© 2000 Oxford University Press
Human Molecular Genetics, 2000, Vol. 9, No. 11 **1665–1670**

Regions of genomic instability on 22q11 and 11q23 as the etiology for the recurrent constitutional t(11;22)

Hiroki Kurahashi[1,+], Tamim H. Shaikh[1+], Ping Hu[3], Bruce A. Roe[3], Beverly S. Emanuel[1,2,§] and Marcia L. Budarf[1,2]

[1]Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA, [2]Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA and [3]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019, USA

Received 17 March 2000; Revised and Accepted 28 April 2000

The constitutional t(11;22)(q23;q11) is the only known recurrent, non-Robertsonian translocation. To analyze the genomic structure of the breakpoint, we have cloned the junction fragments from the der(11) and der(22) of a t(11;22) balanced carrier. On chromosome 11 the translocation occurs within a short, palindromic AT-rich region (ATRR). Likewise, the breakpoint on chromosome 22 has been localized within an ATRR that is part of a larger palindrome. Interestingly, the 22q11 breakpoint falls within one of the 'unclonable' gaps in the genomic sequence. Further, a sequenced chromosome 11 BAC clone, spanning the t(11;22) breakpoint in 11q23, is deleted within the palindromic ATRR, suggesting instability of this region in bacterial clones. Several unrelated t(11;22) families demonstrate similar breakpoints on both chromosomes, indicating that their translocations are within the same palindrome. It is likely that the palindromic ATRRs produce unstable DNA structures in 22q11 and 11q23 that are responsible for the recurrent t(11;22) translocation.

INTRODUCTION

Geneticists have long been interested in understanding the mechanisms and consequences of genomic rearrangement. Non-random chromosomal changes have been demonstrated in association with numerous human acquired and inherited diseases. For example, cytogenetic and molecular studies of human tumors have clearly demonstrated that there are non-random, acquired rearrangements which play an etiological role in tumorigenesis. In addition, recurrent constitutional translocations, inversions and deletions suggest that there may be preferred chromosomal sites for recombination or rearrangement in the human genome. Despite the fact that chromosome 22 represents only ~1.9% of the haploid autosome length (1), numerous rearrangements of this chromosome have been associated with both a variety of malignant diseases and developmental abnormalities (2). Numerous clinically significant human diseases associated with translocations, duplications or deletions of 22q11 are included, suggesting non-random involvement of this region in chromosomal rearrangement and/or instability.

The constitutional t(11;22)(q23;q11) is the only known recurrent, non-Robertsonian translocation in humans. Balanced translocation carriers have no clinical symptoms and clustered breakpoints have been reported in numerous unrelated families (3,4). Carriers are often identified after the birth of unbalanced offspring with supernumerary der(22)t(11;22) syndrome. Patients with supernumerary der(22) syndrome have a distinctive phenotype which consists of severe mental retardation, preauricular tag or sinus, ear anomalies, cleft or high arched palate, micrognathia, heart defects and genital abnormalities in the male (5). This syndrome arises through 3:1 meiotic malsegregation of the balanced translocation in meiosis (3). To date, a molecular etiology for the recurrent nature of this translocation has not emerged.

The t(11;22) breakpoint on 22q11 localizes to one of the chromosome 22-specific low copy repeats (LCRs), which have been identified at multiple loci on 22q11 (3,6,7). Although chromosome 22 has been almost entirely sequenced, the LCR where the t(11;22) breakpoint resides (LCR-B in ref. 7) still contains a gap (6,7). Further, the duplicated nature of the LCR did not allow us to determine the precise location of the breakpoint. The chromosome 11 side of the 11;22 translocation breakpoint has previously been localized within a sequenced BAC clone (3,4). To analyze the breakpoint further, junction fragments from the der(11) and the der(22) of a t(11;22) balanced carrier have been cloned. Here we show that palindromic AT-rich sequences surround the breakpoints on chromosomes 11 and 22. Computer analysis of the DNA sequence flanking the breakpoints predicts formation of hairpin or cruciform structures. It is likely that these unstable DNA structures in 22q11 and 11q23 facilitate the recurrent t(11;22) translocation.

