GENOMICS 7, 37–46 (1990)

Chromosomal Localization of *ZFX*—A Human Gene That Escapes X Inactivation—and Its Murine Homologs

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Received September 5, 1989; revised January 3, 1990

The *ZFY* gene, found in the sex-determining region of the human Y chromosome, encodes a zinc-finger protein that may be the pivotal sex-determining signal. A closely related gene, *ZFX*, is found on the human X chromosome, and it may also function in sex determination. *ZFX* is one of a few genes on the human X chromosome that are known to escape X inactivation. We report the localization of *ZFX*, by meiotic linkage analysis and physical mapping, distal to *POLA* but proximal to *DXS41* (p99-6), near the boundary of bands Xp21.3 and Xp22.1. (Our results suggest the following order of loci in Xp21–p22: cen–*DMD*–[*GK*, *AHC*]–*DXS67* (pB24)–*POLA*–*ZFX*–[*DXS41* (p99-6), *DXS274* (CRI-L1391)]–*DXS43* (pD2)–pter.) These findings contradict the model that escape from X inactivation is limited to genes near the short-arm telomere (i.e., in Xp22.3). Instead, escape from X inactivation is likely a property of several noncontiguous segments of the X chromosome. Curiously, in mouse, the homologous *Zfx* gene maps to X chromosome band D, near the center from which an X-inactivating signal is thought to spread. As judged by comparative mapping, it appears that an X-chromosomal segment that spans the *ZFX* and *DMD* genes has remained grossly intact during the divergence of mouse and human from a common ancestor. Conservation of this chromosomal segment may extend to marsupials, where homologs of the *ZFX* and *DMD* genes have been observed in proximity, but on an autosome. While autosomal homologs of *ZFX* have not been observed in other placental mammals, a locus derived from a processed *Zfx* transcript is found on mouse chromosome 10 band B3 or B4.

INTRODUCTION

In humans and mice, the presence of the Y chromosome determines whether an embryo develops as a male or female. Analysis of humans with partial Y chromosomes reveals that only a minute portion of the short arm of the chromosome is involved in sex determination. Within this sex-determining region, we identified a gene encoding a protein with 13 Cys–Cys/His–His "zinc fingers" (Page *et al.*, 1987a). This Y-encoded zinc-finger protein, which we have named ZFY (Page 1988), may be the primary sex-determining signal.

A related gene, *ZFX*, is found on the short arm of the human X chromosome and has been postulated to play a role in sex determination (Page *et al.*, 1987a). Similarities in gene structure and exon nucleotide sequence suggest that *ZFX* and *ZFY* are derived from a common ancestral gene (Schneider-Gädicke *et al.*, 1989a). *ZFX* and *ZFY* encode similar proteins that have a large acidic domain and 13 zinc fingers and that probably function as transcriptional activators (Schneider-Gädicke *et al.*, 1989a,b; see also Mardon and Page, 1989; Ashworth *et al.*, 1989; Mardon *et al.*, 1990). In contrast to X–Y nucleotide similarity in the pseudoautosomal region, the *ZFX–ZFY* similarity is not maintained by crossingover between the X and Y chromosomes, as evidenced by the fact that the introns of *ZFX* and *ZFY* do not cross-hybridize; nucleotide similarity is essentially limited to the exons (Schneider-Gädicke *et al.*, 1989a). Homologs of human *ZFX* and *ZFY* are found on the X and Y chromosomes of all placental mammals tested (Page *et al.*, 1987a), suggesting that the divergence from a common ancestral gene began prior to the radiation of placental mammals. As judged by transcriptional analysis of cell lines, the human *ZFX* gene escapes X inactivation (Schneider-Gädicke *et al.*, 1989a). The few other genes known to

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0888-7543/90 $3.00

escape X inactivation have all been mapped to the most distal portion of the X-chromosome short arm (e.g., Xp22.3), fostering the notion of a single, discrete portion of Xp that escapes X inactivation (Fialkow, 1970; Race and Sanger, 1975; Shapiro *et al.*, 1979; Mohandas *et al.*, 1980; Migeon *et al.*, 1982; Goodfellow *et al.*, 1984; localizations reviewed by Davies *et al.*, 1987).

In light of the evolutionary relationship of *ZFX* to the putative sex-determining gene on the Y chromosome and the observation that *ZFX* escapes X inactivation, we set out to determine its location on the human X chromosome more precisely, using meiotic linkage, deletion analysis, and *in situ* hybridization. We have also mapped the mouse *Zfx* gene and a *Zfx*-derived autosomal locus (*Zfa*) by *in situ* hybridization.

MATERIALS AND METHODS

DNA Hybridization Probes

Plasmid pDP1039 (Page *et al.*, 1987a) consists of a 1.2-kb genomic *Hin*dIII fragment cloned from the human X chromosome into the *Hin*dIII site of Bluescript. Plasmid pDP1068 consists of a 2.1-kb genomic *Eco*RI fragment cloned from a male BALB/c mouse into the *Eco*RI site of Bluescript. Other DNA probes used are listed in Table 1.

