6

Chromosome Bands

A decisive step forward in human cytogenetics was the discovery of banding techniques that reveal distinctive and reproducible patterns of transverse bands along the chromosomes. These permit accurate identification of all the chromosomes, recognition of a host of structural rearrangements, and identification of the breakpoints in most of these. Chromosome bands have great theoretical and practical significance. They are fundamental units of chromosome organization and play a key role in gene regulation, as described in Chapter 7. They have made possible the rapid identification of an enormous range of karyotypic abnormalities and the construction of increasingly comprehensive physical and genetic linkage maps of the chromosomes. New banding techniques continue to enrich our understanding of the complex human genome. Banding techniques and their applications have been reviewed extensively (for example, Verma and Babu, 1995; Bickmore and Craig, 1997).

O. J. Miller et al., *Human Chromosomes*

Q-banding

Caspersson et al. (1970) set out to develop a DNA-binding fluorochrome that would show base specificity in intensity of fluorescence; they achieved their goal with the guanine-alkylating agent quinacrine mustard. When chromosomes are stained with quinacrine mustard (or quinacrine) and examined with a fluorescence microscope, they show bands of different fluorescent intensity, or brightness, called Q-bands (Fig. 6.1). Quinacrine, like most chromosome stains (including acridine orange and methylene blue, the DNA-staining component of the complex Giemsa stain), is a tricyclic hydrocarbon whose three rings lie in one plane. This thin, flat molecule is just the thickness to fit (intercalate) between adjacent base pairs in DNA.

The extremely detailed subdivision of human chromosomes into hundreds of differentially stained bands came as a huge surprise, because no one thought there were as large regional differences in base composition as these results seemed to imply. However, in 1972, Weisblum and de Haseth provided a partial explanation. They found that synthetic polynucleotides rich in AT base pairs fluoresce brightly with quinacrine, whereas those rich in GC base pairs quench fluorescence. The degree of GC interspersion is thus the critical factor: Only stretches with three or more AT base pairs in a row fluoresce brightly. That is, two stretches of DNA with the same ratio of GC to AT base pairs but different degrees of interspersion of GC with AT base pairs can have different intensities of quinacrine fluorescence. The DNA in Q-bright bands is thus rich in AT clusters, while the DNA of Q-dull bands could either be GC-rich or simply have closely interspersed AT and GC base pairs. Other methods have shown that adjacent bands do, in fact, differ to some extent in their base ratios (Chapter 7). Most striking are the brilliant Q-bands containing highly AT-rich satellite DNAs. The one on the distal long arm of the Y chromosome is particularly striking and is usually visible as a fluorescent Y-body in nuclei (Fig. 6.1) and sperm (Fig. 17.1).

With Q-banding, the chromosomes are stained without any pretreatment, so their morphology is retained. The fluorescence intensity of the bands can be measured (Caspersson et al., 1970). However, slides stained with quinacrine do not last, as the fluorescence fades rather quickly. Chromosomes are usually analyzed in detail only from photographs, although one could now use a sensitive charge-coupled device (CCD) digital camera and computer-assisted analysis, as is used for fluorescence in situ hybridization, or FISH (Chapter 8). However,

