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MOLECULAR MECHANISMS FOR CONSTITUTIONAL CHROMOSOMAL REARRANGEMENTS IN HUMANS

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■ **Abstract** Cytogenetic imbalance in the newborn is a frequent cause of mental retardation and birth defects. Although aneuploidy accounts for the majority of imbalance, structural aberrations contribute to a significant fraction of recognized chromosomal anomalies. This review describes the major classes of constitutional, structural cytogenetic abnormalities and recent studies that explore the molecular mechanisms that bring about their de novo occurrence. Genomic features flanking the sites of recombination may result in susceptibility to chromosomal rearrangement. One such substrate for recombination is low-copy region-specific repeats. The identification of genome architectural features conferring susceptibility to rearrangements has been accomplished using methods that enable investigation of regions of the genome that are too small to be visualized by traditional cytogenetics and too large to be resolved by conventional gel electrophoresis. These investigations resulted in the identification of previously unrecognized structural cytogenetic anomalies, which are associated with genetic syndromes and allowed for the molecular basis of some chromosomal rearrangements to be delineated.

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INTRODUCTION

Constitutional chromosomal rearrangements refer to those that are inherited from a carrier parent or occur de novo in the gametes that result in the zygote, whereas acquired chromosomal abnormalities refer to those that arise during development or during the life of the organism. Cytogenetic imbalance is detected in about 0.6% of all newborn infants, 25% of all miscarriages and stillbirths, and 50–60% of first trimester miscarriages. Most often, the recognized imbalance is caused by aneuploidy, resulting from a meiotic nondisjunction event. Although balanced structural chromosomal rearrangements are found in about 1 in 500 individuals in the population, cytogenetically visible, unbalanced structural anomalies account for about 3% of all recognized chromosome abnormalities. The first unbalanced translocations associated with an abnormal phenotype were described prior to chromosomal banding methods (14, 86, 89). The first chromosomal deletions identified in humans were described shortly thereafter, including the deletion of distal 5p, associated with the cri-du-chat syndrome (62) and the distal deletion of 4p, subsequently named the Wolf-Hirschhorn syndrome (138). Banding methods developed in the 1970s enabled the identification of the chromosomes and the specific bands involved in rearrangements (15, 142). Some chromosomal rearrangements affect segments smaller than the current band resolution obtained using routine or high-resolution karyotyping (2–5 Mb). Molecular cytogenetic methods, such as fluorescence in situ hybridization (FISH), are required to visualize these submicroscopic defects. The frequency of submicroscopic rearrangements that cause specific human traits remains to be determined.

Chromosomal rearrangements in humans are diverse, frequent, and often result in phenotypic abnormalities. Thus, humans are an excellent experimental model to study these rearrangements. Chromosomal rearrangements are identified through the study of children with congenital anomalies, developmental delay/mental

retardation, abnormalities of growth, couples with recurrent miscarriages, and during routine prenatal studies. These studies identified common rearrangements and allowed molecular investigations of the mechanisms leading to these anomalies. Correlations between chromosomal rearrangements and clinical manifestations (genotype/phenotype correlations) have allowed for development of useful diagnostic tools and provided information relevant to prognosis.

Recently, the underlying molecular structure of several constitutional chromosomal rearrangements have been delineated. Molecular tools such as the polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE), and FISH have uncovered the molecular bases of several common chromosome abnormalities. A recurrent theme is emerging in which homologous, nonallelic sequences, which include region-specific repeat gene clusters, are intimately involved in causing chromosomal rearrangements. In this review, we examine the genome architecture that likely contributes to the formation of a range of chromosomal rearrangements.

TYPES OF CHROMOSOMAL REARRANGEMENTS

Interchromosomal Rearrangements

At the level of the chromosome, interchromosomal rearrangements involve two different chromosomes and may occur between nonhomologous chromosomes. These include Robertsonian translocations and reciprocal translocations. At the molecular level, interchromosomal rearrangements may also include deletions and duplications that arise through recombination between two homologous chromosomes, even though they appear at the cytogenetic level to involve only a single chromosome or chromosome pair (homologues). For this discussion, rearrangements between homologous chromosomes are considered in the next section on intrachromosomal rearrangements.

Robertsonian translocations are whole arm exchanges between the acrocentric chromosomes. In humans, chromosomes 13, 14, 15, 21, and 22 are acrocentric. Robertsonian translocations are the most common, recurrent chromosomal rearrangement and are found in 1 in 1000 individuals (38). Although all potential combinations of acrocentic chromosomes have been found to participate in Robertsonian translocations, the distribution of these translocations is not random (126). Specifically, rob(13q14q) and rob(14q21q) are the most common, constituting about 85% of all Robertsonian translocations.

Reciprocal translocations are rearrangements that result from a single break in each of the two participating chromosomes. All chromosomes have been reported to participate in reciprocal translocations. Reciprocal translocations occur in about 1 in 625 individuals in the population. Most reciprocal translocations are considered "private" in that they are found only in related family members. However, the t(11;22)(q23;q11.2) is a relatively common, recurrent reciprocal translocation.

Intrachromosomal Rearrangements

Intrachromosomal rearrangements are cytogenetic aberrations that appear to involve a single chromosome. These include interstitial and terminal deletions, interstitial duplications, marker chromosomes, inversions, and isochromosomes. Some rearrangements may involve a single homologue (sister chromatid exchanges) while others may involve both homologous chromosomes (recombination between homologues).

Based on a literature review of deletions and duplications of the human chromosomes that resulted in malformation, any region of the genome may be subject to rearrangement, but certain parts of the genome are more susceptible than others (9, 10). Interestingly, some regions of the genome have never been observed to have deletion or duplication. These regions may contain a critical dosage-sensitive gene(s), the deletion or duplication of which would be lethal. The apparent lack of rearrangement for some segments may also indicate regions of the genome that are less susceptible to the mechanisms resulting in structural chromosome aberrations. However, these observations could also potentially be explained by a bias in ascertainment either for particular chromosomal rearrangements that are more easily visualized using routine cytogenetic techniques, or for clinically recognizable syndromes.

Interstitial deletions and duplications result from exchange within a chromosome arm, retaining the original telomere. A number of interstitial deletions are responsible for known genetic syndromes (Table 1) [reviewed in (113)]. Recently, interstitial duplications have been associated with specific syndromes and some may reflect the reciprocal recombination product for several of the common interstitial deletions seen in the human genome (Table 1) [reviewed in (113)]. Terminal deletions for most chromosomes have been identified. Those commonly seen or extensively characterized are listed in Table 1.

