22

Chromosome Changes in Cell Differentiation

The most striking achievement of embryogenesis is the differentiation of the genetically identical descendants of a fertilized egg into hundreds of different types of cells and eventually into a highly complex human being. Cell differentiation is accompanied by changes in chromatin structure or, less commonly, ploidy level; these mediate the changes in gene expression required for cell differentiation. Thus, the chromatin structure in stem cells of the germline is readily distinguished from that of differentiating oocytes and spermatocytes, and that of stem cells in the bone marrow, intestine, or skin from that of the more differentiated cell types they give rise to. These changes in chromatin conformation and ploidy level are brought about by a number of quite different mechanisms (reviewed by Miller, 1997).

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Programmed DNA Loss

Programmed DNA loss is an important mechanism of cell differentiation, although one used by only a few cell lineages. The best known example is erythropoiesis, in which red blood cell precursors undergo heterochromatinization and condensation of their chromatin and finally extrude the entire nucleus. More restricted, and precise, DNA loss occurs in the differentiation of T and B lymphocytes. This involves DNA double-strand breakage, excision of large portions of the T cell receptor gene array and B cell immunoglobulin heavy and light chain gene arrays, and V(D)J rejoining of the remaining DNA to yield a very large number of uniquely modified genes in T and B cells, with any one cell expressing only one allele of a now unique gene. The resulting T cell population has an enormous variety of distinctive receptors, while the B cell population can generate an equally enormous variety of different antibodies (Tonegawa, 1983). It is interesting that the unrearranged kappa light chain gene array is heavily methylated and inactive, but after V(D)J rejoining is completed, the rearranged kappa chain gene is demethylated and becomes capable of transcription (Lichtenstein et al., 1994).

Facultative Heterochromatin: Chromatin Structure and Gene Expression

Facultative heterochromatinization during differentiation is widespread. It is functionally equivalent to programmed loss of the DNA sequences involved but has the advantage of being reversible. The best known examples are X inactivation (Chapter 18) and genomic imprinting (Chapter 21). Both of these involve an epigenetic differentiation process that leads to differential expression of the two parental alleles at a given locus, or at multiple loci, and asynchronous DNA replication of the two alleles, the inactive one becoming late replicating. The olfactory receptor (OR) gene family provides a third example. There are several hundred to a thousand OR genes, scattered in multiple clusters on numerous chromosomes (Chapter 31). Several studies have shown that a single olfactory neuron expresses no more than a very tiny number of these OR genes (Malnic et al., 1999), perhaps only one from each cluster. Highly sensitive reverse transcription–polymerase chain reaction (RT-PCR) analyses indicate that each olfactory neuron expresses only one allele of a given OR gene, and from early

embryonic life the two alleles replicate asynchronously, like imprinted or X-linked genes (Chess et al., 1994).

At the most general level, there are two classes of genes: housekeeping and tissue-specific. Housekeeping genes are constitutively expressed in most cell types. Their protein products carry out the various metabolic, structural, and other housekeeping functions required by all cells. In contrast, a restricted array of tissue-specific genes are expressed in a particular cell or tissue and shut down in other cell types. Such gene inactivation is associated with a more condensed chromatin structure, in which the DNA of the gene is not accessible to the transcription factors needed to transcribe RNA from it. The mechanisms involved in such chromatin changes appear to involve methylation of cytosine residues in CpG dinucleotides within the promoter regions just upstream (5′) of genes. When methylated, these sites can bind a specific protein, which in turn binds a histone deacetylase, HDAC1. This enzyme removes acetyl groups from core histones, producing a denser chromatin structure (Chapter 5). Phosphorylation of core histones also leads to a tighter chromatin conformation, but this mechanism is more important for chromosome condensation during mitosis and meiosis than for interphase gene regulation (Chapter 2).

