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Mouse testicular protein variants, including tubulin, detected by isoelectrofocusing two-dimensional gel electrophoresis

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Summary

We have utilized isoelectrofocusing two-dimensional polyacrylamide gel electrophoresis (IEF-2D-PAGE) to determine the frequency of variant proteins detected in mouse testes, and to examine an inbred strain with spermatogenic abnormalities. The observed frequency of variants, $4{\cdot}2 \pm 1{\cdot}0\,\%$, is comparable to results reported for liver. A variant for a protein, identified as tubulin by immunoblotting, was observed in an inbred strain (PL/J) which has defective spermatogenesis, but was also observed in another inbred strain without spermatogenic defects. The gene symbol *Tbn* is proposed for this β-tubulin locus.

1. Introduction

Two-dimensional gel electrophoreses (isoelectrofocusing two-dimensional polyacrylamide gel electrophoresis, IEF-2D-PAGE) of mouse testicular proteins have been performed for a variety of reasons. Initial developmental studies focused on identifying protein changes occurring at various stages of spermatogenesis (Boitani *et al.* 1980; Kramer & Erickson, 1982; Stern, Gold & Hecht, 1983). Similar studies were also undertaken for rat (DePhilip, Tres & Kirzenbaum, 1982) and human testicular proteins (Narayan *et al.* 1983). Post-meiotic stage-specific proteins detected by IEF-2D-PAGE, following *in vitro* translation of isolated mRNA, provided evidence for post-meiotic gene expression (Fujimoto & Erickson, 1982).

Subsequent studies focused on genetic variation in testicular proteins detected by IEF-2D-PAGE. The large number (8) of *t*-complex-encoded testicular proteins identifiable as variants by IEF-SD-PAGE (Silvers *et al.* 1983) could be due to a special concentration of genes expressed in testes which map within the 12–15 cM *t*-complex region of mouse chromosome 17 (Erickson, Lewis & Buttey, 1981; Lyon, 1984, 1986), or to a generally high level of protein polymorphism for testicular proteins. A 10% frequency of variants among human pachytene spermatocyte proteins was noted between individuals by IEF-2D-PAGE, but family studies were not performed to demonstrate that the variants were inherited (Narayan *et al.* 1983). We have now performed an IEF-2D-PAGE analysis of testicular proteins from inbred strains of mice and their F_1 progeny to determine the frequency of inherited variants, as we have done previously for liver proteins of the same strains (Baier, Hanash & Erickson, 1983; Neel *et al.* 1985). Sixteen polypeptides, among 378 scored, exhibited variants. We also searched for protein alterations in the PL/J strain that produces headless sperm, apparently due to abnormalities of centriole localization (Burkhart & Malling, 1981). A β-tubulin variant was observed in PL/J strain. However, the occurrence of the same variant in the 129/SvSn strain indicated that it was not uniquely related to the apparent meiotic abnormalities.

2. Materials and methods

Testes from three strains of mice, A/J, 129/SvSn and BL10.A, were analysed for electrophoretic variants. Representatives of the c^{ch}/c^{3H} (c^{3H}) strain were crossed with each of the three strains. $c^{3H} \times$ A/J, $c^{3H} \times$ 129/SvSn, and $c^{3H} \times$ C57BL/6J (congenic with BL10.A) genotypes were obtained. Testes were excised from mice eight weeks after birth. The testicular capsule was removed and the remaining testes immediately frozen at -80 °C for subsequent analysis.

Testes from an additional strain of mouse, PL/J, were examined for electrophoretic corroboration of a tubulin variant thought to be peculiar to this mouse (Erickson & Karolyi, unpublished data).

IEF-2D-PAGE was performed on samples from pure strains, crosses and PL/J simultaneously. For each sample, approximately 5 mg of testicular tissue

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was solubilized in 90 μl of a 9 M urea–ampholyte mixture to which 10 μl of phenylmethyl sulphonyl-fluoride (15·5 g/l ethanol) had been added as a proteolytic inhibitor. The urea–ampholyte solution consisted of (per litre) 9 mol urea, 2%pH 3·5–10 ampholytes, 2% Nonidet P-40 surfactant and 2% (vol/vol) 2-mercaptoethanol in distilled deionized water. Samples were vortexed intermittently over a 15 min interval until the tissue–urea mixture appeared homogeneous, indicating sufficient solubilization of proteins. Unsolubilized material, including DNA, was pelleted by centrifugation in a Microfuge for 3 min and the supernatant containing the solubilized proteins was removed for electrophoresis.

For each testis sample, 15 μl and 25 μl aliquots of the supernatant were applied to isoelectric focusing gels. Twenty gels were electrophoresed simultaneously. First-dimension gels contained 1·8%pH 3·0–10 ampholytes (Ampholines: LKB, Serva) and 0·13%pH 5–7 ampholytes (LKB). Isofocusing was done at 1200 V for 16 h and at 1500 V for an additional 2 h. SDS gels containing an 11–13·5% g/dl acrylamide gradient were used for the second dimension; further details may be found in Neel *et al.* (1984). Gels were then either stained using the silver-staining technique of Merril *et al.* (1981), or were used for immunoblotting. For immunoblotted gels, duplicate gels were available for silver-staining.