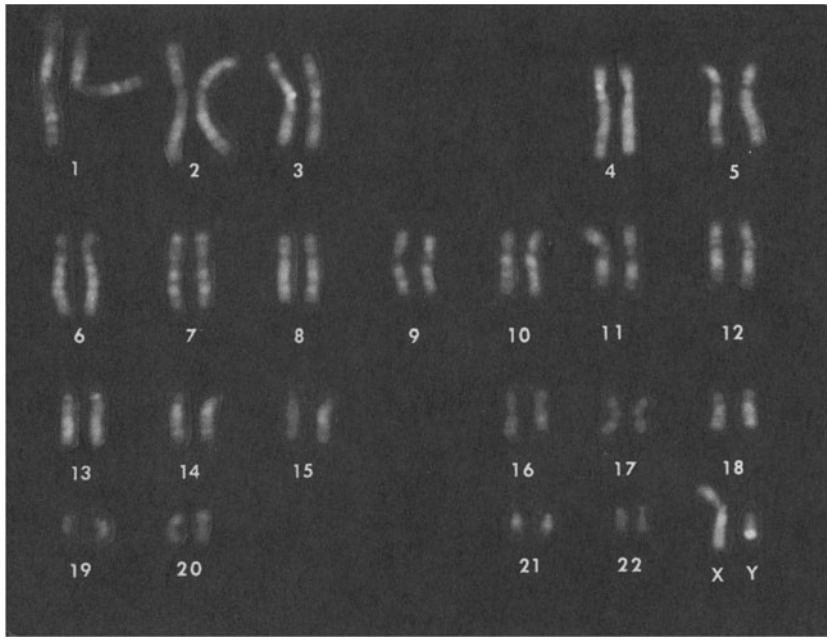

Figure 6.1. Q-banded karyotype of a cell from an XY male (Breg, Quinacrine fluorescence for identifying metaphase chromosomes, with special reference to photomicrography. Stain Technology 47:87–93, copyright 1972, Williams & Wilkins).

for routine work Q-banding has been largely replaced by nonfluorescent G- and R-banding techniques.

C-banding

C-banding was discovered by accident when chromosome spreads were heated to denature the DNA for in situ hybridization. The Giemsa staining of the chromosomes was greatly reduced except in the centromeric regions of most chromosomes and the distal part of the Y (Fig. 6.2). C-banding can be produced in a number of ways. The most common is to treat chromosomes briefly with acid and then with an alkali such as barium hydroxide prior to Giemsa staining. Prominent C-bands are found on chromosomes 1, 9, 16, and the distal Y. Differences in the types of satellite DNAs in the various C-bands are respon-

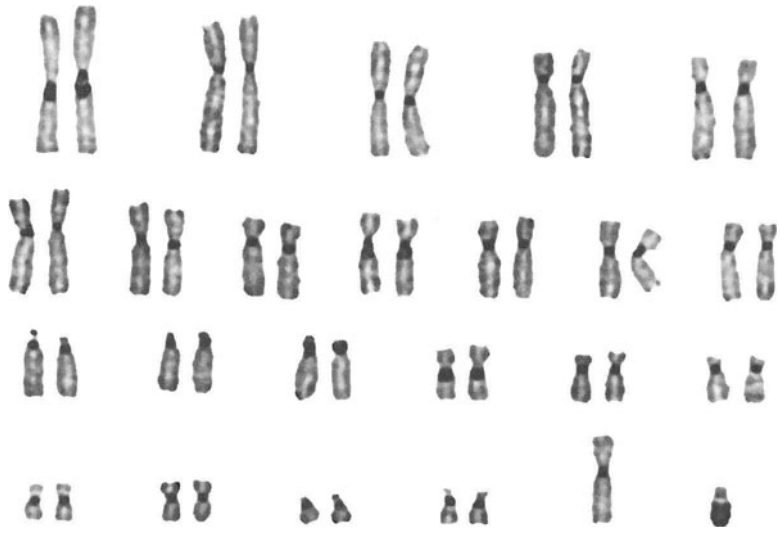

Figure 6.2. C-banded karyotype of an XY cell (courtesy of Arvind Babu).

sible for the differences observed using various stains. For example, the distal end of the Y chromosome is brightly fluorescent with Q-banding, whereas the centromeric heterochromatin of chromosomes 1, 9, and 16 is dark. Giemsa staining at pH 11 (the G11 technique) stains the C-band of chromosome 9 only.

C-bands vary considerably in size in the population. The greatest variability is demonstrated in the satellites and short arms of the acrocentric chromosomes. These variations are generally called *heteromorphisms*, because the term *polymorphism* is restricted by geneticists to a heritable variant that has a frequency of at least 1 per cent in the population. Inversions that involve the large blocks of heterochromatin of chromosomes 1 and 9 and the Q-bright centric band on chromosome 3 are fairly common heteromorphisms. Even extreme variations in the sizes of C-bands do not affect the phenotype. The reason for this is that C-banding stains constitutive heterochromatin, which contains no genes and is never transcribed. Changes in the size of a C-band do not take place very often, since the C-band variants are generally constant from one generation to the next and show normal Mendelian inheritance. Chromosomal heteromorphisms have been used as markers in gene mapping, paternity testing, and distinguishing monozygotic from dizygotic twins or the parent of origin of trisomy and triploidy. They have aided our understanding of hydatidiform moles and the

origins of the different cell lines in chimeras, including persons with bone marrow transplants. However, most of these applications have been supplanted by more precise molecular cytogenetic methods.

