

1 Human Chromosomes: Structural and Functional Aspects

HEIDEMARIE NEITZEL AND MARC TRIMBORN

Abstract

Understanding the structural and molecular basis of the mitotic chromosome remains a basic challenge in cell biology and cytogenetics. The chromosomal behavior during cell division was first described in 1882. At the beginning of the last century, the chromosome theory of inheritance combined the cytological observations with the principles of Mendelian inheritance deduced from breeding experiments. This fundamental theory explained for the first time that the chromosomes are the units of heredity which are arrayed linearly on chromosomes as well as genetic linkage, chromosomal recombination, and the independent assortment of alleles localized on different chromosomes. The chromosome theory of inheritance was the prerequisite for the important improvements in the fields of experimental and clinical cytogenetics. After the definition of the exact number of human chromosomes $2n = 46$ in 1955, it was demonstrated that different human aneuploidies are the leading cause of fetal loss, birth defects, and mental retardation and that duplications or deletions of even smaller chromosomal segments have profound consequences on normal gene expression, resulting in severe developmental and physiologic abnormalities. In the 1980s and 1990s, the field of conventional cytogenetics was revolutionized by the introduction of molecular cytogenetic techniques resulting in the recognition of the importance of subtle cytogenetic aberrations, such as microdeletions and microduplications. Our insights into cell cycle progression, which is coordinated by a complex network of checkpoints to monitor chromosome structure, with DNA repair and spindle formation led to the identification of Mendelian disorders affecting chromosome integrity, some of them associated with high genomic instability and a markedly increased cancer risk. It was the thorough observation of chromosomal changes by many researchers during the last four decades which paved the way for our understanding of the underlying mechanisms of many congenital disorders, as well as of chromosome surveillance, DNA repair, and cancer susceptibility.

1.1 History of Chromosome Research

“Chromosomes have attracted many microscopists not only because these sausage-like bodies represent vehicles of genetic material (and hence, are biologically important) but also because they are hypnotically beautiful objects” (Hsu 1979).

The first cytologist who described chromosome behavior during cell division and how chromosomes move during mitosis was Walter Flemming (1882) in 1882. His terms “prophase,” “metaphase,” and “anaphase” are still used to describe the different steps of mitosis. In 1888 the structures were termed “chromosomes” (Greek *chroma* meaning “color” and *soma* meaning “body”) by the German anatomist Heinrich Waldeyer, because they were particularly well stained with a certain nuclear dye. Flemming’s work and the rediscovery of Mendel’s laws were the basis of the chromosome theory of inheritance. Using the fruit fly *Drosophila melanogaster* as a model organism, Thomas Hunt Morgan and his students at Columbia University, who included such important geneticists as Alfred Sturtevant, Hermann Muller, and Calvin Bridges, made many important contributions to genetics. They showed that genes, strung on chromosomes, are the units of heredity which are arrayed linearly on chromosomes. They described the independent assortment of alleles localized on different chromosomes, X-linked inheritance, genetic linkage, and chromosomal recombination.

The chromosome theory of inheritance combined the cytological observations with the principles of Mendelian inheritance deduced from breeding experiments (Fig. 1.1). The two homologous chromosomes in somatic cells correspond to two alleles, one of each inherited from the mother and the father. The chiasmata observed in meiosis I corresponds to the recombination or crossing over events. The segregation of homologous chromosomes during meiosis correlates with the segregation of alleles into separate gametes.

In 1953 Francis Crick and James Watson described the double-helix structure of DNA and concluded in their paper, published in *Nature*, “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material” (Watson and Crick 1953). Subsequently, Matthew Meselson and Franklin Stahl (1958) demonstrated that DNA replicates semiconservatively, with each strand in a DNA molecule from the parent generation pairing with a new strand in the daughter generation.

In 1955 Tjio and Levan (1956) defined the exact number of human chromosomes $2n = 46$ and in 1959 Lejeune et al. (1959) described the first aneuploidy in humans, demonstrating that children with Down syndrome have three instead of two copies of chromosome 21. Although more than 100 years has passed since the first observation of the chromosome (Flemming 1882) a number of researchers have continued to study chromosomes and their behavior during the cell cycle. Important improvements were made during

