J Mol Evol (1991) 32:310–315

Journal of Molecular Evolution

The Molecular Evolution of ZFY-Related Genes in Birds and Mammals

J. Lanfear* and P.W.H. Holland

Molecular Zoology Laboratory, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

Summary. We report the isolation and nucleotide sequence determination of clones derived from five ZFY-related zinc-finger genes from birds and mammals. These sequences are analyzed with reference to the previously published human genes, ZFX and ZFY, and mouse genes, Zfx, Zfa, Zfy-1, and Zfy-2. The analysis indicates that ZFY-related genes are highly conserved in birds and mammals, and that the rate of nucleotide substitution in the Y-linked genes is not as high as predicted. However, the mouse Zfy-1 and Zfy-2 genes are markedly divergent members of the ZFY gene family; we suggest this relates to X-inactivation of the mouse gene Zfx.

Key words: DNA evolution — ZFY — Divergence — Polymerase chain reaction — Sex determination — Zinc fingers

Introduction

ZFY is a human gene located on the Y chromosome near the pseudoautosomal boundary (Page et al. 1987). Although ZFY was initially thought to be the testis-determining factor, TDF, this suggestion has been recently refuted (Koopman et al. 1989; Palmer et al. 1989). ZFY consists of four exons; the last of which encodes 13 zinc-finger domains with an unusual odd and even double repeat periodicity. There is also a closely related gene on the X-chromosome designated ZFX (Schneider-Gädicke et al. 1989). The gene family is more complex in mice with two closely related genes on the Y-chromosome (Zfy-1 and Zfy-2), one gene on the X-chromosome (Zfx), and a retroposon derived from Zfx on chromosome 10 (Zfa; Ashworth et al. 1989; Mardon and Page 1989). Southern analysis suggests that in all other eutherian mammals, the number of ZFY-related genes and their organization is similar to humans (Page et al. 1987). Sequences capable of hybridizing to ZFY-related gene probes are also present in marsupials (Sinclair et al. 1988), chicken (Page et al. 1987), reptiles (Bull et al. 1988), fish (Ferreiro et al. 1989), and echinoderms (J.L., unpublished); however, in each case these are probably located autosomally and are not sex-linked.

The six ZFY-related gene sequences published to date allow the mammalian gene family members to be classified into two distinct subfamilies; one comprising Zfy-1 and Zfy-2, the other comprising ZFY, ZFX, Zfx, and Zfa (Page et al. 1987; Ashworth et al. 1989, 1990; Mardon and Page 1989; Schneider-Gädicke et al. 1989; Mardon et al. 1990). To elucidate the evolutionary basis for this division, it is necessary to compare homologous DNA sequences from vertebrates other than mouse and human. In addition, because rates and modes of molecular evolution may be affected by sex linkage (Miyata et al. 1987), it will be interesting to compare the nucleotide sequences of autosomal ZFY homologues in nonmammalian vertebrates. To enable these comparisons, we have employed the polymerase chain reaction (PCR; Saiki et al. 1988) to isolate genomic DNA clones derived from ZFY-related genes from two additional species of mammal (hamster and crab-eating fox) and two species of bird (great tit and lesser black-backed gull). Nucleotide sequence analyses reveal striking evolutionary conservation in the zinc-finger region of ZFY-related genes from birds and mammals, irrespective of their chromosomal location. Furthermore, the great-tit Zf, gull Zf, hamster ZFX, fox ZFX, and fox ZFY sequences are all characteristic of the ZFY/ZFX gene subfamily, indicating that the mouse Zfy-1 and Zfy-2 genes underwent rapid. recent sequence divergence.

