though small rises in renin secretion rate were noted in the periods after perfusate change in two of the six preparations, these changes did not achieve significance and were only transient. Previous studies with isolated superfused glomeruli have shown that iso-osmotic replacement of NaCl with sucrose will suppress renin secretion (*23*). The stimulation of renin secretion that we observe when the macula densa was perfused with the iso-osmotic low-NaCl solution is therefore the opposite of that predicted if this solution were presented as the superfusion fluid. It indicates that the response is specific to exposure in the compartment containing the macula densa.

Our experiments show that renin secretion is affected by the composition of the tubular fluid at the macula densa. Changes in the NaCl concentration of the tubular fluid elicited an immediate change in the rate of renin release. This effect was demonstrable in an in vitro preparation where influences from renal nerves and local hemodynamic effects can be excluded. These results directly demonstrate that tubular fluid composition at the macula densa influences renin release and that a fall, rather than a rise, in NaCl concentration is the local tubular signal for stimulation of renin secretion.

REFERENCES AND NOTES

1. T. Inagami, in *Renal Hormones*, J. W. Fisher, Ed. (Academic Press, London, 1986), vol. 3, pp. 217–245.
2. T. K. Keeton and W. B. Campbell, *Pharmacol. Rev.* **31**, 81 (1980).
3. F. S. Wright and J. Schnermann, *J. Clin. Invest.* **53**, 1695 (1974).
4. P. C. Churchill, M. C. Churchill, F. D. MacDonald, *Am. J. Physiol.* **235**, F611 (1978).
5. J. Schnermann, J. Briggs, G. Schubert, *ibid.* **243**, F160 (1982).
6. N. Goormaghtigh, *La Fonction Endocrine des Arterioles Renales* (Librairie R. Fonteyn, Louvain, France, 1944).
7. E. Hackenthal, *Ann. Endocrinol.* **47**, 145 (1986); G. H. Gibbons, V. J. Dzau, E. R. Farhi, A. C. Barger, *Annu. Rev. Physiol.* **46**, 291 (1984).
8. K. Thurau, *Am. J. Med.* **36**, 698 (1964).
9. ———, A. Gruener, J. Mason, H. Dahlheim, *Kidney Int.* **22** (suppl. 12), S55 (1982).
10. A. Gillies and T. Morgan, *Pfluegers Arch.* **375**, 105 (1978).
11. T. Morgan and A. Gillies, *ibid.* **368**, 13 (1977).
12. P. P. Leyssac, *Acta Physiol. Scand.* **62**, 436 (1964).
13. A. J. Vander and R. Miller, *Am. J. Physiol.* **207**, 537 (1964).
14. A. J. Vander, *Physiol. Rev.* **47**, 359 (1967).
15. P. P. Leyssac, *Kidney Int.* **30**, 332 (1986).
16. R. E. Shade, J. O. Davis, J. A. Johnson, R. T. Witty, *Circ. Res.* **31**, 719 (1972).
17. J. P. Briggs and J. Schnermann, *Annu. Rev. Physiol.* **49**, 251 (1987).
18. M. B. Burg and M. A. Knepper, *Kidney Int.* **30**, 166 (1986).
19. J. P. Briggs, J. P. Kokko, H. R. Jacobson, *Proc. Ninth Int. Congr. Nephrol.* 406A (1984); K. L. Kirk, P. D. Bell, D. W. Barfuss, M. Ribadeneira, *Am. J. Physiol.* **248**, F890 (1985).
20. S. Lykkegaard and K. Poulsen, *Anal. Biochem.* **75**, 250 (1976).
21. O. Skøtt, *Pfluegers Arch.* **407**, 41 (1986).
22. S. Itoh, O. A. Carretero, R. D. Murray, *Kidney Int.* **27**, 762 (1985).
23. O. Frederiksen, P. P. Leyssac, S. L. Skinner, *J. Physiol. (London)* **252**, 669 (1975).
24. R. Greger and W. Hampel, *Pfluegers Arch.* **389**, 175 (1981).
25. All experiments were performed with a Krebs-Ringer bicarbonate buffer of the following composition: 115 m*M* NaCl, 25 m*M* $NaHCO_3$, 10 m*M* sodium acetate, 0.96 m*M* NaH_2PO_4, 0.24 m*M* Na_2HPO_4, 5 m*M* KCl, 1.2 m*M* $MgSO_4$, 1 m*M* $CaCl_2$, and 5.5 m*M* glucose, for a total measured osmolality of 300 mOsm/kg. The solution was bubbled with 5% CO_2 plus 95% O_2 to a *p*H of 7.4. When used as dissection medium 2% fetal calf serum was added. Human albumin (0.3%) was present in the bath solution used for superfusion. This solution was used without albumin as the tubular perfusate in control periods. In the hypo-osmolar, low-NaCl perfusate the NaCl concentration was reduced by 95 m*M*. The resultant osmolality was 122 mOsm/kg. The iso-osmolar, low-NaCl solution was identical to the hypo-osmolar, low-NaCl perfusate, except that the osmolality was corrected to 300 mOsm/kg by addition of mannitol.
26. G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, IA, 1967).

