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An updated two-dimensional gel database of rat liver proteins useful in gene regulation and drug effect studies

We have improved upon the reference two-dimensional (2-D) electrophoretic map of rat liver proteins originally published in 1991 (N. L. Anderson *et al.*, *Electrophoresis* 1991, *12*, 907–930). A total of 53 proteins (102 spots) are now identified, many by microsequencing. In most cases, spots cut from wet, Coomassie Blue stained 2-D gels were submitted to internal tryptic digestion [2], and individual peptides, separated by high-performance liquid chromatography (HPLC), were sequenced using a Perkin-Elmer 477A sequenator. Additional spots were identified using specific antibodies.

Figure 1 shows the current annotated 2-D map of F344 rat liver, analyzed using the Iso-DALT system (20 × 25 cm gels) and BDH 4–8 carrier ampholytes. Both the map itself and the master spot number system remain the same as shown in the original publication. Table 1 lists the important features of each identification shown, including the gel position, pI, and M_r for the most abundant or most basic form of each protein. Using this extended base of identified spots, a series of four improved calibration functions has been derived for the pI and SDS-M_r axes (the first two of which are shown in Fig. 2A and B). Both forward and reverse functions are derived, so that one can compute the physical properties of a spot with a given gel location, or inversely compute the gel position expected for a protein having given physical properties:

$$Y_{\text{RATLIVER}} = f_{M_r\text{–RATLIVER Y}}(M_{r\text{SEQUENCE-DERIVED}}) \quad (1)$$

$$X_{\text{RATLIVER}} = f_{\text{pI–RATLIVER X}}(pI_{\text{SEQUENCE-DERIVED}}) \quad (2)$$

$$M_{r\text{GEL-DERIVED}} = f_{\text{RATLIVER Y–M}_r}(Y_{\text{RATLIVER}}) \quad (3)$$

$$pI_{\text{GEL-DERIVED}} = f_{\text{RATLIVER X–pI}}(X_{\text{RATLIVER}}) \quad (4)$$

A spreadsheet program (in Microsoft Excel) was developed to facilitate flexible computation of pIs from amino acid sequence data, and the results were entered into a relational database (Microsoft Access). A table of spot positions and sequence-derived pI's and M_r's was fitted with a large series of analytic equations using Tablecurve (Jandel Scientific), and the four conversion Eqs. (1)–(4), relating computed pI and gel X coordinate, or computed molecular weight and gel Y coordinate, were selected, based on criteria of simplicity, goodness of fit and favorable asymptotic behavior. Table 2 lists the equations and coefficients. Application of Eqs. (3) and (4) to a spot's X and Y coordinates, given in [1], produce improved M_r estimates, and allow computation of pI directly in pH units, instead of in terms of positions relative to creatine phosphokinase (CPK) charge standards. The inverse Eqs. (1) and (2) were used to compute the gel positions of a series of pI and M_r tick marks. These tick marks were plotted with SigmaPlot (Jandel), together with fiducial marks locating several prominent spots, and the resulting graphic was aligned over the synthetic gel image (computed by Kepler from the master gel pattern) using Freelance (Lotus Development). Maps were printed as Postscript output from Freelance, either in black and white (as shown here) or in color, where label color indicates subcellular location (available from the first author upon request). We have also used the rat liver 2-D pattern as presented here to calibrate the patterns of other samples. Using mixtures of rat liver and mouse liver samples, for example, we made composite 2-D patterns that allow use of the rat pattern to standardize both axes of the mouse pattern. This was accomplished by deriving transformations relating the rat and mouse X, and separately the rat and mouse Y, axes (Table 2, lower half; Fig. 2C and D) based on a series of spots that coelectrophorese in these closely related species. These functions were then applied to derive equations relating the mouse liver X and Y to pI and SDS-M_r (Eqs. 5 and 6 below). The resulting standardized 2-D pattern for B6C3F1 mouse liver is shown in Fig. 3.

