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Identification and isolation of transcribed human X chromosome DNA sequences

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ABSTRACT

A human X chromosome specific phage library has been used as a source of X-specific genomic DNA clones which hybridize with cellular RNA. Random cDNA clones were mapped for X chromosome sequence localization and 8 were identified as hybridizing to X chromosome Hind III fragments. All eight also hybridized with autosomal Hind III fragments. The X chromosome genomic sequences corresponding to two of these cDNA clones were isolated from a phage library constructed with the Hind III endonuclease digest products of X enriched DNA. One genomic DNA segment, localized to the short arm of the X, shared sequence homology with at least one region of the human Y chromosome. The methodology developed represents a rapid means to obtain a specific genomic DNA clone from a single chromosome when multiple different genomic loci homologous to an expressed DNA sequence exist.

INTRODUCTION:

Random DNA fragments originating from the human X chromosome have been recently isolated (1,2,3). Such fragments represent DNA markers for various regions of the human X and are useful tools for preliminary elucidation of genetic structure and function of the human X chromosome. Further study of X-chromosomal function requires the isolation and analysis of X chromosome DNA sequences which are actively transcribed in males and on one female homologue but which are repressed in inactive X chromosomes (4). Sequences constituting at least parts of several X chromosome-encoded genetic loci have been cloned, e.g. (5,6,7,8,9). Isolation of each of these cloned genetic sequences required prior availability of the gene product. This is not currently feasible for most genes on the X chromosome, for which the protein product has not been isolated or identified. In this paper, we demonstrate the ability to identify and isolate

genomic DNA fragments, from regions of the X chromosome, which hybridize with cellular RNA. Large numbers of such regions represent a necessary first step in elucidation, at a molecular level, of the function of segments localized throughout the human X chromosome.

MATERIALS AND METHODS

Cell lines and DNA isolation

Most cell lines and all DNA isolation procedures were as previously described (3). One additional human-rodent hybrid cell DNA sample containing human X chromosome representation from Xp21-->Xqter (a generous gift of Dr. T. Mohandas) was utilized to localize fragments of X chromosome DNA proximal or distal to Xp21 in a manner analogous to that previously described (3).

Enzyme reactions

Restriction endonuclease digestions were performed as described by the manufacturers (New England Biolabs, Boehringer Mannheim or Bethesda Research Laboratories). Radiolabeling of DNA was accomplished using either the nick translation activity of E.coli DNA polymerase I (10; Boehringer Mannheim), or T4 DNA polymerase (11; Bethesda Research Laboratories).

Gel electrophoresis and hybridization of DNA

For most experiments, 3 ug of restriction endonuclease cleaved DNA was loaded into a horizontal agarose gel slot in 5% Ficoll, 10mM Tris, 5mM EDTA, 0.1% SDS and 0.01% bromphenol blue. Accurate measurement of DNA was determined by a fluorometric assay using the dye 4', 6-diamidino-2-phenylindole (DAPI) (12). Gel electrophoresis and Southern hybridization analysis (13) were as previously described (3,14).

cDNA construction and plasmid screening

HeLa cytoplasmic poly A+ RNA was a gift of Dr. R. Singer, University of Mass., Amherst. Isolation of total cellular poly-A+ RNA and double-stranded cDNA synthesis were as described for human fetal heart tissue (15) and for lymphocytes (16). The HeLa and lymphocyte cDNA libraries were constructed by annealing dC-tailed double-stranded cDNA into PstI-cleaved, dG-tailed plasmid, pAT153 (17). The fetal heart cDNA library was

constructed in analogous fashion in the plasmid pKT218 (18). The lymphocyte library was a gift of Dr. Derek Woods. Plasmid DNA used in the Southern blot hybridization analyses was prepared from 1.5 ml of individual 24 hour cultures by an alkaline lysis procedure (19). Ten percent of the yield (0.2 ug) from each plasmid DNA preparation was loaded into a horizontal agarose gel slot (30 slots per gel of a 0.8% agarose gel). Following electrophoresis of the marker dye, bromphenol blue, for 3 cm, the DNA samples were stained with ethidium bromide, nicked by exposure to ultra-violet light, and transferred to nitrocellulose (13). The filters were hybridized with ^{32}P-labeled total human DNA: cDNA clones which hybridized were elimitated from further screening as they presumably contained repeated sequences. The plasmids which did not hybridize in this screen with human DNA (86% of those examined) were radiolabeled and hybridized to nitrocellulose strips containing appropriate human or rodent-human hybrid DNA samples.