Western immunoblotting was performed on PL/J, A/J and 129/SvSn simultaneously. Immunotransfer was done at 45 V for 15 h in transfer buffer consisting of (per litre) 0·19 mol glycine and 0·025 mol Tris base in 20% methanol. Blots were washed for one hour in a 0·05 mol Tris base incubation buffer (pH = 7·4), with changes of buffer every 15 min. An affinity-purified sheep antitubulin antibody (the gift of Dr Michael Welsh) was then added in a 1:5000 dilution (μg antibody:ml incubation buffer) and allowed to incubate overnight. A one-hour wash in 100 ml 0·05 mol Tris base incubation buffer was repeated between applications of the antibodies, with changes of buffer every 15 min. Rabbit anti-bovine, horseradish peroxidase-conjugated antibody was applied in a 1:1000 dilution and allowed to incubate overnight. After washing, immunoblots were developed in 0·29 mol/l 4-chloro, 1-naphthol in 16% methanol for 20 min, then rinsed in distilled water.

Because gels expand approximately 4–5 cm during Merril staining, simple visual comparison of stained gels and immunoblots would not be appropriate for analysis. As an aid to analysis of immunoblots, reference points were set up on both the gels and the immunoblots. The first-dimension 'noodles' were marked at the acidic end with Ponceau-red for the testes samples that were to be immunoblotted. When unstained 2D gels were ready for immunotransfer onto nitrocellulose, holes were punched in the gels to be silver-stained (duplicates of those used for immunoblotting) and the nitrocellulose in the same orientation with respect to pH and molecular weight axes (see Fig. 3). These holes, about 2 mm in diameter, served as markers for comparison between stained gels and immunoblots.

3. Results

Approximately 750 spots were consistently visualized on each gel within an effective focusing range of pH 4·0–7·5 and molecular weight 15–85 kDa. Some 393 polypeptide spots were chosen for analysis of polymorphic loci. Spots selected had sufficient intensity for intensity difference due to heterozygosity to be visualized. Spots were scored for their presence, absence or change in intensity for all gels excluding PL/J. At least two sets of gels were available for the inbred strains of mice. When visual genotyping of a homozygote was ambiguous, gel duplicates were relied upon for verification. Spots exhibiting substantial variability, unrelated to the genotype, were eliminated from the analysis. Only 15 such spots were excluded.

A total of 378 spots were included in the analysis. Of these, 16 spot loci were found to be polymorphic. Suspected polymorphisms were corroborated by analyses of testes from crosses. One of the 16 polymorphic loci was observed with an unpaired allele, i.e. no corresponding variant allele was visualized. The remainder of the polymorphic loci presented a normal spot and a variant spot of roughly equal intensity in their respective homozygous states, and both spots at approximately half-intensity when heterozygous (Figs 1 and 2).

One of the polymorphic loci (spot 15) was observed in the region of β-tubulin (MW = 50 kDa, pI ~ 5·0). The variant associated with this locus was seen not only in PL/J but also in 129/SvSn (Fig. 3). This variant focuses at the same molecular weight as in normal tubulin, but migrates approximately one charge unit more basically. Western immunoblots confirmed the identity of this polypeptide as tubulin. Both normal (A/J) and variant (PL/J and 129/SvSn) tubulin alleles, as determined by IEF-2D-PAGE, reacted with the affinity-purified tubulin antibody. The degree of reactivity was approximately equivalent for both alleles. Despite standardization of electrophoretic procedure, technical differences in gel preparation and electrophoresis (variable ampholyte focusing, 'noodle'-stretching when applying the first dimension to the second dimension, etc.) between gels and, subsequently, their immunoblots, were unavoidable; thus, differentiation between normal and variant alleles on the immunoblots was not possible. However, blot reactivity was confirmed as being within tubulin range for both alleles by triangulating the holes in the gels and immunoblots and adjusting for relative size differences of blots and gels. A description of other polypeptides for which variants were observed is presented and summarized in Table 1.