Southern Blot Analysis of Genomic DNAs

Mammalian genomic DNAs were digested with restriction endonucleases, subjected to agarose gel electrophoresis, transferred to nylon membranes, and hybridized with radiolabeled DNA probes as previously described (Page *et al.*, 1987a; Donis-Keller *et al.*, 1987). Hybridizations were typically carried out at 42°C in 50% formamide, 5× SSC (1× SSC = 0.15 *M* NaCl, 15 m*M* Na citrate, pH 7.4), 1× Denhardt's (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 20 m*M* sodium phosphate, pH 6.6, 0.005% denatured salmon sperm DNA, 1% SDS (sodium dodecyl sulfate), 10% dextran sulfate. Following hybridization, transfer membranes were typically washed three times for 15 min each at 60°C in 0.1× SSC, 0.1% SDS and exposed at −80°C for 2 to 6 days with X-ray film backed by an intensifying screen.

Meiotic Linkage Analysis

Estimation of map distances and tests of gene order were performed with the CRI-MAP computer programs (Barker *et al.*, 1987; Donis-Keller *et al.*, 1987). Much of the genotype data analyzed derives from the CEPH (Centre d'Etude du Polymorphisme Humain) database (version 2, published). Unpublished pERT87-15 genotype data for many of the CEPH families were generously provided by Giovanni Romeo and Stefania Fadda. In addition, a number of families segregating X-linked diseases had been typed for pD2, RC8, CRI-L1391, p99-6, and pERT87-30 (A.C. and colleagues, unpublished results); 19 such families (comprising a total of 200 individuals) informative for *ZFX* and at least two other loci were included in the present analyses. *ZFX* (pDP1039/*Msp*I) genotype data for the CEPH families will be submitted to CEPH; genotype data for the disease families are available from A.C.

In Situ Hybridization

In situ hybridization to human chromosomes was carried out as previously described (Andersson *et al.*,

TABLE 1

Human DNA Markers Used in This Study or for Which Previously Published Data Were Analyzed

Locus	Probe	Physical location	Ref.	Number of informative meioses analyzed	Source of meiotic data
DXYS20	pDP230	Xp22.3, Yp	(42)	118	(42)
DXYS28	pDP411a	Xp22.3, Yp	(42)	86	(42)
DXS278	CRI-S232	Xp22.3	(28)	138	(28)
DXS143	dic56	Xp22.2–22.3	(33)	216	CEPH (v. 2)
DXS9	RC8	Xp22.2–22.3	(39)	126	CEPH (v. 2); this paper
DXS43	pD2	Xp22.2	(3)	354	CEPH (v. 2); this paper
DXS274	CRI-L1391	X	(19)	178	CEPH (v. 2)
DXS41	p99-6	Xp22.1	(3)	364	CEPH (v. 2); this paper
ZFX	pDP1039	Xp21.3–22.1	(41); this paper	270	This paper
POLA	pcD-KB pol α	Xp21.3–22.1	(51, 53)	—	—
DXS67	pB24	Xp21.3	(11)	—	—
DXS28	C7	Xp21.3	(12)	—	—
DXS164	pERT87	Xp21.2	(37)	316	Romeo and Fadda; this paper
DXS275	CRI-R393	X	(19)	98	CEPH (v. 2)
DXS7	L1.28	Xp11.3	(14)	257	CEPH (v. 2)

1988). Metaphase cells were obtained from phytohemagglutinin-stimulated 3-day cultures of whole blood from a normal male. Slides were incubated with ^{3}H-labeled plasmid pDP1039 (sp act 1.1×10^7 cpm/μg; concentration 20 to 60 ng/ml) at 42°C in 50% formamide, 2× SSC, 10× Denhardt's, 0.01% denatured salmon sperm DNA, 10% dextran sulfate. The slides were washed three times for 2 min each at 39°C in 50% formamide, 2× SSC and developed after 7 to 40 days of exposure. Metaphase chromosomes were stained with 0.25% Wright's stain and photographed. Destaining, trypsinization, and G-banding were performed as previously described (Andersson *et al.*, 1988).

In situ hybridization to mouse (DBA/2J) chromosomes was carried out as previously described (Tedder *et al.*, 1988). Chromosomes were prepared from 3-day cultures of concanavalin-A-stimulated spleen lymphocytes. Slides were incubated with ^{3}H-labeled plasmid pDP1068 (sp act 8×10^7 cpm/μg; concentration 10 ng/ml) at 42°C, washed (conditions as listed above) three times for 2 min each at 39°C in 50% formamide, 2× SSC, and exposed for 4 to 5 weeks. After staining for Q banding, the slides were examined by fluorescence microscopy to identify chromosomes and using transmitted light to localize autoradiographic grains.

