

## Chapter 2

# Chromosome Heteromorphism

The term heteromorphism is especially applicable to normal variants observed by chromosome banding techniques. However, normal variations in morphology in certain regions of the human genome were noted even before the advent of chromosome banding. In the first Conference on Standardization in Human Cytogenetics in Denver in 1960 [1], chromosomes were divided into Groups A–G based on their relative sizes and positions of the centromeres. The X chromosome was categorized in group C. The Y was distinguishable from the G-group by its lack of satellites and somewhat distinctive morphology. At the London Conference in 1963 [2], prominent secondary constrictions were identified near the centromeres in the chromosome no. 1 pair in group A, in a chromosome pair (no. 9) in the group C and in a pair (no. 16) in the group E. By the Chicago Conference in 1966 [3], it was generally recognized that these regions and the Y varied in length, and that there were morphological variations in the short arms of group D and G chromosomes.

In the early 1970s, Q-, G- and C-banding techniques became widely used. Q- and G-banding introduced a new era in which individual chromosomes could be definitively identified. With this capability, it also became possible to localize regions that were variable in size and staining to specific chromosomes. In particular, Q- and C-banding revealed distinct classes of heteromorphism which were not necessarily detectable in non-banded chromosomes, but could be shown to be heritable in banded chromosomes. The most distinctive heteromorphism by Q-banding was the brightly fluorescent distal long arm of the Y chromosome. The size of this brightly fluorescent segment varied from being almost negligible in size to being the longest segment on the Y long arm. Q-banding also revealed variations in the staining of chromosomes 3, 4, 13 to 15, 21 and 22 [4–8].

Although G-banding techniques became widely used for chromosome identification, C-banding revealed size variations of heterochromatin (h) around the centromeres of every chromosome that could be more easily quantitated than in

non-banded chromosomes. The h regions of chromosomes 1, 9, 16 and in the distal long arm of the Y chromosome, evident in non-banded chromosomes, were especially visible by C-banding [8–12]. A system to describe variations in intensity and size observed by Q- and C-banding was incorporated into the cytogenetic nomenclature (Table 2.1).

Additional specialized techniques quickly followed, including R-banding [13], silver staining for nucleolar organizing regions (NORs) [14], G-11 staining [15], and staining with various fluorescent DNA-binding fluorochromes, either singly or in combination. Some of these techniques revealed additional subclasses of variants [16] so that a complex system of characterizing variants by band intensity and staining technique was proposed in a Paris Conference Supplement (1975) [17]. However, the system was not widely used, and is not included in subsequent versions of ISCN [18].

Early molecular studies showed C-band heteromorphism to be composed of different fractions of DNA, referred to as satellite DNAs, based on their differing AT/GC content and buoyant densities in CsCl or Cs<sub>2</sub>SO<sub>4</sub> gradients [19–21]. Alkaline Giemsa and DA/DAPI [22, 23] techniques stain components of 1qh, 9qh, D-G-group short arms, 16qh and distal Yqh. In situ hybridization studies revealed different but overlapping distributions of satellite DNA fractions to the various heterochromatic regions in the human karyotype, with a loose correlation between alkaline Giemsa staining and sites of the “classical” satellite III [24–28]. In the 1980s and early 1990s, molecular techniques more accurately characterized various satellite DNA sequences [29], while fluorescent in situ hybridization (FISH) [30, 31] allowed for virtually any sufficiently large DNA sequence to be visually localized to specific chromosomal sites. Current, FISH and molecular technologies define satellite DNAs somewhat differently, but sequences in the satellite III family localize to similar chromosome regions.

FISH and DNA sequencing have shown considerable shuffling of satellite sequences. These technologies provided the means to characterize heteromorphism detected by classical techniques with greater accuracy and precision and also to identify new chromosomal variants. A handful of what might be termed “FISH variants” has been reported.

**Table 2.1** Descriptive numerical codes indicating size and intensity of C and Q band heteromorphisms [8, 17]

C/Q-band size	Q-band intensity
1 Very small	1 Negative (absent or little fluorescence)
2 Small	2 Pale (distal 1p)
3 Intermediate	3 Medium (two broad bands on 9q)
4 Large	4 Intense (distal half of 13q)
5 Very large	5 Brilliant (distal Yq)

## 2.1 Chromosome Banding Techniques and Mechanisms

### 2.1.1 *Q*-banding

Caspersson and colleagues at the Karolinska Institute, together with an American team of biochemists at Harvard Medical School headed by S. Farber and G. Foley set out to test or design fluorescent molecules that would preferentially bind to specific nucleotide pairs in DNA. They hoped to be able to detect the DNA spectrophotometrically. One molecule tested was quinacrine mustard dihydrochloride (QM), a nitrogen mustard analog of the anti-malarial drug, quinacrine. The dye, first applied to *Vicia faba* and *Trillium erectum*, revealed brightly fluorescent bands that distinguished the individual plant chromosomes. These findings led Caspersson et al. [32] to apply the QM staining to human chromosomes, with the discovery that the end of the long arm of the Y chromosome was brightly fluorescent—bright enough that the human Y chromosome could be easily detected in interphase as well as in metaphase cells. With refinements, QM produced banding patterns that were specific for each human chromosome (Fig. 2.1). Several investigators showed that the AT-rich regions of DNA corresponded to the bright fluorescent bands obtained with quinacrine mustard [33–35]. Weisblum and DeHaseth [33] showed that rather than preferential binding, this difference in intensity of fluorescence reflected a difference in quenching of the QM molecule. AT-richness alone, however, is not the sole determinant of the intensity of Q-banding. The actual differences in relative percentages of AT versus GC in different regions are not as great as might be implied. The periodicity of interspersed GC, within short, highly repetitive AT-rich sequences, as well as the presence of specific nucleoproteins, appears to play a significant role [36].

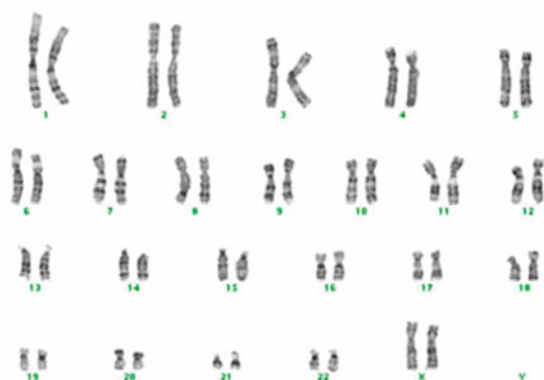
**Fig. 2.1** Normal Q-banded metaphase from 46, XX, female showing heritable variations in size and intensity of staining (arrows), especially of centromeric region of chromosome 3s, and of centromere, short arm, stalk and satellite regions of acrocentric chromosomes



### 2.1.2 G-banding

G-banding, introduced in 1971 by Sumner et al. [37] overcame two significant problems of Q-banding (stability and cost) and thus became the more widely used banding technique in clinical laboratories. G-banding acronyms GTG, GTW, GTL and GAG all represent variations used to obtain the same banding pattern that can be seen and analyzed by standard light microscopy. While the original G-banding method used acid fixation with saline treatment followed by Giemsa staining (GAG) [38], the application of proteolytic enzymes such as trypsin [39, 40] or pancreatin [41, 42] were simpler and improved the banding pattern. The blood stains, Wright's or Leishman's, are often used instead of Giemsa, depending on the laboratory's experience and preference. G-banding patterns are identical, however, irrespective of how they are obtained (by enzymatic or chemical pretreatment) or the blood stain used (Fig. 2.2). Similar to bright Q-bands, dark-staining G-bands are AT-rich regions of chromosomal DNA that are more condensed, and replicate their DNA later than GC-rich regions which are less condensed (Table 2.2) [43]. DNA-binding proteins thought to be involved in maintaining chromosomal structural integrity form the nuclear matrix and include topoisomerases that have a basic role in the control of gene activity [44–46]. It may be that nuclear matrix proteins that hold AT rich regions together make them less easily available for DNA replication and at the same time allow dye to bind only in monomer form so that they stain more intensely. Conversely, GC-rich regions that are gene-rich and transcriptionally active may be more loosely bound and consequently bind dye in polymer form, with less intense staining. Giemsa, Leishman, Wright or Romanowski blood stains all contain mixtures of thiazin dyes, each of which can produce banding under the right conditions. It is evident from the variety of treatments that produce G-banding that more than one mechanism is involved. The most reliable and widely used treatment is mild proteolytic digestion with trypsin [39, 40]. However, the precise role of nucleoproteins in G-banding has not been determined [47–50]. Extraction of