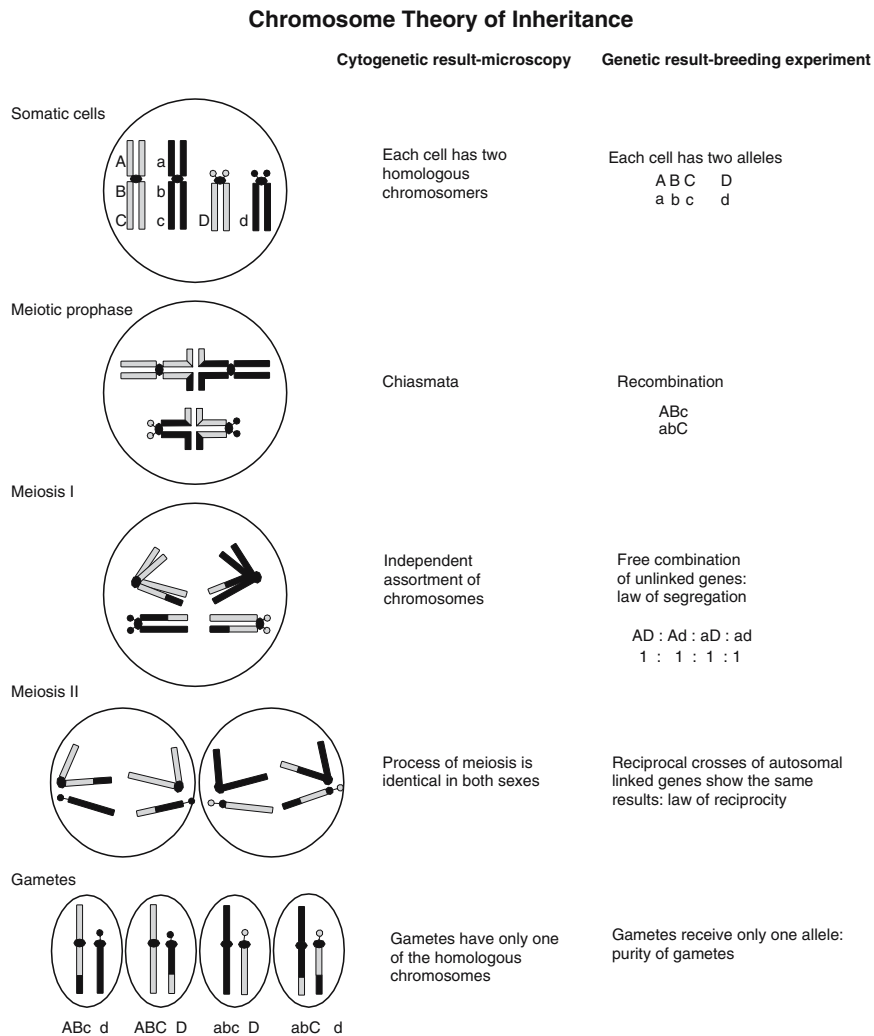


Fig. 1.1 Chromosome theory of inheritance (for details, see the text)

the 1960s and 1970s. The establishment of lymphocyte cultures as an easily accessible source for chromosome preparations (Nowell 1960) and the development of the differential banding methods in the 1970s led to considerable improvement in the fields of experimental and clinical cytogenetics (Caspersson et al. 1969; Drets and Shaw 1971; Dutrillaux and Lejeune 1971; Patil et al. 1971; Sumner et al. 1971). In the 1980s and 1990s, the field of conventional cytogenetics was again revolutionized by the introduction of molecular cytogenetic techniques such as fluorescence in situ hybridization (FISH), spectral karyotyping, and comparative genomic hybridization (CGH), leading

to considerable progress especially in clinical cytogenetics (Cremer et al. 1986; Pinkel et al. 1986; Kallioniemi et al. 1992; Schrock et al. 1996). As a result, there was an increased appreciation of the importance of “subtle” constitutional cytogenetic aberrations, such as microdeletions and imprinting disorders.

1.2 Composition and Compartmentalization of Human Chromosomes

Human DNA is composed of 60% single-copy DNA sequences and 40% repetitive DNAs. The characteristic of human and all other mammalian genomes is its compartmentalization, which finds its expression under the light microscope as G-, R-, T-, and C-bands (Korenberg and Rykowski 1988; Holmquist 1989, 1992). The different chromatin domains differ not only in their AT/GC content but also in their gene content, their replication timing, and their repetitive elements. G-, R-, and T-bands are defined as euchromatin containing most of the protein coding sequences which make up approximately 2% of the total DNA. The highest gene density is found in the telomeric T-bands, followed by the Giemsa-light bands or R-bands. These two compartments harbor predominantly the housekeeping genes which are essential for the metabolism of each single cell. R- and T-bands are accompanied by short repetitive nonviral retroposons, so-called short interspersed nucleotide elements (SINEs). These repeats are propagated in the genome by retroposition and can be classified as either autonomous or nonautonomous elements. The most prominent nonautonomous SINE member is the Alu family in humans and the B1 family in mice. The most abundant members of the autonomous retroposons are the long interspersed nucleotide elements (LINEs), which are several kilobases long and preferentially located in the dark G-bands where the gene density is much lower (Chen and Manuelidis 1989; Ostertag 2001). The C-bands equate to the constitutive heterochromatin, are devoid of protein coding sequences, and are thought to be genetically inert. Constitutive heterochromatin is mainly composed of satellite DNA containing simple repetitive elements, organized as complex tandem arrays (Waye and Willard 1989; Willard 1989; Fig. 1.2).

The different compartments are characterized by differences in their replication timing during S phase. Incorporation of the synthetic thymidine analog bromodeoxyuridine during S phase leads to a characteristic banding pattern which shows that the T- and R-bands replicate in the first half of S phase, while the dark G-bands replicate in the second half. At the very end of S phase the constitutive heterochromatin is replicated as well as the inactivated X chromosome in mammalian females designated as facultative heterochromatin.

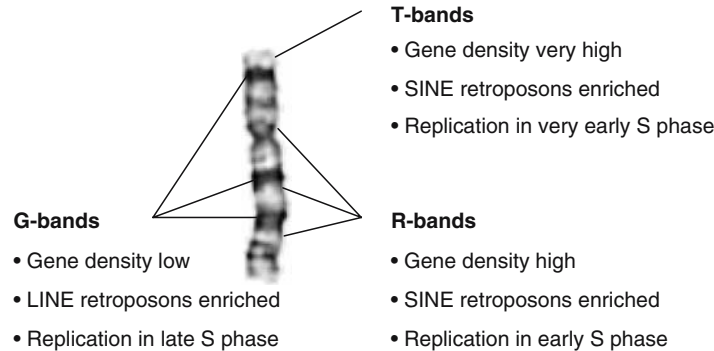


Fig. 1.2 Compartmentalization of human chromosomes. *LINE* long interspersed nucleotide element, *SINE* short interspersed nucleotide element

The differences in gene content of the different compartments can be impressively illustrated by the gene mapping data of the Human Genome Project (Fig. 1.4). The smallest human autosome, chromosome 21, which has a broad Giemsa-dark band comprises 46.9 Mbp of DNA and accommodates 352 genes. In contrast, chromosome 22 consists mainly of R-band material, it is 50 Mbp in physical length, only slightly bigger than chromosome 21, and harbors 742 genes (National Center for Biotechnology Information, Map Viewer, build 36).

