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The Sex-Determining Region of the Mouse Y Chromosome Encodes a Protein with a Highly Acidic Domain and 13 Zinc Fingers

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Summary

The *ZFY* gene, located in the sex-determining region of the human Y chromosome, appears to encode a zinc-finger protein. Two homologous genes, *Zfy-1* and *Zfy-2*, are found in the sex-determining region of the mouse Y chromosome. One or both genes may serve as the primary sex-determining signal in mice. Both *Zfy-1* and *Zfy-2* are transcribed in the adult testis. Nucleotide sequence analysis of a *Zfy-2* cDNA suggests that it encodes a 783 amino acid protein with two domains: the amino-terminal portion is highly acidic, with 25% of its residues being glutamic or aspartic acid, while the carboxy-terminal domain contains 13 zinc fingers. The presence in Zfy-2 of an acidic domain in combination with a putative nucleic acid binding domain suggests that Zfy-2 activates transcription in a sequence-specific fashion.

Introduction

In mice and humans, the developmental fate of the bipotential, embryonic gonad hinges upon the presence or absence of one or more genes on the Y chromosome (Jacobs and Strong, 1959; Ford et al., 1959; Welshons and Russell, 1959). Analysis of human individuals with Y chromosome deletions has identified a small segment of the human Y that contains the entirety of this sex-determining gene or genes (Page et al., 1987), often referred to as the "testis-determining factor," or *TDF*. In particular, an essential portion of the gene maps to a 140 kilobase (kb) segment of the human Y that has been cloned by chromosomal walking. This sex-determining region appears to encode a protein with 13 Cys–Cys/His–His "zinc fingers" (Page et al., 1987), a nucleic acid binding motif first described in Xenopus transcription factor IIIA (Miller et al., 1985; Brown et al., 1985). This Y-encoded zinc-finger protein, which we have named ZFY (Page, 1988), may be the pivotal sex-determining signal. A closely related gene, *ZFX*, exists on the human X chromosome. Homologs of the human *ZFY* and *ZFX* genes are found on the Y and X chromosomes of all placental mammals tested (Page et al., 1987).

In contrast to most other placental mammals, the mouse Y chromosome carries not one but two homologs of the human *ZFY* gene. These two mouse loci, *Zfy-1* and *Zfy-2*, are closely related. Indeed, the presence of two *Zfy* loci on the mouse Y is the result of an intrachromosomal duplication that occurred during mouse evolution (Mardon et al., 1989). Both *Zfy* loci map to the sex-determining region of the mouse Y chromosome (Page et al., 1987). However, *Zfy-1* and *Zfy-2* are not *both* required for testis determination; testis differentiation can occur in the absence of *Zfy-2* (Mardon et al., 1989). It is not known whether *Zfy-1* and *Zfy-2* encode functionally redundant proteins, whether they have different functions, or whether either is a pseudogene.

We report that both *Zfy-1* and *Zfy-2* are transcribed in mouse adult testis. Analysis of a *Zfy-2* cDNA clone suggests that it encodes a 783 amino acid protein with a large, highly acidic domain and 13 zinc fingers. By analogy to proteins such as yeast GAL4 and GCN4 and the human glucocorticoid receptor (Hope and Struhl, 1986; Ma and Ptashne, 1987; Hollenberg and Evans, 1988), this finding of acidic and putative nucleic acid binding domains suggests that Zfy-2 is a sequence-specific activator of transcription.

Results and Discussion

Zfy Genes Are Transcribed in Adult Testis

By Northern analysis, we examined mouse tissues—embryonic, newborn, and adult—for the presence of transcripts from *Zfy-1* or *Zfy-2*. The insert of plasmid pDP1055 originates from the mouse *Zfy-1* genomic locus, but it readily cross-hybridizes to *Zfy-2* under stringent conditions. (At lower stringency, probes derived from mouse *Zfy* loci also cross-hybridize to the *Zfx* gene on the mouse X chromosome and to a related locus on mouse autosome 10. Such cross-hybridization does not occur at high stringency [Mardon et al., 1989; Page et al., unpublished data].) Probe pDP1055 was hybridized under stringent conditions to oligo-dT-selected (poly(A)$^+$) RNAs from the following 36 sources: whole embryos, both male and female, from 12, 14, 16, and 18 days post coitum; and newborn and adult brain, gonad, heart, kidney, liver, lung, and spleen, both male and female. Our results were as follows (Figure 1):

