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Mouse Chromosome Engineering for Modeling Human Disease

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Abstract

Chromosomal rearrangements occur frequently in humans and can be disease-associated or phenotypically neutral. Recent technological advances have led to the discovery of copy-number changes previously undetected by cytogenetic techniques. To understand the genetic consequences of such genomic changes, these mutations need to be modeled in experimentally tractable systems. The mouse is an excellent organism for this analysis because of its biological and genetic similarity to humans, and the ease with which its genome can be manipulated. Through chromosome engineering, defined rearrangements can be introduced into the mouse genome. The resulting mouse models are leading to a better understanding of the molecular and cellular basis of dosage alterations in human disease phenotypes, in turn opening new diagnostic and therapeutic opportunities.

Keywords

chromosome rearrangement; copy number change; mouse model; loxP

INTRODUCTION

The most overt differences between the genomes of humans and mice are the numbers and gene content of their chromosomes. These alterations reflect the generation and fixation of structural alterations in mammalian genomes. In particular, duplications and inversions provide the raw material for the forces of evolution. Duplications of one or a group of genes allow genetic variants to be tested in one copy of a gene(s), enabling new functions to emerge, whereas inversions can lock sets of allelic variants into large haplotype blocks, enabling these to diverge as a group without genetic assortment until the inversion increases in frequency in the population.

The human population contains a huge reservoir of chromosomal variants. Most are existing alterations inherited from previous generations, although in every generation additional de novo changes are generated (102a). Most of these alterations are not recognized as they do not cause disease, thus the frequency of both existing as well as de novo changes is underascertained. However, these variants are detected when they cause disease. Embryonic development is a particularly stringent test for a “normal” genome, thus de novo alterations are detected in about half of the 15–20% of recognized pregnancies that result in miscarriage (<http://www.medgen.ubc.ca/wrobinson/mosaic/changes.htm>). Approximately 2–3% of newborns carry some type of germline chromosomal abnormality, the most common cases involving extra or missing sex chromosomes, followed by trisomies (especially of

chromosome 21). Chromosomal rearrangements can also cause disease when they occur somatically, as is often observed in human cancers.

The size, gene content, and type of germline structural alteration determines whether this has phenotypic consequences or simply provides an opportunity for evolution. Large deletions are potentially the most deleterious type of genomic change because they uncover dosage affects and unmask recessive mutations. Examples include Smith-Magenis syndrome (SMS), associated with a 3.7-Mb deletion on 17p11.2, and DiGeorge syndrome (DGS), associated with a 3-Mb deletion on 22q11. Large duplications tend to have milder phenotypic consequences, unless the duplicated region is very large, as seen in Down syndrome (DS), caused by a duplication of chromosome 21. Inversions and translocations tend to have less severe affects, as relatively few genes are typically affected even by very large alterations. For example, an inversion found in 1-3% of the population involving the heterochromatic region of chromosome 9 [inv(9)(p11q13)] has no known clinical significance (reviewed in 7).

Some chromosomal rearrangements such as the deletions associated with DGS, SMS, and Williams syndrome are generated de novo at a relatively high rate. Many DNA rearrangements arise as a result of the structural characteristics of the genome itself, such as the presence of low copy repeat sequences, which act as substrates for homologous recombination between nonsyntenic regions of chromosomes (reviewed in 66, 105). In addition to duplications and deletions, other types of chromosomal rearrangements include inversions and translocations, which can also have a detrimental effect on the individual if the breakpoint occurs in a gene that results in disruption of its normal function or results in the splicing together of two genes to create a chimeric/hybrid gene. Disease can arise both as a consequence of germline alterations as well as changes that occur somatically. Many translocations may not affect gene function, but can disrupt normal meiotic chromosome segregation, leading to sterility.

Many other disease-associated chromosomal rearrangements have been described, but these are comparatively rare and/or their associated phenotypes are quite variable so they have yet to be classified as “syndromes.” Most constitutional deletions have been identified using conventional cytogenetic techniques, limiting the detection of disease-associated deletions to several million base pairs. The current estimate of chromosomal rearrangements that exist in the human population is likely an under-representation for several reasons. The presence of a chromosomal rearrangement could be missed due to underascertainment of the condition due to a mild phenotype. For example, SMS arises from a deletion on 17p11.2; however, the reciprocal duplication leads to the dup(17)(p11.2p11.2) syndrome, which has a less severe phenotype than SMS (83), as an excess of genetic information (created by a duplication) is usually less detrimental to the organism than a deficiency (created by a deletion). Limitations of the screening technologies themselves often mean that cases of chromosomal rearrangements remain unidentified as some rearrangements are too small to be visualized by traditional cytogenetic techniques.

Recently, bacterial artificial chromosome (BAC) array comparative genomic hybridization (CGH) provided an opportunity to scan the genome at a significantly higher resolution. This has resulted in the detection of disease-associated copy number changes that were previously undetected by cytogenetics (reviewed in 3). This same technology has revealed that large genomic alterations involving the loss or gain of millions of base pairs are common polymorphisms in the human and mouse populations (2, 101). Although it is assumed that most of these copy number polymorphisms (CNPs) do not have developmental or physiological consequences to the individual with the CNP, a subset of these alterations are not neutral and may be disease associated in either a positive or negative sense. The

significance of these copy number changes may not be readily apparent from human studies, but they can be assessed in mice with similar genomic alterations.

Despite the shuffling of the linkage orders in the human and mouse genomes, many conserved linkage groups can be recognized between the two genomes. This conservation enables us to model the chromosomal rearrangements involved in human diseases via chromosome engineering (87, 130, 131). Mouse models that carry engineered chromosomal deletions have been successfully used to model the human chromosomal deletions that are responsible for DGS (59, 60, 69), Prader-Willi syndrome (PGS) (117), and SMS (122, 123). Deletion syndromes are very difficult to analyze in humans because one must rely on rare deletions to subclassify the phenotype. In contrast, specific subdeletions can be generated in mice, enabling specific associations to be drawn between aspects of the phenotype and genes in the deleted region. This approach was instrumental in identifying the causative gene for the principal cardiovascular defect in DGS (45, 59, 69).

Thus, the mouse has proved an invaluable model organism in allowing understanding of the genomic landscape that results in diseases in humans. This is due to its genetic and physiological similarities to humans, as well as the ease with which its genome can be manipulated and analyzed. It is the development of the Cre/*loxP*-based strategy to introduce defined chromosomal rearrangements into the genome (known as “chromosome engineering”) that has allowed the generation of accurate mouse models of human diseases arising from chromosomal rearrangements.

CHROMOSOMAL REARRANGEMENTS FOUND IN HUMANS

Deletions

A chromosomal deletion (Figure 1) can be either interstitial (occurring within one of the chromosome arms) or terminal (occurring at one of the ends of a chromosome arm). The consequence of a deletion depends on the size of the deleted segment, which genes are affected, and the alleles on the homologous chromosome. Thus, a deletion can result in a phenotype in four ways, by disrupting the normal function of a gene at the deletion breakpoint, by causing a reduction in dosage of one or a combination of the deleted genes, by uncovering recessive alleles on the other homolog, or by a “position effect” [cases associated with chromosomal rearrangements outside the transcription and promoter regions of the gene (reviewed in 47a)]. The most overt examples of uncovering of recessive alleles occur on the X chromosome.