**Fig. 2.2** Normal female karyotype by GTG banding



**Table 2.2** Techniques for recognition of different classes of chromatin and properties of chromosome bands (modified from Sumner [43])

Class: heterochromatin	Euchromatin	Special regions
C-banding	G-banding	Ag-NOR staining
G-11 banding	Q-banding	Cd-banding
Q-banding	R-banding	Immunofluorescent
Distamycin/DAPI	T-banding	Staining with CREST
	Replication banding	Serum
<i>Properties of chromosome bands:</i>		
Positive G-/Q-bands, negative R-bands, pachytene chromomeres		Negative G-/Q-bands, positive R-bands, pachytene interchromomere regions
Early chromatin condensation		Late chromatin condensation
Late DNA replication		Early DNA replication
AT-rich DNA		GC-rich DNA
Tissue-specific genes		Housekeeping genes
Long intermediate repetitive DNA sequences (LINEs)		Short intermediate repetitive DNA sequences (SINEs)

histones also seems to have little effect [51–54]. In fact, very little protein is lost from chromosomes in various G-banding treatments [48]. Furthermore, it is evident that there is an underlying structural integrity of the chromosome that is revealed in the “chromomere pattern” of very long chromosomes in meiosis [55, 56]. This pattern in non-banded meiotic chromosomes is identical to the pattern of G-banded metaphase chromosomes (see ISCN 2009) [18].

The relationship between DNA structure and the binding of components making up Giemsa dye mixtures is also not totally understood. Treatments that loosen the integrity of underlying DNA structure appear to be most effective, suggesting that certain Giemsa components bind to condensed DNA in monomeric form and to looser DNA structures in polymeric form. The more the individual dye components become stacked, the greater the shift to lower absorption spectra (purple or pink). In monomer form, the shift is to the blue end of the spectrum. Such a shift in color, based on the ability of a dye to become stacked in polymer form, is referred to as metachromacy. Some Giemsa components are more metachromatic than others. Methylene blue, Azure A, Azure B, and thiazin show varying degrees of metachromacy, determined by the number of methyl groups present in the dye molecule [57–59]. Eosin, which is also a component of Giemsa dyes, shows no metachromacy, but it appears to have a differential staining effect when combined with the other components.

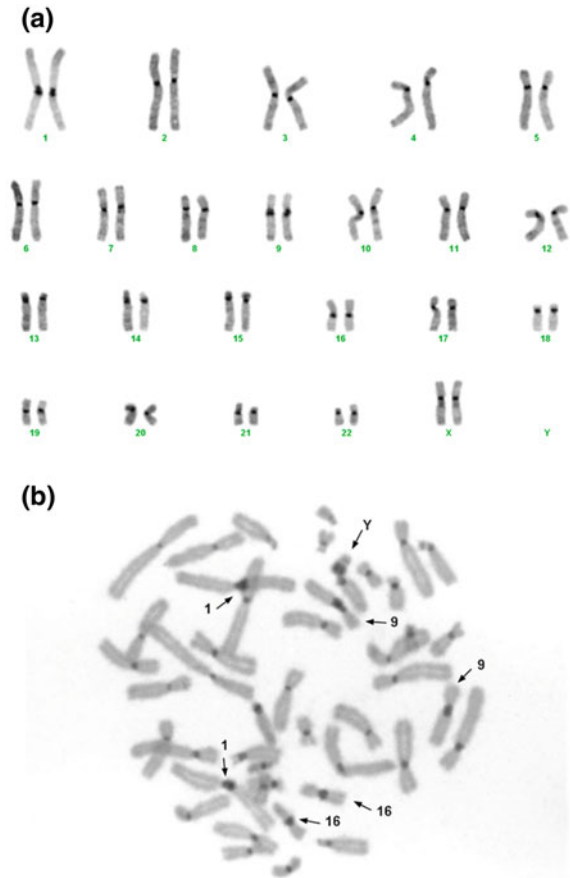
### 2.1.3 *R-banding*

Dutrillaux and Lejeune [60] introduced a banding technique involving treatment of chromosomes in saline at high temperature (87 °C) that resulted in a reverse pattern of G- or Q-bands. They called this “reverse (R) banding” and, since the method involved staining with Giemsa, it is described as a “RHG” banding. R-bands are most useful in identifying abnormalities involving the terminal regions of chromosomes, which are stained lighter by G- and Q-banding. Alternate methods to produce R-banding use various fluorescent chemicals, such as acridine orange and chromomycin A3/methyl green [22, 61, 62] (Table 2.2). However, because of technical difficulties or fluorescent requirements, R-banding is still not used in many laboratories.

### 2.1.4 *C-banding*

During experiments with in situ hybridization of tritium-labeled satellite DNA to mouse chromosomes, Pardue and Gall [64] noted that constitutive heterochromatin at the centromeres of mouse chromosomes stained darker than in other chromosomal regions. In 1971, Arrighi and Hsu [64, 65] developed a modified technique in which they applied Giemsa staining to preparations that were first denatured with 0.07 M NaOH and then incubated in two times the standard saline concentration (2xSSC) for several hours. In a more recent modification, Sumner [66] substituted barium hydroxide for sodium hydroxide, producing the same C-banding pattern (CBG-banding), but with less distortion of the chromosome morphology. Both procedures result in intense staining of the heterochromatin around the centromeres, whereas the rest of the chromosome stains pale blue (Fig. 2.3). Arrighi and Hsu initially postulated that this differential staining was due to faster re-annealing of repetitive DNA in heterochromatin than in the less repetitive DNA sequences elsewhere. McKenzie and Lubs [67] produced C-banding by simply treating chromosomes with HCl and a prolonged incubation in 2xSSC. Studies by Comings et al. [48] demonstrated considerable extraction of nucleoprotein and DNA from non-heterochromatic regions by various C-banding treatments, while heterochromatic regions were resistant to such extraction. Furthermore, they demonstrated that hybridization of repetitive sequences in solution was not required for enhanced staining, but in fact, those regions re-associated instantaneously when they were removed from the NaOH solution. Subsequent incubation in 2xSSC extracted additional non-heterochromatic DNA. Since incubation that produces C-banding is done for times ranging from a couple of hours to overnight, it is unlikely that much single stranded DNA remains to bind Giemsa components. The differential staining is more likely due to the greater amount of double stranded DNA remaining in the heterochromatic regions.

**Fig. 2.3** **a** Normal female karyotype by CBG banding by barium hydroxide treatment followed by Giemsa staining (c41). **b** CBG-banded metaphase from normal male. *Arrows* point to 1, 9, 16 and Y chromosomes, which typically show the greatest amount of heteromorphism in different individuals



### 2.1.5 Cd Banding

The technique, first described by Eiberg [68], reveals pairs of dots at presumed centromere locations; hence, the term “centromere dots” (Cd). The technique involves the usual hypotonic treatment of chromosomes followed by a series of fixations, starting with a 9:1 ratio of methanol to acetic acid, followed by a 5:1 ratio and then the standard 3:1 ratio. One week old slides are then incubated in Earle’s balanced salt solution (pH 8.5–9.0) at 85 °C for 45 min, followed by staining in a dilute solution of phosphate-buffered Giemsa (0.0033 M, pH 6.5). The technique appears to specifically stain only active centromeric regions and not inactive centromeres, secondary constrictions or other variable heteromorphic regions [69, 70]. It has been used identify the active centromere(s) in dicentric, pseudodicentric and Robertsonian translocations. The mechanism of this technique suggested by Eiberg was that it represented a specific DNA-protein complex. Evans and Ross [71]