RESULTS

In the human mapping studies reported here, we made use of plasmid pDP1039, the insert of which derives from a *ZFX* intron and does not cross-hybridize to the Y chromosome.

Meiotic Linkage Mapping of the Human ZFX Gene

Probe pDP1039 detects an X-linked restriction fragment length polymorphism (RFLP). When hybridized to *Msp*I-digested genomic DNAs of unrelated human individuals, pDP1039 detects fragments of 5.1 or 5.3 kb. To confirm that the 5.1- and 5.3-kb fragments are Mendelian alleles, we hybridized probe pDP1039 to *Msp*I-digested DNAs from 21 three-generation families with large sibships (White *et al.*, 1985; Dausset, 1986). A typical family is shown in Fig. 1. As expected, the RFLP exhibited codominant X-linked inheritance in all informative families. No polymorphism was detected when pDP1039 was hybridized to DNAs of eight unrelated individuals digested with either *Taq*I, *Eco*RI, *Hin*dIII, *Bgl*II, *Pst*I, *Pvu*II, or *Xba*I (not shown). The 5.1- and 5.3-kb alleles appear to occur at about equal frequency in Caucasians of Western European origin. Of 63 unrelated X chromosomes, 33 carried the 5.3-kb allele, while the other 30 carried the 5.1-kb allele.

We then searched for genetic linkage in female meiosis between *ZFX* and other X-linked RFLPs. Many of the same three-generation families had previously been typed for several pseudoautosomal and strictly X-linked RFLPs, from which genetic linkage maps of Xp were constructed by multipoint analysis (Drayna and White, 1985; Page *et al.*, 1987b; Donis-Keller *et al.*, 1987; Knowlton *et al.*, 1989). We also included in the analysis X-linked RFLP data from 19 families segregating X-linked diseases (A.C. and colleagues, unpublished results). We constructed a linkage map of distal Xp using the method of sequential incorporation (Barker *et al.*, 1987), beginning with the loci *DXS143* and *DXS43*. Remaining loci were placed in each possible position with respect to these two, and multipoint likelihoods (at the maximum likelihood distances) were computed for each placement. A locus was added to the map (and used in placing subsequent loci) if one placement was favored over all alternatives at 100:1 odds or greater.

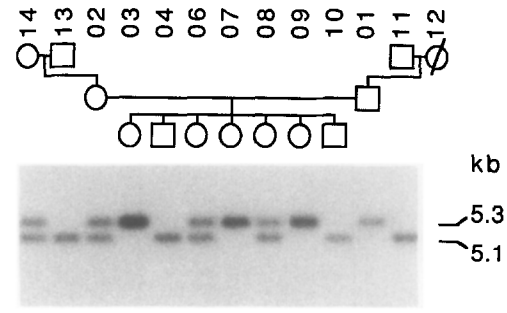

FIG. 1. X-linked inheritance of *Msp*I RFLP at human *ZFX* locus. Probe pDP1039 was hybridized to *Msp*I-digested DNAs from members of family K1349. Allelic 5.1- and 5.3-kb *Msp*I fragments are detected. The numbers above the pedigree identify the individuals; individual 12, the paternal grandmother, is deceased.

Iteration of this process produced a "framework" map of 6 loci together with a list of possible placements (not excluded at 100:1 odds) for the other 6 loci. We then constructed a tentative order for all 12 loci by putting each locus in its most likely position with respect to the framework. The maximum likelihood map for the 12 loci, with a schematic indication of possible alternative orders, is shown in Fig. 2. Table 2 summarizes the support for this order relative to all alternative orders obtainable from it by permuting up to 7 adjacent loci. *ZFX* maps 16 cM distal to pERT87 (*DXS164*, part of the *DMD* gene, in band Xp21.2) and 9 cM proximal to p99-6 (*DXS41*, in band Xp22.1). Thus, analysis of female meiosis maps *ZFX* between *DMD* and *DXS41*, in the region Xp21.2–p22.1.

Physical Mapping of the Human ZFX Gene

Using human–rodent somatic cell hybrids containing partial human X chromosomes, we had previously mapped *ZFX* to Xp21.2–p22.3 (Page *et al.*, 1987a). To refine this physical mapping, we hybridized pDP1039 to restriction-digested genomic DNA from human–ro-

