Am. J. Hum. Genet. 50:725–741, 1992

Common Sequence Motifs at the Rearrangement Sites of a Constitutional X/Autosome Translocation and Associated Deletion

Joseph P. Giacalone* and Uta Francke*,†

*Department of Genetics and †Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford

Summary

Reciprocal chromosome translocations are common de novo rearrangements that occur randomly throughout the human genome. To learn about causative mechanisms, we have cloned and sequenced the breakpoints of a cytologically balanced constitutional reciprocal translocation, t(X;4)(p21.2;q31.22), present in a girl with Duchenne muscular dystrophy (DMD). Physical mapping of the derivative chromosomes, after their separation in somatic cell hybrids, reveals that the translocation disrupts the DMD gene in Xp21 within the 18-kb intron 16. Restriction mapping and sequencing of clones that span both translocation breakpoints as well as the corresponding normal regions indicate the loss of approximately 5 kb in the formation of the derivative X chromosome, with 4–6 bp deleted from chromosome 4. RFLP and Southern analyses indicate that the de novo translocation is of paternal origin and that the father's X chromosome contains the DNA that is deleted in the derivative X. Most likely, deletion and translocation arose simultaneously from a complex rearrangement event that involves three chromosomal breakpoints. Short regions of sequence homology were present at the three sites. A 5-bp sequence, GGAAT, found exactly at the translocation breakpoints on both normal chromosomes X and 4, has been preserved only on the der(4) chromosome. It is likely that the X-derived sequence GGAATCA has been lost in the formation of the der(X) chromosome, as it matches an inverted GAATCA sequence present on the opposite strand exactly at the other end of the deleted 5-kb fragment. These findings suggest a possible mechanism which may have juxtaposed the three sites and mediated sequence-specific breakage and recombination between nonhomologous chromosomes in male meiosis.

Introduction

Fitness, survival, and selection connote competition among members of species for available resources. In man, a more stringent selection pressure operates prenatally, one in which abnormal embryos and fetuses are lost through spontaneous abortion and miscarriage. It has been estimated that more than 20% of all human conceptuses contain chromosome rearrangements (Chandley 1981). More than 60% of abortuses less than 7 wk of age have chromosome abnormalities, with the frequency decreasing with increasing fetal age at the time of death (Boué et al. 1985). Reciprocal translocations, in which there is no cytologically apparent loss of chromosome material, are the most common de novo structural chromosome rearrangements, with frequency estimates of 2.6/10,000–14/10,000 live births (Warburton 1984; Nielsen and Wohlert 1991). Such translocations, termed "constitutional" when present in all of an individual's cells, are presumed to arise during the meiotic or mitotic divisions of gametogenesis in the contributing parent. Constitutional translocations may be distinguished from acquired, somatic translocations, present in a subset of an individual's cells, such as occur in lymphoid neoplasias where cellular oncogenes are activated via their juxtaposition with immunoglobulin or

Received August 19, 1991; revision received October 31, 1991.
Address for correspondence and reprints: Uta Francke, M.D., Howard Hughes Medical Institute, Beckman Center, Stanford University Medical Center, Stanford, CA 94305–5428.

0002-9297/92/5004-0008$02.00

T-cell receptor genes. These translocations occur in cell types where DNA rearrangements are part of normal cellular differentiation programs (reviewed in Haluska et al. 1987). In contrast, constitutional translocations appear to occur randomly throughout the genome when they are ascertained through fetal wastage rather than through live-born unbalanced offspring, where selection for viability introduces a bias (Boué et al. 1985).

Carriers of inherited balanced translocations are phenotypically normal except for the rare instances of translocation breakpoints within genes or their controlling regions. Most translocation carriers are detected because of fertility problems or abnormal offspring resulting from unbalanced segregation of the translocation chromosomes during meiosis. De novo translocations, not present in the parents, are more often associated with mental retardation and physical abnormalities (Jacobs 1974; Funderburk et al. 1977). Therefore, detection of de novo translocations in utero poses difficult counseling problems. An apparently balanced translocation under the microscope may in fact represent a deletion of many kilobases of DNA, undetectable because of the low resolution of cytogenetic analysis yet large enough to produce a severe phenotype. Until recently, it has been virtually impossible to analyze constitutional translocation breakpoints at the DNA sequence level, because of the low likelihood of a translocation occurring close enough to previously cloned DNA sequences to allow cloning of the breakpoints themselves. Girls affected with X-linked progressive muscular dystrophy and a constitutional translocation with a breakpoint in the dystrophin gene have provided the first opportunity for such studies. Cloning and sequencing of one such translocation breakpoint has been reported elsewhere (Bodrug et al. 1987). The characterization of various constitutional translocation breakpoints may help to elucidate the mechanisms by which such translocations occur.

Duchenne muscular dystrophy (DMD) is a degenerative neuromuscular X-linked recessive disorder with an incidence of approximately 1/3,500 live male births. Clinical features include delay in motor development, elevated serum creatine kinase levels, and progressive muscle weakness, resulting in death early in the third decade of life (reviewed in Moser 1984). DMD and the less severe allelic form, Becker muscular dystrophy (BMD), are caused by mutations in the dystrophin gene located in band Xp21 (e.g., see Darras et al. 1988; Koenig et al. 1989). Of all patients with DMD and BMD, more than 60% have deletions of portions of the dystrophin gene, and some have duplications. The deletions are not randomly dispersed throughout the gene but have breakpoints clustered in one of two large introns (Den Dunnen et al. 1989; Blonden et al. 1991).

The localization of the DMD gene to the X chromosome and the fact that affected males do not reproduce imply that females should not be affected with the disease. However, heterozygotes may have minor symptoms of DMD but are severely affected only when the X chromosome that carries the normal allele is preferentially inactivated. Nonrandom X inactivation leading to "manifesting carriers" has been associated with MZ twinning (Richards et al. 1990), can occur for no obvious reason (Francke et al. 1989), and may be due to constitutional X/autosome translocations involving band Xp21. Such translocations have been identified in more than 20 females diagnosed with DMD or BMD phenotypes (reviewed in Boyd et al. 1986). Karyotypic studies of their lymphocytes and fibroblasts indicate that the structurally normal X chromosome is almost exclusively the inactive, late-replicating X. This finding is thought to result from selection against cells with gross genetic imbalance in which the derivative X chromosome is inactivated and the other part of the X, translocated to the autosome and unlinked to the X-inactivation center at Xq13, is incapable of being inactivated. In addition, inactivation of the der(X) chromosome may spread into the transposed autosomal segment and result in effective monosomy for autosomal genes (Mohandas et al. 1982). The gross imbalance of expressed sequences is most likely a cell-lethal condition in early embryonic life, resulting in selection against such cells and leaving those cells in which the der(X) remains active—and the normal X remains inactive—to form the individual. Thus, if the translocation disrupts an X chromosomal gene, e.g., the dystrophin gene, and if the intact copy of the gene on the normal X is inactivated, the female translocation carrier will express the disorder.

We have studied a girl diagnosed with DMD and found to have a constitutional reciprocal translocation involving chromosome bands Xp21 and 4q31. We have mapped, cloned, and sequenced the translocation breakpoints and have found a deletion of approximately 5 kb of X chromosome material, composed mainly of L1 repetitive sequences, from the der(X) chromosome. It is intriguing that we found small regions of sequence homology at the two ends of the deleted DNA, as well as at the sites of breakage on

both chromosomes X and 4. We relate these results to previous analyses of translocations and deletions and to models for the mechanisms by which chromosome rearrangements may arise.

Subject, Material, and Methods

Case Report

US 090479 was born at term after uncomplicated pregnancy and delivery. She weighed 3,250 g and was 50 cm in length. At 12 mo of age she walked with assistance, and at 16 mo she walked independently. At 3 years 8 mo she was evaluated for increasing weakness and was found to have bilateral hypertrophy of the calves, hyperlordosis of the lumbar spine, clumsy gait, and a positive Gower maneuver. Serum levels of creating kinase (CK) were 7,035 and 8,190 U/liter on two occasions, and aldolase was 250U/liter, all greatly elevated. A clinical diagnosis of DMD was made. Muscle histology and clinical progression over the years were consistent with this diagnosis. Chromosome analysis of lymphocytes and fibroblasts revealed a balanced reciprocal translocation: 46,X,t(X;4)(p21; q31). The parents are not consanguineous. Maternal and paternal ages were 28 and 31 years, respectively, at birth of the affected daughter. Both parents and two sisters are unaffected and have normal karyotypes. CK levels were repeatedly normal in the mother and older sister. Therefore, both the translocation and the DMD phenotype have arisen de novo in the patient. Since the translocation breakpoint in Xp21 falls into the region of the DMD gene, the hypothesis was made that the translocation caused the DMD phenotype by disrupting the gene (clinical data provided by Professor W. Rosenkranz [Graz, Austria]).

Somatic Cell Hybrids

To create hybrid series 31, a lymphoblastoid cell line was established by Epstein-Barr virus transformation of lymphocytes from patient US (LCL194) and was fused with Chinese hamster cell line V79/380-6 that is deficient in hypoxanthine phosphoribosyltransferase activity. A total of 5×10^6 cells of each type were mixed in suspension, exposed briefly to a 45% solution of polyethyleneglycol 6000, diluted, and plated into 20 cell culture dishes, as described elsewhere for experiment 28 (de Martinville et al. 1985). After 2–3 wk 11 HAT medium–resistant colonies were isolated from nine independent fusion plates. Karyotyping of the colonies was done after trypsin-Giemsa banding according to a method described elsewhere (Francke et al. 1985). Two of the hybrids had no intact human chromosomes. All others had retained the der(X) chromosome. The inactive normal X was not present in any of the hybrids; the normal chromosome 4 was present in one; and the der (4) was present in two hybrid cell lines. In clones with both translocation chromosomes present, the der(X) bearing the human HPRT gene was eliminated by subcloning in counterselection medium containing 2×10^5 M 8-azaguanine (de Martinville et al. 1985).

