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Sequence Analysis of the Breakpoint Regions of an X;5 Translocation in a Female with Duchenne Muscular Dystrophy

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Summary

X;autosome translocations in females with Duchenne muscular dystrophy (DMD) provide an opportunity to study the mechanisms responsible for chromosomal rearrangements that occur in the germ line. We describe here a detailed molecular analysis of the translocation breakpoints of an X;autosome reciprocal translocation, t(X;5)(p21;q31.1), in a female with DMD. Cosmid clones that contained the X-chromosome breakpoint region were identified, and subclones that hybridized to the translocation junction fragment in restriction digests of the patient's DNA were isolated and sequenced. Primers designed from the X-chromosomal sequence were used to obtain the junction fragments on the der(X) and the der(5) by inverse PCR. The resultant clones were also cloned and sequenced, and this information used to isolate the chromosome 5 breakpoint region. Comparison of the DNA sequences of the junction fragments with those of the breakpoint regions on chromosomes X and 5 revealed that the translocation arose by nonhomologous recombination with an imprecise reciprocal exchange. Four and six base pairs of unknown origin are inserted at the exchange points of the der(X) and der(5), respectively, and three nucleotides are deleted from the X-chromosome sequence. Two features were found that may have played a role in the generation of the translocation. These were (1) a repeat motif with an internal homopyrimidine stretch 10 bp upstream from the X-chromosome breakpoint and (2) a 9-bp sequence of 78% homology located near the breakpoints on chromosomes 5 and X.

Introduction

Duchenne muscular dystrophy (DMD) and its allelic, milder counterpart, Becker muscular dystrophy (BMD), are X-linked recessive neuromuscular disorders that affect ~1/3,000 boys (Moser 1984). The DMD gene has been localized to Xp21 and has been cloned, and the protein product, dystrophin, has been identified (Hoffman et al. 1987; Worton and Thompson 1988). A few rare examples of females with DMD have been described, the majority of whom have been associated with a skewed X-inactivation pattern (Boyd 1992; Pegoraro et al. 1994). Of particular importance have been those in whom the manifestation of the disorder has been associated with de novo X;autosome translocations. The autosomal breakpoint of one of these translocations was found to lie in the rRNA genes on chromosome 21, a discovery that was exploited to obtain some of the first clones from the DMD locus (Ray et al. 1985). The positions of the autosomal breakpoints of translocations associated with DMD are distributed throughout the genome, but all the X-chromosome breakpoints involve Xp21 (Boyd 1992). Of the 24 translocation patients reported, 14 have been studied in some depth, and in each case the X-chromosome breakpoint lies within the 2,300-kb dystrophin gene (Cockburn et al. 1992). Although these females carry two X chromosomes and therefore have two dystrophin gene loci, they manifest DMD because of a skewed X-inactivation pattern. Whereas one gene is disrupted by the translocation and lies on the active X, the other lies on the intact, inactive X chromosome (Therman et al. 1974; Jacobs et al. 1981). The parental origin of the translocation chromosome has been determined for eight of the translocations, and all have been found to involve the paternally derived X chromosome (reviewed by Robinson et al. 1990; Giacolone and Francke 1992). Given that X;autosome translocations are not transmitted through male meiosis, the DMD translocations are likely to have arisen as a result of a postmeiotic rearrangement during spermatogenesis (Bodrug et al. 1991). Interestingly, two of the translocation breakpoints are known to lie in an autosomal transcribed locus, the 28S ribosomal gene in the case of the t(X;21) (Bodrug et al. 1987) and the protein tyrosine phosphatase receptor-type f polypeptide locus (PTPRF) in the case of the t(X;1) (Cockburn 1991).