G-banding, R-banding, and T-banding

G-banding was discovered by accident during attempts to improve C-banding. G-bands are obtained when the chromosomes are pretreated with a salt solution at 60°C or with a proteolytic enzyme, such as trypsin, before staining with Giemsa or a comparable chromatin stain. G-banding (Fig. 4.2) yields essentially the same information as Q-banding (Fig. 6.1). The bands that fluoresce brightly with quinacrine stain intensely with Giemsa stain (are G-dark), whereas the Q-dull regions are G-light. Each method has its advantages. G-banding is permanent and therefore more suitable than the evanescent Q-banding for routine work. By either technique, some 300 bands are readily distinguishable in the haploid genome at metaphase (Paris Conference: 1971 [1972]) and 850–1250 at prometaphase and prophase (ISCN, 1995). Interestingly, the Q-bright, G-dark bands correspond to the chromomeres seen at the pachytene stage of meiosis (Chapter 9). Chromosome bands have shown remarkable constancy during mammalian evolution (Chapter 30).

Reverse banding, or R-banding, was discovered by Dutrillaux and Lejeune (1971). Their technique involves pretreatment with hot (80–90°C) alkali and subsequent staining with Giemsa stain or the fluorochrome acridine orange. Acridine orange intercalates between base pairs in double-stranded DNA and fluoresces bright yellow. It can also bind to single-stranded (heat-denatured) DNA by base stacking, and then it fluoresces pale red. As the name indicates, the R-banding pattern (Fig. 6.3) is the reverse of the Q- or G-banding pattern; in other words, the bands that are intense by R-banding are faint by Q- or G-banding, and vice versa. R-banding stains chromosome ends more distinctively and is often useful for the study of structural changes involving chromosome ends that might go undetected with Q- or G-banding. A modification of R-banding, called T-banding, stains mainly regions near the tips of many chromosomes (Dutrillaux, 1973).

All Q-, G-, and R-bands, whether they fluoresce or stain strongly or weakly, contain abundant DNA and genes, and all bands are counted in determining the total number of bands. However, for ease of communication, one generally refers to Q-bright bands as Q-bands, G-dark (intensely stained) bands as G-bands, and

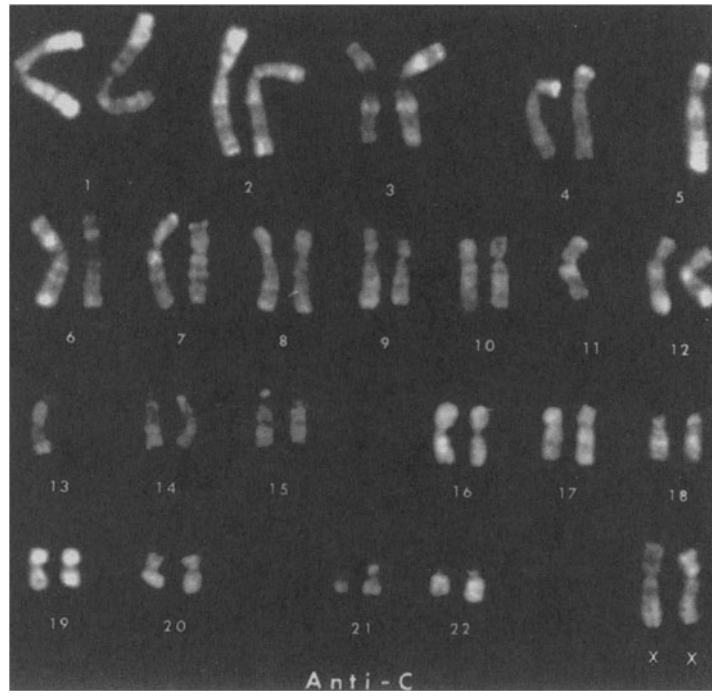

Figure 6.3. R-banded karyotype produced by immunofluorescence detection of anti-cytosine antibodies after denaturing GC-rich DNA in an XX metaphase spread by photooxidation (Schreck et al., 1973).

