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The Spreading of X Inactivation into Autosomal Material of an X;autosome Translocation: Evidence for a Difference between Autosomal and X-Chromosomal DNA

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Summary

X inactivation involves initiation, propagation, and maintenance of genetic inactivation. Studies of replication timing in X;autosome translocations have suggested that X inactivation may spread into adjacent autosomal DNA. To examine the inactivation of autosomal material at the molecular level, we assessed the transcriptional activity of X-linked and autosomal loci spanning an inactive translocation in a phenotypically normal female with a karyotype of 46,X,der(X)t(X;4)(q22;q24). Since 4q duplications usually manifest dysmorphic features and severe growth and mental retardation, the normal phenotype of this individual suggested the spreading of X inactivation throughout the autosomal material. Consistent with this model, reverse transcription–PCR analysis of 20 transcribed sequences spanning 4q24-qter revealed that three known genes and 11 expressed sequence tags (ESTs) were not expressed in a somatic-cell hybrid that carries the translocation chromosome. However, three ESTs and three known genes were expressed from the t(X;4) chromosome and thus "escaped" X inactivation. This direct assay of expression demonstrated that the spreading of inactivation from the adjoining X chromosome was incomplete and noncontiguous. These findings are broadly consistent with the existence of genes known to escape inactivation on normal inactive X chromosomes. However, the fact that a high proportion (30%) of tested autosomal genes escaped inactivation may indicate that autosomal material lacks X chromosome–specific features that are associated with the spreading and/or maintenance of inactivation.

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Introduction

X-chromosome inactivation in somatic cells of female mammals compensates for X-linked gene-dosage differences that exist between the sexes, such that XX females and XY males express many X-linked genes at the same levels. In normal females, this process occurs randomly, early in embryogenesis, and transcriptionally silences the majority of genes on one of two X chromosomes (Lyon 1961). Although the existence of and biological necessity for a phenomenon such as X-chromosome inactivation is widely accepted, the chromosomal mechanism by which this occurs is not well understood. It has been established that the initiation of X inactivation requires the presence, in cis, of the X-inactivation center (XIC) in Xq13.2 (Mattei et al. 1981; Therman and Sarto 1983; Brown et al. 1991*a*), and expression of XIST, a gene located within the XIC and transcribed only from inactive X chromosomes (Brown et al. 1991*b;* McCarry and Dilworth 1992; Richler et al. 1992; Buzin et al. 1994), has been shown to be essential and sufficient for X-inactivation initiation (Lee et al. 1996; Penny et al. 1996; Herzing et al. 1997; Lee and Jaenisch 1997). Once inactivated, the expression status of genes on the X chromosome is stably maintained in somatic-cell lineages. Some epigenetic changes associated with inactive X chromosomes include hypermethylation, especially of the CpG islands in housekeeping genes, and histone H4 underacetylation (Jeppesen and Turner 1993; Tilghman and Willard 1995). The role that these various phenomena play in X-chromosome inactivation, as well as the manner in which X inactivation proceeds along the chromosome, remains unclear.

X inactivation extends along the chromosome, in cis, such that the majority of genes along the inactivated X chromosome are transcriptionally silenced, whereas genes on the active X chromosome remain unaffected. Although most genes are silenced, more than a dozen genes have been shown to escape inactivation and are expressed from both X chromosomes (Disteche 1995; Miller et al. 1995; Brown et al. 1997). Most of these genes are located either in or near the pseudoautosomal

region or elsewhere on the short arm (Disteche 1995). This observation is particularly notable because most of Xp is a more recent evolutionary addition to the "ancestral" X chromosome (Ellis et al. 1990; Graves and Watson 1991), and therefore, may have features more consistent with autosomal DNA. As proposed by Graves and Watson (1991), it is possible that the noncontiguous regions within Xp that escape X inactivation may not possess the properties required to respond to a signal from an inactivation-control center. Such studies imply that autosomal DNA may be less susceptible to the process of X inactivation and that X-chromosomal DNA possesses special properties allowing for chromatin alteration.

