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The *UTX* gene escapes X inactivation in mice and humans

Andy Greenfield[1,3,*], Laura Carrel[4], David Pennisi[1], Christophe Philippe[5,+], Nandita Quaderi[6,†], Pamela Siggers[3], Kirsten Steiner[7], Patrick P. L. Tam[7], Anthony P. Monaco[5], Huntington F. Willard[4] and Peter Koopman[1,2]

[1]Centre for Molecular & Cellular Biology and [2]Department of Anatomical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia, [3]MRC Mammalian Genetics Unit, Harwell, Didcot, Oxfordshire OX11 ORD, UK, [4]Department of Genetics, Center for Human Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA, [5]The Wellcome Trust Centre for Human Genetics, Oxford OX3 7BN, UK, [6]Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, London W2 1PG, UK and [7]Embryology Unit, Children's Medical Research Institute, Wentworthville, NSW 2145, Australia

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We recently have identified a ubiquitously transcribed mouse Y chromosome gene, *Uty*, which encodes a tetratricopeptide repeat (TPR) protein. A peptide derived from the UTY protein confers H-Y antigenicity on male cells. Here we report the characterization of a widely transcribed X-linked homologue of *Uty*, called *Utx*, which maps to the proximal region of the mouse X chromosome and which detects a human X-linked homologue at Xp11.2. Given that *Uty* is ubiquitously transcribed, we assayed for *Utx* expression from the inactive X chromosome (Xi) in mice and found that *Utx* escapes X chromosome inactivation. Only *Smcx* and the pseudoautosomal *Sts* gene on the mouse X chromosome have been reported previously to escape inactivation. The human *UTX* gene was also found to be expressed from Xi. We discuss the significance of these data for our understanding of dosage compensation of X–Y homologous genes in humans and mice.

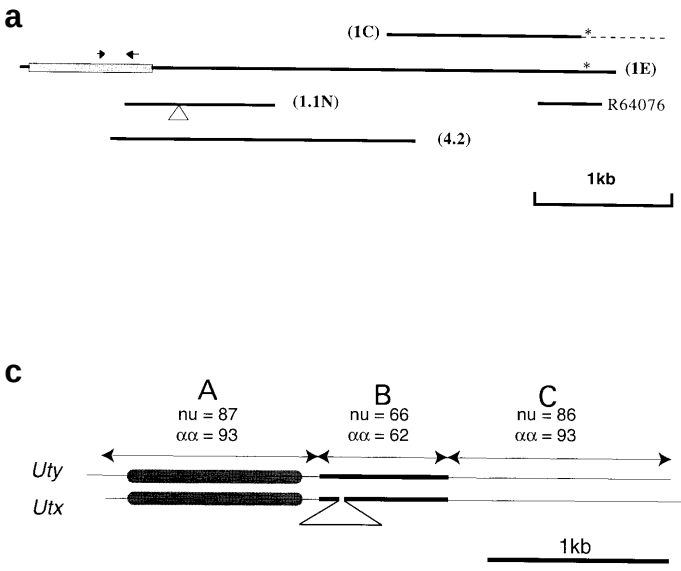
INTRODUCTION

The mammalian Y chromosome contains genes essential for male development, most notably the testis determining gene *SRY* and genes active during spermatogenesis. The observation that most Y-linked genes share an X-linked homologue has led to the widely held view that the mammalian Y chromosome represents a small subset of genes originating from the X chromosome, this subset varying somewhat from species to species. In this view, Y-linked genes are in a dynamic state of degradation, resulting in eventual loss, unless conserved due to their acquisition of male-specific functions (1). One potential driving force behind this evolution of the Y chromosome is X chromosome inactivation. Only a single copy of most X-linked genes is required for normal female development, and males thus have an extra, superfluous copy of any Y-linked gene which may diverge from the X-linked copy. Those few genes that escape X chromosome inactivation are predicted to have a widely expressed, functionally equivalent Y-linked homologue. There are, however, exceptions to this prediction (reviewed in ref. 1). Another complication is presented by the observed phenotypic difference between mice with an XO genotype and 45,X humans.