RNA Isolation and Hybridization

Total cellular RNA was extracted from HeLa cells according to the procedure of Glisin et al (20). Poly A+ RNA was selected on oligo (dT) cellulose columns (P.L. Bio-chemicals, Inc.) according to the procedure of Aviv and Leder (21). Poly A+ RNA from Hela cells was fractionated on 1% agarose, 6% formaldehyde gels according to the procedures of Lehrach et al (22) and Goldberg (23). RNA from the gels was transferred to nitrocellulose filters in 10 X SSC, in a manner similar to that of a modified DNA blot (13) procedure (14). Hybridization to immobilized RNA, with DNA fragments labeled via T4 DNA polymerase, was carried out in 50% formamide at 42^{o}C according to the procedure of Wahl et al (24).

Outline for Isolation of Expressed Sequences on the X Chromosome

A scheme for the identification and isolation of DNA fragments originating from the X chromosome which are complementary to RNA transcripts is depicted in Figure 1. Individual cDNA clones from the cDNA libraries described above were prepared as plasmid DNA, and those of low sequences complexity were identified by lack of hybridization to human DNA

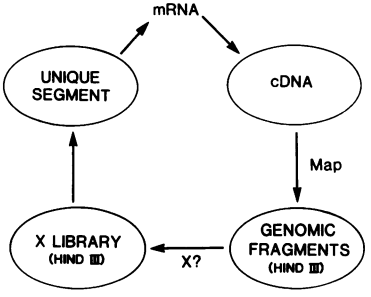

Figure 1: Schematic representation of the steps necessary to isolate the specific chromosomal locus hybridizing with an expressed sequence.

(25). Each cDNA insert was radiolabeled and used as a hybridization probe to Hind III cleaved genomic DNA in Southern blot analyses (13). Those clones that hybridized with an X chromosome-specific Hind III restriction endonuclease fragment were identified by both quantitative and qualitative criteria. The quantitative criterion specified that a specific Hind III fragment show increased representation in DNA from a 49, XXXXY lymphoblast line relative to DNA from a 46,XY cell line. The qualitative criterion specified the presence of this Hind III fragment in DNA from a rodent-human hybrid cell containing primarily the human X, in addition to rodent chromosomes (3). A cDNA clone which satisfied these criteria was hybridized to plaques (26) from an X-enriched recombinant phage library, which had been constructed from the complete Hind III cleavage products of DNA from flow-sorted human X chromosomes (3). Most genomic Hind III fragment smaller than 9 kb previously identified as X in origin are likely to be represented in this library. Once such a phage was identified and plaque-purified, the desired human Hind III DNA insert was transferred to a plasmid vector, and the subcloned insert further fragmented by restriction endonuclease cleavage. Derivative genomic fragments, found on screening to be free of highly repeated sequences, thus constituted few-copy DNA sequences from the human X chromosome within or adjacent to sequences which are potential probes of cellular RNA synthesis.

RESULTS

Over 300 individual cDNA clones were screened for the presence of human repeated DNA sequences by hybridization of

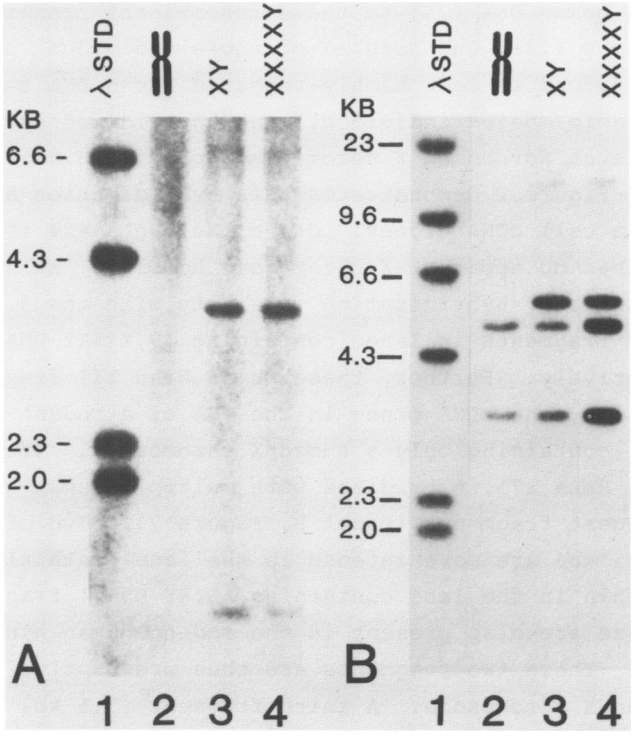

Figure 2: Hybridization analysis of two cDNA clones constructed from Hela cell mRNA.