$$M_{r\text{MOUSELIVER}} = f_{\text{RATLIVER Y–M}_r}(f_{\text{MOUSELIVER Y–RATLIVER Y}}(Y_{\text{MOUSELIVER}})) \quad (5)$$

$$pI_{\text{MOUSELIVER}} = f_{\text{RATLIVER X–pI}}(f_{\text{MOUSELIVER X–RATLIVER X}}(X_{\text{MOUSE LIVER}})) \quad (6)$$

A slightly more complex approach can be used to standardize samples that have few or no spots co-electrophoresing with rat liver proteins. In this case, a 2-D gel is prepared with a mixture of the two samples, and four functions (forward and backward, each for X and Y) are derived relating each sample's own master pattern to the composite. The required functions are then applied in a nested fashion to yield the desired result (using rat plasma as an example):

$$M_{r\text{RATPLASMA}} = f_{\text{RATLIVER Y–M}_r}(f_{\text{RATPLASMA+LIVER Y–RATLIVER Y}}(f_{\text{RATPLASMA Y–RATPLASMA+LIVER Y}}(Y_{\text{RATPLASMA}}))) \quad (7)$$

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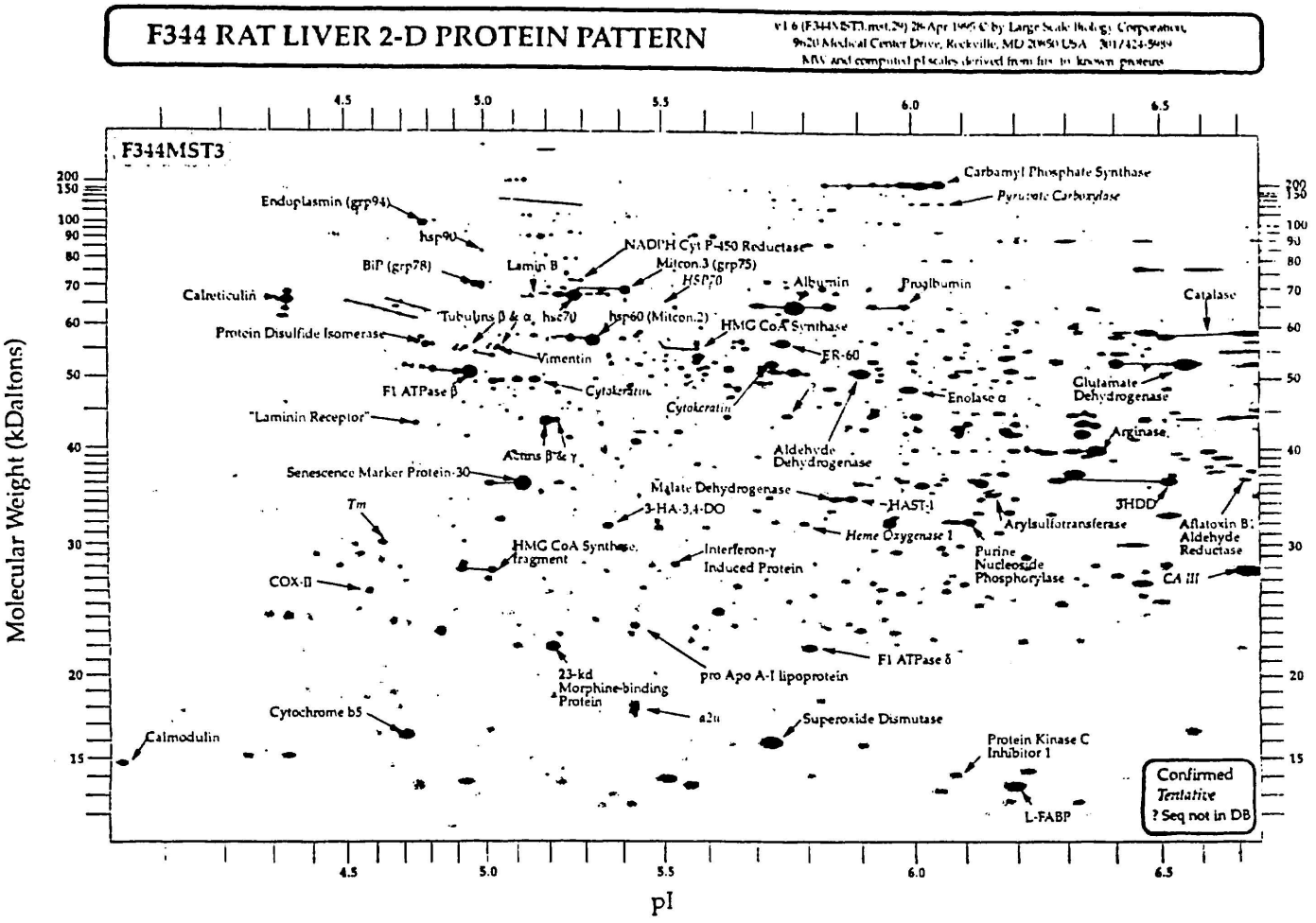

