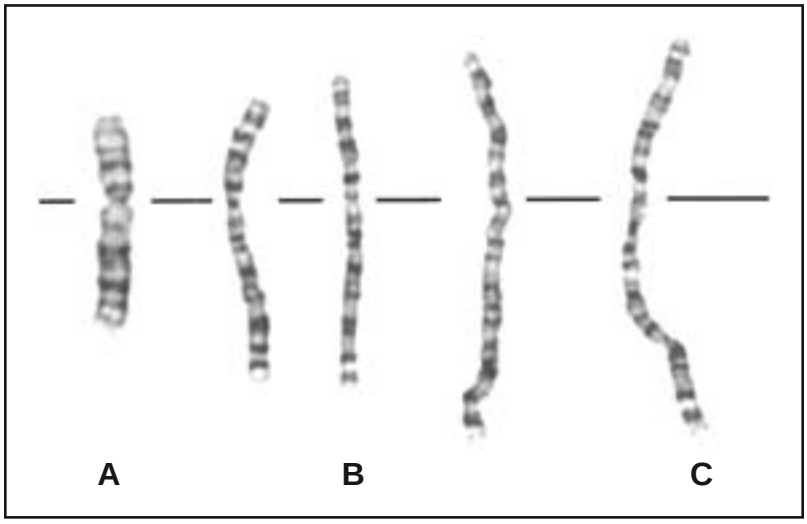

Figure 11. G-band pattern of chromosome 2 in different stages of contraction. Chromosome A represents the 400-band level; chromosome B, the 550-band level; and chromosome C, the 850-band level. The band level is based on the total number of bands on the chromosomes of a haploid set of chromosomes.

SPECIALIZED METHODS TO VISUALIZE CHROMOSOMES

Sister Chromatid Exchange

Chromosomes can be stained to observe the exchange of genetic material between sister chromatids.[28-30] Sister chromatid exchange (SCE) staining is accomplished in cell cultures by incorporating BrdU (in place of thymidine) into replicating cells for 2 cell cycles. As a result of semiconservative DNA replication, chromosomes have one chromatid with BrdU in one strand of DNA, whereas the other chromatid has BrdU in both strands of DNA. This produces an acridine fluorescence pattern in which one chromatid fluoresces more brightly than the other chromatid (Figure 16). Sister chromatid exchanges appear as an interchange between sister chromatids of brightly and dully fluorescent segments. Exchanges between sister chromatids should not be confused with crossing over because the former occurs in somatic cells and involves chromatids of the same chromosome, whereas the latter occurs in germ cells and involves exchange of genetic material between homologous chromosomes.

The biologic importance of SCEs is uncertain, but some mutagens and carcinogens increase their frequency.[31] Thus, the SCE staining method is sensitive for detecting the

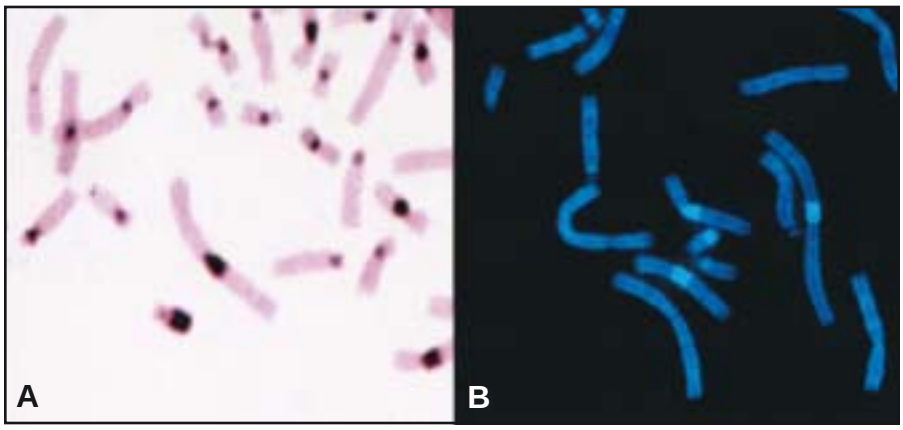

Figure 12. Representative partial metaphases illustrating C-bands with barium hydroxide using Giemsa (A) and distamycin A and 4′,6-diamidino-2-phenylindole dihydrochloride (B).

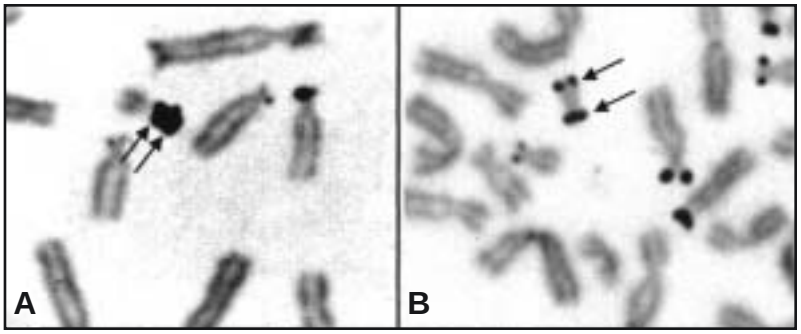

Figure 13. Representative nucleolar organizing region–banded partial metaphases illustrating double satellites (arrows) (A) and an isodicentric chromosome with satellites at both ends (arrows) (B).

effects of certain carcinogens on the chromosomes.[32,33] Also, SCE studies are useful for diagnosing Bloom syndrome because patients with this genetic disorder have a 10-fold higher SCE frequency than healthy individuals.[34,35]

Fragile Sites and Chromosome Breakage

Certain uncondensed portions of DNA in chromosome structure can be visualized as gaps in the staining pattern, and these gaps are prone to chromosome breakage. Gaps that are consistently seen at the same chromosome locus are called *fragile sites*. Fragile sites can be induced by modifying the culture media in ways that interfere with DNA synthesis and are best visualized in chromosomes by using nonbanding or Q-banding methods (Figure 8). Some fragile sites are present in healthy individuals.[36] Other fragile sites are associated with specific medical conditions such as fragile X syndrome, which is associated with a fragile site at Xq27.3.[37-39]

All humans experience increased chromosome breakage when exposed to cytotoxic agents. However, certain autosomal recessive disorders result in the inability to repair DNA associated with certain kinds of chromosome damage. Collectively, these conditions are referred to as *chromosome breakage syndromes*. For example, the chromosomes of patients with Fanconi anemia are especially sensitive to bifunctional alkylating agents such as diepoxybutane or mitomycin C.[40-42] In patients with Fanconi anemia, these agents produce excessive chromosome breakage, including a specific type of chromosome anomaly called *radial configurations* (Figure 17).