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Molecular Analysis of a Constitutional X-Autosome Translocation in a Female with Muscular Dystrophy

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The gene responsible for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) maps to the X chromosome short arm, band Xp21. In a few females with DMD or BMD, the Xp21 region is disrupted by an X-autosome translocation. Accumulating evidence suggests that the exchange has physically disrupted the DMD/BMD locus to cause the disease. One affected female with a t(X;21)(p21;p12) translocation was studied in detail. The exchange points from both translocation chromosomes were cloned, restriction-mapped, and sequenced. The translocation is reciprocal, but not conservative. A small amount of DNA is missing from the translocated chromosomes; 71 to 72 base pairs from the X chromosome and 16 to 23 base pairs from the 28*S* ribosomal gene on chromosome 21.

DUCHENNE MUSCULAR DYSTROPHY (DMD) and the less severe Becker muscular dystrophy (BMD) (*1*) are X-linked neuromuscular diseases that are allelic (*2*). The biochemical defect is unknown, but the gene has been localized to band p21 on the short arm of the X chromosome. The localization was established by linkage analysis (*3*), by the finding of cytologically detectable deletions of the Xp21 region in males with DMD and other phenotypes (*4*), and by the finding of X-autosome translocations involving band Xp21 in a group of 20 females with DMD or BMD (*5*). In these females, the position of the autosomal exchange point is variable, whereas the position of the X chromosome exchange point is consistently in band Xp21, which suggested that the translocations may disrupt the DMD/BMD locus. The disease is expressed in these translocation carrier females because of the preferential inactivation of the normal X chromosome (*5*). In one of the females (*6*), the autosomal breakpoint of the translocation occurred in a block of tandemly repeated ribosomal RNA genes (rDNA) on the short arm of chromosome 21 (*7*). The isolation of a human-specific rDNA probe located near the translocation breakpoint allowed the identification and cloning of a novel restriction fragment, XJ1, from the translocation junction

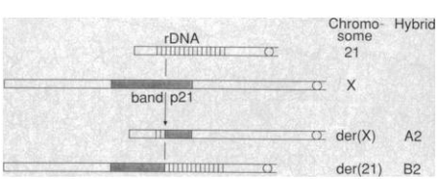

Fig. 1. Schematic diagram of the short arms of the chromosomes involved in the reciprocal translocation. The translocation-derived chromosomes have been segregated in somatic cell hybrids (*7, 8*) as the only human chromosomes on a mouse A9 background. Hybrid A2 has been fully described (*7*) and hybrid B2 is a subclone of the hybrid line C2-T10 reported earlier to contain the der(21) chromosome (*7*).