1.3 The Human Karyotype and Clinical Cytogenetics

In humans, the normal diploid number of chromosomes is 46, consisting of 22 pairs of autosomal chromosomes numbered 1–22 and one pair of sex chromosomes (XX in females and XY in males) (Fig. 1.3). The genome is estimated to contain approximately 25,000 genes which are distributed along 23 chromosome pairs. As mentioned already, even the smallest autosome, chromosome 21, contains 352 genes. Thus, it is not surprising that duplications or deletions of chromosomes, or even small chromosome segments, have profound consequences on normal gene expression, leading to severe developmental and physiologic abnormalities.

Deviations in the number or structure of the 46 human chromosomes are astonishingly common, despite their severe deleterious consequences. Chromosomal disorders occur in an estimated 10–25% of all pregnancies. They are the leading cause of fetal loss and, among pregnancies continuing to term, are the leading known cause of birth defects and mental retardation. The most common chromosomal aberrations are trisomies for various chromosomes, indicating that chromosome segregation at meiosis is an extremely error-prone process in

Institut für Humangenetik, Charité, Campus Virchow-Klinikum

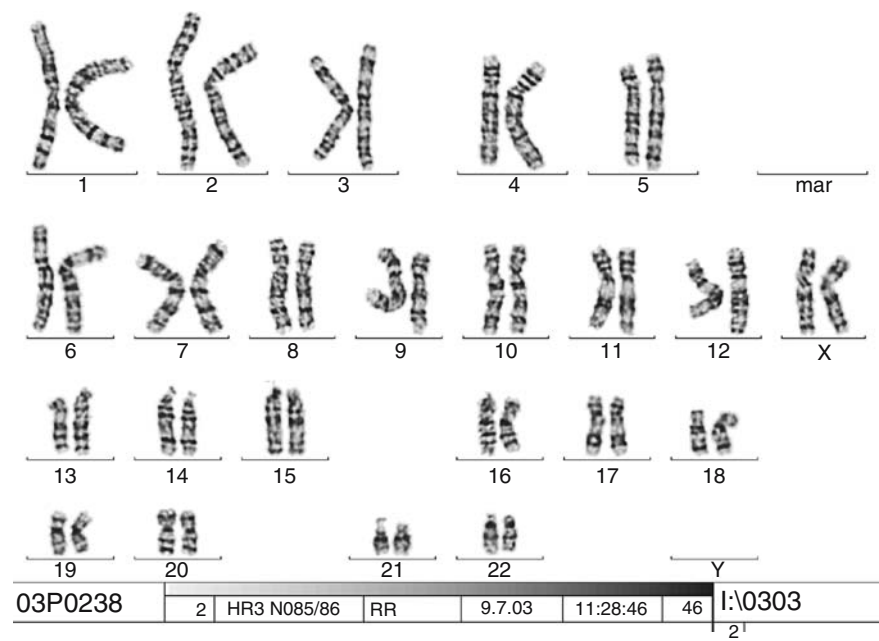


Fig. 1.3 Human female karyotype 46,XX after G-banding

humans. Most chromosomal aneuploidies originate from female meiosis and contribute significantly to pregnancy failures, particularly in women of advanced maternal age. The most common autosomal trisomy is trisomy 21, with a frequency of approximately 1 in 700 live births. In contrast, trisomy 22 results in spontaneous miscarriage due to the extent of imbalance since chromosome 22 carries twice as many genes (742 genes) as chromosome 21. In addition to trisomy 21, only two other autosomal trisomies with low gene density, trisomy 13 (551 genes) and trisomy 18 (432 genes), occur in live births with a prevalence of 1 in 10,000 and 1 in 20,000, respectively. However, most conceptions with trisomy 13 and trisomy 18 result in fetal loss during pregnancy or are associated with death in infancy, typically occurring during the first year of life. Autosomal trisomies of all other chromosomes are not compatible with survival to term, indicating that dose imbalance of the number of autosomal genes has a severe effect on developmental processes and survival (Fig. 1.4). This is further illustrated by the fact that all full autosomal monosomies do not survive to term. Karyotype-phenotype correlation studies on the basis of clinical findings demonstrate that monosomies for an autosomal segment cause more, and more severe alterations to the phenotype and restrict survival more than do trisomies for the same segment.

In contrast to autosomal imbalances, sex chromosome trisomies such as 47,XXX and 47,XXY have few phenotypic complications owing to the

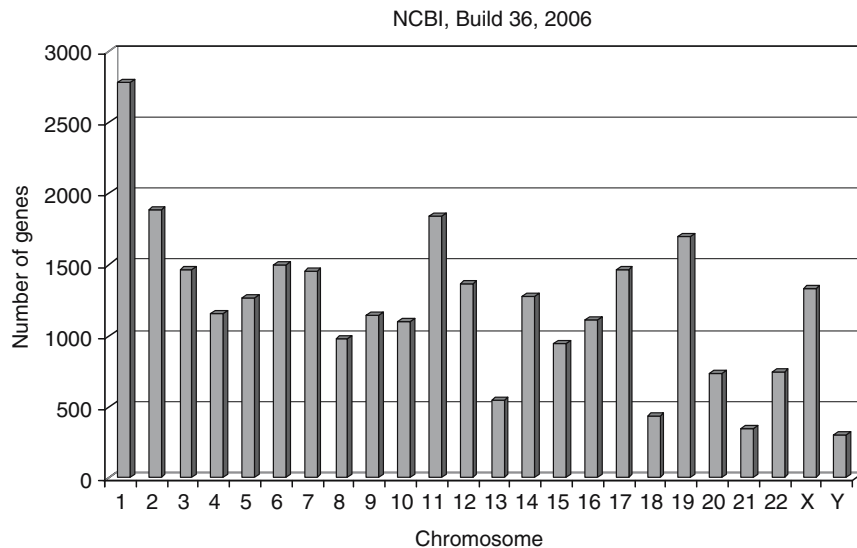


Fig. 1.4 Number of genes mapped to human chromosomes (National Center for Biotechnology Information, NCBI, Map Viewer, build 36)