It has long been recognized that the inactive X becomes heterochromatic, forms the cytologically identifiable Barr body (Barr and Bertram 1949; Van Dyke et al. 1987), and replicates later in the S-phase of the cell cycle than does the active X chromosome (Latt 1973). Thus, the phenomenon of X-inactivation progression has been linked cytologically to the timing of DNA synthesis. Using this difference in the timing of DNA synthesis, late-replication banding analyses of X;autosome translocations in both mouse and human have revealed that translocated autosomal material becomes later replicating compared with its intact autosomal counterpart and thus appears more like the adjacent inactive X chromosome (Eicher 1970; Disteche et al. 1979; Mattei et al. 1982; Keitges and Palmer 1986). Additionally, late-replication studies have suggested that the spreading of inactivation into autosomal sequences may be incomplete and discontinuous (Disteche et al. 1984; Keitges and Palmer 1986; Schanz and Steinbach 1989). The indirect, subjective nature and low resolution of late-replication banding, however, makes it difficult to draw more than general conclusions about the spreading of X inactivation into autosomal DNA.

The spreading of X inactivation into autosomal segments of unbalanced X;autosome translocations has been inferred from late-replication data and the attenuated phenotypes of translocation carriers. However, direct analysis of only a few human translocated autosomal genes has been conducted. In one study, the inactivation of superoxide dismutase in a carrier of a t(X;21) was deduced on the basis of correlations between the levels of enzyme activity and gene dosage in the proband as compared with those in controls (Couturier et al. 1979). One similar study of esterase D levels for an X;13 translocation demonstrated that the X-inactivation signal did not spread into the autosome (Mohandas et al. 1981*a*), whereas, in a study of another t(X;13) isolated in a somatic-cell hybrid, the esterase D gene was inactivated (Mohandas et al. 1982). The spreading of X inactivation into autosomal material has not been systematically evaluated by use of a transcriptional assay, nor has more than one gene been studied for any given translocation. In the present study, to determine the inactivation status of autosomal genes translocated onto the X chromosome and to assess the response of autosomal DNA to X inactivation, we have measured expression patterns of 20 loci that span the length of the translocated chromosome 4 material in a nondysmorphic female with an unbalanced 46,X,der(X)t(X;4)(q22;q24) karyotype.

Subject and Methods

Case Report

The proband, a 32-year-old female, presented with a chief complaint of secondary amenorrhea. Her physical exam was within normal limits and was negative for any dysmorphic facial features or physical anomalies. Persistent menstrual irregularity and low estrogen levels prompted a karyotype analysis, which revealed an unbalanced (X;4) translocation.

Cytogenetic Studies

Peripheral blood lymphocytes from the proband were transformed with Epstein-Barr virus, as described elsewhere (Neitzel 1986). To define the translocation breakpoints, the patient's lymphoblasts were cultured for high-resolution chromosome studies, according to a modified method of Yunis (1976). Cells were grown for ~72 h, were synchronized by the addition of thymidine (300 μ/ml) for the last 16.5 h of culture, and were harvested after the addition of ethidium bromide (8.0 mg/ml) and colcemid (0.044 mg/ml) for the last 45 and 25 min of culture, respectively. Late-replication studies were performed according to the method of Dutrillaux et al. (1976).

Somatic-Cell Hybrids

The patient's der(X) chromosome was isolated in a mouse/human somatic-cell hybrid by use of methods that have been described elsewhere (Brown and Willard 1989). The inactive X-chromosome content of the hybrids was confirmed by use of reverse-transcription–PCR (RT-PCR) with primers for XIST (Brown et al. 1991*b*). Two-color FISH with whole-chromosome paints for both the X chromosome and chromosome 4 (Oncor) was also performed, exactly as specified by the manufacturer; >50 metaphase spreads were analyzed for each clone.

The hybrids were single-cell subcloned to obtain a pure population of cells containing the der(X), while excluding any normal X chromosome or normal chromosome 4. The resultant clone (TVS-B2) was verified to be free of intact chromosome 4 and normal X chromosome, by use of PCR of genomic DNA with primers

for loci on chromosomes 4p (D4S403 and D4S400) and Xq (DXS1200 and DXS984) (table 1), regions not present on the der(X). The chromosome 4 breakpoint (4q24) was confirmed by use of primers for D4S1557, WI6869 (which did not produce product when TVS-B2 DNA was used), and NFKB1 (which did amplify). FISH was repeated as detailed above. TVS-A2, a hybrid retaining a normal chromosome 4 in addition to the der(X), also was obtained, to be used as an internal control for later experiments.