Q-dull, G-light, or R-intense bands as R-bands. That is, the terms Q-, G-, R-, T-, and C-band commonly refer to bands that are more intensely stained or fluorescent by the particular method used.

High-Resolution and Replication Banding

Prometaphase and prophase chromosomes are much longer than metaphase chromosomes, but they too can be banded. Such high-resolution banding can increase the number of visible bands to 850–1250 (Yunis, 1980; ISCN, 1995). For high-resolution G- or R-banding, dividing cells are blocked in the S phase with amethopterin (methotrexate). When the block is released with thymidine-rich medium, the cell cycle is synchronized in a large fraction of the cells, which

can then be studied at the desired stage. Prophase chromosomes show some natural banding, but this can be enhanced with banding techniques. The longer the chromosomes are, the more bands they show. However, longer chromosomes overlap more, and the analysis becomes tedious. The most important uses of high-resolution banding are the recognition of subtle structural changes (Chapters 14–16) and the mapping of genes by in situ hybridization (Chapters 8 and 29).

Chromosome banding comparable to that achieved with other banding techniques can be induced using BrdU to study DNA replication timing (Chapter 3). The replication bands are detected either in the standard way (Latt, 1973) or by using anti-BrdU antibodies (Fetni et al., 1996). The R-bands replicate during the first half of the S period. The Q- or G-bands replicate during the second half of S. Completely reciprocal replication R- and G-bands are seen at high resolution (550–1250 bands per haploid genome) when BrdU is incorporated during either early or late S phase (Fig. 6.4). The individual bands show a constant order of replication (Drouin et al., 1994). Differential staining of the two chromatids in the C-band regions has been observed after BrdU incorporation during one cycle of replication. This *lateral asymmetry* of staining may reflect the presence of one T-rich strand and one A-rich strand in some satellite DNAs (Galloway and Evans, 1975), because BrdU is incorporated in place of T and subsequent photolysis destroys the BrdU-substituted DNA. Lateral asymmetry of C-bands may not be seen with high-resolution replication banding, for reasons that are unclear (Drouin et al., 1994).

Banding with Other Fluorochromes and Nonfluorochromes

Some fluorochromes bind specifically to AT base pairs, for example DAPI (4′,6-diamidino-2-phenylindole) and its close relatives, DIPI and Hoechst 33258. Some fluorochromes bind specifically to GC base pairs, for example, chromomycin. Nonfluorescent dyes may also show base-specific binding. Methyl green and distamycin A bind to AT base pairs, while actinomycin D binds preferentially to GC base pairs. The AT-specific fluorochromes produce a fluorescent G-banding pattern (lacking the intense Q-brightness of AT-rich satellite DNA), but not a very precise one, because the intensity of fluorescence exactly parallels the AT-richness of each band. Similarly, GC-specific fluorochromes produce a mediocre R-band pattern. The fluorescent G-band patterns are