Nature and Mechanism of Genomic Imprinting

Imprinting is thought to occur during gametogenesis, but is this always the case? There is biallelic expression (absence of imprinting) of the *SNRPN* and *UBE3A* genes in all tissues except the brain. This means that an early imprint has been removed or that, like X inactivation, imprinting occurs during embryogenesis and may show tissue-specific differences in timing or occurrence. It could also mean that there is a way around the repressive effect of the imprint. The insulin-like growth factor 2 (*IGF2*) gene is imprinted in fetal liver but shows biallelic expression in adult liver. The reason for this discrepancy is that *IGF2* is transcribed from both alleles from promoter P1, used in adult liver, but is transcribed only from one allele from promoters P3 and P4, used in fetal liver (Vu and Hoffman, 1994). It is unclear whether a similar explanation accounts for other examples of apparently tissue-specific imprinting. Rodent-human somatic cell hybrids provide one model system in which to study genomic imprinting, because the imprint on a single human chromosome, 11 or 15, say, is maintained in the hybrid cells (Gabriel et al., 1998).

What triggers imprinting of any gene is not known. However, details of the process of imprinting are beginning to be worked out. For example, the critical region for Angelman and Prader–Willi syndromes appears to contain a specific imprinting center, comparable to that involved in X inactivation. A cluster of genes in the Prader–Willi critical region is inactivated on the maternal chromosome 15, possibly by the untranslated RNA product of one member of the maternal gene cluster (Wevrick et al., 1994). Similarly, the maternal *IGF2* gene is inactivated by the untranslated RNA product of the maternal *H19* gene. In each case, the *cis*-acting RNA may form a complex with RNA-binding and other proteins, such as histone deacetylase. There is evidence that the removal of acetyl groups from core histones may be a general mechanism for imprinting and gene or X-chromosome inactivation (Jeppesen, 1997). DNA methylation also plays a role both in X inactivation and in imprinting, as well as in gene inactivation (Chaillet et al., 1995).

Deletions in the Prader–Willi syndrome have in common the loss of the *SNRPN* gene and result in the inappropriate silencing of the *IPW* and *ZNF127* genes in *cis* (on the same chromosome) that are 150 and 1000 kb away, flanking *SNRPN*. A man with the deletion cannot reset the imprinting program and therefore passes on to his children a maternal imprint. Thus, the region around the *SNRPN* gene produces a signal necessary for resetting the program. Dittrick et al. (1996) found, in a brain cDNA (bd) library, clones that hybridized to a series of transcripts present in the female germ line but absent in the male germ line. These transcripts arose from alternative upstream start sites, called BD exons, of the *SNRPN* gene. Dittrick et al. (1996) proposed a model showing how alternative transcripts of the *SNRPN* gene can lead to imprint switching. Their model, as interpreted by Bartolomei and Tilghman, 1997 (Fig. 22.1) also relates the locations of the close but not overlapping Angelman and Prader–Willi critical regions to the different imprints that normally occur in the male and female germlines.

The silencing of either maternal or paternal alleles is usually associated with methylation of the promoter region of the allele, rendering it inactive and late replicating. Thus, the paternal *H19* gene in the 11p15 region is methylated and silent, while the maternal allele is unmethylated and expressed. However, the *IGF2* gene in the same region, which is expressed only from the paternal allele, is imprinted only indirectly, and not by methylation. The maternal H19 gene produces an untranslated RNA product, which acts in *cis*, like the *XIST* RNA on the inactive X, to inactivate several nearby genes, including *IGF2*. The paternal

