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A mouse Y Chromosome pseudogene is related to human ubiquitin activating enzyme *E1*

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Abstract. A 2041 bp DNA fragment isolated from the *Sxr* (sex reversed) region of the mouse Y Chromosome (Chr) was sequenced and characterized. The sequence, pY8/b, contains four exons that are highly similar to 525 contiguous bases from the cDNA of human ubiquitin activating enzyme *E1*. Two of the exons contain stop codons, indicating that pY8/b is not part of a functional gene. Sequences related to pY8/b were amplified from the Y Chr of the inbred mouse strain, C57BL/6J. These sequences may be portions of the recently discovered functional equivalent of pY8/b. Despite a high degree of similarity with the human *E1* gene, the functional equivalent of pY8/b is not the mouse *E1* gene, because unlike *E1*, the functional equivalent of pY8/b is expressed in a tissue-specific manner. These data are discussed with respect to theory on the evolution of the mammalian Y Chr, and in particular, to the prediction that functional genes on the Y Chr have a male-specific function.

Introduction

The Y Chr is unique among mammalian chromosomes because, with the exception of a small region that recombines with the X Chr in male meiosis, it is the only chromosome that does not recombine. Muller (1914) theorized that the Y Chr of *Drosophila* diverged from the X Chr as a result of two processes: crossover suppression between the ancestral pair of sex chromosomes, and the subsequent loss of gene function on the Y Chr. One prediction of Muller's hypothesis is that the non-recombining portion of the Y Chr should contain few functional loci. A second is that the Y Chr should have residual loci in the non-recombining region that are homologous to loci on the X Chr. These loci would reflect the ancestral homology between the X and Y Chrs.

Although Muller's hypothesis pertains to the evolution of the *Drosophila* Y Chr, it is also applicable to the evolution of the mammalian Y Chr. Recent advances in the molecular analysis of the Y Chr in mammals have allowed a direct test of the above predictions. For example, although numerous sequences have been isolated from the non-recombining region of the human and mouse Y Chrs, only three mouse and three human functional genes have been identified. These include the zinc finger genes, *Zfy-1* and *Zfy-2* in the mouse, and *ZFY* in human (Page et al. 1987; Mardon et al. 1989; Mardon and Page 1989; Mitchell et al. 1989; Nagamine et al. 1989); the male sex determining locus, *Sry* in mouse, and *SRY* in human (Sinclair et al. 1990; Gubbay et al. 1990; Koopman et al. 1991); and the human ribosomal protein gene, RPS4Y (Fisher et al. 1990). The mouse *Zfy-1* and *Zfy-2* loci appear to be homologous to a locus on the mouse X Chr, *Zfx* (Mitchell et al. 1989; Nagamine et al. 1989), and the human *ZFY* and *RPS4Y* genes appear to have homologs on the human X Chr, *ZFX* and *RPS4X*, respectively (Schneider-Gadicke et al. 1989; Palmer et al. 1990; Fisher et al. 1990). The ribosomal protein gene, *Rps4*, has also been mapped to the mouse X Chr (Hamuas et al. 1992). The presence of an X-specific homolog of *Zfy* (*ZFY*) in both mouse and human suggests ancestral homology between the mammalian X and Y Chrs.

To investigate further the evolutionary consequences of crossover suppression of the mammalian Y Chr, we sequenced and analyzed a DNA clone, pY8/b, reported to be a candidate Y-specific gene from the *Sxr* region of the mouse Y Chr (Bishop et al. 1987). The *Sxr* region on the Y Chr is a non-recombining region that contains both the testis determining locus, *Tdy*, and the H-Y transplantation antigen, *Hya* (reviewed in Eicher and Washburn 1986); it is located on

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the short arm of the Y Chr (McLaren et al. 1988; Roberts et al. 1988). We report that pY8/b contains part of a pseudogene related to the human ubiquitin activating enzyme E1. We provide evidence from amplification experiments that pY8/b-related sequences are located on the Y Chr in the inbred mouse strain C57BL/6J, and we discuss the relevance of these findings to the evolution of mammalian sex chromosomes.

Materials and methods

Source of pY8/b

pY8/b is a 2041 bp *Eco*RI fragment isolated from a flow sorted Y Chr library derived from a male of the inbred mouse strain designated 163H (Baron et al. 1986). This clone was selected as a candidate Y-specific gene because it hybridized with testis cDNA from male mice, but did not hybridize with liver cDNA from either male or female mice (Bishop et al. 1987). When used as a hybridization probe to *TaqI* restricted genomic DNA from male and female C57BL/6J, pY8/b detects four male-specific fragments. The same four fragments are also present when XX mice carrying the *Sxr* region of the male Y Chr are probed with pY8/b. Thus, pY8/b is from the *Sxr* region of the mouse Y Chr (Bishop et al. 1987).