Since the translocation breakpoints associated with DMD lie within the well-characterized dystrophin gene, they have provided an excellent opportunity to analyze

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Table 1

PCR Primers and Conditions

Primer	Sequence (5′ to 3′)	Product Size (bp)	Annealing Temperature (°C)	[$MgCl_2$] (mM)
g[a]	TATGATCCCAGATATTTGTGACAC	550	55	2
i[a]	GACCAGTTTGATTTTCAAAGTCTT			
1A	TTTAATAGTCTGTAATTTGG	349	53	1.5
1B	GTTATTGTGAATATCTCAGT			
2A	AAGCAATTACAAGCGGACTC	374	53	1.5
2B	ATGGTAAGTTGGTTGGTTTA			
3A	ATTGTGCAGTGTTCTCGATG	64	53	1.5
3B	CCTCCATAGTTGATAAATGG			

[a] Adapted from Bodrug et al. (1991).

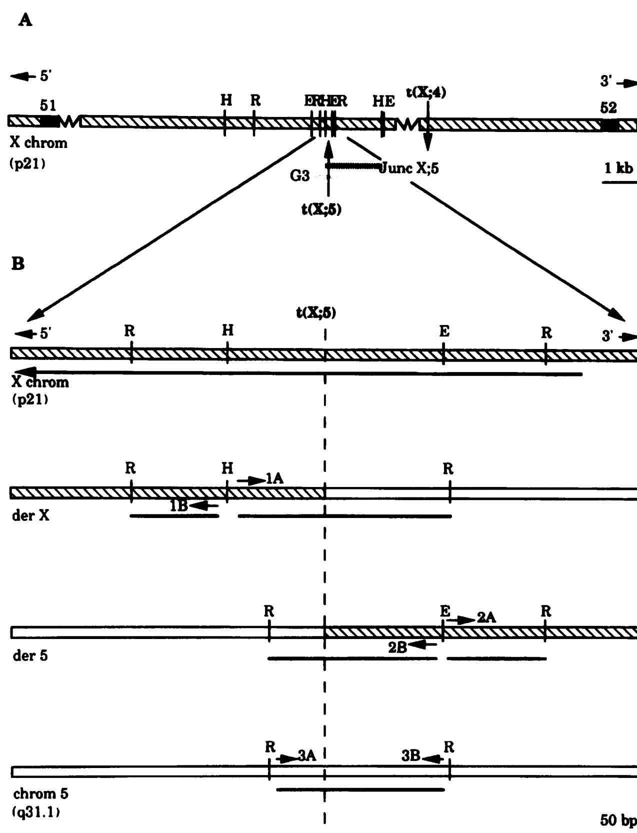

Figure 1 *A*, Schematic representation of intron 51 with the X;5 breakpoint region depicted in detail. The 5′-to-3′ direction of the gene is indicated, as are exons 51 and 52. Subclone JuncX;5, which was used to identify altered fragments in Southern blot analysis of patient DNA, is shaded below the intron, as is subclone G3, which has a 311-bp overlap with JuncX;5. Both t(X;4) and t(X;5) are indicated by arrows. *B*, Breakpoint regions on all chromosomes, drawn to a larger scale. The positions of the IPCR primers used to enable cloning of the junction fragments and the breakpoint region on chromosome 5 are indicated. Hatched bars represent DNA from the X chromosome; unhatched bars represent chromosome 5 DNA; and blackened bars indicate the extent of DNA sequenced on each chromosome. E = *Eco*RI; H = *Hin*dIII; and R = *Rsa*I.

sequence rearrangements associated with the appearance of germ-line translocations. To date, the breakpoint regions of five DMD translocations have been cloned and sequenced. All five translocations arose by nonhomologous recombination with up to 5 bp of junctional homology present at the site of chromosomal exchange. However, unlike the endpoints of the deletions and duplications within the dystrophin locus observed in DMD males (Hu et al. 1991; Love et al. 1991), the translocation breakpoints in DMD females are generally associated with minor genomic rearrangements, e.g., small deletions/duplications (1–72 bp) and insertions (1–3 bp) at the site of the translocation breakpoint. The only larger rearrangement detected was associated with the t(X;4) analyzed by Giacalone and Francke (1992), where 5 kb were deleted at the site of the translocation breakpoint on the X chromosome.