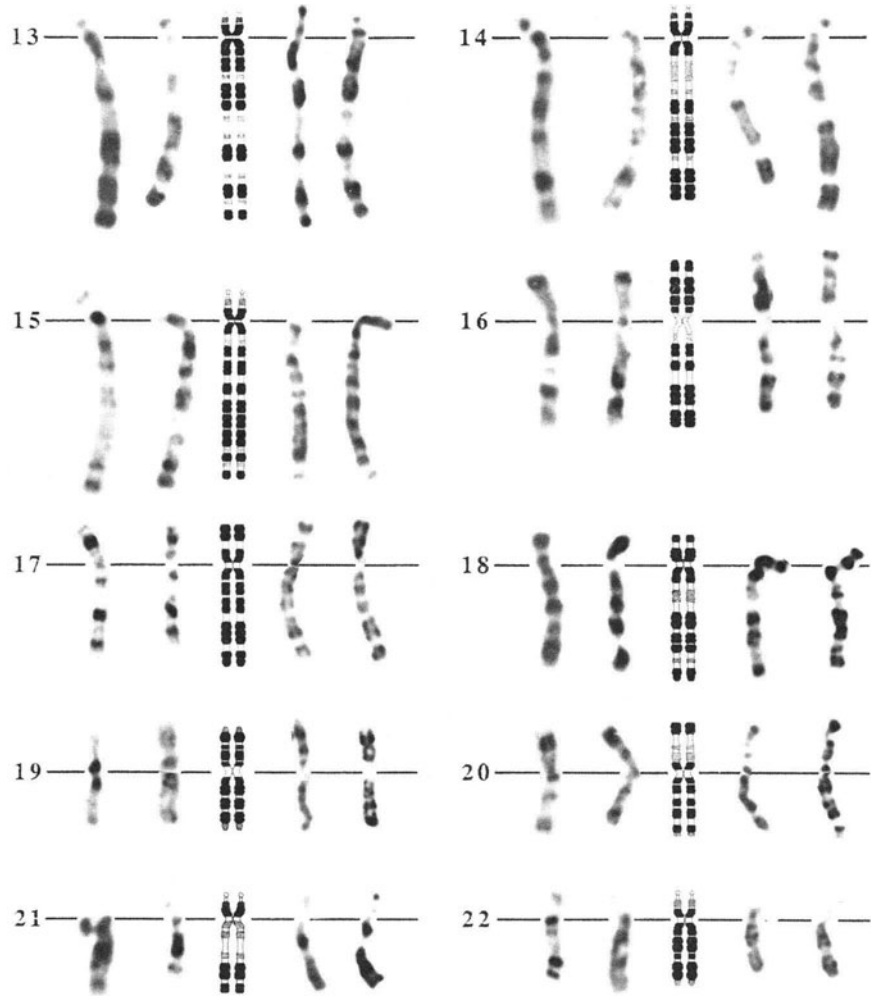

Figure 6.4. High-resolution (850–1250 bands) partial karyotypes. From left to right: GBG (G-bands by BrdU using Giemsa stain), GB-AP (G-bands by BrdU using anti-BrdU and peroxidase), RBG 1250 ideogram, RB-AP (R-bands by BrdU using anti-BrdU and peroxidase), and RBG (R-bands by BrdU suing Giemsa stain) (Fetni et al., 1996, reproduced, with permission of S. Karger AG, Basel).

enhanced when the chromosomes are stained jointly with an AT-specific fluorochrome (DAPI) and a GC-specific nonfluorescent dye (actinomycin D). In the same way, very precise fluorescent R-band patterns are produced when the GC-specific fluorochrome chromomycin is used in combination with the nonfluo-

rescent dye methyl green. The reason such *counterstaining* is effective is that the nonfluorescent dye quenches fluorescence of the fluorochrome by a process of energy transfer that takes place only when the different types of dye molecules are extremely close together.

These counterstain-enhanced banding methods (Schweitzer, 1981) can yield beautifully sharp bands. They have also confirmed earlier evidence that G-bands contain abundant stretches of uninterrupted AT base pairs and that R-bands contain similar uninterrupted stretches of GC base pairs. Furthermore, when chromosomes are stained with two AT-specific chemicals, the fluorescent DAPI and the nonfluorescent distamycin A, a subset of fluorescent C-bands is observed, particularly those on chromosomomes 9, 15, and the Y, the same ones that are revealed using antibodies to methylated DNA (see below).