In males, deletions on the X chromosome result in structural and functional nullisomy for genes located within the deleted region and thus males are typically characterized by full expression of the phenotype. One example is red-green color blindness, which is a result of deletions in the L and M (long and middle wavelength-sensitive) pigment genes on the X chromosome. These genes are similar in sequence and structure, which predisposes them to intergenic homologous recombination resulting in deletions and the formation of L/M hybrid genes that affect color vision (reviewed in 27). In females, deletions on the X chromosome often do not result in a phenotype due to the presence of a second copy of the allele on the other X chromosome. However, females may show a phenotype if a nonrandom X-inactivation occurs resulting in functional nullisomy, or if they are heterozygotes for loss-of-function alleles. (For example, women with heterozygous ornithine transcarbamylase deficiency may have no symptoms or have episodic, symptomatic hyperammonemia, which can be fatal.)

Deletions on the autosomal chromosomes cause structural monosomy and in some cases, depending on the gene(s) affected, this may cause haploinsufficiency [a state that occurs

when loss of function of one gene copy leads to an abnormal phenotype because a single copy of the normal gene(s) cannot provide sufficient protein production as to assure normal function]. Examples of this include deletions of 8q24.1 in Langier-Giedion syndrome (characterized by cone-shaped epiphyses in the hands and multiple cartilaginous exostoses) (64) and deletions of 17p13.3 in Miller-Dieker syndrome (a developmental defect of the brain caused by incomplete neuronal migration and characterized by a smooth brain surface) (30). In addition, deletions of a chromosomal region subject to imprinting (where only the paternally derived allele or the maternally derived allele of a gene is actively expressed) may result in functional nullisomy if the nondeleted gene locus is the inactivated one. This occurs with PWS and Angelman syndrome (AS) (detailed in Prader-Willi syndrome, below; reviewed in Reference 15). Functional nullisomy can also result from germline deletions followed by a secondary somatic event, such as a mutagenic exposure resulting in a point mutation/deletion of the corresponding allele(s) on the intact chromosome. For example, WAGR syndrome patients (characterized by developmental abnormalities such as aniridia, genitourinary malformations, and mental retardation) who have a germline chromosomal deletion on 11p13 are at high risk of developing Wilms tumors due to loss of the *WT1* tumor suppressor gene (TSG) located in this region on the intact chromosome (12).

Somatic chromosomal deletions also play a major role in human disease. In many cancers, chromosomal deletions can be the initiating lesion and deletions are one of the most frequently observed somatic genetic alterations observed. Nonrandom recurrent losses (or gains) of chromosomal segments in tumors result from mutational selection during the development and progression of abnormal cell growth. Chromosomal deletions can result in tumorigenesis if the lost chromosomal region harbors a TSG and, according to the “two-hit” model for tumorigenesis (49), there is a second mutagenic event resulting in loss of the allele on the intact chromosome. However, some TSGs are haploinsufficient, such as *p27^{kip1}*, and in such cases a chromosomal deletion involving this locus would be sufficient to predispose to tumorigenesis (82).

Insertions

Chromosomal insertions are generated by excision of a DNA segment from one chromosomal location and insertion into another (also known as insertional translocation; chromosomal translocations are discussed in Translocations, below) (see Figure 1). An insertion can occur in the same chromosome (intrachromosomal); however, the vast majority involve insertion into a different chromosome (interchromosomal). When the DNA segment inserts in the reverse orientation to the centromere, this is known as an inverted insertion (chromosomal inversions are discussed in Inversions, below). Chromosomal insertions occur at an estimated frequency of 1 in 5000 newborn infants (26), and balanced carriers usually show no phenotype but are at risk of producing offspring with chromosomal abnormalities due to complications during meiosis (depending on the size and nature of the insertion). The interstitial insertion of genetic material from one chromosome into another can generate gene-gene fusions (which are also seen with chromosome translocations) as well as loss-of-function mutations at the site of insertion and excision. Phenotypes can be caused by the function of the fusion product. For example, the genomic inversion and insertion of part of the *AF10* gene into the *MLL* gene results in the *MLL-AF10* gene fusion found in acute myeloid leukemia patients (16).

Duplications

Duplications are the doubling of a section of the genome. Duplication of a whole chromosome results in trisomy (Figure 1), the most well-known of which involves chromosome 21 and results in DS. However, some individuals with only a partial duplication of chromosome 21 still show the Down phenotype, enabling clinicians to try to

narrow down the critical region responsible for the phenotype (reviewed in 103). Trisomy 16 is estimated to occur in >1% of pregnancies, making it the most common trisomy in humans; however, it is the most common chromosomal cause of miscarriages as the condition is not compatible with life (mosaic trisomy 16 individuals may survive but they can have multiple abnormalities) (reviewed in 5).

Segmental duplications describe a situation where a portion of a chromosome is duplicated (Figure 1). These can arise in the offspring of balanced translocation carriers or can occur during meiosis if there is crossing over between sister chromatids that are out of alignment (resulting in one chromatid with duplicated genes and the other chromatid having the corresponding deletion). Although not usually as problematic as deletions, duplications (particularly of large segments) can result in a phenotype, usually as a result of an increase in gene copy number. An example is Charcot-Marie-Tooth disease type 1A (CMT1A), a common inherited neurological disorder that results from a segmental duplication of a portion of chromosome 17 carrying the dosage-sensitive gene producing the peripheral myelin protein 22 (*PMP22*), a critical component of the myelin sheath; overabundance of this gene causes the structure and function of the myelin sheath to be abnormal and patients experience weakness and atrophy of the lower leg muscles (93). Segmental duplications can also result in a phenotype if the genes at the breakpoint of the duplication are disrupted or inappropriately expressed. For example, glucocorticoid-remediable aldosteronism (GRA), characterized by hypertension with variable hyperaldosteronism and high levels of abnormal adrenal steroids, is caused by a segmental duplication on chromosome 8q24 arising from an unequal crossing over that fuses the regulatory region of *CYP11B1* (11- β -hydroxylase gene) to the coding sequence of *CYP11B2* (aldosterone synthase gene), resulting in misregulated expression of aldosterone synthase (58).

Translocations

Translocations are interchromosomal rearrangements involving two different chromosomes and consist of the transfer of chromosomal material between chromosomes. Translocations usually occur following failure to correctly repair breaks in one or two chromosomes. There are two types of translocations, namely Robertsonian translocations and reciprocal translocations (Figure 1). Robertsonian translocations (~1 in 1000 live births) involve whole arm exchanges and occur following breakage at or near the centromere of two acrocentric chromosomes (those chromosomes in which one chromosome arm is very long and the other short; this is the case for human chromosomes 13, 14, 15, 21, and 22 and all mouse chromosomes), followed by fusion of the large fragments of the two chromosomes to form a dicentric chromosome (one with two centromeres). Although the acentric fragments (chromosomes without a centromere) are usually lost at a subsequent cell division, the material lost is relatively small and often does not encode any genes; thus, this does not normally give rise to a phenotype. Consequently, most Robertsonian translocations are regarded as “balanced.” Reciprocal translocations (~1 in 500 live births) result from a single break on each of the two participating chromosomes. Similarly, carriers of reciprocal translocations can be clinically normal and this is considered a “balanced translocation.” Although the carrier of a balanced translocation may show no phenotype, unbalanced gametes may be produced during gametogenesis, resulting in an unbalanced genome, which often causes fetal loss or overt developmental problems if the embryo survives to term.