mechanism of X chromosome inactivation, or as in 47,XYY owing the low number of Y-linked genes, most of which are involved in testicular development or spermatogenesis.

The introduction of molecular cytogenetic techniques such as FISH revolutionized the field of conventional cytogenetics, leading to considerable progress especially in clinical cytogenetics (Cremer et al. 1986; Pinkel et al. 1986; Kallioniemi et al. 1992; Schrock et al. 1996; Fig. 1.5). Subsequently, numerous deletion and duplication syndromes have been described that are too small to be detected under the microscope using conventional cytogenetic methods, such as G-banding. The molecular cytogenetic methods have expanded the possibilities for precise genetic diagnoses, which are extremely important for clinical management of patients and appropriate counseling of their families. Depending on the size of the deletion or duplication, specific FISH probes are employed to identify the aberration (Tonnie 2002).

Since most microdeletion/microduplication syndromes are defined by a common deleted/duplicated region, the abnormal dose of genes located within these regions can explain the phenotypic similarities among individuals with a specific syndrome. Consequently, detailed genotype–phenotype correlations provide a unique resource towards the genetic dissection of complex phenotypes such as congenital heart defects, mental and growth retardation, and specific cognitive and behavioral components of humans. For example, the Williams–Beuren syndrome (WBS), a neurodevelopmental disorder caused by a microdeletion at 7q11.23, provides one of the most convincing models of a relationship that links genes with human cognition and behavior. Detailed

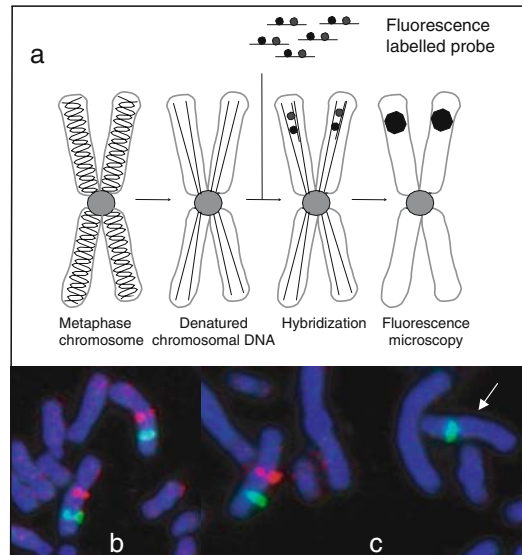


Fig. 1.5 **a** Principle of fluorescence in situ hybridization (FISH), **b** FISH with the Elastin probe for Williams-Beuren syndrome (WBS) and a control region in a normal individual, and **c** in a patient with WBS deletion showing the missing signal in one of the homologous chromosomes (*arrow*)

molecular characterization of the deletion alongside well-defined cognitive profiling in WBS provides a unique opportunity to investigate the neuromolecular basis of complex cognitive behavior and develop integrated approaches to study gene function and genotype-phenotype correlations (Osborne 1999; Mervis and Klein-Tasman 2000).

Furthermore, the molecular analysis of these chromosomal aberrations has led to a growing understanding of their mechanisms of origin, indicating that certain regions of the human genome are especially prone to structural rearrangements due to the presence of repetitive sequence elements. Interaction between chromosome-specific repetitive DNA leads to gain, loss, or inversion of the intervening sequence by nonallelic homologous recombination between misaligned repetitive elements. Where a particular region contains dose-sensitive or imprinted genes, this can lead to a specific genetic disease: loss of 7q11.23 results in Williams syndrome (Osborne 1999), loss of 22q11 results in Di George syndrome/velocardiofacial syndrome (Cuneo 2001), and loss of 15q11-q13 results in either Prader-Willi syndrome or Angelman syndrome (Cassidy et al. 2000; Horsthemke and Buiting 2006; Thomas et al. 2006). The available data demonstrate that the majority of rearrangements, approximately three or four, are interchromosomal. Therefore, they are likely to have arisen as the result of unequal meiotic crossing over between repetitive elements on different chromosome homologues. The remaining intrachromosomal rearrangements are also likely to be

meiotic, although for these cases a postzygotic error during mitosis cannot be excluded (Thomas et al. 2006).

1.4 Cell Cycle and Chromosome Cycle

The cell cycle consists of four distinct phases: G₁ phase, S phase, G₂ phase, and M phase, or mitosis. G₁, S, and G₂ comprise together the interphase. G₁ phase is a growth phase with high metabolic activity increasing the amount of cytoplasm and important organelles for preparing the cell for duplicating its DNA in S phase. In G₂ phase, the cell continues with growth and metabolism in preparation for undergoing mitosis. During M phase the replicated chromatids segregate to the two daughter cells.