RT-PCR Analysis

RNA from the somatic cell–hybrid clones and appropriate controls was isolated with RNAzol (Biotecx), by means of standard protocols. The RNAs were reverse transcribed with random hexamer primers, both in the presence and in the absence (designated "RT+" and "RT−," respectively) of enzyme MuMLV (GIBCO-BRL), to control for contaminating DNA sequences. The resultant cDNA was amplified with PCR using primers for the individual loci that were to be tested (table 1). PCR with these primers created product when human fibroblast cDNA was used but did not amplify mouse cDNA.

Detection of CpG Islands

To search for CpG islands for nuclear factor kappa B1 (NFKB1), carboxypeptidase H precursor (CPH), a homologue of a *Drosophila* tumor suppressor (FAT), and basic fibroblast growth factor (FGFB) (see GenBank), two methods were used to assay likelihood of an island. Cs and Gs were counted in the 500 bp of sequence 5′ to the translation start sites. A straight ratio of Cs and Gs/total bases was calculated, and a value of >50% was consistent with the presence of an island. In addition, the observed/expected CpG ratio (Gardiner-Garden and Frommer 1987) was calculated, and a value of >0.6 was used as the cut-off for identification of a CpG island. An island for facioscapulohumeral muscular dystrophy–region gene 1 (FRG1) has been reported elsewhere (van Deutekom et al. 1996).

Results

Cytogenetic Studies

The patient had an unbalanced 46,X,der(X) t(X;4)(q22;q24) karyotype with phenotype limited to secondary amenorrhea. Chromosomal analysis initially identified the breakpoints at Xq22 and 4q26. High-resolution analysis more precisely determined that the chromosome 4 breakpoint was in 4q24 (fig. 1*B*). Late-replication banding studies of 20 metaphase spreads revealed that the translocated chromosome 4 DNA replicated later than the normal chromosome 4 (fig. 1*C*).

X-Linked Gene Expression in der(X) Hybrid

To determine whether the X-linked genes on the inactive der(X) chromosome were expressed or inactivated in a manner consistent with intact inactive X chromosomes, genes of known inactivation status were assayed. XIST transcripts were detected in the cDNA of hybrid TVS-B2, confirming that this hybrid contained an inactive chromosome. cDNA from this hybrid amplified with PCR primers for three genes—MIC2, UBE1, and XE169—that normally escape the X-inactivation process and are expressed from both active and inactive X chromosomes (Brown et al. 1997). Four genes that are normally transcriptionally silent on inactive X chromosomes—PDHA1, ARAF1, AR, and PGK1 (Brown et al. 1997)—failed to show any product when PCR was performed on the TVS-B2 cDNA. This same cDNA produced the expected product when used with primers for a gene that escapes inactivation, such as MIC2, showing the cDNA to be of adequate quality and quantity (data not shown). Overall, the eight X-linked genes tested demonstrated expression behavior consistent with normal inactive X chromosomes.

The Spreading and/or Maintenance of Inactivation in Translocated Chromosome 4 DNA

The expression patterns of 20 known genes or expressed sequence tags (ESTs) on the chromosome 4 portion of the der(X) chromosome were analyzed, by the same approach as has been described above. The chromosome 4 loci selected for expression analysis (table 1) were chosen from databases, without regard to any properties other than their presence on the der(X) chromosome. These 20 loci were located throughout the entire region of the translocated chromosome 4 cDNA. All 20 known genes or ESTs generated a clear PCR product when cDNA from control human fibroblasts was used but not when mouse fibroblasts were used, indicating that these sequences were expressed in fibroblasts and were suitable, therefore, for testing their possible expression in the der(X) hybrid, TVS-B2. Fourteen of the sequences did not produce PCR product when cDNA from hybrid TVS-B2 was used but did amplify in fibroblast controls (figs. 2 and 3). For example, PCR with primers for the 3′ region of the genes NFKB1, CPH, and FAT did not create product when TVS-B2 cDNA was used (fig. 2). The hybrid containing the der(X) (fig. 2, lane 1) and the mouse sample (fig. 2, lane 3) are both missing the band, whereas the hybrid containing the der(X) and a normal chromosome 4 (fig. 2, lane 2) and the human fibroblast control (fig. 2, lane 4) both manifested the appropriate band. This same cDNA strongly expressed the X-linked gene MIC2 when run in a coincident PCR reaction (data not shown), thus controlling for adequate levels of transcript.