Although there is generally minor overall loss or gain of genetic material in a balanced translocation, a phenotype can still arise, as chromosomal translocations are common occurrences in cancer, particularly lymphoid malignancies (52). There are two principle consequences of translocations that can result in a phenotype: The translocation brings a gene under the influence of different regulatory elements, resulting in altered expression due to a position effect, or a fusion gene encoding a chimeric protein (reviewed in 86) is

generated. Examples of the former situation include the T-cell receptor or immunoglobulin heavy-chain enhancer influencing the expression of a proto-oncogene and thereby activating it, with subsequent effects on cell growth, differentiation, and/or apoptosis. For example, in one form of Burkitt's lymphoma the tip of chromosome 8 is exchanged with the tip of chromosome 14, which places the *c-myc* oncogene next to an immunoglobulin heavy locus, resulting in overexpression of Myc. On the other hand, the fusion of the *BCR* and *c-ABL* genes in the Philadelphia chromosome in chronic myelogenous leukemia (CML) typifies the situation in which breakage on each chromosome occurs within introns of genes, producing fusion genes. The Philadelphia chromosome (found in 95% of CML cases) is a reciprocal translocation between the long arms of chromosomes 22 and 9 [t(9;22)]. The translocated portion of 9q contains the protooncogene *abl*, which is translocated to a specific site on 22q [designated the break point cluster region (BCR)], resulting in unregulated tyrosine kinase activity of the consequent bcr-abl fusion protein (reviewed in 88).

Inversions

Inversions occur when a single chromosome breaks in two places and the material in between is reconstructed upside down. There are two classes of inversions: paracentric inversions, which do not involve the centromere and are restricted to one arm of the chromosome, and pericentric inversions, in which the inverted portion includes the centromere (see Figure 1). As there is no net gain or loss of genetic material (all genes are present, but in a different order on the chromosome), individuals with an inversion may not show a phenotype. However, heterozygous individuals can show reduced fertility due to the production of nonviable gametes, whereas homozygous individuals show no loss of fertility. A phenotype can also arise if the breakpoint occurs within a gene. For example, the pericentric inversion of chromosome 16 [inv(16)(p13q22)] is a characteristic karyotypic abnormality associated with acute myeloid leukemia, most commonly of the M4Eo subtype (55, 62). On 16q the inversion occurs near the end of the coding region for CBF β , and on 16p a smooth muscle myosin heavy-chain (SMMHC) gene (*MYH11*) is interrupted; the resulting inframe fusion mRNA connects the first 165 amino acids of CBF β with the tail region of SMMHC (63).

Other examples of diseases caused by inversions include the genomic disorders, hemophilia type A and Hunter syndrome (Mucopolysaccharidosis type II). Patients with hemophilia A frequently show an inversion of a portion of the factor VIII gene (exons 1-22), caused by homologous recombination between repeat sequences of an intronless gene located within intron 22 and at the 5' end of the factor VIII gene (54). Similarly, inversion of the iduronate-2-sulfatase (*IDS*) gene, due to recombination with *IDS*-related sequences (such as the *IDS-2*, located 20 kb distal to and in the opposite direction of *IDS*), is a common cause of Hunter syndrome (11, 116).

Other Abnormalities

Marker chromosomes—A marker chromosome is an extra piece of a chromosome of unidentified origin (often renamed as an “extra structurally abnormal chromosome,” a “supernumerary chromosome” or an “accessory chromosome”). Marker chromosomes are present in ~0.05% of the human population, and in ~30% of carriers an abnormal phenotype is observed. The effect of this extra genetic material depends on the size and particular chromosome involved, but is often associated with developmental abnormalities and malformations (reviewed in 110). An example includes the supernumerary inverted duplicated chromosome 15 [+inv dup(15)], also known as an isodicentric chromosome 15 [idic(15)]. Inv dup(15) chromosomes are variable with respect to the size and genetic composition of the chromosome and in their phenotypic effects. Patients with small inv dup(15) may have no phenotypic abnormalities, whereas patients with large inv dup(15),

which can include the Prader-Willi region, may have multiple abnormalities, including autistic behavior (reviewed at <http://www.idic15.org/references.html>). Isochromosome 17q [i(17q)] is one of the most common isochromosomes found in cancer. Mechanistically, it appears to be mediated by a nonallelic homologous recombination (NAHR) event between low-copy repeats (LCRs) in opposite orientations located within the two sister chromatids (4a).

Ring chromosomes—A ring chromosome can occur when the ends of the short and long arm break off and join to each other, resulting in the loss of genetic material at the ends of the chromosome prior to the fusion. Ring chromosomes can pass through cell division if they carry a centromere; however, they are susceptible to breakage, disruption, and entanglement during mitosis and, consequently, although only a small amount of genetic material may be lost, the phenotype can be severe. Ring chromosomes of almost all chromosomal origins have been reported (a list can be found at http://www.chromodisorder.org/sytrix/card_list.php3?dbid=82&id=241).

Isochromosomes—Isochromosomes are formed by transverse splitting of the centromere so that both arms are from the same side of the centromere, are of equal length, and possess identical genes. Thus, there is an overall deletion of one arm and duplication of the other (one daughter cell receives both long arms and the other both short arms). Isochromosomes have been reported in 10% of cancers (70).

USING THE MOUSE TO MODEL HUMAN CHROMOSOMAL REARRANGEMENTS

Progressing Human Genetics in the Mouse

Determining the specific DNA sequence associated with a chromosomal rearrangement that causes disease in humans is important in understanding the mechanistic basis of the disease. For some types of rearrangements, such as translocations, common disease-associated breakpoints can be rapidly identified (35, 40), providing a rapid and high-resolution means to identify the affected genes, particularly in cases of gene-gene fusions. However, it can be much harder to pinpoint the specific gene in other types of rearrangements such as duplications and deletions, particularly if they are large and affect many genes. In principle, examining large numbers of patients can help to resolve this difficulty; however, even for relatively common syndromes such as DGS, many of the deletions are recurrent because of genomic architecture that leads to genomic instability, which limits the resolution of genetic analysis to a large, commonly deleted region with several genes. For rare syndromes, the number of cases is often too low or the breakpoints and phenotypes too variable to come to a firm conclusion about common genomic features. However, databases such as DECIPHER offer a potential route forward to at least identify minimal regions for genomic analysis (<http://www.sanger.ac.uk/PostGenomics/decipher/>). By combining, in a single database, genetic and phenotypic data from multiple laboratory groups, it is possible to connect information from rare individual cases and draw common conclusions that would not be possible from single case reports.

Where human genetic analysis has reached its natural limits, genetic manipulation of mice has begun to make significant progress in unraveling the mechanistic basis of several human chromosomal diseases. The anatomical, physiological, and genomic parallels between humans and mice, coupled with the ability to make directed changes in the mouse genome, enable a constellation of genetic variants to be generated in mice to thoroughly evaluate their contribution to the observed human phenotype. In addition to the anatomical and physiological similarities between the two species, this strategy relies on the arrangement of

genes on the chromosomes in large blocks with similar gene order (synteny between the mouse and human chromosomes).

Technologies for Generating Chromosomal Rearrangements

Chromosomal rearrangements in mice occur spontaneously at a low rate, or they can be induced by exposure to chemical mutagens [such as cyclophosphamide, ethylene oxide, chlorambucil, and ethylnitrosourea (ENU) or physical mutagens (such as X-rays) (24, 57, 96, 111, 114)]. Although these mutagens have generated some valuable mouse models for human diseases, such as the segmental trisomy 16 (Ts65Dn) mouse, a model for DS (see-Down syndrome, below) (24, 89), their usefulness is limited because the endpoints and type of rearrangements are random and cannot be predetermined.