By traditional definition, marker chromosomes are small structurally abnormal chromosomes of unidentifiable origin. They are designated as mar followed by the chromosomal origin, if known, in parentheses [e.g. mar(22)]. With the advent of molecular techniques, such as FISH, the chromosomal origins of most marker chromosomes have been identified. The most common marker chromosomes in the human population are those derived from chromosomes X (112), 15 (44, 60, 133), and 22 (30, 71, 72). Inversions have been reported for essentially every human chromosome (53, 70). Pericentric inversions involve a break in each arm of the chromosome and inversion of the segment containing the centromere (53). Paracentric inversions occur after a two-break event in a single chromosome arm and inversion and rejoining of the segment between the two breakpoints (70). Some inversions are found more frequently than others (53). The most common, recurrent inversion in humans is the pericentric inversion associated with the heterochromatin of chromosome 9; $>2\%$ of the population carries the inv(9)(p11q12). Recent investigation suggests stretches of homologous sequence in the long and short arms of chromosome 9 in the region of the common inversion breakpoints that may have acted as substrates for recombination

TABLE 1 Selected chromosomal rearrangements in humans

Chromosome anomaly	Syndrome/disorder	Estimated frequency[a]
INTERSTITIAL DELETIONS		
del(7)(q11.23q11.23)	Williams	1 in 20,000–50,000
del(8)(q24.1q24.1)	Langer-Giedion	—[b]
del(11)(p13p13)	WAGR	1 in 60,000–100,000
del(15)(q12q12)	Prader-Willi or Angelman	1 in 20,000
del(17)(p11.2p11.2)	Smith-Magenis	1 in 25,000
del(17)(p12p12)	HNPP[c]	—
del(20)(p11.23p11.23)	Alagille	1 in 70,000
del(22)(q11.2q11.2)	DiGeorge/velocardiofacial	1 in 4,000
TERMINAL DELETIONS		
del(1)(p36.3)	Monosomy 1p	1 in 10,000
del(4)(p16)	Wolf-Hirschhorn	1 in 50,000
del(5)(p15)	Cri-du-chat	1 in 50,000
del(16)(p13.3)	Rubinstein-Taybi	1 in 125,000
del(17)(p13.3)	Miller-Dieker	—
INTERSTITIAL DUPLICATIONS		
dup(7)(p12p13)	Russell-Silver	—
dup(15)(q12q12)	Variable features with autism	—
dup(17)(p11.2p11.2)	Mild developmental delay	—
dup(17)(p12p12)	Charcot-Marie-Tooth disease type 1A	1 in 2,500
dup(X)(q22q22)	Pelizaeus-Merzbacher disease	—

[a]Wilms tumor, aniridia, genitourinary dysplasia, mental retardation.
[b]Dash denotes incidence is not known, either due to rarity of anomaly or under recognition/ascertainment.
[c]Hereditary neuropathy with liability to pressure palsies.

and inversion formation (84). Isochromosomes are mirror image chromosomes that are comprised of two copies of either a short arm or a long arm. Isochromosomes are observed most often for the X chromosome (48, 139) and the acrocentric chromosomes (111, 115, 117, 118). These rearrangements are distinguished from translocations in that isochromosomes are comprised of genetically identical arms, derived from a single chromosome (115, 117).

FREQUENCY OF CHROMOSOMAL REARRANGEMENTS IN THE POPULATION

Mutation Rates and de novo Occurrence

The known and estimated population frequencies for chromosomal rearrangements are given in Tables 1 and 2. For most chromosomal rearrangements, the mutation rates are not known. However, most microdeletions occur de novo and thus, the population incidence is an approximation of the frequency. The best estimates of mutation frequencies have been determined for Robertsonian translocations

TABLE 2 Frequencies of chromosome rearrangements

Rearrangement	Frequency in the general population
Robertsonian translocations	1 in 1000
Reciprocal translocations	1 in 625
Marker chromosomes	1 in 2000
Terminal deletions	At least 1 in 5000[a]
Interstitial deletions	At least 1 in 4000[b]
Interstitial duplications	At least 1 in 4000[c]

[a]The most common terminal deletion is likely the del(1)(p36). The estimated frequency is 1 in 10,000. At least 50% were not detected by initial chromosome analysis. Thus, the frequency may be twice the estimate, or 1 in 5000.

[b]Recognized interstitial deletions range in frequency between greater than 1 in 50,000 to 1 in 4000. Thus, the frequency is at least 1 in 4000 and may actually be more frequent than recognized.

[c]Based on the estimated frequency of interstitial deletions and the fact that many interstitial duplications may be the reciprocal recombination product of deletions.

and the duplication in 17p12 that causes Charcot-Marie-Tooth disease, type 1A (CMT1A). Mutation rates for Robertsonian translocations (ROB) have been estimated from literature surveys of spontaneous abortions (46) or livebirths (46, 90), and population surveys of newborns with translocation-type Down syndrome or Patau syndrome (trisomy 13) (41, 42, 55, 94). Since it was technically impossible to distinguish isochromosomes of chromosome 21 or 13 from ROBs, the proportion of de novo rearrangements resulting from isochromosomes was unknown at the time these mutation rates were calculated. Thus, in calculating the mutation rates for ROB, all events leading to ROB-type rearrangements were considered together. It is now known that nearly 90% of homologous rearrangements of chromosomes 13 and 21 are isochromosomes (115, 117, 118). ROBs were found to have exceptionally high mutation rates ($\sim 3.9 \times 10^{-4}$ mutations per gamete per generation) (46), one to two orders of magnitude higher than the estimates for mutation rates for human autosomal dominant genetic disease. In fact, the likelihood of encountering a de novo ROB ascertained from a child with translocation Down syndrome or Patau syndrome is about 50% (41). For the majority of ROB, the de novo translocation formation occurs during oogenesis (81).

The duplication associated with CMT1A occurs de novo in about 10% of cases (6). Based on the frequency of this duplication in the population (i.e. the frequency of CMT1A caused by the duplication), the de novo mutation rate for this duplication is estimated to be about 1×10^{-4} mutations per gamete per generation (67). As stated for the mutation frequency for ROB, this mutation rate is orders of magnitude higher than the spontaneous mutation frequency usually associated with single-gene disorders. In contrast to de novo ROBs occurring primarily in oogenesis, the de novo duplication in CMT1A occurs preferentially ($\sim$10:1) during spermatogenesis (8, 66, 83).

Identification of cytogenetic aberrations through congenital anomalies or mental retardation can result in the detection of common, visible, cytogenetic anomalies, but will not ascertain rearrangements below the resolution of routine banding methods or rearrangements that do not result in an adverse outcome or those with subtle clinical phenotypes. Recent studies utilizing either whole genome microsatellite screening or FISH with probes to the chromosome ends are aimed at identifying rearrangements below the detection threshold of routine cytogenetic analysis (34, 109, 122). The FISH probes consist of sets of telomere probes to each unique chromosome end (57, 77). These have been used to detect submicroscopic abnormalities in patients with developmental delay/mental retardation (43, 58, 101). Together, these approaches have allowed for estimates of microrearrangements in the mentally retarded population (~6%–18%). However, since none of these studies were applied to apparently normal individuals, a population incidence of structural anomalies resulting in a normal phenotype is not known. Recently, a polymorphism involving the telomeric region of 13q was identified (115a). It is suspected that many more polymorphisms involving the telomeric regions likely exist.

A recurrent theme in this review is that certain regions of the genome are more susceptible to rearrangement than others. Some regions are so susceptible to rearrangement that in rare cases, structural anomalies may occur on both homologues of a chromosome pair (91, 135). Likewise, certain chromosomal rearrangements, such as the deletion of 1p, are so frequent in their occurrence that they are repeatedly identified through multiple approaches, such as routine chromosome analysis (121), surveys with genome-wide microsatellite analysis (122), and FISH with telomere probes to all chromosome ends (58, 101).

There is a tendency to assume that duplication occurs at a lower frequency than deletion of the same region because fewer patients with duplication are reported in the literature. However, study of the human cytogenetic database revealed that only 2.1% of possible autosomal bands were not involved in any duplication, whereas 11% of possible autosomal bands were not involved in any deletion (9, 10). This finding may be due to the decreased tolerance for haploinsufficiency versus trisomy in the human genome. Large regions of genomic duplication are compatible with survival, including complete trisomy for chromosomes 21, 18, and 13 (which represent approximately 1.9%, 2.9%, and 3.7% of the human genome, respectively). Thus, the underascertainment of duplication in our population may not reflect the absolute disease frequency, but rather may reflect difficulties in (*a*) recognition of patients with mild clinical manifestations and (*b*) visualizing a broader band by cytogenetic analysis.