The chromosomes at G₁, S, and G₂ phases can be directly visualized after premature chromosome condensation (PCC) (Rao et al. 1982; Fig. 1.6). Fusion between mitotic and interphase cells results in a rapid chromosome condensation, with dissolution of the nuclear membrane due to the activity of the mitosis-promoting factor (MPF) in the interphase cells. The morphology of PCC chromosomes varies according to the stage of the interphase cell at the time of fusion. Thus, the PCC at G₁ phase are very long with single chromatids and those at G₂ phase are elongated with slender double chromatids. PCC chromosomes at S phase are characterized by their fragmented, pulverized appearance. The gaps of S-PCC represent the sites of DNA replication.

In all phases of the cell cycle, the surveillance of the chromosomal integrity is crucial for the genetic processes. Therefore, a complex network of checkpoints has evolved to monitor chromosome structure and coordinate cell cycle progression with DNA repair and spindle formation. Checkpoints are coordinated series of responses that delay progress through the cell cycle at a particular phase or transition in response to the lack of appropriate conditions

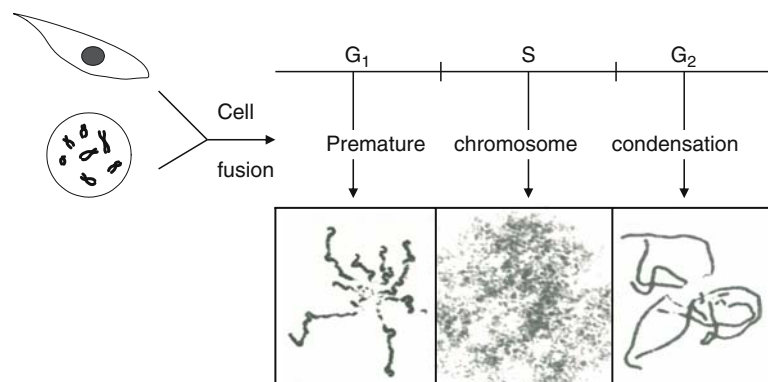


Fig. 1.6 Visualization of G₁-, S-, and G₂-phase chromosomes after premature chromosome condensation

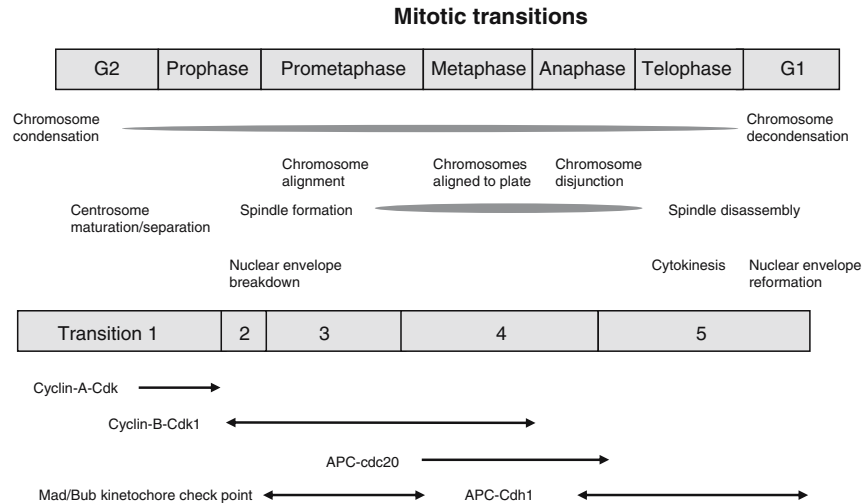


Fig. 1.7 Mitotic transitions according to the classification of Pines and Rieder (2001)

for progression. Delay allows enough time for crucial processes such as DNA repair and spindle attachment to be completed before continuing the cell-division cycle or initiating apoptosis (Hartwell and Weinert 1989; Hartwell and Kastan 1994; Nurse 1997; Clarke and Gimenez-Abian 2000; Morrison and Rieder 2004).

Especially, cell entry into mitosis is under the control of a tightly regulated network of protein kinases, cyclins, and protein phosphatases. According to Pines and Rieder (Pines and Rieder 2001) G2 phase and mitosis can be subdivided into five transitional phases which are characterized not only by the structural and behavioral changes of the chromosomes and the spindle, but also, at the molecular level, by the activation and inactivation of cell cycle regulators such as the cyclin-dependent kinases (Cdks) and the anaphase-promoting complex (APC) (Fig. 1.7). In vertebrates, the G2–M transition is initiated by the increase of cyclin-A-Cdk2 throughout the G2 phase of the cell cycle, resulting in chromosome condensation in the absence of significant cyclin-B1-Cdk1 activity. Subsequently, the cyclin-B1-Cdk1 complex, also known as mitosis-promoting factor (MPF), is activated as a result of its dephosphorylation by Cdc25 and rapidly accumulates in the nucleus, followed by the breakdown of the nuclear envelope and the entry of the cell into metaphase.

1.5 Shaping the Metaphase Chromosome

Chromosomes are complex and highly dynamic structures containing DNA, histones, and non-histone proteins. Understanding the structural and molecular basis of mitotic chromosome condensation remains a basic challenge in

cell biology. During cell cycle progress towards mitosis, the chromosome undergoes progressive morphological conversion. From prophase to metaphase, apparently amorphous interphase chromatin is reorganized into individual chromosomes, with a pair of separate sister chromatids. This process, referred to as chromosome assembly, implying chromosome condensation and sister-chromatid resolution, is an essential prerequisite for the faithful segregation of duplicated genetic information into two daughter cells.