DNA Purification and Southern Analysis

Genomic DNA from lymphocytes, lymphoblastoid cell lines (LCLs) or somatic cell hybrid lines were purified according to a method described by Baas et al. (1984). Restriction-enzyme digests were performed according to manufacturers' (New England Biolabs and Boehringer Mannheim) specifications, except for the addition of spermidine (2–4 mM) to most reactions in buffers of $\geq$50 mM NaCl. Digested DNA was separated on agarose gels and was blotted onto nitrocellulose (BA85; Schleicher and Schuell) or nylon (Hybond-N; Amersham) membranes according to a method described by Southern (1975), with minor modifications. Gels were treated with 0.25 N HCl for 15 min prior to denaturation (0.5 N NaOH, 1.5 N NaCl; 20 min), neutralization (0.5 M Tris pH 6.8, 1.5 M NaCl; 20 min), and capillary transfer in 20 × SSC. Filters were prehybridized in 4 × SSC, 5 × Denhardt's, 0.5% SDS and hybridized in 4 × SSC, 5 × Denhardt's, 100 mM sodium phosphate pH 6.8, 1% SDS, 10 μg heat-denatured salmon sperm DNA/ml. Clones pERT 87-1, -8, -15, and -18 were supplied by A. Monaco and L. Kunkel. These probes and others generated in the course of these experiments were labeled by the random oligonucleotide primer technique of Feinberg and Vogelstein (1983). Hybridizations were performed using a minimum of 10^6 cpm/ml hybridization solution. After hybridization, filters were rinsed in 2 × SSC, 1% SDS for 10 min at room temperature and then were washed twice for 10 min at room temperature in 0.2 × SSC, 0.1% SDS preheated to 65°C. Autoradiography was performed at −40°C by using Kodak XAR X-ray film and intensifying screens.

Cosmid Library Construction and Screening

A cosmid library of LCL 194 was made using the vector pCos2EMBL (Poustka et al. 1984) which contains an R6K origin of replication, kanamycin-resistance gene, two adjacent cos sites separated by a

unique *Pvu*II site, and a tetracycline-resistance gene with a unique *Bam*HI site, the cloning site for this library. The vector was first digested with *Pvu*II, phosphatased, then digested with *Bam*HI, resulting in two cosmid "arms." DNA from LCL 194 was subjected to a partial *Mbo*I digest. DNA from time points giving fragments predominantly in the 35–40-kb size range was precipitated and phosphatased using calf intestinal alkaline phosphatase (Boehringer Mannheim). An aliquot of the phosphatased fragments was tested in a ligation reaction and were found not to ligate. Ten micrograms of this DNA and 6.5 μg of pCos2 EMBL prepared as above were ligated overnight at 15°C in a total volume of 250 μl. Twenty-microliter aliquots of this ligation were packaged in vitro by using extracts prepared from strains BHB 2688 (freeze-thaw lysate) and BHB 2690 (sonic extract) (Scalenghe et al. 1981). Packagings were pooled and run over a CsCl step gradient, and fractions were collected and titered. Phage-containing fractions were used to infect DH 1 cells. Approximately 4×10^5 transformants were generated. Primary transformant colonies were collected and stored at −80°C. Screenings were performed on replatings of the frozen stocks. The cosmid library was screened essentially as described in Sambrook et al. (1989), except that we treated colony-containing filters three or four times with a solution of 25% sucrose, 50 mM Tris pH 8.0, 1.5 mg lysozyme/ml for 1–2 min at 4°C, instead of with 10% SDS. After denaturation, neutralization, and baking, filters were hybridized as described for Southern blots.

Cosmid Mapping, Subcloning, and Sequencing

Individual cosmids were mapped using an indirect end-labeling method. Cosmids were linearized with *Sal*I, which resulted in the cosmid insert flanked by two fragments of the vector. Partial restriction digests of these linearized molecules were Southern blotted and hybridized to the vector fragments from one or the other end. The results were combined with results from complete digestions of the cosmids by the same enzymes, to give the final cosmid map. For sequencing, restriction fragments were subcloned into M13mp18 or mp19, and sequencing was performed by the dideoxy chain-termination method of Sanger et al. (1977) by using the Sequenase system (U.S. Biochemicals). Sequencing reactions were run on a BRL S2 or BioRad SequiGen sequencing apparatus, using the Sequagel gel system (National Diagnostics). Both strands were sequenced, except for the breakpoint in cosmid cNX, which was sequenced several times in one direction only.

Results

Cytogenetic Analysis of the t(X;4) Translocation

High-resolution chromosome analysis of peripheral lymphocytes from this individual by using trypsin-Giemsa banding techniques (Francke and Oliver 1978) revealed an apparently balanced translocation with breakpoints at subbands Xp21.2 and 4q31.22. Examples of the derivative chromosomes der(X) and der(4), their normal homologues, and the interpretation of the sites of breakage and rejoining are displayed in figure 1. Lymphocytes were immortalized by Epstein-Barr virus transformation. Chromosome analysis of this lymphoblastoid cell line (LCL 194) revealed the identical translocation and no additional abnormalities.

Construction and Characterization of Somatic Cell Hybrids

To facilitate physical mapping of the translocation breakpoint, the translocation chromosomes were separated from each other and from their normal homologues in somatic cell hybrids. Hybrid series 31 was created by fusing HPRT-deficient Chinese hamster cell line V79/380-6 and LCL 194 and selecting for hybrid cells in HAT medium. Selected series 31 hybrids containing the derivative chromosomes without either of the normal chromosomes X or 4 were expanded and karyotyped prior to DNA extraction. The hybrids whose DNAs were used in this study have the following human chromosome content: hybrid 31-2A—der(X), 1,6,11(partial),12,15,16,18,19,20,21,22; hybrid 31-3B—der(4), der(X), 7,9,13,14,15,18,19; hybrid 31-3B-7b aza—der(4),13,14,18; and hybrid 31-6A—der(X), 3,5,6,10,13,14,16,20,22.

Molecular Mapping of Translocation Breakpoints

The pERT series of clones was isolated by Kunkel et al. (1985) by virtue of being deleted from the DNA of a male who had DMD and several other X-linked disorders and who was found to have a small cytologically detectable deletion of Xp21 (Francke et al. 1985). To map the translocation breakpoint on the X we tested three probes—pERT 87-1, 87-8, and 87-15—that span about 57 kb within the DMD gene. pERT 87-1 is the most centromeric and lies within intron 12 of the dystrophin gene. pERT 87-8 is located 16 kb distal to 87-1 in intron 13, and pERT 87-15 is 38 kb distal to 87-8 within intron 17 (Monaco and Kunkel 1987, and personal communication; Darras et al. 1988). When the three probes were hybridized to a filter with *Eco*RI fragments from series 31 hybrids, probes 87-1 and 87-8 gave similar patterns

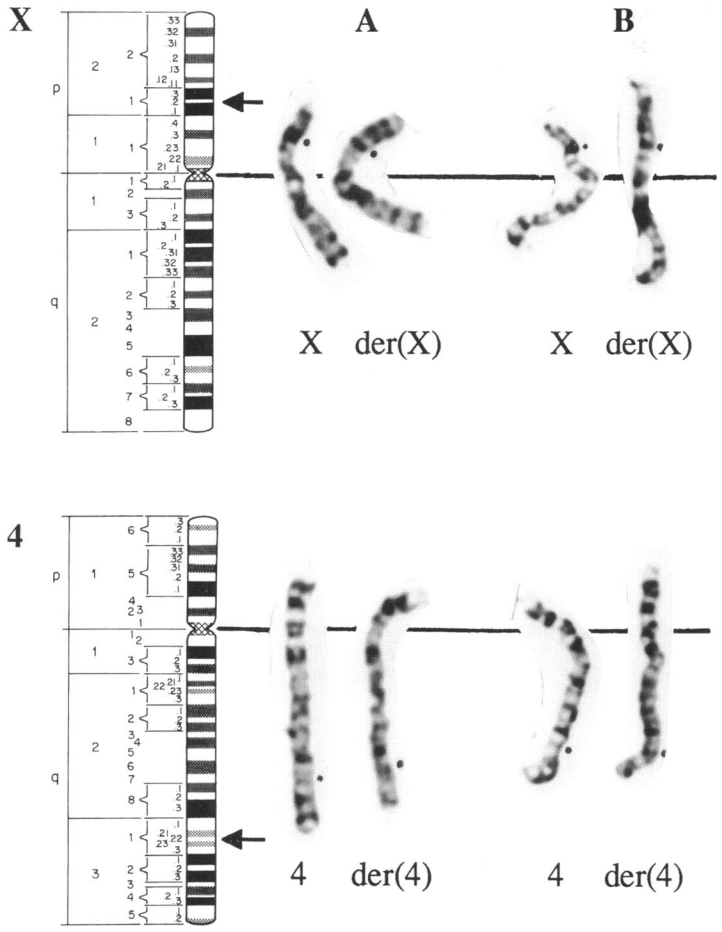
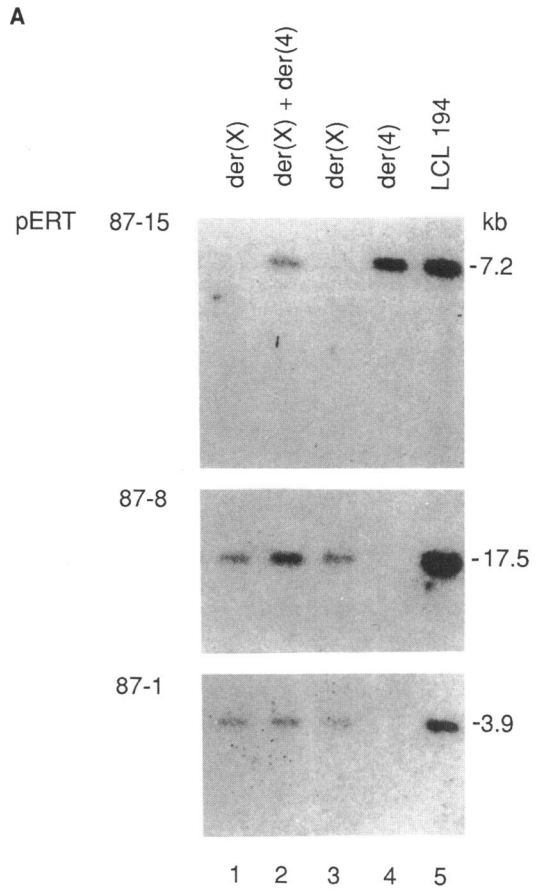