In humans, 99% of 45,X embryos die *in utero*, and those females that survive exhibit Turner stigmata (2,3). In mice, only 35% of XO individuals die *in utero*, and XO adult females, whilst exhibiting some reproductive deficiencies such as shortened reproductive lifespan and reduced litter size, are fertile and near normal phenotypically (4–6). The impact of X monosomy on viability and development has been attributed to the dosage deficiency of a gene or genes on the X chromosome which are normally expressed from both copies of the X chromosome and which are supplemented by functionally equivalent Y-linked homologues in males (7,8). To date, 17 genes localized to both the pseudoautosomal region and the X-specific portion of the X chromosome have been shown to be expressed from the inactive human X chromosome (9). Only two murine X-linked genes which escape X inactivation have been described: *Smcx* in the unique region (10,11) and *Sts* in the pseudoautosomal region (12). This discrepancy between mice and humans in the number of genes known to escape X inactivation has been used to explain the difference in viability of XO embryos between the two species (13). The validity of this explanation clearly depends on how many additional mouse X-linked genes are shown to escape X inactivation.

We recently have described a mouse Y-linked gene, *Uty*, which is widely expressed and encodes a tetratricopeptide repeat (TPR) protein (14). TPR motifs are found in a variety of functionally distinct proteins and are believed to mediate protein–protein

*To whom correspondence should be addressed. Tel: +44 1235 824 544; Fax: +44 1235 834 776; Email: a.greenfield@har.mrc.ac.uk
Present addresses: +Laboratoire de Génétique Médicale, CHU, Allée du Morvan, 54511 Vandoeuvre les Nancy cedex, France and †Telethon Institute of Genetics and Medicine, San Raffaele Biomedical Science Park, Milan, Italy



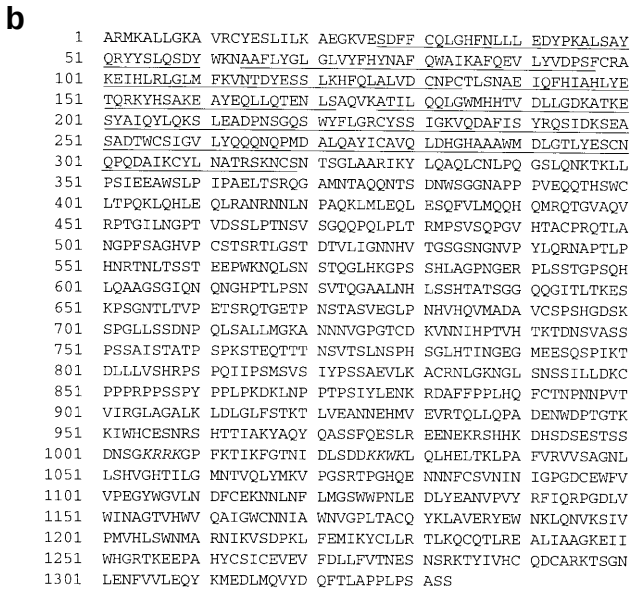


Figure 1. Isolation and characterization of mouse *Utx*. (**a**) Isolation of mouse *Utx* cDNAs. Screening of mouse embryonic cDNA libraries resulted in the isolation of four *Utx* cDNA clones. The relative positions and sizes of these are indicated. Clones 1C and 1E differ at their 3′ termini, diverging at a position (indicated by the broken line in clone 1C) shortly before in-frame stop codons in both clones (marked by asterisks). Clone 1.1N contains an additional 156 bp of sequence (indicated by the triangle) not found in 1E or 4.2. This does not disrupt the ORF. RT-PCR across this region confirms the existence of alternative splicing products (data not shown). The region of clone 1E encoding the TPR domain is boxed. The primers used to study *Utx* expression (Utx-12, -13) are indicated as arrows. The region of clone 1E corresponding to human EST R64076 is also indicated. (**b**) Predicted protein sequence of the *Utx* gene product. The translated 4001 bp ORF of clone 1E is shown. The eight TPR motifs are underlined; the last four are contiguous. Two potential nuclear localization signals (amino acid residues 1005–1008 and 1026–1029, respectively) are shown in italics. (**c**) Sequence comparison of *Uty* and *Utx*. A diagrammatic representation is shown of the alignment of the ORFs of *Uty* and *Utx*. The ORFs have been divided into three segments on the basis of their similarity. Segments are as follows: A, *Uty* nucleotides 39–1395, *Utx* 1–1146; B, *Uty* nucleotides 1396–2120, *Utx* 1147–2414; C, *Uty* nucleotides 2121–3558, *Utx* 2415–4001. The nucleotide (nu) identities and amino acid (αα) similarities are shown above each segment. The region of relative divergence is marked with a bold line. The *Utx*-specific region (nucleotides 1229–1756) is indicated by a non-aligned segment, and the region corresponding to the TPR domain is marked by a shaded box.