Figure 1. Master 2-D gel pattern of Fischer 344 rat liver proteins, annotated with 53 protein identifications and computed p*I* and M_r axes. Tentative identifications are in italic type.

Table 1. Proteins identified in the 2-D pattern of F344 rat liver

MSN[a]	Protein ID[b]	Protein name	Identification comments	Gel X[c]	Experimental pI[d]	Gel Y[c]	Experimental M_r[d]
126	HADO-HUMAN[e]	3-HA-3,4-DO: 3-hydroxy-anthranilate-3,4-dioxy-genase	Internal sequence	871.95	5.36	921.35	30 207
137, 159, 288, 258	DIDH_RAT	3HDD: 3-hydroxysteroid dihydrodiol reductase	Ab (T.M. Penning) and pure protein	1857.52	6.51	822.52	34 406
173	MUP_RAT	a_{2u} globulin	Presence in liver microsome lumen, abundance in kidney, pI, M_r	919.16	5.43	1313.81	19 549
38	ACTB_HUMAN	Actin β	Analogy with other mammalian patterns (*e.g.* human) through coelectrophoresis	763.40	5.19	693.64	41 586
68	ACTG_HUMAN	Actin γ	Analogy with other mammalian patterns (*e.g.* human) through coelectrophoresis	779.42	5.21	692.26	41 677
693	AFAR_RAT	Aflatoxin B1 aldehyde reductase	Internal sequence	1993.32	6.72	818.60	34 593
28, 21, 33	ALBU_RAT	Albumin	Coelectrophoresis with principal plasma protein	1262.81	5.86	445.64	66 354
43	DHAM_RAT	Aldehyde dehydrogenase	*N*-Terminal sequence and AAA	1317.72	5.91	589.03	49 602
96	ARGI_RAT	Arginase	Internal sequence	1730.72	6.34	756.02	37 819
117	SUAR_RAT	Arylsulfotransferase	Internal sequence	1547.96	6.14	849.08	33 186
1163, 1161, 1162, 20	GR78_RAT	BIP (GRP-78)	Ab (F. Witzmann)	665.33	5.01	397.39	74 564
185	CAH3_RAT	CA-III	Uncertain; by comparison with mouse	1996.60	6.72	1017.02	26 887
123	CALM_HUMAN	Calmodulin	Analogy with human cellular patterns through coelectrophoresis	23.05	4.03	1433.25	17 419
3, 201, 48, 39, 22, 24	CRTC_RAT	Calreticulin	Ab (Lance Pohl)	310.59	4.34	433.80	68 206