Polymorphism 1. This is a large and fairly intense

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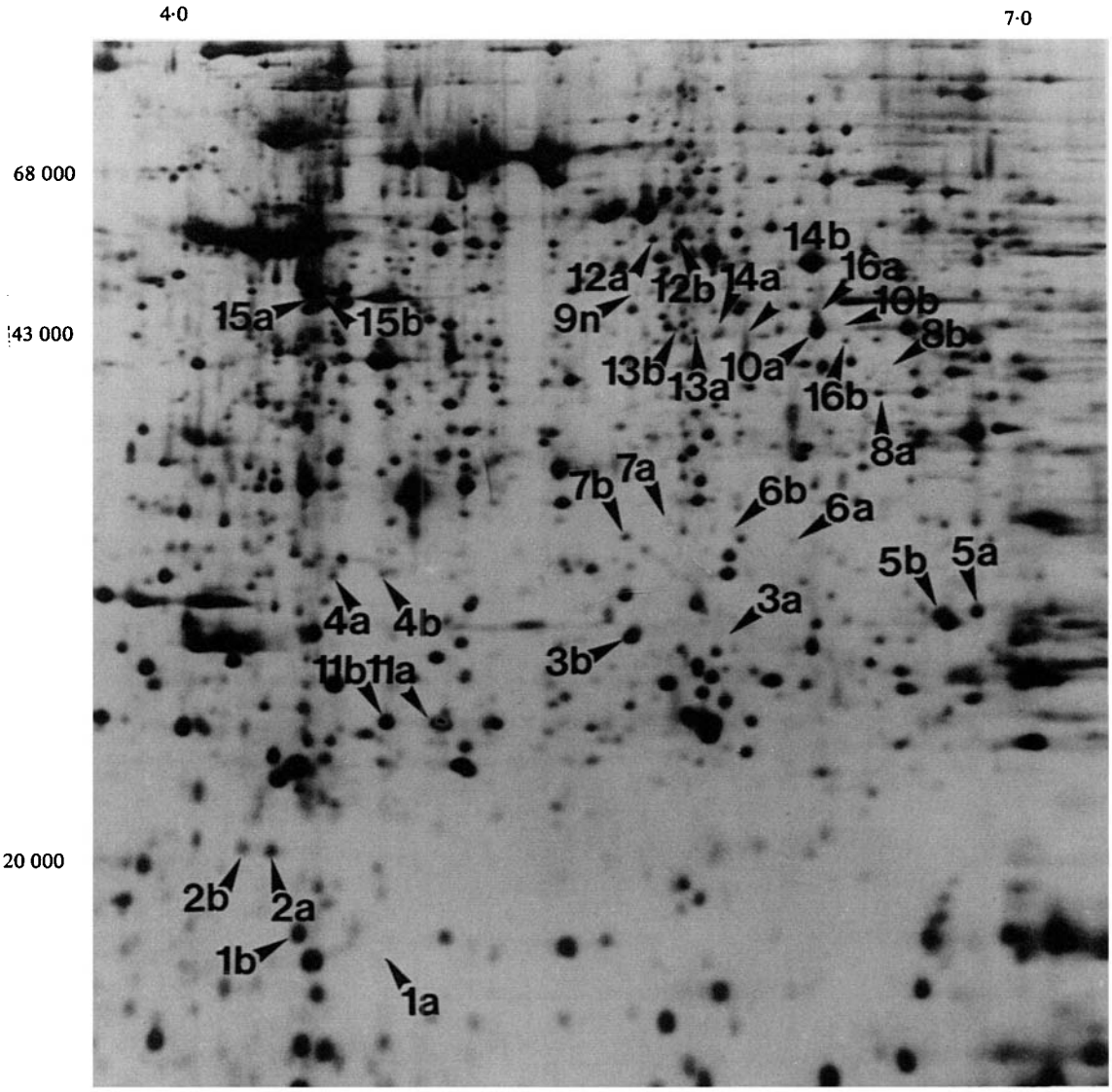

Fig. 1. A 2D gel of the testicular proteins from the F_1 cross of C57BL/6J × c^{ch}/c^{3H} mice. The numbered arrows indicate the loci of the sixteen spots shown to be polymorphic in this study. 'a' and 'b' denote the normal and variant alleles, respectively; 'n' denotes an unpaired variant. Approximate molecular weight (daltons) and IEF values were determined by running IEF and M.W. standards.

spot that focuses in the lower molecular weight region of the gels. Its variant is slightly smaller and migrates two charge units more acidically at a slightly higher molecular weight.

Polymorphism 2. The variant of the normal spot of this locus is of equal intensity and migrates one charge unit more acidically. In the cross heterozygous for this locus, the variant is slightly decreased in intensity as compared to the normal spot.

Polymorphism 3. This is a large, intense spot observed with an equally intense variant which focuses differently in both charge and molecular weight dimensions: approximately three charge units more acidically and at a slightly higher molecular weight.

Polymorphisms 4 and 6. The normal spots of these loci focus at the same molecular weight as their respective variant alleles. Their variants exhibit migration differences of approximately one and three charge units, respectively, towards the basic and acidic ends, respectively, of the pI-axis.

Polymorphism 5. This spot focuses as a very intense, conspicuous spot on the basic periphery of the gel's effective isofocusing range. Its variant migrates two charge units more acidically, though at the same molecular weight, and abuts two other dark spots of equal size.

Polymorphism 7. This is a small, fairly intense spot located in the central portion of the gels. Its variant focuses one charge unit more acidically than the normal spot and at a slightly lower molecular weight. In heterozygotes, both normal and variant spots appear at less than half the intensity of their homozygous counterparts.