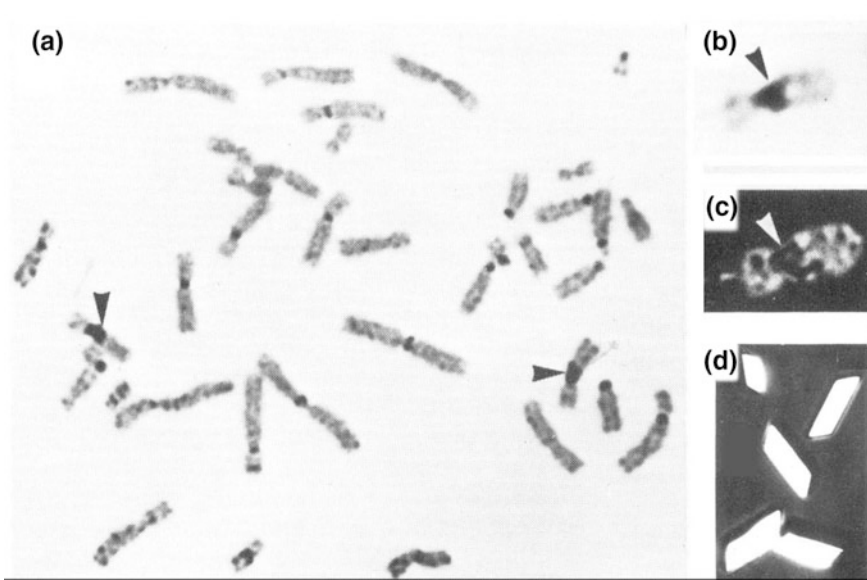
suggested that the Cd-positive regions represent kinetochores. Nakagome et al. [69, 70] and Maraschio et al. [72] studied dicentric and pseudodicentric chromosomes and showed that the Cd-positive regions appeared to correspond only to active centromeres. Although Cd banding is now mainly of historical interest, the discovery of CREST autoantibodies that detected a family of proteins associated with centromeres by Earnshaw and Rothfield [73], led to numerous studies with fluorescent antibodies in the 1980s and 1990s that indicated the presence or absence of specific centromeric proteins (CENP-A, -B and -C) associated with active or inactive centromeres. Since then, much has subsequently been learned about the structure and organization of centromeres [74] (See Sect. 2.6.1 for a more detailed discussion).

### 2.1.6 *G-11 Banding*

G-11 staining is used to selectively stain some heterochromatic regions on human chromosomes a deep magenta color in contrast to the pale blue color of the remainder of the chromosome. These include chromosomes 1, 3, 5, 7, 9, 10, 19 and Y. However, there is variability in the intensity of staining at the pericentromeric and satellite regions of acrocentric chromosomes. Such variability is dependent on the individual characteristics of these chromosomes. The G-11 technique utilizes modified Giemsa staining at an alkaline pH and is useful in the study of human heteromorphic variants and pericentromeric inversions, especially on chromosome 9. G-11 banding received its name from attempts to obtain differential banding of specific chromosome regions by staining in Giemsa at different pH values. The standard pH of the staining solution in G-banding procedures is 6.8–7.0. Patil et al. [75] showed that if the alkalinity of some Giemsa mixtures was raised to 9.0, G-banding could be achieved without any other special treatment. Bobrow et al. [76] subsequently showed that if alkalinity was raised to pH 11, subcomponents of C-bands, especially the secondary constriction (qh region) of chromosome 9 stained a deep magenta color in contrast to the pale blue color of the euchromatic regions. Various components of Giemsa studied by Wyandt et al. [77] showed that comparable G-11 bands were achieved with mixtures of eosin Y and azure B in the right proportions at pH 11. When mixed in equimolar amounts, most of the Azure B and Eosin Y precipitated as large highly reflective trapezoidal crystals of azure-eosinate. Finer crystals appeared to be precipitated at magenta colored sites on chromosomes (Fig. 2.4a–d).

Jones et al. [20] first showed that satellite III DNA, isolated on a silver cesium sulfate gradient, hybridized to the heterochromatic regions of chromosome 9 and to the acrocentric chromosomes. Buhler et al. [28] showed that magenta-staining by G-11 banding, which appears to be especially specific for 9qh, 15p and Yq, corresponded to sites of hybridization of a specific class of highly repetitive DNA,





**Fig. 2.4** **a** Metaphase showing G-11 banding with inset **b** showing enlarged 9 by bright-field and inset **c** showing same chromosome 9 by phase contrast microscopy. Inset **d** shows large trapezoidal azure-eosinate crystals by phase contrast microscopy [Modified from Wyandt et al (1976). *Exp Cell Res*, 102:85–94]

satellite III. Other classes of satellite DNA's, I–VII, were found to be distributed in chromosome 9 and in other chromosomes [21], but satellite III was found mainly in these three chromosomes.

### 2.1.7 Silver Staining (*Ag-NOR*)

Silver staining is a method to stain the nucleolar organizer regions (NORs) on the human acrocentric chromosomes. Early on, chromatic secondary constrictions on chromosomes were associated with nucleolar organizing. Hendersen et al. [78] used in situ hybridization of tritium-labeled rDNA to human metaphases to show presence of rDNA in the satellite regions in some combination of the acrocentric chromosomes. Pardue and Hsu [79, 80] used a similar technique to show hybridization of 18S and 28S rDNAs to secondary constrictions, purported to be nucleolar organizing, in Indian muntjac and several other small mammals. Howell and colleagues used an ammoniacal silver staining technique, which they initially called Ag-SAT, to specifically stain satellite III regions on human acrocentric chromosomes [14, 81], and subsequently developed a simplified technique using a colloidal developer for better results [82]. Goodpasture and Bloom [83] also used ammoniacal silver staining to study the same mammalian cell lines that had been

**Fig. 2.5** Metaphase showing typical Ag-NOR staining



used to localize ribosomal cistrons by in situ hybridization [79, 80] and showed that silver staining detected the very same reported NOR sites, which they then referred to as Ag-NORs. Goodpasture et al. [84] also pointed out that it was the stalk regions in human acrocentric chromosomes that were Ag-positive and not the satellites. Miller et al. [85] and many others [86–96] subsequently showed the Ag-positive regions to be NOR regions that were actively producing rRNAs. They also showed that this technique was most likely staining proteins. NORs vary in size and number in different individuals and even between cells, but specific patterns are heritable. The modal number of active NORs per cell varies between 6 and 10, depending on age [93, 95] and ethnic group (See Sect. 4.2). Nearly all studies show that chromosomes involved in satellite association are more likely to be silver stained, and that smaller or Ag-negative staining NOR regions are less frequently observed in satellite association (See Sect. 4.2 for additional discussion). Silver staining is an important banding method to study heteromorphic variations in the size and number of NORs, and to characterize marker chromosomes or other structural rearrangements involving the acrocentric chromosomes. Figure 2.5 shows a metaphase with typical Ag-NOR staining.

## 2.2 Other DNA-Binding Fluorochromes

A variety of different DNA binding fluorochromes will produce chromosome banding patterns or enhancement of AT or GC rich regions depending on absorption and emission spectra and how they are used in combination (Table 2.3). For instance, the combination of distamycin A (DA) and DAPI produces bright qh

**Table 2.3** Fluorescent DNA ligands used in human chromosome staining, base affinity and type of banding when used with counter stain (adapted from Verma and Babu) [22]

Primary dye	Affinity	Counter stain	Banding
DAPI	AT	Distamycin A <sup>a</sup>	DAPI/DA
DIPI	AT	Netropsin	DAPI/DA
		Pentamidine	DAPI/DA
Hoechst 33258	AT	Distamycin A <sup>a</sup>	DAPI/DA
		Netropsin	DAPI/DA
		Actinomycin D <sup>b</sup>	QFH-bands
		Chromomycin A <sub>3</sub>	QFH-bands
7-aminoactinomycin D	GC	Methyl green <sup>a</sup>	R-bands (enhanced)
Chromomycin A <sub>3</sub>	GC	Distamycin A <sup>a</sup>	R-bands (enhanced)
Mithramycin	GC	Malachite green <sup>a</sup>	R-bands (enhanced)
Olivomycin	GC	Distamycin A	R-bands (enhanced)
		Netropsin	R-bands (enhanced)
		Methyl green <sup>a</sup>	R-bands (enhanced)
Coriphosphin		Methyl green	R-bands (modified)
Quinacrine/quinacrine	GC (low) mustard		Q-bands

<sup>a</sup>Non-fluorescent with AT affinity<sup>b</sup>Non-fluorescent with GC affinity

regions on chromosome 1, 9 and 16 that correspond to G-11 bands and probably to satellite III DNA. The use of various fluorochromes and their mechanisms of action have been described by others [22] and will not be described in detail here.