interaction (15). The 5.5 kb *Uty* transcript encodes an 1186 amino acid protein with eight TPR motifs at its N-terminus. Here we describe the isolation of an X-linked homologue of this gene, called *Utx* (ubiquitously-transcribed TPR gene on the X chromosome), which is expressed from the inactive X chromosome in both mice and humans.

RESULTS

Isolation and mapping of *Utx* cDNAs

We previously have described the detection of presumptive X-linked restriction fragments by Southern analysis of male (XY) and female (XX) genomic DNA with a *Uty* probe (14). Two mouse embryo cDNA libraries were screened with *Uty* probes in order to isolate the X-linked transcript, and four overlapping cDNAs were obtained (Fig. 1a). The single, 4001 bp open reading frame (ORF) of the largest *Utx* cDNA (1E) remains open at the 5′ end. We have been unable to isolate more of the transcript by conventional methods such as library screening and RACE-PCR. However, comparison with the ORFs of murine *Uty* and the recently reported human *UTX* gene (16) suggests that only some 200 bp of ORF at the 5′ end remains to be cloned. The *Utx* ORF is predicted to encode a member of the TPR protein family (Fig. 1b), exhibiting strong similarity to *Uty* at the both the nucleotide and amino acid level (Fig. 1c).

In order to confirm the chromosomal origin of the *Utx* transcript, a *Utx* cDNA clone (1.1N) was hybridized to *Taq*I-digested genomic DNA samples from progeny of an interspecific backcross, previously typed for a range of X-linked markers (17). A single hybridizing fragment was detected, differing in size between the two species [*Mus musculus* (C57BL/6) = 8.5 kb and *Mus spretus* = 7.5 kb] (data not shown). Haplotype analysis of 50 backcross progeny mice positions the gene between the X-linked markers *DXMit50* (3/50 recombinants, 6 ± 3.3 cM) and *DXMit72* (2/50 recombinants, 4 ± 2.8 cM) in the proximal region of the mouse X chromosome (Fig. 2a). In order to obtain a localization with respect to known genes in the proximal region of the mouse X chromosome, segregation of 1.1N was also analysed in 24 interspecific backcross progeny mice already typed for the genes *Otc*, *Ube1x* and *Hprt* and known to harbour recombination breakpoints between them (18). Haplotype analysis of these recombinant mice showed *Utx* to map between *Otc* and *Ube1x* (data not shown). Extrapolating from the two sets of mapping data, *Utx* can be positioned in a small interval on the mouse X chromosome proximal to *Ube1x* and distal to marker *DXMit72* (Fig. 2b). This interval contains the markers *Maoa* and *Maob*, and places *Utx* in band A2–A3 (19).

Expression

Reverse-transcriptase polymerase chain reaction (RT-PCR) with *Utx*-specific primers yielded a product in all male and female tissues tested in 13.5 days post-coitum (dpc) mouse embryos (Fig. 3a). Northern analysis of female mouse poly(A)$^+$ RNA samples using cDNA clone 1.1N detected a transcript of 5.0–5.5 kb in several adult tissues (Fig. 3b).