- As expected, no *Zfy* transcripts were detected in female tissues.
- Interestingly, we did not detect any transcripts in whole male embryos or in dissected newborn male tissues. If the *Zfy* genes are involved in gonadal sex determination, then one would expect to find them expressed in the embryo during or prior to sex differentiation of the bipotential gonad, which is histologically detectable as early as 10–12 days post coitum. The negative results in our Northern analysis of whole embryos, however, do not rule out *Zfy* gene expression during gonadal sex determination. *Zfy* expression, for example, may be limited to a narrow window of time or to a small fraction of cells in the embryo (e.g., the genital ridge).
- Of the remaining tissues, *Zfy* transcripts were detected only in adult testis, where transcripts of approximately 2.9 kb and 3.2 kb were found (Figure 1). Transcription

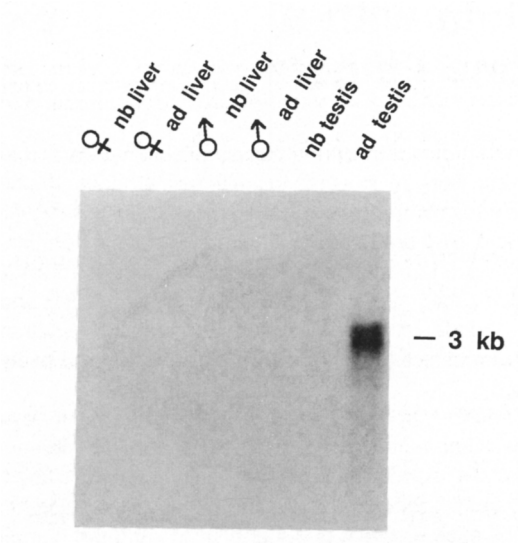

Figure 1. The *Zfy* Genes Are Transcribed in Adult Testis

Probe pDP1055, a mouse *Zfy-1* genomic clone, was hybridized to poly(A)$^+$ RNAs prepared from tissues of FVB/N mice (4 hr exposure). nb, newborn; ad, adult. A doublet of about 3 kb is seen in adult testis. Negative results were obtained, even after exposure for 7 days, with all other tissues tested (some shown here), including: newborn and adult ovary; newborn and adult brain, heart, kidney, liver, lung, and spleen, both male and female; and whole embryos, both male and female, from 12, 14, 16, and 18 days post coitum.

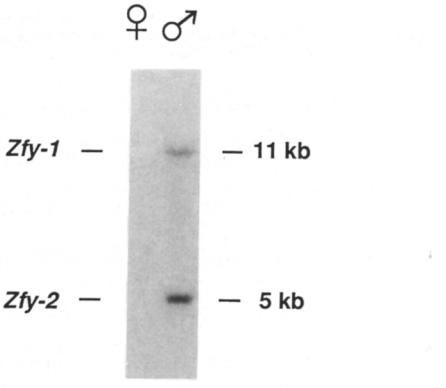

Figure 2. An Adult Testis cDNA Hybridizes to Both *Zfy* Genes

A 1.2 kb HindIII fragment from the 3′ terminus of the pDP1122 (*Zfy-2*) cDNA was hybridized to EcoRI-digested genomic DNAs of female and male BALB/c mice. The 11 kb and 5 kb EcoRI fragments detected derive from, respectively, *Zfy-1* and *Zfy-2* (Page et al., 1987; Mardon et al., 1989). (Identical results were obtained with female and male FVB/N mice [data not shown].)

of the *Zfy* genes in the adult testis—but apparently not in the newborn testis—suggests the possibility of a role in reproductive function in the adult male. In any case, one or both mouse *Zfy* genes are transcriptionally active.

Both *Zfy-1* and *Zfy-2* Are Transcribed

In order to further characterize the *Zfy* transcripts, an adult testis cDNA library was screened with probe pDP1055 at high stringency. Two clones, pDP1121 and pDP1122, resulted from this screen. They contain cDNA inserts of 1.5 kb and 2.8 kb, respectively. We confirmed that the insert of pDP1122 derives from the Y chromosome; when hybridized to EcoRI-digested mouse genomic DNAs, a fragment from pDP1122 detects only male-specific sequences (Figure 2). These are the 11 kb and 5 kb fragments containing the zinc-finger domains of *Zfy-1* and *Zfy-2*, respectively (Mardon et al., 1989). In addition, the 2.8 kb insert of pDP1122 was hybridized to Northern blots of the 36 mouse tissues described above. The 3 kb doublet previously seen with pDP1055 was detected, again only in adult testis (data not shown). These results establish that pDP1122 is a clone from one of the two mouse *Zfy* loci. Similar results were obtained with the insert of pDP1121.