Antibody Banding

The first direct demonstration that chromosome bands reflect differences in base composition of the DNA in different bands came from immunocytochemical studies using antibodies to adenosine and cytosine (Miller et al., 1973). These antibodies bind to the bases in single-stranded (denatured) but not double-stranded DNA. When AT-rich DNA is denatured by heat or UV-irradiation, anti-A produces Q- or G-banding. When GC-rich DNA is denatured by photo-oxidization of G residues, anti-C produces sharp R-banding (Fig. 6.3). R-banding is also produced by antibodies to double-stranded GC polymers (Magaud et al., 1985) and antibodies to acetylated forms of histone proteins (Jeppesen and Turner, 1993). When chromosomal DNA is denatured by UV-irradiation, antibodies to 5-methylcytosine (anti-5MeC) produce C-banding (Miller et al., 1974); this was the first evidence that CpGs in the AT-rich satellite 2 and 3 DNAs are highly methylated. The same regions bind antibodies to the methylated CpG-binding protein, MeCP2 (Lewis et al., 1992) and antibodies to the heterochromatin-binding protein, HP1 (Wreggett et al., 1994).

Weak R-banding is produced by antibodies to Z-DNA, suggesting that a tiny amount of this special form of DNA is present in R-bands (Viegas-Pequignot et al., 1983). Left-handed DNA, or Z-DNA, is transiently present in cells. It tends to form just behind advancing RNA polymerase II molecules. Most genes have about three short regions near the 5′ promoter region of the gene that can take a Z conformation when strongly supercoiled. This may have functional

significance, because specific proteins bind to Z-DNA. One such protein, double-stranded RNA adenosine deaminase, is an RNA editing enzyme that deaminates adenosine in the RNA to produce inosine, which is translated as if it were guanine. This can lead to the incorporation of a different amino acid in the resultant protein (Herbert and Rich, 1996).

Nuclease Banding

A large number of restriction endonucleases have been identified that cut DNA only at specific sequences that are 4–8 base pairs long and palindromic, that is, the sequence is the same, from 5′ to 3′, in each strand. Thus, *Hae*III cuts at GGCC sites, *Eco*RI at GAATTC, *Msp*I at CCGG or $C^{5Me}CGG$, and *Hpa*II at CCGG but not $C^{5Me}CGG$. Miller et al. (1983) showed that the generally abundant sites of a number of restriction enzymes are not distributed at random along the chromosomes. Some restriction enzymes produce a standard G-band or C-band pattern on fixed chromosomes, but some produce unusual patterns. The most informative involve the C-bands, which contain several types of satellite DNA. If the very short repeating sequence of a particular satellite DNA contains the cutting site for a particular restriction enzyme, the DNA will be cut into tiny fragments and lost from the chromosomes. If the satellite DNA lacks the site, it will remain intact and stain strongly against a background of generally reduced staining. A series of restriction enzymes can be used to divide the C-bands into several classes (Miller et al., 1983), and analyze heteromorphisms (Babu, 1988). *Mse*I cuts at TTAA sites and removes DNA mainly from G-bands and some R-bands, leaving the C-bands, T-bands, and some R-bands (Ludeña et al., 1991).

Nucleases have also been used to produce banding, or labeling, that is related to the functional state of the DNA rather than its DNA sequence. By comparing the effects of *Hpa*II and *Msp*I on fixed chromosome spreads, Miller et al. (1983) demonstrated that amplified but inactive ribosomal RNA genes are hypermethylated by showing they are easily cut by *Msp*I but resist cutting by *Hpa*II. Molecular studies have shown that transcriptionally active (or potentially active) genes are in DNAase I–sensitive chromatin in nuclei, reflecting its more open conformation (Chapter 5). Remarkably, variations in nuclease sensitivity are still present in metaphase chromosomes. Kerem et al. (1984) showed that the active X chromosome is much more DNAase I sensitive than the inactive X and that R-bands are, in general, more DNAase I sensitive than G-bands. To do this, they

used nick-translation to incorporate BrdU at sites of nuclease nicking of DNA in chromosome spreads and labeled antibodies to detect the BrdU.