Table 1. continued

MSN[a]	Protein IDb)	Protein name	Identification comments	Gel X[c]	Experimental pI[d]	Gel Y[c]	Experimental M_r[d]
1184, 1186, 114, 174, 1185, 167, 157	CPSM_RAT	Carbamyl phosphate synthase	2-D of pure protein; comfirmed by *N*-terminal sequence and AAA	1453.56	6.05	181.64	160 640
54, 61	CATA_RAT	Catalase	Internal sequence	2000.81	6.73	499.64	58 968
136	COX2_RAT	COX-II	Ab (J. W. Taanman), confirmed by internal sequence	452.57	4.61	1062.67	25 504
87	CYB5_RAT	Cytochrome B5	2-D of pure protein; Ab; confirmed by AAA	515.68	4.73	1370.55	18 493
41	CK-RAT[e]	Cytokeratin	Location in cytoskeletal fraction	1165.12	5.75	569.09	51 448
29	CK-RAT[e]	Cytokeratin	Location in cytoskeletal fraction	743.11	5.15	605.23	48 187
5, 11	ENPL-RAT[e]	Endoplasmin	Ab (F. Witzmann)	567.73	4.83	263.37	112 194
60	ENOA_RAT	Enolase A	Internal sequence and AAA	1399.78	6.00	623.54	46 674
27	ER60_RAT	ER-60	*N*-Terminal sequence (R. M. Van Frank)	1184.20	5.77	523.51	56.169
17	ATPB_RAT	F1 ATPase β	*N*-Terminal sequence and AAA	629.06	4.95	588.83	49 620
196	ATP7_RAT	F1 ATPase δ	Internal sequence	1227.24	5.82	1184.65	22 310
79	F16P_RAT	Fructose-1,6-bis-phosphatase	Uncertain; by comparison with ID in Garrison and Wager (JBC 257:13135-13143)	924.54	5.44	737.77	38 858
62, 78	DHE3_RAT	Glutamate dehydrogenase	*N*-Terminal sequence and internal sequence	1887.39	6.55	566.92	51 655
125	HAST-RAT[e]	HAST-I: N-hydroxyaryl-amine sulfotransferase	Internal sequence	1297.94	5.89	861.55	32 638
307	HO1_RAT	Heme oxygenase 1	Uncertain; available data from internal sequence	1219.39	5.81	915.71	30 423
413, 1250, 933	HMCS_RAT	HMG CoA synthase, cytosolic	Ab (J. Germershausen)	1033.48	5.59	538.13	54 571
133, 144, 235	HMCS_RAT	HMG CoA synthase, mitochondrial (frag)	Ab (J. Germershausen), *N*-terminal sequence (Steiner/Lottspeich)	666.40	5.02	1019.42	26 811
8, 23, 1307	HS7C_RAT	HSC-70	Positional homology (with human, *etc.*) through coelectrophoresis	811.87	5.27	425.76	69 521
15, 25, 110	P60_RAT	HSP-60	Ab (F. Witzman); confirmed by *N*-terminal sequence and AAA	845.09	5.32	520.03	56 561
971	HS70-RAT[e]	HSP-70	Ab (F. Witzman)	976.11	5.51	437.14	67 674
1216, 1215, 90	HS90-RAT[e]	HSP-90	Ab (F. Witzman)	659.86	5.00	329	90 107
256	INGI-HUMAN	Interferon-γ induced protein	Internal sequence	993.85	5.54	1006.04	27 237
415, 734	LAMB-RAT[e]	Lamin B	Positional homology with human through coelectrophoresis, nuclear location	737.10	5.14	425.19	69 615
80	LAMR-RAT[e]	"Laminin receptor"	Internal sequence	534.02	4.77	697.62	41 327
227	FABL_RAT	L-FABP (liver fatty acid binding protein)	Ab (N. M. Bass)	1586.09	6.18	1483.43	16 622
134	MDHC_MOUSE	Malate dehydrogenase	Internal sequence	1270.85	5.86	861.96	32 620
18, 35, 226	GR75-RAT[e]	Mitcon:3; grp75	Positional homology with human through coelectrophoresis	905.67	5.41	413.67	71 589
175, 251	NCPR_RAT	NADPH P450 reductase	2-D of pure protein	824.69	5.29	393.21	75 366
1168, 1170, 1171	PDI_RAT	PDI: Protein disulfide isomerase	*N*-Terminal sequence (R. M. van Frank), Ab	564.30	4.83	528.47	55 618
47, 93	ALBU_RAT	Pro-Albumin	Microsomal lumen location, p*I*, M_r relative to albumin	1391.03	5.99	446.68	66 195
236	APA1_RAT	Pro-APO A-I lipoprotein	Coelectrophoresis with plasma protein	920.41	5.43	1137.51	23 467
320	IPK1_BOVIN	Protein kinase C inhibitor 1	Internal sequence; homology with bovine protein	1480.01	6.08	1458.81	17 007
152	PNPH_MOUSE	Purine nucleoside phosphorylase	Internal sequence	1507.19	6.10	911.16	30 599
1179, 1180, 1181, 1182, 1183	PYVC-RAT[e]	Pyruvate carboxylase	Tentative; 2-D of pure protein (J. G. Henslee, *JBC*, 1979); reported in *Biochim. Biophys. Acta 1022*, 115–125	1485.10	6.08	223.52	131 589
55, 103	SM30_RAT	SMP-30: Senescence marker protein-30	Internal sequence	721.71	5.11	830.10	34 051
135	SODC_RAT	Superoxide dismutase	AAA; comfirmed by internal sequence (R. M. Van Frank)	1161.24	5.74	1388.68	18 173
172	TPM-RAT[e]	Tm: tropomyosin	Location in cytoskeleton, 2-D position relative to human, Ab	476.24	4.66	957.86	28 865
277, 56	TBA1_RAT	Tubulin α	Positional homology with human through coelectrophoresis, cytoskeletal location	688.22	5.06	537.67	54 620
50, 1225	TBB1_RAT	Tubulin β	Positional homology with human through coelectrophoresis, cytoskeletal location	621.29	4.93	535.48	54 855
1224	VIME_RAT	Vimentin	Positonal homology with human through coelectrophoresis, cytoskeletal location	673.00	5.03	539.50	54 426