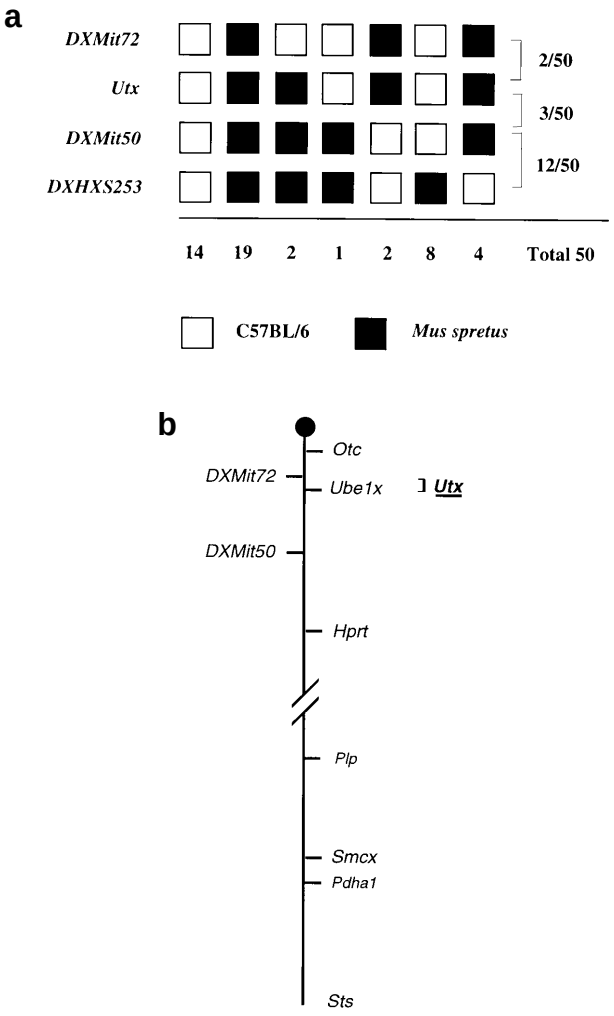


Figure 2. Genetic mapping of *Utx* on the mouse X chromosome. (**a**) Haplotype analysis of 50 interspecific backcross animals previously typed for X-linked markers (17). Each solid box represents an *M.spretus* allele, each open box an *M.musculus* (C57BL/6) allele. Numbers beneath each column indicate the number of mice in each recombinant class. Minimization of double recombinants suggests a location of *Utx* between the markers *DXMit72* and *DXMit50*. (**b**) Genetic map of the mouse X chromosome indicating the relative positions of the *DXMit72–DXMit50* interval and the anchor loci *Hprt*, *Ube1X* and *Otc* in the proximal region. The distal segment of the map also shows the position of the two genes previously shown to escape X inactivation in the mouse, *Smcx* (10) and the pseudoautosomal gene *Sts* (12). The distance between *Hprt* and *Plp* is ~38 cM (19).

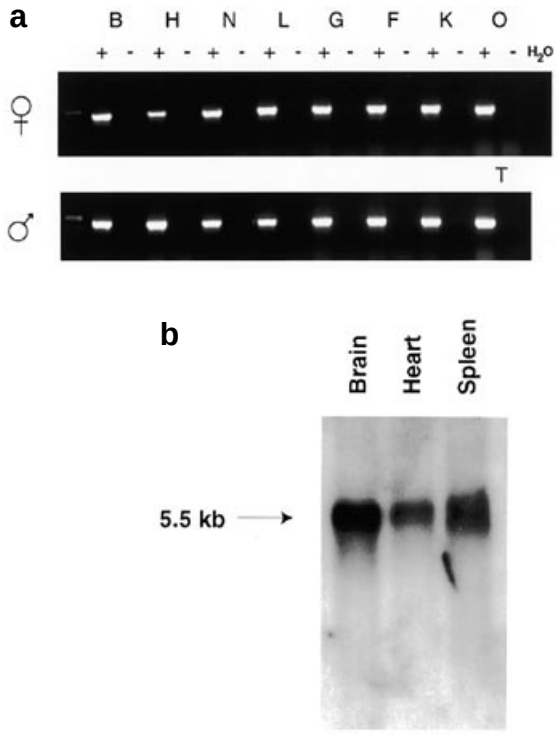


Figure 3. Expression of murine *Utx*. (**a**) RT-PCR with *Utx*-specific primers gives a 460 bp product in every male and female fetal tissue tested. RNA was derived from pooled tissues from 13.5 dpc fetuses (C3H/101 F1 animals). PCR (35 cycles) was performed with total RNA, both with (+) and without (–) reverse transcription, from the following tissues: brain (B), heart (H), lung (N), liver (L), gut (G), forelimb (F), kidney (K), ovary (O) and testis (T). The first track contains markers. (**b**) Hybridization of *Utx* clone 1.1N to poly(A)$^+$ RNA from three adult female tissues reveals a transcript of ~5.5 kb. No significant variation in transcript levels is apparent. Loading was controlled by hybridization with a β-actin probe (data not shown). Autoradiographic exposure was for 3 days.