Figure 1 Cytogenetic characterization of t(X;4) translocation in DMD female. High-resolution G-banding ideograms of chromosomes X and 4 are from Francke (1981). Arrows point to the breakpoints in subbands Xp21.2 and 4q31.22. Trypsin-Giemsa-banded translocation derivatives der(X) and der(4) and their normal homologues X and 4 are aligned at the centromeres. Chromosomes under A and B are from two different cells. Dots indicate the sites of breakage and rejoining in the formation of the translocation. On the der(X) chromosomes, the regions between the dots and the ends of the short (upper) arms represent region 4q31.22–4qter. On the der(4) chromosomes, the regions between the dots and the end of the long (lower) arms represent region Xp21.2-Xpter. The reciprocal exchange of chromosome material involves a slightly larger region from chromosome 4 than from chromosome X, so that der(X) is longer than X and der(4) is shorter than 4. However, there is no evidence for any loss of material.

(fig. 2*A*). The sequences homologous to both probes are present in hybrids containing the der(X) chromosome (fig. 2*A*, lanes 1 and 3) but are absent from the hybrid containing the der(4) chromosome (fig. 2*A*, lane 4). This places them proximal to the translocation breakpoint on the X chromosome. Probe 87-15 gave the opposite hybridization pattern. The sequence is absent from the der(X)-containing hybrids and is present in the der(4) hybrid and thus maps distal to the translocation breakpoint. The breakpoint in the 38-kb interval between pERT 87-8 and 87-15 could be further defined in *Pst*I-digested DNA from the series 31 hybrids. Probe 87-8 recognizes a 9.0-kb *Pst*I fragment in controls and in the der(X)-containing hybrids (data not shown), indicating that the breakpoint is ≥9 kb from 87-8. In contrast, pERT 87-15 hybridizes to a 12.5-kb fragment in controls (fig. 2*B*, lane 1) and to two fragments in LCL194 (fig. 2*B*, lane 2). In addition to the 12.5-kb fragment, there is a novel 11.0-kb fragment which is also present in hybrid 31-3B-7b aza (fig. 2*B*, lane 6), indicating that it is derived from the der(4) translocation chromosome. Since no 11.0-kb *Pst*I fragment has been observed in normal controls or in the patient's parents, we conclude that it is not due to



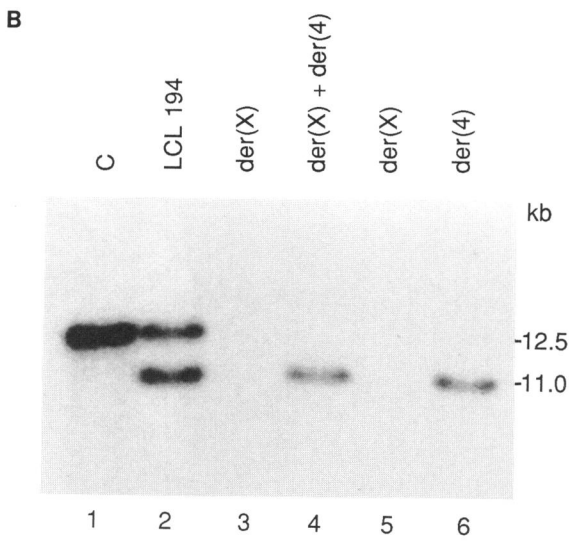


Figure 2 Mapping of t(X;4) translocation breakpoint within pERT87 (DXS164) locus. The derivative chromosomes present in each hybrid cell line are indicated above the lanes. *A*, Southern blots containing *Eco*RI-digested hybrid cell line DNAs hybridized to pERT probes 87-15 (*top*), 87-8 (*middle*), or 87-1 (*bottom*). Lane 1, Hybrid 31-2A. Lane 2, Hybrid 31-3B. Lane 3, Hybrid 31-6A. Lane 4, Hybrid 31-3B-7b aza. Lane 5, LCL 194 from t(X;4) DMD female. The hybridization pattern places 87-1 and 87-8 proximal and 87-15 distal to the translocation breakpoint. *B*, Southern blot containing *Pst*I-digested DNA samples hybridized to pERT 87-15 Lane 1, Normal female control. Lane 2, LCL194. Lane 3, Hybrid 31-2A. Lane 4, Hybrid 31-3B. Lane 5, Hybrid 31-6A. Lane 6, Hybrid 31-3B-7b aza. The identification of a new 11.0-kb fragment originating from the der(4) chromosome indicates that the translocation breakpoint lies within 11 kb of pERT 87-15.

a polymorphism but results from the juxtaposition of a new *Pst*I site in the translocated chromosome 4 material. The translocation breakpoint must lie in this junction fragment and within 11.0 kb of pERT 87-15.

To clone the breakpoints, pERT 87-15 was used to screen a cosmid library constructed from LCL 194. Two cosmids, cD4 and cNX, were isolated and restriction mapped (fig. 3). As cosmid cD4 contained the 11.0-kb *Pst*I fragment hybridizing to pERT 87-15, and as cNX contained the corresponding 12.5-kb *Pst*I fragment, and as it is within these fragments that the restriction maps of the cosmids diverge, we hypothesized that cosmid cD4 represents the der(4) chromosome and that cNX represents the normal X chromosome. The 4.5-kb *Bam*HI-*Pst*I fragment of cD4 within which the maps of cD4 and cNX diverge was subcloned, designated pD4, and restriction mapped (fig. 4). Subfragments of this clone were tested for localization to either the X chromosome or chromosome 4. The 2.1-kb *Hin*dIII-*Bam*HI fragment from one end of pD4 was hybridized to a small panel of hybrid DNA samples designed to distinguish clones mapping to chromosomes 4 or X. The sequence in this fragment is clearly X chromosome specific, as it is present in a hybrid which contains only an X chromosome as its human component (fig. *5A*, lane 5). It is also present on the der(4) chromosome (fig. *5A*, lane 9), which places it, as expected, distal to the translocation breakpoint. The 800-bp *Pst*I-*Pvu*II subfragment from the other end of pD4, however, is not present in hybrids containing an X chromosome (fig. *5b*, lanes 4 and 5) but is present in a hybrid containing a normal 4 but no X (fig. *5B*, lane 3) and in the hybrid containing the der(4) (fig. *5B*, lane 9). Independent hybridization