[+]These authors contributed equally to this work

[§]To whom correspondence should be addressed at: The Children's Hospital of Philadelphia, 1002 Abramson Research Center, 3516 Civic Center Boulevard, Philadelphia, PA 19104, USA. Tel: +1 215 590 3856; Fax: +1 215 590 3764; Email: beverly@mail.med.upen.edu

RESULTS AND DISCUSSION

Recent studies have utilized fluorescence *in situ* hybridization (FISH) to examine clustering of the chromosome 11 and 22 breakpoints of the t(11;22). The chromosome 11 breakpoint of the 11;22 translocation has previously been localized within BAC clone b442e11 (3). This BAC clone has been completely sequenced (GenBank accession no. AC007707). To further sublocalize the breakpoint, multiple PCR primer pairs were designed from the BAC sequence and used to PCR amplify DNA from a somatic cell hybrid, Cl4/GB, containing the der(22) from a t(11;22) carrier (8). The c14h primer pair amplifies the corresponding genomic segment from the hybrid DNA while the c14i primer pair does not (Fig. 1a). Thus, the breakpoint is located between these PCR units. Based on the BAC sequence, these PCR products are separated by ~500 bp in b442e11 and they flank a previously described 130 bp AT-rich region (ATRR) (4). However, PCR amplification of the ATRR from human genomic DNA yields a PCR product which is 200 bp longer than that amplified from b442e11, suggesting that the BAC has sustained a 200 bp deletion within the ATRR. PCR amplification of 10 additional genomic DNAs from normal individuals yields the longer PCR product, indicating that the shorter product is unlikely to be a polymorphism. The sequence of the longer PCR product demonstrates that the deleted segment contains a palindromic ATRR in the vicinity of the breakpoint (Fig. 1b).

To identify the junction fragments, Southern hybridization was performed using either the c14i or c14h PCR products to probe DNAs from several t(11;22) carriers (Fig. 2, lanes 2 and 3) and the Cl4/GB hybrid (Fig. 2, lane 1). The sequence of the BAC indicates that both c14i and c14h reside on the same 4 kb *Hin*dIII fragment (Fig. 1a). When hybridization was performed with the proximal probe, c14i, in addition to the normal 4 kb fragment, a novel 4.5 kb rearranged fragment from the der(11) was detected in *Hin*dIII-digested DNA from the balanced carriers (Fig. 2a). When hybridization to the same membrane was performed with the c14h probe, a 9 kb rearranged fragment from the der(22) was detected (Fig. 2b). Several additional restriction enzymes were used to confirm the results and rearranged fragments in *Eco*RI- or *Bst*YI-digested DNA were detected with the c14h probe.

To further characterize the breakpoint region, a phage library was constructed from the *Hin*dIII-digested genomic DNA of a translocation carrier whose rearranged fragments had been identified by Southern analysis (GB in Fig. 2). The resulting library was screened with the c14i probe to obtain the der(11) junction fragment (Fig. 3a). Sequence comparison of the der(11) junction fragment with the normal chromosome 11 sequence confirmed that the breakpoint was within the palindromic ATRR on 11q23 (Fig. 3b). The 11q23 homologous portion of the junction fragment was followed by a longer ATRR and additional DNA sequence, which was presumably from 22q11. BLAST (9) database searches with ~500 bp of this sequence indicate that it shares high homology (99%) with a previously identified duplicated sequence (10), which is included within larger LCRs on 22q11 (6,7).

The duplicated sequence present at the junction of the t(11;22) has previously been implicated in the t(17;22) breakpoint of a patient with neurofibromatosis 1 (NF1) (10). The 17;22 translocation in this individual disrupts the *NF1* gene on chromosome 17 and resides within LCR-B on chromosome 22 (B.S. Emanuel, unpublished data). The sequence of the chromosome 22 portion of the der(17) junction fragment from the t(17;22) and the der(11) junction fragment from the t(11;22) are similar to each other. This suggested that the t(11;22) and the t(17;22) might occur by a similar mechanism and may involve similar breakpoints on chromosome 22. Based on this hypothesis, a PCR primer (JF22.1) was designed from the chromosome 22 sequence of the der(22) junction fragment of the t(17;22)