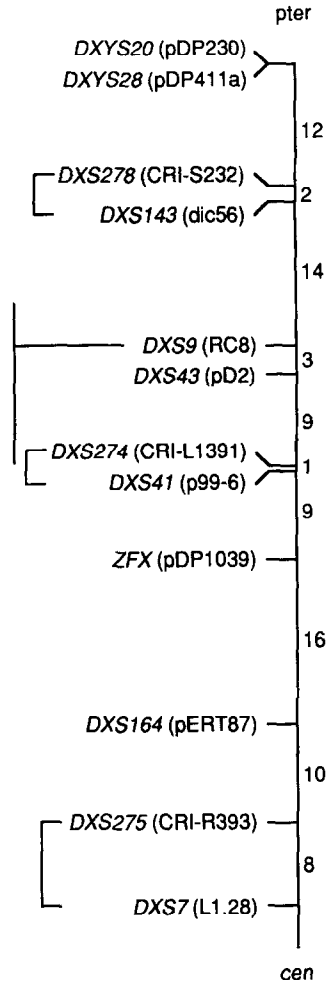

FIG. 2. Meiotic linkage map of the distal short arm of the human X chromosome. The most likely order of the loci is shown. Map distances between pairs of loci are given in Kosambi centimorgans. The order of loci given is favored over alternative orders by odds of at least 100:1, except where (i) brackets indicate pairs of loci whose order is not established at the level of 100:1 odds, or (ii) the bar indicates that the location of *DXS9* with respect to *DXS274* and *DXS43* is not established at the level of 100:1 odds.

dent hybrid Sin176, which retains a human X chromosome with a large interstitial short-arm deletion; one breakpoint falls near the Xp21.3/p22.1 boundary (Ingle *et al.*, 1985). Sin176 was also tested for the presence of six other human X loci, all in Xp21 or Xp22. The results are summarized in Table 3. The presence of *ZFX* in Sin176 argues that *ZFX* maps near or distal to the boundary of bands Xp21.3 and p22.1. *ZFX* must be distal to *POLA* (DNA polymerase α), which is deleted in Sin176. It was previously shown that, in another interstitial Xp deletion (patient KC; Francke, 1984), *DXS43, DXS41,* and *POLA* are present, but *DXS67, DXS28,* and *DXS164* are deleted (de Martinville *et al.*, 1985; Wang *et al.*, 1985; Francke *et al.*, 1987). These results, together with those in Table 3, unambiguously order *ZFX* and *POLA* with respect to the other loci in Table 3. Given that glycerol kinase deficiency (*GK*) and adrenal hypoplasia (*AHC*) have previously been mapped between *DMD* and *DXS28* (Francke *et al.*, 1987), these results suggest the following gene order: cen–*DMD*–[*GK, AHC*]–*POLA*–*ZFX*–pter.

The results of chromosomal *in situ* hybridization with pDP1039 are summarized in Fig. 3. Of a total of 196 autoradiographic grains located on chromosomes, 39 (20%) were found on Xp. (At least one grain was found on Xp in 23 of the 45 metaphase spreads examined.) A distinct peak was centered on bands Xp21–p22.1. This assignment is entirely consistent with the linkage and deletion mapping studies described above. In aggregate, the physical and genetic studies map *ZFX* distal to *POLA* and proximal to *DXS41* (p99-6), near the border shared by bands Xp21.3 and p22.1.

Physical Mapping of the Mouse Zfx Gene and Its Autosomal Homolog

Homologs of the human *ZFY* gene are found on the Y and X chromosomes of all placental mammals tested, including mice (Page *et al.*, 1987a). In most placental mammals, we detect two *ZFY*-related loci: one on the Y chromosome and one on the X chromosome. By contrast, in the mouse there are four *ZFY*-homologous loci (Page *et al.*, 1987a; Page, 1988): *Zfy-1* and *Zfy-2* map to the sex-determining region of the mouse Y chromosome, *Zfx* is on the mouse X chromosome, and a fourth locus, *Zfa,* is autosomal. As judged by comparative nucleotide sequence analysis, *Zfy-1* and *Zfy-2* are very closely related (Mardon and Page, 1989; Ashworth *et al.*, 1989). Their coexistence on the mouse Y chromosome is the result of an intrachromosomal duplication that occurred during rodent evolution (Mardon *et al.*, 1989). Similarly, *Zfx* and *Zfa* are very closely related; sequences cloned from the two loci cross-hybridize under extremely stringent conditions, whereas they cross-hybridize to *Zfy-1* and *Zfy-2* only under more moderate conditions. The mouse *Zfa* locus is the result of retroposition of a processed *Zfx* transcript during rodent evolution (Mardon *et al.*, 1990). We have shown by *in situ* hybridization that both *Zfy-1* and *Zfy-2* are located near the centromere of the mouse Y chromosome (Mardon *et al.*, 1989). Here we report the localization by *in situ* hybridization of *Zfx* and *Zfa.*

In this experiment we made use of plasmid pDP1068, which derives from *Zfa.* As judged by Southern analysis (not shown), pDP1068 strongly cross-hybridizes to *Zfx* under stringent conditions. Under more moderate conditions, pDP1068 also cross-hybridizes to *Zfy-1* and *Zfy-2.*

The results of *in situ* hybridization with pDP1068 are summarized in Fig. 4. In a total of 55 metaphase