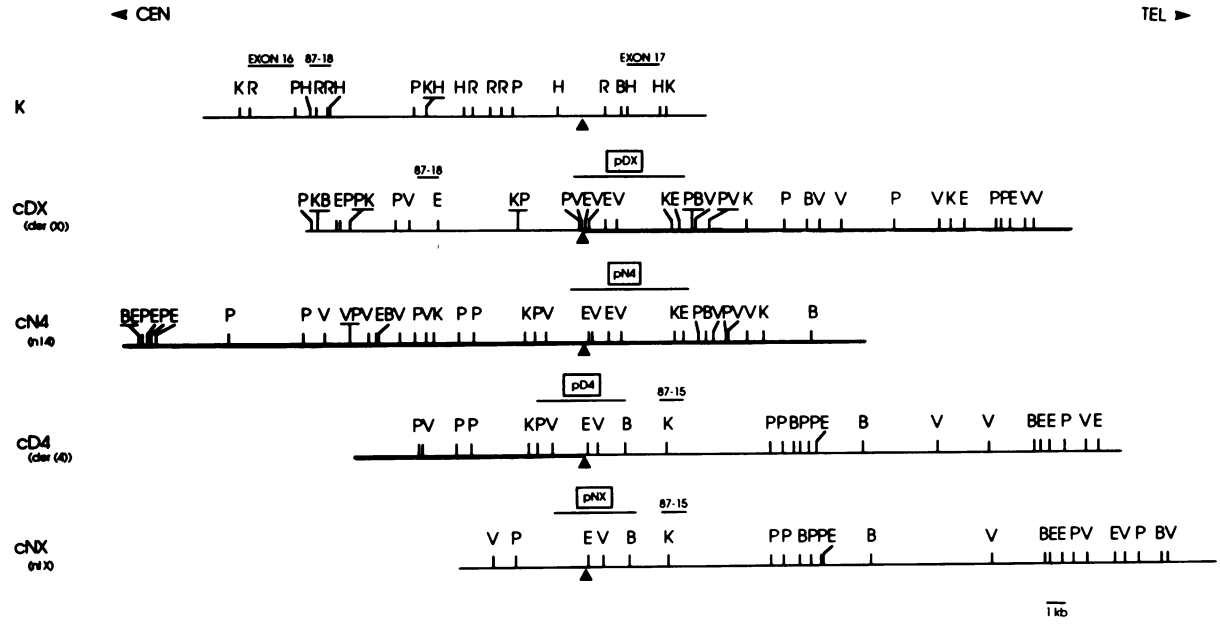


Figure 3 Restriction maps of cosmids spanning t(X;4) translocation breakpoints and of corresponding normal chromosomes 4 and X. Restriction maps of cosmids representing der(X) (cDX), normal 4 (cN4), der (4) (cD4), and normal X (cNX) are aligned. "K" is L. Kunkel and A. Monaco's map of this region of the X chromosome. Maps are arranged to align the translocation breakpoints, marked by arrowheads. The locations of pERT 87-15 and 87-18 and of *Hin*dIII fragments that contain exons 16 and 17 are indicated. For each cosmid, the breakpoint-containing subclones are marked. The centromere is located to the left, and the telomere is to the right. Thicker lines indicate chromosome 4 material, and thinner lines indicate X chromosome material. Restriction sites are as follows: B = *Bam*HI; E = *Eco*RV; H = *Hin*dIII; K = *Kpn*I; P = *Pst*I; R = *Eco*RI; and V = *Pvu*II.

to a general mapping panel also places this clone on chromosome 4 (data not shown). This confirms that cosmid cD4 represents the der(4) chromosome.

Using the chromosome 4–specific *Pst*I-*Pvu*II fragment of pD4 as a probe, we rescreened the LCL 194 cosmid library to isolate a cosmid representing the normal chromosome 4, designated cN4 (fig. 3). Restriction mapping of this cosmid indicated both virtual identity with the chromosome 4–specific region of cosmid cD4 and divergence of restriction sites on the telomeric side of the breakpoint, as expected. The isolation of a der(X) cosmid was complicated by the fact that approximately 12 kb of DNA proximal to the breakpoint consists of a block of L1 repetitive sequences (Monaco and Kunkel 1987). We used the single-copy probe pERT 87-18, which lies approximately 13 kb proximal to the translocation breakpoint, to screen the cosmid library and isolated cosmid cDX that is likely to represent the der(X) chromosome (fig. 3). The restriction sites on the telomere side of the breakpoint are virtually identical to the corresponding sites on the chromosome 4 cosmid cN4. On the X-specific portion, many of the sites around pERT 87-18 match the X chromosome map (K) provided by Kunkel and Monaco. However, in cosmid cDX, the pERT 87-18 sequence lies approximately 5 kb closer to the breakpoint than it does on the normal X, implying that this intervening X-specific DNA may have been deleted from the translocation chromosome. To clarify this, fragments of all four cosmids surrounding the breakpoints were subcloned, restriction mapped, and sequenced.

Subcloning and Sequencing of Translocation Breakpoints

Comparison of the restriction maps of the cosmid subclones (fig. 4) reveals that the chromosome 4 material is not notably deleted, as all the restriction sites from the normal 4 subclone are present on either the der(4) or the der(X) subclones. Also, the X chromosome region distal to the breakpoint is conserved on the der(4) chromosome. However, the restriction maps of the X chromosome region proximal to the breakpoint differ in the normal X subclone and the der(X) subclone. This is to be expected if the X chromosome DNA on the der(X) subclone were derived

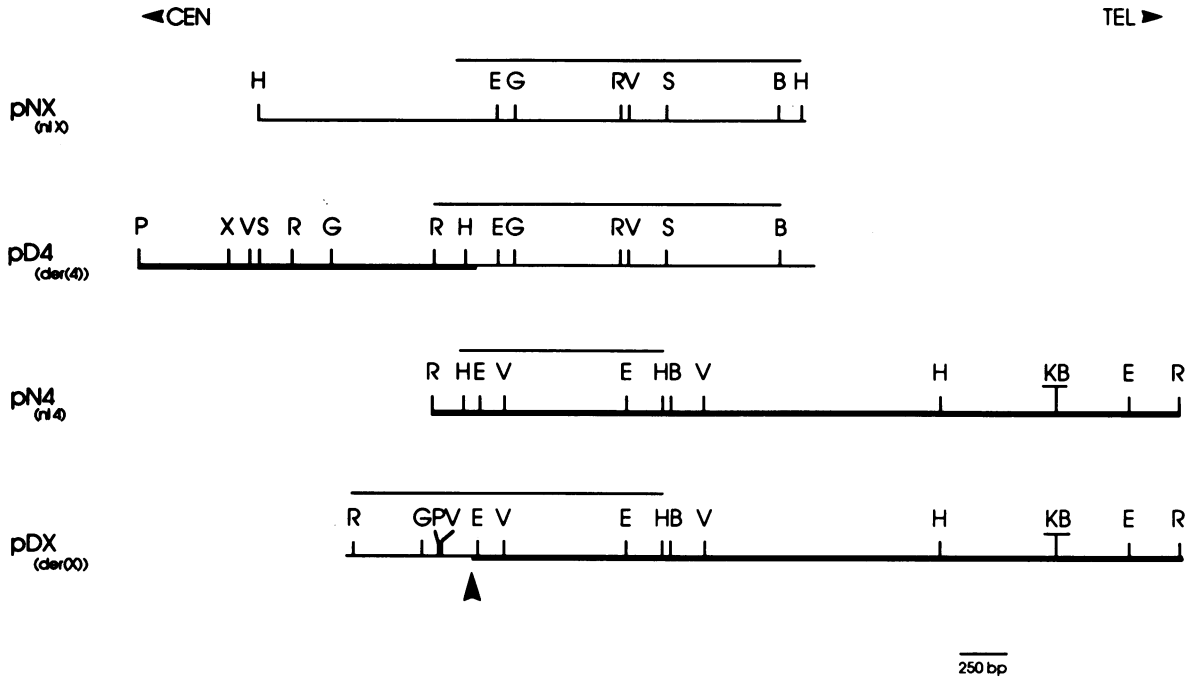


Figure 4 Restriction maps of subclones derived from cosmids containing t(X;4) breakpoint regions. Subclone pNX is derived from the normal X cosmid cNX, pD4 is from the der(4) cosmid cD4, pN4 is from the normal cosmid cN4, and pDX is from the der(X) cosmic cDX. Thicker lines indicate chromosome 4 material, and thinner lines indicate X chromosomal material. The translocation breakpoint is indicated by an arrowhead. Overlining indicates the regions which have been sequenced. Centromere is to the left, and telomere is to the right. Restriction sites are as follows: B = *Bam*HI; E = *Eco*RV; H = *Hin*dIII; G = *Bgl*II; K = *Kpn*I; P = *Pst*I; R = *Eco*RI; and V = *Pvu*II.

from a region 5 kb proximal to the breakpoint, with the intervening DNA having been deleted.

To complete the analysis, it was necessary to isolate the region of the X chromosome within which the proximal end of the deletion lies. A Southern blot containing an *Eco*RI digest of the normal X cosmid cNX was hybridized with the X-specific 400-bp *Eco*RI-*Pst*I fragment of the der(X) subclone pDX (fig. 5). A 2.5-kb *Eco*RI fragment of cosmid cNX was identified. Subcloning, restriction mapping, and sequencing revealed a cluster of *Bgl*II, *Pst*I, and *Pvu*II sites identical to that found in the X-specific region of pDX near the breakpoint (fig. 4). Also, >400 bp of sequence from this clone, from the *Bgl*II site to the breakpoint, are identical to that of the corresponding region of pDX (data not shown), indicating that this clone represents the normal X chromosome DNA within which the proximal deletion breakpoint falls.

The cloning of both translocation breakpoints, the corresponding regions from the normal X and the normal 4, and the proximal end of the deleted DNA enabled us to sequence the rearrangement sites themselves. Sequenced regions are indicated by overlining in figure 4. The sequence surrounding the translocation breakpoints on the derivative chromosomes and the normal homologues are compared in figure 6. It is surprising that the pentamer GGAAT is present in the same orientation on both the normal chromosomes X and 4, exactly at the breakpoint site. One copy of this pentamer has been deleted in the formation of the derivative chromosomes. Although figure 6 suggests that it is the X chromosome copy which has been deleted, the recombination between the chromosomes X and 4 at this site could have occurred anywhere within the 5-bp motif. The reason we favor the idea that the X chromosome pentamer has been deleted is that four of the five bases in the pentameric motif are part of a 6-bp sequence, GAATCA, which occurs again, as an inverted repeat, on the other strand at the distal end of the deleted 5-kb segment (marked by arrows in fig. 6). At least four bases of chromosome 4 DNA—the –GACT– immediately adjacent to the pentamer—have been lost in the formation of the der(X). The next two bases, –TG–, are also present on the X chromosome