Figure 1. Genomic structure and restriction map of the t(11;22) breakpoint region on 11q23. (**a**) Location of the breakpoint on 11q23. The portion of 11q proximal to the breakpoint and present in the der(11) is depicted as a white box and the portion distal to the breakpoint and present in the der(22) is depicted by a gray box. The breakpoint-spanning BAC b442e11 is also shown. In addition to the Apo gene cluster, b442e11 contains two other genes. The first, *ZNF259* (GenBank accession no. NM_003904), is a zinc finger gene homologous to rodent *ZPR1* and is located ~24 kb proximal to the breakpoint. The second, *KIAA0999*, exists only as a cDNA sequence in GenBank (accession no. AB023216) and is located ~30 kb distal to the breakpoint. Both genes are oriented telomere to centromere and no genes appear to be disrupted by the translocation. The PCR-based markers used to further localize the breakpoint are shown as small hatched boxes. The breakpoint was localized using a somatic cell hybrid (Cl4/GB) containing the der(22) of a balanced t(11;22) carrier (8). H, *Hin*dIII. (**b**) Sequence of the region flanking the t(11;22) breakpoint on 11q23. The orientation of the sequence is centromere to telomere. PCR primers are indicated by arrows: full arrows designate c14irev and c14ifor; dashed arrows designate c14hrev and c14hfor. The ATRR between c14i and c14h is boxed. The sequence deleted in b442e11 but present in genomic DNA is shown in bold.

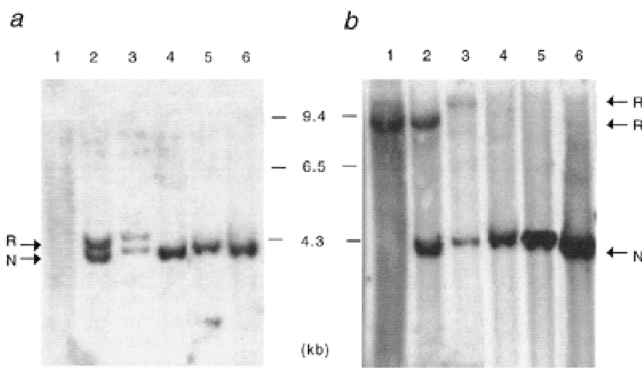
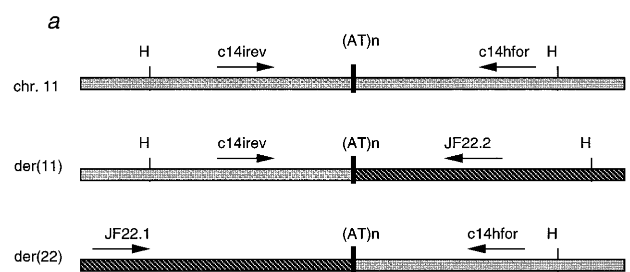


Figure 2. Identification of t(11;22) junction fragments using Southern hybridization. Lane 1, somatic cell hybrid Cl4/GB; lane 2, balanced carrier GB; lane 3, balanced carrier SK; lanes 4–6, normal controls. Size markers are indicated in the center. (**a**) Autoradiogram of *Hin*dIII-digested genomic DNA probed with c14i. The novel 4.5 kb rearranged fragment (R) from the der(11) in both balanced carriers is indicated. The normal 4 kb band (N) was detected in both balanced carriers as well as the three normal controls (lanes 4–6). The somatic cell hybrid Cl4/GB contains only the der(22) of the t(11;22) and is therefore negative for any fragments hybridizing to probe c14i, which is present on the normal 11 and der(11) chromosomes. (**b**) Autoradiogram of *Hin*dIII-digested DNA probed with probe c14h. The same blot as used in (a) was used. An ~9 kb rearranged fragment (R) was detected in Cl4/GB (lane 1) and both the balanced carriers (lanes 2 and 3). The normal 4 kb band (N) was detected in both balanced carriers as well as the three normal controls (lanes 4–6). Since the somatic cell hybrid Cl4/GB contains the der(22) but not the normal chromosome 11, it is positive only for the novel ~9 kb fragment.

(Fig. 3a) (10). Primers JF22.1 from chromosome 22 and c14hfor from 11q23 were used to amplify genomic DNA from a t(11;22) balanced carrier and normal controls. A PCR product was obtained only from DNA of the translocation carrier, which on sequence analysis was confirmed to be the der(22) junction fragment. Analysis of the der(22) junction fragment demonstrated that the 11q23 breakpoint is located within the palindromic ATRR (Fig. 3c). Interestingly, the chromosome 22 breakpoint also occurs within an ATRR flanked by sequences homologous to the previously described duplicated sequence on chromosome 22 (Fig. 3c) (10). Additional analysis of the sequence of the der(22) and der(11) junction fragments demonstrates that the 500 bp sequence flanking the t(11;22) breakpoint on chromosome 22 is also palindromic (Figs 3c and 4c).