We were interested in whether these cDNAs derive from *Zfy-1* or from *Zfy-2*. We obtained the complete nucleotide sequence of the 2.8 kb cDNA insert of pDP1122 as well as 135 nucleotides of pDP1121 (Figure 3). Although the nucleotide sequences of the two cDNAs are very similar, they are not identical. Within the 135 nucleotide region of comparison, pDP1121 and pDP1122 differ by 2 bp substitutions. This difference suggested that pDP1121 and pDP-1122 derive from different loci—one from *Zfy-1* and the other from *Zfy-2*. In order to confirm this inference and assign the cDNAs to particular genes, we sequenced the corresponding 135 bp regions of *Zfy-1* and *Zfy-2* genomic clones. In this region, the *Zfy-1* genomic sequence is identical to that of the pDP1121 cDNA, while the *Zfy-2* genomic sequence is identical to that of the pDP1122 cDNA. Thus, pDP1121 is from *Zfy-1* and pDP1122 is from *Zfy-2*.

Because both loci were represented in the cDNA library, we conclude that both *Zfy-1* and *Zfy-2* are transcribed in adult testis. The 3 kb doublet on Northern blots of adult testis (Figure 1) may comprise a single transcript from each of the two genes. Alternatively, one or both genes may produce multiple transcripts. (Using a probe derived from the most 5′ 300 bp of the *Zfy-2* cDNA, an additional adult testis-specific transcript of 700 bases is detected [data not shown]. This transcript is not detected using pDP1055 [Figure 1], which hybridizes to the 3′ portions of the *Zfy* genes. We have not further characterized this short transcript.)

Zfy-2 Encodes a Protein with 13 Zinc Fingers

Nucleotide sequence analysis of pDP1122, the *Zfy-2* cDNA, revealed a single long open reading frame. The first AUG in this frame (position 1 in Figure 3) occurs in a sequence context that is highly favorable for initiation of translation (Kozak, 1986). Beginnning at this putative initiation codon, the open reading frame apparently encodes a protein 783 amino acids in length, with a predicted molecular weight of 89,000. The AUG codon is preceded by a 5′ leader of 319 nucleotides containing multiple stop codons in all three reading frames. A 3′ untranslated sequence of 126 bases is followed by 32 adenosines. There is no canonical (AATAAA) polyadenylation signal (Fitzgerald and Shenk, 1981) in the 3′ untranslated region, but we note that the sequence AATATAAA occurs 22 nucleotides 5′ of the poly(A) track.