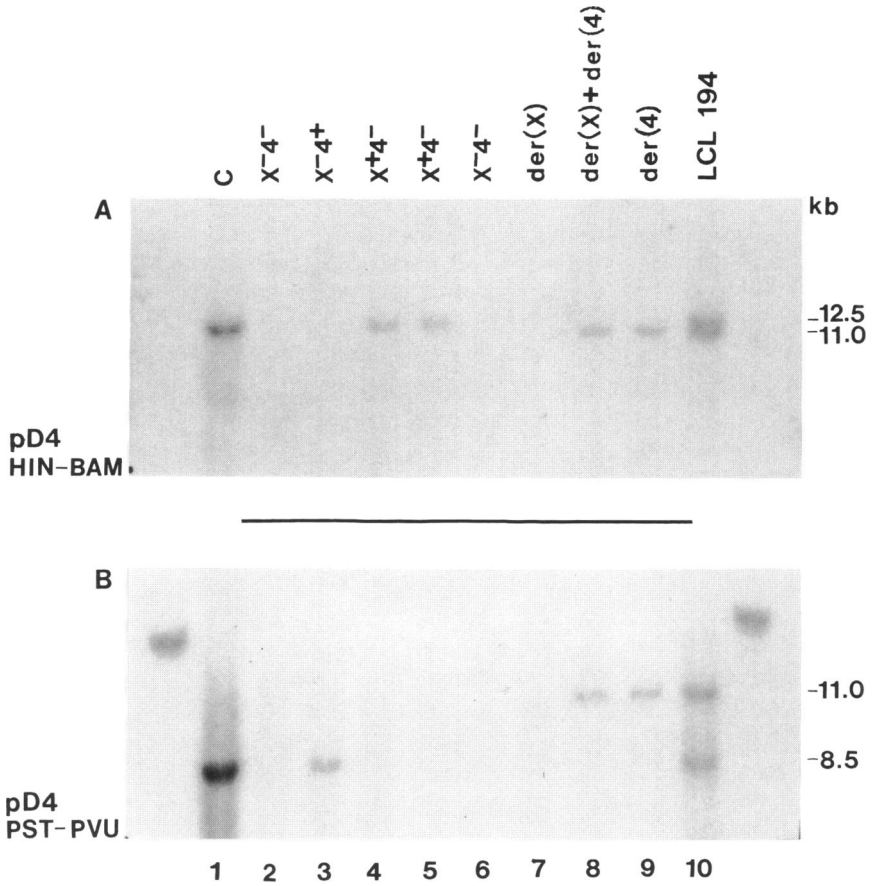


Figure 5 Demonstration that cosmid cD4 is derived from der(4) chromosome and spans breakpoint. Fragments of cosmid cD4 subclone pD4 (see fig. 4 and text) were hybridized to a small somatic cell hybrid panel to determine whether this subclone contains sequences from both chromosomes 4 and X. *A*, Hybridization of 2.1-kb *Hin*dIII-*Bam*HI fragment. *B*, Hybridization of 800-bp *Pst*I-*Pvu*II fragment. DNA samples digested with *Pst*I are as follows: Lane 1, Normal female control. Lanes 2–6, Hybrids containing or lacking chromosomes X or 4, as indicated above each lane. Lanes 7–9, Hybrids containing either or both of the t(X;4) translocation derivatives. Lane 10, LCL 194, t(X;4) cell line. Hybridization patterns indicate that the *Hin*dIII-*Bam*HI fragment is X specific, that the *Pst*I-*Pvu*II fragment is derived from chromosome 4, and that both fragments hybridize to the same 11.0-kb *Pst*I fragment, on der(4), that contains the breakpoint.

at the proximal end of the deletion (overlined in fig. 6). This makes it impossible to know which dinucleotide pair was retained and which was lost. The possible implications of these small regions of homology at the sites of the translocation are discussed below.

Parental Origin of Translocation, and Confirmation of De Novo Deletion

The possibility remains that the X-chromosome DNA deleted in the der(X) chromosome was not lost during the translocation event as we hypothesize but that the deletion was preexisting in the parental chromosome that was involved in the translocation. Even though it is considered unlikely, since no deletion polymorphism has been found by screening populations with the surrounding pERT probes, we evaluated this possibility by first determining the parental origin of the translocation by using X-specific RFLPs detected by the pERT 87 probes. Probe pERT 87-1 recognizes an *Xmn*I RFLP with two alleles, one of 8.7 kb and one of 7.5 kb (fig. 7*A*). Since patient US (fig. 7*A*, lane 3) and her mother (fig. 7*A*, lane 2) are both heterozygous and since her father (fig. 7*A*, lane 1) is hemizygous for the 8.7-kb allele, US must have inherited the 8.7-kb allele from her father and the 7.5-kb allele from her mother. Hybridization to DNA from series 31 somatic cell hybrids indicates that the der(X) chromosome contains the 8.7-kb allele (fig. 7*A*, lane

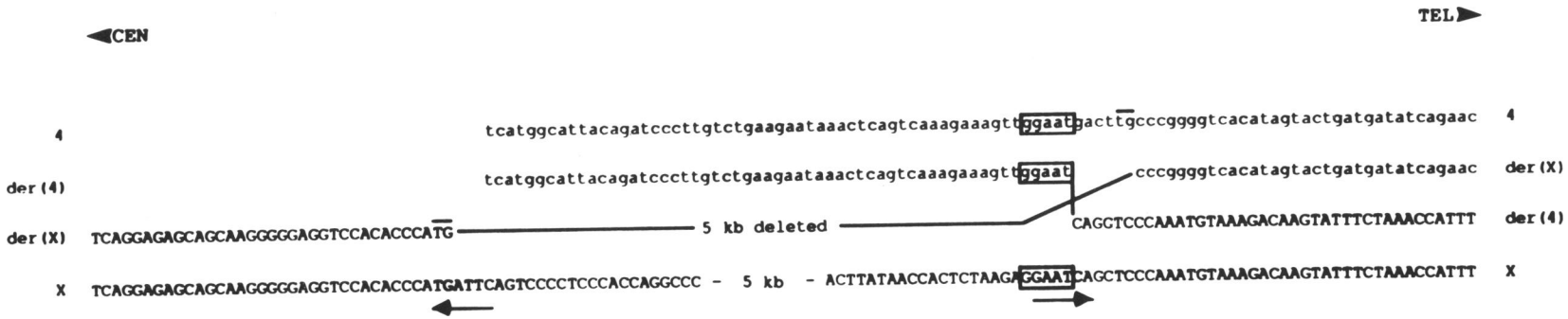


Figure 6 Sequence surrounding translocation breakpoints. Fragments of subclones shown in fig. 5 and containing the translocation breakpoints were cloned into M13 and sequenced. Uppercase letters indicate X chromosome sequence, and lowercase letters indicate chromosome 4 sequence. The sequence GGAAT is present at the breakpoints of both chromosomes X and 4 (boxes). Arrows highlight the 6-bp sequence, GAATCA, which is present as inverted repeats at the two ends of the deleted 5 kb of X chromosome material. The four bases adjacent to the boxed pentamer on the normal chromosome 4 have been lost in the translocation. The next two bases, TG (overlined) are present on both chromosomes 4 and X, one copy of which has been deleted from the der(X) chromosome.

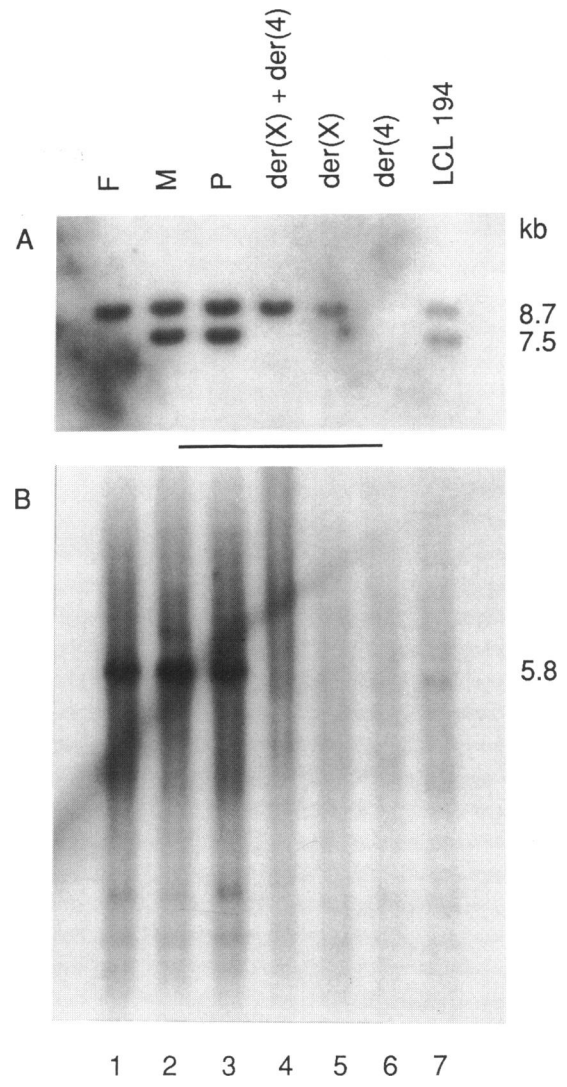


Figure 7 Determination of parental origin of translocation and de novo nature of deletion. *A*, *Xmn*I-digested DNA from patient US, from her parents, and from somatic cell hybrids separating her translocation chromosomes was hybridized with pERT 87-1, which recognizes two *Xmn*I alleles, one of 8.7 kb and one of 7.5 kb. Lanes 1–3, Leukocyte DNA of father (F), mother (M), and patient US (P). Lane 4, Hybrid 31-3B. Lane 5, Hybrid 31-2A. Lane 6, Hybrid 31-3B-7b aza. Lane 7, LCL 194. US and her mother are both heterozygotes, and her father is hemizygous for the 8.7-kb allele. US's 8.7-kb allele must be paternal, and it is this allele which is present on the der(X) chromosome (lane 5). Thus the translocation is of paternal origin. *B*, Same samples as in panel *A*, in the same order and hybridized with *Hin*dIII-*Eco*RV fragment of pNX, which is part of deleted X chromosome material. Hybridization shows that neither of the translocation chromosomes contains the 5.8-kb *Xmn*I band recognized by this probe (lanes 4–6). However, the father's DNA (lane 3) does contain this fragment. Thus the deletion was not preexisting in the paternal X chromosome involved in the translocation.