Segregation Ratios in Familial Rearrangements

The segregation ratios for most interstitial deletions and duplications are presumed to be about 50%, based on the expectation that the condition is not lethal and that the homologous chromosomes disjoin properly in meiosis. The duplication seen in CMT1A segregates as an autosomal dominant trait (69). Although rare, autosomal

dominant inheritance has been observed for a limited number of contiguous gene deletion syndromes including Williams syndrome (73, 79, 110) and VCFS (61).

In some rare cases, mosaicism has been identified for deletions (52, 145) and duplications (63, 123). The finding of mosaicism may indicate that the rearrangement occurred in mitosis rather than in meiosis. Two cases of mosaicism for the deletion associated with Smith-Magenis syndrome have been identified (52, 145). These cases may represent a mitotic event between sister chromatids that results in mosaicism for the deletion. In cases of mosaicism for the duplication associated with CMT1A, the normal cell line likely reflects reversion of the duplication to normal (63). These somatic events stress the frequent propensity of such rearrangements to occur.

Segregation distortion is well documented for the reciprocal translocation (11;22). The carrier frequency among progeny of t(11;22) carriers is 71% (144). Both male and female carriers of this translocation are at significant risk for unbalanced offspring (4% to 7% and 6% to 10%, respectively), due to 3:1 meiotic segregation (34a, 144). The segregation ratios of male carriers of ROB have been examined. There is a significant excess of carrier offspring from the expected 50% (37). The segregation ratios range from 0.55 to 0.66 (40, 78). Carriers of ROB are at significant risk for chromosome malsegregation and aneuploid offspring. The risk depends on the chromosomes involved in the translocation and the sex of the carrier parent. Risks for chromosomally abnormal offspring range from ~1% for rob(13q14q) male and female carriers, to 10% for female carriers of rob(14q21q), to nearly 100% for male and female rob(21q21q) carriers (124).

METHODS OF STUDYING CHROMOSOMAL REARRANGEMENTS

Routine and High-Resolution Karyotype Analysis

Chromosome banding techniques, developed in the early 1970s, have allowed for the unequivocal identification of each of the 23 pairs of human chromosomes. Even prior to banding, large structural abnormalities, such as large telomeric deletions and translocations, could be identified based on chromosome morphology. The advent of banding techniques transformed clinical cytogenetics into the important medical diagnostic tool that it is today. However, some deletions and duplications are too subtle to be detected by routine analysis. Instead, the detection of deletions and duplications often requires high-resolution studies. High-resolution chromosome analysis was developed by Yunis (142). A typical metaphase cell may contain about 400 to 500 bands per haploid genome; however, high-resolution preparations can identify up to 2000 bands (143). High-resolution analysis is not practical for every case, as the extended prometaphase chromosomes are often overlapped and entangled after the cell preparation. The use of high-resolution cytogenetic analysis is therefore restricted for cases in which a particular chromosomal region needs to be studied in greater detail.

Fluorescence In Situ Hybridization

For more targeted investigations of certain areas of the genome, fluorescence in situ hybridization (FISH) has become an important molecular cytogenetic technique. FISH enables one to interrogate regions of the human genome too small to be visualized by conventional cytogenetic techniques. FISH has become the standard approach for commonly known microdeletions and microduplications [reviewed in (113, 114)]. For FISH, a DNA probe is labeled with a nucleotide that is conjugated with a fluorescent molecule or a reporter molecule (termed direct labeling or indirect labeling, respectively). After hybridization to a metaphase or interphase cell and detection of the signal, the probe can be visualized in the nucleus with the aid of fluorescence microscopy [reviewed in (128)]. FISH has been used with a variety of DNA probes including yeast artificial chromosomes (YAC), bacterial artificial chromosomes (BAC), cosmids, and plasmids to identify microdeletions and microduplications. Analysis of microdeletions typically occurs in metaphase cells, while microduplication analysis usually involves FISH on interphase nuclei, in order to visualize two closely spaced signals (45, 69, 116).

Pulsed Field Gel Electrophoresis

Pulsed field gel electrophoresis (PFGE) separates large fragments of DNA ($>$20 kb) that cannot be resolved by conventional agarose gel electrophoresis. Prior to electrophoresis, the DNA is prevented from shearing through preservation and enzymatic digestion in solid agarose. The agarose-embedded DNA, digested with a rare cutting restriction endonuclease, is separated by PFGE. Through Southern analysis and hybridization, patient-specific junction fragments have been identified in some deletions and duplications (103). Junction fragments result from the juxtaposition of DNA sequences that normally reside in different locations. Specifically, junction fragments, unique to the patient, have been identified in CMT1A duplications (16, 69, 87, 96, 103, 137); the deletion, representing the reciprocal recombination product and responsible for hereditary neuropathy with liability to pressure palsies (HNPP) (16, 104, 127); the deletion of 17p11.2 in Smith-Magenis syndrome (17); and the reciprocal recombination product–duplication 17p11.2 (17, 91, 92). Patient-specific junction fragments of apparently the same size suggest that a precise recombination mechanism is involved in forming these rearrangements, since the identical sized junction fragment, in multiple individuals, represents a recurrent event.

OBSERVATION OF STRUCTURE

Robertsonian Translocations

The nonrandom distribution of ROBs in the population suggests that there is a specific mechanism or underlying genomic architecture or sequence that promotes the exchange between certain acrocentric chromosomes. Therman et al (126)

hypothesized that sequences are shared on the short arms of chromosomes 13, 14, and 21 that participate in homologous recombination to form ROB. This yet-to-be-identified sequence is postulated to be in opposite orientation on chromosome 14, in comparison to chromosomes 13 and 21, which facilitates the formation of rob(13q14q) and rob(14q21q), but impedes the formation of rob(13q21q). This hypothesis is supported by the abundance of rob(13q14q) and rob(14q21q) in the population and the relative infrequency of rob(13q21q).

The acrocentric short arms contain tandem arrays of β-satellite, satellite III, and satellite I DNA, in addition to the 18S and 28S rRNA genes. The sequences on these chromosomes have variable distributions, such that some repetitive DNAs are shared across all acrocentrics, some are found on a few acrocentrics, or some are even unique to one acrocentric chromosome (28, 54). The placement of the breakpoints provides further evidence for a precise mechanism involved in forming the "common" ROBs rob(13q14q) and rob(14q21q), distinct from the "rare" ROBs (all remaining possible combinations). The majority of ROBs are dicentric (i.e. retain both centromeres from the two participating acrocentric chromosomes). Based on FISH with specific repetitive satellite sequences for the acrocentric chromosome short arms, the breakpoints in the common ROBs are found to occur between the same satellite sequences on chromosome 14 in 38 of 40 rob(13q14q) and 20 of 20 rob(14q21q) (39, 82). In contrast, for the rare ROBs, the breakpoints appear to occur randomly throughout the participating acrocentric short arms, with some breakpoints even occurring in the long arms of one of the participating chromosomes (82, 125). The finding that the majority of de novo rob(13q14q) and rob(14q21q) formed during maternal meiosis provides additional evidence for a precise mechanism in the formation of the common ROB (81). A study of 15 common de novo ROBs showed that all but one formed during oogenesis. The one paternally derived rob(13q14q) had a distinct breakpoint from the majority of rob(13q14q) studied (81, 82). Therefore, this one exception likely arose through a "random" error during spermatogenesis, distinct from the mechanism in oogenesis that gives rise to the majority of common ROBs (81).