The der(11) junction fragment was amplified by PCR with primers JF22.2 and c14irev (Fig. 3b) from five t(11;22) balanced carriers representing three generations of GB's family. Sequence analysis demonstrated that their breakpoints are identical, suggesting that the translocated chromosome is stably transmitted through generations. The der(11) breakpoints of two additional translocation carriers from two separate unrelated t(11;22) families, one of which was also analyzed by Southern hybridization (SK in Fig. 2), were studied by PCR and subsequent sequencing and their breakpoints are similar to the one detected in the first family. In these additional families the breakpoints localize within the palindromic ATRR of chromosome 11. Comparable results were obtained from the der(22) junction fragment from all three t(11;22) families (data not shown). The size variation observed in the rearranged fragments by Southern blot analysis (Fig. 2b) presumably resulted from restriction fragment length polymorphisms in the different families tested.



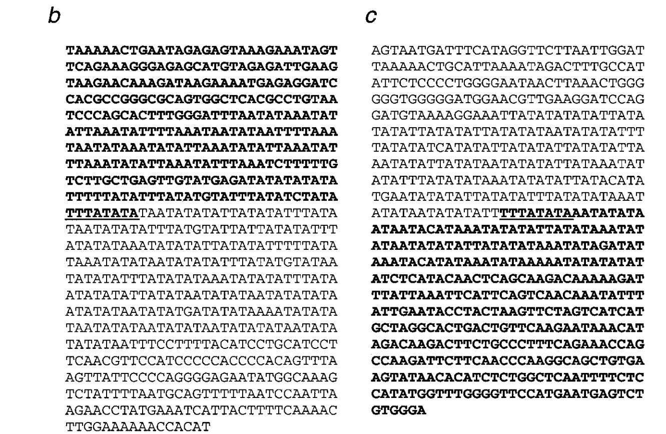


Figure 3. Sequence and genomic structure of regions flanking the t(11;22) breakpoint. (**a**) Genomic structure and restriction map of the normal chromosome 11 and the der(11) and der(22) junction fragments. Chromosome 11 is indicated by gray boxes and chromosome 22 by hatched boxes. The t(11;22) breakpoint is indicated by a thick vertical line. Thin vertical lines indicate restriction sites. H, *Hin*dIII. PCR primers are indicated with their orientation shown by arrows. (**b**) Sequence of the der(11) junction fragment from balanced carrier GB. This sequence was obtained by sequencing the 4.5 kb *Hin*dIII junction fragment cloned into the λZAP phage vector. (**c**) Sequence of the der(22) junction fragment from balanced carrier GB. This sequence was obtained after PCR with primers c14hfor and JF22.1. In (b) and (c) the sequences from chromosome 11 are shown in bold and those from chromosome 22 are shown in plain type. Eight base pairs which cannot be definitely assigned to chromosome 11 or 22 are underlined. The translocation breakpoint on 11q23 is within the palindromic ATRR. The sequence of the chromosome 22 portion of the junction fragments demonstrates that the breakpoint is within an ATRR that is part of a larger palindrome. Two additional unrelated t(11;22) families were analyzed by PCR to obtain junction fragments. The der(11) junction fragment was amplified with primers c14irev and JF22.2 and the der(22) junction fragment was amplified with primers JF22 and c14hfor. The PCR products were sequenced. The sequences obtained from these two families were similar to those obtained from balanced carrier GB as shown in (b) and (c).

Chromosome 22-specific duplicated blocks referred to as LCRs have been identified at multiple loci on 22q11 (6). The t(11;22) breakpoint on 22q11 localizes to one of these repeats, previously reported as LCR-B or LCR22-3a (3,7,11) (Fig. 4a). Although chromosome 22 has been almost entirely sequenced, a small number of gaps remain because of incomplete clone coverage across 22q (6). LCR-B, where the t(11;22) breakpoint resides, contains one such gap which we estimate to be ~90 kb (Fig. 4b). The sequence of the chromosome 22 portion of the der(11) junction fragment could not be identified within clones localized to LCR-B on chromosome 22. This suggests that the t(11;22) breakpoint maps within the gap between cHK89 and b562f10 (Fig. 4b). It has been suggested that this