The human genome contains 6×10^9 bp of DNA per diploid cell, corresponding to approximately 1.7 m of DNA which is organized in chromatin fibers. The basic unit of chromatin is the nucleosome, which consists of 146 bp of DNA wound around an octamer of histone proteins, two each of histones H2A, H2B, H3, and H4 accounting for the first sixfold to sevenfold linear compaction of the DNA (Bednar et al. 1998).

The binding of linker histone H1 to linker DNA sequences, so-called scaffold-associated regions (SARs), localized between nucleosomes leads to further chromatin compaction – the 30-nm fiber – generating another sixfold to sevenfold compaction. It has been proposed that the 30-nm chromatin fiber is accomplished by a unique three-dimensional zigzag folding pattern rather than by supercoiling. In mitosis, the 30-nm fiber must compact another 200- to 500-fold to achieve the final 10,000- to 20,000-fold linear compaction of the mitotic chromosome (Swedlow and Hirano 2003; Fig. 1.8). Early studies suggested the presence of a chromosome scaffold composed of non-histone proteins that serve as the backbone of the mitotic chromosome. Two major scaffold proteins, topoisomerase II and SC2, a structural maintenance of chromosomes (SMC) family member, have been shown to be part of the scaffold and involved in condensation. *cis* sites for condensation are expected to lie along the chromosomal axis *in vivo*. Scaffold models propose that the SARs are the *cis*-acting DNA sequences that serve as binding sites for DNA topoisomerase II, a major component of the chromosome scaffold, resulting in radial DNA loops of approximately 50–100 kb in length (Earnshaw et al. 1985; Earnshaw and Heck 1985). Yet the radial loop model has remained highly controversial.

The highly organized chromatin can be modified further by various mechanisms, such as posttranslational modifications of histones, ATP-dependent chromatin remodeling, and the exchange of histone proteins.

1.6 Cohesion and Condensins

The discovery of SMC proteins led to rapid progress in our understanding of chromosome organization and behavior. The identification and characterization of cohesion and the condensins, SMC-containing complexes, demonstrated them to be key regulators that function in chromosome assembly and segregation during mitosis (Hirano 2005, 2006; Losada and Hirano 2005; Nasmyth and Haering 2005). SMC proteins are ubiquitous in organisms from

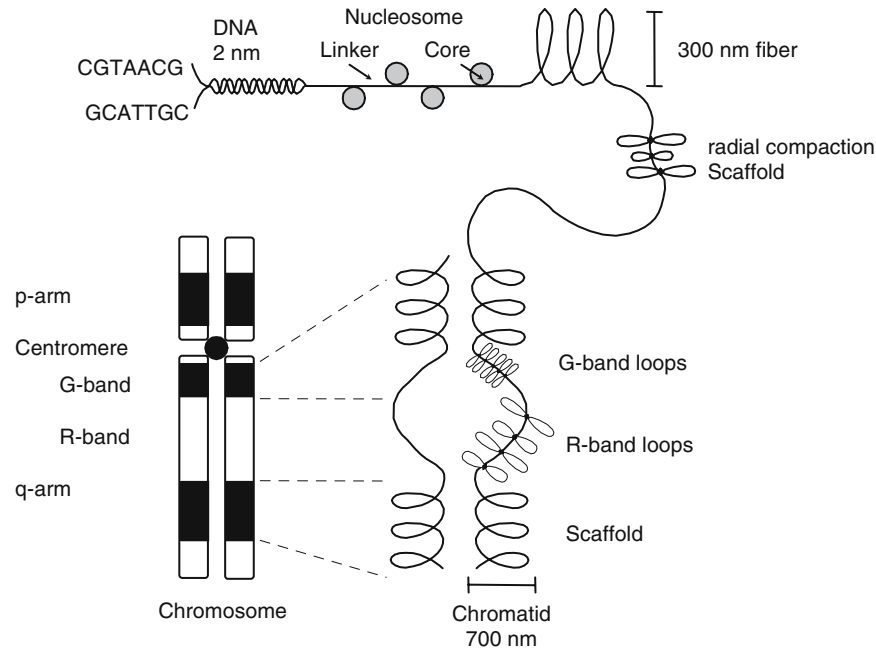


Fig. 1.8 Model of chromatin compaction from DNA double helix to metaphase chromosome

bacteria to humans. The SMC proteins SMC1 and SMC3 constitute the core of the cohesin complex and bind the non-SMC proteins Scc1 and Scc3 to form a ringlike structure that mediates sister-chromatid cohesion. A model of the interaction of cohesion predicts that the establishment of sister-chromatid cohesion is accomplished when a replication fork passes through the cohesin ring that is preloaded during the G1 phase of the cell cycle. The sister chromatids generated by DNA replication become aligned along the entire length of their arms and at the kinetochore. In vertebrates, most cohesin dissociates from chromatin at prophase, and only a small population, enriched in the pericentromeric region, remains on the chromosomes until metaphase. Cohesion is essential for the congression and alignment of chromosomes from prometaphase to metaphase. At the onset of anaphase, loss of cohesion triggers the separation of sister chromatids, allowing them to be pulled apart to opposite poles of the cell.