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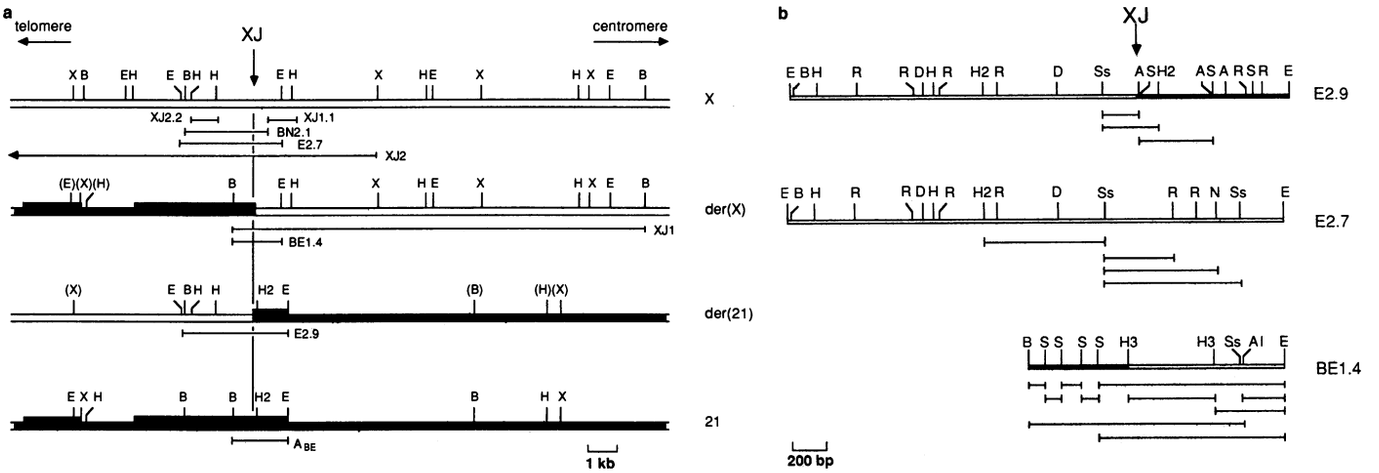


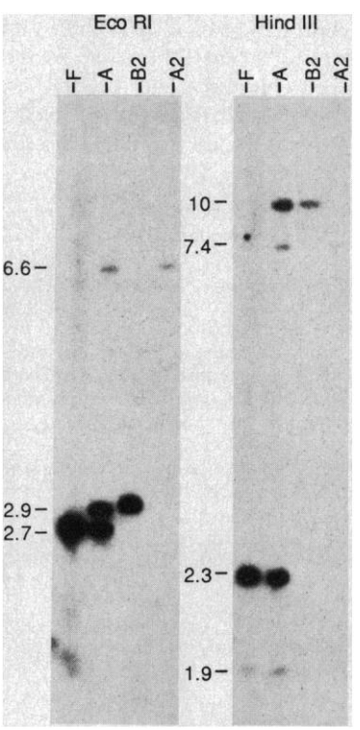
Fig. 2. (**a**) Restriction maps of the translocation-derived chromosomes and the corresponding regions of the X chromosome and chromosome 21. The solid region represents DNA from chromosome 21, with the thicker portion representing the 18*S* and 28*S* rRNA genes, whereas the open region represents DNA from the X chromosome. The restriction map of the X chromosome was obtained by restriction enzyme digests of phage clones XJ1 and XJ2, the latter derived from a human 4X library that had been screened with XJ1.1 (*9*). The restriction map of the der(21) chromosome was obtained in part from digests of the Eco RI–junction fragment clone E2.9 and in part from restriction enzyme digests of the hybrid B2 followed by Southern blot analysis. The restriction maps of regions of the der(X) chromosome (*8*) and chromosome 21 (*31*) have been published. In each restriction map a restriction site without parentheses represents data obtained from digests of cloned regions, whereas a restriction site with parentheses represents data obtained by sizing bands on Southern blots. Some restriction sites that have not been confirmed on the der(X) and der(21) chromosomes are not indicated on the restriction map. Abbreviations: B, Bam HI; E, Eco RI; H, Hind III; H2, Hind II; X, Xba I. (**b**) Detailed restriction maps of the translocation junction fragments E2.9 cloned from the hybrid B2 and BE1.4 subcloned from XJ1 and derived from the hybrid A2. The corresponding map of the X chromosome is from clone E2.7 isolated from DNA of the father of the translocation patient. Below each map are the fragments of DNA that have been subcloned into one or more of pSP65, pGEM3, pBSM13, M13mp18, or M13mp19. Sequencing of subclones from BE1.4 was performed on single-strand phage DNA by Sanger dideoxy chain termination sequencing. Sequencing of subclones from E2.7 and E2.9 was done by Sanger dideoxy sequencing of NaOH denatured double-stranded plasmid DNA (*32*). Abbreviations as in (a) plus AI, Alu I; A, Ava I; D, Dra I; H3, Hae III; R, Rsa I; S, Sma I; Ss, Sst I.

of the X-derived chromosome [der(X), Fig. 1] (*8*). Chromosome walking from XJ1 has extended the cloned portion of the X chromosome in both directions from the site of the translocation (*9, 10*).