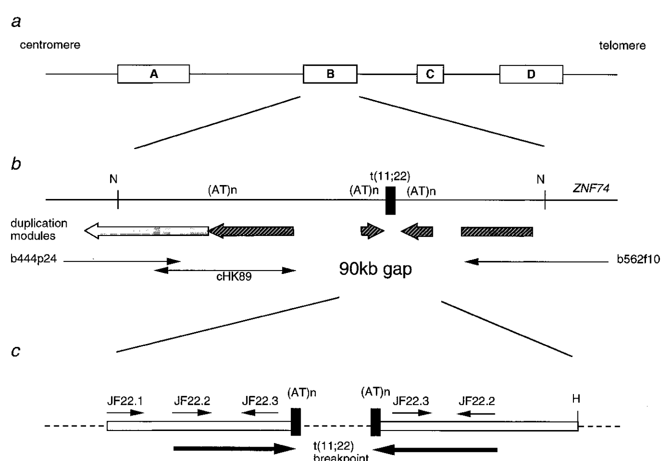


Figure 4. Genomic structure of the normal chromosome 22 in the region flanking the t(11;22) breakpoint. (**a**) Four copies of the chromosome 22-specific low copy repeats (LCRs) on 22q11 are shown. These four LCRs are designated A–D based on their order centromere to telomere (7). (**b**) The existing clone contig across LCR-B is shown. Clone cHK89 initially appeared to close the gap between b444p24 and b562f10. Detailed sequence analysis shows that it extends only ~30 kb from b444p24. A gap exists between cHK89 and b562f10, which is estimated to be ~90 kb based on the size of the *Not*I fragment (145 kb), indicated by thin vertical lines (N). The duplicated modules in LCR-B are indicated by either hatched or gray arrows. The data obtained from the junction fragments suggest that in the breakpoint region there are two copies of the duplicated modules in a head-to-head orientation. N, *Not*I. (**c**) The structure of normal chromosome 22 as derived from the der(22) and der(11) junction fragments is shown. Sequence of the region proximal to the breakpoint was derived from the der(22) junction fragment, while the distal sequence was derived from the der(11). H, *Hin*dIII. The breakpoint is flanked on either side by ATRRs which are part of long palindromes (bold arrows).

region contains sequences that are unclonable in bacteria (3,7,11). The identification of palindromic sequences flanking the t(11;22) breakpoint lends support to this hypothesis (Fig. 4c), since palindromes have been previously shown to be unstable in *Escherichia coli* (12). Copies of JF22.2 are present on either side of the breakpoint in a head-to-head orientation. Thus, although we expected to generate a breakpoint-spanning product from the normal chromosome 22 by PCR with the single JF22.2 primer (Fig. 4c), none was detected. One reason for the failure to generate this PCR product may be that the duplicated sequences on chromosome 22 flanking the t(11;22) breakpoint are not tractable to PCR. Alternatively, it is possible that the t(11;22) involves a more complex rearrangement that may have deleted a region of genomic DNA flanking the breakpoint. Finally, because the sequence of this region is presently unknown (6), the distance separating the primers may be greater than can be amplified in genomic DNA. It is clear that the region of 22q11 involved in the t(11;22) is a hotspot for various constitutional translocations (10,13). This suggests that the genomic structure of the region flanking the 22q11 breakpoint of the t(11;22) may be unstable, making it susceptible to rearrangement.

It has been observed that unusual DNA structures can promote genetic instability in prokaryotic as well as eukaryotic genomes (12,14–16). Palindromic DNA can lead to the formation of single-stranded hairpin or double-stranded cruciform structures. These hairpin or cruciform structures are putative substrates for nucleases and mismatch repair enzymes. Experiments in bacteria and yeast have shown that palindrome-mediated instability increases as hairpin structures are cleaved to create deletions and double-stand breaks (DSBs), respectively (14,16). In yeast, DSBs are hotspots for recombination during meiosis (17). Recent reports suggest that palindrome-mediated hairpins show instability in mammalian cells because of breaks generated at the hairpin structure by a hairpin-nicking enzyme (15,18).