## 2.3 Sister Chromatid Exchange Staining (SCE)

Sister chromatid exchanges (SCE) are the result of an interchange of DNA between replication products at homologous loci [97]. SCEs at low levels are normally seen in humans and can be demonstrated in somatic cells by incorporating a thymidine analog, 5-bromodeoxyuridine (BrdU) into replicating DNA for two successive cell cycles and subsequent photodegradation of the resulting chromosomes. Staining of metaphases with Hoechst 33258 [98] or with Giemsa following this procedure results in faint staining of one chromatid and strong staining of the other chromatid. A reversal of staining intensity of the two chromatids occurs where there has been an exchange (Fig. 2.6). Because of the semi-conservative nature of DNA replication, after two complete pulses of BrdU substitution, one chromatid has both halves of the DNA helix BrdU-substituted (bifilarly labeled) while the other chromatid has only one half of the DNA helix BrdU-substituted (monofilarly labeled). The latter is

**Fig. 2.6** Metaphase showing sister chromatid exchanges (arrows)



the basis of the differences in staining of sister chromatids that allows for the detection of SCEs, mainly in non-heterochromatic regions. The technique has been extensively used for testing the mutagenic potential of various chemicals [99], to study cell cycle kinetics [100, 101] and to diagnose Bloom syndrome, in which there is a ten-fold increase in SCE per cell [102].

## 2.4 Replication Banding

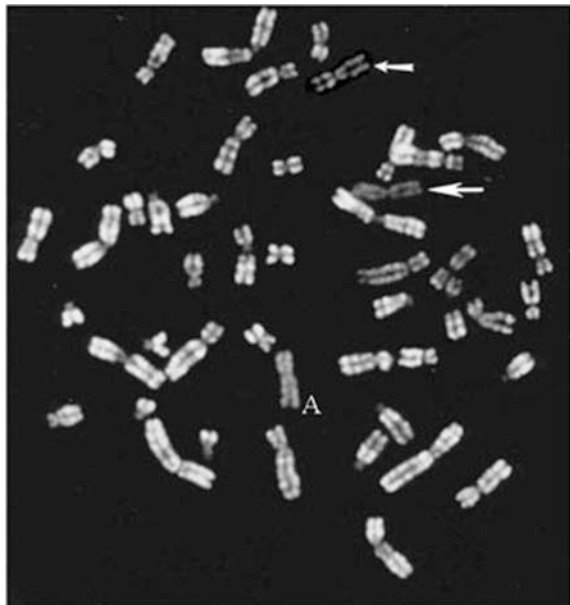
Replication banding is most useful in identifying the early and late replicating X-chromosomes in females or in patients with sex chromosome abnormalities. It is well known that one of the X-chromosomes in females is inactive, resulting in dosage compensation [103]. It is also known that X chromosome inactivation is random and that the inactive X chromosome initiates and completes DNA synthesis later than the active X and other chromosomes [104–108]. Replication banding, obtained by the incorporation of 5-bromodeoxyuridine (BrdU) and a subsequent staining with Giemsa or other stains [98], allows for the distinction of the active and inactive X-chromosomes. Variations in replication banding can also be achieved. In the “T pulse” procedure, BrdU is made available at the beginning of the cell cycle and is then replaced with thymidine for the last 5–6 h before the harvest. With the RBG technique (R-bands by BrdU and Giemsa), active or early replicating chromosome regions represented by the active X chromosome stain light. The “B pulse” is the opposite. Thymidine, made available at the beginning of the cell cycle, is replaced with BrdU the last 5–6 h before harvest. Subsequent Giemsa

staining will result in early-replicating chromosome regions appearing dark because they have incorporated thymidine, while the inactive or late replicating chromosome regions appear pale due to the BrdU-incorporation.

**Banding patterns:** The equivalent of Q- and G- or R-banding patterns is achieved depending on whether a B or T pulse is used. If a B-pulse is used, a Q or G-banding pattern is achieved and if a T-pulse is used, an R-banding pattern is achieved. Subtle changes in pattern toward the earliest R-bands or latest G-bands can be achieved by shortening the length of the BrdU pulse. A short T-pulse at the very end of the S-period can produce what are referred to as T-bands (bright or dark bands at the terminal ends of some chromosome arms). These bright bands with a T-pulse also correspond to early replicating, GC-rich regions, whereas dull bands correspond to late-replicating AT-rich regions. The exception to this is the late-replicating X chromosome whose bright bands do not differ in AT: GC content from the less intensely stained bands at the same locations on the early-replicating X (Fig. 2.7).

**Lateral asymmetry:** An interesting variation of the BrdU labeling technique is the method of detecting lateral asymmetry. The latter is due to an interstrand compositional bias in which one half of the DNA helix is predominantly T-rich and the complementary half is correspondingly A-rich [109]. Since BrdU substitutes for thymidine and not adenine, after one complete pulse of BrdU, the BrdU-rich strand stains less intensely than the T-rich complement, resulting in a block of heterochromatin that is more intensely stained on one chromatid than on the other (See Fig. 6.1c, Sect. 6.1). A more equal distribution of thymidine in both strands in either euchromatin or heterochromatin, without interstrand compositional bias,

**Fig. 2.7** Metaphase with 47, XX,i(Xq) showing replication banding with a late T-pulse showing active X (A), lighter staining inactive X (*small arrow*) and extra i(Xq) (*large arrow*)



results in both chromatids staining similarly. Variation in the size and location of such blocks forms the basis of a subclass of variants in chromosomes 1, 9, 15, 16 and Y [110–112].

## 2.5 High Resolution Banding and Special Treatments

Other treatments and methods that have particular bearing on characterizing heteromorphisms include treatments such as methotrexate added to cultures of synchronized cells in G2 [113], which is used for high resolution chromosome banding. Ethidium bromide intercalates into GC rich regions during cell culture, a property that is also used to produce elongated chromosomes for high resolution banding analysis [114, 115]. 5-azocytidine and a number of DNA analogs, such as FudR, produce very long secondary constrictions, such as shown by Balicek [116], or can enhance so-called “fragile sites” on chromosomes. Most of these are common fragile sites that can be induced in vitro in cells from anyone (See Chap. 7 on Fragile Sites). Other “rare” fragile sites are induced only in cells from certain individuals and are heritable.

## 2.6 Satellite DNA in Heteromorphic Regions

Genes and gene related sequences (promoters, introns, etc.) constitute about 25% of the human haploid genome; only about 3% of the genome is transcribed. Repetitive sequences comprising most of the remainder are the basis of both heteromorphisms observed at the chromosomal level and polymorphisms detected at the molecular level. Tandem repeated DNA sequences are classified by the length of the individual repeated unit and by total size [29].

Satellite DNA makes up approximately 10% of the genome [117, 118]. Consisting of large tandem repeated DNA sequences, it is located mainly in heterochromatic blocks in the pericentromeric regions of human chromosomes, the short arms of acrocentric chromosomes and the distal long arm of the human Y chromosome [119, 120]. *Alpha satellite* DNA is the principle component found at the centromere of every human chromosome. Other satellite DNAs distributed to various chromosomal locations include:

1. *Beta satellite* DNA, a 68 bp monomer that consists of different subsets that have been shown to be chromosome specific by FISH [121]
2. *Gamma satellite* DNA, a 220 bp monomer, observed at the centromeres of chromosomes 8 and X [122]
3. Additional families that include a 48 bp satellite DNA on the acrocentric chromosomes, and the Sn5 family found in the pericentromeric regions of chromosome 2 and the acrocentric chromosomes [123].

Human satellite DNA fractions, consisting of heterogeneous mixtures of repetitive DNA sequences isolated from main band DNA by buoyant densities on CsCl (cesium chloride) [124] or CsSO<sub>4</sub> (cesium sulfate) gradients [21, 24, 124], are referred to as *classical satellites I, II and III* [25, 125]. In situ hybridization of these fractions to human chromosomes labels locations that correspond to heterochromatin viewed by C-banding or by the fluorescent dyes DAPI and distamycin [25–27, 126].