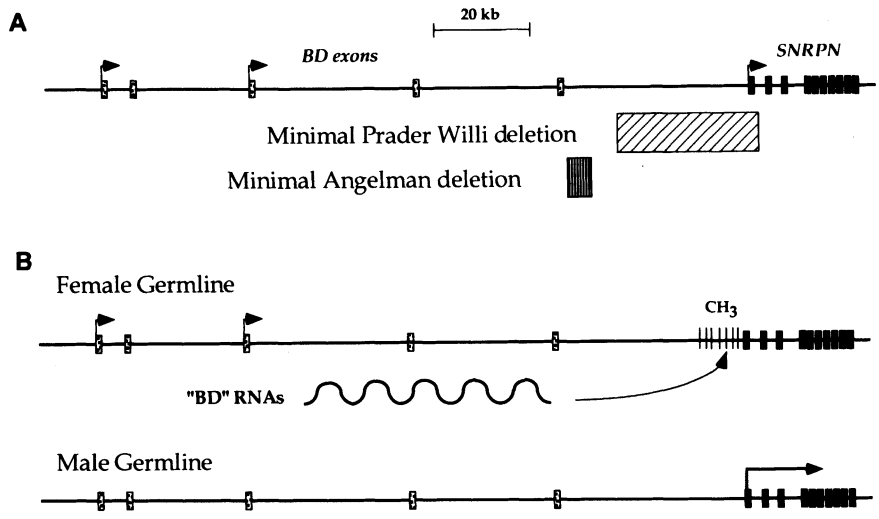

Figure 22.1. Imprinting effects in the Prader–Willi/Angelman region. (A) Exons (black) of the *SNRPN* gene and upstream BD exons (clear) of the *SNRPN* gene are shown, with transcription start sites indicated by arrows. (B) Model showing transcription from BD start sites in the female germline leads to methylation and inactivation of SNRPN, while absence of such BD transcription in the male germline allows *SNRPN* transcription (Bartolomei and Tilghman, 1997, with permission from Annu Rev Genet, v31, copyright 1997, by Annual Reviews, www.annualreviews.org).

H19 is inactive, so the paternal *IGF2* can be expressed (Bartolomei and Tilghman, 1997).

Tissue-Specific Differences in X Inactivation

X inactivation does not occur in early embryonic cells but requires some as yet unknown step in cell differentiation. The process is not yet understood despite intensive study both in humans and in model systems, including induced differentiation of undifferentiated but totipotent embryonic stem cells. The time of X inactivation varies from tissue to tissue; it does not occur in the male germ line until adulthood, and even then not until primary spermatocytes have differenti-

ated from the spermatogonial stem cells. The single X chromosome then remains inactive throughout spermatogenesis. X inactivation is reversed at a certain stage of differentiation of the female germline, again by mechanisms that are poorly understood, and both X chromosomes remain active throughout oogenesis (Fig. 18.2). In the extraembryonic tissues, the paternal X chromosome is preferentially inactivated, perhaps reflecting a paternal imprint that is erased in cells of the embryo itself (Chapter 18).

Erasing an imprint, or reactivating an inactive X chromosome, can be done by demethylation of the relevant DNA, comparable to that described above for immunoglobulin genes after V(D)J rearrangements have been completed. There are other examples of tissue-specific genes that are demethylated only in the cell type in which they are expressed (Kafri et al., 1993). A human enzyme capable of carrying out such demethylations has been identified. The gene for this demethylase is transcribed into mRNA in a wide variety of tissues, although demethylase activity has been demonstrated only in lung cancer cells (Bhattacharya et al., 1999). The high level of this enzyme in some cancers may be responsible for their reduced level of DNA methylation. The resultant alterations in gene expression may contribute to the genomic destabilization so important in the development of cancers (Chapter 26).

Germline-Specific Gene Expression and Sex-Specific Imprinting

In some respects, the germline behaves just like any other tissue whose differentiation requires the activation of specific genes and the inactivation of many others. A growing number of genes have been identified whose expression is limited to the male and/or female germline. One of the first of these was the autosomal phosphoglycerate kinase (*PGK*) gene. It is expressed only in the male germline, and only from the primary spermatocyte stage, when the X-linked *PGK* housekeeping gene is inactivated, along with most of the other genes on the X chromosome. The enzyme PGK is essential for cell survival. Another gene, that for cyclin A1, is expressed in both male and female germlines at the time of meiosis. The cyclin A1 mRNA level rises dramatically in late pachytene spermatocytes and falls to an undetectable level soon after meiosis is over. Somatic cells and germline stem cells, such as spermatogonia, express only cyclin A2 (Sweeney et al., 1996). Cyclins A1 and A2 activate the cyclin-dependent kinases CDK2 and CDK1 to drive the cell through S and G2 (Chapter 2).

One of the most recently identified genes with male germline–specific expression is the *DMRT1* gene. *DMRT1* has been mapped to 9p23.3–p24.1 by a breakpoint that disrupts the gene and caused XY male-to-female sex reversal, indicating its importance for male sex determination. A surprising finding is that *DMRT1* shares homology with a major sex-determining gene in both the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* (Raymond et al., 1998). Further evidence of the high degree of functional homology between even quite distantly related eukaryotes is provided by another gene with germline-specific expression: *diaphanous* (*DIA*) named for the homologous *Drosophila* gene, whose mutation produces male and female sterility (Bione et al., 1998). The human *DIA* gene maps to Xq21, as shown by disruption of the gene by a t(X;12)(q21;p13) translocation in a woman with secondary amenorrhea due to premature ovarian failure (lack of oocytes) at age 17. *DIA* is expressed only from the active X chromosome (Bione et al., 1998).