Table 1

Primers Used

Gene	Product	Primers[a] (5′→3′)	Product Size (bp)
X chromosome:			
DXS984	STS	F: TTTCTGTCTGCCAAGTGTTT R: TACTGNGCCCCTACTCCATTC	177
DXS1200	STS	F: TACACACCAAACAACAGAGCCT R: CTAGGGGCACTTGAAAACAA	278
Chromosome 4:			
D4S400	STS	F: ATCCCTCACAGCACATCAAA R: GTCTACACTGGGTGCTTTTC	168
D4S403	STS	F: AGGTGGCCCTGAGTAGGAGT R: TTTGAGGGAATGATTTGGGT	226
NIB 329	EST	F: GCTTTAGTACACATGATTCACA R: ACTGTGTGCAGTTTTTGAAA	81
NIB 1375	EST	F: AAAGCAATTAATGACCTCCC R: GTGCAATACAATTCCTGCAT	110
NIB 1478	EST	F: AAGGGGAAAAAAAGCAAGAT R: TGCTTCCTAAAAACAGACCA	256
NIB 1531	EST	F: CAACTAGAAGCGTGACACCT R: CATGCATGTGCTTTTGTATG	115
NIB 1562	EST	F: AGCCATTGTACACATTGCAG R: TCTTGTTCCCATGGTGATAA	92
SGC31331	EST	F: AAGAAAGGGTAAGAAGGCTTAGA R: AAACTCTAGCCTTGCCACAC	150
WI-6176	EST	F: TCTGATTAACTCATCACAGTACTCG R: GTAGCGCCTTGTTTGAATACC	226
WI-6702	EST	F: TATTCAATTGCCTCTGAGTACTGC R: GTTACTGTCATCCCTCTATGGTTC	249
WI-7583	EST	F: TGGCTATCCTTTAATGATGCG R: AAAAAAACGAAATGCAACATCC	344
WI-9414	EST	F: TTTTTTTAATGTCGTGAAATAAGCA R: TCTGCCTGTCTAGACATATTCAGG	189
WI-12857	EST	F: GTTTCATCAGACACTGGTGTTACA R: GAACAATCTTTTAAAAGCAAAAATG	219
WI-14754	EST	F: AAGACAAAGCATACACAACCAGC R: AAGCTCCTGCTTTTGGCTTT	141
WI-16186	EST	F: ATTCGCCTCACAAAAATGTG R: TCCCAGGCCCAGTTCAGAT	150
WI-16900	EST	F: TTCTCAAGGAATAGTGCCCTC R: ATGCCAAGTGGGGTTCTGAT	150
bFGF	Basic fibroblast growth factor	F: GACTCAGTCGGAACAAATTGG R: TCATCAGTTACCAGCTCCCC	366
NFKB/WI-7764	Nuclear factor kappa beta	F: CCGTGTAAACCAAAGCCCTA R: CAGAGGGACAACAGCAATGA	299
IL15/WI-9355	Interleukin 15	F: TTAATTAACAAACATCACTCTGCTGC R: TTCTAAGAGTTCATCTGATCCAAGG	122
CPH/WI-7540	Carboxypeptidase H precursor	F: GTGACCAATGTCACATAATGAATGC R: CATTCAGAATGCAATTCTGGG	325
FAT	Homologue of *Drosophila* tumor suppressor	F: GCTGTGCCATTTCCCAAC R: GATAATGGCACTGACACCACC	245
FRG1	Facioscapulohumeral muscular dystrophy–region gene 1	F: TCTACAGAGACGTAGGCTGTCA R: CTTGAGCACGAGCTTGGTAG	180

[a] F = forward; and R = reverse. All primer sequences were obtained from the Genome Database, the Human Transcript Map, or the Whitehead Institute, except for FRG1, which is from van Deutekom et al. (1996). PCR was performed under standard conditions (30 cycles of 95°C for 2 min, 94°C for 15 s, 55°C for 15 s, and 72°C for 40 s; 72°C for 7 min; and 4°C indefinitely), except in the cases of WI-7540 (35 cycles [same conditions]) and FRG1 (30 cycles of 95°C for 2 min, 94°C for 40 s, 60°C for 1 min, and 72°C for 1.5 min; 72°C for 7 min; and 4°C indefinitely).