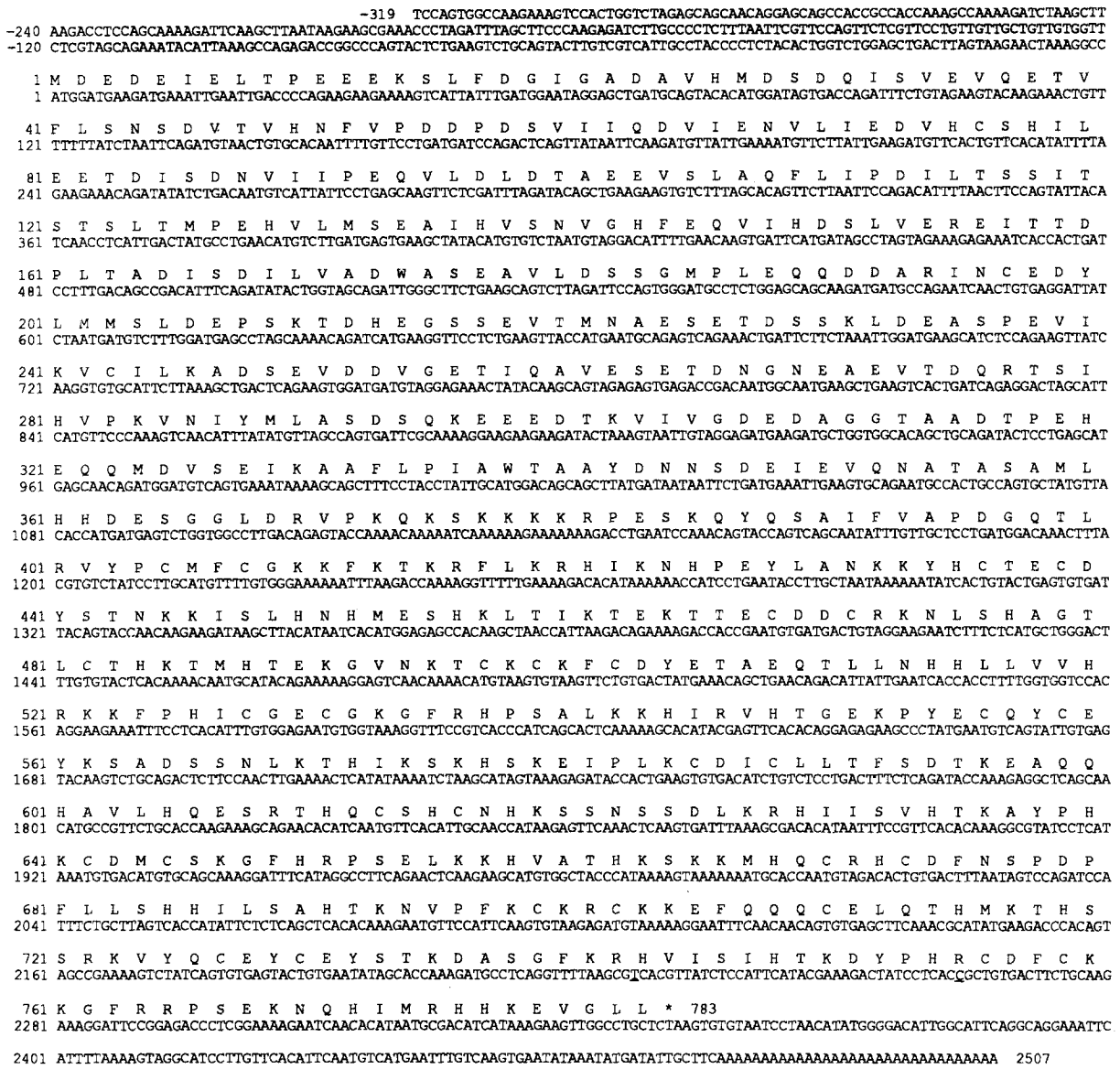

Figure 3. Nucleotide Sequence of a *Zfy-2* cDNA and Predicted Amino Acid Sequence of the Encoded Protein

The complete nucleotide sequence of the cDNA insert of plasmid pDP1122 is shown. The predicted 783 amino acid sequence is given above the corresponding nucleotide sequence. Numbering of nucleotides and amino acids begins with the first in-frame AUG codon. There is a putative 5′ untranslated region of 319 nucleotides. Comparative sequencing of the portion of pDP1121 (a *Zfy-1* cDNA) corresponding to nucleotides 2165–2300 revealed differences only at positions 2226 (T to C, silent) and 2263 (C to A, arginine to serine), which are underlined.

The carboxy-terminal half of the predicted mouse Zfy-2 protein comprises 13 putative zinc fingers, each with two cysteines and two histidines (Figure 4). This carboxy-terminal half, residues 391–783, corresponds with remarkable precision to the predicted translation of a single exon of the human *ZFY* gene (plasmid pDP1007; Page et al., 1987). Indeed, the amino acid sequence of the zinc-finger domain of mouse Zfy-2 is 80% identical to that of human ZFY, with no insertions or deletions (Figure 4). In particular, the human ZFY and mouse Zfy-2 proteins appear to have nearly identical carboxyl termini, differing at only 5 of the last 69 residues (Figure 4).

The tandem array of zinc fingers in the human ZFY protein is marked by a second-order repeat, consisting of a pair of fingers (Page et al., 1987). While such a two-finger repeat has not been observed, to our knowledge, in other proteins with multiple Cys–Cys/His–His fingers, it is readily apparent in the zinc-finger domain of mouse Zfy-2 (Figure 4). More precisely, the zinc-finger domain of either the human or mouse protein can be seen as consisting of six and one-half tandem repeats of a 57 residue or 63 residue unit. This repeat unit comprises two fingers and two "linkers," each seven or more residues in length. Though all the fingers share the Cys–Cys/His–His backbone, the amino acid consensus sequence of the even-numbered fingers (2, 4, 6, . . . 12) is quite distinct from that of the odd-