The male and female germlines differ remarkably not only in how they conduct meiosis and genetic recombination (Chapter 10) but also in their ability to imprint genes. Imprinting occurs during both spermatogenesis and oogenesis, but different genes are inactivated in the two processes, even when the imprinted genes are in the same region, as seen in the Prader–Willi and Angelman syndrome imprints in 15q11–q13 (Chapter 21). The mechanisms involved in differential imprinting in spermatogenesis and oogenesis, like those of imprinting itself, remain to be discovered.

Embryonic Inactivation of All but One Centromere per Chromosome

When a dicentric chromosome arises in the germline or early embryo, one of the two centromeres is usually inactivated during embryogenesis by what can be called a *differentiation* process. Only one primary constriction is visible (hence the name that is sometimes used, *pseudodicentric*), but a second C-band may mark the position of the inactive centromere (Fig. 22.2b,c). Inactivation of a centromere is usually a fixed event in differentiation, with the same centromere inactive in all somatic cells. Dicentric chromosomes in which the centromeres are some distance apart can be maintained only if one centromere is inactivated. This happens with most isodicentric and dicentric chromosomes, including Robertsonian translocations. The inactivation is not absolute in many of these dicen-

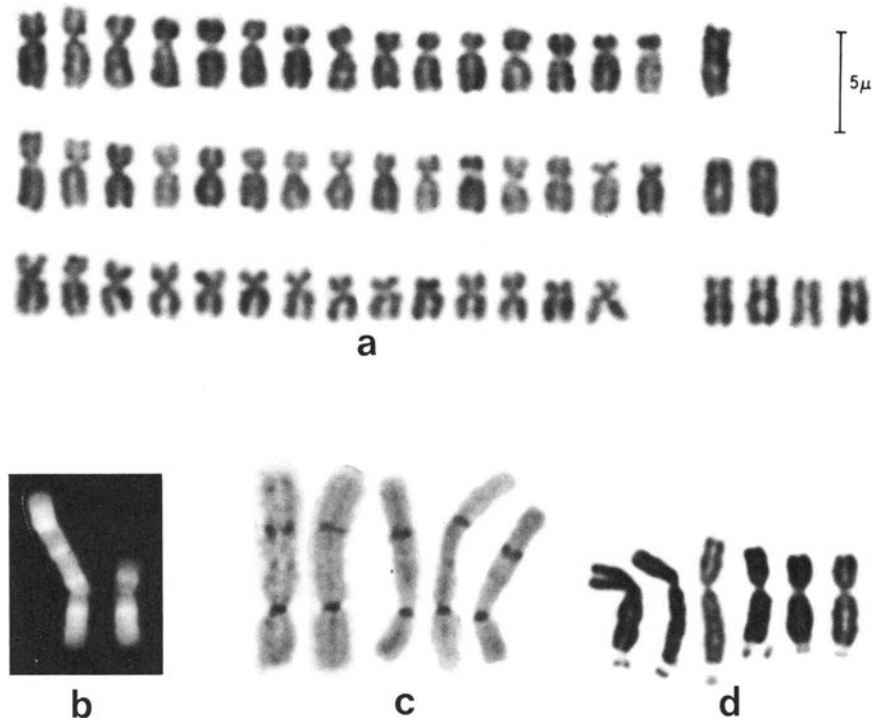

Figure 22.2. (a) The C group, with X chromosomes lacking a functional centromere, from three cells. (b) Q-banded idic(Xp–) joined at Xp telomeres, and normal X. (c) C-banded idic(Xp–) from five cells, showing two C-bands but only one primary constriction (active centromere). (d) X chromosome from six cells, expressing a fragile site (a–c, courtesy E. Therman; D, courtesy S. Roberts).