FLUORESCENCE IN SITU HYBRIDIZATION

In 1969, Pardue and Gall[43] used radioactive DNA probes for highly repetitive sequences of mice and showed that these sequences hybridized with centromeric regions of

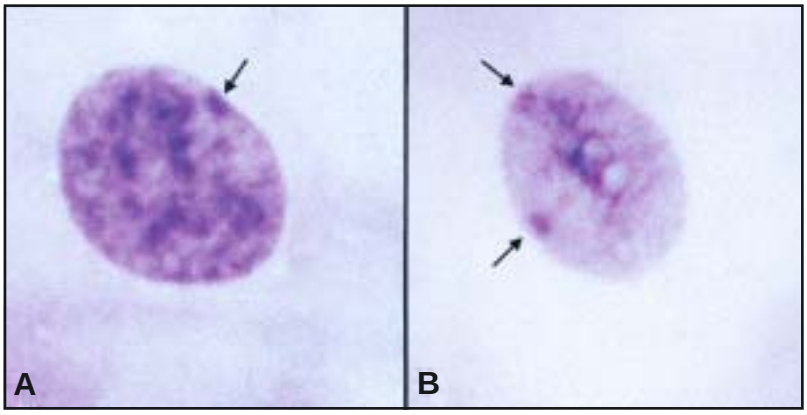

Figure 14. Dark staining chromatin structures on nuclear borders are late-replicating X chromatin. In older literature, these structures were referred to as *Barr bodies*, but the official cytogenetic name is X-chromatin bodies. A, One X-chromatin body in a cell with 2 X chromosomes (arrow) from a healthy female. B, Abnormal cell with 3 X chromosomes showing 2 X-chromatin bodies (arrows).

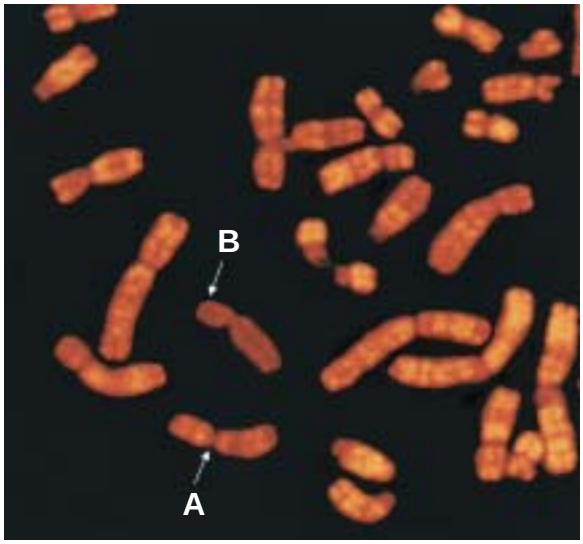

Figure 15. Representative metaphase showing the different replication band patterns using acridine orange stain for an early-replicating X chromosome (A) and a late-replicating X chromosome (B). The late-replicating chromosome in this metaphase lacks a small portion of the p-arm.

chromosomes. By 1986, Pinkel et al[44,45] developed a method to visualize chromosomes using fluorescent-labeled probes that is called *fluorescence in situ hybridization* (FISH). FISH experts refer to their field as molecular cytogenetics because their work crosses the fields of molecular genetics (DNA probes) and cytogenetics (evaluation of chromosomes).[46]

FISH can be performed on either metaphase or interphase cells and involves denaturing genomic DNA by using heat and formamide. Slide preparations are flooded with chromosome-specific DNA sequences attached to colored fluors and incubated at 37°C. During this time, probe DNA anneals with complementary DNA sequences in the chromosomes. The presence or absence of FISH signals is observed with a fluorescence microscope.

FISH probes are generally classified by where they hybridize in the genome or by the type of chromosome anomaly they detect. These techniques are useful in the work-up of patients with various congenital and malignant neoplastic disorders, especially in conjunction with conventional chromosome studies.

Centromere-Specific FISH Probes

Although most centromeric regions of human chromosomes consist of highly repetitive α-satellite DNA, there are unique α-satellite DNA sequences for most chromosomes. FISH probes for the repetitive centromeric DNA hybridize to all centromeres,[47] whereas FISH probes for unique DNA sequences hybridize to specific chromosomes (Figure 18).[48,49] Although FISH probes are available to detect each centromere, in most instances chromosome 13 centromeres cannot be distinguished from chromosome 21 centromeres, and chromosome 14 centromeres cannot be distinguished from chromosome 22 centromeres. Centromere FISH probes can be used to detect numeric anomalies, verify the presence of centromeres in structurally abnormal chromosomes, and serve as internal controls for other FISH probe strategies.

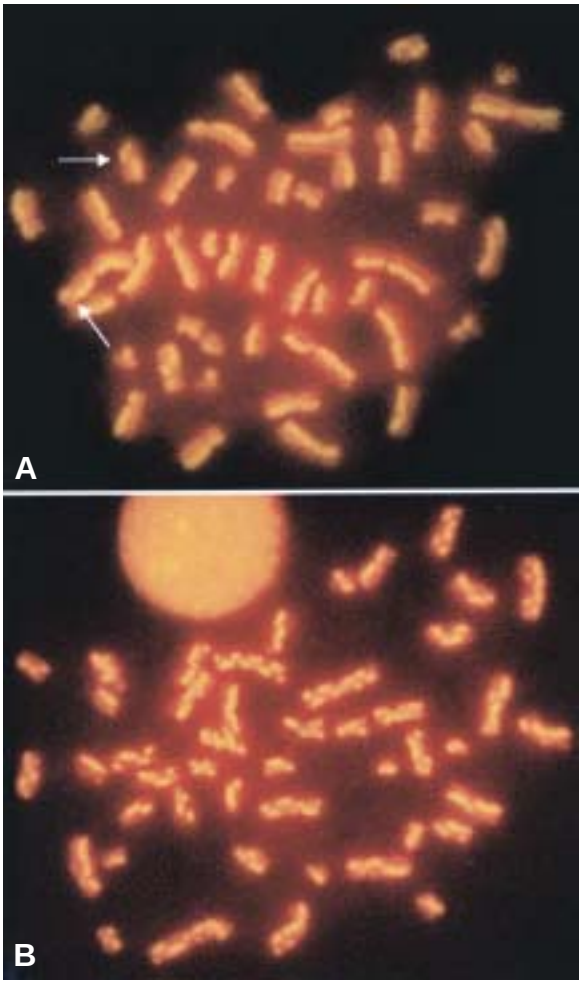

Figure 16. Metaphases with chromatids differently stained by acridine orange to reveal sister chromatid exchanges. A, Metaphase from a healthy individual. Arrows mark 2 chromosomes that have sister chromatid exchanges. B, Metaphase with increased sister chromatid exchanges in a cell from a patient with Bloom syndrome.