These limitations have been overcome by the development of chromosome engineering techniques, based on gene targeting in embryonic stem (ES) cells and the *Cre/loxP* site-specific recombination. With this system, the type of chromosomal rearrangement depends on the orientation of the *loxP* sites and the relative position of selection cassettes used to recover cells that have undergone the long-range *Cre/loxP* recombination event (Figure 2) (1, 87, 108, 119, 132). In this technique, gene targeting is used to insert the *loxP* sites sequentially into two different loci in the ES cell genome (Figure 3 shows an example of the type of gene targeting vectors used). The doubly targeted ES cells are then exposed to Cre to induce recombination between the *loxP* sites and generate the rearranged chromosome. ES cell clones identified as carrying the desired chromosomal rearrangement(s) are used to generate chimaeras, from which the progeny that carry the engineered chromosome are derived (Figure 4). The use of chromosomal engineering to study human chromosomal disorders such as deletions and duplications relies on the conservation of the order of genes in the two genomes. The relative orientation of the genes in the two species is also important when generating translocations. If one gene is inverted (with respect to the centromere) in one species, interchromosomal *Cre/loxP* recombination will generate an acentric fragment and dicentric chromosome instead of a balanced translocation.

An extension of the chromosome engineering strategy is the generation of nested deletions (a series of variably sized, overlapping deletions surrounding a predetermined genomic locus). To avoid generating individual targeting vectors for nested endpoints, random retroviral integration of a second *loxP* site can be used, thus generating a library of ES cell clones with the same targeted endpoint and a collection of random endpoints (Figure 5) (112). Nested chromosomal deletions can also be generated by X-ray and UV-induced mutagenesis (6, 115, 129).

One elegant aspect of the *Cre/loxP* system is that it allows a chromosomal rearrangement to be generated in a temporally and/or spatially controlled manner. For example, chromosomal rearrangements can be generated somatically from a chromosome (or chromosomes) with targeted unrecombined *loxP* sites. The rearrangement can be generated somatically by breeding this (these) chromosome(s) into a background where Cre is expressed under the control of a tissue-specific or chemically inducible promoter (through the use of Cre-deleter mice). This is particularly useful if the chromosomal rearrangement would otherwise cause a developmental anomaly or embryonic lethality, as can occur with large deletions or fusion-gene elements created by translocations. The somatic generation of recombination events also enables the stochastic nature of mutations that occur in cancer to be mimicked.

Deletion and duplications can also be generated in vivo using Cre-mediated targeted meiotic recombination (TAMERE) technology. In this technology, the *loxP* sites are bred together in the same mouse in *trans*. Following chromosome pairing in meiotic prophase and Cre expression through the *Sycp1/Cre* transgene (which produces Cre in male spermatocytes

during the stages when chromosome pairing occurs), recombination between the *loxP* sites occurs and generates chromosomes with deletions and duplications (42). The advantage of this technology is that pre-existing chromosomes with targeted *loxP* sites can be bred together. However, the size limits of this technology are essentially unknown.

MOUSE MODELS OF HUMAN DISEASE

Deletions

DiGeorge syndrome—DiGeorge syndrome (DGS) is characterized by a triad of cardiac malformations (notably, interrupted aortic arch), immune deficiency, and hypocalcemia. There can also be dysmorphic facial features. The cause of DGS has been identified as a microdeletion of chromosome 22q11.2 (31, 100). It is classified along with velo-cardiofacial syndrome (Shprintzen syndrome) and cono-truncal anomaly face syndrome, which are also caused by the same 22q11 deletion. Approximately 90% of patients have a typically deleted region of ~3 Mb, which encompasses ~30 genes, with 10% of patients having a smaller 1.5-Mb deletion. Yet, despite the genetic homogeneity of the syndrome, the distinct clinical features of the disorder are incompletely penetrant and show variable expressivity. To further dissect the molecular genetic basis of DGS, chromosome engineering has been used to generate deletions in the mouse genome that encompass subsets of the genes deleted in patients (summarized in Figure 6) (reviewed in 61). The gene content of the human 22q11 region is syntenic to a region of mouse chromosome 16 (65).

The first mouse deletion to be generated encompassed 1.2 Mb (18 of the 24 genes deleted in patients with the 1.5-Mb deletion), and heterozygous mice showed cardiovascular defects similar to those seen in human patients (60). Furthermore, the cardiovascular defects could be corrected when the normal gene dosage was restored by generating mice that carried both the deletion and the reciprocal duplication, showing that a haploinsufficient gene(s) within the deleted region was involved in heart development. Two other deletions were also generated that partially overlapped with the 1.2-Mb deletion, one encompassing 150 kb (47) and the other encompassing 550 kb (85). However, mice heterozygous for both of the smaller deletions had normal heart development, excluding a role for these particular regions in DGS and indicating that the gene responsible for the cardiovascular defects lay in the remaining deletion interval containing eight genes (Figure 6).

Using a candidate gene approach, mice with null mutations in specific genes in that region were generated [*Comt* (38) and *Pnut11* (59)], but did not show any cardiovascular defects. To narrow down which of the remaining six genes in the critical region was responsible for the cardiovascular phenotype, deletion mice (69) were bred with transgenic mice carrying large genomic DNA fragments from within the critical region. This led to the identification of a region containing four genes (*Gnb1l*, *Tbx1*, *Gp1bb*, and *Pnut11*) that was sufficient to correct the cardiovascular defects (Figure 6) (59, 69). Finally, a candidate gene approach that involved generating a *Tbx1* null mouse showed that the cardiovascular defects seen in *Tbx1*^{+/-} mice were identical to those found in the 1.2-Mb deletion mice, providing compelling evidence that *Tbx1* was responsible for the phenotype (59, 69). Interestingly, the phenotype of *Tbx1* null mice is reminiscent of severe forms of *del22q11* syndrome (45), indicating that human *TBX1* might be involved in both cardiovascular and noncardiovascular aspects of the *del22q11* syndrome phenotype (reviewed in 4, 61).

Smith-Magenis syndrome—Smith-Magenis syndrome (SMS) is a multiple congenital anomaly/mental retardation syndrome characterized by distinct craniofacial and skeletal anomalies and neurobehavioral features (39). An interstitial deletion of 3.7 Mb on chromosome 17p11.2 derived from nonallelic homologous recombination between flanking low-copy repeats has been identified in most patients (16a). The predicted reciprocal

duplication leads to the dup(17)(p11.2p11.2) syndrome with a less severe phenotype than SMS (83). SMS is thought to be a contiguous gene syndrome caused by haploinsufficiency of one or more genes in the associated deletion region. From analysis of patients with unusual-sized deletions, the smallest region of overlap, or SMS critical region (SMCR), has been confined to a ~950-kb interval containing ~20 genes (120).

Human 17p11.2 is syntenic to the 32-34-cM region of mouse chromosome 11, with the order, number, and orientation of the genes being highly conserved, thus making it feasible to establish a mouse model for SMS (9). Chromosome engineering was used to generate mice containing a ~2-Mb deletion of the syntenic region of the human SMS common deletion (*Df(11)17*) (122). *Df(11)17*+ mice partially recapitulate the SMS phenotype with craniofacial abnormalities, obesity, seizures, and neurobehavioral abnormalities including an abnormal circadian rhythm (122, 123). Retrovirus-mediated chromosome engineering was used to generate ES cells with subdeletions in the same region. Mice carrying these subdeletions showed obesity and craniofacial abnormalities, suggesting that the genes responsible are located within the small deletions (126). Interestingly, the craniofacial phenotype was strongly affected by the size of the deletions (126), suggesting a role for segmental aneuploidy in expression of the phenotype. In contrast to this, recent work using a candidate gene approach has focused on the *Rai1* gene, which lies in the SMCR and is mutated in some SMS patients (10, 107). *Rai1* haploinsufficiency in mice causes craniofacial phenotypes and obesity similar to that seen in humans (8), suggesting a role for a single-gene haploinsufficiency in expression of the phenotype. Thus, for both humans and mice, it is not yet clear whether segmental aneuploidy or single-gene haploinsufficiency is responsible for expression and penetrance of the full phenotype.