The DMD translocation, t(X;5)(p21;q31.1), analyzed here was first reported by Nevin et al. (1986) and the X-chromosome breakpoint has been positioned between exons 51 and 52 of the dystrophin locus by somatic-cell hybrid analysis (Cockburn et al. 1992). We report here both the refinement of this localization to 10–31 kb proximal to the t(X;4) translocation breakpoint studied by Bodrug et al. (1991) and the subsequent isolation and sequencing of clones that traverse the breakpoints on the der(X), the der(5), and the breakpoint regions on the normal homologues. These sequence data demonstrate that the translocation arose by nonhomologous recombination and is associated with an insertion junction (Roth et al. 1985).

Patient, Material, and Methods

Case-Report Update

The patient who carries the t(X:5)(p21;q31) is now age 16.5 years and has been followed regularly since first reported by Nevin et al. (1986). At age 12 years, although able to walk and despite reasonably good muscle power, she began to tire more easily, and, by the age

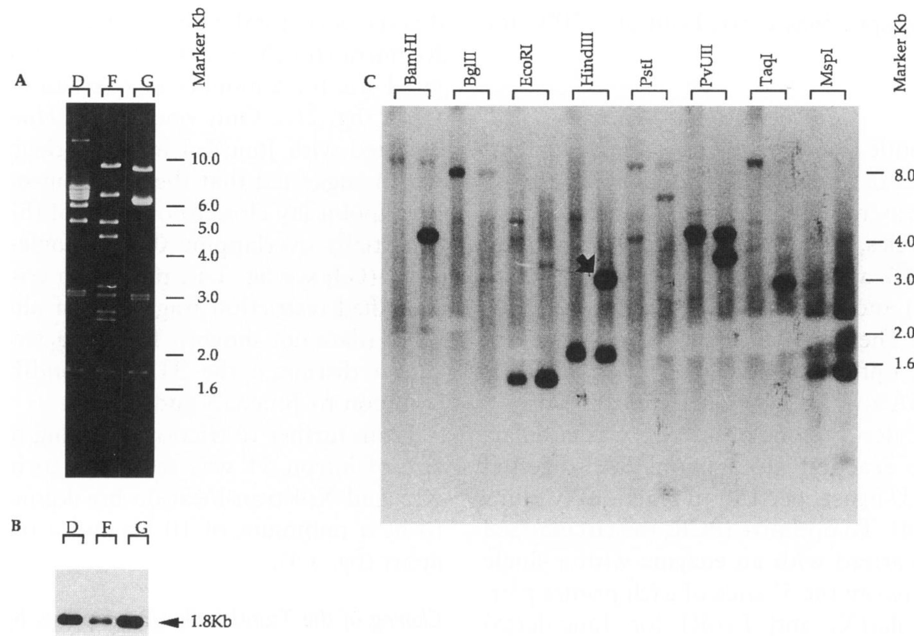

Figure 2 *A*, *Hin*dIII digests of cosmids that span intron 51: D and F (which contain the 7.0-kb Hd41 fragment and exon 52) and G (which contains the 3.1-kb HD40 fragment and exon 51). *B*, Hybridization of the 1.8-kb *Hin*dIII fragment JuncX;5 purified from cosmid G to the digests depicted above, showing that this is the same fragment present in D and F. C, Identification of junction fragments after hybridization of JuncX;5 to a Southern blot of pairs of enzyme digests of control female DNA (*left*) and patient (HEM) DNA (*right*). Restriction fragments of altered size (arrowed only in *Hin*dIII digests) are present in six of the eight digests. Only one altered fragment is present in most lanes, indicating that the breakpoint lies close to one end of JuncX;5.

of 14 years, she was totally wheelchair bound. Although confined to a wheelchair, she is remarkably uncontracted at the hip and knee and maintains a straight back. Since age 13 years, she has had episodes of supraventricular tachycardia, and during the past year she has had four major episodes necessitating hospitalization. She remains alert and bright and in remarkably good health.

Cell and Hybrid Lines

The lymphoblastoid cell line, HEM, established from the patient described above, has been described elsewhere (Boyd and Buckle 1986). The somatic cell hybrid, HEMAG-N2, which retains the der(X) chromosome and a few autosomes has also been described elsewhere (Boyd et al. 1988; note that there is an error in table 2 of this reference; i.e., HEMAG-N2 contains the der(X), not the der(5) as stated).