Two autoradiographs are presented. The genomic DNA samples in panel A and B are the same and were separated by electrophoresis and transfered to nitocellulose (13; see methods). The DNA samples are the following: lane 1, ^{32}P-labeled Hind III cleaved λ phage DNA; lane 2, 3 ug of Hind III cleaved hamster-human hybrid DNA with only an intact human X chromosome among rodent chromosomes; lane 3, 3 ug of Hind III cleaved 46,XY DNA; lane 4, 3 ug of Hind III cleaved 49,XXXXY DNA. The autoradiograph in panel A is the hybridization pattern of cDNA clone Hela #24 following "nick translation" (10) ^{32}P-labeling of the entire cDNA clone bearing plasmid. The autoradiograph in panel B is the hybridization pattern of cDNA clone Hela #71 radiolabeled with ^{32}P via T4 DNA polymerase (11). The autoradiograph in panel A is representative of the entire cDNA hybridization analysis using probes radiolabeled by "nick translation", panel B represents a blot subsequently carried out with a more sensitive labeling procedure, to more clearly demonstrate X chromosome sequences.

^{32}P-labeled human DNA (25) to these recombinant plasmids on Southern blots (13). One hundred of those individual cDNA clones which were judged to lack highly-repeated human DNA sequences were then radiolabeled individually and hybridized on Southern blots to search for human X chromosome-specific Hind III DNA fragments. Figure 2 demonstrates this hybridization analysis for two HeLa cell cDNA clones. One clone, HeLa #24 (Panel A, Figure 2), has no apparent X chromosome homology, as judged by the equivalence of hybridization intensity with the 3.8 and 1.6 kb Hind III fragments in lanes containing 49,XXXXY DNA and 46,XY DNA, respectively. Further, these human Hind III fragments are not detected by the cDNA probe in the DNA of a rodent-human hybrid cell containing only a human X chromosome. The second cDNA clone, HeLa #71, hybridizes with multiple human genomic DNA Hind III digest fragments (panel B, Figure 2). Two of these (5.0 and 3.5 kb) are more intense in the lane containing 49, XXXXY DNA than in the lane containing 46,XY DNA. Fragments of the same size are also present in the rodent-human hybrid cell DNA sample. These two fragments are thus presumptively assigned to the human X chromosome. A third fragment (5.5 kb) and a fourth fainter (16 kb) fragment do not appear to be of X chromosomal origin.

A summary of the entire cDNA hybridization analysis, based on data similar to the two examples presented in Figure 2, is presented in Table 1. Fifty six of 100 cDNA clones hybridized to Southern blots gave analyzable hybridization patterns; the remainder either failed to hybridize due to technical difficulties or hybridized to more than 20 Hind III fragments. Eight of the 56 analyzable cDNA clones hybridized to at least one Hind III genomic fragment which, by the criteria of Fig. 2B, localized to the human X. However, all of these eight cDNA clones also hybridized with one or more human DNA fragments localized elsewhere than the X (as demonstrated for cDNA clone Hela #71 in Figure 2B). Moreover, thirty six clones (out of the 56 successfully tested) hybridized to a rodent Hind III restriction fragment that was not observed in the human DNA samples. The intensity of hybridization with rodent specific Hind III fragments varied from very faint to equivalent to that

Table 1

Summary of cDNA Clone Hybridization

Genomic Hind III Fragment Hybridization Pattern*		Number of Clones Hybridizing			
		HeLa	Fetal Heart	Lymphocyte	Total
General**	no signal	22	0	8	30
	repeated	7	5	2	14
	3 - 20 bands	21	4	2	27
	1 - 2 bands	14	7	8	29
	Total	64	16	20	100
Specific	Hamster homology***	23	8	5	36
	Hybridization with Human X DNA****	7	1	0	8

*Based on autoradiographs of nitrocellulose filter imprints of Hind III-cleaved DNA samples of 49,XXXXY DNA, 46,XY DNA and hamster-human hybrid DNA containing only the human X, among rodent chromosomes. The filter is hybridized with a single ^{32}P-labeled cDNA plasmid. No signal designates lack of apparent hybridization; repeated is defined as either greater than 20 Hind III fragments hybridizing or lane smear without discrete bands.

**This analysis includes only those plasmids which did not hybridize with radiolabeled human DNA (see Methods)

***Rodent homology is defined as hybridization to a hamster Hind III fragment of a different size than that observed in the human lanes.

****X chromosome hybridization is defined as a fragment which hybridizes more strongly to 49,XXXXY DNA than 46,XY DNA and is present at the same size in the hybrid cell DNA sample.

observed for human DNA fragments.