Palindromic sequences have been identified near the breakpoints of the t(11;22) translocation at 11q23 and 22q11. Using mfold (http://BiBiServ.TechFak.Uni-Bielrfeld.DE/fold/), software that predicts secondary structure in RNA and single-stranded DNA, sequence derived from a normal individual who does not carry the t(11;22) was submitted to analysis. A hairpin/cruciform structure was predicted for the palindromic ATRR flanking the breakpoint on 11q23 (Fig. 5a). Longer ATRR-containing palindromic sequences also flank the breakpoint on 22q11 (Fig. 4c), suggesting a similar hairpin/cruciform structure. It is interesting to note that the breakpoints on both chromosomes are within the ATRRs. AT-rich sequences have a lower melting temperature and this may further facilitate hairpin formation at physiological temperatures. These observations have led us to propose a model for the formation of the recurrent, constitutional t(11;22) (Fig. 5b). In this model the palindromic sequences flanking the breakpoint on both 11q23 and 22q11 would facilitate the formation of hairpin/cruciform structures. The proposed hairpin-nicking activity would create DSBs on both chromosomes (15). Illegitimate reciprocal exchange between the disrupted chromosomes 11 and 22 would lead to formation of a translocation between them (Fig. 5b). The identification of unstable DNA sequences near the t(11;22) breakpoints would appear to be an important first step towards designing and testing different models for the formation of this recurrent translocation. This would include analysis of the orientation or position of chromosomes 11q23 and 22q11 during meiosis, as well as attempts to recapitulate the rearrangement in a yeast assay system. Further, these data indicate a precise molecular location for the 11;22 translocation breakpoints within the 11q23 and 22q11 regions. This finding lends itself to the analysis of additional translocations occurring at these chromosomal sites.

MATERIALS AND METHODS

Samples

All samples used in this study were derived from patients participating in a research study of the t(11;22) as reported previously (3). Genomic DNA from the patients was isolated as described (3). The somatic cell hybrid (Cl4/GB) containing the der(22) is derived from GB, one of the balanced carriers used in this study, and has been reported previously (8).

Southern analysis

The isolation of BAC clone b442e11 has been described previously (3). b442e11 was entirely sequenced at the University of Oklahoma Advanced Centre for Genome Technologies (http://