TABLE 2

Relative Odds (with Respect to the Order Shown in Fig. 2) for Alternative Orders with up to 7 Adjacent Loci Permuted

	Permuted order										
Relative odds	[*DXYS20*, *DXYS28*]	*DXS278*	*DXS143*	*DXS9*	*DXS43*	*DXS274*	*DXS41*	*ZFX*	*DXS164*	*DXS275*	*DXS7*
1:35	—	*DXS143*	*DXS278*	—	—	—	—	—	—	—	—
1:1.1	—	—	—	*DXS43*	*DXS9*	—	—	—	—	—	—
1:38	—	—	—	*DXS43*	*DXS274*	*DXS9*	—	—	—	—	—
1:1.1	—	—	—	*DXS43*	*DXS9*	*DXS41*	*DXS274*	—	—	—	—
1:1	—	—	—	—	—	*DXS41*	*DXS274*	—	—	—	—
1:380	—	—	—	—	—	—	*ZFX*	*DXS41*	—	—	—
$1:3.2 \times 10^9$	—	—	—	—	—	—	—	*DXS164*	*ZFX*	—	—
1:11	—	—	—	—	—	—	—	—	—	*DXS7*	*DXS275*
1:40	—	*DXS143*	*DXS278*	*DXS43*	*DXS9*	—	—	—	—	—	—
1:41	—	*DXS143*	*DXS278*	*DXS43*	*DXS9*	*DXS41*	*DXS274*	—	—	—	—
1:34	—	*DXS143*	*DXS278*	—	—	*DXS41*	*DXS274*	—	—	—	—
1:380	—	—	—	*DXS43*	*DXS9*	—	*ZFX*	*DXS41*	—	—	—
1:11	—	—	—	—	—	*DXS41*	*DXS274*	—	—	*DXS7*	*DXS275*

Note. Orders are listed only if they have odds within 1:1000 of the Fig. 2 order, with the exception that the order with *ZFX* and *DXS164* interchanged is also included. The order of *DXYS20* and *DXYS28* with respect to each other was found to have little or no effect on the relative odds of these permuted orders. Thus, these two loci are shown as a complex.

cells examined, 36% of the hybridization sites (26 of 76 sites) were on chromosome 10, 20% (15 of 76 sites) were on the X chromosome, and 7% (5 of 76 sites) were on the Y chromosome. The peaks of hybridization were located at chromosome 10 bands B3 and B4, at X chromosome bands C and D, and near the Y centromere. The weak hybridization signal near the Y centromere is probably due to cross-hybridization of the probe to *Zfy-1* and *Zfy-2*. We conclude that *Zfx* maps to X-chromosome band C or D, while *Zfa* maps to chromosome 10 band B3 or B4.

DISCUSSION

A Gene Escaping X Inactivation near the Xp21.3/22.1 Boundary

The studies reported here provide a precise localization of the *ZFX* gene on the short arm of the human X chromosome. First, meiotic linkage analysis (Fig. 2) maps *ZFX* 16 cM distal to *DXS164* (the pERT87 probes, within the *DMD* gene; Xp21.2) and 9 cM proximal to *DXS41* (p99-6; Xp22.1). Second, deletion mapping on partial human X chromosomes (Table 3 and Page *et al.*, 1987a) localizes *ZFX* to Xp21.3–p22.3, distal to *POLA*. Third, as judged by *in situ* hybridization (Fig. 3), *ZFX* maps to Xp21–p22.1. These mutually consistent results map *ZFX* near the boundary of bands Xp21.3 and Xp22.1 and suggest the following order of loci: cen–*DMD*–[*GK, AHC*]–*DXS67* (pB24)–*POLA*–*ZFX*–[*DXS41* (p99-6), *DXS274* (CRI-L1391)]–*DXS43* (pD2)–pter. Our findings are in good agreement with the studies of Affara and colleagues (1989) and Müller and Schempp (1989), who concluded on the basis of *in situ* hybridization that *ZFX* maps to Xp21 or Xp21.3, respectively.

In mammals, dosage compensation of X-linked genes is accomplished by transcriptional inactivation of genes on all but one X chromosome per cell (Lyon, 1988). While most genes on the X chromosome are presumed to be subject to such inactivation, transcriptional analysis reveals that the human *ZFX* gene escapes X in-

TABLE 3

Deletion Mapping with Hybrid Sin176 (Retaining Xpter–p22.1;p11–qter) Places *ZFX* Distal to *POLA*

Locus	Probe	Presence/absence
DXS43	pD2	+
DXS41	p99-6	+
ZFX	pDP1039	+
POLA	pcD-KB pol α	−
DXS67	pB24	−
DXS28	C7	−
DXS164	pERT87-30	−

Note. As described by Ingle *et al.* (26), human/hamster hybrid Sin176 carries a human X chromosome with an interstitial deletion of the short arm. This X chromosome retains band Xp22.1 and may or may not retain a small portion of distal Xp21.3. The probes, all in Xp22.2–p21.2, were hybridized to restriction-digested DNA from Sin176. (The deletion of *DXS28* was previously reported by Ingle *et al.* (26).) The loci are listed according to their order, from most distal to most proximal, in Xp22–p21 (other data supporting this order are reviewed by Davies *et al.* (15)).