In aggregate, the fifty six individual cDNA clones hybridized to a total of 205 Hind III fragments in the genomic DNA of a single human subject. Fourteen of these 205 fragments (7%) hybridized more intensely to 49,XXXXY DNA than to 46,XY DNA, and were also present in the hybrid cell DNA sample containing the X as its only consistent human component. These 14 fragments were thus provisionally localized to the human X chromosome. Four other Hind III fragments, out of the total of 205, exhibited variation in size between the two human DNA samples. The cDNA clones hybridizing to these 4 fragments apparently recognize a restriction fragment length polymorphism (RFLP) (27) of an autosomal genomic DNA sequence. Further characterization of one of the four cDNA clones (HeLa #75) indicate that the observed Hind III "RFLP" provisionally localizes to human chromosome #6 and is inherited in a Mendelian fashion, with a minor allele frequency of 0.29 (Kunkel et al, unpublished observations).

Confirmation of the provisional X chromosomal localization of genomic DNA fragments detected by cDNA clones was accomplished by isolation and analysis of their correlate X chromosome-specific genomic DNA fragments. This isolation was necessitated by the finding that all of the cDNA clones localized provisionally to the X chromosome also hybridized with DNA restriction fragments on other chromosomes (Figure 2, panel B). Four cDNA clones (Hela #71, Hela #79, Hela #82 and Heart #21), recognizing a total of nine presumed X chromosome-derived Hind III fragments, were radiolabeled. Each radiolabeled cDNA probe was used separately in a hybridization screen (26) of approximately 50,000 recombinant phage from an X chromosome-enriched Hind III complete digest library (3). Following plaque purification of 8 to 12 homologous phage from each screen, phage DNA was prepared and cleaved with Hind III. Six of the expected nine possible Hind III fragments initially hybridizing with these four cDNA clones were identified in multiple phage by both similarity of insert size with the desired Hind III fragment and continued hybridization homology to the cDNA clone originally used in the hybridization screen. Some additional phage, homologous to cDNA probes but with

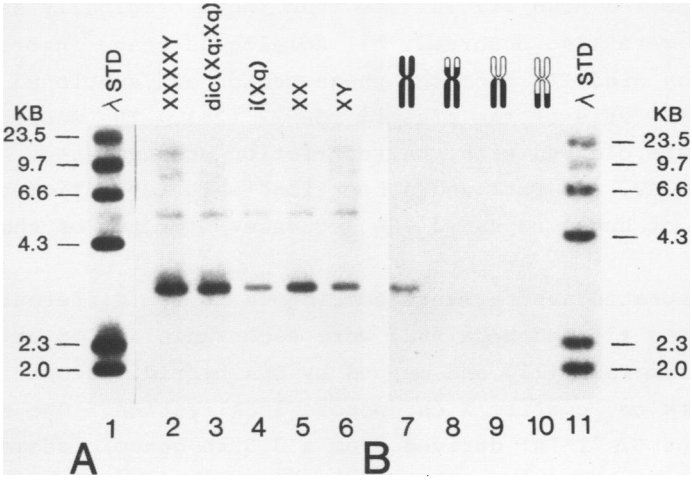

Figure 3: Autoradiograph of the hybridization of the derived subclone λ71-7A DNA to various DNA samples which localize this sequence to the short arm of the human X chromosome

The 3.5 kb Hind III fragment derived from the enriched human X library and homologous to the human cDNA clone Hela #71 was cleaved with the restriction endonucleases Hind III and PstI to release a fragment of 1.8 kb, which had previously been shown to be free of human repeated sequences by lack of hybridization to ^{32}P-labeled total human DNA. This 1.8 kb fragment was radiolabeled with ^{32}P via T4 DNA polymerase (11) and used as a hybridization probe with various Hind III cleaved genomic DNA samples (3 ug) immoblized on nitrocellulose (13; see methods). The genomic DNA samples were the following: Lane 2, 49,XXXXY DNA; lane 3, 46,X,dicX(q24) [one representation of Xq24 -> Xqter, 3 representations of Xq24 -> Xpter]; lane 4, 46,X,i(Xq) DNA, [one representation of Xcen -> Xpter and 3 representations of Xcen -> Xqter]; lane 5, 46,XX DNA; lane 6, 46,XY DNA; lane 7, a rodent-human hybrid cell line DNA sample with only a human X among rodent chromosomes; lane 8, a rodent-human hybrid cell line DNA sample with human X chromosome representation only from Xp21 -> Xqter, among other human chromosomes, and rodent chromosomes; lane 9, a rodent-human hybrid cell line DNA sample with human X chromosome representation only from Xq1 -> Xqter; lane 10, a rodent-human hybrid cell line DNA sample with human X chromosome representation only from Xq24 -> Xqter; lanes 1 and 11 ^{32}P-labeled Hind III cleaved λ phage DNA as molecular weight markers (sizes given along margins). The symbols across the top represent either a karyotypic short hand of the X chromosomes present in each human cell line or, in the case of rodent-human hybrid cells, the darkened portion represents that portion of the human X chromosome retained in these hybrids. The data presented in panels A and B were obtained at different times.

different sized Hind III inserts than those originally assigned to the X, were also observed. All homologous phage inserts were released by Hind III from the phage vector and subcloned into the Hind III site of the plasmid pBR322 (28). The subcloned inserts were cleaved with the restriction endonucleases Bam HI, EcoRI, Eco RV, and PstI and subportions were identified that were free of human repeated DNA sequences, for two of these inserts.