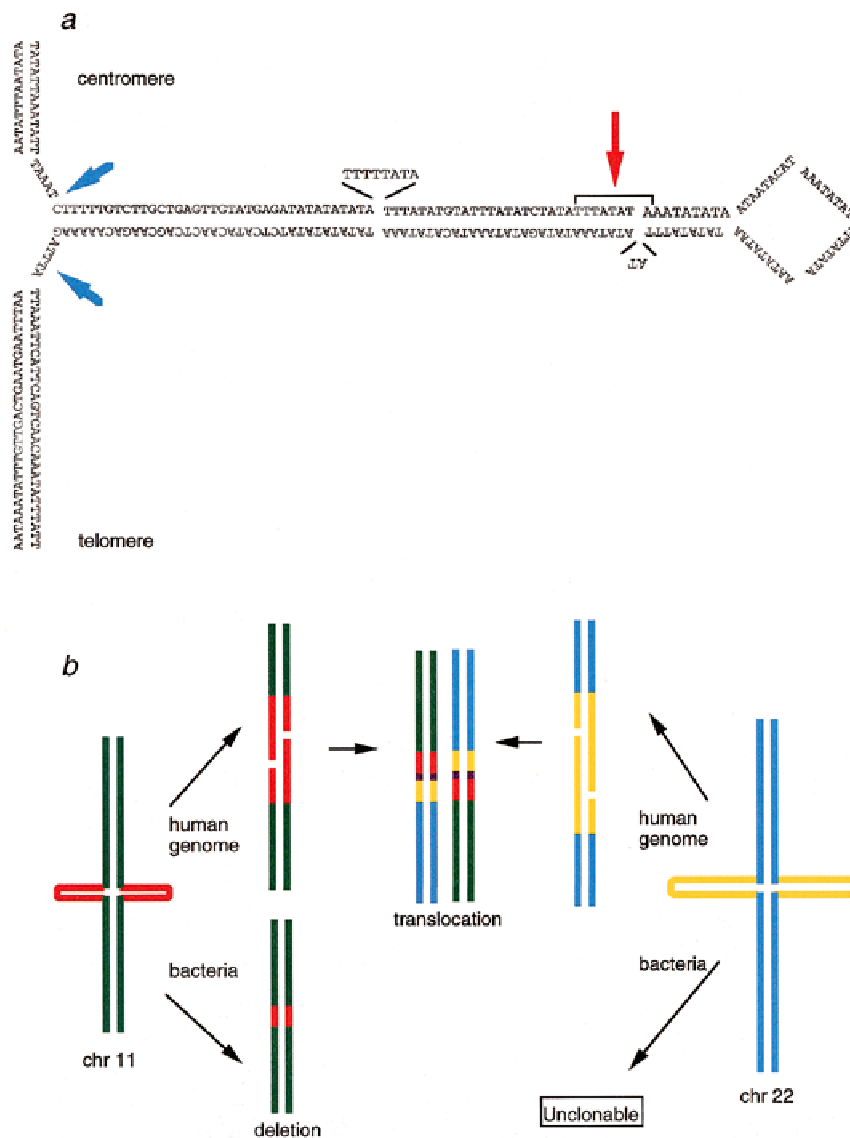


Figure 5. Proposed model for secondary structure formation and the mechanism involved in the translocation. (**a**) Putative hairpin structure of palindromic ATRR from 11q23 in DNA from a normal control as predicted by secondary structure prediction software *mfold* (http://BiBiServ.TechFak.Uni-Bielefeld.DE/mfold/). There are two mismatched regions in the hairpin. The mismatched regions in the stem of the hairpin may be more sensitive to nuclease activity causing nicks at these regions. This hypothesis is supported by the observation that the 11q23 breakpoints of the t(11;22) localize in the vicinity of one of the two mismatched regions in the stem of the putative hairpin (red arrow and bracket). The breakpoints of the region deleted in b442e11 are indicated by blue arrows. The base of the hairpin may be prone to breakage in bacterial cells, which may explain the deletion in b442e11. (**b**) Model for the mechanism involved in the translocation. Chromosome 11 is shown as green lines and the putative hairpin/cruciform caused by the palindromic ATRR is shown in red. Chromosome 22 is shown as blue lines and the putative hairpin/cruciform caused by the palindrome on 22q11 is shown in yellow. DSBs can occur at mismatched regions within the putative hairpins/cruciforms on both chromosomes. Illegitimate reciprocal exchange can then occur between the disrupted chromosomess 11 and 22 and, after DNA repair (purple lines), can lead to a translocation between chromosomes 11 and 22. Since palindromes are known to be unstable in *E.coli* (12), the smaller palindrome from 11q23 may be deleted during culture, as observed in the deletion in b442e11, and the larger palindrome on 22q11 might lead to large deletions in bacterial clones and therefore prove to be unclonable.

www.genome.ou.edu/) and has been deposited in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) (accession no. AC007707). The probes used for Southern analysis, c14h and c14i, were generated by PCR from b442e11. The primers used were as follows: c14hfor, 5′-AACACTCCCACTGACAGCTA-3′; c14hrev, 5′-GGTTTGGGGTTCCATGAATG-3′; c14ifor, 5′-CTCTACATGCTCTCCCTTTC-3′; c14irev, 5′-GGAAGT-TAGAGAAAACTGAGAA-3′. PCR conditions were as described earlier (3). Probes were radiolabeled with [α-^{32}P]dCTP using the random primer method. Southern hybridization was performed using standard methodology.

Isolation of junction fragments of the t(11;22)

Genomic DNA from translocation carrier GB was digested with *Hin*dIII and electrophoresed in low melting point agarose. The area corresponding to the rearranged junction fragment was excised and the DNA was extracted from the gel slice and

ligated to *Hin*dIII-cut λZAP vector arms (Stratagene, La Jolla, CA). The resulting phage library was processed and hybridized as per the manufacturer's protocol. The probe used for library screening was c14i.

The der(22) junction fragment was obtained by PCR with the following primers: JF22.1 (5′-GGTGTAGTCCCAGTGT-GAGT-3′) from chromosome 22; c14hfor from chromosome 11. Other nested primers from chromosome 22 used for PCR and sequencing included JF22.2 (5′-CCTCCAACGGATC-CATACT-3′) and JF22.3 (5′-TGCATCCTTCAACGTTCCA-3′) (the chromosome 22 primers correspond to a, c and b in ref. 10, respectively). PCR products were purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA). PCR products were either sequenced directly or after cloning into the TA cloning vector (Invitrogen, San Diego, CA). Sequences were analyzed with BLAST (http://www.ncbi.nlm.nih.gov/blast) (9), RepeatMasker2 (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker/) (A. Smit and P. Green, unpublished data) and mfold (http://BiBiServ.TechFak.Uni-Bielefeld.DE/mfold/).

ACKNOWLEDGEMENTS

We would like to thank Gloria Balaban and Stephanie St Pierre for their active support of our research efforts and Elaine H. Zackai and Livija Celle for assistance in obtaining patient samples for this study. This work was supported by grants CA39926 (B.S.E.), HD 26979 (B.S.E.) and HG00313 (B.A.R.) and funds from the Charles E.H. Upham endowed chair from the Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine (B.S.E.).