Sequencing and analysis

pY8/b was sequenced in both directions using sequence-specific synthetic oligonucleotide primers and the Sanger dideoxy sequencing technique (Sanger et al. 1977). The nucleotide sequence was determined from double stranded products using Sequenase Version 2.0 polymerase under the conditions recommended by the manufacturer (United States Biochemical). The computer software program EuGene (release 3.2) was used to identify open reading frames of pY8/b and to search GenBank (release 67.0) and PIR (release 27.0) for similar nucleotide and peptide sequences, respectively. Regions of nucleotide and peptide similarity were identified (Lawrence et al. 1986; Lawrence and Goldman 1988) and percent similarities were calculated.

Amplification experiments

The pY8/b sequence was used to select three primer pairs for polymerase chain reaction (PCR) amplifications (Saiki et al. 1985) of related sequences in C57BL/6J inbred mice. The first primer pair spans the region between nucleotides 9 and 385; the second, the region between nucleotides 1640 and 1950; and the third, the region between nucleotides 1746 and 2030 (Fig. 1). Nine amplification experiments were conducted, one with each primer pair at each of three annealing temperatures (50°C, 48°C, and 38°C). Three samples were included in each experiment: a 1.5 μg sample of female C57BL/6J genomic DNA, a 1.5 μg sample of male C57BL/6J genomic DNA, and a blank to which no DNA was added. We used 5 μl of each 10 μM primer in each 100 μl reaction. The concentrations of *Taq* DNA polymerase, reaction buffer, and deoxynucleoside triphosphates per 100 μl reaction were as recommended by Perkin-Elmer Cetus. The amplification protocol was as follows: 25–35 cycles, with denaturation at 95°C for 1 min, annealing at 50°C, 48°C, or 38°C for 1 min, and elongation at 72°C for 1 min 15 s. PCR products were separated by size on a 3% Nusieve gel, stained with ethidium bromide, and visualized under UV light. To control for possible contamination of our template DNA, these experiments were repeated using two additional female and two additional male samples of C57BL/6J genomic DNA.

Southern blot preparation/DNA labeling/hybridization conditions

PCR products were transferred from the Nusieve gel to nylon membranes in 0.4 M NaOH following the protocol of Reed and Mann (1985). Nylon membranes were prewashed at 65°C for 1 h in 0.1% SSC and 0.5% SDS, and prehybridized at 65°C for 4 h in 100 ml of 4 × SSCP, 10× Denhardt's solution, and 1% SDS. Membranes were hybridized overnight at 65°C in 20 ml of 4 × SSCP, 2 × Denhardt's solution, 1% SDS, and approximately 1.0×10^6 cpm/ml denatured probe labeled with [α-^{32}P]-dCTP to a specific activity of $>1.0 \times 10^9$ cpm/μg using random primer labeling (Feinberg and Vogelstein 1983, 1984). Following hybridization, the nylon membranes were rinsed in a solution of 0.5 × SSC, 0.1% SDS at 53°C (low stringency conditions) or 0.1 × SSC, 0.1% SDS at 65°C (high stringency conditions), and exposed to Kodak XAR-5 film for 4 h at room temperature.

Results

Sequence analysis

The pY8/b sequence is 2041 nucleotides in length (Fig. 1) and contains four separate domains that, when spliced together, are highly similar to 525 contiguous bases from the published cDNA of human ubiquitin activating enzyme E1 (Handley et al. 1991; Table 1, Fig. 2). Domain 1 encompasses 72 bases (positions 372–443), contains two stop codons, and is 86% identical at the nucleotide level and 83% identical at the amino acid level with a portion of E1 (positions 2064–2135). Domain 2 encompasses 198 bases (positions 680–877), contains three stop codons, and is 74% identical at the nucleotide level and 79% identical at the amino acid level with a portion of E1 (positions 2136–2330). Domain 2 contains an additional GAC codon at positions 791–793 that is absent in the E1 cDNA. Domain 3 encompasses 75 bases (positions 1438–1512), contains no stop codons, and is 84% identical at the nucleotide level and 81% identical at the amino acid level with a portion of E1 (positions 2331–2405). Domain 4 includes 183 bases (positions 1617–1799), contains no stop codons, and is 84% identical at the nucleotide level and 75% identical at the amino acid level with a portion of E1 (positions 2406–2588).

Amplification experiments

Amplification experiments using the first primer pair (which spans the region between nucleotides 9 and 385, Fig. 1) and annealing temperatures of 50°C, 48°C, and 38°C consistently resulted in the amplification of two male-specific fragments, 376 bp and 410 bp in length (Fig. 3a, top panel). No DNA products were amplified from female genomic DNA. Thus, both fragments are located on the Y Chr.