Present address and offprint requests to: J. Lanfear, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, UK

Table 1. Details of all clones analyzed

Species and sex	Notation	Band sizes produced by amplification	Number of recombinant colonies	Number of colonies screened (I)	Number of clones sequenced	Number containing artifacts	Number containing contaminants	Number with sequences homologous to ZFY	Number of different genes present
Chinese hamster (female)	ZFX-(CHA)	360	98	98	5	0	0	5	1
Crab-eating fox (male)	ZFX/Y-(FOX)	360	16	16	10	1	0	9	2
Great tit (female)	ZF-(PM)	360	180	115	29	14	6	9	1
Great tit (male)	ZF-(PM)	360	25	25	6	3	0	3	1
Lesser gull (male)	ZF-(LF)	360	200	8	4	1	0	3	1

Materials and Methods

Materials. Male and female crab-eating fox (*Dusicyon thous*) DNA was prepared from blood. Hamster DNA was isolated from the CHO (Chinese hamster ovary) cell line, and was provided by Mark Meuth (ICRF, Clare Hall, UK). The avian DNAs were isolated from blood samples collected under license from wild birds by standard methods (Griffiths and Holland 1990). The two oligonucleotide primers (positive strand 5'-CACATTTGTGGNGAATGNGGNAAAGG-3' and negative strand 5'-GGGGTATTCACACTNTACACG-3') matched to the cysteine pair region of fingers 5 and 9, respectively, and were synthesized on a Milligen/Biosearch 7500 DNA synthesizer.

Methods. PCR-mediated DNA amplification reactions were carried out on a Techne PHC-2 programmable dri-block using the Amplitaq enzyme (Perkin-Elmer Ltd.) and the cycle parameters recommended by the enzyme supplier. The major amplified products (360 bp) were purified via preparative agarose gel electrophoresis, kinased with T4 polynucleotide kinase, blunt-ended using Klenow fragment, ligated into phosphatased SmaI-digested pUC13 vector (Gibco BRL), and transformed into competent *Escherichia coli* DH5α. Multiple clones were sequenced from each transformation, using T7 DNA polymerase and 7-deaza-dGTP sequencing mixes (Pharmacia). Direct sequencing was carried out as described by Winship (1989). The parameter K_s^* (the number of nucleotide substitutions in conserved codons per silent site) was calculated by hand (Miyata and Yasunaga 1980; Miyata et al. 1987).

Results and Discussion

Amplification and Cloning of ZFY-Related Genes

Using the primers described above in the PCR, we have amplified and cloned DNA fragments homologous to human ZFY from a Chinese hamster ovarian cell line, male crab-eating fox (*Dusicyon thous*), male and female great tit (*Parus major*), and a male lesser black-backed gull (*Larus fuscus*). All species generated DNA fragments of the expected size (360 bp). Table 1 shows the details of all recombinant clones analyzed. In total, 262 recombinant clones were analyzed (54 of these via DNA sequencing) representing five novel ZFY-related genes.

Table 2. Percentage similarity of nucleotides (upper right) and amino acids (lower left) between the DNA and predicted protein sequences of ZFY-related genes

	ZFY	ZFX	ZFY-(FOX)	ZFX-(FOX)	ZFX-(CHA)	Zfx	Zfa	Zfy-1	Zfy-2	Zf-(PM)	Zf-(LF)
ZFY	—	92	90	90	92	90	89	83	83	82	82
ZFX	95	—	94	96	93	92	91	83	83	83	84
ZFY-(FOX)	94	99	—	95	90	90	89	81	81	82	84
ZFX-(FOX)	93	98	99	—	89	91	92	84	84	83	84
ZFX-(CHA)	88	93	94	93	—	94	94	83	83	81	82
Zfx	94	97	98	97	98	—	98	83	83	82	84
Zfa	91	94	95	94	91	98	—	82	82	81	83
Zfy-1	82	84	83	83	79	84	81	—	99	78	77
Zfy-2	82	84	83	83	79	84	81	99	—	78	77
Zf-(PM)	91	93	92	91	88	91	88	82	82	—	95
Zf-(LF)	91	93	94	93	89	93	90	80	80	98	—