Non-repeated subfragments homologous to two different cDNA clones (HeLa #71 and HeLa #82) were each radiolabeled utilizing T4 DNA polymerase (11) and mapped by DNA hybridization experiments to confirm X chromosome localization. One such subfragment (λ 71-7A) derived from a 3.5 kb genomic segment homologous to cDNA HeLa #71 (originally hybridized in Figure 2B) hybridized (Figure 3) to a 3.5 kb Hind III fragment in total human DNA and is localized to Xp21 --> Xpter. The 3.5 kb Hind III fragment hybridizing with probe λ 71-7A is detected in digests of human-rodent hybrid cell DNA when an intact human X chromosome is present (lane 7, Figure 3), but not when there is representation in the hybrid cell DNA of proximal Xp(Xp21-->Xqter, lane 8) or only human X chromosome long arm material (lanes 9 and 10, Figure 3). This same 3.5 kb fragment hybridizes with equivalent intensity to 46,XY DNA (lane 6) and to DNA isolated from a human cell line (46,X,i(Xq)) with one copy of Xp and three copies of Xq (lane 4); these intensities are both less than that observed with 46,XX DNA (lane 5). Another subcloned genomic fragment, homologous to cDNA HeLa #82, has been localized in an analogous manner to Xq24 -> Xqter (data not shown). Human genomic subclones which are free of repeated DNA sequences have not yet been identified for the other two cDNA clones originally used to screen the X chromosome-enriched phage library.

Once a genomic subfragment, ascertained by its hybridization to a cDNA probe, has been identified as X chromosomal in origin, its potential as a probe for transcription was examined by testing for hybridization homology with HeLa cell Poly A+ RNA. Experiments were undertaken to determine the transcriptional activity of two cDNA clones and their X chromosome genomic loci;

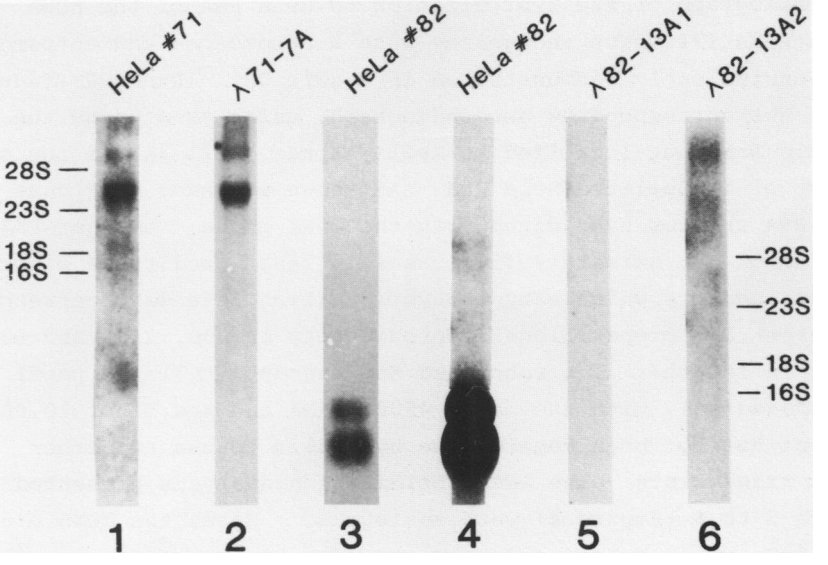

Figure 4: Autoradiograph of the hybridization of two Hela cDNA clones and their derived X chromosome genomic subclone to HeLa RNA samples.