Amplification experiments using the second primer pair (which spans the region between nucleotides 1640 and 1950, Fig. 1) and annealing temperatures of 50°C and 48°C, resulted in two male-specific fragments, 310 bp and 350 bp in length. No products were amplified from female genomic DNA at these temperatures (not shown in figure). At a lower annealing temperature of

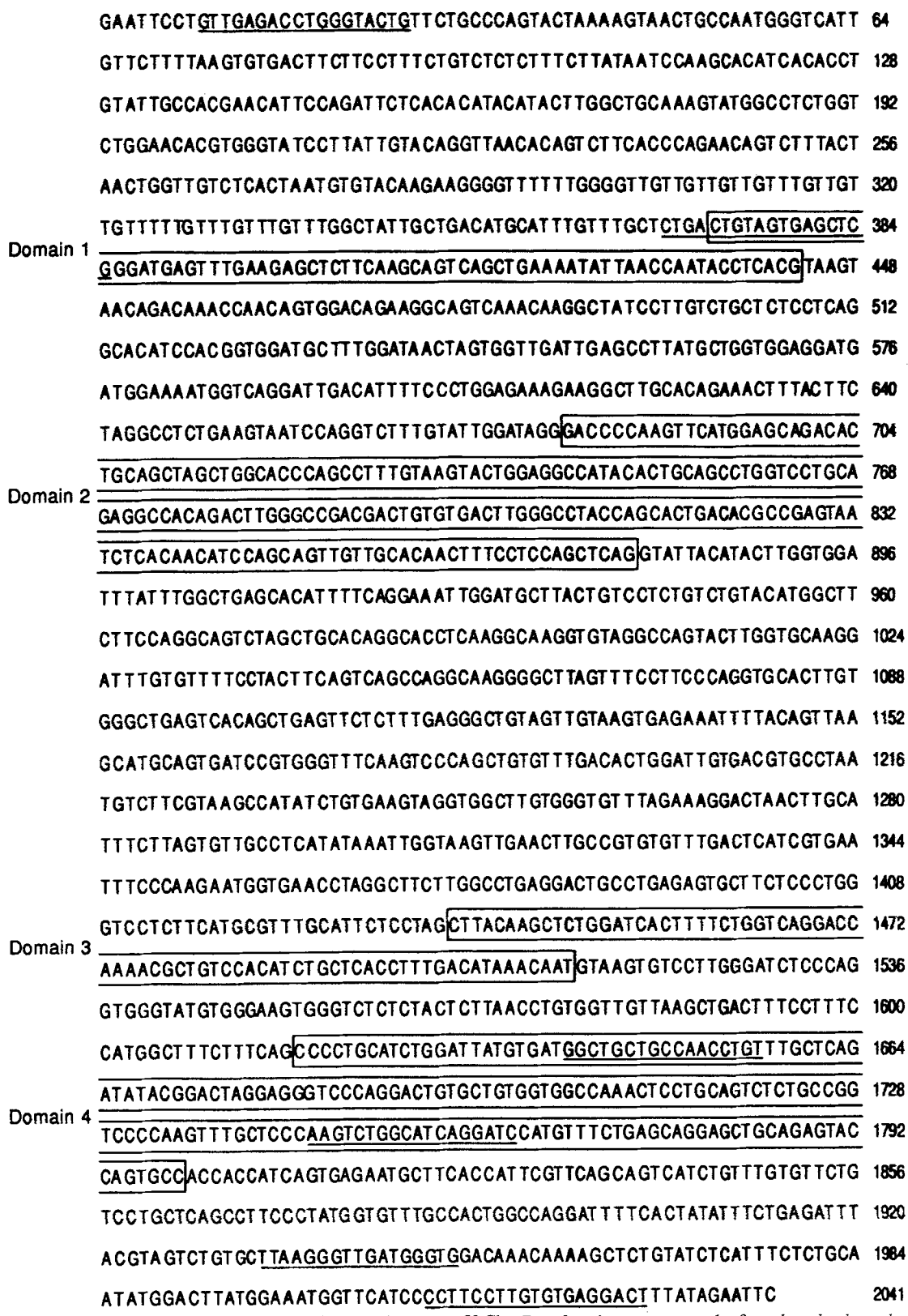

Fig. 1. The nucleotide sequence of pY8/b from the mouse Y Chr. **Boxed regions** represent the four domains homologous to 525 contiguous bases of human ubiquitin activating enzyme E1. Sequences used as primers in the amplification experiments are **underlined;** in each case the second primer of each pair is the reverse complement of the actual sequence used.

38°C, the same two male-specific fragments were amplified, and four female-specific fragments were detected; these were approximately 1500 bp, 1100 bp, 315 bp and 285 bp in length (Fig. 3b, top panel).

Amplification experiments using the third pair of primers (which spans the region between nucleotides 1746 and 2030) and annealing temperatures of 50°C, 48°C, or 38°C resulted in two male-specific fragments,

Table 1. Percent similarity at the nucleotide and amino acid level between portions of pY8/b from mouse and ubiquitin activating enzyme E1 from human.

Domain	Nucleotide Positions		Percent Identity	
	Mouse pY8/b	Human E1	Nucleotide	Amino Acid
1	372–443	2064–2135	86	83
2	680–877	2136–2330	74	79
3	1438–1512	2331–2405	84	81
4	1617–1799	2406–2588	84	75

284 bp and 320 bp in length, and two fragments shared between male and female, approximately 1300 bp and 1000 bp in length (Fig. 3c, top panel).