Isochromosomes

Acrocentric Isochromosomes are structurally abnormal chromosomes that result from a duplication of a single chromosome arm. Isochromosomes cannot be distinguished from ROB based on their chromosome morphology and staining. Instead, molecular methods, such as DNA polymorphism analysis, is used to delineate the origin of homologous acrocentric rearrangements (2, 93, 106, 115, 117, 118). Isochromosomes of every acrocentric chromosome have been described. Isochromosomes of chromosome 21 account for about 34% of the rearrangements seen in Down syndrome (23). Isochromosomes of chromosomes 13 and 15 are seen in a significant proportion of Patau syndrome (47) and Prader-Willi or Angelman syndrome (105), respectively. The majority of homologous acrocentric rearrangements ($\sim$90%) are isochromosomes and not ROBs, based on the

inheritance of homozygous markers, near the centromere, from the heterozygous parent of origin (2, 106, 115, 117, 118).

The parental origins of the de novo isochromosomes are roughly equally divided between maternally derived and paternally derived rearrangements (115, 117). This is in sharp contrast to ROB formation, which is almost exclusively maternal in origin (81). The finding of complete homozygosity along the lengths of both arms of isochromosomes lends support to the hypothesis that most isochromosomes form postzygotically (106). However, a number of isochromosomes show heterozygosity for distal polymorphic markers, which indicates that recombination occurred during meiosis and supports a meiotic model for the formation of some isochromosomes (5, 117, 118). Both dicentric and monocentric isochromosomes have been found. This observation led to the hypothesis that isochromosomes form through a U-type exchange between sister chromatids in the short arm or within the centromeric α-satellite DNA, which results in dicentric or monocentric isochromosomes, respectively (115, 118). A U-type exchange mechanism between sister chromatids would explain the occurrence of both dicentric and monocentric rearrangements, whereas centromere misdivision, proposed originally as the mechanism for isochromosome formation, would result in only monocentric isochromosomes (Figure 1) (115). The recombination substrates through which the U-type exchange between sister chromatids occurs are not known, although the finding of repetitive satellite DNA arrays throughout the short arms of the acrocentric chromosomes presumably provides ample homologous sequence substrate for misalignment during exchange events.

The X Chromosome The most common isochromosome seen in the human population is the isochromosome of the long arm of the X chromosome ($\sim$1 in 13,000). Greater than 15% of individuals with Turner syndrome have an isochromosome of Xq in a population of their cells. Despite the relative abundance of Xq isochromosomes in the population, very few studies have been conducted on their molecular basis (48, 139). However, based on the two published studies, the majority of isochromosomes of Xq were derived from a single X chromosome (i.e. are true isochromosomes and not a translocation between two different X chromosomes) (48), were equally likely to be of maternal or paternal origin (48), and were dicentric, with the breakpoints in the proximal short arm (48, 139). Since no consistent breakpoints were observed among the isochromosomes studied, there does not appear to be a particular single sequence substrate involved in generating these rearrangements (48, 139). Nevertheless, multiple copies of a repeated sequence at various loci on Xp could result in the observation of different breakpoints. Based on the observations of complete homozygosity along the length of Xq in these patients, with no evidence of recombination between genetic markers, a U-type exchange between sister chromatids was proposed (48). Since most of these patients lacked a normal 46,XX cell line, a meiotic origin of the isochromosome was postulated (48).

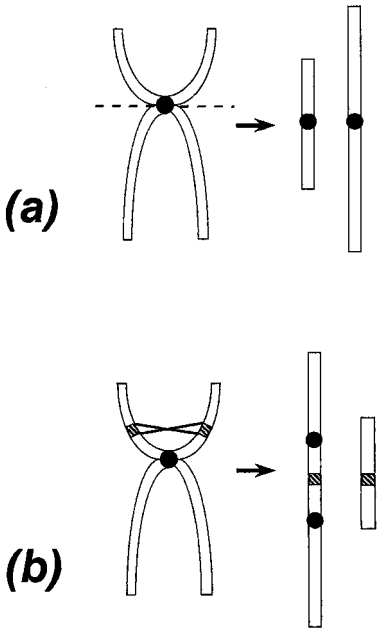

Figure 1 Two mechanisms for isochromosome formation. Chromosomes are represented as open lined structures with the centromere as a solid circle. The hatched boxes denote the sequence substrates for recombination. (*a*) Misdivision of the centromere results in two monocentric products, one isochromosome of the long arm and one isochromosome of the short arm. (*b*) U-type exchange between sister chromatids in the short arm results in a dicentric isochromosome and an acentric fragment that is lost in subsequent cell divisions. Not shown is a U-type exchange between sister chromatids within the centromere that would result in two monocentric isochromosomes, not distinguishable from misdivision of the centromere.

Marker Chromosomes

Marker chromosomes are commonly found as extra, supernumerary chromosomes during routine cytogenetic analysis (~1 in 2000 individuals in the general population). Marker chromosomes of chromosome 15 origin are the most common autosomal marker in the human population (~40% of all marker chromosomes) and can be found in phenotypically normal individuals and in the mentally retarded population (60). These bisatellited markers, which consist of two copies of the short arm (satellite DNA) of chromosome 15 in an inverted orientation, are often referred to as inv dup(15). The inconsistency in phenotype among individuals who carry inv dup(15) led investigators to study the size and parental origin of these marker chromosomes. FISH identified two sizes of inv dup(15) markers, large and small (60). All inv dup(15) were dicentric. For the small markers, no chromosome 15q euchromatin could be identified between the two centromeres (60).

The large markers contained two copies of the Prader-Willi syndrome/Angelman syndrome critical region at 15q12 (60). For the most part, patients with the small marker chromosomes were phenotypically normal, whereas patients with the large marker chromosomes were mentally retarded (60). The 15q12 region contains dosage-sensitive and imprinted genes relevant to human disease. Tetrasomy of the region would be expected to result in an abnormal phenotype based on clinical observations in patients with deletion, duplication, and triplication of 15q12 (107).

Parallels can be drawn between inv dup(15) and the small, supernumerary markers of chromosome 22 origin. Bisatellited markers derived from chromosome 22 [mar(22)] have been associated with the cat eye syndrome (72). Although the phenotype is variable, the variability has not been attributed to the different sized mar(22) (72). Most patients had most features of the syndrome, regardless of the size of their marker chromosome. FISH and restriction fragment length polymorphisms (RFLPs) characterized the mar(22) in cat eye syndrome as heterogeneous, with variability in size and appearance (72). Most markers had two copies of the sequences investigated, while some markers appeared asymmetric, with three copies of some loci between the centromeres.

Deletions

Terminal Deletions of the distal segments of all chromosomes have been described. Although most deletions appear to be unique to the individual, some terminal deletions occur (or are ascertained) more frequently than others. This preponderance of certain terminal deletions may reflect the relative viability of monosomy for the region or may reflect an underlying genomic structure that is unstable or more prone to rearrangement. Some of the more recognized, common terminal deletions include 1p-, 4p-, 5p-, 9p-, 11q-, 17p-, 18q-, and 22q-. The Monosomy 1p36 syndrome has been extensively investigated (121, 140). Like most terminal deletions that have been studied, the breakpoints do not occur at a single site, but are variable in size. However, for some terminal deletions, there are clusterings of breaks at a number of locations (19, 140). For the Monosomy 1p36 syndrome, 78% of deletions occur on the maternally inherited chromosome 1 (140).