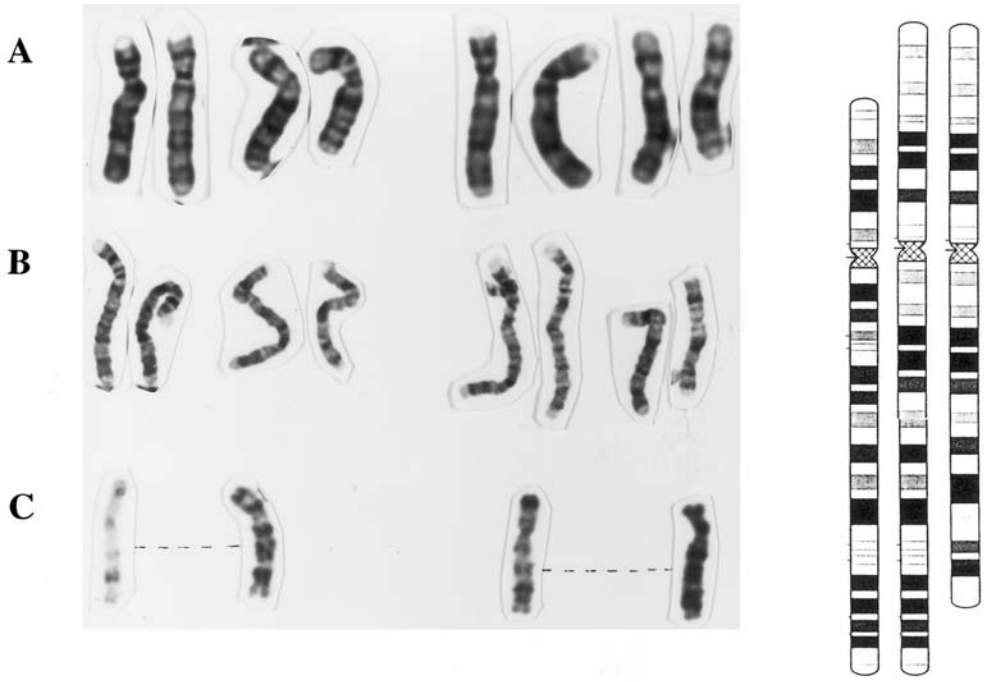

Figure 1 Two representative G-banded partial karyotypes from the routine study (*A*) and two G-banded partial karyotypes from the high-resolution study (*B*). For each partial karyotype, chromosomes shown are (*from left to right*) a pair of normal chromosomes 4, t(X;4), and a normal X chromosome. In panel *C*, late-replication banding of the t(X;4) (*left*) and of a normal chromosome 4 (*right*) is shown; the broken lines indicate the breakpoint on the t(X;4) (*lighter banding*), compared with the same region on chromosome 4. Ideograms of a normal chromosome 4, the translocation chromosome, and a normal X chromosome also are shown (*far right*).

In contrast to these 14 sequences, six other genes or ESTs from chromosome 4 were well expressed in hybrid TVS-B2. For example, bFGF, interleukin 15 (IL15), and FRG1 were expressed at high levels in TVS-B2 (fig. 2, lane 1). These autosomal genes thus escaped inactivation. The six genes and ESTs expressed in this somatic-cell hybrid were interspersed with genes/ESTs that were transcriptionally silent (fig. 3). Of the 20 genes/ESTs tested, 30% (6/20) were transcribed and thus escaped X inactivation.