Satellite DNA fractions have been further separated by restriction enzyme analysis into *classical satellites 1, 2 and 3*, found primarily in the large h regions of chromosomes 1, 9, 16 and Y [127–129]. Although satellites 1, 2 and 3 are incorporated within density gradient fractions, they are distinct from satellites I, II and III in that each is a simple 5 bp component within satellites I, II, and III, respectively [127]. By in situ hybridization, satellite 1 is localized to the pericentromeric regions of chromosomes 3 and 4, and the short arms of the acrocentric chromosomes, both proximal and distal to the rDNA of acrocentric stalk regions. Satellite 2 is localized to the large heterochromatic regions of chromosomes 1 and 16, with less prominent domains in the pericentromeric regions of chromosomes 2 and 10. Satellite 3 is localized to the h regions of chromosomes 1, 9, Y and the acrocentric chromosome short arms, proximal to the ribosomal DNA [127]. It is also found in the pericentromeric region of chromosome 10 [130].

### 2.6.1 Alpha Satellite DNA

The fundamental unit of alpha satellite DNA is a monomer of ~171 bp. Monomers are organized in tandem into higher-order repeats (HORs), ranging from 2 to >30 [131]. HORs at each centromere are in turn tandem repeated up to several hundred times to form an array of several million base pairs. HORs that are specific for each chromosome and hence useful as FISH probes that typically show less than 5% divergence. In addition to HORs, an alpha satellite DNA motif specifically serves as the binding site for centromeric Protein B (CENP-B), which is found in most mammalian centromeres and was initially thought to be involved in the assembly of essential kinetochores proteins, such as CENH3 (CENP-A), CENP-C and CENP-E. Although, the CENP-B-alpha-satellite motif is involved in normal functional human centromere/kinetochore formation [132, 133], more recent investigations indicate that epigenetic factors, rather than sequence directed mechanisms, are involved [133–143]. The discreet nature of a functional centromere in mammals and humans is determined by the presence of a specific nucleosome histone complex, which contains a variant H3 histone (CENP-A, also called CENH3), that is present in all mammalian centromeres, including spontaneously occurring neocentromeres (Dalal et al.) [135]. The roles of alpha satellite motifs, various centromeric proteins and epigenetic factors in centromere/kinetochore function are still active areas of investigation [136–143].

### 2.6.2 *Satellites I–IV*

Early on, satellites I–IV were shown to make up about 5% of the human genome and to be AT rich compared with main band DNA. Satellites I–IV are distinguished by their respective restriction endonuclease digestion products (using EcoRI and HaeIII). Satellite I is resistant to digestion by both enzymes, whereas satellites II–IV were identified by characteristic ladders of different size fragments, which include:

1. Fragments that are various multiples of a 170 bp monomer
2. Fragments with no obvious size relationship to the ladder fragments
3. A 3400 pb male-specific fragment.

Restriction enzyme digestions with HinfI and TaqI can distinguish additional different fragments for the remaining satellites [144].

The evolution of Satellite III, a family of repetitive sequences localized primarily to the short arms of chromosomes 13–15, 21 and 22, between the centromeric and rDNA sequences, and to heterochromatin of the long arms of 1, 9 and Y, has been studied by Jarmuz et al. [145]. Subfamilies of satellite III are divided into two groups based on the percentage of the characteristic penta bp sequence motifs, GGAAT and GGAGT. GGAAT is predominant in the first group, whereas both sequences are approximately equally present in the second group. Subfamilies pR-1, pR2, and pR4, representing the first group, are present on all acrocentric chromosomes. Group 2 is represented by subfamilies pTRS-47, pR-2 and pR-4. pR-1 and pTRS-47 are present on chromosomes 14 and 22. pE-2 is present on chromosomes 13, 14 and 21. pW-1 is present on chromosomes 13 and 21. pTRS-63 is present on chromosome 14. The distribution of these various subfamilies has been correlated with their evolution in various Homidae and, more or less, with the distribution of 18s and 24s rRNA genes. Although not associated with rDNA, satellite III sequences are found on the Y chromosome from gibbons to humans. By PCR, amplification of products specific to pW-1, pR-1 and pTRS-63 are found. Bands for pR-2, pTRS-47, pR-4 and pE-2 are also found, but do not contain satellite III when analyzed by sequencing. By FISH, weak signals for pR-1 and pR-4 are found, but none for pTRS-47 or pTRS-63.

Transcription of non-coding satellite III RNAs has been reported as a general stress response in human cells [146]. Heat shock transcription factor 1 (HSF1), which binds to and transcribes *hsp* genes in satellite III, is key in the protection of cells against the deleterious effects of stress [147].

### 2.6.3 *Beta Satellite*

Beta satellite consists of a 68 pb monomer [119] that is typically replicated as part of a higher order repeat (HOR or duplicon) that spans the transition from euchromatic genes in the short arm to predominantly heterochromatic satellite regions in



chromosomes 1q12, 3q12, 9q12, Yq11 and the pericentromeric/centromeric regions of acrocentric chromosomes 13–15, 21 and 22. In these regions, beta units are closely linked with LSau restriction sites [119] as part of a complex repeat (D4Z4-like unit) that is 3.3 kb in length and is localized on 4q35 and 10q26. Duplicons involving beta satellite repeats appear to have evolved independent of alpha satellite DNA localized to similar regions in old world monkeys, approximately 35 million years ago [148]. Chromosome 4q35 appears to be the ancestral locus for the D4Z4 sequence. Beta satellite appears to tentatively trace back to the orangutan.

A deletion of D4Z4 duplicons in 4q35 has been associated with causing fascioscapulohumeral muscular dysdystrophy [149]; however, loss of D4Z4 sequences on 10q26 has not been found to cause disease [149, 150].

#### **2.6.4 Minisatellites**

Levy and Warburton [29] classify minisatellites into AT and GC rich. Tandem repeated GC rich sequences [151] are present at many different loci which vary in the size of the individual repeat (6 to ~100 bp) as well as in total length (100 bp to several kilobases). The widely variable number of tandem repeats (VNTRs) at these loci has made them a useful tool in forensic science for individual identification by DNA fingerprinting, and as highly polymorphic, multiallelic markers for linkage studies [152]. While most minisatellites are GC-rich, AT-rich minisatellites in humans are remarkably different from the GC-rich minisatellites [153–157]. The common features of these alleles include a predicted tendency to form hairpin structures and a domain organization with similar variant repeats commonly existing as blocks within arrays [152]. These loci may also share some mechanisms of mutation, with transient single-stranded DNA, forming stable secondary structures which promote interstrand misalignment and subsequent expansions or contractions in repeat number [157]. Telomeres are a special subset of minisatellites. The majority of hypervariable minisatellite DNA sequences are not transcribed, however some have been shown to cause disease by influencing gene expression, modifying coding sequences within genes and generating fragile sites [152].

#### **2.6.5 Microsatellites**

Microsatellites consist of units of two to four nucleotides repeated one to a few dozen times. Polymorphic alleles of such sites consist of a differing number of repeats, also referred to as short tandem repeat polymorphisms (STRPs). Several hundred thousand STRP loci are distributed throughout the genome, with many

alleles for each locus in the population. Microsatellite polymorphisms are not usually implicated in disease, but are useful markers for determining the identity of a particular individual [158, 159].

## 2.7 Single Nucleotide Polymorphisms (SNPs)

The most common polymorphisms are single nucleotide polymorphisms (SNPs). In contrast to STRPs, SNPs usually only have two alleles for any specific location. They occur approximately once every 1000 bp, with approximately 3 million differences between any two genomes or an estimated 10 million alleles in all human populations. A subset of approximately 1 million of the most frequent SNPs has been chosen for a high-density map called the “HapMap” or haplotype map of the human genome [160].