Predicted protein product of *Utx*

The predicted *Utx* gene product shows strong similarity to the predicted product of the mouse *Uty* gene (14). Figure 1c shows an alignment of the ORFs of *Utx* and *Uty*, indicating a central region of divergence between the two genes where similarity drops to ~66%, at both the nucleotide and amino acid level. *Utx* and *Uty* share strong homology outside of the variant region (Fig. 1c). In addition, a region of ~500 bp of the 4kb *Utx* ORF appears to have no equivalent in the *Uty* composite cDNA described (14). RT-PCR employing primers in this region was used to confirm that it is exonic and not a contaminating intron (data not shown). This region is conserved in the human *UTX* cDNA (16).

Database searches with the *Utx* sequence identify other members of the TPR protein family, the best match to a gene product of known function being the yeast glucose repressor protein, Ssn6p (20), which acts as a transcriptional repressor. This best match is identical to that previously described for *Uty* (14).

Conservation of *UTX* on the human X chromosome

In order to identify an X-linked human homologue of *Utx*, primers were designed (hUTX-1 and hUTX-2) from the sequence of a human expressed sequence tag (EST; R64076) which shows 95% identity at the nucleotide level to murine *Utx*. An RT-PCR product, derived from human female fibroblast cDNA using these primers, was used in Southern analysis of a panel of rodent–human somatic cell hybrids carrying derivative X chromosomes (data not shown). This hybridization analysis indicated a location at Xp11.23–Xp11.3 between the t75-2ma-1b and SIN76 breakpoints (21). To confirm this localization of human *UTX*, high density cosmid filter arrays from a flow-sorted X chromosome library (22,23) were then screened with mouse cDNA clone 4.2 (Fig. 1a). A single positive cosmid was isolated and mapped by fluorescent *in situ* hybridization (FISH) on normal human metaphase spreads to Xp11.2 (data not shown).

A human Y-linked homologue

Southern analysis of male and female human genomic DNA with a murine *Uty* probe revealed male-specific fragments and fragments shared between males and females (data not shown). Hybridization of this probe to a series of contiguous yeast artificial chromosome (YAC) clones from the human Y chromosome (24) showed strong hybridization to yOX YAC clone 237, indicating a localization of human *UTY* to band 5D/5E of Yq (data not shown). Recently, *UTY* was shown to map to band 5C on the basis of deletion mapping (16). Band 5 of Yq is known to contain a gene(s) functioning in spermatogenesis (25) and a Y-specific growth gene (26).

Escape from X inactivation

In order to assay for expression from the inactive mouse X chromosome, females carrying the T(X;16)16H (T16H) translocation were used. As previously described (13,27–29), T16H females undergo non-random inactivation of the normal, paternally derived X chromosome. We exploited two variants between the normal X chromosome, derived from *M.castaneus*, and the T16H translocation X chromosome in order to assay for expression of *Utx* from Xi. *Utx* RT-PCR products derived from parental mice and F1 females carrying the T16H translocation were either analysed by gel electrophoresis (Fig. 4a) or sequenced directly (Fig. 4b). In the case of both variants analysed, the T16H and *M.castaneus Utx* alleles were detected in F1 T16H females, indicating that *Utx* escapes X chromosome inactivation in the mouse.

To determine whether the human *UTX* gene was expressed from the inactive X chromosome, RT-PCR using the hUTX primers described above was employed. A series of mouse–human somatic cell hybrids that retain independent active or inactive X chromosomes (30) were analysed for *UTX* expression. As shown in Figure 4c, the control gene *PGK1* is subject to inactivation, and although *PGK1* is expressed in the two hybrids carrying active human X chromosomes, no expression was seen in any of the five independent hybrids retaining inactive X chromosomes. In contrast, *UTX* expression was observed in both active and inactive X hybrids, demonstrating that the human gene escapes X inactivation. These data confirm the recent report of *UTX* expression from human Xi (16).