The molecular mechanism resulting in telomeric deletions is unknown, but is likely to reflect the DNA sequences found adjacent to human telomeres. Each chromosome end is capped with the repetitive sequence (TTAGGG)n (75). Directly adjacent, proximally located, to the telomere cap is a group of repetitive sequences, which have been referred to as telomere-associated repeats (TAR) (11, 134). TARs are polymorphic in their chromosomal distribution. Each TAR region can be subdivided into a distal region of shorter sequence repeats (<2 kb) that are shared by many chromosomes and a more proximal region containing longer sequence repeats (10–40 kb) that are shared by a smaller subset of chromosomes (32). Thus, some chromosome ends contain the same TARs, whereas other subtelomeric

regions do not have TARs at all. The polymorphic distribution is thought to be the result of exchange events between nonhomologous chromosomes (11).

Certain TAR sequences are common to multiple chromosomes, suggesting that nonhomologous regions of the genome have undergone exchanges. However, the frequency at which these exchanges occur is thought to be low and nonrandom (32); otherwise, homogenization of the subtelomeric sequences among chromosomes would result, which has not been observed. The function or purpose of TAR DNA is unclear. The TAR may be a "buffer" between the terminus and the adjacent, gene-rich, unique sequence (32). Further speculation has implied that the TAR may be the site of recombination, to acquire a telomere, if a chromosome break should occur (22). The polymorphic TARs between nonhomologous chromosomes may promote pairing of these regions and more proximal recombination may result in translocations and deletions. A nonreciprocal outcome would lead to unbalanced derivative chromosomes, which, at the level of the light microscope, might appear as terminal deletions. In fact, derivative chromosomes have been identified with FISH and very distal "telomeric" probes for deletions of distal 1p (3). These derivative chromosomes may represent the product of recombination between homologous sequences on nonhomologous chromosomes.

Other mechanisms may result in cytogenetically defined terminal deletions and these include breakage due to fragile sites (49, 50), double-strand DNA breaks of unknown cause that are "healed" by the addition of the telomeric sequence $(TTAGGG)_n$(33, 59, 136), and "terminal" deletions that are really interstitial deletions (3). In Jacobsen syndrome, the breakpoints in terminal deletions of 11q23 tend to cluster in a defined region in most patients (50, 85). This region also contains the proto-oncogene *CBL2*, which contains a CCG trinucleotide repeat (50). Expansion of this repeat may result in the folate-sensitive fragile site *FRA11B* that is potentially the site of breakage for terminal deletions of 11q23. Analysis of five Jacobsen syndrome patients and their parents found two cases of deletion to be derived from a *FRA11B*-expressing chromosome (49). However, for the remaining three families investigated, the site of breakage was proximal to *FRA11B* (49). Despite this finding, given the low incidence of both this fragile site and Jacobsen syndrome in the population, the association between *FRA11B* expression and deletion at 11q23 is unlikely to be due to chance and indicates that a proportion of cases may be caused by this mechanism. Additionally, six CCG-trinucleotide repeats localize to the Jacobsen syndrome breakpoint region. A nonrandom clustering of chromosome deletion breakpoints within CCG-repeats was found in several Jacobsen syndrome patients (48a).

Interstitial Interstitial deletions have been recently recognized to cause a number of genetic syndromes (Table 1) [reviewed in (113)]. For most of these syndromes, the majority of patients share a common sized deletion (12, 17, 18, 35, 36, 51, 76, 141). This finding is consistent with the hypothesis that the deletions arise through a common mechanism and that the DNA sequences at the breakpoints may be involved in the rearrangements.

The common deletion in 7q11.23 found in Williams syndrome is about 1.6 Mb in size (88) and is present in greater than 90% of patients (79). The deletion size in most patients with Prader-Willi syndrome or Angelman syndrome is about 4 Mb (76); however, two different proximal breakpoints have been identified in both the maternally derived deletions of Angelman syndrome and the paternally derived deletions of Prader-Willi syndrome (21). The common Smith-Magenis syndrome deletion within 17p11.2 is approximately 5 Mb (17, 129) and is found in the vast majority of patients (17, 51). For most patients with DiGeorge syndrome/VCFS, the deletion in 22q11.2 is about 3 Mb (12, 74). Some patients have an alternate distal deletion breakpoint, resulting in a smaller, 1.5-Mb deletion (12, 30). Although altered sized deletions, or even rarer unique deletions, can be found in patients with these syndromes, the finding of the same-sized deletions in the majority of patients points to a specific mechanism giving rise to most of these structural rearrangements.

MECHANISMS OF CHROMOSOMAL REARRANGEMENT FORMATION

Unequal Crossing Over as a Mechanism for Rearrangement

Crossing over between nonallelic, directly repeated, homologous segments between sister chromatids (intrachromosomal) or between homologous chromosomes (interchromosomal) would be expected to produce two reciprocal products: a tandem or direct duplication and a deletion (Figure 2). To investigate the possibility of unequal crossing over as a mechanism for rearrangement, polymorphic markers flanking the rearranged site can be examined in family members and individuals with de novo rearrangements. Comparison of multiple markers between the individual with the de novo rearrangement and their parents is usually sufficient to determine the haplotypes of each chromosome contributing to the rearrangement. To establish phase, chromosomes of grandparents or siblings are included in the analysis. In this manner, rearrangements arising from a single chromosome (between sister chromatids) can be distinguished from rearrangements arising from recombination between homologous chromosomes. This type of analysis has been used to investigate the chromosomal basis of deletions associated with Williams syndrome (4, 27, 130), neurofibromatosis (66a), Prader-Willi and Angelman syndromes (13, 107, 108), and DiGeorge syndrome (4) and duplications involving 17p11.2 (92) and CMT1A (95, 137).

In Williams syndrome, deletions occur equally from either the maternally or paternally derived chromosome 7 (79). Haplotype analysis has demonstrated a high frequency of recombination between the markers flanking the common deletion interval in Williams syndrome, consistent with unequal crossing over between homologous chromosomes in meiosis causing the deletion (27, 130). De novo deletions on the maternally inherited chromosome 15 cause Angelman syndrome, while