Discussion

Although the process of X inactivation appears to be absolutely dependent on the presence, in cis, of both the XIC and the XIST gene (Willard 1996), the degree to which it can be influenced by the nature of the chromosomal DNA is uncertain. At least at the cytological level, X inactivation can spread over quite long distances, into autosomal material, in both murine and human X;autosome translocations (e.g., see Disteche et al. 1979; Mattei et al. 1982; Keitges and Palmer 1986). In addition, ectopic copies of the mouse Xist gene can lead to inactivation of at least some autosomal loci (Lee and Jaenisch 1997). Our results demonstrate that the inactivation signal is capable of spreading over stretches of autosomal DNA >100 Mb in length. In analyzing the expression patterns of autosomal genes translocated onto an X chromosome, we found that, much like normal inactive X chromosomes, most sequences were silenced by the spreading of the inactivation signal. Clearly, the ability of the X-inactivation message to spread into translocated autosomal DNA demonstrates that whatever characteristics make DNA susceptible to the general inactivation process are not unique to the X chromosome. However, approximately one third of chromosome 4 genes and ESTs tested were expressed from the inactive der(X). These results suggest either that some autosomal sequences may be resistant to the X-inactivation signal or that autosomal DNA lacks chromosome X–specific elements, such as those hypothesized by Gartler and Riggs (1983), that allow for efficient spreading and/or maintenance of X inactivation.

One third of genes and ESTs assayed in our study were found to escape inactivation. When compared with the X chromosome, the proportion of inactivation-escaping genes observed in the autosomal DNA was more similar to that seen in certain regions of Xp (Miller et al. 1995) but was higher than that reported for either other areas of Xp or all of Xq. This suggests that susceptibility to

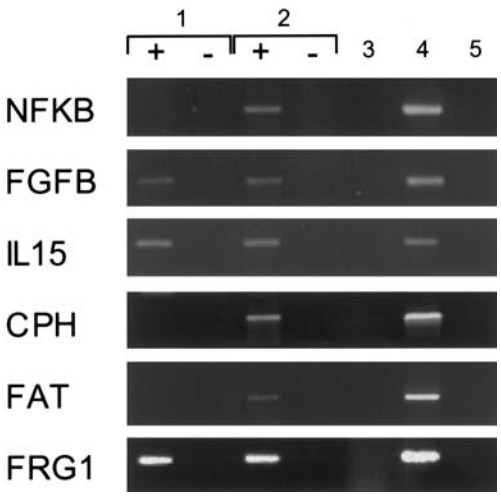

Figure 2 Expression pattern of six autosomal genes translocated onto an X chromosome; a plus sign (+) denotes RT+; and a minus sign (−) denotes RT− controls. Lane 1, Somatic-cell hybrid TVS-B2 containing the der(X) without either a normal X chromosome or a normal chromosome 4. Lane 2, Hybrid TVS-A2 containing der(X) and a normal chromosome 4. Lane 3, Mouse parental line tSa1S9. Lane 4, Normal human fibroblast. Lane 5, Water.

X inactivation may correlate with the evolutionary origin of chromosomal DNA. The long arm of the X chromosome, with the XIC, is considered to be the "ancestral X," since it has been linked in mammals for >170 million years (Watson et al. 1990). It contains very few genes that escape inactivation (Brown et al. 1997). In contrast, 15 genes on the short arm are expressed from inactive X chromosomes (Disteche 1995; Brown et al. 1997). The majority of genes that escape X inactivation are clustered in and around the pseudoautosomal region. Since the genes in the pseudoautosomal region have functional Y homologues, this area presumably has an "autosomal" chromatin conformation. It has been hypothesized that the area just proximal to the pseudoautosomal region, an area that also contains a high proportion of genes that escape inactivation, was once pseudoautosomal but, recently in human evolution, was disrupted by a pericentric inversion on the Y chromosome (Fraser et al. 1987; Yen et al. 1988). This area may not have lost autosomal chromatin elements or may not have accumulated ancestral X-chromatin properties appropriate to allow for complete inactivation and/or maintenance. In toto, Xp is a newer evolutionary addition to the X chromosome, the "autosomally derived" X (Graves and Watson 1991), and Xp contains numerous regions that escape X inactivation (Willard et al. 1993; Miller et al. 1995; Carrel et al. 1996). It would follow, then, that autosomal material newly translocated to the X chromosome, as in the case discussed in the present report, might be even less susceptible to spreading or maintenance of inactivation.