Polymorphism 8. The normal allele of this locus

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(a)

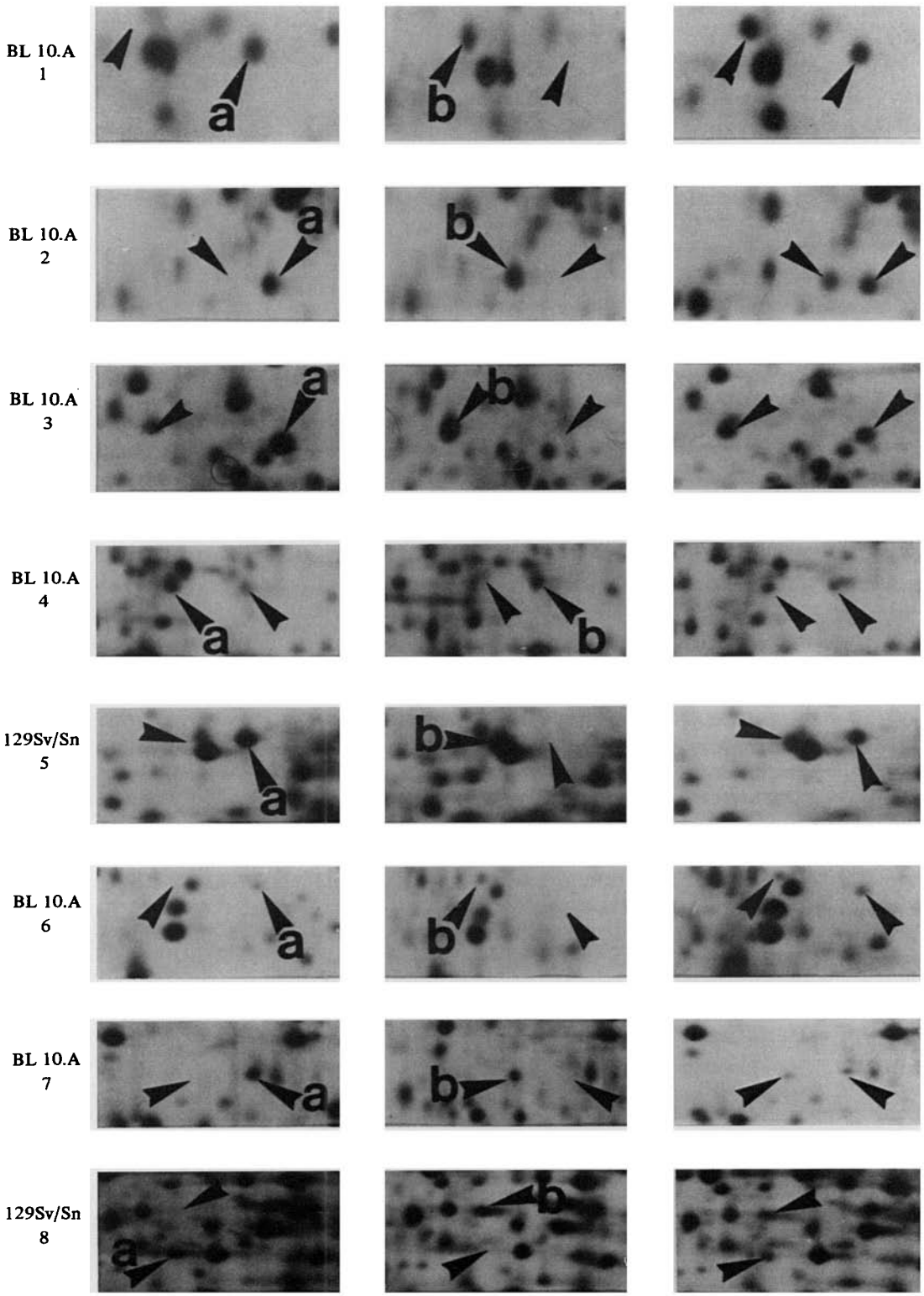

Fig. 2.(*a*). For legend see opposite.