5), thereby proving that the translocation is of paternal origin.

In order to determine whether the X deletion is present on the paternal X chromosome, a filter containing the identical samples used for the RFLP analysis digested with *Xmn*I was hybridized to the 1.2-kb *Eco*RV-*Hin*dIII fragment of clone pNX (fig. 4), which represents part of the DNA presumed to be deleted from the der(X) chromosome. This probe contains about 30 bp of X chromosome DNA from the far side of the translocation breakpoint, which sequencing data have shown not to be deleted from the translocation chromosomes, as well as a portion of an L1 repetitive sequence as deduced from the K map (fig. 3). This fragment hybridized to a 5.8-kb *Xmn*I fragment present in both parents (fig. 7*B*, lanes 1 and 2) as well as in the patient's leukocyte DNA (fig. 7*B*, lane 3) and in her LCL 194 (Fig. 7B, lane 7). This fragment is missing from both translocation chromosomes in series 31 hybrids (fig. 7*B*, lanes 4–6) and is not replaced by any new fragments. We interpret these results as indicating that this *Xmn*I fragment is present on the father's X chromosome as well as on the patient's normal X but has been deleted from the translocation chromosomes. Similar results have been obtained when the same X-specific probe has been hybridized to *Pst*I and *Kpn*I digests of LCL 194 and series 31 hybrid DNAs. Although we can neither test the entirety of the suspected deleted region, as it is composed mostly of L1 sequences, nor rule out the possibility that the father is a germ-line mosaic wherein a portion of his X chromosomes would have the deletion, the existing evidence is consistent with the hypothesis that deletion and translocation arose simultaneously.

Discussion

Many deletion mutations in different human genes have been characterized at the DNA sequence level, and theories have been proposed for potential mechanisms by which such deletions may occur (Krawczak and Cooper 1991). Since there have been few opportunities to study constitutional chromosomal translocations, the mechanisms by which they occur are still obscure. Fine-structure analysis of a variety of constitutional translocations might provide clues that will allow the formulation and testing of hypotheses. Toward this end, we have undertaken the molecular characterization of a chromosome translocation in a female with DMD and a t(X;A) translocation.

We have assigned the translocation breakpoints on

a high-resolution cytogenetic map as t(X;4)(p21.2; q31.22) and have mapped the X breakpoint within the pERT 87 (DXS164) locus that is part of the dystrophin gene. The translocation that appeared balanced cytogenetically is associated with the deletion of approximately 5 kb of X chromosome DNA from intron 16 of the DMD gene. This region is missing from the der(X) chromosome, while 4–6 bp of chromosome 4 material have been lost in the formation of the der(X) translocation chromosome. On the basis of previous analyses of the overall gene structure (Koenig et al. 1988; Den Dunnen et al. 1989; A. P. Monaco and L. M. Kunkel, personal communication), we have assigned the translocation breakpoint to the 18-kb intron separating exons 16 and 17, which on the genomic level (Koenig et al. 1987) corresponds to about 500 kb from the 5′ end of the gene. This region of the DMD gene encodes the rodlike central portion of the dystrophin molecule, a domain composed of 24 repeated segments of 109 amino acids. A 50-amino-acid proline-rich segment which lies between repeats 3 and 4 may function as a "hinge" necessary for the higher-order folding of dystrophin dimers (Koenig and Kunkel 1990). The breakpoint in the t(X;4) translocation lies within the intron which separates the exons coding for the third repeat (exon 16) and this hinge segment (exon 17). There is no evidence, however, for this region being prone to rearrangements. More than 60% of individuals with DMD or BMD have deletions of portions of the gene, with deletion breakpoints clustered in two large introns, the 110-kb intron 7 and the 170-kb intron 44 (Wapenaar et al. 1988; Den Dunnen et al. 1989; Blonden et al. 1991; Love et al. 1991). Both of these regions are hundreds of kilobases from the t(X;4) breakpoint. Also, analysis of approximately two dozen females with DMD and t(X;A) translocations demonstrates that these translocation breakpoints are distributed more or less randomly throughout the gene (Boyd and Buckle 1985; Boyd et al. 1986, 1988; Bodrug et al. 1990).

The finding of a translocation and relatively large deletion in our patient raises the question of whether the 5-kb deletion of X chromosome DNA and the t(X;4) translocation were independent events or occurred simultaneously. We have shown that the deletion was not preexisting on the parental chromosome that was involved in the translocation, although, as mentioned above, we cannot rule out germ-line mosaicism in the father. Statistically, the likelihood of two rare events occurring independently at the same site is much lower than the likelihood of these two events occurring together, in a dependent fashion. It has been estimated that the frequency of t(X;A) translocations is less than 1/10,000. In a study of 34,910 newborns, 49 reciprocal translocations between autosomes were identified while no t(X;A) translocations were found (Nielsen and Wohlert 1991). Similarly, although deletions within the dystrophin gene are common in DMD/BMD patients, in the general population no deletion of material around pERT 87-15 has yet been described. The notion that, in our patient, deletion and translocation arose together as the result of a complex rearrangement event is further supported by our finding of a common sequence motif, GAAT, that is shared by the GGAAT pentamer at the translocation breakpoints on chromosomes X and 4 and by the hexamer GAATCA at the ends of the deleted material (discussed below).

Further insight may be gained by considering the parental and cellular origin of the translocation/deletion. Our finding that the translocation is derived from the patient's father is consistent with previous reports that de novo structural chromosome rearrangements are far more often paternally than maternally derived (Olson and Magenis 1988). Spermatogonia undergo numerous cycles of replication and mitotic divisions prior to meiosis, thus providing ample opportunities for mutations and rearrangements to occur, in contrast to oogenesis, in which far fewer cell divisions occur. Although this difference may explain the predominance of paternal origin of point mutations and most chromosome rearrangements, it is not likely to apply to de novo t(X;A) translocations, because a t(X;A) that arises in a premeiotic cell is unlikely to be propagated. Male carriers of t(X;A) translocations are almost invariably sterile, because gametogenesis is blocked in meiosis I (Chandley 1988; Martin 1988; Quack et al. 1988). Thus, we favor the view that the t(X;4) under study here occurred during a meiotic division in the patient's father. However, we cannot exclude the less likely possibility that the translocation occurred in a mitotic division during spermatogenesis or that it is of postzygotic origin resulting in mosaicism for the t(X;4) in the affected female. All her leukocytes studied contained the translocation, and the severity of her progressive muscular dystrophy can only be explained if all or the vast majority of her muscle tissue carries the translocation. Thus, at the phenotypic or cellular level there is no evidence for mosaicism in this individual.

The paternal origin of this translocation is also intriguing in that it brings to 13/13 the number of de

novo t(X;A) translocations in which parental origin has been determined and which are paternal in origin (Chamberlin and Magenis 1980; Pai et al. 1980; Bjerglund-Nielsen and Nielsen 1984; Kean et al. 1986; Ribeiro et al. 1986; Bodrug et al. 1990; Robinson et al. 1990). If each parental X were equally likely to become involved in a translocation, the chances of all 13 being paternally derived is exceedingly small ($1/3^{13}$). There is one striking aspect of male meiosis which may predispose to t(X;A) translocations. Whereas in female meioisis both X chromosomes are completely synapsed, in male meiosis only the distal portion of the X short arm, the pseudoautosomal region, is involved in synapsis with the Y short arm. This leaves the bulk of the X chromosome unpaired during the initial stages of meiosis, when synaptonemal complex formation is occurring. Heterologous pairing is frequently observed in early stages of meiosis. Thus, the unpaired section of the X chromosome may become synapsed with other chromosomes before being segregated into the "sex vesicle." A translocation event arising from such interaction might explain the apparent predominance of t(X;A) translocations derived from males compared with females.

The objective of this study was to find, at a molecular level, a potential reason for a large-scale chromosomal rearrangement to have occurred. Sequence analysis of the translocation breakpoints was undertaken in an attempt to identify features of both chromosomes X and 4 which would have targeted these specific sites for aberrant interaction with each other. Analysis of this t(X;4) translocation did not uncover any known features that could be primarily responsible for the translocation or deletion. No repetitive sequences were present either at the breakpoints on chromosome X or 4 or at the ends of the deleted region. Although the deleted 5 kb contain L1 sequences, these sequences are not present at the ends of the deleted segment. We also do not find either an unusually high concentration of AT nucleotides or alternating purine/pyrimidine residues which might take on a Z-DNA configuration around the translocation/deletion breakpoints, both of which situations have been implicated in a predisposition to rearrangements (Boehm et al. 1989; Love et al. 1991). Also, there are no sequences, such as multiple topoisomerase II cleavage sites (Gasser and Laemmli 1987), which might indicate that the rearrangement sites function as chromosome scaffold attachment sites, a chromosome configuration which might serve to bring distant DNA sequences into close proximity. A constitutive aphidicolin-inducible fragile site, FRA 4C, has been assigned to 4q31.1, but we cannot determine its proximity to the translocation breakpoint on chromosome 4.