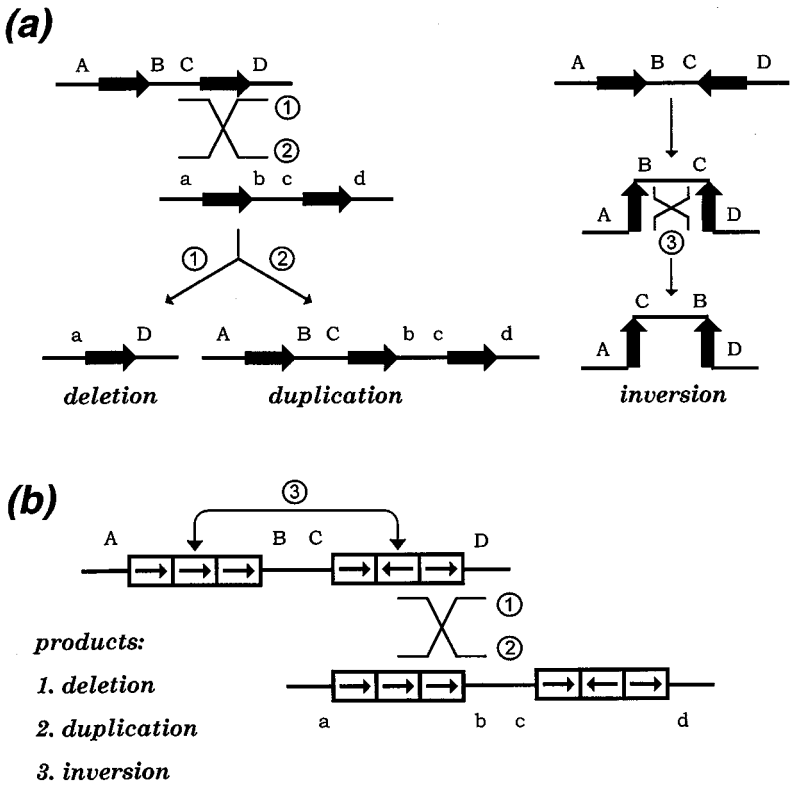

Figure 2 Molecular mechanisms for chromosomal rearrangements. (*a*) Horizontal lines represent chromosomal segments with low copy region specific repeat (LCR) sequences or repeat gene clusters represented as thick horizontal arrows. The letters A, B, C, D and a, b, c, d represent unique sequence flanking the repeats on homologous chromosomes, respectively. Homologous recombination between nonallelic directly repeated sequences (i.e. unequal crossing over) results in deletion [1] and duplication [2] rearrangements. If the repeat sequence substrate utilized for homologous recombination are represented in an inverted order within the genome architecture, then an inversion [3] chromosome results. (*b*) Horizontal open rectangles represent repeat gene clusters with the arrows denoting the direction of the repeated sequences. Note that with this more complex genome architecture, deletion [1], duplication [2], or inversion [3] may occur depending on which portion of the repeat gene cluster is utilized as the substrate for homologous recombination.

deletions on the paternally inherited chromosome 15 cause Prader-Willi syndrome. Although both interchromosomal and intrachromosomal recombination has been found for paternally derived deletions of 15q12 in Prader-Willi syndrome, haplotype analysis suggests that unequal exchanges between sister chromatids occur more frequently than mispairing and recombination between homologous chromosomes for this deletion (13, 107). Limited studies in Angelman syndrome have

shown that all cases studied resulted from an apparent meiotic recombination event between homologous chromosomes that led to the deletion of 15q12 (107). Segregation analysis of the haplotypes in families with de novo del(22)(q11.2q11.2) showed evidence for meiotic recombination between homologues for the majority of cases studied, consistent with unequal crossing over as a common mechanism leading to deletions in DiGeorge syndrome/VCFS (4). Both maternally and paternally derived deletions in proximal chromosome 22 contribute to DiGeorge syndrome/VCFS (4). Collectively, unequal crossing over between homologous chromosomes in meiosis, resulting in interstitial deletions, occurs with equal frequency in either oogenesis or spermatogenesis. However, for those deletions that are the result of unequal crossing over between sister chromatids, the majority (~64% when combining data from several studies) occur on the paternally derived chromosome.

To gain additional insight, the reciprocal recombination products, duplications of the same region, have been examined. Intriguingly, in seven patients with de novo duplications of 17p11.2, five of six informative cases were paternal in origin (92). Although duplications in four cases were the result of interchromosomal rearrangements between homologous chromosomes 17 in either the mother (n = 1) or the father (n = 3), both duplications that arose through an intrachromosomal sister chromatid exchange were paternally inherited (92). Furthermore, the duplication seen in the majority of patients with CMT1A is found de novo in about 10% of cases (6). Segregation analysis of haplotypes in de novo cases has shown that unequal crossing over occurs most frequently in paternal gametogenesis (~87%) (8, 66, 83). The recombination between homologues occurs at variable locations within a 99% identical, flanking 24-kb repeat sequence; although there is a hot spot for strand exchange located near a *mariner* transposon-like element (64, 97, 99, 100). The finding of breakpoints clustered in a low-copy repeat sequence supports the hypothesis that CMT1A, and the reciprocal deletion causing HNPP are the result of unequal crossing over during meiosis and are dictated by the intrinsic genome sequence that is prone to rearrangement of this region. Thus, misalignment of nonallelic homologous sequences and unequal sister chromatid exchange appear to occur more frequently in spermatogenesis, resulting in either deletions or duplications.

Breakpoint Cluster Regions Point to a Common Mechanism

Robertsonian Translocations The breakpoints on the common ROBs cluster between two known repetitive classes of DNA (39, 82). For rob(13q14q) and rob(14q21q), the breakpoint on chromosome 14 occurs between two satellite III subfamilies of repetitive DNA for 97% of ROBs studied (Figure 3). On the centromeric side of the chromosome 14 breakpoint, the satellite III subfamily, pTRS-47, is retained, whereas on the telomeric side of the breakpoint, the satellite III subfamily, pTRS-63, is lost from the translocation. The breakpoint on either chromosome 13 or 21, for rob(13q14q) or rob(14q21q), respectively, is between a

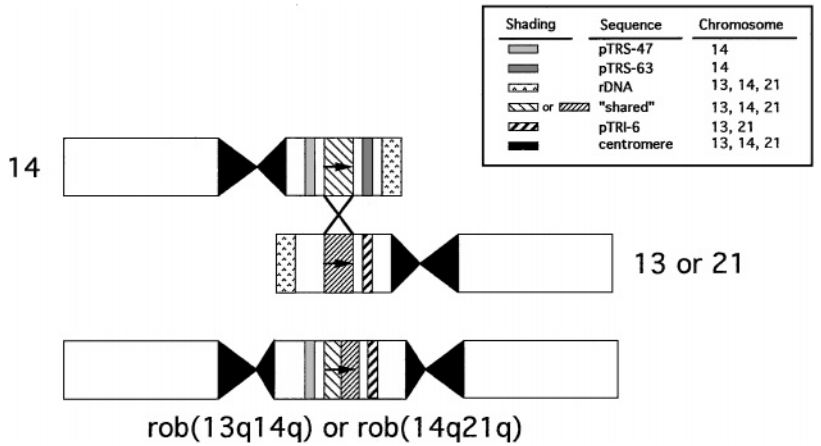

Figure 3 Formation of the common Robertsonian translocations: rob(13q14q) and rob(14q21q). Chromosomes are represented by open rectangles with centromeres as solid triangles. The key to the shading for different repetitive DNA families is given in the inset. The short arms of the participating acrocentric chromosomes contain a number of repeat sequences, satellite subfamilies, and the 18S and 28S ribosomal RNA genes. Our working hypothesis is that a sequence is shared between chromosomes 13, 14, and 21 that is in opposite orientation on chromosome 14, as compared to chromosomes 13 and 21, and facilitates exchanges resulting in rob(13q14q) and rob(14q21q), but not rob(13q21q). During ROB formation, a chromosome 14 satellite III sequence, pTRS-47, is retained on the translocation, whereas another chromosome 14 satellite III sequence, pTRS-63, and all distal loci are lost. Likewise, a chromosome 13 and 21 satellite I sequence, pTRI-6, is retained on the translocation, while the rDNA sequences are lost. The result is a dicentric translocation with breakpoints located within a consistent region on the participating acrocentric short arms.

satellite I repetitive subfamily, pTRI-6, and the rRNA genes. As described previously, the breakpoints in the rare ROBs occur randomly throughout the short arms and proximal long arms of the participating acrocentric chromosomes (82). The clustering of breakpoints within the common ROBs suggests a specific mechanism unique to the rob(13q14q) and rob(14q21q).