Microdeletion and Microduplication FISH Probes

Certain microdeletions and microduplications are difficult, sometimes impossible, to detect using routine cytogenetic analysis. FISH probes for these small chromosome

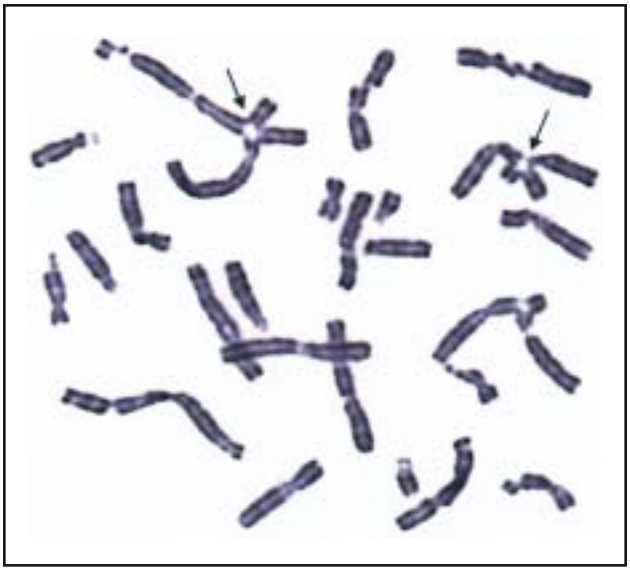

Figure 17. Mitomycin C exposure in a partial metaphase from a patient with Fanconi anemia. Metaphase shows chromosome breakage and radial formations (arrows).

anomalies may involve the actual gene or a critical region surrounding the gene (Figure 19).[50] A good FISH strategy to detect these anomalies uses a probe of one color for a control target and a probe of another color for the disease target. With this strategy, many microdeletion and microduplication syndromes have been identified (Figure 20).[51-53]

Telomere and Subtelomere FISH Probes

Early FISH probes for telomeric regions of chromosomes hybridized to the repetitive TTAGGG sequences.

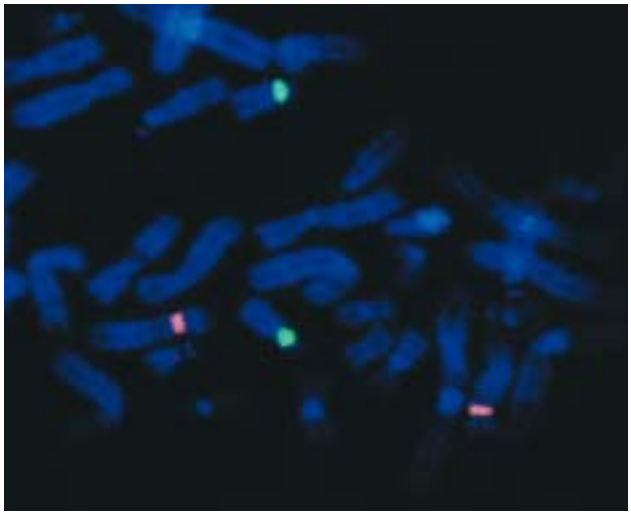

Figure 18. Centromeric fluorescence in situ hybridization probes for chromosome 12 (red) and 18 (green) in a partial metaphase cell.

Since these DNA sequences are the same at the ends of all chromosomes, all telomeres are visible at the same time (Figure 4). By using FISH probes for unique DNA sequences adjacent to each telomere, it is possible to identify accurately the "ends" of each chromosome except for the short arms of the acrocentric chromosomes.[54,55] These subtelomeric probes can be used to detect subtle chromosome deletions, translocations, inversions, and other chromosomal rearrangements involving the terminal ends of chromosomes. Subtelomeric abnormalities have been identified in individuals with dysmorphic features and/or birth defects (Figure 21).

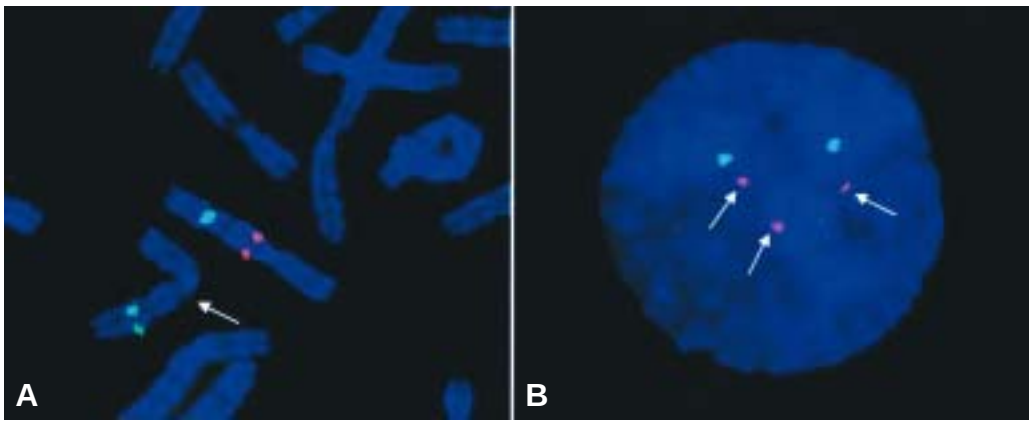

Figure 19. A, Partial metaphase showing a microdeletion of the elastin gene (*ELN*) (arrow) associated with Williams syndrome. B, Interphase cell shows a microduplication of the peripheral myelin protein 22 gene (*PMP22*) (arrows) associated with Charcot-Marie-Tooth syndrome.