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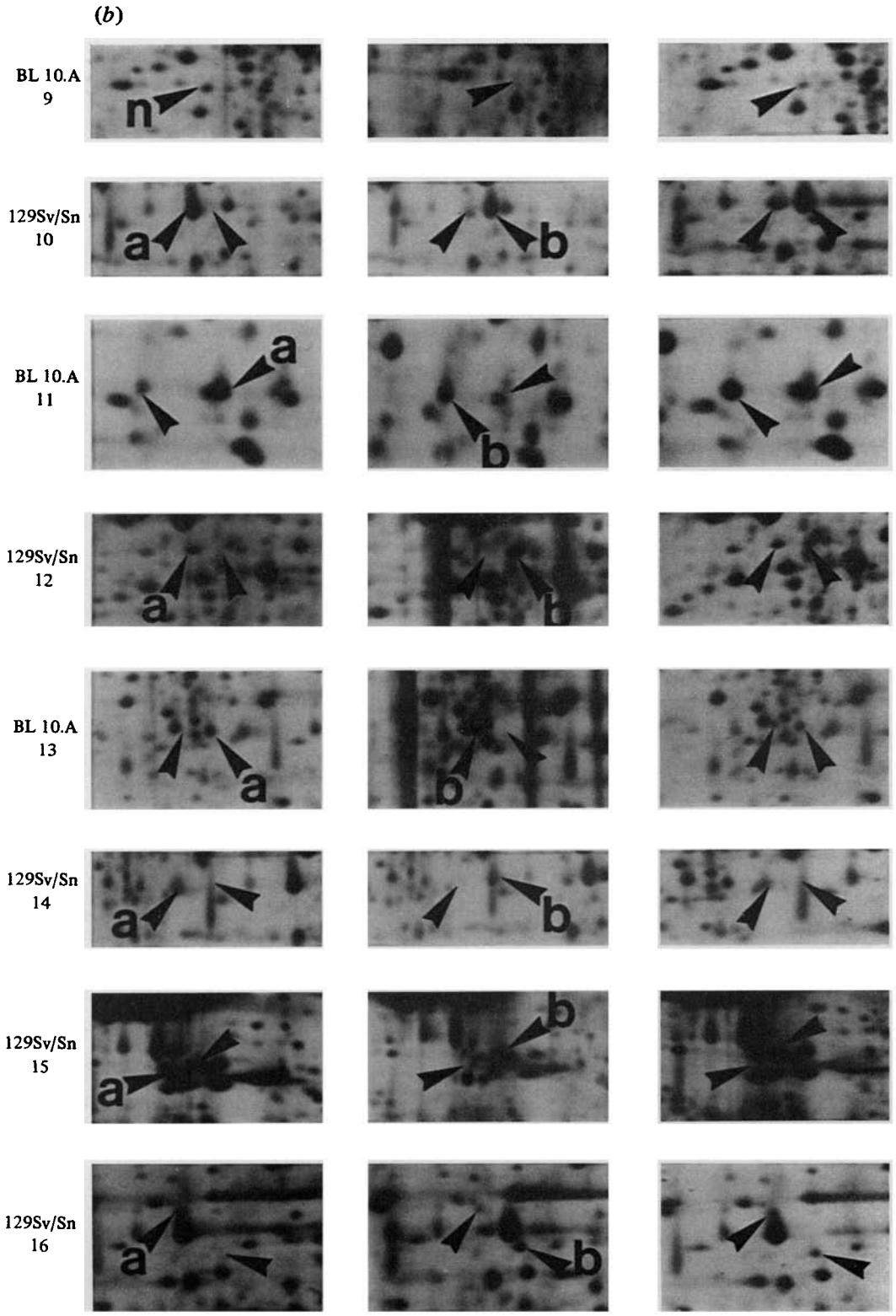

Fig. 2. Illustration of the sixteen variants in this study. The first column represents A/J, the template strain; the centre column the variant strain indicated at the left; and the right column the F_1 heterozygous cross.

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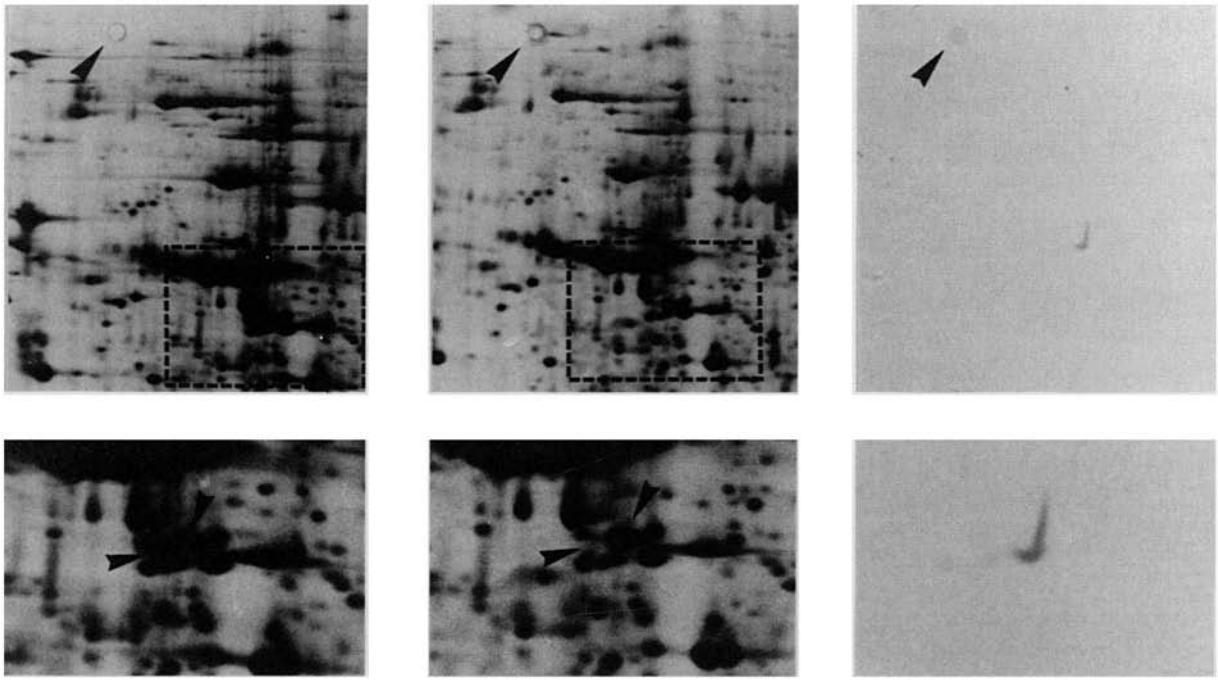