tric RTs (Chapter 16), and the same is true in some other dicentrics. Sullivan and Willard (1998) studied five stable dicentric X chromosomes made up of two copies of Xq separated by variable lengths of Xp. All cells of the one with 32 Mb of Xp had only one active centromere. The other four, with only 4–12 Mb of Xp, had two active centromeres in 82–87% of cells. In one interesting subject, who had a t(15;20)(pter;pter) chromosome, the centromere from chromosome 15 was always inactivated in lymphocytes, but either centromere could be inactivated in fibroblasts (Rivera et al., 1989). The inactivation process may be irreversible, as in a 6p;19p translocation, in which the chromosomes were attached end to end and the centromere of chromosome 6 was inactive. The dicentric chromosome had a tendency to break at the point of fusion, producing complex

mosaicism (Drets and Therman, 1983). The resultant chromosome 6 behaved like an acentric fragment, indicating that the centromere of this chromosome was not reactivated. The mechanism of centromere inactivation is unknown. Is it related to the mechanism that prevents sister chromatids from developing separate functional centromeres at the first meiotic division?

The same process of centromere inactivation may account for the absence of a functional centromere on some X chromosomes, especially in older women. These chromosomes look and act like acentric fragments, drifting at random in anaphase and giving rise to cells in which one X is missing and to cells in which Xs accumulate (Fig. 22.2A). This may account for the increased frequency of 45,X cells in older women (Fitzgerald and McEwan, 1977; Nakagome et al., 1984). Centromere inactivation has rarely been observed in monocentric autosomes (Therman et al., 1986).

Endoreduplication, Polyploidy, and Polyteny

Normal mitosis is characterized by alternating chromosome reproduction and segregation of daughter chromosomes. However, the two processes can be uncoupled in a variety of ways. Here we shall be concerned only with those modifications of mitosis that occur as a part of normal cell differentiation; those important in carcinogenesis are discussed in chapters 25 and 26. Virtually all these modifications are characterized by an absent or defective spindle, and in most cases they result in an increased number of chromosome sets, or *polyploidy*. The most important are the similar processes of endoreduplication and endomitosis.

Polyploidy always occurs in the differentiation of some types of cells, such as trophoblast cells of the placenta and platelet-producing megakaryocytes of the bone marrow. It occurs less often, and to a lesser degree, during the differentiation of a few other types of cells. Polyploidy most commonly arises by *endoreduplication*, in which the chromosomes replicate two or more times between mitoses instead of once. If two rounds of replication have occurred, the diplochromosomes in the next mitosis consist of four chromatids (Fig. 15.2). After three or four rounds of replication without an intervening mitosis, polytene chromosome bundles consisting of 8 or 16 chromatids, respectively, may be produced. Endoreduplication may occur in many tissues, especially in adverse conditions. In some human fibroblast cultures 3–5% of the dividing cells may be tetraploid

and a few even octoploid, whereas such divisions are rare in cultured lymphocytes. Diplochromosomes (with four chromatids) occur only at the first division after endoreduplication, so they are found in only a fraction of the tetraploid cells.

Some bone marrow cells undergo repeated rounds of endoreduplication without cell division to form platelet-producing megakaryocytes with 8C–128C amounts of DNA. Normal megakaryocyte differentiation is induced by the activation of the cytokine receptor c-MPL by the binding of its specific ligand, thrombopoietin (Drachman and Kaushansky, 1997). Differentiation can also be induced in cultured erythroleukemia cells by exposing them to a phorbol ester, such as TPA (12-0-tetradecanoyl phorbol-13-acetate). The failure of mitosis to occur in these cells may be due to a marked fall in the cyclin-dependent kinase CDK1 and a lack of cyclin B1/CDK1 kinase (metaphase promoting factor, MPF) activity, so that the completion of S does not trigger mitosis (Chapter 2; Datta et al., 1996). Without the activation of the anaphase promoting factor, APC, cyclin E is not targeted for destruction by the ubiquitin system, and repeated rounds of replication can occur.