Table 1. continued

MSN[a]	Protein IDb)	Protein name	Identification comments	Gel X[c]	Experimental pI[d]	Gel Y[c]	Experimental M_r[d]
113	Unknown	?: not in sequence databases	Internal sequence	1191.28	5.78	680.42	42 469
104	BBPL_RAT	23 kDa morphine-binding protein	Internal sequence	773.31	5.20	1182.41	22 363

a) Master spot number (MSN) from [1]
b) SwissPROT identifier
c) Coordinates of the most basic or most abundant assigned spot on the F344 master gel pattern
d) pI and M_r of the most basic or most abundant assigned spot, derived from the calibration functions included here
e) SwissPROT style proposed identifier
Abbreviations: AAA, amino acid analysis; Ab, antibody

Table 2. Equations and coefficients

Function	Equation (f)	r2	a	b	c	d	e
Rat gel Y = f(computed M_r)	$y = a + b\exp(-x/c)$	0.988181021	178.74803	1967.7892	32363.958		
Rat gel X = f(computed pI)	$y = a + bx + cx/\ln x + d/x + e/x^{1.5}$	0.99247216	−8685665.5	−904497.94	3856926.1	18276844	−27154534
Computed M_r = f(rat gel Y)	$y = a + bxc$	0.9960177	−8464.5809	19095881	−0.9086255		
Computed pI = f(rat gel X)	$y = a + bx + cx^2 + dx^2 \ln x + ex^3$	0.99176499	4.044686	−0.00114238	0.0000323	−0.00000455	0.00000000176
Mouse gel Y = f(rat gel Y)	$y = a + bx + cx^{1.5} + dx^{0.5} \ln x + ex/\ln x$	0.99951069	11861.44	678.91666	−0.78964914	1567.5639	−6953.9592
Mouse gel X = f(rat gel X)	$y = a + bx^2 \ln x + cx^{2.5} + dx^3$	0.99926349	58.935923	0.00091353	−0.000213688	0.00000159	
Rat gel Y = f(mouse gel Y)	$y = a + bx^2 \ln x + cx^{2.5} + dx^3$	0.99950032	69.740526	0.00050772	−0.000130392	0.00000116	
Rat gel X = f(mouse gel X)	$y = a + bx + cx^2 \ln x + dx^{2.5} + ex^3$	0.9992832	−198.07189	2.0899063	−0.000671191	0.000145189	−0.000000986