ZFY-related genes: ZFY and ZFX from human; Zfx, Zfy-1, Zfy-2, and Zfa from mouse; ZFY-(FOX) and ZFX-(FOX) from the crab-eating fox; ZFX-(CHA) from the Chinese hamster; Zf-(PM) from the great tit; and ZF-(LF) from the lesser black-backed gull

```
ZFY      Primer 1 5'  TTC CGA CAC CCG TCG GAA CTG AGA AAG CAC ATG CGA ATC CAT ACC GGC GAG AAG CCA TAC
ZFX                   --T --T --- --- --A --G --C -A- --- --- --- --- --- --- --T --G --- --- --G ---
ZFY-(FOX)             --T --T --- --- --A --G --C -A- --- --- --- --- --- --C --T --- --- --- --- ---
ZFX-(FOX)             --T --T --- --A --A --G --C -A- --- --- --- --- --- --C --T --G --- --- --G ---
Zfy-1                 --- --T --- --A --A -C- --C -A- --- --- --A --- G-T --C --A --A --- --- --C --T
Zfy-2                 --- --T --- --A --A -C- --C -A- --- --- --A --- G-T --C --A --A --- --- --C --T
Zfx                   --- --C --T --- --A --G --C -A- --G --- --- --- --- --- --T --A --A --- --- --T
Zfa                   --- T-- --T --A --A --G --C -A- --- --- --- --- --- --- --T --A --A --- --C --T
ZFX-(CHA)             --- --A --- --- --A --G --- -A- --- --- --- --- --- --- --- --G --- --- --C --T
Zf-(PM)               --- --- --T --- --- --G --C -AG --- --- --- --G --- --C --T --T --- --- --C ---
Zf-(LA)               --T --- --T --- --A --G --C -AG --- --- --- --- --- --C --T --T --A --A --C ---


ZFY                   CAA TGC CAG TAC TGT GAA TAT AGG TCT GCA GAC TCT TCT AAC TTG AAA ACA CAT ATA AAA
ZFX                   --- --- --- --- --C --- --- --- --- --- --- --- --- --- --- --- --G --- G-C ---
ZFY-(FOX)             --G --- --A --- --C --- --- --A --- --- --- --- --- --- --A --- --- --- G-G ---
ZFX-(FOX)             --G --- --- --- --C --- --- --C --C --- --- --- --- --- --- --- --- --- G-- ---
Zfy-1                 G-- --T --- --T --- --G --C -A- --- --- --- --- --C --- --- --- --T --- --- ---
Zfy-2                 G-- --T --- --T --- --G --C -A- --- --- --- --- --C --- --- --- --T --- --- ---
Zfx                   G-- --- --- --- --C --- --C --- --- --- --T --- --- --- --- --C --- G-- ---
Zfa                   G-- --- --- --- --C --- --C --- --C A-- --T --- --- --- --- --- --C T-- G-- ---
ZFX-(CHA)             G-- --- --- --- --C --- --C --- --C --- --T --- --- --- --- --- --- --- G-- ---
Zf-(PM)               --G --- --- --T --- --- --C C-A --- --C --- --- --C --- --- --- --C --- G-- --G
Zf-(LA)               --G --- --A --T --C --- --C C-- --- --C --- --- --- --- --- --- --T --- G-- --G


ZFY                   ACA AAG CAT AGT AAA GAG ATG CCA TTC AAG TGT GAC ATT TGT CTT CTG ACT TTC TCA GAT
ZFX                   --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G ---
ZFY-(FOX)             --C --- --- --- --- --- --- --T --- --- --- --- --C --- --- --- --- --- --- ---
ZFX-(FOX)             --T --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- --- ---
Zfy-1                 T-T --- --- --- --- --- --A --- C-G --- --- -G- --C --- --C --- --- --- --- ---
Zfy-2                 T-T --- --- --- --- --- --A --- C-G --- --- --- --C --- --C --- --- --- --- ---