The primary rationale for cloning the der(X) translocation junction was based on the presumption that the X-derived portion of the junction clone might contain sequences from within the DMD/BMD gene. This would be true only if the translocation had physically disrupted the DMD/BMD locus by splitting the gene or by separating it from a nearby regulatory locus. An alternative possibility was that during the formation of the translocation there was a substantial but cytologically undetectable deletion (of up to a few million base pairs) that might have deleted a DMD/BMD gene located at a considerable distance from the XJ1 cloned region. To determine the extent of any deletion at the translocation breakpoint, we cloned the translocation junction from the 21-derived chromosome [der(21), Fig. 1]. A comparison of restriction maps and nucleotide sequence of this clone and of the previously cloned junction from the der(X) chromosome (Fig. 1) with the normal X and 21 has revealed no major deletion at the site of the translocation.

Cloning of the junction fragment from the der(21) chromosome contained in a somatic cell hybrid, B2 (Fig. 1), was first attempted with rDNA probes. This proved impractical as the 40 to 60 copies of ribosomal genes on this chromosome (*7*) gave a hybridization signal that obscured the detection of any novel junction fragment. The alternative strategy was to walk from XJ1 on the centromeric side of the der(X) junction along the normal X chromosome in a telomeric direction until probes were obtained that recognized sequences on the der(21) chromosome. The first walk clone in the telomeric direction (XJ2) yielded the clone

Fig. 3. Visualization of the der(21) and der(X) translocation junctions by means of an X-derived probe from the region of the X chromosome spanning the translocation breakpoint. DNA was isolated from lymphoblast cultures of the patient carrying the translocation (A), the patient's father (F) with an unrearranged X chromosome, and the hybrids A2 and B2. DNA was digested with Eco RI or Hind III, separated by electrophoresis on an 0.8% agarose gel and transferred to nitrocellulose. The fragment BN2.1 derived from the junction region of the X chromosome contained some intermediate repeat sequences. For use as a probe the fragment was labeled by nick-translation then preannealed with 1 mg of sheared (500 bp) human placental DNA to a Cot of 65 (*33*). Prehybridization of the nitrocellulose filter was at 42°C in 50% formamide, 3× standard saline citrate (SSC), 0.05*M* $NaPO_4$, 10× Denhardt's solution, 0.15 mg ml^{-1} of sheared herring sperm DNA, and 1% (w/v) glycine. Samples were hybridized for 15 hours in the same solution plus 10% dextran sulfate and 4.8×10^7 cpm ml^{-1} of ^{32}P-labeled DNA. The filter was washed two times for 30 minutes each in 0.1% SDS and 0.1× SSC at 65°C. Autoradiography was at −70°C with an intensifying screen for 10 days.



BN2.1 (top panel, Fig. 2a) that spanned the translocation junction. When used as a probe on a Southern blot containing the patient's DNA (Fig. 3), fragments from both translocation chromosomes, as well as the normal X, were visualized. This indicated immediately that no major deletion had occurred at the site of the translocation. As seen in Fig. 3, the Eco RI and Hind III bands from the patient's normal X chromosome are identical to the bands from her father's normal X chromosome. The other two bands are derived from the der(X) and der(21) translocation chromosomes. This was confirmed by the presence of the same size bands in DNA of two somatic cell hybrids, A2 and B2, containing the der(X) and der(21) chromosomes (Fig. 3). From these data and similar data with other restriction enzymes, a restriction map of the der(21) translocation chromosome was constructed (Fig. 2a). Comparison of the restriction maps of the der(X) and der(21) translocation chromosomes with the restriction maps of the unrearranged X chromosome and chromosome 21 shows colinearity, indicating little or no deletion of material at the site of the translocation (Fig. 2a).