The poly A+ RNA samples were separated on the basis of molecular weight by electrophoresis, transferred to nitrocellulose and hybridized as described in the Methods section. Identical amounts of HeLa poly A+ RNA (2ug) were loaded to each lane. The radiolabeled DNA used as a hybridization probe for each lane is listed at the top of each lane and are the following: Panel 1, HeLa #71; panel 2 the derived 1.8 kb subclone λ71-7A; panels 3 and 4, HeLa #82; two exposures of the same autoradiograph are given to demonstrate the fainter large transcripts; panels 5 and 6 were hybridized with two different genomic fragments (λ82-13A1, 1.6 kb; λ82-13A2, 0.5 kb), both were derived by Bam HI and Hind III cleavage of the same 7 kb Hind III fragment homologous to cDNA Hela #82 and localized to Xq24 -> Xqter. All hybridization probes were radiolabeled via T4 DNA polymerase (11). The size markers indicated in the margins are rRNA and were visualized on the ethidium bromide stained gel prior to transfer of RNA to nitrocellulose. Panels 1 and 2 are identical filters prepared at the same time, as are panels 3 to 6.

first, HeLa #71 and the subclone λ71-7A (derived from Xp21-->Xpter) and second HeLa #82 and the subclone λ82-13A2 (derived from Xq24 -> Xqter). Poly A+ total RNA samples from HeLa cells were separated by electrophoresis and transferred to nitrocellulose paper (22,23,24). Panel 1 (Figure 4) is an

autoradiograph of the hybridization to HeLa RNA of the cDNA clone HeLa #71 which recognized both X chromosome and autosomal loci equivalently (demonstrated in Figure 2B). Panel 2 (Figure 4) is the corresponding autoradiograph, using as a probe the genomic fragment localized to Xp21-->Xpter (λ71-7A) to the same amount of immobilized HeLa RNA. As shown in panel 1 (Figure 4), four RNA species hybridized with the cDNA probe: a major RNA species of approximately 4500 bases in length and three minor RNA transcripts which vary in hybridization intensity between different RNA preparations (approximately 10,000, 2400 and 800 bases in length). The subcloned Xp fragment (λ71-71, panel 2) hybridized only with the major 4500 b RNA and the minor 10,000 b RNA but has not been observed to hybridize to the two other minor transcripts. The RNA hybridization analyses presented in panels 3 to 6 (Figure 4) were analogous. Here, the cDNA probe HeLa #82, which hybridized very strongly to two transcript sizes of 1250 b and 1700 b, also hybridized weakly to two larger transcripts of 6600 b and 7900 b (detected on the heavy exposure, panel 4). The derived genomic subclone, λ 82-13A2, hybridized with only extremely large transcripts (above 10,000 bases in length; panel 6, Figure 4). For comparison, another unique sequence subclone λ 82-13A1, derived from the same 7 kb Hind III fragment as λ 82-13A2, detected no RNA homology. Neither subclone hybridizes with the two abundant RNA transcripts, which hybridized with the original cDNA clone HeLa #82. The data suffice to show, for both cDNA clones (HeLa #71 and HeLa #82), that the cloned cDNA is homologous to HeLa RNA and that X-specific genomic fragments identified by these clones hybridized only to a subset of those HeLa transcripts recognized by the original cDNA clone.

Further hybridization studies (Figure 5) with the λ71-7A subcloned fragment indicate that this Xp21-->Xpter locus exhibits hybridization homology with the human Y chromosome. This subfragment still exhibits weak homology (see Figure 3) with the autosomal locus seen in Figure 2B (a 5.5 kb fragment), and it can also be seen to hybridize with DNA sequences located on the human Y chromosome (Figure 5). The λ 71-7A subfragment hybridizes to additional fragments in two placental DNA samples

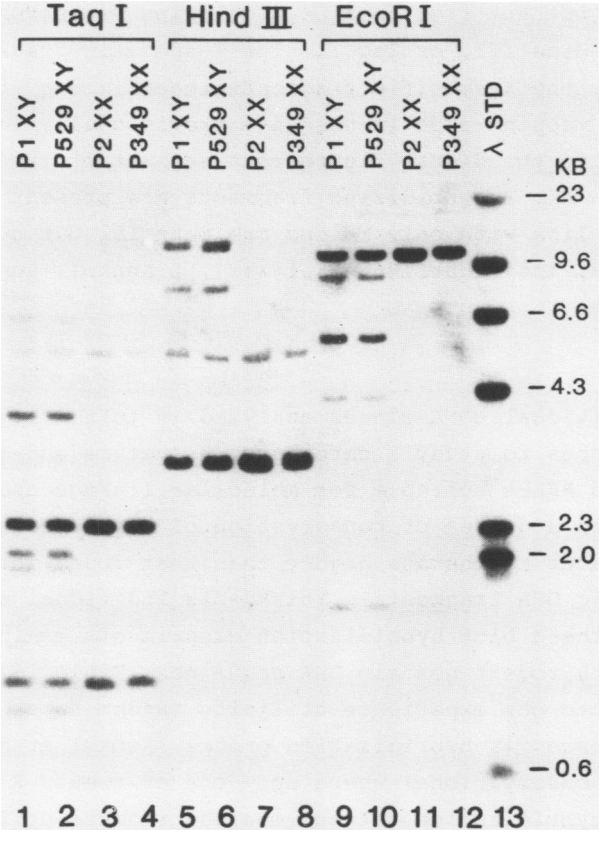

Figure 5: Autoradiograph of the hybridization of the derived subclone λ71-7A to various endonuclease digests of either male or female placental DNA