The PCR products from the three amplification experiments shown in Fig. 3 were hybridized with radioactively labeled pY8/b using the Southern blot technique (Fig. 3a-c, bottom panel). All male-specific PCR products hybridized at both low and high stringencies, confirming our assumption that these Y-specific PCR products are related to pY8/b. In contrast, the female-specific fragments, and the fragments shared between male and female C57BL/6J did not hybridize with pY8/b at either low or high stringencies, suggesting that they are not related to pY8/b.

Discussion

The high similarity between four domains in pY8/b and 525 contiguous bases of the cDNA from human ubiquitin activating enzyme *E1* (Handley et al. 1991) suggests that pY8/b contains exons of a gene related to *E1*. However, the presence of stop codons in the first and second pY8/b domains indicates that these exons are part of a nonfunctional gene. pY8/b was originally isolated from the Y Chr because it hybridizes with a cDNA synthesized from mouse testis mRNA (Bishop et al. 1987). This suggests that pY8/b has a functional equivalent in the mouse genome.

Our amplification data indicate that there are at least two pY8/b related sequences on the Y Chr of C57BL/6J. This finding is consistent with the recent discovery of a functional gene related to pY8/b on the Y Chr in the mouse (Mitchell et al. 1991). It is doubtful, however, that the functional equivalent of pY8/b on the Y Chr is the mouse *E1* gene. Ubiquitin activating enzyme E1 is a regulatory molecule that catalyzes the activation of ubiquitin. Ubiquitin has been isolated from a variety of eukaryotic organisms, and from a wide variety of tissues (reviewed in Wilkinson 1988). One would expect that E1 would also be found in a wide variety of mammalian tissues, and indeed, Northern blot analysis has shown that E1 is present in several human tissues as a single transcript approximately 3.5 kb in length (Handley et al. 1991). In contrast, the functional equivalent of pY8/b on the Y Chr appears to be expressed in a tissue-specific manner, that is, when pY8/b was used as a hybridization probe to poly(A)$^+$ RNA from adult male mouse liver and testis, it identified a testis-specific transcript approximately 3.5 kb in length (Bishop et al. 1987). This tissue specificity suggests that the Y-specific functional equivalent of pY8/b is not the mouse *E1* gene. Rather, it may be a member of a family of *E1*-like regulatory genes.

Our amplification and Southern blot experiments indicate that there are no pY8/b-related sequences in the female C75BL/6J genome. However, Mitchell and co-workers (1991) identified a ubiquitously expressed gene on the X Chr which they hypothesize to be the mouse *E1* gene. If pY8/b is related to the purported mouse *E1* gene on the X Chr, one would expect to amplify a pY8/b-related sequence in both females and males. Our failure to detect this gene using PCR can be explained in the following way. Most of the primers used in our experiments were constructed from introns, which are evolutionarily less conserved than exons. It is probable, therefore, that our primers were designed from a region of the pseudogene that is not conserved in the functional *E1* gene on the X Chr.

The presence of a pair of related functional genes in the nonpseudoautosomal portion of the X and Y Chrs is an intriguing find because, like the apparent homology between mouse *Zfx* and *Zfy-1*/*Zfy-2*, and between human *ZFX* and *ZFY*, and *RPS4X* and *RPS4Y*, it suggests ancestral homology between the mammalian X and Y Chrs. Because recombination is absent between such loci, they are effectively non-allelic genes, and divergence at both the nucleotide and amino acid level resulting in functional differences is expected. Studies of the zinc finger genes in mouse have determined that *Zfx* and *Zfy-1*/*Zfy-2* are 70% identical at the amino acid level and differ markedly in timing and specificity of expression (Mardon et al. 1990; Nagamine et al. 1990). In contrast, studies of the zinc finger genes in human have determined that *ZFX* and *ZFY* are on average 92% identical at the amino acid level and both genes are ubiquitously expressed (Schneider-Gadicke et al. 1989; Lau and Chan 1989; Palmer et al. 1990). The ribosomal protein genes, *RPS4X* and *RPS4Y*, located on the human X and Y Chrs are 93% identical at the amino acid level (Fisher et al. 1990). The *E1*-related genes on the X and Y Chrs in mouse are 85% identical at the amino acid level and differ in specificity of expression (Mitchell et al. 1991).

These differences between residual homologs on the X and Y Chrs may be best understood using an evolutionary perspective. For example, Charlesworth (1991) hypothesized a two-step process for the suppression of crossing-over between the X and Y Chrs which has implications for the function of Y-linked loci. First, suppression of crossing-over evolved between genes controlling male and female sex function; and second, genetic exchange was restricted between other sex chromosome-linked genes for which allelic variants were advantageous in males but disadvantageous in females.