Chromosomal engineering has also been used to generate mice carrying the reciprocal duplication *Dp(11)17* of the syntenic region on mouse chromosome 11 that spans the genomic interval commonly deleted in SMS patients. *Dp(11)17*+ animals are underweight yet do not have the seizures, craniofacial abnormalities, or reduced fertility seen in the *Df(11)17*+ mice (122). Furthermore, *Df(11)17/Dp(11)17* mice have no apparent phenotype, suggesting that most of the observed phenotypes in SMS and dup(17)(p11.2p11.2) syndrome result from gene dosage effects (122). Thus, these mouse models represent a powerful tool to analyze the consequences of gene dosage imbalance in this genomic interval and to further investigate the molecular genetic basis (segmental aneuploidy versus single-gene haploinsufficiency) of both SMS and dup(17)(p11.2p11.2).

Prader-Willi syndrome—Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two oppositely imprinted neurobehavioral disorders. PWS is characterized by hypotonia, cognitive impairment, difficult behaviors, and hyperphagia leading to profound obesity and hypogonadism (44), whereas AS is characterized by severe mental retardation, ataxic gait, absent speech, and a happy demeanor (19). PWS is caused by paternal deficiency of human chromosome 15q11-q13 (caused by a 4-Mb deletion on the paternal chromosome or by maternal uniparental disomy), whereas AS results from maternal deficiency for the same chromosomal region, although some patients only show mutations in the *UBE3A* gene, suggesting that this is the AS gene (reviewed in 74). The absence of a similar class of patients with PWS suggests that PWS may represent a contiguous gene syndrome, caused by loss of two or more paternally expressed genes. Six paternally expressed transcripts have been mapped to the PWS/AS region (*SNRPN*, *ZNF217*, *NDN*, *IPW*, *PARI*, *PAR5*), and imprinting of these genes appears to be controlled by an imprinting center (IC). Paternal microdeletions have been identified in three PWS families and involve the *SNRPN* gene. These microdeletions abolish small nuclear ribonucleoprotein polypeptide N (*SNRPN*) expression and are associated with a lack of expression of other paternally expressed genes in the region (14, 91, 99, 113).

To evaluate the role of SNRPN and the IC in PWS and whether the phenotypic features seen in humans result from the loss of expression of a single imprinted gene or multiple genes, studies were conducted in mice. The central part of mouse chromosome 7 (7C) is homologous to human 15q11-q13, with conservation of both gene order and imprinted features (73). Mice harboring an intragenic deletion in *Snrpn* are phenotypically normal, suggesting that mutations of SNRPN are not sufficient to induce PWS (117, 127). In contrast, mice with a larger deletion involving both *Snrpn* and the putative IC lack expression of several of the imprinted genes in the PWS region (thus modeling PWS at the molecular level) and manifest some phenotypes common to PWS infants (such as failure to survive after birth due to inadequate feeding, but not hypogonadism or obesity) (127). Similarly, mice carrying a large chromosomal deletion extending from *Snrpn-Ube3a* show postnatal lethality in ~80% of the mice when inherited paternally, but no signs of hypogonadism or obesity (117). A radiation-induced deletion (*p^{30PUB}*) generated at the *p* locus in mice (which includes the *Ipw* locus) is not associated with neonatal lethality when paternally inherited (46), suggesting that the region associated with lethality lies between *Snrpn* and *Ipw*. More recently, expression profiling studies on these mice have suggested that the *Pwcr1/MBII-85 snoRNA* is the candidate most likely needed for the neonatal survival of mice (28). Thus, these mouse models have proven valuable for investigating the molecular mechanisms of imprinting in this region of the genome and for getting closer to determining the gene(s) responsible for the PWS phenotype.

Duchenne muscular dystrophy—Duchenne muscular dystrophy (DMD) is a severe X-linked degenerative muscle disorder that eventually affects all voluntary muscles, the heart, and respiratory muscles such that survival beyond the early 30s is rare. DMD is caused by mutations in the 2.4-Mb dystrophin (*DMD*) gene resulting in a lack of dystrophin protein (50). *DMD* is a highly complex gene, composed of 85 exons including 7 unique first exons linked to independent tissue-specific promoters, generating at least 3 full-length and 4 shorter dystrophin isoforms (72). Studies of dystrophin function have benefitted from analysis of the *mdx* mouse, a naturally occurring animal model for DMD in which a point mutation forms a premature stop codon, leading to a loss of full-length dystrophin, although small isoforms still remain (106). However, although *mdx* skeletal muscle shows muscular hypertrophy and dystrophy, the life span of the mouse is not substantially reduced, which is a major difference compared to DMD patients. Mouse strains that also lack the small isoforms have been generated [such as the *mdx^{3cv}* mouse (22) and the *DMD^{mdx-βgeo}* mouse (124), which show severe loss of most dystrophin isoforms] and these mice display essentially the same muscle pathology as *mdx* mice, but each with some additional phenotypes (although the phenotypic discrepancy between the mouse strains has not been fully explained).

Understanding the function of dystrophin isoforms at the molecular level is very important as it may provide insights into the regulation and function of the dystrophin gene, and it may also aid in the development of therapies to relieve the symptoms of muscular dystrophy. Therefore, two new mouse strains were developed using chromosome engineering technology to delete the entire dystrophin gene: the *DMD-null* mouse, in which dystrophin expression was absent in all tissues, and the *DMD-floxed* mouse, in which the *DMD* gene is floxed and the brain-type dystrophin (*Dp427c*) gene is specifically inactivated (because the targeting vector introducing the *loxP* site into the 5' end of the dystrophin gene replaced the brain-type promoter and exon 1 of *Dp427c*) (53). *DMD-null* mice were viable but exhibited male sterility and the muscle showed severe degeneration and regeneration of myofibers, which are typical symptoms of DMD. *DMD-floxed* mice were viable and phenotypically normal, despite the absence of brain (cortical)-type dystrophin (*Dp427c*) expression. Using this mouse, *DMD* gene deletion could be induced in a temporally and spatially controlled

manner by regulated expression of Cre and together with transgenic experiments could help to clarify the function of the individual dystrophin isoforms.

Luca region (3p21.3) deleted in lung cancers—Chromosomal deletions appear to be the earliest and most frequent somatic genetic alteration during carcinogenesis and are often the location of TSGs. Chromosomal deletions can inactivate a TSG either by revealing a gene mutation through loss of heterozygosity (as proposed in the two-hit theory) (49), by inducing haploinsufficiency (through quantitative hemizygous deletion and associated loss of expression), or by homozygous deletion. Lung cancer is the most common cause of cancer death in the world (1.18 million deaths annually) (81) and deletions are frequently seen on the short arm of chromosome 3 (3p) (125). Allele loss at 3p21.3 is an early, preneoplastic event in the development of breast and lung cancers (67, 125), with further genetic damage required to drive these tumors to malignancy (18). A 120-kb minimal region of loss at 3p21.3 (containing 8 genes) was defined by overlapping homozygous deletions in lung and breast cancer cell lines (56, 102) and is believed to harbor a TSG(s).