Fig. 3. Illustration of the tubulin alleles detected by 2D gels and of PL/J immunoblot reactivity. Above, the relevant one-sixth of the gel; below, an enlargement of the tubulin area which is outlined on the gels only (the immunoblot is at the same enlargement). Since gels expand 4–5 cm during Merril staining, the relative distance between marker and spot is not exact in this figure. Arrows indicate the tubulin alleles and one of the reference points set up on the 2D gels and immunoblots.

focuses near the basic periphery of the gel; its variant focuses the equivalent of 2 kDa above it. Both spots have a small tail extending along the pI-axis towards the basic periphery of the gel.

Polymorphism 9. This high-molecular-weight spot focuses in an area of low density of spots. Despite the advantage of easy spot identification due to the lack of crowding, no variant was detected. This unpaired variant appeared in a cross at half-intensity.

Polymorphism 10. This large, intense, teardrop-shaped spot focuses nearly under a slightly small spot which sometimes resolves into a streak that runs along the pH axis towards the basic end of the gel. Its variant migrates approximately one charge unit more basically, to focus directly under the slightly smaller spot/streak. Both alleles in homozygous form are of equal size and intensity. In heterozygotes, however, the normal allele is diminished in size by approximately 30%, the variant allele maintaining its apparent size as in homozygotes.

Polymorphism 11. Both alleles of this polymorphism are associated with a cluster of spots. The normal allele is one of a group of three spots; its variant migrates two charge units more acidically than the normal spot, and at a slightly higher molecular weight.

Polymorphism 12. The variant of this high-molecular-weight spot migrates approximately one charge unit more basically into a semicircular arrangement of similarly-sized spots. Both normal and variant spots are of equal size and intensity in their respective homozygotes and they decrease to approximately half intensity when heterozygous.

Polymorphism 13. The alleles of this spot locus are the same molecular weight, one charge unit apart. The normal allele is a small, dark spot; the variant allele is the same size and equally intense, appearing within a triangle of small, intense spots, one charge unit more acidically than the normal allele.

Polymorphism 14. The normal allele of this polymorphism is a diffuse though coherent spot with two small tails extending upwards and basically, respectively, and abuts a spot half its size to the left. Its variant is a spot of equal intensity and size that rests on top of another spot that is slightly stretched along the molecular-weight axis. Unfortunately, in heterozygotes the presumed half-intensity alleles are visually absorbed by the spots that they abut, and distinct alleles are not discernible.

Polymorphism 15. This polymorphism represents tubulin and its variant, which were described above.

Polymorphism 16. The normal and variant spots are situated in proximity to another polymorphic spot. The spot corresponding to the normal allele is a small, intense spot with a slightly higher molecular weight than the spot corresponding to the normal allele for polymorphism 10. The variant spot of polymorphism 16 is located at a lower molecular weight and focuses

Table 1. *Summary characterization of polymorphic loci detected in this study*

Designation	Strain	Approximate MW (kDa)	pI
1a	A/J, 129/SvSn	19	4·8
1b	BL10.A, c^{ch}/c^{3H}	20	4·6
2a	A/J, 129/SvSn, c^{ch}/c^{3H}	22	4·5
2b	BL10.A	22	4·4
3a	A/J, 129/SvSn	28	6·1
3b	BL10.A, c^{ch}/c^{3H}	28	5·7
4a	A/J, 129/SvSn, c^{ch}/c^{3H}	31	4·7
4b	BL10.A	31	4·8
5a	A/J, BL10.A	29	6·9
5b	129/SvSn, c^{ch}/c^{3H}	29	6·7
6a	A/J, 129/SvSn, c^{ch}/c^{3H}	33	6·3
6b	BL10.A	33	6·0
7a	A/J	33	5·8
7b	129/SvSn, BL10.A, c^{ch}/c^{3H}	33	5·7
8a	A/J, BL10.A, c^{ch}/c^{3H}	39	6·5
8b	129/SvSn	41	6·5
9*n*	A/J, c^{ch}/c^{3H}	50	5·7
10a	A/J, BL10.A, c^{ch}/c^{3H}	44	6·3
10b	129/SvSn	44	6·4
11a	A/J, c^{ch}/c^{3H}	26	5·0
11b	129/SvSn, BL10.A	26	4·8
12a	A/J, 129/SvSn, c^{ch}/c^{3H}	58	5·7
12b	BL10.A	58	5·8
13a	A/J, 129/SvSn, c^{ch}/c^{3H}	44	5·9
13b	BL10.A	44	5·8
14a	A/J, BL10.A	44	6·0
14b	129/SvSn, c^{ch}/c^{3H}	44	6·1
15a	A/J, BL10.A, c^{ch}/c^{3H}	50	4·6
15b	129/SvSn, PL/J	50	4·7
16a	A/J, BL10.A, c^{ch}/c^{3H}	45	6·3
16b	129/SvSn	41	6·4