Zfx                   --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Zfa                   --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ZFX-(CHA)             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-- --- --- --- ---
Zf-(PM)               --T --A --C --- --G --A -C- --G --G --- --- --T --- --- T-C -A- --- --T --- ---
Zf-(LA)               --T --A --C --- --G --A -C- --- --- --- --C --T --- --- T-C -A- --- --T --- ---


ZFY                   ACC AAA GAA GTG CAG CAA CAT ACT CTT GTC CAC CAA GAA AGC AAA ACA CAT CAG TGT TTG
ZFX                   --- --- --G --- --- --- --- G-- --- A-- --- --- --- --- --- --- --C --- --- ---
ZFY-(FOX)             --- --- --G --- --- --- --- G-- --C A-- --- --- --- --- --- --- --C --- --- ---
ZFX-(FOX)             --- --- --G --- --- --- --- G-- --- A-- --- --- --- --- --- --- --C --- --- ---
Zfy-1                 --- --- --G -CT --- --- --- G-C G-- C-G --- --- --- --- -G- --- --- --A --- -CA
Zfy-2                 --- --- --G -CT --- --- --- G-C G-- C-G --- --- --- --- -G- --- --- --A --- -CA
Zfx                   --- --- --G --- --A --- --- G-- --- --- --- --- --G --- --- --T --C --- --- ---
Zfa                   --- --- --G --- --A --- --- G-- --- --- --T --- --G --- --- --T --C --- --- ---
ZFX-(CHA)             --- --- --- --- --A --- --- G-- --- A-- --T --- --G --- --- --- --C --- --- ---
Zf-(PM)               --- --- --G C-A --- --G --- --A --- A-G --T --- --- --T --- --- --- --- --- ---
Zf-(LA)               --- --- --G C-A --- --G --- --G --- A-G --T --- --- --T --- --- --- --- --- ---


ZFY                   CAT TGC GAC CAC AAG AGT TCA AAC TCA AGT GAT TTG AAA CGA CAT GTA ATT TCA GTT CAT
ZFX                   --- --- --- --- --- --- --G --- --- --- --- --- --- --- --C A-- --- --- --- --C
ZFY-(FOX)             --- --T --- --- --- --- --G --- --- --C --- --- --- --- --C A-- --- --- --- --C
ZFX-(FOX)             --C --- --- --- --- --- --- --- --- --- --- --- --- --- --C A-- --- --G --- --C
Zfy-1                 --- --- A-- --T --- --- --- --- --- --- --- --A --G --- --C A-- --- --C --- --C
Zfy-2                 --- --- A-- --T --- --- --- --- --- --- --- --A --G --- --C A-- --- --C --- --C
Zfx                   --- --T --T --- --- --- --- --- --- --- --- --- --- --- --C A-- --- --- --- ---
Zfa                   --- --T --- --- --- --- --- --- --- --- --- --- --- --- --C A-- --- --- --- ---
ZFX-(CHA)             --- --- --- --- --- --- --G --- --- --- --- --- --- --- --C A-- --- --- --- --C
Zf-(PM)               --- --T --- --T --- --C --- --- --G --- --- C-- --- --- --C A-T --- --- --C --C
Zf-(LA)               --- --T --- --T --- --C --- --- --G --- --- C-C --- --- --C A-- --- --- --C --C


ZFY                   ACG AAA GAC TAT   3' Primer 2
ZFX                   --- --- --- --C
ZFY-(FOX)             --- --G --- --C
ZFX-(FOX)             --- --G --- --C
Zfy-1                 --A --G -CG ---
Zfy-2                 --A --G -CG ---
Zfx                   --- --G --- ---
Zfa                   --- --G --- ---
ZFX-(CHA)             --A --G --- ---
Zf-(PM)               --A --- --- ---
Zf-(LA)               --- --- --- ---
```