DISCUSSION

We have identified and characterized an X-linked gene which escapes X chromosome inactivation in both mice and humans. In mice, *Utx* is only the second gene from the X-specific portion of the X chromosome known to be expressed from the inactive X (Xi), the other being *Smcx* (10,11,29,31). In contrast, its human counterpart *UTX* is an addition to an already long list of genes which are expressed from human Xi (9,32).

The existence of human genes that escape X chromosome inactivation is widely believed to explain the 45,X Turner syndrome phenotype. Such X-linked 'Turner' genes are predicted to have a functionally equivalent Y-linked homologue (7,8). Thus in 46,XX and 46,XY individuals, a normal phenotype would result from an effective double dose of each of these genes, whilst in 45,X individuals, haploinsufficiency would result in death *in utero* or characteristic Turner stigmata in survivors. While several candidates have been proposed, including the recently identified *SHOX* gene (33), the gene(s) responsible for Turner syndrome have not been identified. *UTX* maps to a region of the human X chromosome (Xp11–22) associated with Turner syndrome in females bearing interstitial deletions (3) and escapes X chromosome inactivation. Whilst it is not yet known whether its Y-linked homologue *UTY* is functionally equivalent, the potential role of *UTX* in Turner syndrome should be investigated further.

The functional equivalence of *UTX* and *UTY* can be addressed directly in the mouse by targeted mutagenesis. Mouse *Utx* and *Uty* show strong overall homology, but the central region of divergence between the two could reflect either a lower requirement for structural conservation in this region or functional non-equivalence between the two proteins and a male-specific role for *Uty*. Comparison of the phenotype of males with females bearing one allele of *Utx* inactivated by homologous recombination should determine whether *Uty* or some other loci can function in the place of *Utx*. These experiments will also determine whether haploinsufficiency of *Utx* contributes to any of the phenotypic characteristics of the XO mouse, such as its reduced ability to support the survival and prenatal growth of embryos (6,34).

Differences in phenotype between 45,X humans and XO mice have been attributed to an absence of X-specific mouse genes escaping X inactivation (13). However, it is now clear that at least two such genes exist. Localized areas of persistent histone H4 acetylation have been detected on both mouse and human Xi, identifying three homologous chromosomal areas with potential transcriptional competence (35). *UTX* maps to one of these homologous regions: Xp11.2 in humans and band A2 in the mouse (Fig. 2). It will be interesting to determine whether these regions contain other genes escaping X chromosome inactivation, particularly on the mouse X chromosome.

Whilst the PCR experiments employed to assay expression of *Utx* from the inactive mouse X chromosome were not designed to be quantitative, expression from the active X chromosome appears to exceed that from Xi (Fig. 4a and b). This observation is similar to that originally described for the expression of *Smcx* from the mouse Xi (10), which was confirmed by quantitative procedures (29,31). It will now be possible to determine whether the stage and tissue specificity which characterize the degree of escape from X inactivation by the murine *Smcx* gene (29,31) are common to *Smcx* and *Utx*, or whether genes active on Xi exhibit unique dynamics of expression. This will allow further insight into the mechanism of spread of inactivation along the X chromosome and the functional significance of the escape of murine X-linked genes from this process.

MATERIALS AND METHODS

cDNA library screening and sequencing

cDNA clones 1E and 1C were isolated from an oligo(dT)-primed 7.5 dpc mouse embryo cDNA library. Clones 1.1N and 4.2 were isolated from a random-primed 11.5 dpc mouse embryo cDNA library (14). Clones and RT-PCR products were sequenced by dideoxy chain termination, and products were analysed on a 373A DNA Sequencer (Applied Biosystems).

Northern and Southern analyses

Total RNA was isolated using Trizol reagent (Gibco, BRL) and mRNA was selected with an Oligotex mRNA mini kit (Qiagen).