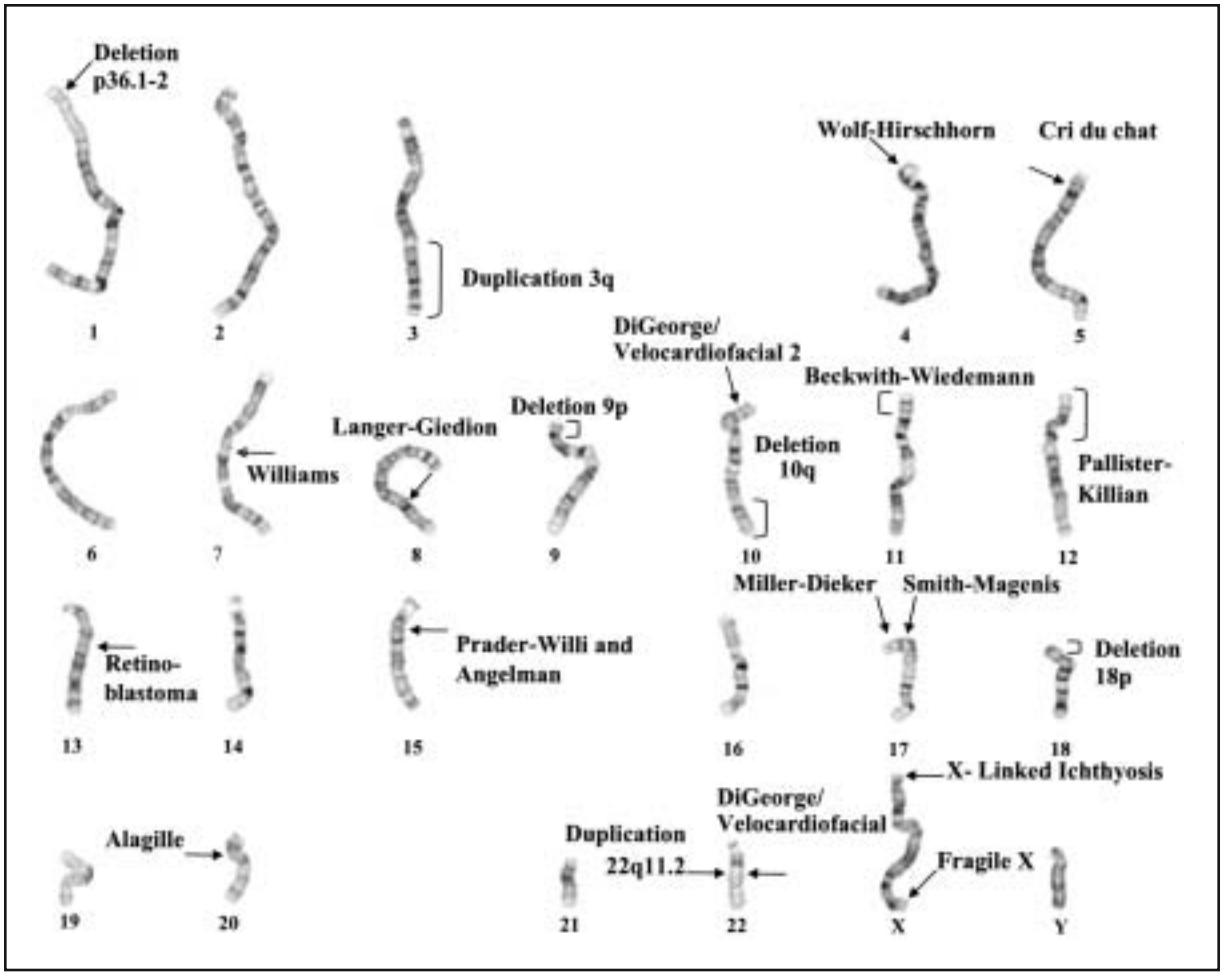

Figure 20. Haploid karyotype indicating the loci of selected congenital cytogenetic disorders.

FISH to Detect Translocations, Inversions, and Amplification in Neoplastic Disorders

Various FISH probes are available for visualizing chromosome anomalies associated with oncogenes.[56] FISH can be used to observe multiple signals of an oncogene in individual cells as a result of gene amplification (Figure 22).[57-59] FISH assays can detect the pathogenic fusion of oncogenes and activating genes that results from certain chromosome translocations and inversions (Figure 23). Various FISH methods are available for visualizing chromosome anomalies associated with neoplastic disorders. More information on these FISH methods and their applications in clinical practice is available.[60,61] FISH is also useful for many neoplastic disorders (Table 1).[56]

Whole Chromosome Paints and Multicolor FISH

Chromosome painting methods are particularly useful for characterizing chromosome anomalies that are difficult to define with conventional cytogenetic studies.[62] Whole chromosome paints for specific chromosomes can be applied to metaphase cells to characterize major structural anomalies (Figure 24).[63,64] Multicolor FISH can be used to fluoresce each of the 24 human chromosomes simultaneously with a different color (Figure 25).

CYTOGENETIC NOMENCLATURE: VISUALIZING CHROMOSOMES WITHOUT PICTURES

The standardization of cytogenetic nomenclature enables cytogeneticists to communicate a visual image of the chromosome makeup of a cell or an individual without using pictures. Although nomenclature has evolved over time, the original basic concepts adopted in the 1960s have remained primarily unchanged. Current cytogenetic nomenclature is published as a single document entitled *ISCN 1995: An International System for Human Cytogenetic Nomenclature*.[2]

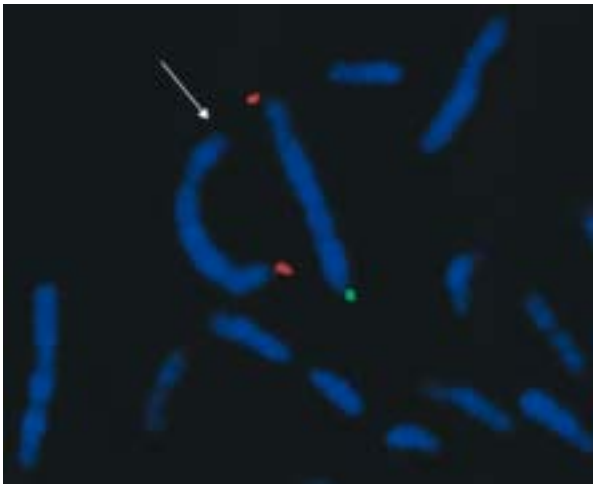

Figure 21. Partial metaphase showing loss (arrow) of the subtelomeric region of chromosome 5 p-arm.

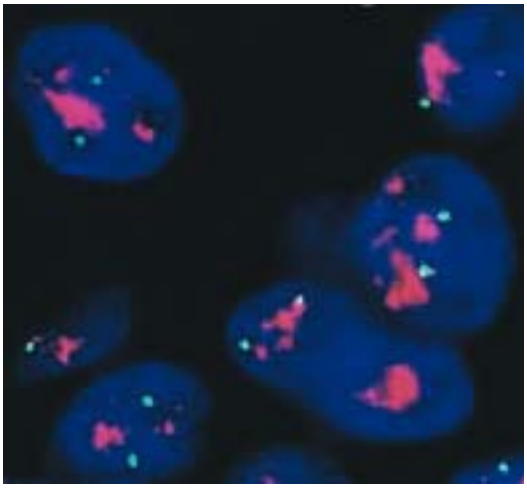

Figure 22. Interphase nuclei showing amplification of *HER2* oncogene in breast cancer. Green signals indicate the centromere for chromosome 17. Clusters of red signals indicate amplification of the *HER2* oncogene.

In the description of a karyotype, the total number of chromosomes (including the sex chromosomes) is listed first, followed by a comma and the sex chromosomes. Thus, nomenclature for a healthy male is 46,XY and a healthy female is 46,XX. The autosomes are specified only when an abnormality is present. For example, 47,XY,+21 indicates a male with trisomy 21 (Down syndrome). Some examples of cytogenetic expressions for chromosome abnormalities are shown in Table 2.

In 1971, nomenclature was expanded to describe banded chromosomes (Figure 26). Each chromosome arm is subdivided first into regions and then into bands.[65,66] The regions and bands are numbered on each arm from the centromere outward to the ends of the chromosome (telomeres). Wherever a band is subdivided, a number is assigned to each subband and is designated after a decimal point. Thus, the expression 1q32.2 (read as "one-q-three-two-point-two" rather than as "one-q-thirty-two point two") indicates a band located on chromosome 1, q-arm, region 3, band 2, and subband 2.

If standard cytogenetic analysis is performed concurrently with FISH, they are reported together and separated by a period. The FISH nomenclature begins with *ish* to indicate in situ hybridization and then the chromosome anomaly, if any, hybridization location, probes used, and

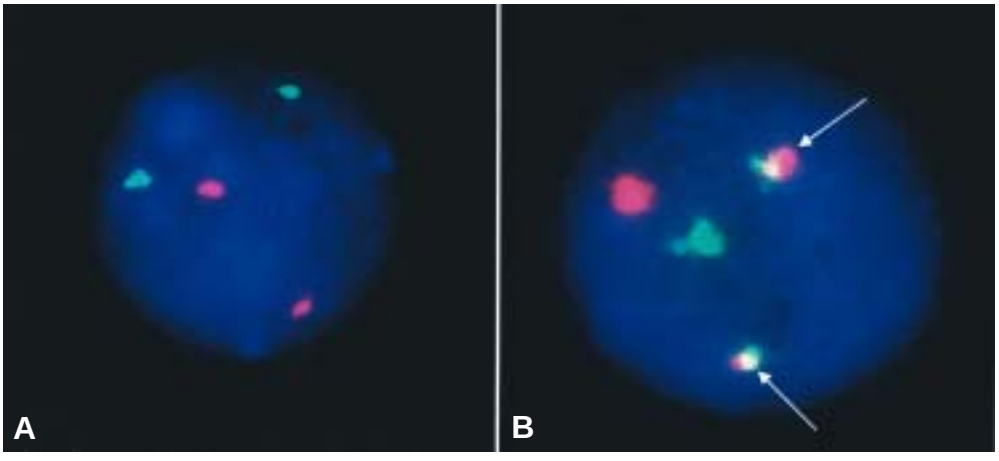

Figure 23. A, Interphase nucleus showing a normal signal pattern for *BCR* and *ABL*. B, Interphase nucleus shows 2 fusion signals (arrows) resulting from a reciprocal translocation involving *BCR* and *ABL* loci in chronic myeloid leukemia.