Thus, the only unique feature of this chromosome rearrangement is a 5-bp sequence, GGAAT, present on chromosomes X and 4 precisely at the translocation breakpoints. On the X chromosome it overlaps a hexamer, GAATCA, that is also present in inverted orientation on the opposite strand at the proximal end of the deleted 5-kb segment. We propose that the three GAAT tetramers (one each at all three sites of rearrangement) were juxtaposed simultaneously, perhaps in the very first stages of synaptonemal complex formation, when homologues are being pulled together by an unknown mechanism. At this point, the recombination machinery may have recognized the aligned GAAT motifs and may have initiated a crossover event by introducing double-stranded breaks in all three rearrangement sites. Although drawn as staggered cuts in figure 8, these could just as easily be blunt-ended breaks. Such breaks would release the 5 kb of X chromosome DNA and would generate four free chromosome ends. Ligation of the fragment containing Xpter-Xp21.2 to the fragment of chromosome 4 containing 4pter-4q31.22 yields the der(4) chromosome. Meanwhile, exonucleolytic deletion of the free ends of the remaining chromosome fragments would result in the loss of one of the copies of the GAAT tetramer and four to six bases of chromosome 4q material. Although the model in figure 8 indicates that 6 bp of 4q DNA are lost in addition to the GAAT overhang, the TG dinucleotide nearest the point of ligation could have been deleted from either the 4q fragment or the X chromosome fragment. In either case, after the chewing back of the ends, the 4qter-4q31.22 fragment ligated to the Xqter-Xp21.2 fragment would generate the der(X) chromosome.

This hypothesis cannot address the question of whether the recombinational machinery responsible for this rearrangement specifically recognized the GAAT tetranucleotide sequence or simply recognized the alignment of two DNA molecules with a small region of sequence homology. The molecular analysis of the t(X;21) DMD translocation (Bodrug et al. 1987) would suggest that the latter is the case. The t(X;21) breakpoint contains a conserved CGGC tetramer present once on the X chromosome and multiple times on chromosome 21. This motif is not present at the t(X;4) breakpoint, nor is the GAAT motif present at the t(X;21) breakpoint. The machinery responsible for these translocations may have little sequence speci-

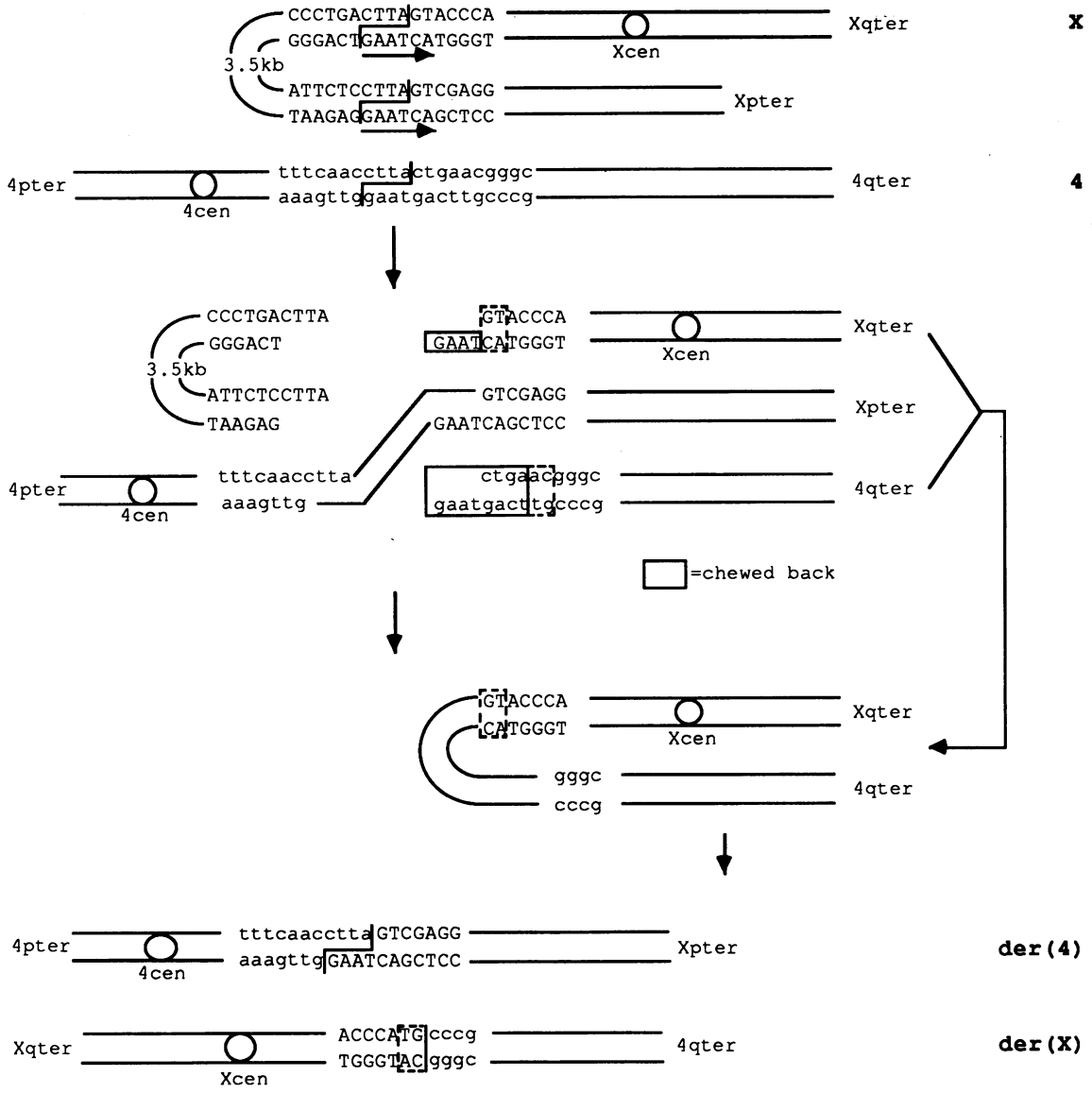


Figure 8 Model of translocation/deletion event. We propose that during meiosis the short arm of the X chromosome (uppercase nucleotides) folded back on itself, aligning the two copies of the hexamer 5′-GAATCA-3′ (horizontal arrows). At the same time, the long arm of chromosome 4 (lowercase nucleotides) was brought into proximity, aligning the 5′-GGAAT-3′ on both chromosomes X and 4. Subsequently, double-stranded breaks were made at all three sites, resulting in the loss of the 5 kb of X chromosome DNA which had been looped out, leaving four other free chromosome ends. Direct ligation of the 4pter-4q31 fragment containing the chromosome 4 centromere to the Xp21-Xpter fragments results in the formation of the der(4) chromosome. Chewing back of the remaining two free ends and subsequent ligation produces the der(X) chromosome. In the formation of the der(X) chromosome, one copy of the TG dinucleotide abutting the breakpoints on both chromosomes X and 4 (broken boxes) has been deleted while one copy has been maintained.

ficity. Besides the conserved, albeit different, tetramers at the breakpoints, no homology to Alu or L1 sequences, to *chi* sequences (GCTGGTGG), or to immunoglobulin recombinatorial sequences were found surrounding the breakpoints of either translocation. Whereas the t(X;4) is associated with a large 5-kb deletion of X chromosome material as well as with several bases from chromosome 4, in the t(X;21) the formation of the der(X) resulted in the loss of only 71 or 72 nucleotides of X sequence. In the formation of the der(21), 16–23 bp of chromosome 21 were lost and three nucleotides were added. The CGGC tetra-

mer is present at both ends of the chromosome 21 deleted material and within two bases of one end of the X deletion, but is not present at the other end. We might hypothesize strand breakage at all three CGGC sites, along with extensive exonucleolytic deletion of X chromosome sequences before both addition of the three nucleotides to the der(X) chromosome and repair of the free chromosome ends.

The best-characterized mammalian systems which involve chromosome rearrangements involve members of the immunoglobulin and T-cell-receptor supergene families. These genes undergo DNA rearrangements as a programmed part of the development of B- and T-cells, respectively, resulting in the loss of chromosomal material. These rearrangements are mediated by the "recombinase" enzyme system, which acts on specific, conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) sequence motifs flanking the immunoglobulin V, D, and J segments to be recombined. Many B- and T-cell neoplasias appear to result from malfunctioning of the recombinase system, resulting in a chromosome translocation between the Ig or T-cell-receptor gene and an oncogene on another chromosome, an oncogene which, by chance, is flanked by a sequence similar to the motif recognized by the recombinase enzymes (reviewed in Haluska et al. 1987). A chromosome translocation has also been described in a transgenic mouse carrying an exogenous immunoglobulin transgene, in which isotype switching occurred via interchromosomal recombination (Gerstein et al. 1990). Although constitutional chromosomal translocations clearly do not occur by the recombinase system active in lymphoid cells, they represent aberrant meiotic recombination. The study of additional constitutional translocation breakpoints, as well as the identification of enzymes involved in meiotic recombination, will aid in understanding the normal processes of meiotic chromosome pairing and recombination and eventually will reveal the mechanisms by which aberrant chromosomal rearrangements—translocations, deletions, and inversions—occur.

Acknowledgments

We are indebted to Professor Dr. W. Rosenkranz (University of Graz, Austria) for patient samples and clinical information, to Brigitte Foellmer and Christine Keller for lymphocyte transformation and hybrid cell culture, to Janet Cowan for chromosome preparations, to Anna-Maria Frischauf and the Lehrach Laboratory at the EMBL (Heidelberg) for helping U.F. to construct the cosmid library during a sabbatical, to Tony Monaco and Louis Kunkel for probes and restriction maps of the DMD gene, and to Hunt Willard for critical reading of the manuscript. J.P.G. submitted part of this work in partial fulfillment of the requirements for the degree of doctor of philosophy at Yale University. Financial support was provided by the Muscular Dystrophy Association of America, the National Institutes of Health, and the Howard Hughes Medical Institute.