Trophoblast cells of the placenta reach ploidy levels of 64C or higher. The mechanism leading to these polyploid cells is not clear. It may involve *endomitosis*, a process in which chromosomes duplicate and periodically condense, going through stages called *endoprophase*, *endometaphase*, and *endoanaphase*, but the nuclear membrane never breaks down (Sarto et al., 1982). Molecular analysis might clarify the events actually involved. In rodents, where trophoblast cells reach ploidy levels of 512C, the mechanism involved appears to be endoreduplication, with cells, and nuclei, increasing stepwise in both size and degree of polyploidy. This is accompanied by the development of polytene chromosomes in which all the sister chromatids synthesized from one homologue remain adherent to one another, even though they do not show the density and fine banding seen in *Drosophila* polytene chromosomes. In situ hybridization with four of five gene probes tested showed a single signal from all the amplified copies of tightly bundled sister chromatids in these interphase nuclei, with no pairing of homologues (Varmuza et al., 1988). Homologous pairing of mitotic chromosomes is generally absent in humans. The mitotic crossing over seen in Bloom syndrome (Chapter 24) is suggestive of somatic pairing, but the other chromosomes in the metaphase spreads do not show somatic pairing.

Cells with far more than one nucleus arise as a regular developmental change in a variety of cells, most notably placental cytotrophoblast and muscle. Their mechanism of production is cell fusion, which is triggered by still unknown

factors. Binucleate and low multinucleate cells can arise by mitosis that is not followed by cell division. This is a common phenomenon in liver (D'Amato, 1989).

Triradials, Multiradials, and ICF Syndrome, a Hypomethylation Disorder

A triradial chromosome, or triradial, is marked by a partial endoreduplication: It has three arms instead of the usual two (Fig. 20.1). Triradials are rare: Stahl-Mauge et al. (1978) found only two triradials in 53,000 cells from normal people. Triradials are much more common after exposure to chromosome-breaking agents and in patients with Fanconi anemia or Bloom syndrome (Chapter 24). The breakpoint in a triradial is often a fragile site. Although several different mechanisms for the origin of triradials have been proposed, most symmetrical triradials probably arise by partial endoreduplication (Kuhn and Therman, 1982), but what triggers this? Treating normal cells with the demethylating agent 5-azacytidine causes partial endoreduplications, sister chromatid exchanges, and decondensation of the pericentromeric heterochromatin of chromosomes 1, 9, and 16 (Hori, 1983). Normally, the DNA in these regions is highly methylated (Miller et al., 1974). Could its demethylation be responsible for all these changes? Studies on a rare genetic disease, ICF syndrome, indicate this is indeed the case and serve to emphasize the importance of regulated DNA methylation in normal differentiation.

The autosomal recessive ICF syndrome of immunodeficiency, centromeric heterochromatin instability, and facial anomalies is characterized by the presence of multiradial chromosomes 1, 9, and 16 and other pericentromeric rearrangements of these chromosomes (Fig. 22.3). The hetetochromatin of these chromosomes is hypomethylated in the ICF syndrome (Miniou et al., 1994), especially satellite 2, a major component of the heterochromatin of chromosome 1 (Hernandez et al., 1997). The ICF syndrome is thus a hypomethylation disorder. The phenotype of normal cells after treatment with 5-azacytidine or 5-azadeoxycytidine is identical to that of ICF cells: There are multiradials with up to seven arms, and 80% of the multiradials involve chromosome 1. The treatment also produces whole-arm deletions, isochromosomes 1, and pericentromeric fusions of chromosomes 1 and 9 or 1 and 16 (Hernandez et al., 1997). The *ICF* gene locus has been mapped to a 9-cM region in 20q11–q13, using homozygosity mapping (Wijmenya et al., 1998). This region contains the *DNA*