Using chromosome engineering (permissible due to the synteny in gene content and order between human 3p21.3 and a region on mouse chromosome 9), a deletion of approximately 370 kb was created in the mouse germline corresponding to the deleted region at 3p21.3 (109). The deletion was homozygous lethal; however, the heterozygotes developed normally despite being haploinsufficient for 12 genes, including the candidate TSG, *Rassf1*. Although disappointing, this might reflect the fact that conversion to homozygosity is relatively infrequent or may even be a cell-lethal event. More recently, a candidate gene approach showed that *Rassf1* is one of the important TSGs in this region, as mice null for the *Rassf1A* isoform of *Rassf1* showed a decreased lifespan and increased incidence of tumorigenesis compared with wild-type mice (118).

Duplications

Down syndrome—Trisomy 21 or Down syndrome (DS) is the most frequently observed aneuploidy among live born infants and produces a variety of developmental anomalies including facial dysmorphism, congenital heart and gut defects, infertility, varying degrees of mental retardation, immunodeficiencies, and increased incidence of leukemia (34). There are two main views of the mechanistic basis of DS. The DS critical region (DSCR) concept proposes that a small number of dosage-sensitive genes have large effects on phenotypes when present in three copies. The developmental instability hypothesis takes into consideration that non-specific, small effects of many genes perturb genetic homeostasis. Although substantial progress toward understanding the molecular and cellular aspects of DS has come through analyzing individuals with segmental trisomy 21 [arising from translocations or duplications resulting in triplication of a subset of the chromosome (51, 68)], it is mouse models of DS that have led the way in providing a real understanding.

Comparative mapping between mice and humans has revealed that human chromosome 21 shares a large region of synteny with mouse chromosome 16 (90). The first trisomy 16 mice (TS16; generated by the mating of normal diploid females with males carrying a Robertsonian translocation) had an extra copy of mouse chromosome 16, containing more than 80% of the genes found on human chromosome 21 (33). However, these mice were embryonic lethal, thus precluding studies on the development of the nervous system and the aging process. In addition, mouse chromosome 16 contains some genes not found on human chromosome 21 (hence TS16 is not trisomic for all human chromosome 21 genes, but is trisomic for other genes not implicated in DS) (Figure 7).

The first segmentally trisomic DS mouse model (Ts65Dn; generated by a radiation-induced reciprocal translocation) (25) contained an extra copy of the region of mouse chromosome

16 conserved in human chromosome 21, including the DSCR, as shown in Figure 7. Ts65Dn mice survive to adulthood and express some characteristics of DS such as developmental delay, hyperactivity, weight problems, craniofacial dysmorphology, impaired learning, and behavioral abnormalities (24, 89, 92). Ts1Cje mice (containing a translocation between chromosome 16 and the very distal region of chromosome 12, serendipitously generated during targeting of the *Sod1* gene) show less severe learning deficits than Ts65Dn mice and no degeneration of basal forebrain cholinergic neurons (97). Ms1Ts65 mice, segmentally trisomic for the *App-Sod1* region (generated by breeding Ts65Dn females to balanced Ts1Cje males) (Figure 7), show that this region had an even smaller contribution to the DS phenotype than that of the *Sod1-Mxl* region (Ts1Cje mice) (79, 98).

More recently, Reeves and colleagues used chromosome engineering to create mice that were trisomic or monosomic for only the mouse chromosome segment orthologous to the DSCR (Ts1Rhr mice) (see Figure 7) and found that these genes were not sufficient to recapitulate the facial phenotype (characteristic dysmorphologies of the craniofacial skeleton seen in DS individuals), a result that could not have been predicted from or observed in human studies (80). This finding has huge implications for understanding the mechanism behind DS as it points to interactions among noncontiguous genes and contradicts the DSCR concept. Reeves and colleagues proposed that “a triplicated gene, the solitary effect of which is inconspicuous, could contribute to a trisomic phenotype in combination with other genes based on the specificity of effects and interactions of these genes” (80).

However, as human chromosomes have orthologs on more than one mouse chromosome, mouse lines aneuploid for individual mouse chromosomes only partially represent the human situation. Thus, in an attempt to construct a mouse model for human trisomy 21, Fisher and colleagues (77) used irradiation microcell-mediated chromosome transfer (XMMCT) to generate a *trans*-species aneuploid mouse line (*Tc1*) that stably transmits a freely segregating, almost complete human chromosome 21. Indeed, *Tc1* mice show phenotypic alterations seen in humans with DS and in other DS mouse models, including changes in behavior, synaptic plasticity, cerebellar neuronal number, heart development, and mandible size. One concern with this model is the instability of the extra chromosome: Different levels of mosaicism of human chromosome 21 were observed in different tissues, confounding phenotypic analysis.

Translocations

The feasibility of generating chromosome translocations in ES cells was first demonstrated by Smith and colleagues (108), reproduced a chromosomal translocation commonly found in mouse plasmocytomas involving the *c-myc* locus on chromosome and the immunoglobulin heavy-chain (*IgH*) locus on chromosome 12. The same Grosveld and colleagues (119) also generated a translocation between the *Dek* on chromosome 13 and the *Can* gene chromosome 2 to mimic human chromosomal translocation t(6;9), which is associated with a specific subtype of acute myeloid leukemia (121). Chromosomal translocations in mice have now been engineered model several translocations found in human leukemias.

Reciprocal translocations involving the MLL gene—The multiple mixed lineage leukemia (*MLL*) gene from human chromosome 11q23 is involved in more than 30 different chromosomal translocations, resulting in an array of different MLL fusion genes associated with acute leukemias (reviewed in 23). The main fusion partners are found in tumors of either lymphoid lineage (*AF4*), myeloid lineage (*AF9*, *ELL*), or both lineages (*ENL*). Thus, it is not known if MLL fusions are oncogenic in any hematopoietic cell type (progenitor or committed; “noninstructive model”) and/or whether they are capable of driving

differentiation down a specific hematopoietic lineage (“instructive model”). It is only through the use of mouse models that these issues have begun to be addressed.

The first attempt to model the reciprocal translocation between chromosomes 9 and 11 [t(9;11)] involved producing an MLL-AF9 fusion gene using a “knock-in” approach whereby human *AF9* sequences were fused into the mouse *Mll* gene so that expression of the *Mll-Af9* fusion gene occurred from endogenous *Mll* transcription control elements (Figure 8) (21). Chimeric and germline mice carrying the fusion gene developed acute myeloid leukemia (21, 29).

However, the most ideal model would recapitulate the de novo generation of the chromosomal translocation somatically. This has been achieved through the use of chromosomal engineering. Rabbitts and coworkers have targeted *loxP* sites into the introns of mouse *Mll* and *Af9* genes (on chromosomes 9 and 4, respectively), corresponding to the breakpoint regions of human leukemias. Mice carrying these alleles were then crossed with mice expressing Cre (under the control of a ubiquitous promoter), and the resulting *Mll-Af9* fusion gene was detected (20). However, tumors were not reported in these mice, possibly due to the restricted expression characteristics of the Cre in these first-generation models.