In Situ Hybridization Banding

In situ hybridization of the very abundant *Alu* repetitive sequences produces an R-banding pattern, while hybridization of LINE1 repeats produces a G-banding pattern. This indicates that each of these two classes of repetitive DNA is more abundant in a different fraction of the genome (Korenberg and Rykowski, 1988). By using the polymerase chain reaction with oligonucleotide primers complementary to parts of the *Alu* or LINE1 sequence, hybridization probes can be generated that enable a banding pattern to be produced simultaneously with localization of gene probes (Lichter et al., 1990). Hybridization of a highly GC-rich fraction of DNA produces T-bands (Fig. 7.2), and so does hybridization of the unmethylated CpG-rich tiny fraction of genomic DNA (Craig and Bickmore, 1994). There is lateral asymmetry of G and C residues in telomeric repeats, with the Gs in one strand and the Cs in the other (Chapter 4). This is the basis for a novel method to detect pericentric inversions, using a single-stranded probe that hybridizes only to one strand of telomeric DNA (Bailey et al., 1996; Chapter 16).

Nomenclature of Banded Chromosomes and Abnormal Karyotypes

At the Paris Conference: 1971 (1972) a system of nomenclature was proposed for banded human chromosomes and chromosome abnormalities. Figure 6.5 shows an *ideogram* of a banded karyotype according to this system. Telomeres, centromeres, and a number of prominent bands are used as landmarks. A section of a chromosome between two landmarks is called a *region*, and these regions are numbered 1, 2, 3, and so on, in both p and q directions, starting from the centromere. The bands within the regions are numbered according to the same rule. Thus, the first band in the second region of the short arm of chromosome 1 is 1p21.

The increasing use of high-resolution banding has led to an extension of this system. For example, 14q32 (Fig. 6.6, left) indicates chromosome 14, long arm