Cosmids and Cosmid Subclones

Cosmids characterized as provisional positives for the dystrophin cDNA clone 44-1 were obtained from the ICRF X chromosome cosmid reference library (Nizetic et al. 1991). 44-1 contains DMD fragment 8 as defined by Koenig et al. (1987) and identifies exon-containing *Hin*dIII fragments 36–41 (Hd36–41), which include exons 48–52 (Koenig et al. 1987; Den Dunnen et al. 1989). Hybridization of cDMD 44-1 to *Hin*dIII digests of these cosmids revealed that ICRFc104C0461 (G) contained the Hd40 (3.1 kb) fragment (exon 51) and that cosmids ICRFc104A1287 (F), ICRFc104E0691 (D), and ICRFc104F0891 (A) contained the Hd41 (7.0 kb) fragment (exon 52). No positive hybridization signal was observed after hybridization of cDMD 44-1 to the other provisional positives (ICRFc104C0691, ICRFc104E0891, ICRFc104F0791, and ICRFc104G04193). Cosmids A, D, F, and G were analyzed further, and relevant subclones were obtained by ligating gel-purified restriction fragments into the appropriate cloning site of pUC19 or M13mp8/M13mp9.

Filter Hybridizations

Genomic DNA from human placenta, the cell line HEM, and the somatic-cell hybrid (HEMAG-N2) were purified using standard procedures. Restriction-enzyme digests were carried out according to the manufacturers' specifications; digests were electrophoresed on 0.7% agarose gels, and the DNA was subsequently transferred to Hybond N+ filter membranes, with 0.4 M NaOH as the transfer buffer. Membranes were hybridized overnight at 63°C–65°C, either with probes labeled with ^{32}P-dCTP by nick-translation or by random priming, using commercially available kits (Amersham). Final washing conditions varied in stringency, 0.1–0.5 × SSC or 20-40 mM NaPi, depending on the probe and hybridization buffers used (Church and Gilbert 1984; Laval et al.

1991). Autoradiography was carried out at −70°C for 4 h–5 d.

PCR

The oligonucleotide primer sequences, annealing temperatures, and sizes of amplification products are given in table 1. PCR was routinely carried out in standard buffers, for 35 cycles, in 30–50-μl reaction volumes. Primer pairs 1A-1B and 2A-2B were used to isolate clones Junc-der(X) and Junc-der(5), respectively, by inverse PCR (IPCR). The Junc-der(X) clone of 450 bp was circularized under optimal conditions (10 μg/ml *Rsa*I-digested HEM DNA and 3 U ligase, per 150-μl reaction). Likewise, the Junc-der(5) clone of 400 bp was circularized in a separate reaction (15.3 μg/ml *Rsa*I-digested HEM DNA and 4 U ligase, per 150-μl reaction) (Collins and Weisman 1984). To optimize IPCR, the circularized products were linearized with an enzyme with a single recognition site between the 5′ ends of each primer pair: *Hin*dIII for Junc-der(X) and *Eco*RI for Junc-der(5) (Cockburn 1991) (fig. 1*B*). IPCR was carried out in 10 mM Tris-HCl (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$, and 0.1 mg gelatine/ml (w/v) (pH 8.3), for 35 cycles (94°C for 1 min, 53°C for 30 s, and 70°C for 1 min).

Cloning and Sequencing

All amplification products were gel-purified prior to being cloned into TA-vectors (Invitrogen). Sequencing was performed by using Sequenase (version 2; USB).

Results

Breakpoint Mapping and Isolation of Junction Fragments

The X-chromosome breakpoint of the t(X;5)-(p21;q31.1) associated with DMD has been mapped previously to intron 51 of the dystrophin gene by using somatic-cell hybrids (Cockburn et al. 1992). The X-chromosome breakpoint of the t(X;4)(p21.2;q35) translocation associated with DMD has also been mapped to this intron and positioned in the same *Hin*dIII fragment as exon 52 (Bodrug et al. 1991). Primers g and i (table 1), designed to prime across the X-chromosome breakpoint region of the X;4 translocation, failed to yield an amplification product when the somatic-cell hybrid HEMAG-N2 was used as a template for PCR (data not shown). Since HEMAG-N2 contains the der(X) of the t(X;5), these data suggest that the X-chromosome breakpoint of the t(X;5) lies proximal to the t(X;4).