References

Baas F, Bikker H, van Ommen GJB, de Vijlder JJM (1984) Unusual scarcity of restriction site polymorphism in the human thyroglobulin gene: a linkage study suggesting autosomal dominance of a defective thyroglobulin allele. Hum Genet 67:301–305

Bjerglund-Nielsen L, Nielsen IM (1984) Turner's syndrome and Duchenne muscular dystrophy in a girl with an X; autosome translocation. Ann Genet 27:173–177

Blonden LAJ, Grootscholten PM, den Dunnen JT, Bakker E, Abbs S, Bobrow M, Boehm C, et al (1991) 242 breakpoints in the 200-kb deletion-prone P20 region of the DMD gene are widely spread. Genomics 10:631–639

Bodrug SE, Ray PN, Gonzalez IL, Schmickel RD, Sylvester JE, Worton RG (1987) Molecular analysis of a constitutional X-autosome translocation in a female with muscular dystrophy. Science 237:1620–1624

Bodrug SE, Roberson JR, Weiss L, Ray PN, Worton RG, Van Dyke DL (1990) Prenatal identification of a girl with a t(X;4)(p21;q35) translocation: molecular characterization, paternal origin, and association with muscular dystrophy. J Med Genet 27:426–432

Boehm T, Mengle-Gaw L, Kees UR, Spurr N, Lavenir I, Forster A, Rabbitts TH (1989) Alternating purine-pyrimidine tracts may promote chromosomal translocations seen in a variety of human lymphoid tumours. EMBO J 8:2621–2631

Boué A, Gropp A, Boué J (1985) Cytogenetics of pregnancy wastage. Adv Hum Genet 14:1–7

Boyd Y, Buckle VJ (1986) Cytogenetic heterogeneity of translocations associated with Duchenne muscular dystrophy. Clin Genet 29:108–115

Boyd Y, Buckle V, Holt S, Munro E, Hunter D, Craig I (1986) Muscular dystrophy in girls with X;autosome translocations. J Med Genet 23:484–490

Boyd Y, Cockburn D, Holt S, Munro E, van Ommen GJ, Gillard B, Affara N, et al (1988) Mapping of 12 translocation breakpoints in the Xp21 region with respect to the locus for Duchenne muscular dystrophy. Cytogenet Cell Genet 48:28–34

Chamberlin J, Magenis RE (1980) Parental origin of *de novo* chromosome rearrangements. Hum Genet 53:343–347

Chandley AC (1981) The origin of chromosomal aberrations in man and their potential for survival and reproduc-

tion in the adult human population. Ann Genet 24:5–11
——— (1988) Meiotic studies and fertility in human translocation carriers. In: Daniels A (ed) Progress and topics in cytogenetics, vol 8: The cytogenetics of mammalian autosomal rearrangements. Alan R Liss, New York, pp 361–382
Darras BT, Blattner P, Harper JF, Spiro AJ, Alter S, Francke U (1988) Intragenic deletions in 21 Duchenne muscular dystrophy (DMD)/Becker muscular dystrophy (BMD) families studied with the dystrophin cDNA: location of breakpoints on HindIII and BglII exon-containing fragment maps, meiotic and mitotic origin of the mutations. Am J Hum Genet 43:620–629
de Martinville B, Kunkel LM, Bruns G, Morlé F, Koenig M, Mandel JL, Horwich A, et al (1985) Localization of DNA sequences in region Xp21 of the human X chromosome: search for molecular markers close to the Duchenne muscular dystrophy locus. Am J Hum Genet 37:235–249
Den Dunnen JT, Grootscholten PM, Bakker E, Blonden LAJ, Ginjaar HB, Wapenaar MC, van Paassen HMB, et al (1989) Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. Am J Hum Genet 45:835–847
Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13
Francke U (1981) High-resolution ideograms of trypsin-Giemsa banded human chromosomes. Cytogenet Cell Genet 31:24–32
Francke U, Darras BT, Hersh JH, Berg BO, Miller RG (1989) Brother/sister pairs affected with early-onset, progressive muscular dystrophy: molecular studies reveal etiologic heterogeneity. Am J Hum Genet 45:63–72
Francke U, Ochs HD, de Martinville B, Giacalone J, Lindgren V, Distèche C, Pagon RA, et al (1985) Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. Am J Hum Genet 37:250–267
Francke U, Oliver N (1978) Quantitative analysis of high-resolution trypsin-Giemsa bands on human prometaphase chromosomes. Hum Genet 45:137–165
Funderburk SJ, Spence MA, Sparkes RS (1977) Mental retardation associated with "balanced" chromosome rearrangements. Am J Hum Genet 29:136–141
Gasser SM, Laemmli UK (1987) A glimpse at chromosomal order. Trends Genet 3:16–22
Gerstein RM, Frankel WN, Shieh C-L, Durdik JM, Rath S, Coffin JM, Nisonoff A, et al (1990) Isotype switching of an immunoglobulin heavy chain transgene occurs by DNA recombination between different chromosomes. Cell 63: 537–548
Haluska FG, Tsujimoto Y, Croce CM (1987) Mechanisms of chromosome translocation in B- and T-cell neoplasia. Trends Genet 3:11–15
Jacobs PA (1974) Correlation between euploid structural chromosome rearrangements and mental subnormality in humans. Nature 249:164–165
Kean VM, Macleod HL, Thompson MW, Ray PN, Verellen-Dumoulin C, Worton RG (1986) Paternal inheritance of translocation chromosomes in a t(X;21) patient with X linked muscular dystrophy. J Med Genet 23:491–493
Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, et al (1989) The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet 45:498–506
Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM (1987) Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 50:509–517
Koenig M, Kunkel LM (1990) Detailed analysis of the repeat domain of dystrophin reveals four potential hinge segments that may confer flexibility. J Biol Chem 265:4560–4566
Koenig M, Monaco AP, Kunkel LM (1988) The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. Cell 53:219–228
Krawczak M, Cooper DN (1991) Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86:425–441
Kunkel LM, Monaco AP, Middlesworth W, Ochs HD, Latt SA (1985) Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. Proc Natl Acad Sci USA 82:4778–4782
Love DR, England SB, Speer A, Marsden RF, Bloomfield JF, Roche AL, Cross GS, et al (1991) Sequences of junction fragments in the deletion-prone region of the dystrophin gene. Genomics 10:57–67
Martin RH (1988) Abnormal spermatozoa in human translocation and inversion carriers. In: Daniels A (ed) Progress and topics in cytogenetics, vol 8: The cytogenetics of mammalian autosomal rearrangements. Alan R Liss, New York, pp 397–417
Mohandas T, Sparkes RS, Shapiro LJ (1982) Genetic evidence for the inactivation of a human autosomal locus attached to an inactive X chromosome. Am J Hum Genet 34:811–817
Monaco AP, Kunkel LM (1987) A giant locus for the Duchenne and Becker muscular dystrophy gene. Trends Genet 3:33–37
Moser H (1984) Duchenne muscular dystrophy: pathogenetic aspects and genetic prevention. Hum Genet 66:17–40
Nielsen J, Wohlert M (1991) Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Århus, Denmark. Hum Genet 87:81–83
Olson SB, Magenis RE (1988) Preferential paternal origin

of *de novo* structural chromosome rearrangements. In: Daniels A (ed) Progress and topics in cytogenetics, vol 8: The cytogenetics of mammalian autosomal rearrangements. Alan R Liss, New York, pp 583–599

Pai GS, Thomas GH, Mahoney W, Migeon BR (1980) Complex chromosome rearrangements: report of a new case and literature review. Clin Genet 18:436–444

Poustka A, Rackwitz HR, Frischauf A-M, Lehrach H (1984) Selective isolation of cosmid clones by homologous recombination in *Escherichia coli*. Proc Natl Acad Sci USA 81:4129–4133

Quack B, Speed RM, Luciani JM, Noel B, Guichaoua M, Chandley AC (1988) Meiotic analysis of two human reciprocal X-autosome translocations. Cytogenet Cell Genet 48:43–47

Ribeiro MCM, Melaragno MI, Schmidt B, Brunoni D, Gabbai AA, Hackel C (1986) Duchenne muscular dystrophy in a girl with an (X;15) translocation. Am J Med Genet 25:231–236

Richards CS, Watkins SC, Hoffman EP, Schneider NR, Milsark IW, Katz KS. Cook JD, et al (1990) Skewed X inactivation in a female MZ twin results in Duchenne muscular dystrophy. Am J Hum Genet 46:672–681

Robinson DO, Boyd Y, Cockburn D, Collinson MN, Craig I, Jacobs PA (1990) The parental origin of *de novo* X-autosome translocations in females with Duchenne muscular dystrophy revealed by M27β methylation analysis. Genet Res 56:135–140

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 1.98–1.99

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Scalenghe F, Turco E, Edstrom JE, Pirrotta V, Melli M (1981) Microdissection and cloning of DNA from a specific region of *Drosophila melanogaster* polytene chromosomes. Chromosoma 82:205–216

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Wapenaar MC, Kievits T, Hart KA, Abbs S, Blonden LAJ, den Dunnen JT, Grootscholten PM, et al (1988) A deletion hot spot in the Duchenne muscular dystrophy gene. Genomics 2:101–108

Warburton D (1984) Outcome of cases of de novo structural rearrangements diagnosed at amniocentesis. Prenatal Diagn 4:69–80