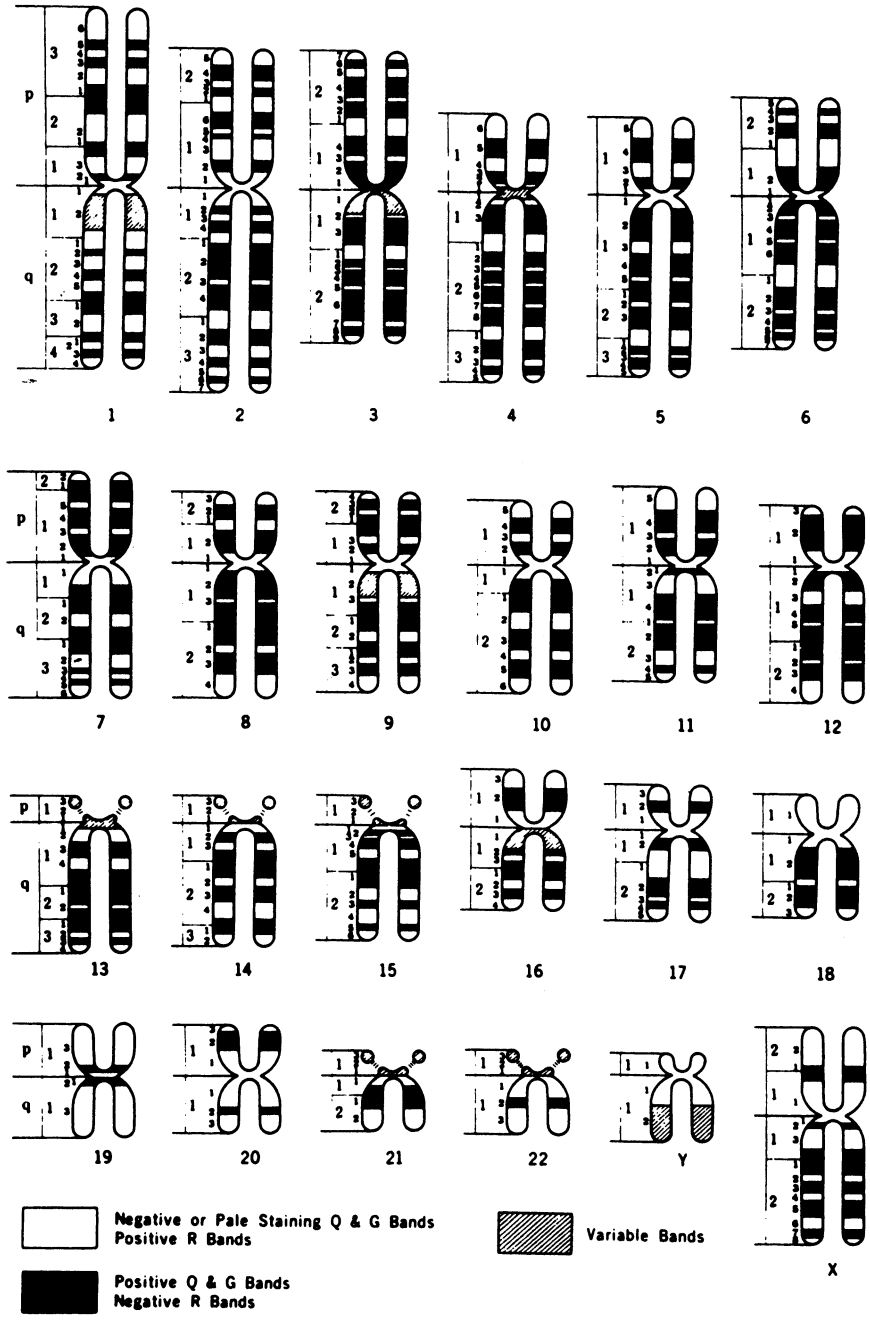

Figure 6.5. Paris conference ideogram of a banded karyotype.

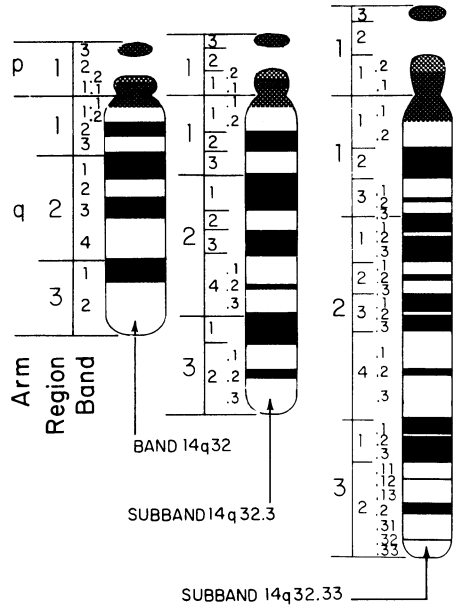

Figure 6.6. Ideograms of chromosome 14 at the 320- (left), 500- (center), and 900-band (right) stages, illustrating the Paris nomenclature. The sub-bands seen at the 500-band stage are designated with decimals, those seen at the 900-band stage with decimals and digits (Yunis, 1980).

region 3, band 2. High-resolution banding reveals three subbands in this band. To indicate a subband a dot is used, followed by the number of the subband (they are numbered sequentially from the centromere). The most distal subband in chromosome 14 (Fig. 6.6, middle) is thus 14q32.3. When the subband is further subdivided by still higher-resolution banding (Fig. 6.6, right), an additional digit is added, the last subband thus being 14q32.33. The most recent update of this international system of nomenclature is ISCN (1995). Francke (1994) has prepared a digitized and differentially shaded ideogram for genomic applications.

For the designation of chromosome abnormalities, two systems, one short and one detailed, are provided. For the actual use of both systems the reader is referred to the current standardization committee report (ISCN, 1995). In the following discussion only a few basic examples of the short system are given. An extra or a missing chromosome is denoted with a plus or a minus sign, respectively, before the number of the chromosome. The designation of a

female with trisomy 13 is 47,XX,+13, and that of a male with monosomy 21 is 45,XY,–21. A female with a deletion of the short arm of chromosome 5 and the cri du chat syndrome is designated 46,XX,del(5p). The karyotype of a female carrier of a Robertsonian translocation (centric fusion) between chromosomes 14 and 21 is 45,XX,der(14;21)(q10;q10). For historical reasons, the designation *rob* is also used: 45,XX,rob(14;21)(q10;q10). The karyotype of a male translocation heterozygote (carrier) in whom chromosome arms 3p and 6q have exchanged segments, the breakpoints being 3pl2 and 6q34, is 46,XY,t(3;6)(pl2;q34).

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