a

Fig. 1. Continued on next page

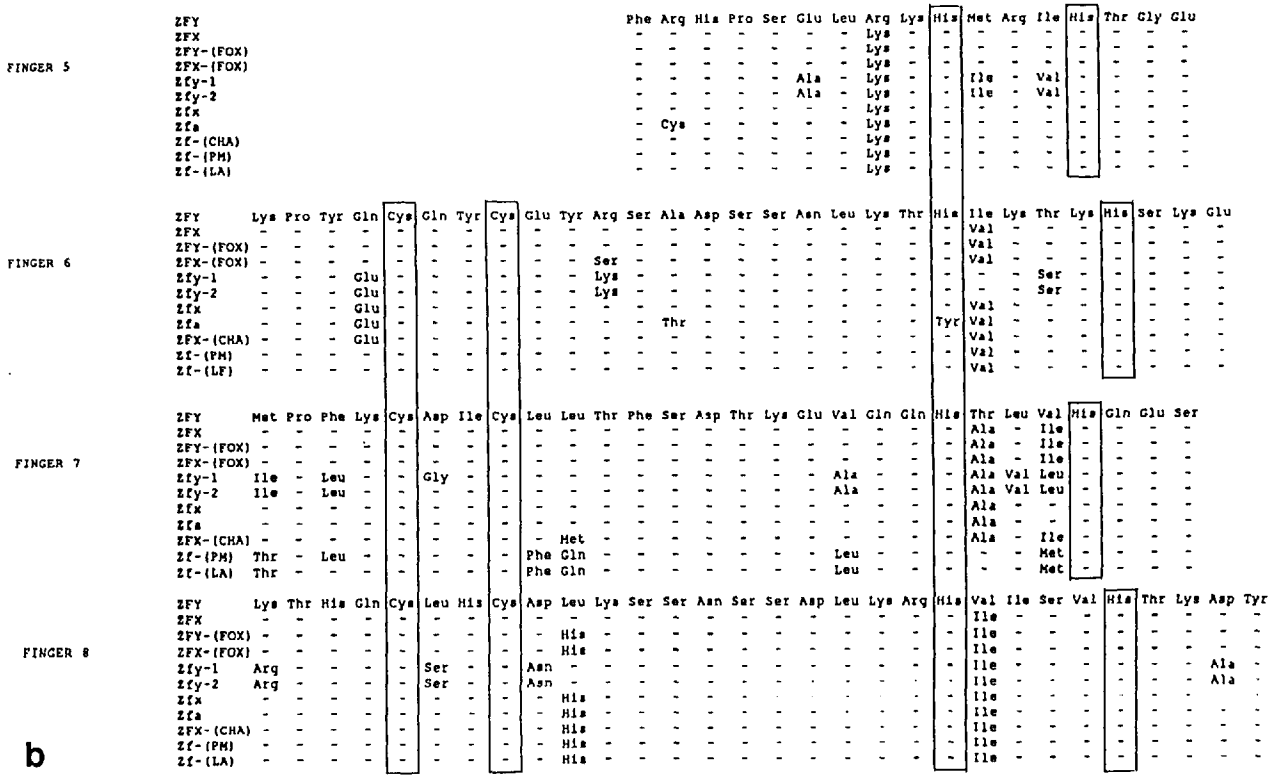

Fig. 1. a Genomic DNA sequence of ZFY, ZFX, Zfx, Zfy-1, Zfy-2, Zfa, ZFX-(FOX), ZFY-(FOX), ZFX-(CHA), Zf-(PM), and Zf-(LF) aligned over the amplified region. Dashes indicate sites identical to ZFY. b Predicted translation products from the above sequences. Dashes indicate identities with ZFY.