One prediction of this hypothesis is that Y-linked loci, other than the sex determining locus, will function differently than their homologs on the X Chr. Y-linked genes would originally have been alleles of homologous X-Y loci that were subject to different

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Domain 1
          ***  ***                              glu                  ser              ile
mouse     CTG  TAG  TGA  GCT  CGG  GAT  GAG  TTT  GAA  GAG  CTC  TTC  AAG  CAG  TCA  GCT  GAA  AAT  ATT  AAC  CAA  TAC  CTC  ACG
human     CTG  CAG  TGG  GCT  CGG  GAT  GAG  TTT  GAA  GGC  CTC  TTC  AAG  CAG  CCA  GCA  GAA  AAT  GTC  AAC  CAG  TAC  CTC  ACA
          leu  gln  trp  ala  arg  asp  glu  phe  glu  gly  leu  phe  lys  gln  pro  ala  glu  asn  val  asn  gln  tyr  leu  thr

Domain 2
                         met       gln           gln                                  ***                    ile  his  cys
mouse     GAC  CCC  AAG  TTC  ATG  GAG  CAG  ACA  CTG  CAG  CTA  GCT  GGC  ACC  CAG  CCT  TTG  TAA  GTA  CTG  GAG  GCC  ATA  CAC  TGC
human     GAC  CCC  AAG  TTT  GTG  GAG  CGA  ACA  CTG  CGG  CTG  GCA  GGC  ACT  CAG  CCC  TTG  GAG  GTG  CTG  GAG  GCT  GTG  CAG  CCG
          asp  pro  lys  phe  val  glu  arg  thr  leu  arg  leu  ala  gly  thr  gln  pro  leu  glu  val  leu  glu  ala  val  gln  pro

                                                        asp                       tyr  gln       ***       ala  glu
mouse     AGC  CTG  GTC  CTG  CAG  AGG  CCA  CAG  ACT  TGG  GCC  GAC  GAC  TGT  GTG  ACT  TGG  GCC  TAC  CAG  CAC  TGA  CAC  GCC  GAG
human     AGC  CTG  GTG  CTG  CAG  CGA  CCA  CAG  ACC  TGG  GCT  GAC  ---  TGC  GTG  ACC  TGG  GCC  TGC  CAC  CAC  TGG  CAC  ACC  CAG
          ser  leu  val  leu  gln  arg  pro  gln  thr  trp  ala  asp  ---  cys  val  thr  trp  ala  cys  his  his  trp  his  thr  gln

          ***       his            gln                                    ala
mouse     TAA  TCT  CAC  AAC  ATC  CAG  CAG  TTG  TTG  CAC  AAC  TTT  CCT  CCA  GCT  CAG
human     TAC  TCG  AAC  AAC  ATC  CGG  CAG  CTG  CTG  CAC  AAC  TTC  CCT  CCT  GAC  CAG
          tyr  ser  asn  asn  ile  arg  gln  leu  leu  his  asn  phe  pro  pro  asp  gln

Domain 3
                              ser  leu                                                  leu                    ile
mouse     CTT  ACA  AGC  TCT  GGA  TCA  CTT  TTC  TGG  TCA  GGA  CCA  AAA  CGC  TGT  CCA  CAT  CTG  CTC  ACC  TTT  GAC  ATA  AAC  AAT
human     CTC  ACA  AGC  TCA  GGA  GCG  CCG  TTC  TGG  TCT  GGG  CCC  AAA  CGC  TGT  CCA  CAC  CCG  CTC  ACC  TTT  GAT  GTC  AAC  AAT
          leu  thr  ser  ser  gly  ala  pro  phe  trp  ser  gly  pro  lys  arg  cys  pro  his  pro  leu  thr  phe  asp  val  asn  asn

Domain 4
                                                                                  ile                 gly
mouse     CCC  CTG  CAT  CTG  GAT  TAT  GTG  ATG  GCT  GCT  GCC  AAC  CTG  TTT  GCT  CAG  ATA  TAC  GGA  CTA  GGA  GGG  TCC  CAG  GAC
human     CCC  CTG  CAT  CTG  GAC  TAT  GTG  ATG  GCT  GCT  GCC  AAC  CTG  TTT  GCC  CAG  ACC  TAC  GGG  CTG  ACA  GGC  TCT  CAG  GAC
          pro  leu  his  leu  asp  tyr  val  met  ala  ala  ala  asn  leu  phe  ala  gln  thr  tyr  gly  leu  thr  gly  ser  gln  asp

          cys       val            lys  leu                leu  pro           lys       ala                    ile  arg
mouse     TGT  GCT  GTG  GTG  GCC  AAA  CTC  CTG  CAG  TCT  CTG  CCG  GTC  CCC  AAG  TTT  GCT  CCC  AAG  TCT  GGC  ATC  AGG  ATC  CAT
human     CGA  GCT  GCT  GTG  GCC  ACA  TTC  CTG  CAG  TCT  GTG  CAG  GTC  CCC  GAA  TTC  ACC  CCC  AAG  TCT  GGC  GTC  AAG  ATC  CAT
          arg  ala  ala  val  ala  thr  phe  leu  gln  ser  val  gln  val  pro  glu  phe  thr  pro  lys  ser  gly  val  lys  ile  his