Table 1. **Common Chromosome Anomalies and Genes Associated With Specific Neoplastic Disorders***

Disorder	Abnormality	Gene(s)
Translocation		
CML, ALL, AML	t(9;22)(q34;q11.2) or variant (Philadelphia chromosome)	*BCR/ABL*
BL, ALL	t(8;14)(p24;q32)	*IgH/c-MYC*
AML, ALL	t(11;var)(q23;var)	*MLL*
AML	t(8;21)(q22;q22)	*ETO/AML1*
ALL (especially pediatric)	t(12;21)(p13;q22)	*TEL/AML1*
APL	t(15;17)(q22;q21)	*PML/RARα*
ALCL	t(2;5)(p23;q35)	*ALK/NPM*
MCL, MM	t(11;14)(q13;q32)	*IgH/CCND1*
FL, DLCL	t(14;18)(q32;q21)	*IgH/BCL2*
Deletions		
MDS, AML	del(5)(q13q33) or variant	…
CLL, LCL, ATL	del(6)(q14q23)	…
AML, MDS, t-AML	del(7)(q22q36)	…
AMM, PV, CML, MM, CLL, NHL	del(13)(q13q22)	…
Glioma	del(1p), del(19q)	…
Urothelial cancer	homozygous del(9)(p21)	…
Numeric abnormalities		
MDS, juvenile CMML	monosomy 7	…
AML, MDS, CMPD, CML	monosomy 8	…
Urothelial cancer	trisomy 3, 7, and/or 17	…
ALL	hyperdiploid karyotype	…
ALL	near haploid karyotype	…
Other		
AML	inv(16)(p13q22) or variant	*MYH11/CBFβ*
MDS, CMPD	i(17q)	*P53*
Breast cancer	17q11.2 amplification	*HER2*

* ALCL = anaplastic large cell lymphoma; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; AMM = agnogenic myeloid metaplasia; APL = acute promyelocytic leukemia; ATL = adult T-cell lymphoma; BL = Burkitt lymphoma; CLL = chronic lymphocytic leukemia; CML = chronic myeloid leukemia; CMML = chronic myelomonocytic leukemia; CMPD = chronic myeloproliferative disease; DLCL = diffuse large cell lymphoma; FL = follicular lymphoma; LCL = large cell lymphoma; MCL = mantle cell lymphoma; MDS = myelodysplastic syndrome; MM = multiple myeloma; NHL = non-Hodgkin lymphoma; PV = polycythemia vera; t-AML = therapy-related AML. Ellipses indicate gene(s) not known.

whether the FISH signal is present, absent, or rearranged. For example, the following nomenclature describes a healthy male karyotype and FISH for the elastin gene (*ELN*) showing a deletion of the 7q11.23 region (Figure 19, A): 46,XY.ish del(7)(q11.23q11.23)(ELN–).

The nomenclature for interphase FISH differs from that for metaphase FISH because results are preceded by *nuc ish*. For example, the following interphase FISH nomenclature indicates 3 copies of *ABL* and 3 copies of *BCR*, but 2 signals of *ABL* and *BCR* are connected (Figure 23, B): nuc ish 9q34(*ABL*x3)(*ABL* con *BCR*x1),22q11.2(*BCR*x3) (*ABL* con *BCR*x1).

The *ISCN 1995*[2] contains rules for describing the size and staining intensity of normal chromosome polymorphisms. The publication describes how to write chromosome breaks and gaps, quadriradials, and many other types of abnormalities. It contains directions for describing clones and subclones in neoplastic disorders. This document is sufficiently broad to offer suggestions for writing cytogenetic descriptions for meiotic chromosomes. The *ISCN 1995* also applies to certain other hominoid primates such as *Pan troglodytes*, *Gorilla gorilla*, and *Pongo pygmaeus*, and their band patterns are included in this document.

VISUALIZATION OF CHROMOSOMES IN CLINICAL PRACTICE

Cytogenetic studies for congenital disorders are used for a wide variety of purposes (Figure 20). Chromosome analysis can often identify the cause of mental retardation and birth defects, allowing for appropriate decision making based on prognoses. Prenatal studies can provide reassurance to families with increased risk for chromosome abnormalities and information to help them make difficult reproductive decisions. Identification of any balanced structural anomaly in an individual with infertility and/or miscarriages provides more options for specific assisted reproductive techniques.

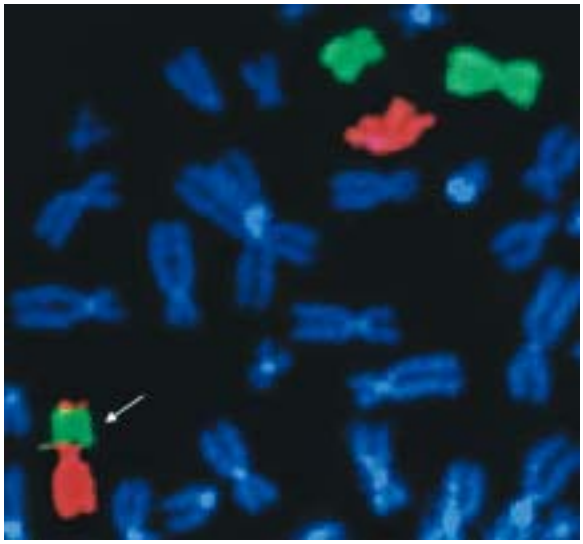

Figure 24. Whole chromosome paints used to identify an insertion of chromosome 11 into chromosome 10 (arrow).

Any congenital cytogenetic abnormality in a patient can have wide-reaching implications for family members. For example, an unbalanced translocation in a newborn with multiple birth defects should be followed up with chromosome studies of the parents to determine whether either carries the balanced version of their newborn's translocation. If one parent carries the translocation, the recurrence risk increases. In addition, their children, siblings, and extended family members may have infertility, risk of miscarriage, or children with birth defects and developmental delays.