More precise localization of the t(X;5) breakpoint was obtained by analysis of cosmid clones from the ICRF X-chromosome reference library (see Patient, Material, and Methods). Cosmids F and G, which contain exon 51, and cosmid D, which contains exon 52, were digested with *Hin*dIII. A common 1.8-kb single-copy *Hin*dIII fragment (fig. 2*A* and *B*) was identified after failing to yield a signal after hybridization of cosmid digests with total human DNA. This 1.8-kb *Hin*dIII fragment (JuncX;5) detected restriction fragments of altered size for a number of restriction enzymes in HEM DNA (fig. 2*C*). Only one altered *Hin*dIII fragment was detected with JuncX;5 in the patient's DNA (fig. 1*A*), which suggested that the X-chromosome translocation breakpoint lay close to one end of this clone. Similarly, a partially overlapping 0.7-kb single-copy *Eco*RI fragment (G3; see fig. 1*A*), present in cosmids D, F, and G identified restriction fragments of altered size in HEM DNA (data not shown). This suggested that the translocation disrupted the 311-bp *Hin*dIII-*Eco*RI fragment common to JuncX;5 and G3.

From further restriction mapping of the cosmids, the size of intron 51 was estimated to be 18–55 kb. The X;5 and X;4 translocation breakpoints were estimated to be a minimum of 10 kb and a maximum of 31 kb apart (fig. 1*A*).

Cloning of the Translocation Breakpoints by Using IPCR

The X-chromosome breakpoint region contained in the clones G3 and JuncX;5 was sequenced, and *Rsa*I sites that flanked the breakpoint were identified (fig. 1). The sizes of the genomic *Rsa*I fragments on the der(X) and der(5) were estimated, by Southern blot analysis, to be an appropriate size for IPCR (~450 bp and ~400 bp, respectively; data not shown). Primers were designed to enable the cloning of the translocation breakpoints of the der(X) and der(5) by IPCR carried out on HEM DNA (figs. 1*B* and 3*A* and *B*). IPCR primers 1A and 1B (table 1), which lie proximal to the breakpoint in the normal X and which flank a *Hin*dIII site, were designed for IPCR amplification of the der(X) *Rsa*I fragment, Junc-der(X) (fig. 3*A*). IPCR primers 2A and 2B, which lie distal to the breakpoint on the normal X and which flank an *Eco*RI site, were designed for amplification of the der(5) *Rsa*I fragment, Junc-der(5) (fig. 3*B*).

HEM DNA was digested and, in separate reactions for the der(X) and der(5), ligated under conditions that favored circularization of each of these *Rsa*I fragments (see Patient, Material, and Methods). After individual linearization, separate IPCR reactions were performed, and the products were cloned by using a TA-cloning kit (Invitrogen). A larger *Rsa*I fragment was coamplified in both reactions (fig. 3*C, top*); that this arose from the normal X-chromosome template was confirmed by sequence analysis (data not shown). Hybridization of JuncX;5 to the amplification products confirmed that they all contained X-chromosome junction sequences (fig. 3*C, bottom*).

Both clones, Junc-der(X) and Junc-der(5), were sequenced, and the exact position of the breakpoint on the normal X chromosome was identified. The new sequence information obtained, assumed to represent sequences that flank the translocation breakpoint on chromosome 5, was used to design primers 3A and 3B for