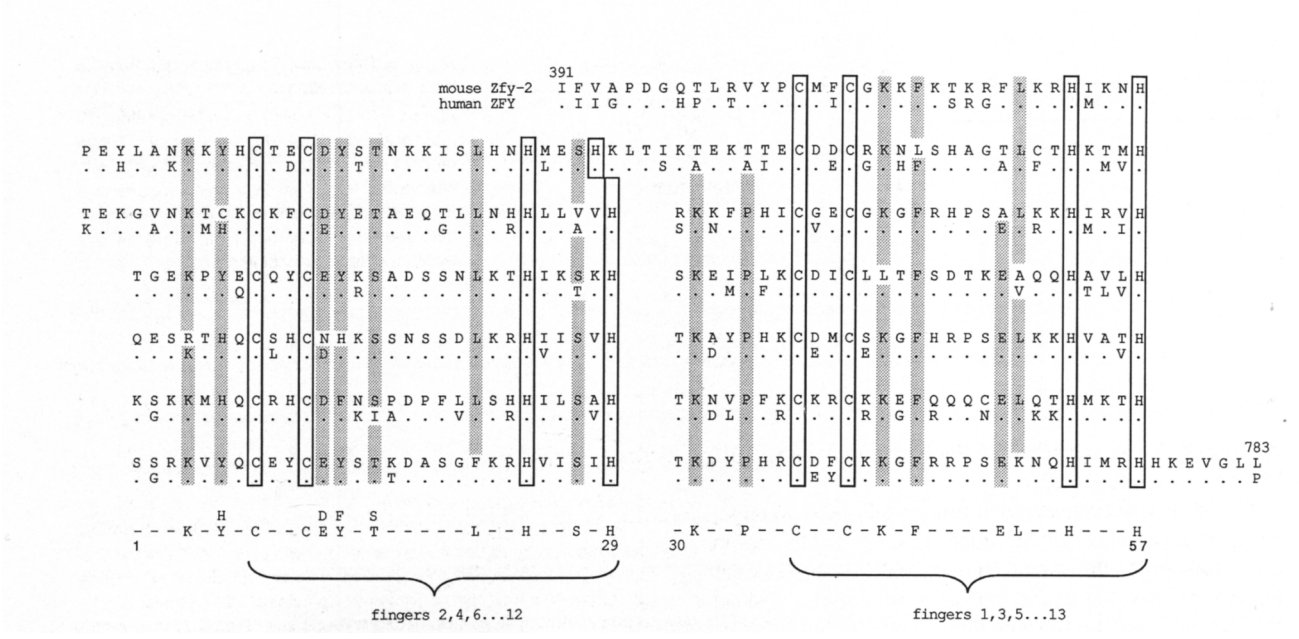

Figure 4. The Zinc-Finger Domains of the Putative Mouse Zfy-2 and Human ZFY Proteins

The predicted amino acid sequence of the carboxy-terminal, zinc-finger domain (residues 391–783) of mouse Zfy-2 is shown. Below mouse Zfy-2 is shown the corresponding portion of human ZFY (Page et al., 1987); dots indicate identical residues. The sequences are aligned on the basis of a 57 amino acid repeat unit, the consensus for which is shown at the bottom of the figure. Each repeat contains an "even-numbered" finger (2, 4, 6, . . . 12; on the left) and an "odd-numbered" finger (1, 3, 5, . . . 13; on the right). The invariant cysteines and histidines, highly characteristic of zinc finger domains (e.g., as in Xenopus transcription factor IIIA [Miller et al., 1985; Brown et al., 1985]) are boxed. Other residues that are highly conserved among the repeats are shaded.

numbered fingers (1, 3, 5, . . . 13). The odd-numbered consensus sequence is much more akin to the majority of Cys–Cys/His–His finger domains in other proteins that have been analyzed (Gibson et al., 1988). This repetitive structure in ZFY/Zfy-2 may have arisen by multiplication of a primordial two-finger unit during the evolution of the gene.

The absence of any insertions or deletions in comparing the zinc-finger domains of human ZFY and mouse Zfy-2 (Figure 4) suggests that a precisely defined architecture is required for function. Although the structure of the complexes formed between proteins with multiple Cys–Cys/His–His fingers and their nucleic acid binding sites is unknown, two general models have been proposed. According to model I, the protein wraps around the DNA double helix, following the major groove, with all fingers making structurally equivalent contacts with the DNA (Fairall et al., 1986; Berg, 1988). In model II, the protein is distributed along one face of the double helix, with every other finger making structurally equivalent contacts (Fairall et al., 1986). Our finding of a two-finger repeat in human ZFY and mouse Zfy-2 is as predicted by model II. (Again, this prediction has been borne out by few if any other multiple-finger proteins.) Nonetheless, the two-finger repeat can be reconciled with model I, in which case one might expect to find a corresponding repeat in the sequence of the nucleic acid binding site. Resolution of these issues must await identification of the DNA or RNA binding site(s) of the ZFY/Zfy proteins and, ultimately, determination of the structure of the complex.

Amino-Terminal Portion of the Zfy-2 Protein Is Highly Acidic

The amino-terminal portion of the predicted Zfy-2 protein is remarkably acidic: 91 of the amino-terminal 369 residues (25%) are aspartic or glutamic acid. With only 25 basic residues (arginine, lysine, and histidine), the net charge on this portion of the protein is at least −66. Thus, two large domains comprise the bulk of the Zfy-2 protein: the amino-terminal half is acidic, and the carboxy-terminal half contains zinc fingers (Figure 5).