Four human DNA samples isolated from human placenta were cleaved with three restriction endonucleases, TaqI, Hind III and EcoRI. Each sample had been tested previously for completion of digestion (no other X chromosome DNA fragments when hybridized to these same cleaved samples showed differences between males and females). Three ug of the following digests (P1, 46,XY; P529; 46,XY; P2, 46,XX; P349, 46,XX) were separated by electrophoresis: lanes 1 through 4, TaqI-cleaved DNA samples; lanes 5 through 8 Hind III-cleaved DNA samples; lanes 9 through 12 EcoRI-cleaved DNA samples; lane 13 contains ^{32}P-labeled phage DNA cleaved with Hind III and the respective molecular weights are given. Y chromosome specific fragments are those additional fragments observed only in the male DNA samples.

from males, but not from females, following digestion of the DNA with EcoRI, Hind III, or Taq I. The Y-specific fragments differ in size from the X-specific fragments recognized by this probe. Preliminary mapping experiments have provisionally localized the Y fragments to the distal fluorescent segment of the human Y chromosome. All Y hybridizing fragments are present in a human female cell line with only Y long arm material but no short arm material (29; individual #6 46X,t(X;Y), L.Kunkel, unpublished observations).

DISCUSSION

The individual cDNA clones analyzed in this study have provided probes to study X chromosome structure and function, have yielded RFLPs suitable for molecular linkage analysis, and have detected a degree of conservation of expressed sequences between hamster and humans higher than that found for random human genomic DNA fragments. Thirty-six individual cDNA clones used in Southern blot hybridization experiments exhibited homology with rodent genomic DNA sequences (Table 1). This is in contrast to our experience utilizing random human genomic unique sequences as hybridization probes (Kunkel et al, unpublished observations) where only one of twenty X fragments show cross-hybridization between man and rodents utilizing the same stringency and hybrid DNA sample used in this study (Table 1). The rodent Hind III fragments are likely to represent expressed loci, segments of which are conserved between man and rodents. Variation in Hind III fragment sizes compared between two human individuals was observed for 4 cDNA clones. One of these 4 clones (HeLa #75) recognizes a useful RFLP (28) with the enzyme Hind III. Hybridization analysis of random cDNA clones against a few human DNA samples may thus represent a useful means of detecting RFLPs within the genome of humans.

The major emphasis of the Southern blotting analysis (13) of cDNA clones presented in Table 1 was to identify Hind III fragments from the X chromosome which hybridized with a particular RNA transcript. As presented in Table 1, homology to the X chromosome was detected for 8 of 56 cDNA clones. None of the eight cDNA clones hybridized solely to DNA from the human X

chromosome. This observation may be unique to X chromosome loci or may be common for many gene loci. The latter possibility is supported by the finding that many of the cDNA clones tested (Table 1) hybridized to 3 or more Hind III fragments in the genomic DNA of a single individual. The multiple sites of hybridization may reflect the presence of pseudogenes (30,31) for which only one locus is expressed, and/or they may represent multiple loci of gene families (32). What does emerge from the data obtained with X chromosome-homologous loci is that the genomic representation of a specific cDNA clone can be complex. The study of specific expression from a particular region of the genome necessitates the cloning of not only the cDNA clone but also the corresponding genomic clones.

Fluorescence activated flow-sorted phage libraries can facilitate the acquisition of a chromosome-specific genomic locus homologous to a cDNA clone with multiple chromosomal loci. Since most phage from such libraries contain inserts from the chromosome of interest, a specific phage, once identified and isolated, is likely to contain the desired genomic locus. The use of a Hind III complete digest library (3) aids in the identification of the desired genomic Hind III fragment during screening. Mapping a non-repeated subclone from a particular phage confirms the chromosomal assignment of the genomic fragment. A non-repeated subfragment has been isolated from two recombinant phage containing X chromosome-derived genomic DNA. Each is homologous to a different HeLa cDNA clone and both have been specifically mapped to a section of the human X chromosome. One presented here (Figure 3) maps to Xp21 -> Xpter, another (not presented) maps to Xq24 -> Xqter. Both are homologous to Poly A+ RNA as shown in (Figure 4) and may therefore be regions of the human X chromosome which are transcribed.