Numbers of ZFY-Related Genes in Each Species

Cloning of the product generated by PCR amplification of fox DNA resulted in the isolation of two types of ZFY-related recombinant clones. This result is as predicted, because the individual was a male and would be expected to possess one X-linked and one Y-linked gene. Sequence comparisons, Table 2, reveal that these two genes are closely related to each other (95% nucleotide sequence identity). Thus, to determine which of the fragments generated derives from the X-chromosome, a PCR was carried out on DNA from a female fox, and the product directly sequenced (data not shown). This allowed one of the genes isolated from the male to be definitively ascribed to the X-chromosome, ZFX-(FOX), and the other to the Y-chromosome, ZFY-(FOX).

In all other species analyzed, a single type of ZFY-related recombinant clone was generated. In the hamster, this is particularly interesting because it reveals that the divergence of mice and hamsters predates the retroposition of Zfx to form Zfa, indicating that the Zfx/Zfa retroposition is of recent origin.

To determine whether the avian homologue of ZFY is located on an autosome or on a sex chromosome, analysis of a female bird is necessary [in birds, the heterogametic sex is female (ZW) whereas males are homogametic (ZZ)]. Therefore, a total of 115 recombinant clones derived from PCR amplification of female great tit DNA was screened, of which 31 were sequenced. Fourteen contained artifactual sequences unrelated to ZFY. These nonhomologous sequences were presumably generated through nonspecific binding of the primers, although why this phenomenon should be so prevalent in this species is unknown. The remaining clones all contained equivalent sequences, which, combined with the Southern blot data from great tits (data not shown) and chickens (Page et al. 1987), definitively ascribes the avian homologue of ZFY to either an autosome or the pseudoautosomal region of the sex chromosomes. Similarly, only one class of ZFY-related recombinant clones was isolated from the male lesser black-backed gull (Table 1).

ZFY Is Highly Conserved in Birds and Mammals

The nucleotide sequences from the five novel ZFY-related genes isolated in this study are aligned with those of six of the published homologues in Fig. 1a. Translations of these sequences are aligned in Fig. 1b, with conserved residues and structural motifs boxed.

As shown in Fig. 1b and Table 2, the three new

mammalian sequences are closely related to both human genes and to the mouse genes Zfx and Zfa. Interestingly, the mouse Y genes, Zfy-1 and Zfy-2, are clearly dissimilar from both ZFY-(FOX) and ZFY-(human), despite the fact that all three taxa diverged at the same time, around 70 million years ago (Benton 1990). This indicates that divergence of the Y-linked genes occurred in the lineage leading to mice and prior to the duplication event that led to the two genes on the mouse chromosome. The avian sequences are also closely related to each other (95% nucleotide similarity), despite the fact that these two species diverged around 50–70 million years ago (Feduccia 1983).

Interestingly, of seven amino acid differences between ZFX and Zf-(PM), six are in the region of finger 7. This localization of differences suggests that finger 7 is either redundant in mammals or birds, or that its role has subtly changed during vertebrate evolution. Finger 7 apart, the level of similarity suggests that ZFX and its avian counterpart may share a common function, as is also supported by the similarity in expression patterns by human ZFX (Schneider-Gädicke et al. 1989) and chicken Zf (Dilella et al. 1989).

The unusual odd and even two-finger periodicity, characteristic of ZFY, is evident in all of its homologues (Fig. 1b). Furthermore, its presence in the great tit and gull ZFY homologues indicates that it is an ancient feature of the gene family, which has been conserved for at least 300 million years (Benton 1990).

Nucleotide Substitution Rates in the ZFY Gene Family

We have estimated the rate of nucleotide substitution in the X-linked and Y-linked ZFY-related genes by calculating the rates of nucleotide substitution at silent nucleotide sites in conserved codons, as proposed by Miyata et al. (1987). Using this measure, Miyata et al. demonstrated that genes on the X-chromosome evolve more slowly than similar genes on autosomes, in accordance with their theoretical predictions. Miyata et al. also predicted that genes on the Y chromosome should experience a rate of nucleotide substitution twice that of autosomal genes, but were unable to adequately test this due to lack of data. Both predictions can be tested for the ZFY-related gene family.