To examine the junction region in more detail, we cloned the 2.9-kb Eco RI–junction fragment E2.9 (Fig. 2a). DNA from the hybrid B2 was digested with Eco RI and fragments of 2.6 to 3.2 kb were purified by gel electrophoresis. The size-fractionated DNA was eluted from the gel block and used to prepare a library in the phage vector λgt10 (*11*). Approximately 10^6 recombinant phage were plated on *Escherichia coli* DB1161 (*12*) and screened with the X-derived unique sequence probe XJ2.2 (Fig. 2a), a process that yielded seven positive clones. Each of the clones also hybridized with the rDNA fragment A_{BE} (Fig. 2a), which confirmed that the clones contained ribosomal gene sequences from chromosome 21. Restriction mapping of a plasmid subclone that contained the insert from one of these phage confirmed the presence of Bam HI, Hind II, and Hind III sites as predicted by the map obtained by Southern blotting (Fig. 2, a and b).

To examine the translocation breakpoint at the nucleotide level, we had to sequence a normal X chromosome for comparison. The most appropriate X chromosome for this analysis was that from the patient's father, since it had been shown by restriction fragment length polymorphism (RFLP) mapping that the patient's translocation originated in her father's X chromosome (*13*). This would eliminate any ambiguities due to individual heterogeneity of X-chromosome sequences. The 2.7-kb Eco RI fragment E2.7 (Fig. 2a) from the region of the junction was selected for cloning and sequencing. DNA from the father was digested with Eco RI and fragments of 2.6 to 3.0 kb were cloned into λgt10, which yielded a library of approximately 10^5 recombinants. The library was screened with two X-derived unique sequence probes, XJ2.2 and XJ1.1 (Fig. 2a), one from each side of the translocation on the X chromosome. Two phage clones positive for both probes were purified and the inserts were subcloned into plasmids and mapped with restriction endonucleases.

The analysis of the der(X) translocation

a

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   1 GTCGACCACA TGGTTGGTGT TACATAACAA GCCATTGAAA ATAAAGCAAG CAAGCAATTA
  61 AGAAGGTACC AAACCCTGCT CAATTCTATT TTGTGGGTTT TGACCCCGTG ATTTAGCCTA
 121 TCATGAAAAA TTCTCATCTT CATTTTGACC CCCCACCCAG ATTTGTGCCA TCTGCAAAAT
 181 TGAAAATTAc CCCTTTGCTG ATGTTATGTA AGGTATTGAT CAATTATTCA GCAGCACAGA
 241 ACCTGTGGTA GCTTACTAAT TATAATAAAA CAATATTTAG AGTTGCTGGT CACTTAATAT
 301 CTACCAAGCA CCTTTTTAAA TACTTTTACA TGTATGTAAC TACATACATA CATGTTAAAC
 361 TTAAATATTT TCAAAACAAT CTTATCAGAT GGATACAATT ATCGCCATTT TACAGATGAG
 421 GAAACTGAAA CACAGTATGA CTAACTTTCT GAGGGCCACA CAGCTTTTAA GTAATACAGC
 481 TAGGATTCAA ACCCAAGCAG TGCATCTCAA GATCTCAGAC TTTTACCACT CTGCCCTCCT
 541 GACTAAAATG TGTATTCTAT ATACAGAGCT CACTCTCCCA AGTCAATATG GTTATTAATT
 601 CCTCTGCCAA CTCTGTCATA TAGATCACTT ATCACCATCT TATCCCGATA CCATAGAACC
 661 TTAGCCACTG TTTTACTCAA ATAGAAATAA ACCATTAAAA AAAAAAAATA GGTGCCACAG
 721 TAAAAGTATG ATCTCTTCAA CCTGATGAGA CCATCACAAC CAGTCTCAAA ACTTCTTTTC
 781 TGAAGAACAA GCTATTAATT TAGATGAATG AGTCAATAAC AAACTTATCG CAGATTTAAA
              ^ ^
 841 CATACAGAAA TTTTCACGGC AAATCTGTAA AATATGAGGT CAATAAATAT CTAATTTATA
                         ^
 901 CTCAATAGCA TTTATACTGT TATAGAAATG ATGCAATATA CAAGACACAT AACTGTACAT
 961 AACACCCTAG CCATTAAATC ATTGTGCAGA TGGCTCCCAC TATCATTGTG ACTTCTGTGC
1021 CTCTTTCCCC ATAGTGTTTG CCAAGCCATA AGCTGATATT TTATTTTACC TTCAGTTCTC
1081 TGTACATTAC AGCTTCTCTG CCTGCAACAT CCTTTTCATC TGGCTCCTTT ACATGTCAGG
1141 TAAGTTACCA CTGCCCCTTC AAAGCCTTTC CTTACGGCCT GAGTTTTGGT TCAATGCATG
1201 ACCTGACTGT AAAACACTAT TTCCCACATC ATGTATGTCT TTCCCTACTC ATCACATTGC
1261 TGAAGAATTG CCCATGTATC TGCTTGTCTC CCATGATAAG GACAATGTCT TTTTCCTTTA
1321 TAACATAATG AGGAGAGCTC
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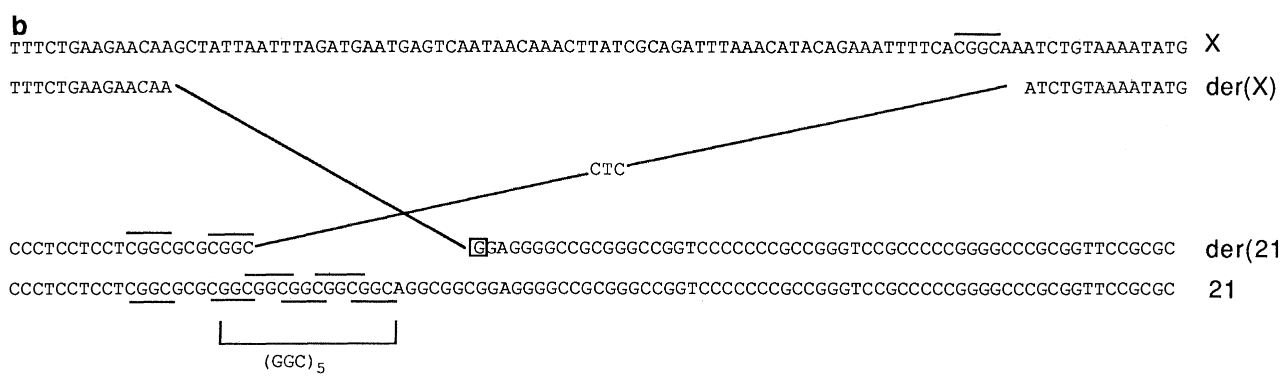