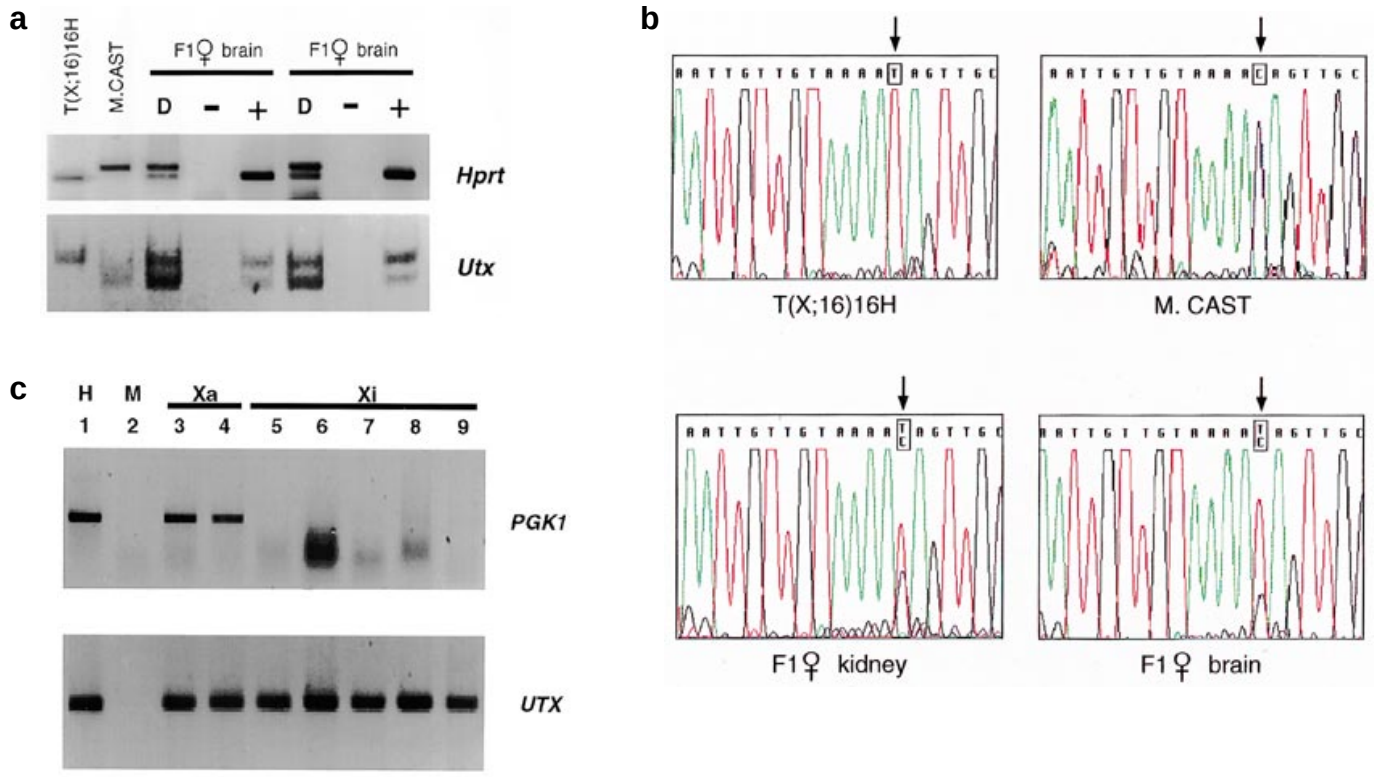


Figure 4. *UTX* escapes X chromosome inactivation in mice and humans. (**a**) Negative image of an ethidium bromide-stained gel showing mouse *Utx* RT-PCR products from brain RNA samples of T(X;16)16H (T16H), *M.castaneus* (M.CAST) and two newborn (T16H×*M.castaneus*) F1 females. F1 females bearing the T16H translocation show non-random inactivation of the paternally derived *M.castaneus* X chromosome. RT-PCR was performed with (+) and without (–) reverse transcriptase to control for genomic DNA contamination. 'D' is a genomic DNA control. Expression of both the T16H and *M.castaneus Utx* alleles is observed in the F1 carrier females, establishing that *Utx* is expressed from the inactive X chromosome. The control *Hprt* gene shows a non-random pattern of inactivation, as expected. Primers Utx-4 and Utx-5 were used for the first 20 cycles of amplification, and nested primers Utx-6 and Utx-7 were then employed for an additional 15 cycles on an aliquot of the first-round product. Products were digested with *Bst*EII to distinguish between the parental alleles. The control gene, *Hprt*, was amplified and alleles differentiated by *Hin*fI digestion as previously described (29). (**b**) Direct sequence analysis of *Utx* RT-PCR products from tissues of a T(X;16)16H female, an *M.castaneus* male (M.CAST) and a (T16H×*M.castaneus*)F1 female. Direct sequence analysis of *Utx* RT-PCR products from two tissues (kidney and brain) of an F1 female T16H carrier shows them to consist of a mixture of products derived from both the T16H allele and the *M.castaneus* allele. These data confirm the expression of *Utx* from the mouse Xi. Primers Utx-12 and Utx-13, which give no amplification product with genomic DNA template, were used for RT-PCR and Utx-15 for sequencing. Analysis of the expression of two control genes, *Hprt* and *Xist*, established that non-random X-inactivation had occurred in these F1 tissues (data not shown). (**c**) Negative image of ethidium bromide-stained gels showing RT-PCR amplification of human *UTX* and *PGK1* from a range of mouse–human somatic cell hybrids. Lanes 1 and 2 are control lanes showing amplification of *UTX* from human (H) and mouse (M) cDNA. Lanes 3 and 4 are from the mouse–human somatic cell hybrids t60–12 (lane 3) and AHA-11aB1 (lane 4), both of which contain an active human X chromosome on a mouse background. Somatic