Reciprocal Translocations Since the majority of constitutional reciprocal translocations are unique (or "private"), very little has been reported regarding the mechanisms of formation. An exception is the t(11;22), the most common, recurrent reciprocal translocation in the human population. Breakpoint studies on both 11q and 22q have demonstrated a common site for rearrangement (31, 119). The breakpoint on chromosome 22 maps within a low-copy region-specific repeat (LCR22) that is associated with the breakpoints seen in del(22)(q11.2) in DiGeorge/VCF syndrome (29). The breakpoint on the long arm of chromosome 11 is consistently found between two genetic markers, in a genomic region of about 185–190 kb (31, 119), narrowed to a 190-bp region harboring an AT-rich

repeat (31), and recently sequenced (58a). The repetitive sequence found in the low-copy repeats on 22q also has multiple copies of an AT-rich sequence (31). These findings suggest that AT-rich regions may be prone to recombination events that lead to rearrangements. Other regions of the genome commonly undergo recombination between repetitive sequences, such as between variable number of tandem repeats (VNTRs) [e.g. steroid sulfatase deletions, reviewed in (68)].

Inv dup(15) Marker Chromosomes The breakpoints associated with inv dup(15) marker chromosomes cluster in two regions, producing two different sized chromosomes. Small inv dup(15) do not contain a copy of the region commonly deleted in the Prader-Willi or Angelman syndromes (44). Large inv dup(15) typically contain two copies of this region of 15q12 (133). The breakpoint of the small inv dup(15) can occur at either of two locations, which are the same alternate proximal breakpoints seen in deletion of 15q12 (44). These two breakpoints occur with equal frequency among these small marker chromosomes. The breakpoint of the large inv dup(15) occurs at the distal breakpoint seen in deletion 15q12 (133). The majority of inv dup(15) are thought to occur through a U-type of exchange between the homologous chromosomes 15. In all cases examined, the de novo inv dup(15) occurred during maternal meiosis. The clustering of breakpoints among deletion 15q12 and inv dup(15) suggests an underlying genomic structure or sequence substrate that is susceptible to rearrangement. Other markers, such as the mar(22) found in cat eye syndrome, also show clustering of breakpoints in one or two major sites (30, 71).

Interstitial Deletions and Duplications In contrast to most terminal deletions that show variable sites for deletion breakpoints (19, 140), most recurrent interstitial deletions have common breakpoints in the majority of patients (Table 3). In cases in which the breakpoints have been sequenced, the majority of the strand exchanges occur in low-copy repeat sequences (64, 97), thus providing direct evidence of common breakpoint regions. The finding of common breakpoints predicts the occurrence of rearrangement-specific junction fragments in de novo chromosome anomalies.

TABLE 3 Known characteristics of low copy, region-specific repeat sequences (LCRs) in the human genome

Rearrangement	Syndrome	Size of repeats (kb)	Distance between repeats (Mb)
del(7)(q11.23q11.23)	Williams	320	1.6
del(15)(q12q12)	Prader-Willi/Angelman	400	3.5
del or dup(17)(p11.2p11.2)	Smith-Magenis	250–400	5.0
del or dup(17)(p12p12)	CMT1A /HNPP	24	1.5
del(17)(q11.2q11.2)	Neurofibromatosis I	15–100	1.5
del(22)(q11.2q11.2)	DiGeorge/velocardiofacial	200	3.0

Precise Recombination Mechanism Illustrated Through Novel Junction Fragments

A seminal finding in the study of the mechanisms of chromosomal rearrangements comes from the detection of consistently sized, patient-specific, novel junction fragments (17, 69, 87, 92). The finding of specific junction fragments by PFGE in multiple independent rearrangement events suggests a precise recombination mechanism giving rise to the chromosomal rearrangements.

Although fairly recently delineated, the mechanism resulting in CMT1A has become the defining situation of a precise recombination mechanism resulting in a recurrent rearrangement. The 1.5-Mb monomer, containing the *peripheral myelin protein 22* (*PMP22*) gene, is flanked by 24-kb low-copy repeat sequences (87, 99). These sequences are 99% identical. Misalignment and recombination between homologous chromosomes 17 during meiosis results in breakpoints that are resolvable by PFGE as novel junction fragments (i.e. not carried by either parent). Through the use of somatic cell hybrids, the junction fragments have been shown to be rearrangement specific (17). Patient-specific junction fragments have been demonstrated for the Smith-Magenis deletion in proximal 17p (17) and in the reciprocal duplication product (92). Patient-specific junction fragments have also been detected in Williams syndrome (88) and DiGeorge/VCFS patients (120), using PFGE and probes specific for the low-copy region-specific repeated flanking loci. In each case, the junction fragment is apparently of the same size and rearranged-chromosome specific, suggestive of a precise recombination mechanism giving rise to these recurrent rearrangements.

Repeat Gene Clusters Flank Common Deletion Sites

Structure of Known Repeat Gene Clusters Involved in Interstitial Deletions
Low-copy repeat gene clusters are now known to flank a number of recurrent interstitial deletion and duplication regions (Table 3). The best-characterized low-copy repeats, termed CMT1A-REPs, are those flanking the 1.5-Mb monomer that is duplicated in CMT1A and deleted in HNPP (16, 69, 87, 99, 100). The proximal and distal CMT1A-REPs are involved in misalignment and unequal crossing over between homologous chromosomes 17.

The low-copy repeats in 17p11.2 that flank the Smith-Magenis syndrome deletion also contain repeat gene clusters (17). The SMS-REPs contain at least four genes (*CLPSMCR*, *TRESMCR*, *KERSMCR*, *SRPSMCR*) (17, 92). Recombination between these SMS-REPs results in the deletion associated with Smith-Magenis syndrome and the reciprocal duplication product (92). Large low-copy repeat gene clusters flank the common deletion seen in Prader-Willi and Angelman syndromes (1, 20, 102). The repeated segments are ~400 kb in size. At least seven genes map to each of the proximal and distal repeats (20). These sequences have been implicated in the deletion associated with Prader-Willi and Angelman syndromes through loss of these repeats on the deleted chromosome as assessed by FISH (20).

The 1.6-Mb deletion associated with Williams syndrome is flanked by low-copy repeats (80, 88). The repetitive sequences are greater than 320 kb in size and contain functional or pseudogenes of *GTF2I* and *NCF1* (88) as well as members of the *PMS2* mismatch repair gene family (80). The deletion results from recombination between telomeric *GTF2I/NCF1* and centromeric *GTF2IP1/NCF1P1* loci. The DiGeorge/VCFS common deletion region of about 3 Mb is flanked by low-copy region-specific repeat gene clusters (LCR22) as well (29). The 200-kb repeats contain tandem repeats of genes/pseudogenes including *GGT, BCRL, V7-rel, POM121-like*, and *GGT-rel*. Sequence analysis showed that these repeats have high sequence homology (26). Greater than 90% of patients have the same 3-Mb deletion (12). A precise recombination mechanism between flanking repeats likely causes the consistently sized deletions (30).

Recently, microdeletions of 17q11.2 containing the *NF1* locus have been characterized (25). These low-copy repeats that flank the 1.5-Mb deletions are 15–100 kb in size and contain at least 4 ESTs and an expressed pseudogene (*SH3GL*). The breakpoints in the majority of NF1 deletion patients cluster at these low-copy repeat sequences.