The K_s^c value for the mouse and human X-linked ZFY-related genes, over the amplified region, is 0.17. This is similar to the K_s^c value for other X-linked genes [0.18 (Myelin phospholipoprotein-PLP), 0.31 (Hypocanthine phosphombosyltransferase-HPRT), 0.33 (Phosphoglycerate kinase 1-PGK1), 0.51 (Ornithine transcarbamoylase-OTC); Miyata et al. 1987]. A Y-linked sequence would be predicted to have a rate of nucleotide substitution three times that of an X-linked sequence (Miyata et al. 1987). The K_s^c value of human ZFY and mouse Zfy-1 over the amplified region is 0.34; this is two times that of the X-linked sequences, not three times as predicted by the model of Miyata et al.

The rate of nucleotide substitution of the Y-linked genes in human and mouse at silent sites is, therefore, lower than expected; yet, paradoxically, the mouse Y-linked genes are particularly divergent from all other mouse, human, fox, great-tit, and lesser black-backed gull ZFY-related genes at the protein level. It is therefore unlikely that the divergence of the mouse sequence is solely due to the random accumulation of nucleotide substitutions.

A possible explanation is connected with the difference in expression patterns between ZFY/X and Zfy-1,2. The two human genes are expressed ubiquitously in adult tissues (Palmer et al. 1989; Schneider-Gädicke et al. 1989) whereas Zfy-1 and Zfy-2 are expressed, respectively, in fetal and adult testis only (Palmer et al. 1989; Koopman et al. 1989). This change in the gene regulation suggests that mouse Zfy-1 and Zfy-2 are not functionally equivalent to human ZFY. Furthermore, as suggested by Burgoyne (1989), mouse Zfx, unlike human ZFX (Schneider-Gädicke et al. 1989; Page et al. 1990), may be subject to X-inactivation. It is proposed here that the observed divergence of sequence and expression patterns of mouse Zfy-1,2 is related to the possible X-inactivation of mouse Zfx.

We suggest that, in most mammals, ZFY and ZFX are functionally equivalent (as suggested by the similarity of these sequences both in human and in fox; Fig. 1). However, in the lineage leading to mice, Zfx has adopted X-inactivation, such that equal gene dosage between the sexes is fulfilled by Zfx alone. This would allow selection to act rapidly on the sequence and regulation of the Y-linked gene, such that it acquired a new, or more specific, function.

An alternative viewpoint is that ZFY and ZFX are not functionally interchangeable. For example, Palmer et al. (1990) pointed out that of 11 differences between ZFX and ZFY, five affect zinc-finger residues implicated in DNA sequence recognition (Lee et al. 1989). However, our analysis of the two fox genes is not consistent with this interpretation because these two genes differ by only one amino acid substitution over the amplified region.

Conclusions

We report the isolation and sequence determination of clones derived from five novel ZFY-related genes, from two species of mammal and two species of

bird. Sequence comparisons between these genes and their homologues from mouse and human indicate that ZFY-related gene sequences have been remarkably conserved since the divergence of birds and mammals (approximately 300 million years) with the exception of the sequence encoding the 7th zinc finger. However, our analyses reveal that the mouse Zfy-1 and Zfy-2 genes have undergone significant divergence away from the ancestral mammalian ZFY gene subfamily. Furthermore, this divergence has occurred despite a lower than predicted mutation rate in Y-linked ZFY-related genes.

Acknowledgments. We thank Dr. D.C. Page (Cambridge, MA) and Dr. R. Lovall-Badge (NIMR, London) for the provision of probes of human and mouse genes used in preliminary work. We also thank Max Telford for helpful discussion of the data, John McVey for advice on PCR and for synthesizing the primers, and Richard Griffiths and Mark Meuth for tissue samples.