A second translocator model (in which de novo chromosomal translocations are created in mice) was generated using the original ES cells carrying the *Mll-loxP* allele (20), but the second *loxP* site was targeted into the *Enl* gene intron, such that recombination between the *loxP* sites created a mouse fusion gene equivalent to the *MLL-ENL* fusion found in human leukemias with t(11;19) (Figure 8) (36). These *Mll-loxP;Enl-loxP* mice were bred to mice in which Cre was expressed under control of the hematopoietic *Lmo2* gene, which allows the translocations to be generated in multipotent stem cells. Reciprocal chromosomal translocations were generated and the mice developed myeloid tumors with rapid onset and high penetrance (36), as did *Mll-loxP;Af9-loxP* mice (32). When the *Mll-loxP;Enl-loxP* mice were crossed to mice specifically expressing Cre in T cells and their progenitors (*Lck-Cre* transgene), it was found that *Mll-Enl* fusions can be oncogenic in the lymphoid lineage (as also seen in human leukemias) (Figure 8) (32). Interestingly, when *Mll-Af9* translocations were induced in a similar T-cell population, no leukemias arose, even though *Mll-Af9* translocations in uncommitted progenitors induced myeloid leukemias (32).

Conditional translocation models have also been generated using invertor mice, in which a *loxP*-flanked cDNA sequence within an intron segment is knocked into the intron of a target gene in the opposite transcriptional orientation, and RNA splicing between the inserted cassette and targeted gene does not occur until the cassette is inverted by Cre expression (37). *MLL-AF4* invertor mice develop lymphoid leukemias when crossed with *Rag-Cre*, *Lck-Cre*, or *CD19-Cre* mice (T.H. Rabbitts, unpublished observations).

Thus, these mouse models allow a direct recapitulation of human cancer-associated translocations and formally show that these translocations result in cancer. More importantly, they have provided a mechanistic insight into the biological implications of these translocations, showing that hematopoietic stem cells are targets for Mll fusions, although Mll fusions are not necessarily oncogenic in every hematopoietic cell. Furthermore, the Mll-associated leukemic precursor cell expressing the *Mll-Enl* fusion can undergo lineage reassignment from the lymphoid lineage into myeloid lineage.

Reciprocal translocation t(8;21)—The translocation between chromosomes 8 and 21, t(8;21)(q22;q22), is the most frequent abnormality seen in approximately 46% of patients with acute myeloid leukemia (M2 subtype). The breakpoints in this translocation involve the acute myeloid leukemia-1 (*AML1*) gene on chromosome 21 and *ETO* (eight twenty one)

gene on chromosome 8, and the translocation results in a fusion gene that contains the 5' region of *AML1* fused to almost all of *ETO* (76).

The first mouse model of this translocation was developed using a knock-in approach whereby gene targeting was used to insert an *Aml-Eto* fusion gene into the *Aml* locus. However, the Aml1-Eto fusion protein caused embryonic lethality due to an absence of normal hematopoiesis (78, 128), thus precluding analysis of the effects of the fusion gene in leukemogenesis. To circumvent this problem, a conditional *Aml1-Eto* knock-in allele was generated, containing a *loxP*-flanked transcriptional stop cassette upstream of the *Aml1-Eto* fusion site. To activate the conditional allele in vivo, these mice were bred with mice expressing *Cre* (under the control of an inducible promoter) (43). However, these mice only developed leukemia after exposure to a mutagenic agent (the DNA alkylating agent, N-ethyl-N-nitrosourea), showing that the Aml1-Eto fusion protein alone was insufficient to induce leukemia.

This highlights that expression of the fusion protein did not directly recapitulate the human cancer-associated chromosomal translocation. To create a more relevant model, *loxP* sites were targeted into introns of the murine *Aml1* and *Eto* genes on mouse chromosomes 16 and 4, respectively. The translocation was generated in vivo by breeding with mice expressing Cre under transcriptional control of the *Nestin* gene. [Nestin-Cre mice do not express Cre early in embryogenesis, thus avoiding the issue of embryonic lethality due to the presence of the Aml1-Eto fusion protein (13).] However, no tumors were reported in these mice, possibly because of the restricted expression characteristics of the Cre transgene used in this study.

Inversions

Chromosomal inversions are useful genetic tools for recovering and maintaining mutations in model organisms such as *Drosophila* and mice, due to the suppression of recombination that occurs in the inverted region (as recombination products between an inverted and a wild-type chromosome do not produce viable gametes) (reviewed in 41). Thus, a dominant marker carried by the inversion can be used to maintain entire unrecombined blocks of chromosome from one generation to the next. This property makes visibly marked inversions useful for recovering and/or maintaining mutations that are not easily recovered or maintained as homozygotes. Chromosomes carrying an inverted segment that is phenotypically marked are known as balancer chromosomes.

Balancer chromosomes—Traditionally, most inversions in the mouse have been generated after chemical mutagenesis or radiation treatment (94, 95). However, these inversions cover only a small fraction of the mouse genome, and most do not carry a phenotypic marker and/or are too large to effectively suppress recombination. Moreover, the few that are available are on a variety of different genetic backgrounds. The development of chromosome engineering, which allowed the generation of designer inversions anywhere in the mouse genome on a defined genetic background, provided a huge step forward for mouse genetics (71). The first specifically engineered mouse balancer chromosome was a 24 cM inversion between *Trp53* and *Wnt3* on mouse chromosome 11 that is recessive lethal and dominantly marked with a K14-Agouti transgene (132). The inversion functions as a balancer chromosome because it can be used to maintain a lethal mutation in the inversion interval as a self-sustaining *trans*-heterozygous stock. Since this first report, balancer chromosomes have been generated for segments on mouse chromosomes 15 (17), 11 (48, 126a), and 4 (75). Balancer chromosome strains provide key advances for mutagenesis screens, for stock maintenance, and for tracking quantitative traits.

CONCLUSION

Chromosomal rearrangements are recognized as frequent occurrences in human disease and can result in a wide range of phenotypes. Recent advances in technology have begun to reveal an extraordinary repertoire of copy number changes previously undetected by cytogenetic techniques. Undoubtedly, many of these will be disease associated, others will be phenotypically neutral, and others will be disease protective. To make genotype-phenotype correlations and to gain mechanistic insight into the gene(s) involved, many of these copy number changes will need to be modeled in mice. The mouse is an excellent model organism for this purpose, not only because of its biological and genetic similarity to humans, but also because of the ease with which its genome can be manipulated. Although chromosome engineering involves several manipulative steps, many laboratories have successfully used this technology to introduce defined chromosomal rearrangements into the mouse genome, which has not only enabled the generation of accurate mouse models of numerous human diseases but has also contributed to the identification of several causal genes. Careful analysis of these models has allowed elucidation of the molecular and cellular basis of many of these disease phenotypes, which will pave the way for progress in diagnosis and curative or preventative therapies.

SUMMARY POINTS

1. Chromosomal rearrangements (deletions/insertions, duplications, translocations, and inversions) are frequent occurrences in humans and can be disease associated (with a wide range of phenotypes) or phenotypically neutral.
2. The repertoire of human chromosomal rearrangements is constantly expanding as new technologies with increased sensitivity allow the discovery of previously undetected rearrangements.
3. The mouse is an excellent system for modeling chromosomal rearrangements because of its biological and genetic similarity to humans and the ease with which its genome can be manipulated.
4. Chromosome engineering allows the introduction of defined chromosomal rearrangements into the mouse genome.
5. This technology has led to the development of accurate mouse models of a number of human diseases, contributed to the identification of several causal genes, and allowed an understanding of the molecular and cellular basis of these disease phenotypes.