Evolution of Repeat Gene Clusters The high degree of sequence conservation between low-copy repeats within various microdeletion/microduplication syndromes suggests that one copy is the progenitor and recent genomic duplication gave rise to additional copies. In spite of the genomic instability associated with these repeated sequences, selective pressure must be maintaining them in the human genome. For CMT1A-REPs, copies are found only in primates and not in rodents (56, 87). A single copy is found in gorillas and orangutans, but two copies are found in chimpanzees and humans (7, 56, 99). In contrast, for the low-copy repeats found on 22q11.2, the duplication predates the divergence of the great apes from the Old World monkeys, estimated to have occurred at least 20 million years ago (mya) (120). Likewise, the presence of multiple copies of the low-copy repeats on 15q12 in the orthologous region of *Macaca fascicularis* (crab-eating macaque) suggests that these genomic duplications occurred over 20 mya (20). For the low-copy repeat sequences flanking the common Williams syndrome deletion region, these repeats are not found in the mouse genome, also indicating a recent evolutionary event that likely occurred after divergence between rodents and humans ($\sim$65 mya), but before the diversification of hominoids ($\sim$15 mya) (24). However, when the genomic duplication resulting in these low-copy region-specific repeats occurred in nonhuman primate species has not been delineated.

Recombination Leads to Chromosomal Rearrangements

The finding of consistently sized deletions, clustering of breakpoints in many types of structural rearrangements, novel junction fragments specific to the rearranged chromosome, and haplotype analysis consistent with meiotic events supports a recombination model for generating chromosomal rearrangements in humans. The

characterization of repeat gene clusters has demonstrated regions of strand exchanges within defined sequences (100). Strand exchange during homologous recombination appears to occur within stretches of sequence identity and suggests potential minimal efficient processing segments (MEPS) for substrates of homologous recombination (97). Many copies of *mariner* transposon-like elements (98), and other potential *cis*-acting recombinogenic sequences, are found throughout the human genome and may be the initiation sites of double-strand DNA breaks. Misalignment of nonallelic repeat sequence substrates and homologous recombination, followed by resolution of the recombination intermediates leads to deletion and duplication of the intervening sequence. The duplication would be predicted to be in tandem, as has been demonstrated for both CMT1A (87, 131) and dup(17)(p11.2) (92). The finding of deletion and the reciprocal duplication event in multiple individuals (17, 92) supports the model of homologous recombination events in meiosis mediated by the low-copy repeat sequences and supports the prediction that other regions of the genome that contain low-copy repeat sequences shown to recombine to form deletions may also produce the reciprocal duplication product. Very few duplications have been identified for 22q11.2 (30), and none has been reported for 7q11.23. Duplications of these small, G-band light regions may be difficult to identify by karyotype analysis, or possibly the phenotype is normal or mild, as to escape clinical detection. Duplications and even triplications have been identified for the 15q12 region (107).

The low-copy repeat elements found to flank these common deletion regions show striking sequence similarity and identity for substantial stretches of sequence (Table 3). For mammalian homologous recombination, the amount of uninterrupted homology dictates whether or not efficient recombination can take place. Homologous recombination appears to require a minimum of 134–232 bp of uninterrupted homology (MEPS) (132). Other regions of the genome, such as the short arms of the acrocentric chromosomes, likely contain genome architecture that predisposes to recombination and translocation formation. The proof awaits sequencing of the regions containing the breakpoints.

A GENERAL MODEL FOR CONSTITUTIONAL CHROMOSOMAL REARRANGEMENTS

The information obtained from the chromosomal rearrangements described in this review allows one to propose a model that could elucidate the molecular mechanisms leading to these rearrangements. In general, three steps appear operative: (*a*) a DNA double-strand break to initiate the process, (*b*) interaction of sequence substrates for recombination, and (*c*) resolution of a recombination intermediate with the formation of a novel recombination product. The details for the proposed steps in the model are most complete for interstitial microdeletion/microduplication syndromes. Mounting evidence, from studying the products of recombination at the DNA sequence level from CMT1A duplication and HNPP deletion patients, suggests a double-strand break mechanism (64, 65, 97). What

initiates or stimulates the double strand break is not clear. However, a *mariner* element (100) and microsatellite sequences (64) have been proposed to be involved based on their close proximity to the site of recombination initiation. For the second step, it has been clearly documented that the recombination occurs in nonallelic regions of homology. Thus, low-copy, region-specific repeat sequences are the substrates for a homologous recombination event. In the third step, the homologous recombination products are resolved from an unequal crossing-over event resulting in deletion or duplication.

Some terminal deletions may represent variations of this model wherein a double strand break results in loss of the chromosome end, which is now healed by either a telomere capture event (which could potentially represent homologous recombination using TARs or other substrates) (72a) or de novo synthesis of a new telomere by telomerase, using the broken DNA end as a substrate.

Theoretically, double-strand breaks could be initiated at aborted DNA transposition events (e.g. *mariners* or other DNA transposons), fragile sites (e.g. Jacobsen syndrome), or microsatellite sequence that may form unusual DNA structures [e.g. possibly the t(11;22)]. Recombination substrates identified to date consist of significant lengths (>20 kb) of sequence homology (LCRs) reflecting a unique genome architecture as possible substrates for recombination. Other shorter sequences of homology (such as repetitive elements with significant stretches of sequence identity) could potentially be utilized for homologous recombination substrates. This may be the basis for rearrangement syndromes with multiple breakpoints, such as what is observed in Pelizaeus-Merzbacher disease associated with *PLP* gene duplication (45). Of course the possibility exists that nonhomologous recombination may be the mechanism for some chromosomal rearrangements, but no direct evidence for this mechanism in recurrent rearrangement syndromes has been described to date.

CONCLUDING REMARKS

Constitutional chromosomal rearrangements occur through a recombination of genomic sequences. It is quite possible that recombination-based diseases occur more frequently than replication-based diseases, because replication errors are often found and repaired by the complex system of repair enzymes. In contrast, recombination errors, especially between misaligned segments of homology, proceed as predicted for "normal" recombination and would not be detected as an error. Thus, replication-based errors can be considered an abnormal process, whereas recombination-based errors occur during a normal process and may not be detected as aberrant.

Several common features of genomic rearrangements leading to chromosomal anomalies are emerging. Homologous segments flanking deletion- or duplication-prone regions provide the substrates for recombination that predisposes to rearrangement. These low-copy, region-specific repeats (LCRs) contain sufficient stretches of sequence identity within similarity for MEPS to allow for homologous

recombination. These LCRs represent relatively recent genome evolutionary events occurring during primate speciation. Futhermore, differences in the frequencies of rearrangements contributed by the paternally or maternally derived chromosomes suggest that differences in male and female meiosis and recombination is a significant factor in generating these rearrangements. Somatic rearrangements leading to loss of heterozygosity associated with malignancy may well reflect unique, region-specific genome structural features and subsequent mitotic recombination events.

Many regions of the human genome likely contain LCRs that predispose to rearrangement. The underlying genomic sequences determine the mechanism for rearrangement. Thus, homologous recombination between heterologous regions likely occurs at a high frequency and chromosomal rearrangements are the direct result of the sequence architecture of the human genome. Physical mapping efforts have been tremendously important in identifying genome structural features and changes resulting from DNA rearrangements. The Human Genome Project will likely continue to uncover regions of low-copy, region-specific repeats that might be predicted to be involved in recurrent rearrangement. However, sequencing alone does not provide an understanding of the complexity of the genomic structure. The application of both FISH and PFGE, methods enabling investigation of genome architectural features too small to be visualized by conventional cytogenetic techniques and too large to be resolved by conventional gel electrophoresis or DNA sequencing, have been instrumental in delineating mechanisms for chromosomal rearrangements. Thus, careful scrutiny of the human genome with physical methods has identified unanticipated genomic structure that is associated with chromosomal rearrangements. More detailed investigation of these structural properties should elucidate the exact nature of this association and provide insights into the general mechanisms underlying chromosomal rearrangements.

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