approximately one charge unit more basically than its normal allele. Both spots appear at half intensity in heterozygous form.

4. Discussion

Several laboratories have searched for genetic variation in mouse liver or kidney by IEF-2D-PAGE (reviewed in Neel *et al.* 1985). The number of variants found in these studies was much lower than the frequency found using one-dimensional electrophoresis of blood and plasma proteins in wild populations of *Mus musculus domesticus* and *M. m. musculus* (Selander, Hunt & Yong, 1969; Hunt & Selander, 1973). This discrepancy in the number of variants found by the two methods in the different tissues can be partly explained by the fact that the same allele of a biallelic polymorphism may be fixed by inbreeding in the different lines (Neel *et al.* 1985). An apparent exception to the relatively low frequency of variants detected by IEF-2D-PAGE was the finding of 8 variant testicular proteins controlled by the *t*-complex; this chromosomal region represents only about 0·5% of the mouse genome (Silver *et al.* 1983). These findings contrasted with 12 variants found among 584 testicular spots compared between 2 inbred strains, but details and genetic control in F_1s were not provided (Silver *et al.* 1983). The discrepancy between the rates of variation found for *t*-complex testicular loci and for those encoded elsewhere in the genome was explained by postulating an origin of the *t*-complex from an alien rodent species, a hypothesis which no longer seems tenable (Figueroa *et al.* 1985). In order to resolve this discrepancy, we have studied the frequency of variant proteins found by IEF-2D-PAGE between several inbred strains and their F_1 hybrids.

The variant proteins which we have found among the 378 scored in these inbred mice (which do not contain a *t*-complex) are mostly of the paired variety. By this we mean that the F_1 exhibits a pair of polypeptides, only one or the other of which is present in a parental strain. One unpaired variant was found – the polymorphic spot detected is present in one strain and in the F_1, but is absent in another strain, which does not contribute a detectable spot to the F_1. This variant could result from an inability to detect the second strain's allele by IEF-2D-PAGE

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(possibly because it co-migrates with another polypeptide) or from the presence of a null allele in the second strain.

The 16 polymorphisms found represent a frequency of $4{\cdot}2 \pm 1{\cdot}0\%$, which is highly comparable to the $4{\cdot}9 \pm 2{\cdot}0\%$ we found in liver (Neel *et al.* 1985), although other authors have found lower frequencies (Klose & Feller, 1981), in liver. The high frequency of IEF-2D-PAGE-detected *t*-complex-encoded testicular protein variants must have some other explanation, perhaps related to the multiple effects of the *t*-complex on male reproduction (Erickson, 1978; Lyon, 1984, 1986).

The analyses of mutations in the structural gene for a testis-specific β-tubulin in *Drosophila* have elucidated multiple functions of this β-tubulin in spermatogenesis (Kemphues *et al.* 1979, 1980, 1982). Although the existence of sperm-specific tubulins in mice is well-documented (Villasante *et al.* 1986), mutations affecting spermatogenesis which might be due to abnormalities of tubulin are unknown (Beatty, 1970). The spermatogenic abnormalities of centriole position in the PL/J strain described by Burkhart & Malling (1981) could reflect tubulin variation, as microtubule orientation by centrioles may affect the centrioles' movement. Although β-tubulin is known to be variable in its position on IEF-2D-PAGE, we found that PL/J testes showed a consistent charge variation for a putative tubulin as compared to the A/J strain. Western blotting confirmed the identification of this spot as a tubulin. However, the variant was also present in the 129/SvSn strain, which is not known to have spermatogenic abnormalities. Thus there is no evidence that this tubulin variation is related to the abnormalities of centriole position in the PL/J strain. We propose the gene symbol *Tbn* for this β-tubulin variant.

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References

Baier, L. J., Hanash, S. M. & Erickson, R. P. (1984). Mouse liver protein variants detected by two-dimensional electrophoresis. In *Electrophoresis '83* (ed. H. Hirai), pp. 189–194. Berlin: de Gruyter.