REFERENCES

1. Morton, N.E. (1991) Parameters of the human genome. *Proc. Natl Acad. Sci. USA*, **88**, 7474–7476.
2. Kaplan, J.C., Aurias, A., Julier, C. and Prieur, M. (l987) Human chromosome 22. *J. Med. Genet.*, **24**, 65–78.
3. Shaikh, T.H., Budarf, M.L., Celle, L., Zackai, E.H. and Emanuel, B.S. (1999) Clustered 11q23 and 22q11 breakpoints and 3:1 meiotic malsegregation in multiple unrelated t(11;22) families. *Am. J. Hum. Genet.*, **65**, 1595–1607.
4. Edelmann, L., Spiteri, E., McCain, N., Goldberg, R., Pandita, R.K., Duong, S., Fox, J., Blumenthal, D., Lalani, S.R., Shaffer, L.G. *et al.* (1999) A common breakpoint on 11q23 in carriers of the constitutional t(11;22) translocation. *Am. J. Hum. Genet.*, **65**, 1608–1616.
5. Zackai, E.H. and Emanuel, B.S. (1980) Site-specific reciprocal translocation, t(11;22)(q23;q11), in several unrelated families with 3;1 meiotic disjunction. *Am. J. Med. Genet.*, **7**, 507–521.
6. Dunham, I., Shimizu, N., Roe, B.A., Chissoe, S., Hunt, A.R., Collins, J.E., Bruskiewich, R., Beare, D.M., Clamp, M., Smink, L.J. *et al.* (1999) The DNA sequence of human chromosome 22. *Nature*, **402**, 489–495.
7. Shaikh, T.H., Kurahashi, H., Saitta, S.C., O'Hare, A.M., Hu, P., Roe, B.A., Driscoll, D.A., McDonald-McGinn, D.M., Zackai, E.H., Budarf, M.L. *et al.* (2000) Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum. Mol. Genet.*, **9**, 489–501.
8. Budarf, M.L., Sellinger, B., Griffin, C. and Emanuel, B.S. (1989) Comparative mapping of the constitutional and tumor-associated 11;22 translocation. *Am. J. Hum. Genet.*, **45**, 128–139.
9. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
10. Kehrer-Sawatzki, H., Haussler, J., Krone, W., Bode, H., Jenne, D.E., Mehnert, K.U., Tummers, U. and Assum, G. (1997) The second case of a t(17;22) in a family with neurofibromatosis type 1: sequence analysis of the breakpoint regions. *Hum. Genet.*, **99**, 237–247.
11. Funke, B., Edelmann, L., McCain, N., Pandita, R.K., Ferreira, J., Merscher, S., Zohouri, M., Cannizzaro, L., Shanske, A. and Morrow, B.E. (1999) Der(22) syndrome and velo-cardio-facial syndrome/DiGeorge syndrome share a 1.5-Mb region of overlap on chromosome 22q11. *Am. J. Hum. Genet.*, **64**, 747–758.
12. Leach, D.R. (1994) Long DNA palindromes, cruciform structure, genetic instability and secondary structure repair. *Bioessays*, **16**, 893–900.
13. Li, M., Budarf, M.L., Chien, P., Barnoski, B.L., Emanuel, B.S. and Driscoll, D.A. (1995) Clustering of DiGeorge/velocardiofacial-associated translocations suggestive of a translocation 'hotspot'. *Am. J. Hum. Genet.*, **57** (suppl.), A119.
14. Nag, D.K. and Kurst, A. (1997) A 140-bp-long palindromic sequence induces double-strand breaks during meiosis in the yeast *Saccharomyces cerevisiae*. *Genetics*, **146**, 835–847.
15. Akgun, E., Zahn, J., Baumes, S., Brown, G., Liang, F., Romanienko, P.J., Lewis, S. and Jasin, M. (1997) Palindrome resolution and recombination in the mammalian germ line. *Mol. Cell. Biol.*, **17**, 5559–5570.
16. Connelly, J.C., Kirkham, L.A. and Leach, D.R.F. (1998) The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc. Natl Acad. Sci. USA*, **95**, 7969–7974.
17. Wu, T.C. and Lichten, M. (1994) Meiosis-induced double-strand break sites determined by yeast chromatin structure. *Nature*, **263**, 515–518.
18. Collick, A., Drew, J., Penberth, J., Bois, P., Luckett, J., Scaerou, F., Jeffreys, A. and Reik, W. (1996) Instability of long inverted repeats within mouse transgenes. *EMBO J.*, **15**, 1163–1171.