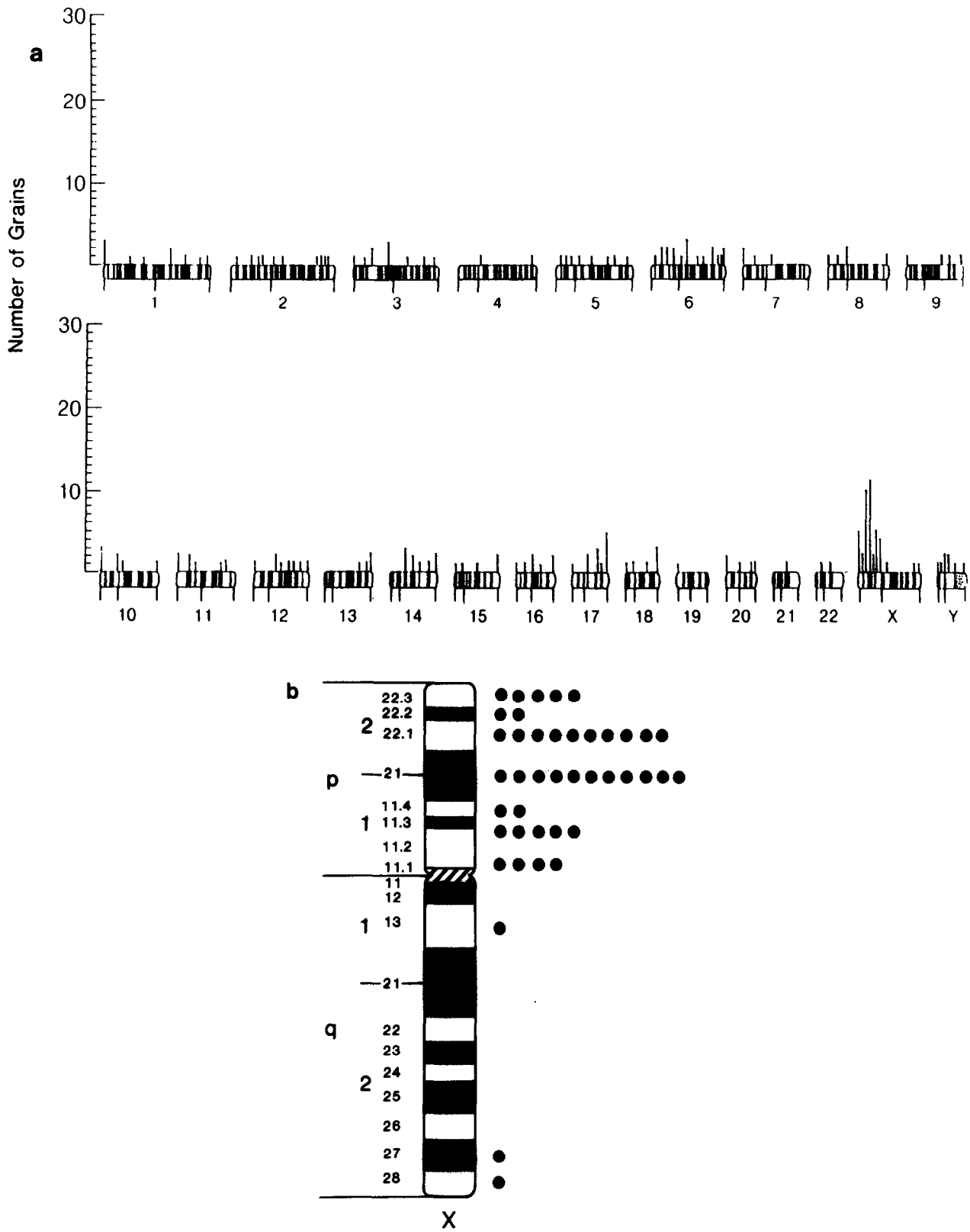

FIG. 3. *In situ* hybridization of pDP1039, a human *ZFX* probe, to human chromosomes. (a) Histogram summarizing the sites of hybridization in the 45 metaphase cells scored. (b) Distribution of grains on a diagram of the X chromosome.