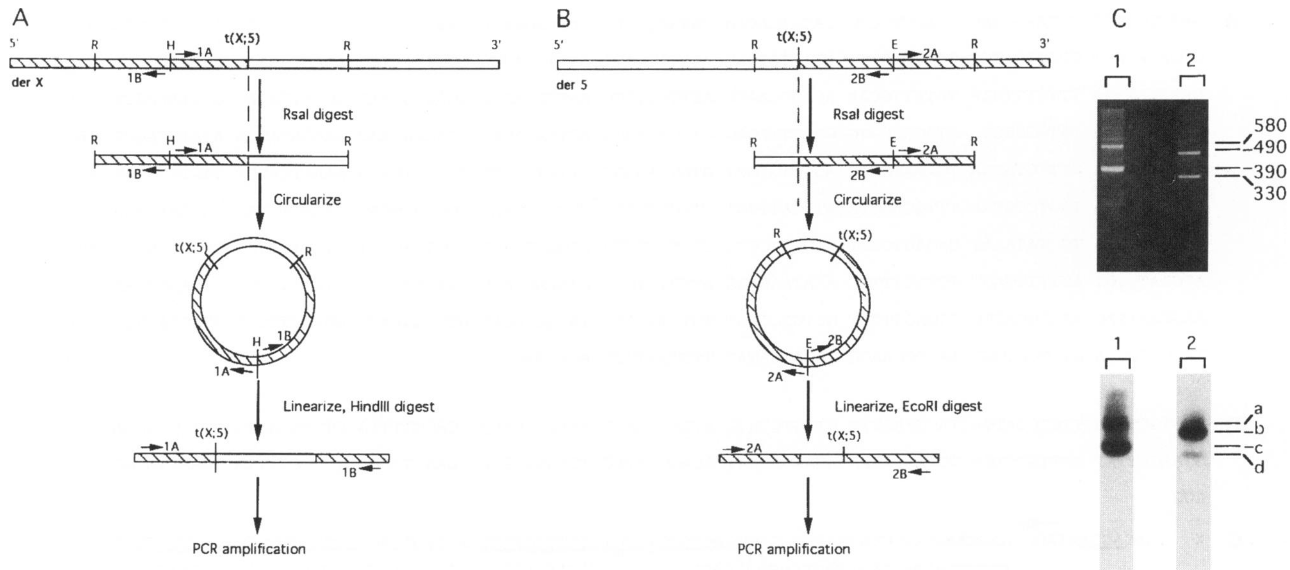

Figure 3 Strategy used for cloning the junction fragments: Junc-der(X) from the der(X) (*A*) and Junc-der(5) from the der(5) (*B*), by IPCR. Hatched bars represent DNA from the X chromosome; and unhatched bars represent chromosome 5 DNA. The 5′-to-3′ direction of the gene is indicated. IPCR primers are indicated by arrows. R = *Rsa*I; H = *Hin*dIII; and E = *Eco*RI. *C, Top,* IPCR amplification of the breakpoint regions on the der(5) (lane 1) and the der(X) (lane 2). Each IPCR reaction gives an additional product (top band in both lanes) that is of the expected size for coamplification of the normal X *Rsa*I breakpoint–containing fragment. The products, a 391-bp Junc-der(5) and a 336-bp Junc-der(X) fragment, were run on an agarose gel and stained with ethidium bromide. *Bottom,* Southern blot of the agarose gel depicted above, hybridized with JuncX;5. Hybridization confirms that all IPCR products contain X-chromosome breakpoint-region DNA. The discrepancy in labeling intensity is due to unequal yields of the IPCR products and the relative amount of X-chromosome material present.

amplification of the autosomal breakpoint region (fig. 1*B*). Amplification with 3A and 3B of female genomic DNA gave a product of the expected size, which was cloned and sequenced. PCR analysis of a monochromosomal hybrid panel (Spurr 1994) by using primers 3A and 3B confirmed that they amplified a sequence from chromosome 5 (data not shown).

Sequence Analysis: Insertions on Both the der(X) and the der(5)

The sequences of the junction fragments and the breakpoint regions on the normal homologues are depicted in figure 4. The translocation is associated with a deletion of three nucleotides (CAA) from the X chromosome. Furthermore, two small insertions are present at the breakpoints, AGGA on the der(X) and TTGTGG on the der(5) (fig. 4*C*). Interestingly, the 4-bp motif AGGA is present 9 bp proximal to the breakpoint on the X chromosome, and the 6-bp motif TTGTGG is present 14 bp distal to the breakpoint on chromosome 5. Whether these cognate sequences were involved in events that led to their insertion at the point of chromosomal exchange, or whether the insertions are of a different origin, is unclear.