cell hybrids containing an inactive human X chromosome are t86-B1maz1b-3a (lane 5), t75-2maz34-4a (lane 6), t48-1a-1DAZ4A (lane 7), t11-4Aaz5 (lane 8) and LT23-1E2Buv5Cl26-7A2 (lane 9). *UTX* is expressed from both the active and inactive X chromosome, whilst *PGK1* shows expression only from the two hybrids containing an active X. The X chromosomal origin of the hUTX-1/2 product was also confirmed by amplification from cell hybrids AHA11aB1, t60-12 and LT23-1E2Buv5Cl26-7A2, which retain an X chromosome as their only human chromosome (lanes 3, 4 and 9). The low molecular weight product in *PGK1* tracks 6–8 is likely to be primer dimer.

RNA and DNA samples were electrophoresed, transferred to nylon filters and hybridized according to standard procedures (36).

RT-PCR

RNA was isolated using Trizol reagent (Gibco BRL) according to the manufacturer's recommendations. cDNA was prepared and PCR reactions were performed as previously described (28,29,37).

PCR primers

The PCR primers used are listed in Table 1.

Sequencing primers

Mouse *Utx* RT-PCR products were sequenced with the primer Utx-15: 5′-CCAAGGACTTCTGGAGATAC-3′.

Table 1. PCR primers

Primer	Sequence
hUTX-1	5′AGACATTGAGGGAAGCTCTC-3′
hUTX-2	5′-TCAAGATGAGGCGGATGGT-3′
Utx-4	5′-ATGAAAATACCAGGAGCTAAAC-3′
Utx-5	5′-AGTGTTTGCTCTGCTACCTG-3′
Utx-6[a]	5′-GATCTGTTTTGAGTGAAATGGTTAC-3′
Utx-7	5′-AAAATTGTGTACTGGCAGATGT-3′
Utx-12	5′-CAGCATGACCGTGGTCCAAT-3′
Utx-13	5′-CCCTGCACTTTGTCCAATGCT-3′

[a]Primer Utx-6 incorporates two mismatches at non-polymorphic bases. Amplification with this primer creates a *Bst*EII site in the *Utx* RT-PCR product of *M.castaneus*.

PCR amplification of human *UTX*

Primers hUTX-1 and hUTX-2 preferentially amplify the human *UTX* gene, though a faint product from mouse of the same size was also observed. Sequence analysis of this mouse transcript identified several nucleotide differences when compared with the human gene. Therefore, to analyse expression specifically from the human X chromosome in somatic cell hybrids, RT-PCR products were digested with *Hin*fI to cleave the mouse product, and a 299 bp human-specific product remains. To confirm further that the product was human in origin, the products amplified from two somatic cell hybrids containing independent inactive X chromosomes were sequenced and shown to be identical to the human X-linked EST R64076.

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