## References

1. Denver Conference (1960) A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet* i:1063–1065; reprinted in Chicago Conference 1966, pp 12–15
2. London Conference on the Normal Human Karyotype (1963) *Cytogenetics* 2:264–268; reprinted in Chicago Conference 1966, pp 18–19
3. Chicago Conference (1966) Standardization in human cytogenetics. Birth defects: original article series, vol 2, no 2. The National Foundation, New York
4. Caspersson T et al (1968) Chemical differentiation along metaphase chromosomes. *Exp Cell Res* 49:219–222
5. Caspersson T, Zech L, Johansson C (1970) Analysis of human metaphase chromosome set by aid of DNA-binding fluorescent agents. *Exp Cell Res* 62:490–492
6. Geraedts JPM, Pearson PL (1974) Fluorescent chromosome polymorphisms; frequencies and segregation in a Dutch population. *Clin Genet* 6:247–257
7. Lin CC et al (1976) Chromosome analysis on 930 consecutive newborn children using quinacrine fluorescent banding technique. *Hum Genet* 31:315–328
8. Lubs HA et al (1977) Q and C-banding polymorphisms in 7 and 8 year old children: racial differences and clinical significance: In: Hook E, Porter (eds) *Population cytogenetic studies in humans*. Academic Press, New York, pp 133–159
9. Craig-Holms AP, Moore FB, Shaw MW (1973) Polymorphism of human C-band heterochromatin I: frequency of variants. *Am J Hum Genet* 25:181–192
10. Muller HJ, Klinger HP, Glasner M (1975) Chromosome polymorphism in a human newborn population II. Potentials of polymorphic chromosome variants for characterizing the idiogram of an individual. *Cytogenet Cell Genet* 15:235–239
11. McKenzie WH, Lubs HA (1975) Human Q and C chromosomal variations: distribution and incidence. *Cytogenet Cell Genet* 14:97–115
12. Magenis RE et al (1977) Heritability of chromosome banding variants. In: Hook EB, Porter IH (eds) *Population cytogenetics. Studies in humans*. Academic Press, New York, pp 179–188
13. Dutrillaux B, Lejeune J (1971) Cytogénétique humaine. Sur une nouvelle technique d’analyse du caryotype humain. *CR Acad Sci* 272:2638–2640

14. Howell WM, Denton TE, Diamond JR (1975) Differential staining of the satellite regions of human acrocentric chromosomes. *Experientia* 31:260–262
15. Bobrow M, Madan K, Pearson PL (1972) Staining of some specific regions of human chromosomes, particularly the secondary constriction of No. 9. *Nat New Biol* 238:122–124
16. Paris Conference (1971) Standardization in human genetics. Birth defects original article series, vol 8, no 7. The National Foundation, New York
17. Paris Conference Supplement (1975) Standardization in Human genetics. Birth defects original article series, vol XI, no 9. The National Foundation, New York
18. ISCN (2009) An international system for human cytogenetic nomenclature. In: Shafer LG, Tommerup (eds). S Karger, Basel
19. Saunders GF et al (1972) Locations of human satellite DNA in human chromosomes. *Nature New Biol* 236:244–246
20. Jones KW, Corneo G (1971) Location of satellite and homogeneous DNA sequences on human chromosomes. *Nature New Biol* 233:268–271
21. Ginelli E, Corneo G (1976) The organization of repeated DNA sequences in the human genome. *Chromosoma (Berl)* 56:55–69
22. Verma RS, Babu A (1995) Human chromosomes. Principles and techniques. McGraw-Hill, New York, pp 72–127
23. Babu A, Macera MJ, Verma RS (1986) Intensity heteromorphisms of human chromosome 15p by DA/DAPI technique. *Hum Genet* 73:298–300
24. Corneo G, Ginelli E, Polli EJ (1968) Isolation of complementary strands of a human satellite DNA. *J Mol Biol* 33:331
25. Miklos GLG, John B (1979) Heterochromatin and satellite DNA in man: properties and prospects. *Am J Hum Genet* 31:264–280
26. Gosden JR et al (1975) The location of four human satellite DNAs on human chromosomes. *Exp Cell Res* 92:148–158
27. Jones KW et al (1973) The chromosomal localization of human satellite DNA III. *Chromosoma (Berl.)* 42:445–451
28. Buhler EM et al (1975) Satellite DNA III and alkaline Giemsa staining. *Humangenetik* 26:329–333
29. Levi B, Warburton P (2004) Molecular dissection of heteromorphic regions. In: Wyandt HE, Tonk VS (eds) Atlas of human chromosome heteromorphisms. Kluwer Academic Publishers, Dordrecht, pp 97–105
30. Hopman AHN et al (1988) Non radioactive in situ hybridization. In: Van Leeuwen FW et al (eds) Molecular neuroanatomy. Elsevier Science Publishers B.V., pp 43–68
31. Lichter P, Ried T (1994) Molecular analysis of chromosome aberrations. In situ hybridization. In: Gosden JR (ed) Methods in molecular biology. Chromosome analysis protocols. Humana Press, Totowa, New Jersey (USA), pp 449–478
32. Caspersson T, Zech L, Johansson C (1970) Analysis of human metaphase chromosome set by aid of DNA-binding fluorescent agents. *Exp Cell Res* 62:490–492
33. Weisblum B, de Haseth PL (1972) Quinacrine, a chromosome stain specific for deoxyadenylate-deoxythymidylate rich regions in DNA. *Proc Natl Acad Sci USA* 63:629–632
34. Ellsman JR, Barr HJ (1972) Quinacrine fluorescence of specific chromosome regions. Late replication and high A: T content in *Samoa leonensis*. *Chromosoma* 36:375–390
35. Comings DE et al (1975) Mechanism of chromosome banding. V. Quinacrine banding. *Chromosoma* 50:111–145
36. Michelson AM, Monny C, Kovoov A (1972) Action of quinacrine mustard on polynucleotide. *Biochimie* 54:1129–1136
37. Sumner AT, Evans HJ, Buckland RA (1971) New technique for distinguishing between human chromosomes. *Nature New Biol* 232:31–32
38. Drets ME, Shaw MW (1971) Specific banding patterns of human chromosomes. *Proc Nat Acad Sci (USA)* 68:2073–2077
39. Seabright M (1971) A rapid banding technique for human chromosomes. *Lancet* 2:971–972

40. Wang HC, Fedoroff (1972) Banding in human chromosomes treated with trypsin. *Nat New Biol* 235:52–54
41. Muller W, Rozenkranz (1972) Rapid banding technique for human and mammalian chromosomes. *The Lancet* 1:898
42. Pearson P (1972) The use of new staining techniques for human chromosome identification. *J Med Genet* 9:264–275
43. Sumner AT (1994) Chromosome banding and identification absorption staining. In: Gosden GR (ed) *Methods in molecular biology. Chromosome analysis protocols*. Humana Press, Totowa, NJ, pp 59–81
44. Saitoh Y, Laemmli (1994) Metaphase chromosome structure: bands arise from a differential folding path of highly AT-rich scaffold. *Cell* 75:609–621
45. Hancock R (2000) A new look at the nuclear matrix. *Chromosoma* 109(4):219–225
46. Nickerson J (2001) Experimental observations of the nuclear matrix. *J Cell Sci* 114(3):463–474
47. Comings DE, Avelino A (1974) Mechanisms of chromosome banding II. Evidence that histones are not involved. *Exp Cell Res* 86:202–206
48. Comings DE et al (1973) The mechanisms of C- and G-banding of chromosomes. *Exp Cell Res* 77:469–493
49. Sumner AT (1973) Involvement of protein disulphides and sulphydryls in chromosome banding. *Exp Cell Res* 83:438–442
50. Korf BR et al (1976) The role of trypsin in the pre-treatment of chromosomes for Giemsa banding. *Hum Genet* 31:27–33
51. Holmquist GP (1992) Chromosome bands, their chromatin flavors and their functional features. *Am J Hum Genet* 51:17–37
52. Brown RL, Pathak S, Hsu TC (1975) The possible role of histones in the mechanism of G-banding. *Science* 189:1090–1091
53. Holmquist GP, Comings DE (1976) Histones and G-banding of chromosomes. *Science* 193:599–602
54. Retief AE, Ruchel R (1977) Histones removed by fixation. Their role in the mechanism of chromosome banding. *Exp Cell Res* 106:233–237
55. Okada TA, Comings DE (1974) Mechanisms of chromosome banding III. Similarity between G-bands of mitotic chromosomes and chromomeres of meiotic chromosomes. *Chromosoma* 48:65–71
56. Jhanwar SC et al (1982) Mid-pachytene chromomere maps of human autosomes. *Cytogenet Cell Genet* 33:240–248
57. Comings DE (1975) Mechanisms of chromosome banding. IV. Optical properties of the Giemsa dyes. *Chromosoma* 50:89–110
58. Comings DE, Avelino E (1975) Mechanisms of chromosome banding VII. Interaction of methylene blue with DNA and chromatin. *Chromosoma* 51:365–379
59. Wyandt HE et al (1980) Mechanisms of Giemsa banding II. Giemsa components and other variables in G-banding. *Hum Genet* 53:211–215
60. Dutrillaux B, Lejeune J (1971) Cytogenetique humaine. Sur une nouvelle technique d'analyse du caryotype humain. *CR Acad Sci (III)* 272:2638–2640
61. Bobrow M, Collacott HEAC, Madan K (1972) Chromosome banding with acridine orange. *Lancet* 2:1311
62. Wyandt HE et al (1974) Colored reverse-banding of human chromosomes with acridine orange following alkaline-formalin treatment: densitometric validation and applications. *Humangenetik* 23:119–130
63. Pardue ML, Gall JG (1970) Chromosomal localization of mouse satellite DNA. *Science* 168:1356–1358
64. Arrighi FE, Hsu TC (1971) Localization of heterochromatin in human chromosomes. *Cytogenetics* 10:81–86
65. Hsu TC, Arrighi FE (1971) Distribution of constitutive heterochromatin in mammalian chromosomes. *Chromosoma* 34:243–253

66. Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75:304–306
67. McKenzie WH, Lubs HA (1973) An analysis of the technical variables in the production of C bands. *Chromosoma* 41:175–182
68. Eiberg H (1974) New selective Giemsa technique for human chromosomes. Cd staining. *Nature* 248:55
69. Nakagome Y et al (1984) The “loss” of centromeres from chromosomes of aged women. *Am J Hum Genet* 36:398–404
70. Nakagome Y et al (1986) The loss of centromeric heterochromatin from an inactivated centromere of a dicentric chromosome. *Jinrui Idengaku Zasshi* 31:21–26
71. Evans HJ, Ross A (1974) Letter: spotted centromeres in human chromosomes. *Nature* 249:861–862
72. Maraschio P, Zuffardi O, Lo CF (1980) Cd bands and centromeric function in dicentric chromosomes. *Hum Genet* 54:265–267
73. Earnshaw WC, Rothfield (1985) Identification of a family of centromere proteins using autoimmune sera from patients with scleroderma. *Chromosoma* 91(3–4):313–321
74. Willard HF (1990) Centromeres of mammalian chromosomes. *Trends Genet* 6:410–416
75. Patil SR, Merrick S, Lubs HA (1971) Identification of each human chromosome with a modified Giemsa stain. *Science* 173:821–822
76. Bobrow M, Madan K, Pearson PL (1972) Staining of some specific regions of human chromosomes, particularly the secondary constriction of No. 9. *Nature New Biol.* 238:122–124
77. Wyandt HE et al (1976) Mechanism of Giemsa banding of chromosomes I. Giemsa-11 banding with azure and eosin. *Exp Cell Res* 102:85–94
78. Hendersen AS, Warburton D, Atwood KC (1972) Localization of ribosomal DNA in human chromosomal complement. *Proc Nat Acad Sci USA* 69(11):3394–3398
79. Pardue ML, Hsu TC (1975) Locations of 18S and 28S ribosomal genes on the chromosomes of Indian muntjac. *J Cell Biol* 64:251–254
80. Hsu TC, Spirito SE, Pardue ML (1975) Distribution of 18 + 28S ribosomal genes in mammalian genomes. *Chromosoma* 53(1):25–36
81. Howell WM, Denton TE (1974) An ammoniacal—silver stain technique specific for satellite III DNA regions on human chromosomes. *Experientia* 30(11):1364–1366
82. Howell WM, Black DA (1980) Controlled silver-staining of nucleolar organizer regions with protective colloidal developer: a 1-step method. *Experientia* 36(8):1014–1015
83. Goodpasture C, Bloom SE (1975) Visualization of nucleolar organizing regions in mammalian chromosomes using silver staining. *Chromosoma* 53(1):37–50
84. Goodpasture C et al (1976) Human nucleolus organizers: the satellites or the stalks? *Am J Hum Genet* 28:559–566
85. Miller OJ et al (1976) Expression of human and suppression of mouse nucleolus organizer activity in mouse-human somatic cell hybrids. *Proc Natl Acad Sci USA* 73:4531–4535
86. Verma RS et al (1983) Preferential association of nucleolar organizing human chromosomes as revealed by silver staining technique at mitosis. *Mol Gen Genet* 190:352–354
87. Mikelsaar AV et al (1977) Frequency of Ag-stained nucleolar organizer regions in the acrocentric chromosomes of man. *Hum Genet* 37(1):73–77
88. Markovic VD, Worton GR, Berg JM (1978) Evidence for the inheritance of silver stained nucleolus organizer regions. *Hum Genet* 41(2):181–187
89. Mikelsaar AV, Ilus T (1979) Populational polymorphisms in silver staining of nucleolus organizer regions in human acrocentric chromosomes. *Hum Genet* 51(3):281–285
90. Ray M, Pearson J (1979) Nucleolar organizing regions of human chromosomes. *Hum Genet* 48(2):201–210
91. Taylor EF, Martin-DeLeon PA (1981) Familial silver staining patterns of human nucleolus organizer regions (NORs). *Am J Hum Genet* 33(1):67–76
92. Morton CC et al (1981) Quinacrine mustard and nucleolar organizer region heteromorphisms in twins. *Acta Genet Med Gemellol (Roma)* 30(1):39–49

93. Denton TE et al (1981) The relationship between aging and ribosomal gene activity in humans as evidenced by silver staining. *Mech Ageing Dev* 15(1):1–7
94. Zakharov AF et al (1982) Polymorphisms of Ag-stained nucleolar organizer regions in man. *Hum Genet* 60(4):334–339
95. Das BC et al (1986) The number of silver-staining NORs (rDNA) in lymphocytes of newborns and its relationship to human development. *Mech Ageing Dev* 36(2):117–123
96. Zurita F et al (1997) Interchromosomal, intercellular and interindividual variability of NORs studied with silver staining and in situ hybridization. *Heredity (Edinb)* 78(3):229–234
97. Perry P, Wolff S (1974) New Giemsa method for differential staining of sister chromatids. *Nature* 261:156–158
98. Latt SA (1973) Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc Natl Acad Sci (USA)*. 70:3395–3399
99. Gebhart E (1981) Sister chromatid exchange (SCE) and structural chromosome aberration in mutagenicity testing. *Hum Genet* 58:235–254
100. Craig-Holmes AP, Shaw MW (1976) Cell cycle analysis of asynchronous cultures using the BudR-Hoechst technique. *Exp Cell Res* 99:79–87
101. Crossen PE, Morgan WF (1977) Analysis of human lymphocyte cell cycle time in culture measured by sister chromatid differential staining. *Exp Cell Res* 104:453–457
102. German J, Crippa LP, Bloom D (1974) Bloom's syndrome III. Analysis of the chromosome aberrations characteristic of this disorder. *Chromosoma* 48:361–366
103. Lyon MF (1961) Gene action in the X-chromosome of the mouse (*Mus musculus L.*). *Nature* 190:372–373
104. German JL III (1962) DNA synthesis in human chromosomes. *Trans NY Acad Sci* 24: 395–407
105. Peterson AJ (1964) DNA synthesis and chromosomal asynchrony. *J Cell Biol* 23:651–654
106. Priest JH, Heady JE, Priest RE (1967) Delayed onset of replication of human X chromosomes. *J Cell Biol* 35:483–486
107. Lyon MF (1972) X-chromosome inactivation and developmental patterns in mammals. *Biol Rev* 47:1–35
108. Latt SA (1975) Fluorescence analysis of late DNA replication in human metaphase chromosomes. *Somat Cell Genet* 1:293–321
109. Angell RR, Jacobs PA (1975) Lateral asymmetry in human constitutive heterochromatin. *Chromosoma* 51:301–310
110. Lin MS, Alfi OS (1978) Detection of lateral asymmetry in the C band of human chromosomes by BrdU-DAPI fluorescence. *Somatic Cell Genet* 4:603–608
111. Angell RR, Jacobs PA (1978) Lateral asymmetry in human constitutive heterochromatin: frequency and inheritance. *Am J Hum Genet* 30:144–152
112. Gosh PK, Rani R, Nand R (1979) Lateral asymmetry of constitutive heterochromatin in human chromosomes. *Hum Genet* 52:79–84
113. Yunis JJ (1976) High resolution of human chromosomes. *Science* 191:1268–1269
114. Yunis JJ (1981) Mid prophase human chromosomes; the attainment of 2000 bands. *Hum Genet* 56:295–298
115. Ikeuchi T (1984) Inhibitory effect of ethidium bromide on mitotic chromosome condensation and its application to high-resolution chromosome banding. *Cytogenet Cell Genet* 38:56–61
116. Balicek P, Zizka J (1980) Intercalar satellites of human acrocentric chromosomes as a cytological manifestation of polymorphisms in GC-rich material. *Hum Genet* 54:343–347
117. Wayne JS, Creeper LA, Willard HF (1987) Organization and evolution of alpha satellite DNA from human chromosome 11. *Chromosoma* 95:182–188
118. Choo KHA, Vissel B, Earle E (1989) Evolution of Alpha satellite DNA on human acrocentric chromosomes. *Genomics* 5:332–344
119. Wayne JS, Willard HF (1989) Human beta satellite DNA: genomic organization and sequence definition of a class of highly repetitive tandem DNA. *Proc Natl Acad Sci (USA)* 86:6250–6254