Such a combination of well-demarcated acidic and DNA binding domains is reminiscent of eukaryotic transcriptional activators such as yeast GAL4 and GCN4 and the human glucocorticoid receptor. In particular, it is the acidic domain that confers the activating function in these factors. While the activating domains of GAL4, GCN4, and the glucocorticoid receptor show no striking sequence similarity to each other, all are characterized by a high proportion of acidic residues (Ma and Ptashne, 1987; Hope and Struhl, 1986; Hollenberg and Evans, 1988).

By analogy, we infer that Zfy-2 activates transcription in a sequence-specific fashion. Specifically, we would suppose that the protein is targeted to particular DNA sequences by the zinc-finger domain. There the acidic domain likely interacts with components of the RNA polymerase II transcriptional machinery so as to facilitate the formation or function of preinitiation transcription complexes (Ptashne, 1988; Sigler, 1988). (However, Zfy-2 may have other functions as well. For example, Xenopus transcription factor IIIA not only activates transcription by

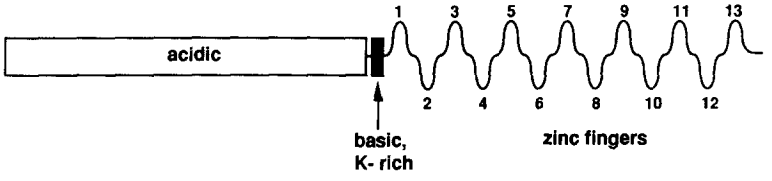

Figure 5. Schematic Diagram of the Putative Mouse Zfy-2 Protein

Almost half the protein—an amino-terminal domain of about 369 residues—is highly acidic. A tandem array of 13 putative zinc fingers, with alternating fingers of two types (i.e., a two-finger repeat), comprises the carboxy-terminal half. Between these two large domains is a small, highly basic region.

binding DNA, it also binds RNA [Pelham and Brown, 1980; Honda and Roeder, 1980].)

If Zfy-2 is directly involved in transcriptional regulation, it should be localized to the nucleus. Of possible note in this regard is the sequence KQKSKKKKRPESKQY (residues 373–387) found between the acidic, amino-terminal domain and the zinc-finger, carboxy-terminal domain (Figure 5). Although no consensus sequence has been identified, a run of basic residues appears to serve as a signal for nuclear localization of several proteins, including SV40 and polyoma large T antigens, human myc, human lamins A and C, yeast *MATα2*, and yeast ribosomal protein L3 (Dang and Lee, 1988; Dingwall and Laskey, 1986; Lanford et al., 1988; Loewinger and McKeon, 1988). A search of the GenBank database revealed two sequences similar to the basic run in Zfy-2: KKKSKKEKDKDSK, in human poly (ADP-ribose) polymerase (Uchida et al., 1987), and KSKKKKKPESSQY, in the Syrian hamster intracisternal A particle genome (Ono et al., 1985). Whether these basic regions serve to localize these proteins to the nucleus remains to be tested.

Functional Relationship of the *Zfy* Genes

The *Zfy-1* and *Zfy-2* genes are very similar. First, their coexistence is the result of an intrachromosomal duplication that occurred during mouse evolution (Mardon et al., 1989). Second, both genes are transcribed in the adult testis (Figures 1 and 3). Third, the amino acid sequences predicted from corresponding portions of these genes are nearly identical; there is only one amino acid substitution in the 45 residue region of comparison (Figure 3). Neither *Zfy-1* nor *Zfy-2* appears to be a pseudogene; both may encode functional proteins.

Are *Zfy-1* and *Zfy-2* functionally distinct? Although both *Zfy* genes are located in the sex-determining region of the mouse Y chromosome (Page et al., 1987), a sex-determining function has not as yet been demonstrated for either gene. It is known that *Zfy-1* and *Zfy-2* are not *both* required for testis determination (Mardon et al., 1989). The similarity between the two genes raises the possibility that they encode functionally redundant proteins. Perhaps either *Zfy* gene alone will suffice to induce male sexual differentiation. Addition of *Zfy-1* and *Zfy-2*, via transgenic manipulation, to XX mouse embryos should resolve the functional relationship of these genes to each other and their role in gonadal sex determination.