Fig. 4. (**a**) Sequence from the X chromosome surrounding the translocation breakpoint. The actual breakpoints occurred after nucleotides 790 or 791 on the der(21) chromosome and nucleotide 862 on the der(X) chromosome. (**b**) Diagram of the origin of the nucleotides on the translocated chromosomes. On the translocation-derived chromosomes there are 71 to 72 nucleotides not accounted for from the X chromosome and 16 to 23 nucleotides not accounted for from chromosome 21. There are also an additional three residues (CTC) on the der(X) chromosome not found on the X chromosome or chromosome 21. The G residue on the der(21) chromosome at the breakpoint, which could have originated from either the X chromosome or chromosome 21, is boxed. The sequence of the rDNA region presented here is from nucleotide 3443 to 3541 from the published 28*S* sequence containing five GGC repeats near the translocation breakpoint (*14*). The CGGC tetranucleotides are underlined or overlined.

breakpoint was continued by subcloning the 1.4-kb Bam HI–Eco RI fragment BE1.4 (Fig. 2a) from XJ1 into the plasmid vector pSP65 (Fig. 2a). Detailed restriction maps of the cloned regions of the der(X) and der(21) chromosomes and of the X chromosome are shown in Fig. 2b, and the fragments indicated were subcloned into plasmid vectors for sequencing as described in the figure legend.

The sequence of 1340 base pairs from the X chromosome of the patient's father is presented in Fig. 4a. Comparison of this sequence with the published 28*S* rDNA sequence (*14*) showed no homologous regions, consistent with the fact that the rDNA genes are extremely GC-rich, whereas the sequence surrounding the translocation is very AT-rich. A comparison of this sequence with those in GenBank did not show any notable homology. Analysis of this sequence for homology to numerous subsequences including the immunoglobulin switch recombination signals (*15*), the chi sequence (*16*), the HO/*MAT* locus recombinase (*17*), an Alu sequence (*18*), and Line sequences (*19*) showed no significant homologies.