The complete X-chromosome and chromosome 5 sequences, as illustrated in figure 4*A* and *B*, were searched for matches to human repetitive DNA (Pythia version 2). A homologue to a subfamily of Alu sequences, Sb0 or Sb1 (consensus sequence: TACTAAAAATAC AAAAA) (Jurka 1993), was identified on the X chromosome (fig. 4*A*, position 49–65). No significant homologies to sequences like LINE elements, χ-like structures, Z-DNA, or topoisomerase II sites were identified on either chromosome involved. Two topoisomerase I sites were identified close to but not directly at the site of breakage on both the X chromosome and chromosome 5 (fig. 4*C*).

Other features of the DNA sequence of the breakpoint region include an imperfect repeat motif, $(\mathrm{GAGTCAGT(G)_C T(C)_G TCTCTCTC\ TCTC(TC)_D TTTT})_2$ (the subscript C and G denote substitution for C and G, respectively; and the subscript D denotes deletion) distal to the breakpoint on the X chromosome (fig. 4). Note that this repeat motif contains an internal stretch of 14–18 pyrimidines. There is a small region (9 bp), immediately distal to the breakpoint on chromosome 5, that is 78% homologous to a region 3 bp distal to the breakpoint on the X chromosome (fig. 4*C*).

Discussion

We have confirmed the assignment of the t(X;5)(p21;q31.1) translocation associated with DMD to the intron flanked by exons 51 and 52, which places the breakpoint in the distal rod domain, in the region of spectrinlike repeat number 20 (Roberts et al. 1993). Intron 51 is also disrupted by the t(X;4) translocation

Figure 4 Sequence of the chromosomes involved in the t(X;5) translocation, shown in 5′-to-3′ orientation. *A*, X-chromosome sequence surrounding the breakpoint. The sequence was obtained from cosmid subclones juncX;5 and G3. Nucleotides 263–870 were also sequenced from the IPCR patient-specific coproduct, obtained with primers 1A and 1B. Because of the uncertainty as to the exact location of the breakpoint, the region is indicated by two arrowheads. The CAA deletion is in boldface. The sequence in lowercase represented by letters indicates a member of a subfamily of Alu sequences (Sb1 or Sb0), position 48–65. *B*, Chromosome 5 sequence surrounding the breakpoint. The sequence was obtained from a cloned PCR product from control female DNA with primers 3A and 3B. The site of the breakpoint is indicated by an arrow. The nucleotides represented by lowercase letters (position 51–61) are discussed in the text. *C*, Schematic representation of the origin of the nucleotides involved in the translocation. There are an additional four nucleotides, AGGA (*boxed*), on the der(X) chromosome and an additional six, TTGTGG (*circled*) on the der(5) chromosome. Both additions are of unknown origin; for discussion, see the text. One of these additions (AGGA) is present once on the X chromosome, and the other (TTGTGG) is present once on chromosome 5 (*thicker underlining*). Distal to the breakpoint on the X chromosome lies a repeat motif, $(GAGTCAGT(G)_CT(C)_GTCTCTCTCTC(TC)_DTTTT)_2$ (the subscript C and G denote substitution for C and G, respectively (*thinner underlining*); and the subscript D denotes deletion). The repeat motif contains a homopyrimidine stretch (*double underlining*). The small region of homology between the X chromosome and chromosome 5 is shaded on both chromosomes, with nonhomologous nucleotides left out on chromosome 5. Topoisomerase I sites are indicated by arrows (consensus sequence 5′-A/T-G/C-T/A-T-3′) (Been et al. 1984). Uppercase letters are used for the X chromosome and der(X); and lowercase letters are used for chromosome 5 and der(5).