The expressed inactive der(X) genes were interspersed with regions of DNA that were subject to inactivation (fig. 3). The finding of noncontiguous spreading of X inactivation is similar to Keitges and Palmer's (1986) observation that late replication may "jump over" some autosomal bands translocated onto an inactive X chromosome. In addition, these findings are broadly consistent with the few early-replicating, hypoacetylated bands on the normal inactive X chromosome (Willard 1983; Schwemmle et al. 1989; Jeppesen and Turner 1993), which have been shown to contain genes that escape X inactivation (Miller et al. 1995). These findings support a regional control model of X inactivation, predicting (*a*) that the X chromosome may have sequences that aid in the spreading and/or maintenance of X inactivation (Gartler and Riggs 1983) or, alternatively, (*b*) that there may be "boundary" elements in the autosomal DNA, as well as a few regions of the "autosomal Xp," that inhibit the spreading of X inactivation (reviewed in Brown and Willard 1993; Miller et al. 1995; Carrel et al. 1996).

Our studies do not allow for the distinction between the failure of initiation of X inactivation of some translocated chromosome 4 regions and the ineffective maintenance of X inactivation. It is plausible that the autosomal DNA lacks features for proper maintenance, such that all of the translocated chromosome 4 loci were initially inactivated but that the inactive chromatin conformation could not be maintained in some regions. It is of interest that our proband exhibited a completely normal phenotype (with the exception of secondary amenorrhea, which may be attributed to her being hemizygous for Xq22-qter), even though approximately one third of translocated chromosome 4 genes may have been expressed in triplicate during development. Duplications of distal 4q are associated with a very severe phenotype, including mental retardation, dysmorphic facial features, growth and psychomotor retardation, and severe congenital anomalies (Schinzel 1984). Perhaps only a proportion of chromosome 4 genes, when duplicated, produce the aberrant phenotype, or, alternatively, it is possible that, in our patient, the majority of chromosome 4 genes were inactivated during critical times of development and subsequently were reactivated.

Several previous studies suggested that X inactivation of autosomal DNA may not be appropriately maintained. Dilution plating of clonal derivatives of a cell line containing an unbalanced X;14 translocation revealed that, the longer the cells were cultured, the more autosomal material that was early replicating, suggesting an instability of inactivation (Schanz and Steinbach 1989). In a murine system, progressive reactivation of an autosomal albino gene that was translocated onto an

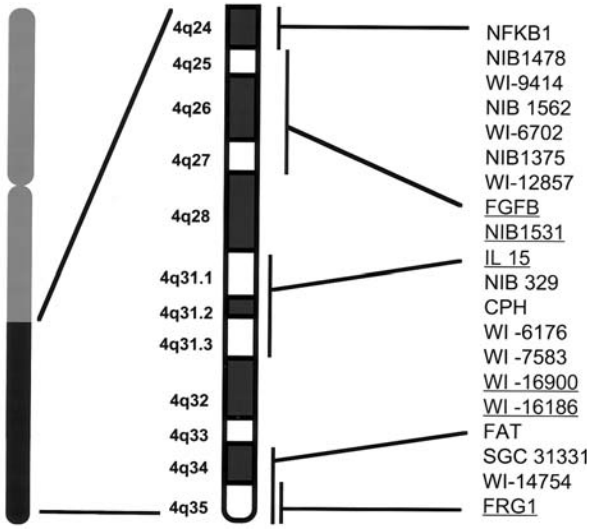

Figure 3 Summary of expression patterns of 20 chromosome 4 loci translocated onto the X chromosome. Expressed loci are denoted by underlining (inactivated loci are not underlined). Genes mapped cytogenetically by use of FISH are denoted by unbroken lines (Genome Database). ESTs and known genes are ordered by use of doubly linked YAC contigs and/or radiation-hybrid panels, as presented in the Human Transcript Map.

X chromosome was observed as the mouse aged and developed pigmented patches (Cattanach 1974). The coat-color changes were thought to reflect an autosomal gene reactivation due to a lack of maintenance of X inactivation. Both of these studies suggested that spreading of inactivation into adjacent translocated autosomal material exhibited a proximity effect, such that genes or autosomal chromosome bands closest to the breakpoint were more likely to maintain inactivation and that the process of maintenance of inactivation was inversely related to distance from the XIC. Our finding that the most distal gene tested, FRG1, was inactivated implies that the proximity hypothesis is not generally applicable (fig. 3).