Beatty, R. A. (1970). The genetics of the mammalian gamete. *Biological Review* **45**, 73–119.

Boitani, C., Geremia, R., Rossi, R. & Monesi, V. (1980). Electrophoretic pattern of polypeptide synthesis in spermatocytes and spermatids of the mouse. *Cell Differentiation* **1**, 41–49

Burkhart, J. G. & Malling, H. V. (1981). Sperm abnormalities in the PL/J mouse strain: a description and proposed mechanism for malformation. *Gamete Research* **4**, 177–183.

DePhilip, R. M., Tres, L. L. & Kirzenbaum, A. L. (1982). Stage-specific protein synthesis during rat spermatogenesis. *Experimental Cell Research* **142**, 489–494.

Erickson, R. P. (1978). *t*-alleles and the possibility of post-meiotic gene expression during mammalian spermatogenesis. *Federation Proceedings* **37**, 2517–2521.

Erickson, R. P., Lewis, S. E. & Butley, M. (1981). Is haploid gene expression possible for sperm antigens? *Journal of Reproductive Immunology* **3**, 195–217.

Figueroa, F., Golubic, M., Nižetic, D. & Klein, J. (1985). Evolution of mouse major histocompatibility complex genes borne by *t*-chromosomes. *Proceedings of the National Academy of Sciences, USA* **82**, 2819–2823.

Fujimoto, M. & Erickson, R. P. (1982). Functional assays for mRNA detect many new messages after male meiosis in mice. *Biochemical and Biophysical Research Communications* **108**, 1369–1375.

Hunt, W. G. & Selander, R. K. (1973). Biochemical genetics of hybridization in European house mice. *Heredity* **31**, 11–33.

Kemphues, K. J., Raff, R. A., Kaufman, T. C. & Raff, E. C. (1979). Mutation in a structural gene for a β-tubulin specific to testis in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **76**, 3991–3995.

Kemphues, K. J., Raff, E. C., Raff, R. A. & Kaufman, T. C. (1980). Mutation in a testis-specific β-tubulin in *Drosophila*: analysis of its effects on meiosis and map location of the gene. *Cell* **21**, 445–451.

Kemphues, K. J., Kaufman, T. C., Raff, R. A. & Raff, E. C. (1982). The testis-specific β-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. *Cell* **31**, 655–670.

Klose, J. & Feller, M. (1981). Genetic variability of proteins from plasma membranes and cytosols of mouse organs. *Biochemical Genetics* **19**, 859–870.

Kramer, J. M. & Erickson, R. P. (1982). Analysis of stage-specific protein synthesis during spermatogenesis of the mouse by two-dimensional gel electrophoresis. *Journal of Reproductive Fertility* **64**, 139–144.

Lyon, M. J. (1984). Transmission ratio distortion in mouse *t*-haplotypes is due to multiple distorter genes acting on a responder locus. *Cell* **37**, 621–628.

Lyon, M. F. (1986). Male sterility of the mouse *t*-complex is due to homozygosity of the distorter genes. *Cell* **44**, 357–363.

Merril, C. R., Goldman, D., Sedman, S. A. & Ebert, M. H. (1981). Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* **211**, 1437–1438.

Narayan, P., Scott, B. K., Millette, C. F. & DeWolf, W. C. (1983). Human spermatogenic cell marker proteins detected by two-dimensional electrophoresis. *Gamete Research* **7**, 227–239.

Neel, J. V., Rosenblum, B. B., Sing, C. F., Skolnick, M. M., Hanash, S. M. & Sternberg, S. (1984). Adapting two-dimensional gel electrophoresis to the study of human germ-line mutation rates. In *Methods and Applications of Two-dimensional Gel Electrophoresis of Proteins* (ed. J. E. Celis & R. Bravo, pp. 259–306. New York: Academic Press.

Neel, J. V., Baier, L., Hanash, S., Erickson, R. P. (1985). Frequency of polymorphisms for alleles encoding for liver proteins of domesticated mice. *Journal of Heredity* **76**, 314–320.

Selander, R. K., Hunt, W. G. & Yong, S. Y. (1969). Protein polymorphism and genetic heterogeneity in two European subspecies of the house mouse. *Evolution* **23**, 379–390.

Silver, L. M., Uman, J., Danska, J. & Garrels, J. I. (1983). A diversified set of testicular cell proteins specified by genes within the mouse *t*-complex. *Cell* **35**, 35–45.

Stern, L., Gold, B. & Hecht, N. B. (1983). Gene expression during mammalian spermatogenesis. I. Evidence for stage-specific synthesis of polypeptides *in vivo*. *Biology of Reproduction* **28**, 483–496.

Villasante, A., Wang, D., Dobner, P., Dolph, P., Lewis, S. A. & Cowan, N. J. (1986). Six mouse α-tubulin in RNAs encode five distinct isotypes: testis-specific expression of two sister genes. *Molecular and Cellular Biology* **6**, 2409–2419.

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