120. Vissel B, Choo KH (1989) Mouse major (gamma) satellite DNA is highly conserved and organized into extremely long tandem arrays: implications for recombination between non-homologous chromosomes. *Genomics* 5:407–414
121. Wier HU, Zitzelsberger HF, Gray JW (1992) Differential staining of human and murine chromatin *in situ* by hybridization with species-specific satellite DNA probes. *Biochem Biophys Res Commun* 182:1313–1319
122. Lee C et al (1995) Human gamma X satellite DNA: an X chromosome specific centromeric DNA sequences. *Chromosoma* 104:103–112
123. Johnson DH, Kroisel PM, Klapper HJ, Rosenkranz W (1992) Microdissection of a human marker chromosome reveals its origin and a new family of centromeric repetitive DNA. *Hum Mol Genet* 1:741–747
124. Corneo G, Ginelli E, Polli E (1970) Repeated sequences in human DNA. *J Mol Biol* 48:319–327
125. Jones KW et al (1973) Satellite DNA, constitutive heterochromatin and human evolution. In: Pfeiffer RA (ed) *Modern aspects of cytogenetics: constitutive heterochromatin in man*. Stuttgart-New York, F.K. Schattauer Verlag, pp 45–61
126. Jones KW et al (1974) The chromosomal location of human satellite DNA I. *Chromosoma* 49:161–171
127. Prosser J et al (1986) Sequence relationships of three human satellite DNAs. *J Mol Biol* 187:145–155
128. Tagarro I, Wiegant J, Raap AK, Gonzalez-Aguilera JJ, Fernandez-Peralta AM (1994) Assignment of human satellite 1 DNA as revealed by fluorescent *in situ* hybridization with oligonucleotides. *Hum Genet* 93:125–128
129. Jeanpierre M (1994) Human satellites 2 and 3. *Ann Genet* 37:63–71
130. Jackson MS, Mole SE, Ponder BA (1992) Characterization of a boundary between satellite III and aliphoid sequences on human chromosome 10. *Nucleic Acids Res* 20:4781–4787
131. Willard HF (1991) Evolution of alpha satellite DNA. *Curr Opin Genet Dev* 1:509–514
132. Cooke CA et al (1990) CENP-B: a major human centromeric protein located beneath the kinetochore. *J Cell Biol* 110:1475–1488
133. Gieni RS, Chan GKT, Hendzel MJ (2008) Epigenetics regulate centromere formation and kinetochore function. *J Cell Biochem* 104:2027–2039
134. Masumoto H et al (2004) The role of CENP-B and alpha-satellite DNA: de novo assembly and epigenetic maintenance of human centromeres. *Chromosome Res* 12(6):543–556
135. Dalal Y et al (2007) Structure, dynamics and evolution of centromeric nucleosomes. *Proc Nat Acad Sci USA* 104(41):15974–15981
136. Perpelescu M, Fukagawa T (2011) The ABCs of CENPs. *Chromosoma* 120(5):425–446
137. Nechemia-Arbely Y et al (2012) Replicating centromeric chromatin: special and temporal control of CENP-A assembly. *Exp Cell Res* 318(12):1353–1360
138. Stellfox MA et al (2013) Putting CENP-A in its place. *Cell Mol Life Sci* 70(3):387–406
139. Padeganeh A et al (2013) Octameric CENP-A nucleosomes are present at human centromeres throughout the cell cycle. *Curr Biol* 23:764–769
140. Henikoff JG et al (2015) A unique chromatin complex occupies young  $\alpha$ -satellite arrays of human centromeres. *Sci Adv* 20(15):e1400234
141. Earnshaw WC (2015) Discovering centromere proteins: from cold white hands to the A, B, C of CENPs. *Nat Rev Mol Cell Biol* 16(7):443–449
142. Fujita R et al (2015) Stable complex formation of CENP-B with the CENP-A nucleosome. *Nucleic Acids Res* 43(10):4909–4922
143. Fachinetti D et al (2015) DNA sequence-specific binding of CENP-B enhances the fidelity of human centromere function. *Dev Cell* 33(3):314–327
144. Frommer M et al (1982) Simple repeated sequences in human satellite DNA. *Nucleic Acids Res* 10(2):547–563
145. Jarmuz M et al (2007) Evolution of satellite III DNAs. *Am J Hum Genet* 80:495–501
146. Valgardsdottir R et al (2008) Transcription of satellite III non-coding RNAs is a general stress response in human cells. *Nucleic Acids Res* 36(2):423–434

147. Herbolme G et al (2013) Dynamics of the full length and mutated heat shock factor 1 in human cells. *PLOS ONE* 8(7):e67566
148. Cardonne MF et al (2004) Evolution of Beta satellite DNA sequences: evidence for duplication-mediated repeat amplification and spreading. *Mol Biol Evol* 21(9):1792–1799
149. Lemmers RJLF et al (2010) Worldwide population analysis of 4q and 10q subtelomeres identifies only four discrete interchromosomal sequence transfers in human evolution. *Am J Hum Genet* 86:364–377
150. Scionti I et al (2012) Large scale population analysis challenges the current criteria for the molecular diagnosis of fascioscapulohumeral muscular dystrophy. *Am J Hum Genet* 90: 628–635
151. Bosi PR, Grant GR, Jeffreys AJ (2002) Minisatellites show rare and simple intra-allelic instability in the mouse germ line. *Genomics* 80:2–4
152. Desmarais E et al (1993) Variant mapping of the Apo(B) AT rich minisatellite. Dependence on nucleotide sequence of the copy number variations. Instability of the non-canonical alleles. *Nucleic Acids Res* 21:2179–2184
153. Buresi C et al (1996) Structural analysis of the minisatellite present at the 3' end of the human apolipoprotein B gene: new definition of the alleles and evolutionary implications. *Hum Mol Genet* 5:61–68
154. Yu S et al (1997) Human chromosomal fragile site FRA16B is an amplified AT-rich minisatellite repeat. *Cell* 88:367–374
155. Hewett DR et al (1998) FRA10B structure reveals common elements in repeat expansion and chromosomal fragile site genesis. *Mol Cell* 1:773–781
156. Jobling MA, Bouzekri N, Taylor PG (1998) Hypervariable digital DNA codes for human paternal lineages: MVR-PCR at the Y-specific minisatellite, MSY1(DYF155S1). *Hum Mol Genet* 7:643–653
157. Butler JM (2004) Short tandem repeat analysis for human identity testing. *Current protocols in human genetics*. Unit 14.8
158. Butler JM (2007) Short tandem repeat typing technologies used in human identity testing. *BioTechniques* 43 suppl:i–iv
159. Payseur BA, Jing P (2009) A genomewide comparison of population structure at STRPs and nearby SNPs in humans. *Mol Biol Evol* 26:1369–1377
160. Manolio TA, Collins FS (2009) The HapMap and genome-wide association studies in diagnosis and therapy. *Ann Rev Med* 60:443–456



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