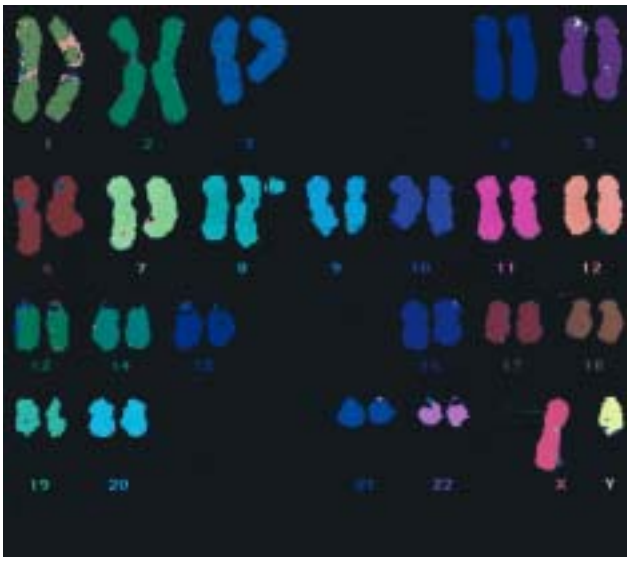

Figure 25. Karyotype prepared from a multicolor fluorescence in situ hybridization metaphase illustrating uniquely colored pairs of chromosomes and identification of a small ring chromosome as chromosome 8.

Table 2. **Examples of Cytogenetic Nomenclature and Interpretation**

Nomenclature	Interpretation
46,XY	Normal male; 46 chromosomes, including 1 X and 1 Y chromosome
46,XX	Normal female; 46 chromosomes, including 2 X chromosomes
45,X	45 chromosomes, including 1 sex chromosome
47,XXY	47 chromosomes, including 2 X chromosomes and 1 Y chromosome
47,XY,+21	47 chromosomes, including 1 X, 1 Y, and 3 chromosomes 21
mos 45,X/46,XX	Mosaic; some cells have 45 chromosomes including 1 X; other cells have 46 chromosomes including 2 X chromosomes
46,X,i(X)(q10)	Isochromosome of X long arm
46,X,idic(X)(q28)	Isodicentric chromosome X; break and fusion point at Xq28
46,XX,del(1)(q21)	Deletion of chromosome 1; break point at 1q21
46,XY,r(2)(p21q31)	Ring chromosome 2; break and fusion points at 2p21 and 2q31
46,XY,inv(2)(p21q31)	Inversion of chromosome 2; break points at 2p21 and 2q31
45,XX,t(9;22)(q34;q11.2)	Translocation involving chromosomes 9 and 22; break points at 9q34 and 22q11.2

Conventional cytogenetic studies are used widely by physicians to diagnose and manage hematologic malignancies. The observation of specific chromosome anomalies can help classify certain neoplasms and provide clues to disease progression (Table 1). Cytogenetic studies are useful for assessing the effectiveness of treatment, monitoring remission, detecting therapy-related leukemia, and establishing success of engraftment after bone marrow transplantation. Although these studies are performed for the purpose of identifying acquired abnormalities, they may identify congenital abnormalities that will have implications for family members.

FISH studies are useful for assessing both congenital and acquired abnormalities. These methods can be applied to virtually any tissue source and in some cases will provide results more rapidly than conventional chromosome studies. FISH is useful for identifying numeric abnormalities and characterizing structurally abnormal chromosomes. Prenatal FISH studies provide timely information about the presence or absence of common numeric abnormalities in a developing fetus. In hematologic disorders, FISH is useful for accurate diagnoses and for assessing response to treatment. For some disorders, FISH alone can provide a definitive result. However, FISH often does not provide the same degree of

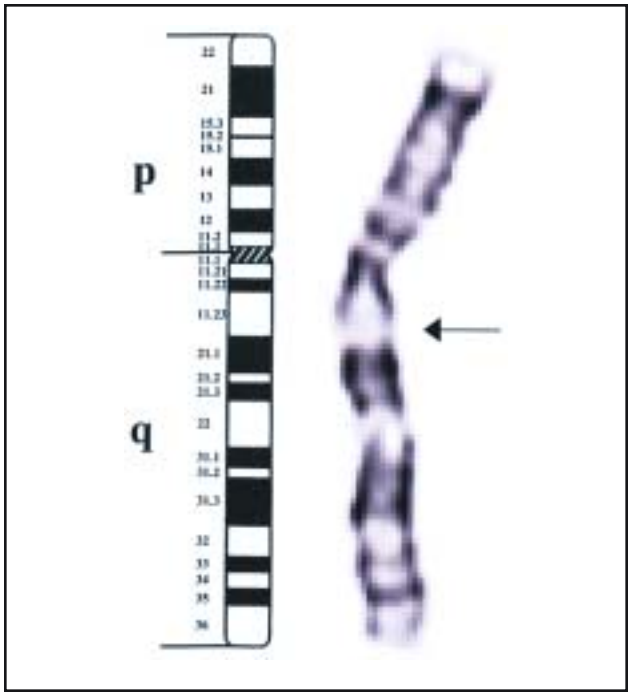

Figure 26. An idiogram from the *International System for Human Cytogenetic Nomenclature* for chromosome 7 at the 550-band stage (left) and aligned with a photograph of a G-banded chromosome 7 (right). Chromosome 7 p-arm is subdivided into 2 regions and the q-arm into 3 regions. Chromosome bands are numbered sequentially from the centromere outward to the ends of the chromosome (telomeres). The arrow indicates 7q11.23, which is read as "seven-q-one-one-point-two-three" rather than "seven-q-eleven-point twenty-three."

interpretive results as routine cytogenetics. Thus, complete chromosome analyses are typically needed.

COMMENT

This article specifically deals with visualizing chromosomes and is not intended to provide comprehensive information about applying cytogenetic studies in clinical practice. In routine clinical practice, physicians should rely on the expertise of genetic professionals, such as medical geneticists, genetic counselors, clinical cytogeneticists, and others. Readers who wish to learn more about the application of cytogenetics to clinical practice should refer to textbooks that deal with subject matter of their particular interest.[60,61]