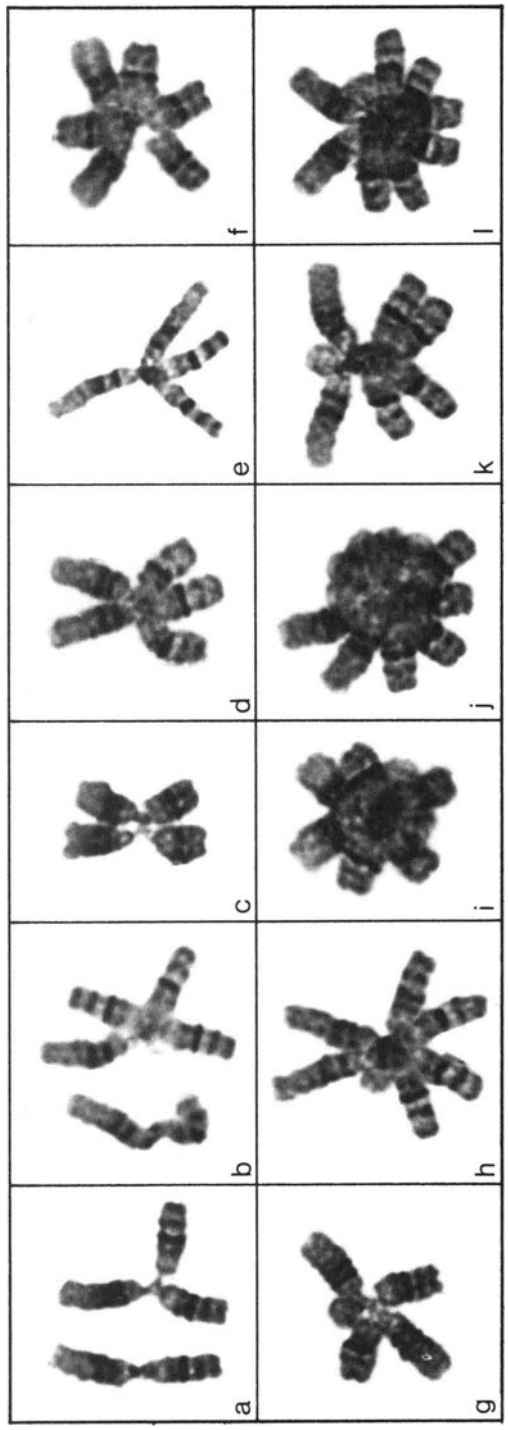

Figure 22.3. Multiradial chromosomes 1 and 16 in cultured lymphocytes from a patient with the ICF syndrome (reproduced from Smeets et al., ICF syndrome: a new case and review of the literature, Hum Genet 94:240–246, copyright 1994, Springer-Verlag).

methyltransferase 3B (*DNMT3B*) gene. Mutations of both alleles of this gene have been identified in each of five unrelated ICF patients (Xu et al., 1999). All five had the typical hypomethylation of satellite 2 on chromosomes 1 and 16 and of satellite 3 on chromosome 9.

References

Bartolomei MS, Tilghman SM (1997) Genomic imprinting in mammals. Annu Rev Genet 31:493–525

Bhattacharya SK, Ramchandani S, Cervoni N, et al. (1999) A mammalian protein with specific demethylase activity for mCpG DNA. Nature 397:579–583

Bione S, Sals C, Manzin C, et al. (1998) A human homologue of the *Drosophila melanogaster diaphanous* gene is disrupted in a patient with premature ovarian failure: evidence for conserved function in oogenesis and implications for human sterility. Am J Hum Genet 62:533–541

Brown CJ, Ballabio A, Rupert JI, et al. (1991) A gene from the region of the X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 349:38–44

Chaillet JR, Bader DS, Leder P (1995) Regulation of genomic imprinting by gametic and embryonic processes. Genes Dev 9:1177–1187

Chess A, Simon I, Cedar H, et al. (1994) Allelic inactivation regulates olfactory receptor gene expression. Cell 78:823–834

D'Amato F (1989) Polyploidy in cell differentiation. Caryologia 42:183–211

Datta NS, Williams JL, Caldwell J, et al. (1996) Novel alterations of CDK1/cyclin B1 kinase complex formation occur during the acquisition of a polyploid DNA content. Mol Biol Cell 7:209–223

Dittrich B, Buiting K, Korn B, et al. (1996) Imprint switching on human chromosome 15 may involve alternate transcripts of the *SNRPN* gene. Nat Genet 14:163–170

Drachman J, Kaushansky K (1997) Dissecting the thrombopoietin receptor: Functional elements of the Mpl cytoplasmic domain. Proc Natl Acad Sci USA 94:2350–2355

Drets ME, Therman E (1983) Human telomeric 6;19 translocation chromosome with a tendency to break at the fusion point. Chromosoma 88: 139–144