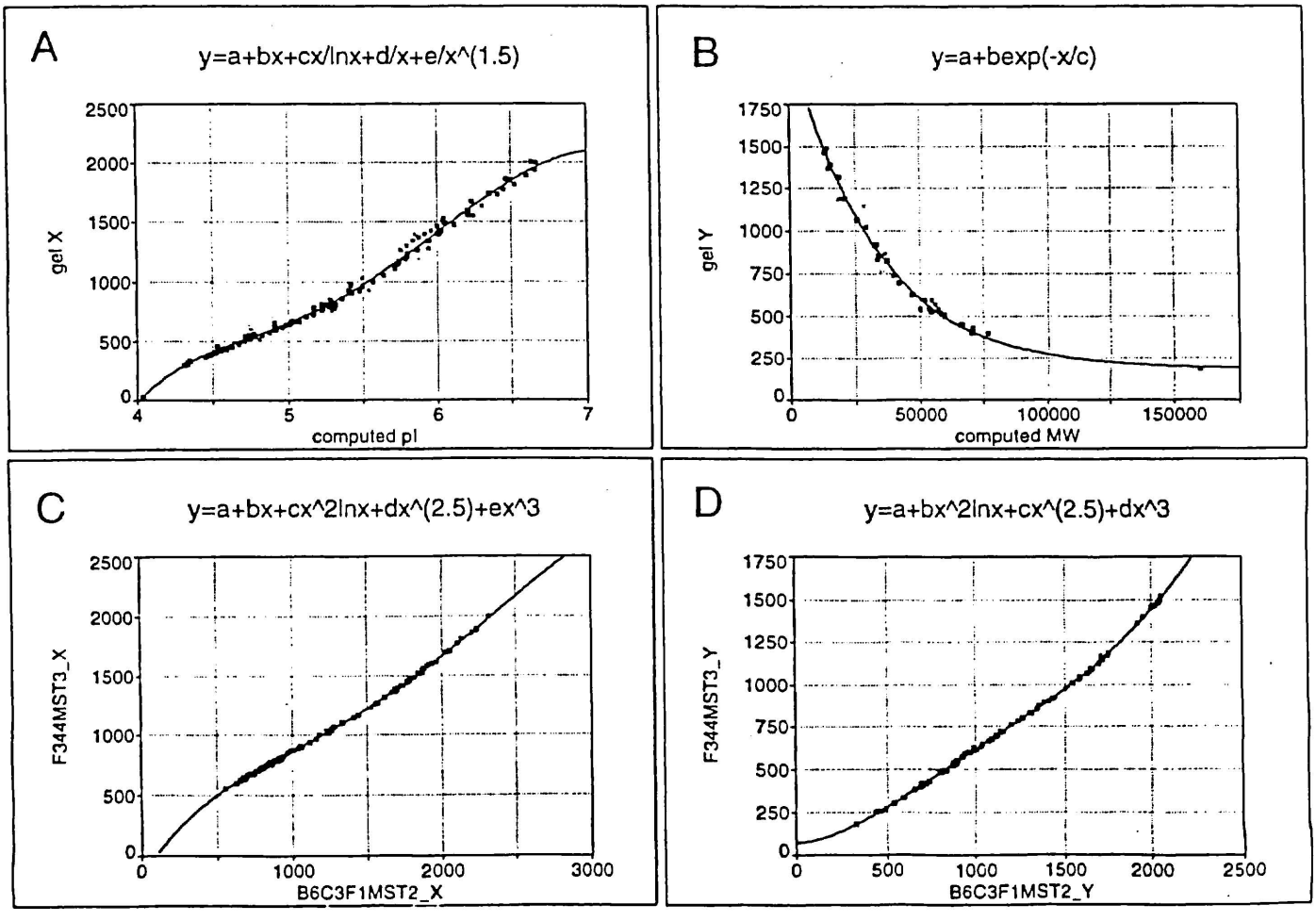

Figure 2. Plots showing fits of selected equations (continuous curves) to data on identified proteins (square symbols). (A) pI computed from sequence data *versus* gel X position for identified spots in F344 rat liver; (B) M_r computed from sequence data *versus* gel Y position for identified spots in F344 rat liver; (C) gel X position for spots in B6C3F1 mouse liver *versus* X position in F3443 rat liver, for coelectrophoresing spots; (D) gel Y position for spots in B6C3F1 mouse liver *versus* Y position in F3443 rat liver, for coelectrophoresing spots. In each case, inverse equations were also computed (Table 2).