               glu                       thr  ser
mouse     GTT  TCT  GAG  CAG  GAG  CTG  CAG  AGT  ACC  AGT  GCC
human     GTT  TCT  GAC  CAG  GAG  CTG  CAG  AGC  GCC  AAT  GCC
          val  ser  asp  gln  glu  leu  gln  ser  ala  asn  ala
```

Fig. 2. The nucleotide sequence of the four domains of pY8/b from mouse compared with the homologous nucleotide sequence of E1 from human. The amino acid sequence from human is given below the E1 sequence. Differences in the amino acid of the mouse are given above the pY8/b sequence. **Asterisks** indicate stop codons. A **dashed line** in the E1 sequence indicates a gap.

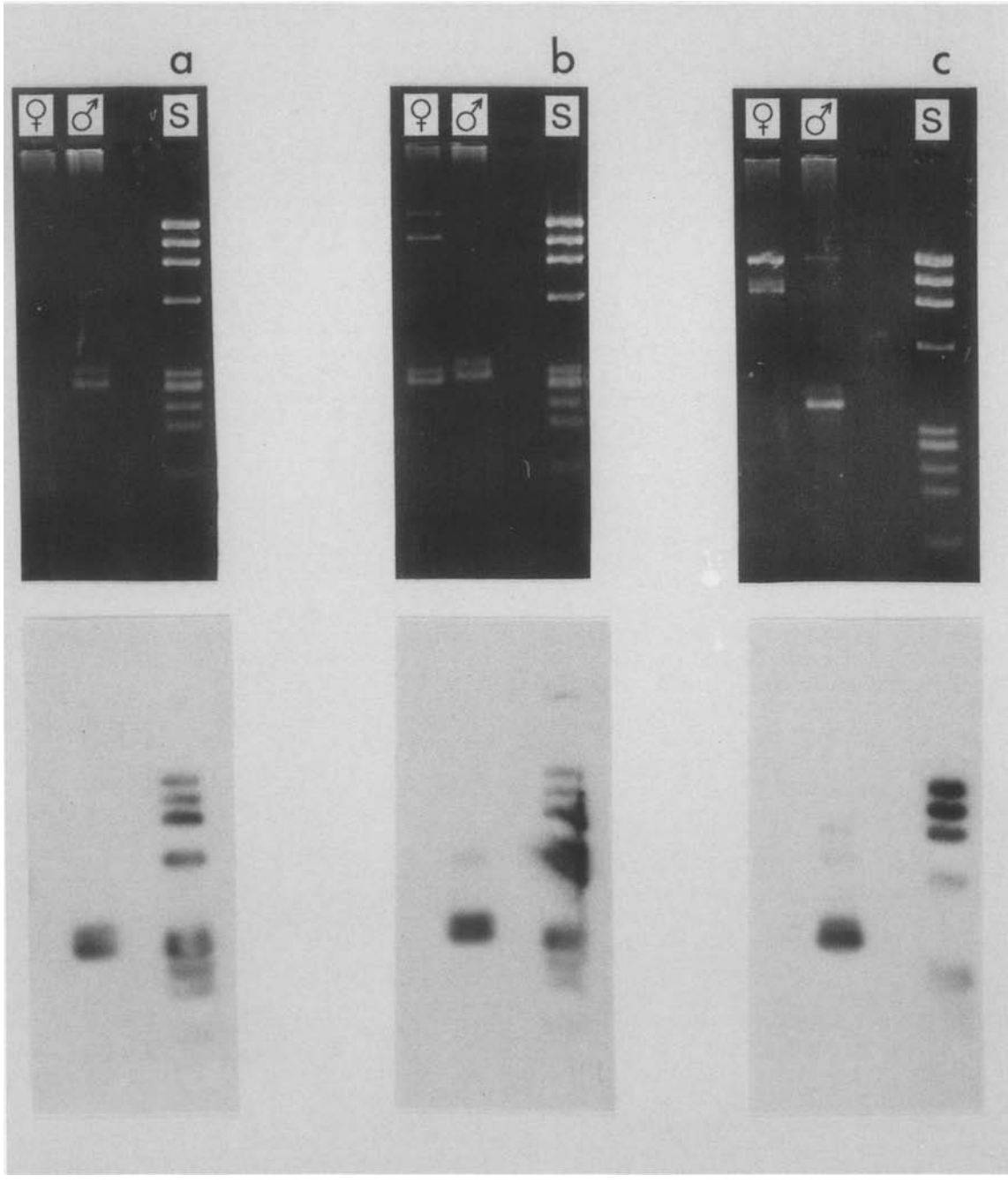

Fig. 3. Results of the amplification experiments using three pairs of primers from pY8/b. PCR products were separated by size on a 3% Nuseive gel and stained with ethidium bromide. Female (♀) and male (♂) genomic DNA was used in each experiment as well as a blank to control for contamination. The size standard (S) is *Hae*III digested phiX174 RF DNA. **Top panel: (a)** Amplification of the male-specific target size fragment, 376 bp in length, and an additional male-specific fragment, 410 bp in length, from C57BL/6J using an annealing temperature of 48°C and primers spanning nucleotides 9–385. Identical results are obtained at annealing temperatures of 38°C and 50°C. **(b)** Amplification of the target size 310 bp fragment and an additional 350 bp fragment in male C57BL/6J, and four fragments, approximately 1500 bp, 1100 bp, 285 bp and 315 bp in length in female C57BL/6J using an annealing temperature of 38°C and primers spanning nucleotides 1640–1950. The presence of female-specific fragments can be explained by hypothesizing that, in the absence of a good match with female template DNA, the primers annealed to other, less well matched genomic sequences unrelated to pY8/b. This is further supported by the fact that the female-specific fragments did not amplify at annealing temperatures of 48°C and 50°C (not shown in figure). **(c)** Amplification of the target size 284 bp male-specific fragment, an additional 320 bp male-specific fragment, and two fragments shared between male and female, 1300 bp and 1000 bp in length using an annealing temperature of 48°C and primers spanning nucleotides 1746–2030. Identical results are obtained at 38°C and 50°C. **Bottom panel:** Corresponding Southern blot hybridized with pY8/b under high stringency conditions. No female-specific fragments or fragments shared between males and females hybridized with pY8/b under low or high stringency conditions.

selection in males and females. With the evolution of crossover suppression between the ancestral X and Y Chrs, alleles fixed on the Y Chr would become independently evolving loci that code for male-specific characters. If *Zfy*, *RPS4Y*, and the *E1*-related locus on the Y evolved from the ancestral mammalian X and Y, then they should function in a male-specific manner. A direct test of this prediction will come from two kinds of studies: phylogenetic studies to determine whether these loci were members of the ancestral mammalian X and Y Chrs, and functional studies of these genes at the protein level.

The zinc finger genes on the sex chromosomes are the first for which there are data of this kind. Although these genes are present on the sex chromosomes in a variety of eutherian orders (Page et al. 1987), they are not present on the sex chromosomes in metatherians (Sinclair et al. 1988), suggesting that they were not part of the ancestral mammalian X and Y Chrs (assuming a single origin for the mammalian XY pair). In addition, the zinc finger genes on the X and Y Chrs in humans are highly similar and ubiquitously expressed, indicating that the zinc finger gene