The study of expression from a specific region of the genome, such as we wish to perform for the human X chromosome, requires a unique DNA sequence from the region which is homologous to cellular RNA. Since both of the subclones presented here (λ71-7A and λ82-13A2) still retain some weak autosomal homology, as do other, known gene sequences (6,8), proof that a specific sequence on the X chromosome is indeed transcribed

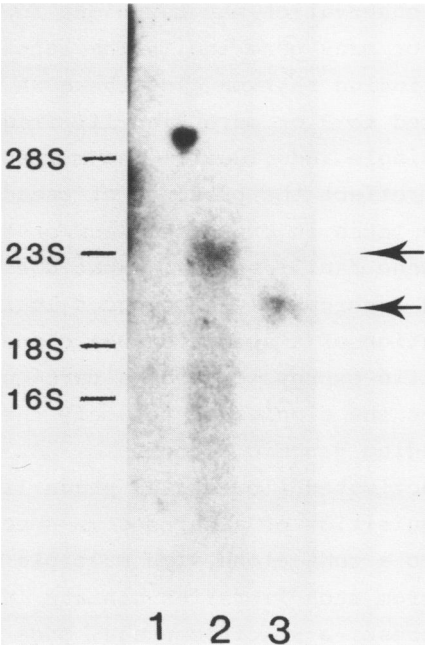

Figure 6: Hybridization analysis of λ71-7A with various RNA samples from hybrid cells.

RNA samples (10 ug) were separated by electrophoesis (see Methods) and transferred to nitrocellulose. The RNA samples were prepared from the following cell lines; lane 1, a rodent-human hybrid cell with an intact inactive human X chromosome among various human autosomes; no active human X and no autosome homologous locus to λ71-7A (the 5.5kb Hind III fragment, figure 2 and 3) were present in this cell line; lane 2, a rodent-human hybrid cell with an intact active human X chromosome among a few human autosomes but no λ 71-7A homologous autosome sequences (the 5.5 kb Hind III fragment, figures 2 and 3); lane 3, a rodent-human hybrid cell with no human X chromosome material, but the autosomal homologous locus to λ71-7A, the 5.5kb Hind III fragment. All hybrid cell lines were tested on Southern blots (13) for the presence or absence of various human Hind III fragments homologous to λ71-7A (Tantravahi and Bruns; unpublished observations). λ 71-7A was radiolabeled utilizing T4 DNA polymerase (11). The rRNA molecular weight markers on the left margin were visualized on the ethidium bromide stained gel. The arrows on the right margin correspond to transcript sizes of nearly 4500 bases in lane 2 and 2400 bases in lane 3. The lane smear is due to technical difficulties in preparing RNA from hybrid cells.

requires further experiments. These include comparative sequence data between the cDNA clone and the multiple genomic loci, or hybridization to RNA isolated from human-rodent hybrid cells containing a single active human X chromosome, but not the human autosomes recognized by the original cDNA. Such later experiments have been initiated for the λ71-7A subclone. Preliminary observations indicate that the 4500 base human transcript (Panel 2, Figure 4) is present in poly A^+RNA of a rodent-human hybrid cell that contains an intact active human X chromosome but no homologous human autosome loci (Figure 6).

The hybridization pattern of one X chromosome genomic subfragment homologous to cDNA is consistent with the initial localization (Figure 3) to the distal region of Xp. This portion of the X chromosome is thought to pair during meiosis with the short arm of the human Y chromosome (33). This same genomic clone also appears to hybridize to DNA located within the distal end of the Y chromosome long arm. The hybridization pattern of this clone, which detects X and Y chromosome homology, is in contrast to that found at another DNA locus on the X chromosome, DXYS1 (34). Whereas probes for DXYS1 hybridize to DNA from Yp and Xq (35, D. Page, personal communication) and exhibits extensive DNA sequence homology between the X and Y chromosome, the Xp localized subfragment λ71-7A has different restriction enzyme sites on the Y than that of the X (Figure 5) and is localized to Yq. Analysis of this cDNA clone HeLa #71 has led to the study of three different chromosomes: an autosomal region, the X chromosome and the Y chromosome. Restriction endonuclease mapping of these regions should delineate the extent of homology; RNA hybridization analysis should yield information concerning possible functional homology between the loci on the X and Y chromosomes.

The methodology presented here allows rapid isolation of multiple X chromosome DNA fragments which hybridize with cellular mRNA. Once uniquely mapped and proven homologous to RNA, these regions serve as possible transcription markers along the X chromosome. Coupled with known cloned loci of X chromosome origin, they should facilitate the delineation of

expression and activity along the entire extent of the human X chromosome.

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