described by Bodrug et al. (1991), which we estimate to lie 10–31 kb away. It is of interest to note that two of the other 14 DMD translocations studied in depth, the t(X;1) and the t(X;21), lie ~50 kb apart (Cockburn 1991). Differences in the distribution of translocation and deletion/duplication breakpoints associated with DMD suggest that the mechanisms involved in generating the two classes of mutation differ (Cockburn et al. 1992). That this is the case is supported by differences in the nature of the features observed at the breakpoint sites in the few cases studied in detail. Whereas the deletion/duplication endpoints analyzed all involve "clean breaks" (Hu et al. 1991; Love et al. 1991), the five X;autosome translocations analyzed to date all have minor additions or deletions at the site of the rearrangement (table 2). These additional nucleotides are thought to originate from a repair process during which the free ends of the DNA strands are filled in prior to ligation, thus enabling nonhomologous recombination to take place (Thode et al. 1990). The sequence data presented here from a sixth X;autosome translocation reveal that this rearrangement has the structure of a typical insertion junction (Roth et al. 1985), with insertion events of unknown origin on both derived chromosomes, 4 bp (AGGA) on the der(X) and 6 bp (TTGTGG) on the der(5).

Table 2

Summary of Features Associated with DMD Translocations

Translocation	Breakpoint Intron	Additions (bp)	Deletion (bp)	Common Motif(s) or Significant Feature(s)	Junctional Homology	Reference
t(X;21)(p21;p12)	7	+3 on X	−(71−72) on X −(16−23) on 21	CGGC on X and 21	G	Bodrug et al. 1987
t(X;4)(p21;q35)	51	+3 on der(X)	−3 on X −(7−8) on 4	Topo I site on 4 Topo II sites on X, 4	A	Bodrug et al. 1991
t(X;2)(p21;q37.3)	63	+2 on der(X)	−(0−2) on X −(1−3) on 2	Topo I site on 2	TG	Bodrug et al. 1991
t(X;4)(p21;q31.2)	16		−5000 on X −4 on 4	GAATCA on X, X	GGAAT on X TG	Giacalone and Francke 1992
t(X;1)(p21;p34)	7	+(2−5)[a] on X	−(4−7) on X	GAA on X, 1 TGC on 1	GAA	Cockburn 1991
t(X;5)(p21;q31)	51	+4 on der(X)	−3 on X	AGGA on X TTGTGG on 5 TCTATAAA on X, 5		Present study

[a] Duplication of 2−5 bp of X-chromosome sequence.

The sequences obtained (fig. 4) from the translocation chromosomes and their normal homologues were analyzed for the presence of any significant features that may have played a role in the etiology of the chromosome rearrangement. This translocation, like the others, arose by nonhomologous recombination. However, a 9-bp region immediately distal to the translocation breakpoint on chromosome 5 was 78% homologous to a 9-bp region 3 bp distal to the translocation breakpoint on the X chromosome, and it is possible that this played a role in the generation or stabilization of a recombination complex.

No χ-like sequences, which are thought to be involved in VDJ-recombination, were found. However, topoisomerase (topo) I sites were identified within 5 bp of the breakpoint on both chromosomes involved in this translocation. Topo I and topo II sites have been found in the vicinity of other translocation breakpoints, and, because of their ability to make transient cuts in DNA, they are thought to play an active role in the generation of chromosome rearrangements (Bae et al. 1988). Hu et al. (1991) studied two DMD gene duplications and identified topo I and II sites within one nucleotide of the breakpoints. Topo I and II sites are also associated with, although not directly at, three breakpoints in two DMD translocations (see table 2). Comparison of the sequence spanning the t(X;5) breakpoint with that present at other translocation, deletion, and duplication breakpoints in the dystrophin gene revealed that, just proximal to the breakpoint on chromosome 5 (see fig. 4*B*), there was a stretch of nucleotides, AAATTTTATGT, similar to the AAATTGTGTTT and AAATTTGCTGTTT sequences identified at breakpoint junctions in two DMD deletion patients (Love et al. 1991).

In conclusion, we have cloned and sequenced the breakpoints of a translocation that disrupts the dystrophin gene in intron 51, and we have shown that the translocation arose by nonhomologous recombination with imprecise reciprocal exchange. Two features were found that may have contributed to the etiology of the exchange event: (1) a repeat motif with an internal homopyrimidine stretch 10 bp upstream from the X-chromosome breakpoint and (2) 9 bp of 78% homology near to the breakpoints on chromosome 5 and on the X chromosome.

Acknowledgments

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