Glossary

SMS	Smith-Magenis syndrome
DS	Down syndrome
Homologous recombination	the union between two homologous lengths of DNA
BAC	bacterial artificial chromosome
CGH	comparative genomic hybridization
CNP	copy number polymorphism

Chromosome engineering	a Cre- <i>loxP</i> -based strategy to introduce defined chromosomal rearrangements into the mouse genome
PWS	Prader-Willi syndrome
TSG	tumor suppressor gene
CML	chronic myelogenous leukemia
Synten	the condition of two or more genes located in the same approximate spacing, order, and orientation on the same chromosome
Gene targeting	the insertion of DNA into specific sites within the genome of ES cells
ES cell	embryonic stem cell
Cre/<i>loxP</i> system	the reaction catalyzed by the P1 bacteriophage recombinase, Cre, that leads to site-specific recombination between two <i>loxP</i> sites
TAMERE	targeted meiotic recombination
AS	Angelman syndrome
IC	imprinting center
DMD	Duchenne muscular dystrophy
DSCR	Down syndrome critical region
XMMCT	irradiation microcell-mediated chromosome transfer

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Figure 1.
Examples of common chromosomal rearrangements found in humans.

Figure 2.

Recombinase reactions catalyzed by Cre. Box: The target site recognized by the P1 bacteriophage recombinase, Cre, is called *loxP* [locus of cross over (*x*) in *P1*]. The red arrow in the core region of the *loxP* site indicates the direction (orientation) of the *loxP* site. (a) A *cis* recombination event between two *loxP* sites (*black triangles*) in the same orientation will lead to deletion (Df) or duplication (Dp) of the flanked DNA sequence. (b) If the *loxP* sites are orientated in opposite directions, the *loxP*-flanking sequence will be inverted. (c) Recombination between two *loxP* sites in *trans* will lead to the reciprocal exchange (translocation) of the regions that flank the *loxP* sites.

Figure 3.

Gene targeting in embryonic stem (ES) cells to enable chromosomal engineering. Insertional targeting vectors, as shown, can be used to insert *loxP* sites (*black triangles*), positive selectable markers (such as the neomycin resistance cassette, *N*, and the puromycin resistance cassette, *P*), and *Hprt* gene fragments (*5'* and *3'*) into the desired endpoints (loci) of ES cells. Expression of the neomycin and puromycin resistance genes allows different targeting events to be selected. The complementary, but nonfunctional, *5' Hprt* and *3' Hprt* fragments are derived from the *Hprt* minigene and recombination between the two *loxP* sites will result in union of the two *Hprt* fragments to generate a functional *Hprt* minigene, which confers resistance in *Hprt*-deficient ES cells to the drug HAT (hypoxanthine, aminopterin, and thymidine), thereby enabling in vitro selection of ES cells carrying the desired chromosomal rearrangement (87).

Figure 4.

A general strategy for chromosomal engineering in mice. (a) A *LoxP* site is inserted into the first endpoint using gene targeting in embryonic stem (ES) cells (involving recombination between the exogenous targeting vector and the endogenous homologous genomic locus). The targeting vector carries a positive selectable marker gene. (b) A second *LoxP* site, linked to a different positive selectable marker gene, is targeted to the second endpoint (either on the same or a different chromosome). This can be achieved either by gene targeting or random insertion (see Figure 5). (c) Double-targeted ES cell clones, identified by molecular techniques such as Southern blotting, are then exposed to Cre, which catalyzes the recombination between the *LoxP* sites. (d) ES cell clones carrying the desired chromosomal rearrangements are identified using molecular techniques such as Southern blotting, fluorescence in situ hybridization (FISH), and/or comparative genomic hybridization (CGH). (e) These ES cell clones are subsequently injected into mouse blastocysts and the embryos transferred into the uteri of pseudopregnant foster mothers. (f) Chimeras that are generated from the blastocyst injection are then mated with wild-type mice to establish germline transmission of the modified allele. (g) The progeny from these matings are molecularly characterized and a mutant mouse line carrying the engineered chromosome(s) is established.

Figure 5.

Nested chromosomal deletions induced with a retroviral vector. The first deletion endpoint is fixed by targeting the *5' Hprt* cassette and a *loxP* site (black arrow) to a predetermined locus. The *3' Hprt* cassette and a second *loxP* site are then randomly integrated into the genome of the embryonic stem (ES) cell using a recombinant retroviral vector. Cre catalyzes recombination between the *loxP* sites and ES cells carrying the deleted chromosomes can be identified (112).

Figure 6.

Diagram of the mouse chromosome 16 deletion mutants made by different groups. (a) *Df1/Dp1* (60), (b) *Lgdel* (69), (c) (47), and (d) (85). The red bar indicates the gene content of the transgene that rescues cardiovascular defects in *Df1/+* and *Lgdel/+* mutants. *Arvcf*, armadillo repeat gene deleted in velo-cardio-facial syndrome; *Comt*, catechol-*O*-methyltransferase; *Dgcr2*, DiGeorge syndrome gene c; *Es2el*, expressed sequence 2 embryonic lethal; *Gsc1*, goosecoid-like; *Hira*, histone cell-cycle-regulation defective homolog A; *Pnutl1*, peanut-like 1; *Ranbp1*, Ran-binding protein 1; *Scl25a1*, solute carrier family 25 member 1; *Tbx1*, T-box 1; *Ufd1l*, ubiquitin fusion degradation 1-like; *Znf 74*, zinc-finger protein 74.

Figure 7.

Triplicated regions in trisomy 16 (TS16) mice. (a) TS16 (33), (b) Ts65Dn (24, 89), (c) Ts1Cje (97), (d) Ms1Ts65 (79, 98), and (e) Ts1Rhr (80). In TS16 mice, the whole chromosome 16 is triplicated and the syntenic regions to human chromosomes are indicated. For Ts65Dn, Ts1Cje, Ms1Ts65, and Ts1Rhr mice, the segmental triplicated region is shown and the genes involved are as indicated (the triplicated region in Ts1Cje spans from *Sod1* to *Mx1*; however, Ts1Cje is not functionally triplicated for *Sod1* because the *Sod1* gene in the translocated segment has been inactivated by the targeting event). *App*, amyloid β (A4) precursor protein; *Cbr1*, carbonyl reductase 1; *Grik1*, glutamate receptor, ionotropic, kainate1; *Sod1*, superoxide dismutase 1; *Gart*, phosphoribosylglycinamide formyltransferase; *Dryk1*, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a; *Mx1*, myxovirus (influenza virus) resistance-1; *Mx2*, myxovirus (influenza virus) resistance-2.

Figure 8. Mouse models of the reciprocal translocations involving the *MLL*. (a) Example of a knock-in approach: To generate *Mll-Af9* fusion gene mice, a targeting vector consisting of the 3' terminal end of human *AF9* gene (yellow) fused within exon 8 of mouse *Mll* gene [at the position of fusions found after t(9;11)(p22;q23) is generated]. This targeting vector is introduced into the mouse genome using gene targeting in embryonic stem (ES) cells and the resulting allele transmitted through the germline. Using this strategy, the fusion protein is expressed at all developmental stages and in all tissues, and chimeric mice carrying the fusion protein develop acute myeloid leukemia. (b) Example of a translocator approach: To generate de novo chromosomal translocations in mice, *loxP* sites are introduced into the relevant genes using gene targeting in ES cells, and chimeric mice carrying the modified alleles are derived. These mice are bred to obtain germline transmission and subsequently interbred with each other before being bred with Cre deleter mice (mice expressing Cre under the control of a specific promoter). Therefore, recombination between the *loxP* sites and subsequent generation of the fusion gene only occurs in tissues/cell lineages where Cre is expressed (as detailed in the table).