on the Y may not necessarily function in a male-specific manner (Schneider-Gadicke et al. 1989; Palmer et al. 1990). Together, these data do not violate the prediction that Y-linked loci will function differently than their residual homologs on the X Chr, because *ZFY* may not have been part of the ancestral mammalian Y Chr. However, the testis-specific expression of *Zfy-1* and *Zfy-2* in adult mice (Nagamine et al. 1990) suggests that these genes function in a male-specific manner in some species. If the male-specific function of *Zfy-1* and *Zfy-2* in the mouse evolved subsequent to the translocation of the zinc finger genes onto the mammalian X and Y Chrs, as the phylogenetic data indicate, then the male-specific function of *Zfy-1*/*Zfy-2* in the mouse cannot be explained by Charlesworth's hypothesis. Rather, a different explanation is needed for the male-specific expression of *Zfy-1*/*Zfy-2* in the mouse. Additional research on the expression of the zinc finger genes across a variety of mammalian taxa should clarify whether differential function of these genes, as seen in the mouse, is indeed an evolutionarily derived trait. More generally, these kinds of studies, investigating the history and function of residually homologous loci from the X and Y Chrs, should significantly further our understanding of sex chromosome evolution.

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References

Baron, B., Metezeau, P., Hatat, D., Roberts, C., Goldberg, M.E., and Bishop, C.E.: Cloning of DNA libraries from mouse Y chromosomes purified by flow cytometry. *Som Cell Mol Gen 12:* 289–295, 1986.

Bishop, C.E., Roberts, C., Michot, J.-L., Nagamine, C., Winking, H., Guénet, J.-L., and Weith, A.: The use of specific DNA probes to analyse the *Sxr* mutation in the mouse. *Development 101(Suppl):* 167–175, 1987.

Charlesworth, B.: The evolution of sex chromosomes. *Science 251:* 1030–1033, 1991.

Eicher, E.M. and Washburn, L.L.: Genetic control of primary sex determination in mice. *Ann Rev Genet 20:* 327–360, 1986.

Feinberg, A.P. and Vogelstein, B.: A technique for radiolabeling DNA restriction endonuclease fragments to a high specific activity. *Anal Biochem 132:* 6–13, 1983.

Feinberg, A.P. and Vogelstein, B.: A technique for radiolabeling DNA restriction endonuclease fragments to a high specific activity. *Anal Biochem 137:* 266–267, 1984.

Fisher, E.M.C., Beer-Romero, P., Brown, L.G., Ridley, A., McNeil, J.A., Lawrence, J.B., Willard, H.F., Bieber, F.R., and Page, D.C.: Homologous ribosomal protein genes on the human X and Y chromosomes: Escape from X inactivation and possible implications for Turner syndrome. *Cell 63:* 1205–1218, 1990.

Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Munsterberg, A., Vivian, N., Goodfellow, P., and Lovell-Badge, R.: A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature 346:* 245–250, 1990.

Hamuas, R.M., Brown, S.D.M., Kerr, J.T., Fisher, E.M., Romero, P., Vinn, A., and Page, D.: The mapping of the locus *Rps4* to the X inactivation region in the mouse. *Cytogenet Cell Genet,* in press, 1992.

Handley, P.M., Mueckler, M., Siegel, N.R., Ciechanover, A., and Schwartz, A.L.: Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. *Proc Natl Acad Sci USA 88:* 258–262, 1991.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P.N., and Lovell-Badge, R.: Male development of chromosomally female mice transgenic for *Sry*. *Nature 351:* 117–121, 1991.

Lau, Y.-F.C. and Chan, K.: The putative testis-determining factor and related genes are expressed as discrete-sized transcripts in adult gonadal and somatic tissues. *Am J Hum Genet 45:* 942–952, 1989.

Lawrence, C.B., Goldman, D.A., and Hood, R.A.: Optimized homology searches of the gene and protein sequence banks. *Bull Math Biol 48:* 569–583, 1986.