Experimental Procedures

Northern and Southern Blotting

RNA was prepared from FVB/N mouse tissues or whole embryos by the guanidine thiocyanate method of Chirgwin et al. (1979) as modified by Okayama et al. (1987). Briefly, frozen tissue was disrupted by high speed polytron treatment in a solution containing 5.5 M guanidine thiocyanate. After ultracentrifugation through a 1.51 g/ml of cesium trifluoroacetic acid cushion, the RNA was resuspended in 4 M guanidine thiocyanate and precipitated with ethanol. Polyadenylated poly(A)$^+$ RNA was obtained by selection on oligo-dT cellulose. For Northern analysis, 5 μg of poly(A)$^+$ RNA was subjected to electrophoresis in 2.2 M formaldehyde, 1% agarose (Maniatis et al., 1982) and transferred to nylon membrane. Southern transfers of mouse genomic DNA were prepared as previously described (Page et al., 1987).

DNA inserts of plasmids were purified from vector sequences, radiolabeled with ^{32}P by random-primer synthesis (Feinberg and Vogelstein, 1984), and hybridized to RNA or DNA blots for 16 hr at 47°C in 50% formamide, 5× SSC (1× SSC = 0.15 M NaCl, 15 mM sodium citrate [pH 7.4]), 5× Denhardt's solution (1× Denhardt's = 0.02% Ficoll 400, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 1% SDS, 20 mM $NaPO_4$ (pH 6.6), and 0.005% denatured calf thymus DNA. The nylon membranes were washed three times for 30 min each at 65°C in 0.1× SSC, 0.1% SDS and exposed at −80°C for 4 hr to 7 days with X-ray film backed by an intensifying screen.

The mouse genomic inserts of plasmids pDP1055 (Mardon et al., 1989) and pDP1125 are homologous to the zinc-finger domain of the human *ZFY* gene (e.g., plasmid pDP1007; Page et al., 1987). *Zfy-1* clone pDP1055 consists of a 1.8 kb HindIII genomic fragment inserted into Bluescript (Stratagene). *Zfy-2* clone pDP1125 contains a 5 kb EcoRI genomic fragment, also in Bluescript. Both *Zfy* genomic clones derive from liver DNA of an FVB/N male mouse.

cDNA Cloning and Nucleotide Sequence Analysis

cDNA libraries were prepared from FVB/N adult testis poly(A)$^+$ RNA essentially by the method of Gubler and Hoffman (1983) except that both first and second strand reactions were carried out in one tube, without intermediate extractions or precipitations (Sartoris et al., 1987). Following ligation to EcoRI–NotI adaptors (Invitrogen), the double-stranded cDNA was size-fractionated by agarose gel electrophoresis. After electroelution, the 1.5–4 kb fraction was ligated to λgt10 (Huynh et al., 1985). Unamplified libraries totalling 5 × 10^6 recombinants were screened using the mouse genomic insert of plasmid pDP1055 as probe. The cDNA inserts of two recombinant phages isolated in this manner were purified and reinserted into the EcoRI site of Bluescript, generating plasmids pDP1121 and pDP1122. The inserts of both plasmids are released from the vector by digestion with either EcoRI or NotI endonuclease, indicating, as expected, the presence of adaptor sequences at both ends of the cDNA inserts. Clone pDP1121 may contain sequences from an unrelated, autosomal locus in addition to those from *Zfy-1*.

Nested deletions for both strands of the 2.8 kb cDNA insert of pDP1122 were generated using the ExoIII nuclease method (Henikoff, 1984); mung bean nuclease was substituted for S1 nuclease. Single-stranded DNA templates generated from these nested deletions were sequenced by dideoxy chain termination (Sanger et al., 1977).

Using a modification of the FASTA algorithm (Pearson and Lipman,

1988), the amino acid sequence predicted from the nucleotide sequence of pDP1122 was compared with the potential translation products of the GenBank nucleic acid database (containing, at the time of analysis, more than 22 million nucleotides).

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References

Berg, J. (1988). Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins. Proc. Natl. Acad. Sci. USA *85*, 99–102.

Brown, R. S., Sander, C., and Argos, P. (1985). The primary structure of transcription factor TFIIIA has 12 consecutive repeats. FEBS Lett. *186*, 271–274.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry *18*, 5294–5299.

Dang, C. V., and Lee, W. M. F. (1988). Identification of the human *c-myc* protein nuclear translocation signal. Mol. Cell. Biol. *8*, 4048–4054.

Dingwall, C., and Laskey, R. A. (1986). Protein import into the cell nucleus. Annu. Rev. Cell Biol. *2*, 367–390.

Fairall, L., Rhodes, D., and Klug, A. (1986). Mapping of the sites of protection on a 5 S RNA gene by the Xenopus transcription factor IIIA. J. Mol. Biol. *192*, 577–591.