The sequences spanning the breakpoints of the two translocation-derived chromosomes and the corresponding sequence of the unrearranged X chromosome are presented in Fig. 4b. For comparison, the sequence of a 28*S* rDNA gene that represented the breakpoint region on chromosome 21 is included (*14*). The translocated chromosomes have lost 71 to 72 nucleotides from the X chromosome and 16 to 23 nucleotides from chromosome 21. A single base uncertainty is generated by the G residue [seen at the breakpoint on the der(21) chromosome], which could have originated from either the X chromosome or chromosome 21. A second uncertainty arises because the trinucleotide GGC, found at the site of the translocation on chromosome 21, is repeated in different rDNA genes from four to six times (Fig. 4b shows five GGC repeats) (*20*). The der(X) sequence has one GGC present at the breakpoint, meaning that three to five GGC trinucleotides are missing in the der(X) chromosome, depending on the number present in the repeat unit actually involved in the translocation. There are three nucleotides (CTC) at the breakpoint of the der(X) chromosome whose origin is uncertain.

The presence of the CGGC tetranucleotide was noted in the immediate vicinity of the breakpoint on the der(X) chromosome, repeated six times on chromosome 21 and one time on the X chromosome (Fig. 4b). Although it is uncertain whether this limited homology could be used as an explanation for the translocation, it may be a recognition site for an enzyme involved in the translocation process. It has been suggested that the tetranucleotide GAGG, which occurs within 11 to 12 nucleotides of a c-*myc*–immunoglobulin (Ig) heavy chain translocation breakpoint in five out of six translocations in murine plasmacytoma lines, may have been recognized by an enzyme with a DNA-cleaving ability (*21*). As such, the tetranucleotide sequence observed here could be involved in the origin of the translocation. This CGGC tetranucleotide is also implicated because the region of the rDNA gene that joins the X chromosome is a region that shows marked variability in length between individuals (*22*). The region is composed of GGC repeats that can generate variable lengths by unequal homologous sister chromatid exchange. It is possible that a similar recombination event involving this region and the CGGC tetranucleotide on the X chromosome led to the translocation. Since an association of the sex vesicle with the nucleolus in human sperm has been described (*23*), it is possible that a normal ribosomal meiotic recombination event went awry. For example, a break in the ribosomal gene distal to a ribosomal CGGC might allow a single strand of rDNA to displace one X strand at the CGGC tetranucleotide, possibly mediated by a recA type of mechanism (*24*).

Although numerous tumor-associated translocations have been studied in molecular detail (*25*), we know of no other mammalian constitutional translocation that has been studied at the nucleotide level. The Ig heavy and light chain genes and the T cell receptor (TCR) gene are frequently involved in translocations associated with malignancies, and it has been suggested that the inherent recombinatorial activity of the Ig and TCR loci may occasionally promote intermolecular recombination rather than intramolecular recombination (*26*). The regions involved in the translocation described here are not tumor-associated nor do they have any relation to known Ig genes or TCR genes. Thus, the causative mechanism of constitutional translocation may not be the same as the mechanism of somatic rearrangement that is involved with Ig or TCR genes. As other constitutional translocations are studied it will be of interest to determine if these occur primarily between regions containing repeat units as seen in a number of Philadelphia chromosome translocations (*27*). It will also be important to determine if long or short regions of homology exist at the site of exchange, and it will be interesting to see if translocations occur preferentially between transcriptionally active regions as seen in our patient.

There are 20 known cases of DMD/BMD females who carry X-autosome translocations with breakpoints at Xp21 (*5*). The t(X;21) translocation is the first one to be studied at the molecular level, and our analysis has demonstrated that there is no major secondary rearrangement at the site of the translocation. Other studies from our laboratory (*28*) have demonstrated that the dystrophic phenotype in the t(X;21) translocation-carrying female can be accounted for because the translocation breaks through an intron of the gene, separating a few exons at the 5′ end on the der(X) chromosome from the remainder of the gene on the der(21) chromosome. Translocation exchange points in other female DMD/BMD patients map both distal and proximal to the t(X;21) translocation studied here (*29*). It is not yet clear whether these translocations also act by disrupting the coding region of the gene, or whether they act by some other mechanism. For example, some of these translocations might act by separating the gene from a cis-acting regulatory element. With the rapid generation of pulsed-field gel electrophoresis maps and more detailed restriction maps of the DMD/BMD locus, combined with the availability of numerous probes spanning this locus (*30*), it is only a matter of time before other translocation breakpoints are cloned and sequenced. This will provide comparative data to help elucidate the mechanism by which these translocations arose, and the mechanism by which they cause the disease.