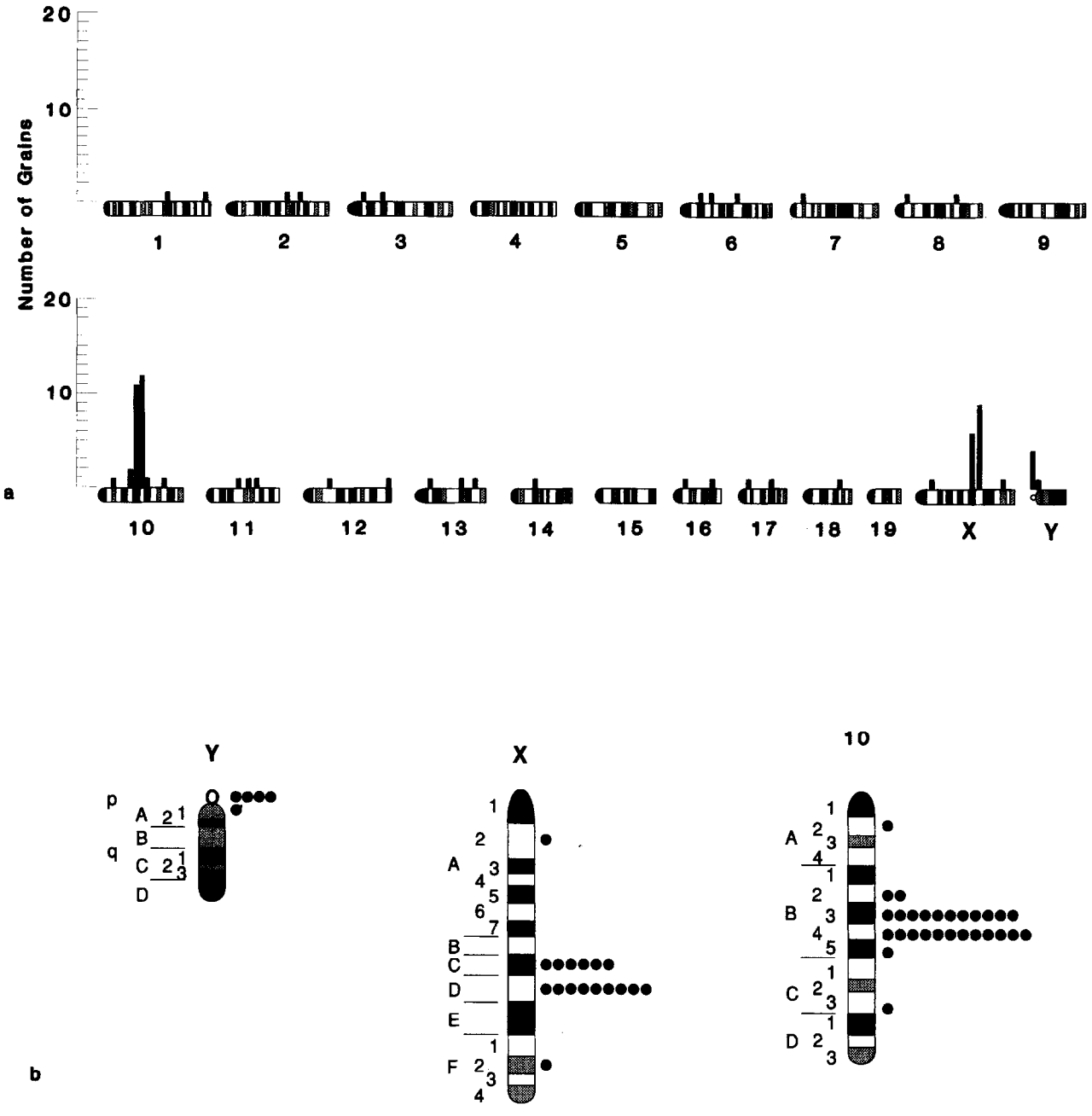

FIG. 4. *In situ* hybridization of pDP1068, a genomic clone from the mouse autosomal homolog of *Zfx*, to mouse chromosomes. (**a**) Histogram summarizing the sites of hybridization in the 55 metaphase cells scored. (**b**) Distribution of grains on diagrams of chromosomes Y, X, and 10.

activation (Schneider-Gädicke *et al.*, 1989a). Three other X-linked genes (*XG, STS,* and *MIC2*) had been shown to escape X inactivation (Fialkow, 1970; Race and Sanger, 1975; Shapiro *et al.*, 1979; Mohandas *et al.*, 1980; Migeon *et al.*, 1982; Goodfellow *et al.*, 1984), but all map to the extreme distal portion of the short arm of the human X chromosome (band Xp22.3; reviewed by Davies *et al.*, 1987). These earlier observations had suggested that a single, discrete portion of Xp escapes X inactivation. However, as *ZFX* maps more proximally, near the Xp21.3/22.1 boundary, escape from X inactivation must not be limited to genes in Xp22.3. Thus, non-inactivation either extends across a large, contiguous portion of Xp or is a characteristic of multiple, noncontiguous portions of the X chromosome. The latter model is consistent with the observation that the *A1S9T* gene, which maps even more proximally on Xp (Xp11.1–p11.3; Brown and Willard,

1990), also escapes X inactivation (Brown and Willard, 1989). *DMD* and *POLA*, which are subject to X inactivation (e.g., Jacobs *et al.*, 1981; Wang *et al.*, 1985), are flanked by genes that escape X inactivation: *A1S9T* is proximal to these loci, while *ZFX, STS, XG,* and *MIC2* are distal. Thus, genes that undergo X inactivation are intermixed with genes that do not—at least on the short arm of the human X chromosome. As previously noted (Schneider-Gädicke *et al.*, 1989a), escape from X inactivation correlates with Y homology.