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

Fitzgerald, M., and Shenk, T. (1981). The sequence 5′-AAUAAA-3′ forms part of the recognition site for polyadenylation of late SV40 mRNAs. Cell *24*, 251–260.

Ford, C. E., Miller, O. J., Polani, P. E., de Alameida, J. C., and Briggs, J. H. (1959). A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet *1*, 711.

Gibson, T. J., Postma, J. P. M., Brown, R. S., and Argos, P. (1988). A model for the tertiary structure of the 28 residue DNA-binding motif ('zinc finger') common to many eukaryotic transcriptional regulatory proteins. Protein Eng. *2*, 209–218.

Gubler, U., and Hoffman, B. J. (1983). A simple and very efficient method for generating cDNA libraries. Gene *25*, 263–269.

Henikoff, S. (1984). Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene *28*, 351–359.

Hollenberg, S. M., and Evans, R. M. (1988). Multiple and cooperative *trans*-activation domains of the human glucocorticoid receptor. Cell *55*, 899–906.

Honda, B. M., and Roeder, R. G. (1980). Association of a 5S gene transcription factor with 5S RNA and altered levels of the factor during cell differentiation. Cell *22*, 119–126.

Hope, I. A., and Struhl, K. (1986). Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell *46*, 885–894.

Huynh, T. V., Young, R. A., and Davis R. W. (1985). Construction and screening cDNA libraries in lambda gt10 and lambda gt11. In DNA Cloning Techniques: A Practical Approach, D. Glover, ed. (Oxford: IRL Press), pp. 49–78.

Jacobs, P. A., and Strong, J. A. (1959). A case of human intersexuality having a possible *XXY* sex-determining mechanism. Nature *183*, 302–303.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell *44*, 283–292.

Lanford, R. E., White, R. G., Dunham, R. G., and Kanda, P. (1988). Effect of basic and nonbasic amino acid substitutions on transport induced by simian virus 40 T-antigen synthetic peptide nuclear transport signals. Mol. Cell. Biol. *8*, 2722–2729.

Loewinger, L., and McKeon, F. (1988). Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. EMBO J. *7*, 2301–2309.

Ma, J., and Ptashne, M. (1987). Deletion analysis of GAL4 defines two transcriptional activating segments. Cell *48*, 847–853.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 202–203.

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

Miller, J., McLachlan, A. D., and Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO J. *4*, 1609–1614.

Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T., and Arai, K. (1987). High-efficiency cloning of full-length cDNA; construction and screening of cDNA expression libraries for mammalian cells. Meth. Enzymol. *154*, 3–28.

Ono, M., Toh, H., Miyata, T., and Awaya, T. (1985). Nucleotide sequence of the Syrian hamster intracisternal A-particle gene: close evolutionary relationship of type A particle gene to types B and D oncovirus genes. J. Virol. *55*, 387–394.

Page, D. C. (1988). Is *ZFY* the sex-determining gene on the human Y chromosome? Phil. Trans. R. Soc. (Lond.) B *322*, 155–157.

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. (1987). The sex-determining region of the human Y chromosome encodes a finger protein. Cell *51*, 1091–1104.

Pearson, W. R., and Lipman, D. J. (1988). Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA *85*, 2444–2448.

Pelham, H. R. B., and Brown, D. D. (1980). A specific transcription factor that can bind either the 5S RNA gene or 5S RNA. Proc. Natl. Acad. Sci. USA *77*, 4170–4174.

Ptashne, M. (1988). How eukaryotic transcriptional activators work. Nature *335*, 683–689.

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

Sartoris, S., Cohen, E. B., and Lee, J. S. (1987). A rapid and improved method for generating cDNA libraries in plasmid and phage lambda vectors. Gene *56*, 301–307.

Sigler, P. B. (1988). Acid blobs and negative noodles. Nature *333*, 210–212.

Uchida, K., Morita, T., Sato, T., Ogura, T., Yamashita, R., Noguchi, S., Suzuki, H., Nyunoya, H., Miwa, M., and Sugimura, T. (1987). Nucleotide sequence of a full-length cDNA for human fibroblast poly(ADP-ribose) polymerase. Biochem. Biophys. Res. Commun. *148*, 617–622.

Welshons, W. J., and Russell, L. B. (1959). The Y chromosome as the bearer of male determining factors in the mouse. Proc. Natl. Acad. Sci. USA *45*, 560–566.

Note Added in Proof

PCR data consistent with transcription of *Zfy-1* and *Zfy-2* in mouse adult testis have recently been reported: Nagamine, C. M., Chan, K., Kozak, C. A., and Lau, Y.-F. (1989). Chromosome mapping and expression of a putative testis-determining gene in mouse. Science *243*, 80–83.