REFERENCES AND NOTES

1. H. M. Kingston, M. Sarfarazi, N. S. T. Thomas, P. S. Harper, *Hum. Genet.* **67**, 6 (1984).
2. L. M. Kunkel *et al.*, *Nature (London)* **322**, 73 (1986).
3. K. E. Davies *et al.*, *Nucleic Acids Res.* **11**, 2303 (1983).
4. A. Clarke *et al.*, *J. Med. Genet.* **23**, 501 (1986).
5. Y. Boyd *et al.*, *ibid.*, p. 484.
6. C. Verellen-Dumoulin *et al.*, *Hum. Genet.* **67**, 115 (1984).
7. R. G. Worton, C. Duff, J. E. Sylvester, R. D. Schmickel, H. F. Willard, *Science* **224**, 1447 (1984).
8. P. N. Ray *et al.*, *Nature (London)* **318**, 672 (1985).
9. M. W. Thompson *et al.*, *J. Med. Genet.* **23**, 548 (1986).
10. P. N. Ray *et al.*, in preparation.
11. T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning*, D. M. Glover, Ed. (IRL, Oxford, 1986), vol. 1, pp. 49–78.
12. A. R. Wyman, L. B. Wolfe, D. Botstein, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2880 (1985).
13. V. M. Kean *et al.*, *J. Med. Genet.* **23**, 491 (1986).
14. I. L. Gonzalez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7666 (1985).
15. T. Honjo, *Annu. Rev. Immunol.* **1**, 499 (1983).
16. G. R. Smith, S. M. Kunes, D. W. Schultz, A. Taylor, K. L. Triman, *Cell* **24**, 429 (1981).
17. J. A. Nickoloff, E. Y. Chen, F. Heffron, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7831 (1986).
18. C. W. Schmid and W. R. Jelinek, *Science* **216**, 1065 (1982).
19. M. Hattori, S. Hidaka, Y. Sakaki, *Nucleic Acids Res.* **13**, 7813 (1985).
20. I. L. Gonzalez, R. D. Schmickel, J. E. Sylvester, unpublished observations.

21. S. P. Piccoli, P. G. Caimi, M. D. Cole, *Nature (London)* **310**, 327 (1984).
22. J. L. Gorski, I. L. Gonzalez, R. D. Schmickel, *J. Mol. Evol.* **24**, 236 (1987).
23. A. Stahl, M. Hartung, M. Devictor, J.-L. Berge-Lefranc, *Hum. Genet.* **68**, 173 (1984).
24. S. M. Honigberg, B. J. Rao, C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9586 (1986).
25. H. E. Varmus, *Annu. Rev. Genet.* **18**, 553 (1984); C. M. Croce and P. C. Nowell, *Adv. Immunol.* **38**, 245 (1986).
26. L. R. Finger, R. C. Harvey, R. C. A. Moore, L. C. Showe, C. M. Croce, *Science* **234**, 982 (1986); F. G. Haluska, S. Finver, Y. Tsujimoto, C. M. Croce, *Nature (London)* **324**, 158 (1986).
27. A. de Klein *et al.*, *Nucleic Acids Res.* **14**, 7071 (1986).
28. A. H. M. Burghes *et al.*, *Nature (London)* **328**, 434 (1987).
29. Y. Boyd *et al.*, *Clin. Genet.* **31**, 84 (1987).
30. A. P. Monaco and L. M. Kunkel, *Trends Genet.* **3**, 33 (1987); R. G. Worton and A. H. M. Burghes, *Int. Rev. Neurobiol.*, in press.
31. J. M. Erickson, C. L. Rushford, D. J. Dorney, G. N. Wilson, R. D. Schmickel, *Gene (Amsterdam)* **16**, 1 (1981); J. E. Sylvester *et al.*, *Hum. Genet.* **73**, 193 (1986).
32. M. Hattori and Y. Sakaki, *Anal. Biochem.* **152**, 232 (1986).
33. P. G. Sealey, P. A. Whittaker, E. M. Southern, *Nucleic Acids Res.* **13**, 1905 (1985).
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