The core component of the two condensin complexes is the monomers SMC2 and SMC4 (Hirano 2005). The SMC cores are bound by different sets of regulatory subunits forming the functional complexes. Vertebrates have two different condensin complexes, condensin I and condensin II, each containing a unique set of regulatory subunits. CAP-G, CAP-D2, and CAP-H bind to

the condensin core to form condensin I (Hirano et al. 1997), whereas condensin II is defined by its regulatory subunits CAP-G2, CAP-D3, and CAP-H2 (Ono et al. 2003). Condensin I (and possibly condensin II as well) has the ability to introduce positive helical tension into double-stranded DNA in vitro (Kimura and Hirano 1997; Kimura et al. 1999; Bazett-Jones et al. 2002; Hagstrom et al. 2002). While the two complexes cooperate to assemble metaphase chromosomes (Ono et al. 2003), their behaviors are regulated differently during the cell cycle (Hirota et al. 2004; Ono et al. 2004; Trimborn et al. 2006). Condensin II is nuclear throughout the cell cycle and participates in an early stage of prophase chromosome condensation within the nucleus, whereas condensin I gains access to chromosomes only after the breakdown of the nuclear envelope. Both complexes finally bind to the central chromatid axes in an alternate pattern. The molecular mechanism underlying the differential regulation of the two condensin complexes remains to be determined, but it was proposed that sequential activation of cyclin A/Cdk and cyclin B/Cdk could be responsible for the successive loading of condensin I and condensin II (Hirano 2005). The loading of condensins is a prerequisite for the proper assembly and segregation of metaphase chromosomes. The diverse functions of the SMC complexes, however, range far beyond chromosome segregation and may include gene regulation and DNA repair.

1.7 DNA Repair

The DNA damage checkpoints cause cell cycle delay before or during the decisive cell cycle transitions of replication and mitosis (G1/S, intra-S, G2/M checkpoints) (Sancar et al. 2004). This involves a number of highly conserved proteins that sense the damage and signal the cell cycle machinery. Central to this network are two protein kinases, ataxia telangiectasia mutated (ATM) and ATM-Rad3 related (ATR). These kinases sense the DNA damage and start signaling cascades which finally result in cell cycle arrest or induce apoptotic pathways. ATM kinase is primarily activated by DNA double-strand breaks (DSBs) induced by ionizing irradiation, whereas ATR kinase responds to UV-induced and replication-specific DNA damage. ATR kinase activation demands the association with the protein ATRIP and two additional complexes RAD17 and 9-1-1. The MRE11-NBS-RAD50 (MNR) complex plays a crucial role in the ATM kinase mediated answer on DNA DSBs. It was generally thought that ATM kinase and ATR kinase work independently. Recent reports, however, indicate that ATM kinase and nuclease activity of meiotic recombination 11 (MRE11) are required for the processing of DNA DSBs to generate the replication protein A (RPA) coated single-stranded DNA that is needed for ATR kinase recruitment (Watson and Crick 1953; Adams et al. 2006; Jazayeri et al. 2006). Critical for the signal transduction following the damage detection are a vast number of mediators, many of them

(e.g., TOPBP1, 53BP1, and BRCA1) containing BRCT domains. By means of these transducers and the checkpoint kinases CHK1 and CHK2, the signal is transferred to the target/effector proteins like CDC25 phosphatases, p53, or SMC1 (Lee 2002; Kastan and Bartek 2004; Sancar et al. 2004; Li and Zou 2005). DNA DSBs are repaired by two distinct but connected pathways: nonhomologous end-joining (NHEJ) and homologous repair (HR). NHEJ rejoins the two ends of a DSB by simple ligation in an error-prone process, while HR uses a homologous template to copy and restore the information disrupted by the break. This promotes error-free repair. Since the information is usually copied from an intact sister chromatid, HR is the preferred pathway in S and G2 phases, while NHEJ is the predominant pathway in the G1 phase (reviewed in Lisby and Rothstein 2004).

1.8 Mendelian Disorders Affecting Chromosome Integrity

1.8.1 Chromosome Instability Disorders

Our insights into DNA repair processes in normal cells have been considerably improved by the identification of the underlying genetic defects of chromosome instability syndromes. Chromosome instability syndromes are a group of inherited disorders associated with high genomic instability and a markedly increased cancer risk. In the following, we focus on two instability syndromes, one implicated in the repair of DSBs and one involved in the repair of DNA cross-links.

Patients affected by Nijmegen breakage syndrome (NBS) have biallelic mutations in the *NBS1* gene, mapped on chromosome 8q21. It encodes a 95-kDa protein called nibrin, a member of the hMre11hRad50 protein complex, involved in the ATM-dependent DNA damage signaling pathway of cellular response to DSBs. The affected patients present with microcephaly, a distinct facial appearance, growth retardation, immunodeficiency, cytogenetic abnormalities, radiosensitivity, and high susceptibility to lymphoid malignancy. In 40% of patients, a malignancy occurs before the age of 21 (Varon et al. 1998, 2001, Varon et al. 2003; Kitagawa and Kastan 2005; Kruger et al. 2007).

Fanconi anemia is a genetically heterogeneous, autosomal recessive or X-recessive chromosome instability disorder with increased hypersensitivity to cross-linking agents. At least 12 genetic complementation groups have been described (FA-A, FA-B, FA-C, FA-D1, FA-D2, FA-E, FA-F, FA-G, FA-I, FA-J, FA-L, FA-M) and all except FA-I have been linked to a distinct gene. All Fanconi anemia proteins act in a single pathway involved in DNA cross-link repair. Most Fanconi anemia proteins form a complex that activates the FANCD2 protein via monoubiquitination, which is prerequisite for the activation of *BRCA2*, a gene which was originally identified in families with

increased breast and ovarian cancer susceptibility. Disruption of any of the Fanconi anemia proteins results in an increased chromosomal instability (Fig. 1.9a). Fanconi anemia patients have a high risk for bone marrow failure, aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, and, later in life, epithelial malignancies. The most life-threatening early event in most complementation groups is bone marrow failure, which occurs typically during the first decade of life (Wang and D'Andrea 2004; Kennedy and D'Andrea 2005; Bagby and Alter 2006; Lyakhovich and Surralles 2006).