References

Ashworth A, Swift S, Affara N (1989) Sequence of cDNA for murine Zfy-1, a candidate for Tdy. Nucleic Acids Res 17: 2864

Ashworth A, Skere B, Switt S, Lovall-Badge R (1990) 2ta is an expressed retroposon derived from an alternative transcript of the 2tx gene. EMBO J 9:1529–1534

Benton MJ (1990) Phylogeny of the major tetrapod groups: morphological data and divergence dates. J Mol Biol 30:409–420

Bull JJ, Hillis DM, O'Steen S (1988) Mammalian ZFY sequences exist in reptiles, regardless of sex-determining mechanism. Science 242:567–569

Burgoyne PS (1989) Thumbs down for zinc-finger? Nature 342: 860–862

Dilella AG, Smith RG, Page DC (1989) Identification and expression of the avian homologue to the putative mammalian sex-determining gene. J Cell Biol 109:Abstract No. 1561

Feduccia A (1983) The age of birds. Harvard University Press, Cambridge MA

Ferreiro C, Medvano JF, Gall GAE (1989) Genome analysis of rainbow trout and sturgeon with restriction enzymes and hybridization with a ZFY gene derived probe to identify sex. Aquaculture 81:245–251

Griffiths RG, Holland PWH (1990) A novel avian W-chromosome DNA repeat in the lesser black-backed gull (*Larus fuscus*). Chromosoma 99:243–250

Koopman P, Gubbay J, Collignon J, Lovell-Badge R (1989) Zfy gene expression patterns are not compatible with a primary role in mouse sex determination. Nature 342:940–942

Lee MS, Gippert GP, Soman KV, Care PA, Wright PE (1989) Three dimensional solution structure of a single zinc finger binding domain. Science 245:635–637

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

Mardon G, Luoh SW, Simpson EM, Gill G, Brown LG, Page DC (1990) Mouse 2tx protein is similar to 2ty-2, containing an acidic activation domain and 13 zinc-fingers. Mol Cell Biol 10:681–689

Miyata T, Yasunaga T (1980) Molecular evolution of mRNA: a method for estimating evolutionary rates of synonymous and amino acid substitutions from homologous nucleotide sequences and its application. J Mol Evol 16:23–36

Miyata T, Hayastrida H, Kuma K, Mitsuyasa K, Yasunaga T (1987) Male-driven molecular evolution: a model and nucleotide sequence analysis. Cold Spring Harbor Symp Quant Biol 52:863–867

Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack HJ, McGillvray B, de la Chapelle A, Brown LG (1987) The sex-determining region of the human Y chromosome encodes a finger protein. Cell 51:1091–1104

Page DC, Disteche CM, Simpson EM, de la Chapelle A, Andersson M, Alitalo T, Brown LG, Green P, Aidekots G (1990) Chromosomal localization of ZFX—a human gene that escapes X inactivation and its murine homologs. Genomics 7: 37–46

Palmer MS, Sinclair AH, Berta P, Ellis NA, Goodfellow PN (1989) Genetic evidence that ZFY is not the testis-determining factor. Nature 342:937–939

Palmer MS, Berta P, Sinclair AH, Pym B, Goodfellow PN (1990) Comparison of human ZFY and ZFX transcripts. Proc Natl Acad Sci USA 87:1681–1685

Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi RH, Horn GT, Mullis KB, Erhlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Schneider-Gädicke A, Beer-Romero P, Brown LG, Nussbaum R, Page DC (1989) ZFX has a gene structurally similar to ZFY, the putative sex determinant, and escapes X inactivation. Cell 57:1247–1258

Sinclair AH, Foster JW, Spencer JA, Page DC, Palmer M, Goodfellow PN, Graves JAM (1988) Sequences homologous to ZFY, a candidate human sex-determining gene, are autosomal in marsupials. Nature 336:780–783

Winship PR (1989) An improved method for directly sequencing PCR amplified material using dimethyl sulphoxide. Nucleic Acids Res 17:1266

Received June 29, 1990/Revised August 23, 1990