Fitzgerald PH, McEwan CM (1977) Total aneuploidy and age-related sex chromosome aneuploidy in cultured lymphocytes of normal men and women. Hum Genet 39:329–337

Gabriel JM, Higgins MJ, Gebuhr TC, et al. (1998) A model system to study genomic imprinting of human genes. Proc Natl Acad Sci USA 95: 14857–14862

Hernandez R, Frady A, Zhang X-Y, et al. (1997) Preferential induction of chromosome 1 multibranched figures and whole-arm deletions in a human pro-B cell line treated with 5-azacytidine or 5-azadeoxycytidine. Cytogenet Cell Genet 76:196–201

Hori T-A (1983) Induction of chromosome decondensation, sister chromatid exchanges and endoreduplications by 5-azacytidine, an inhibitor of DNA methylation. Mutat Res 121:47–52

Jeppesen P (1997) Histone acetylation: a possible mechanism for the inheritance of cell memory at mitosis. BioEssays 19:67–74

Kafri T, Gao X, Razin A (1993) Mechanistic aspects of genome-wide demethylation in the preimplantation monse embryo. Proc Natl Acad Sci USA 90:10558–10562

Kuhn EM, Therman E (1982) Origin of symmetrical triradial chromosomes in human cells. Chromosoma 86:673–681

Lichtenstein M, Keine G, Cedar H, et al. (1994) B cell–specific demethylation: a novel role for the intronic kappa chain enhancer sequence. Cell 76: 913–923

Malnic B, Hirono J, Sato T, et al. (1999) Combinatorial receptor codes for odors. Cell 96:713–723

Miller OJ (1997) Chromosome changes in cell differentiation. Genetics 146: 1–8

Miller OJ, Schnedl W, Allen J, et al. (1974) 5-Methylcytosine localised in mammalian constitutive heterochromatin. Nature 251:636–637

Miniou P, Jeanpierre M, Blanquet V, et al. (1994) Abnormal methylation pattern in constitutive and facultative (X inactive chromosome) heterochromatin of ICF patients. Hum Mol Genet 3:2093–2102

Nakagome Y, Abe T, Misawa S, et al. (1984) The "loss" of centromeres from chromosomes of aged women. Am J Hum Genet 36:398–404

Raymond CS, Shamu CE, Shen MM, et al. (1998) Evidence for evolutionary conservation of sex-determining genes. Nature 391:691–695

Rivera H, Zuffardi O, Maraschio P, et al. (1989) Alternate centromere inactivation in a pseudodicentric (15;20)(pter;pter) associated with a progressive neurological disorder. J Med Genet 26:626–630

Sarto GE, Stubblefield PA, Therman E (1982) Endomitosis in human trophoblast. Hum Genet 62:228–232

Smeets DFCM, Moog U, Weemaes CMR, et al. (1994) ICF syndrome: a new case and review of the literature. Hum Genet 94:240–246

Stahl-Maugé C, Hager HD, Schroeder TM (1978) The problem of partial endoreduplication. Hum Genet 45:51–62

Sullivan BA, Willard HF (1998) Stable dicentric X chromosomes with two functional centromeres. Nat Genet 20:227–228

Sweeney C, Murphy M, Kubelka M, et al. (1996) A distinct cyclin A is expressed in germ cells of the mouse. Development 122:53–64

Therman E, Trunca C, Kuhn EM, et al. (1986) Dicentric chromosomes and the inactivation of the centromere. Hum Genet 72:191–195

Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302: 575–581

Varmuza S, Prideaux V, Kothary R, at al. (1988) Polytene chromosomes in mouse trophoblast giant cells. Development 102:127–134

Vu TH, Hoffman AR (1994) Promoter-specific imprinting of the human insulin-like growth factor-II gene. Nature 371:714–717

Wevrick R, Kerns JA, Francke U (1994) Identification of a novel paternally expressed gene in the Prader–Willi syndrome region. Hum Mol Genet 3:1877–1882

Wijmenya C, Heuvel LPWJ van den, Strengman E, et al. (1998) Localization of the ICF syndrome to chromosome 20 by homozygosity mapping. Am J Hum Genet 63:803–809

Xu G-L, Bestor TH, Bourc'his D, et al. (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402:187–191