These two examples might be sufficient to demonstrate that any mechanism impairing chromosome surveillance and chromosomal integrity has profound effects on cancer formation and progression.

1.8.2 PCC Syndrome

The first description of a disorder in humans affecting the fundamental process of chromosome condensation was reported in 2002 (Neitzel et al. 2002). The patients' chromosomes display PCC (PCC syndrome) in early G2 phase commencing as soon as 1 h after completion of S phase and also delayed decondensation after mitosis (Fig. 1.9b).

In 2004, it was demonstrated that PCC syndrome is caused by mutations in the *MCPH1* gene encoding microcephalin (Trimborn et al. 2004). Microcephalin encompasses 835 amino acids and contains one N-terminal and two C-terminal BRCT domains (BRCA1 C-terminus) linking its function to DNA checkpoint control and/or DNA repair. The clinical phenotype is characterized by microcephaly, growth retardation, and mental retardation. These findings implicated microcephalin as a novel regulator of chromosome condensation and linked the apparently disparate fields of neurogenesis and chromosome biology.

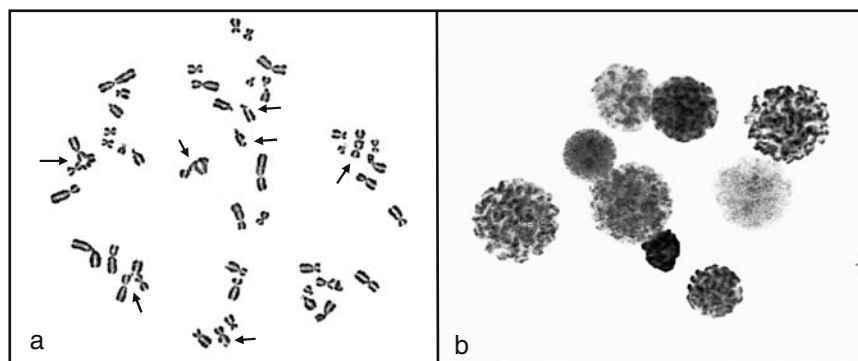


Fig. 1.9 Chromosomal instability syndromes: a increased chromosomal breakage in lymphocytes of a Fanconi anemia patient; b aberrant chromosome condensation in a patient with *MCPH1* autosomal recessive primary microcephaly

The misregulation of chromosome condensation in *MCPH1* deficiency is mediated by the SMC protein condensin II (Trimborn et al. 2006).

In patient cells with *MCPH1* deficiency, knockdown of condensin II subunits leads to a pronounced reduction of cells with the condensation defects in both G1 and G2 phases of the cell cycle. In contrast, knockdown of condensin I subunits does not reverse the cellular phenotype. Consistently, condensin I stays in the cytoplasm in the prophaselike cells of *MCPH1* patients. These results offer a molecular explanation for the aberrant chromosome condensation in *MCPH1* deficiency. In normal cells, microcephalin acts as a negative regulator of condensin II which prevents PCC until the onset of prophase and allows timely decondensation after mitosis.

1.8.3 Further Syndromes Affecting Structural Maintenance of the Chromosome

In the last few years a growing number of genes that regulate genome surveillance and cell cycle progression have been linked to developmental and progressive neurological diseases. Some of these are involved in chromosome dynamics, spindle formation, and the centrosome cycle. Mutations in the gene *NIPBL*, the human counterpart of *Scs2*, were shown to cause Brachmann/de Lange syndrome (Krantz et al. 2004; Tonkin et al. 2004), which is associated with growth retardation, microcephaly, and limb malformations. *Scs2* is crucial for sister-chromatid cohesion and replication licensing (Furuya et al. 1998; Ciosk et al. 2000; Gillespie and Hirano 2004). Expression of *NIPBL* in developing limbs of human embryos was shown by in situ hybridization (Tonkin et al. 2004). A dual role for Nipped-B, the *Drosophila* homologue of *NIPBL*, in sister-chromatid cohesion and developmental regulation connected with limb formation was recently confirmed (Rollins et al. 2004). Mutations in yet another cohesin factor gene *ESCO2* cause Roberts syndrome (Vega et al. 2005), characterized by premature centromere separation. Brachmann/de Lange syndrome and Roberts syndrome share clinical symptoms, such as growth retardation, microcephaly, and intriguingly limb malformations.

BUB1B is mutated in mosaic variegated aneuploidy (MVA), an autosomal recessive disorder characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple different chromosomes and tissues (Hanks et al. 2004; Hanks and Rahman 2005). *BUB1B* encodes a key protein in the mitotic spindle checkpoint (Sudakin et al. 2001) and is involved in sister-chromatid cohesion (Kitajima et al. 2005). Affected individuals present with severe intrauterine growth retardation, various congenital abnormalities, microcephaly, developmental delay, and a high risk of malignancy.

These few examples illustrate the relevance of genes involved in the surveillance of the chromosomal integrity, cell cycle progression, and DNA repair. Deficiencies of these genes due to mutation do not only result in

multiple congenital abnormalities of almost all tissues but also in a high risk for the development of various malignancies. Furthermore, these examples demonstrate impressively the importance of the field of human cytogenetics: in all cases the cytogenetic observation of distinctive chromosomal features, such as increased chromosomal instability, PCC, increased somatic nondisjunction, or premature centromere division, preceded the identification of the underlying gene defects.

Hsu's annotation in 1979 that chromosomes are "hypnotically beautiful objects" still holds true. Beyond it, the thorough observation of chromosomal changes by many researchers during the last four decades paved the way for our understanding of the underlying mechanisms of many congenital disorders, as well as of chromosome surveillance, DNA repair, and cancer susceptibility.

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