A Role in Sex Determination?

Numerous XY-viable deletions of the human X chromosome have been described. Given the possibility that *ZFX* functions in sex determination, it would be of great interest to know whether a *ZFX*-deleted XY embryo would develop as a male or as a female. Several XY-viable deletions causing glycerol kinase deficiency and adrenal hypoplasia, and in some cases muscular dystrophy, include *DXS28* and *DXS67* (Francke *et al.*, 1987), both of which map proximal to *ZFX* (Table 3). It is unlikely that any such deletion extends to include *ZFX* since DNA polymerase α (*POLA*), which is probably required for cell viability, maps between [*GK, AHC*] and *ZFX*. We are not aware of any XY-viable deletion that includes *ZFX*.

Comparative Mapping in Human, Mouse, and Marsupials

Homologs of the human *ZFX* gene are found on the X chromosomes of all placental mammals tested (Page *et al.*, 1987a). As determined by *in situ* hybridization, *Zfx* maps to band C or D of the mouse X chromosome (Fig. 4), placing it near the *Dmd* gene (the homolog of the human *DMD* gene) in band B or C and proximal to *Pgk-1* (in band D) (Disteche *et al.*, 1989). These results are in agreement with deletion analysis of partial mouse X chromosomes, which maps *Zfx* between the T16H and T14RL translocation breakpoints, distal to *Dmd* and near *Pgk-1* (Mitchell *et al.*, 1989; Nagamine *et al.*, 1989). These findings are consistent with meiotic linkage analysis which reveals that, on the mouse X chromosome, *Zfx* is about 6 cM distal to *Dmd* and about 8 cM proximal to *Pgk-1* (Mitchell *et al.*, 1989; Keer *et al.*, submitted).

This comparative mapping of *ZFX* in human and mouse is consistent with the growing body of information on conservation of local gene order between the X chromosomes of the two species (Avner *et al.*, 1988; Brockdorff *et al.*, 1987; Davisson, 1987; Lyon, 1988). Specifically, it appears that a segment spanning the *DMD* and *ZFX* genes has remained grossly intact during the divergence of the human and mouse X chromosomes (Mitchell *et al.*, 1989). Indeed, in marsupials, homologs of *DMD* and *ZFX/ZFY* map in proximity, but on an autosome (Sinclair *et al.*, 1988). Thus, the *DMD–ZFX* linkage may predate the divergence of placental mammals and marsupials which occurred at least 130 million years ago (Air *et al.*, 1971).

GK, AHC, and *POLA* all map between *DMD* and *ZFX* on the human X chromosome. We predict that homologs of these genes will be found between *Dmd* and *Zfx* on the mouse X chromosome. Perhaps homologs of *GK, AHC,* and *POLA* are also located near the *DMD* and *ZFX/ZFY* homologs on marsupial autosomes.

In humans, two genes in this linkage group (*DMD* and *POLA*) are known to be subject to X inactivation, while *ZFX* escapes X inactivation. Curiously, on the mouse X chromosome, *Zfx* maps close (Mitchell *et al.*, 1989; Keer *et al.*, submitted) to the X-inactivation center (reviewed by Gartler and Riggs, 1983). It will be of interest to see whether mouse *Zfx* is subject to X inactivation.

Note. Recent studies demonstrate that a portion of the Y chromosome near but not including *ZFY* induces male differentiation (Palmer *et al.*, 1989).

ACKNOWLEDGMENTS

We thank David Adler for computer-generated chromosome idiograms and Rebecca Mosher and Douglas Vollrath for comments on the manuscript. We thank Louis Kunkel, Sam Latt (deceased), Jean-Louis Mandel, and Teresa Wang for DNA probes. We are especially grateful to Giovanni Romeo and Stefania Fadda for allowing us to analyze their unpublished pERT87-15 genotype data. This work was supported by grants from the National Institutes of Health (HD22532 to D.C.P., GM40543 to Helen Donis-Keller, GM30476 to C.M.D.), the Lucille P. Markey Charitable Trust, the March of Dimes, the American Cancer Society, the Sigrid Jusélius Foundation, the Folkhälsan Institute of Genetics, and the Academy of Finland.

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