Lawrence, C.B. and Goldman, D.A.: Definition and identification of homology domains. *CABIOS 4:* 25–33, 1988.

Mardon, G. and Page, D.C.: The sex-determining region of the mouse Y chromosome encodes a protein with a highly acidic domain and 13 zinc fingers. *Cell 56:* 765–770, 1989.

Mardon, G., Mosher, R., Disteche, C.M., Nishioka, Y., McLaren, A., and Page, D.C.: Duplication, deletion and polymorphism in the sex-determining region of the mouse Y chromosome. *Science 243:* 78–80, 1989.

Mardon, G., Luoh, S.-W., Simpson, E.M., Gill, G., Brown, L.G., and Page, D.C.: Mouse *Zfx* protein is similar to *Zfy-2:* Each contains an acidic activating domain and 13 zinc fingers. *Mol Cell Biol 10:* 681–688, 1990.

McLaren, A., Simpson, E., Epplen, J.T., Studer, R., Koopman, P., Evans, E.P., and Burgoyne, P.S.: Location of the genes controlling H-Y antigen expression and testis determination on the mouse Y chromosome. *Proc Natl Acad Sci USA 85:* 6442–6445, 1988.

Mitchell, M.M., Simon, D., Affara, N., Ferguson-Smith, M., Avner, P., and Bishop, C.E.: Localization of murine X and autosomal sequences homologous to the human Y located testis-determining region. *Genetics 121:* 803–809, 1989.

Mitchell, M.M., Woods, D.R., Tucker, P.K., Opp, J.S., and Bishop, C.E.: A candidate spermatogenic gene from the mouse Y chromosome may be a ubiquitin activating enzyme. *Nature 354:* 483–486, 1991.

Muller, H.J.: A gene for the fourth chromosome of *Drosophila*. *J Exp Zool 17:* 325–336, 1914.

Nagamine, C.M., Kaimin, C., Kozak, C.A., and Lau, Y.-F.C.:

Chromosome mapping and expression of a putative testis-determining gene in the mouse. *Science 342:* 80–83, 1989.

Nagamine, C.M., Chan, K., Hake, L.E., and Lau, Y.-F.C.: The two candidate testis-determining Y genes (*Zfy-1* and *Zfy-2*) are differentially expressed in fetal and adult mouse tissues. *Genes Develop 4:* 63–74, 1990.

Palmer, M.S., Berta, P., Sinclair, A.H., Pym, B., and Goodfellow, P.N.: Comparison of human *ZFY* and *ZFX* transcripts. *Proc Natl Acad Sci USA 87:* 1681–1685, 1990.

Page, D.C., Mosher, R., Simpson, E.M., Fisher, E.M.C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., and Brown, L.G.: The sex-determining region of the human Y chromosome encodes a finger protein. *Cell 51:* 1091–1104, 1987.

Reed, K.C. and Mann, D.A.: Rapid transfer of DNA from agarose gels to nylon membranes. *Nucl Acids Res 13:* 7207–7221, 1985.

Roberts, C., Weith, A., Passage, E., Michot, J.L., Mattei, M.G., and Bishop, C.E.: Molecular and cytogenetic evidence for the location of *Tdy* and *Hya* on the mouse Y chromosome short arm. *Proc Natl Acad Sci USA 85:* 6446–6449, 1988.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, A., and Arnheim, N.: Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science 230:* 1350–1354, 1985.

Sanger, F., Nicklen, S., and Coulson, A.R.: DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA 74:* 5463–5467, 1977.

Schneider-Gadicke, A., Beer-Romero, P., Brown, L.G., Nussbaum, R., and Page, D.C.: *ZFX* has a gene structure similar to *ZFY,* the putative human sex determinant, and escapes X inactivation. *Cell 75:* 1247–1258, 1989.

Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A.-M., Lovell-Badge, R., and Goodfellow, P.N.: A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature 346:* 240–244, 1990.

Sinclair, A.H., Foster, J.W., Spencer, J.A., Page, D.C., Palmer, M., Goodfellow, P.N., and Graves, J.A.: Sequences homologous to *ZFY,* a candidate human sex-determining gene, are autosomal in marsupials. *Nature 336:* 780–783, 1988.

Wilkinson, K.D.: Purification and structural properties of ubiquitin. *In* M. Rechsteiner (ed.); *Ubiquitin,* pp. 5–38, Plenum Press, New York, 1988.