REFERENCES

1. Waldeyer W. Ueber Karyokinese und ihre Beziehungen zu den Befruchtungsvorgängen. *Arch Mikr Anat*. 1888;32:1-122.
2. Mitelman F, ed. *ISCN 1995: An International System for Human Cytogenetic Nomenclature*. Basel, Switzerland: S Karger; 1995.
3. Earnshaw WC, Tomkiel JE. Centromere and kinetochore structure. *Curr Opin Cell Biol*. 1992;4:86-93.
4. Powell FC, Winkelmann RK, Venencie-Lemarchand F, Spurbeck JL, Schroeter AL. The anticentromere antibody: disease specificity and clinical significance. *Mayo Clin Proc*. 1984;59:700-706.
5. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci U S A*. 1988;85:6622-6626.
6. Painter TS. Studies in mammalian spermatogenesis, II: the spermatogenesis of man. *J Exp Zool*. 1923;37:291-336.
7. Tjio JH, Levan A. The chromosome number of man. *Hereditas*. 1956;42:1-6.
8. Lejeune J, Gautier M, Turpin R. Étude des chromosomes somatiques de neuf enfants mongoliens. *Compt Rend*. 1959;248:1721-1722.
9. Ford CE, Jones KW, Polani PE, de Almeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*. 1959;1:711-713.
10. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature*. 1959;183:302-303.
11. Jacobs PA, Baikie AG, Court Brown WM, MacGregor TN, Maclean N, Harnden DG. Evidence for the existence of the human "superfemale." *Lancet*. 1959;2:423-425.
12. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. *J Natl Cancer Inst*. 1960;25:85-109.
13. Dewald GW. Modern methods of chromosome analysis and their application in clinical practice. *Clin Lab Annu*. 1983;2:1-29.
14. Caspersson T, Zech L, Johansson C. Differential binding of alkylating fluorochromes in human chromosomes. *Exp Cell Res*. 1970;60:315-319.
15. Seabright M. A rapid banding technique for human chromosomes. *Lancet*. 1971;2:971-972.
16. Yunis JJ. High resolution of human chromosomes. *Science*. 1976;191:1268-1270.
17. Pardue ML, Gall JG. Chromosomal localization of mouse satellite DNA. *Science*. 1970;168:1356-1358.
18. Arrighi FE, Hsu TC. Localization of heterochromatin in human chromosomes. *Cytogenetics*. 1971;10:81-86.
19. Arrighi FE, Saunders PP, Saunders GF, Hsu TC. Distribution of repetitious DNA in human chromosomes. *Experientia*. 1971;27:964-966.
20. Comings DE, Avelino E, Okada TA, Wyandt HE. The mechanism of C- and G-banding of chromosomes. *Exp Cell Res*. 1973;77:469-483.
21. Comings DE. Mechanisms of chromosome banding and implications for chromosome structure. *Annu Rev Genet*. 1978;12:25-46.
22. Matsui S, Sasaki M. Differential staining of nucleolus organisers in mammalian chromosomes. *Nature*. 1973;246:148-150.
23. Goodpasture C, Bloom SE, Hsu TC, Arrighi FE. Human nucleolus organizers: the satellites or the stalks? *Am J Hum Genet*. 1976;28:559-566.
24. Goodpasture C, Bloom SE. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma*. 1975;53:37-50.
25. Latt SA. Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc Natl Acad Sci U S A*. 1973;70:3395-3399.
26. Latt SA. Fluorescence analysis of late DNA replication in human metaphase chromosomes. *Somatic Cell Genet*. 1975;1:293-321.
27. Grzeschik KH, Kim MA, Johannsmann R. Late replicating bands of human chromosomes demonstrated by fluorochrome and Giemsa staining. *Humangenetik*. 1975;29:41-59.
28. Latt SA. Microfluorometric analysis of deoxyribonucleic acid replication kinetics and sister chromatid exchanges in human chromosomes. *J Histochem Cytochem*. 1974;22:478-491.
29. Kato H. Spontaneous sister chromatid exchanges detected by a BUdR-labelling method. *Nature*. 1974;251:70-72.

30. Korenberg JR, Freedlender EF. Giemsa technique for the detection of sister chromatid exchanges. *Chromosoma*. 1974;48:355-360.
31. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature*. 1975;258:121-125.
32. Wolff S, Rodin B, Cleaver JE. Sister chromatid exchanges induced by mutagenic carcinogens in normal and xeroderma pigmentosum cells. *Nature*. 1977;265:347-349.
33. Carrano AV, Thompson LH, Lindl PA, Minkler JL. Sister chromatid exchange as an indicator of mutagenesis. *Nature*. 1978;271:551-553.
34. Chaganti RS, Schonberg S, German J. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc Natl Acad Sci U S A*. 1974;71:4508-4512.
35. Dicken CH, Dewald G, Gordon H. Sister chromatid exchanges in Bloom's syndrome. *Arch Dermatol*. 1978;114:755-760.
36. Yunis JJ, Soreng AL. Constitutive fragile sites and cancer. *Science*. 1984;226:1199-1204.
37. Lubs HA. A marker X chromosome. *Am J Hum Genet*. 1969;21: 231-244.
38. Sutherland GR. Heritable fragile sites on human chromosomes, II: distribution, phenotypic effects, and cytogenetics. *Am J Hum Genet*. 1979;31:136-148.
39. Sutherland GR. Heritable fragile sites on human chromosomes, I: factors affecting expression in lymphocyte culture. *Am J Hum Genet*. 1979;31:125-135.
40. Auerbach AD, Adler B, Chaganti RS. Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. *Pediatrics*. 1981;67:128-135.
41. Cervenka J, Arthur D, Yasis C. Mitomycin C test for diagnostic differentiation of idiopathic aplastic anemia and Fanconi anemia. *Pediatrics*. 1981;67:119-127.
42. Kuffel DG, Lindor NM, Litzow MR, Zinsmeister AR, Dewald GW. Mitomycin C chromosome stress test to identify hypersensitivity to bifunctional alkylating agents in patients with Fanconi anemia or aplastic anemia. *Mayo Clin Proc*. 1997;72:579-580.
43. Pardue ML, Gall JG. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. *Proc Natl Acad Sci U S A*. 1969;64:600-604.
44. Pinkel D, Gray JW, Trask B, van den Engh G, Fuscoe J, van Dekken H. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. *Cold Spring Harb Symp Quant Biol*. 1986;51(pt 1):151-157.
45. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci U S A*. 1986;83:2934-2938.
46. Dewald GW. Interphase FISH studies of chronic myeloid leukemia. *Methods Mol Biol*. 2002;204:311-342.
47. Jabs EW, Wolf SF, Migeon BR. Characterization of a cloned DNA sequence that is present at centromeres of all human autosomes and the X chromosome and shows polymorphic variation. *Proc Natl Acad Sci U S A*. 1984;81:4884-4888.
48. Henegariu O, Bray-Ward P, Artan S, Vance GH, Qumsyieh M, Ward DC. Small marker chromosome identification in metaphase and interphase using centromeric multiplex fish (CM-FISH). *Lab Invest*. 2001;81:475-481.
49. Dewald GW, Schad CR, Christensen ER, et al. Fluorescence in situ hybridization with X and Y chromosome probes for cytogenetic studies on bone marrow cells after opposite sex transplantation. *Bone Marrow Transplant*. 1993;12:149-154.
50. Ledbetter DH, Ballabio A. Molecular cytogenetics of contiguous gene syndromes: mechanisms and consequences of gene dosage imbalance. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. Vol 1. 7th ed. New York, NY: McGraw-Hill; 1995:811-839.
51. Crifasi PA, Michels VV, Driscoll DJ, Jalal SM, Dewald GW. DNA fluorescent probes for diagnosis of velocardiofacial and related syndromes. *Mayo Clin Proc*. 1995;70:1148-1153.
52. Nickerson E, Greenberg F, Keating MT, McCaskill C, Shaffer LG. Deletions of the elastin gene at 7q11.23 occur in approximately 90% of patients with Williams syndrome. *Am J Hum Genet*. 1995;56:1156-1161.
53. Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR. Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nat Genet*. 1992;2:292-300.
54. Jalal SM, Harwood AR, Sekhon GS, et al. Utility of subtelomeric fluorescent DNA probes for detection of chromosome anomalies in 425 patients. *Genet Med*. 2003;5:28-34.
55. Knight SJ, Lese CM, Precht KS, et al. An optimized set of human telomere clones for studying telomere integrity and architecture. *Am J Hum Genet*. 2000;67:320-332.
56. Dewald GW, Ketterling RP, Wyatt WA, Stupca PJ. Cytogenetic studies in neoplastic hematologic disorders. In: McClatchey KD, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2002:658-685.
57. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-182.
58. Persons DL, Bui MM, Lowery MC, et al. Fluorescence in situ hybridization (FISH) for detection of HER-2/neu amplification in breast cancer: a multicenter portability study. *Ann Clin Lab Sci*. 2000;30:41-48.
59. Cell Markers and Cytogenetics Committees, College of American Pathologists. Clinical laboratory assays for HER-2/neu amplification and overexpression: quality assurance, standardization, and proficiency testing. *Arch Pathol Lab Med*. 2002;126:803-808.
60. Gersen SL, Keagle MB, eds. *The Principles of Clinical Cytogenetics*. Totowa, NJ: Humana Press; 1999.
61. Gardner RJM, Sutherland GR. *Chromosome Abnormalities and Genetic Counseling*. 2nd ed. New York, NY: Oxford University Press; 1996.
62. Jalal SM, Law ME, Stamberg J, et al. Detection of diagnostically critical, often hidden, anomalies in complex karyotypes of haematological disorders using multicolour fluorescence in situ hybridization. *Br J Haematol*. 2001;112:975-980.
63. Jalal SM, Law ME, Dewald GW. *Atlas of Whole Chromosome Paint Probes: Normal Patterns and Utility for Abnormal Cases.* Rochester, Minn: Mayo Foundation for Medical Education and Research; 1996.
64. Kraker WJ, Borell TJ, Schad CR, et al. Fluorescent in situ hybridization: use of whole chromosome paint probes to identify unbalanced chromosome translocations. *Mayo Clin Proc*. 1992;67:658-662.
65. Paris Conference (1971): standardization in human cytogenetics. *Cytogenetics*. 1972;11:313-362.
66. Paris Conference (1971), supplement (1975): standardization in human cytogenetics. *Cytogenet Cell Genet*. 1975;15:201-238.