It has been well established that methylation of GC-rich promoter regions of X-linked housekeeping genes is crucial for maintenance of inactivation status (Mohandas et al. 1981*b*). Many genes on the X chromosome do not have CpG-rich promoters, and it has been hypothesized that X inactivation may be less tightly controlled in these tissue-specific genes, such that they are more likely to reactivate (Gartler and Goldman 1994). We analyzed 500-bp regions 5′ to the translation start sites of three chromosome 4 genes that were inactivated (NFKB1, CPH, and FAT) and two genes that escaped inactivation (FGFB and FRG1). All five of these genes had clear CpG islands, by accepted criteria (Gardiner-Garden and Frommer 1987). Although the number of genes examined is relatively small, these results suggest that the ability of autosomal genes to undergo or maintain X inactivation is not controlled solely by the presence or absence of a CpG island.

Human/rodent somatic-cell hybrids have been an important tool in the study of expression patterns of X-linked genes. The system allows the isolation of either an active or inactive X away from its homologue and, as such, has been used to establish the X-inactivation status of many X-linked genes (Brown and Willard 1993; Brown et al. 1997). Although there are rare documented cases of spontaneous reactivation of genes normally subject to inactivation in somatic-cell hybrids occurring at frequencies of $<1 \times 10^{-6}$ (reviewed in Gartler and Goldman 1994), in the vast majority of cases, the somatic cell–hybrid system accurately reflects the expression patterns of X-linked genes in human somatic cells (Brown et al. 1997). It is, of course, formally possible that the expression patterns observed in the present study are specific to the somatic cell–hybrid model system, rather than a faithful reflection of patterns seen in human diploid cells (Brown et al. 1997; Disteche 1997). Although direct analysis of gene expression in human cells (e.g., when expressed polymorphisms or RNA in situ hybridization is used) would be necessary to evaluate this in detail, it is worth noting that most X-linked genes retain their inactivation patterns in somatic-cell hybrids. Thus, the fact that approximately one-third of chromosome 4 genes are expressed in this system likely indicates that they are, in some way, distinct from most X-linked genes, regardless of whether they are actually expressed from the inactivated autosome in the patient's cells.

Since the der(X) originated from a single parental chromosome 4, imprinting of the apparent "inactivated" genes must be considered. It is possible that some genes on chromosome 4q are imprinted and are only expressed from either the maternal or paternal homologue, which may give results consistent with "inactivation." However, to date there is no evidence for imprinted loci on chromosome 4 (Ledbetter and Engel 1995), and one case with documented maternal uniparental disomy of chromosome 4 was reported as normal (Lindenbaum et al. 1991).

The hypothesis that autosomal DNA translocated onto an X chromosome can be subject to the spreading of X inactivation has been tested in this X;autosome translocation isolated in a somatic-cell hybrid, by expression studies that detect the presence or absence of any measurable gene transcript from 20 autosomal loci. Further studies characterizing the expression patterns of genes on this and other X-autosome transactions will address other aspects of the mechanism of X inactivation, such as the role that the X-chromosome breakpoint plays in allowing the inactivation signal to spread, the variability of gene-expression patterns in different tissues, and the epigenetic changes that accompany the

maintenance of X inactivation. Our results imply that there is something truly different about the ancestral X chromosome, as compared with autosomal DNA. It may be that the proposed boundary elements, which insulate regions of DNA from the X-inactivation signal, are lost over time as DNA "ages" from "autosomal X" to "ancestral X." Alternatively, it may be that X-chromosomal "stabilizing sequences" are accumulated during evolution (Gartler and Riggs 1983). Analysis of the qualities that autosomal DNA shares with Xp material but not with the long arm of the X chromosome may help explain some of the mechanistic complexities of X-chromosome inactivation.

Acknowledgments

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Electronic-Database Information

URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for NFKB1 [M58603], CPH [G115892], FAT [X87241], and FGFB [M27968])
Genome Database, http://gdbwww.gdb.org/gdb/gdbtop.html
Human Transcript Map, http://www.ncbi.nlm.nih.gov/SCIENCE96/
Whitehead Institute, http://www-genome.wi.mit.edu/

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