Primer on Medical Genomics Part XII will appear in the March issue.

Glossary of Terms Frequently Used in Cytogenetic Studies

Acrocentric chromosome—Chromosome whose centromere is located very near one end.
α Satellite DNA—Repetitive portion of DNA located in the heterochromatin associated with chromosome centromeres.
Amplification—Increase in the number of copies of a specific DNA sequence.
Aneuploidy—Chromosome number that is not an exact multiple set, a gain or loss of chromosomes.
Autosome—Chromosome other than a sex chromosome; humans have 22 pairs of autosomes.
Barr body—See X-chromatin body.
BrdU (5-bromodeoxyuridine)—Thymidine analogue.
C-bands—Bands most commonly produced by treating chromosome preparations with hydrochloric acid, barium hydroxide, salt solution, and heat; the dark bands contain highly repetitive, late replicating DNA sequences; they are observed in the pericentric regions of all human chromosomes and the distal long arm of the Y chromosome.
Centromere—Visible constriction on metaphase chromosomes where 2 sister chromatids are joined.
Chromatid—Longitudinal half of a chromosome resulting from DNA replication.
Chromatin—Composite of DNA and proteins that comprise chromosomes.
Chromatid break—Distinctly disconnected portion of a chromatid.
Chromatid gap—Nonstaining area of a single chromatid with minimal malalignment of the chromatid.
DA and DAPI-bands (distamycin A and 4′,6-diamidino-2-phenylindole dihydrochloride)—Bright fluorescent bands in the C-band regions of chromosomes 1, 9, 16, and Y and also the short arm of chromosome 15.
Deletion—Loss of a chromosome segment.
Dicentric chromosome—Chromosome with 2 centromeres.
Diploid—In humans, a set of 23 maternal and 23 paternal chromosomes that together make up the normal chromosome complement of an individual.
Duplication—Extra copy of a chromosome segment.
Euchromatin—Early replicating, genetically active regions of chromosomes; light staining G-bands.
FISH—Fluorescence in situ hybridization.
FISH probe—Fluorochrome-tagged molecule attached to a specific DNA sequence.
Fragile site—Nonstaining gap of varying width that usually involves both chromatids.
G-bands—Series of dark and light bands along each chromosome that are most often produced by trypsin and Giemsa stain; the light bands are guanine-cytosine (GC)-rich, and the dark bands are adenine-thymine (AT)-rich.
Haploid—In humans, a single set of 23 chromosomes.
Heterochromatin—Late-replicating, generally inactive regions of chromosomes; dark-staining G-bands.
High-resolution bands—Increased number of bands visualized in decondensed chromosomes.
Hybridization—Process of binding a specific DNA probe to a complementary DNA sequence on a chromosome.
Inversion—Reversal of the linear sequence of chromosome bands.
ish—In situ hybridization, the designation used in cytogenetic nomenclature to indicate hybridization to metaphase chromosomes.
Isochromosome—Abnormal chromosome with 2 copies of one arm and loss of the other arm forming a mirror-image banding pattern.
Karyotype—Chromosomes arranged systematically from largest to smallest in accordance with rules from the *International System for Human Cytogenetic Nomenclature*.
Kinetochore—Structure that forms at the centromere during cell division as a binding site for spindle fibers.
Metacentric chromosome—Chromosome whose centromere is located in the middle.
Microdeletion—Loss of a chromosome segment that is often difficult to visualize.
Microduplication—Extra copy of a chromosome segment that is often difficult to visualize by routine chromosome analysis.
Monosomy—Absence of a chromosome from a diploid cell.
Multicolor FISH—Method in which each chromosome pair has a unique color.
nuc ish—Designation used in cytogenetic nomenclature to indicate in situ hybridization in interphase cells.
Nucleolar organizing region (NOR)—Site of ribosomal RNA in the stalks of satellites on human acrocentric chromosomes.
Nucleolus—Dark-staining body in the nucleus composed primarily of RNA and protein.
Nucleosome—Basic structural unit of chromatin consisting of DNA and histones.
Polymorphism—Normal differences in chromosome banding patterns among humans.
Polyploid—Three or more haploid sets of chromosomes.
Q-bands—Banding technique that uses a fluorochrome (usually quinacrine) to produce distinct bright and dull fluorescing-banding patterns; the bright bands are AT-rich and the dull bands are GC-rich.
RBA—Replication bands visualized with acridine orange stain.
Radial—Exchange between chromatids, usually of different chromosomes, that produces an abnormal chromosome with more than the usual 2 arms.
Satellite stalk—Narrow, constricted region between the centromere and the satellite knob on acrocentric chromosomes.
Sister chromatid exchange (SCE)—Exchange of genetic material between chromatids of the same chromosome.
Spindle fibers—Functional elements that pull sister chromatids apart during cell division.
Submetacentric chromosome—Chromosome whose centromere is between the middle of the chromosome and telomere.
Subtelomere probe—FISH probe that binds to unique DNA sequences proximally and adjacent to the telomere.
Telomere—End of the chromosome.
Translocation—Transfer of a chromosome segment to another chromosome or region; exchanges of portions of chromosomes, usually involving 2 or more chromosomes.
Trisomy—Presence of an additional chromosome in a diploid cell.
Whole chromosome paint—Set of FISH probes that hybridize to most or all of an entire chromosome.
X-chromatin body—Dark-staining structure on the periphery of the female interphase nucleus; the inactive X chromosome.