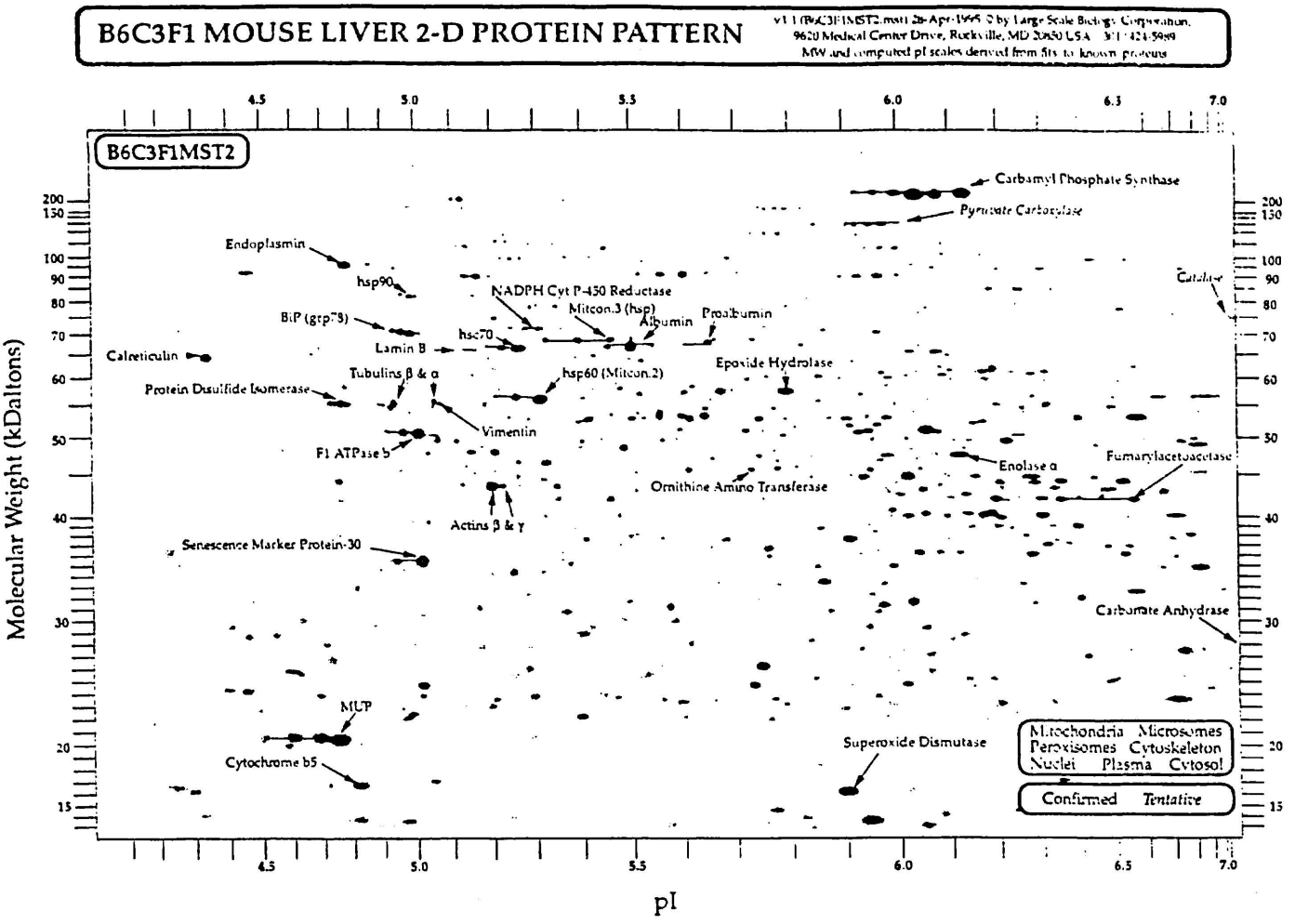

Figure 3. Master 2-D gel pattern for B6C3F1 mouse liver, standardized using the F344 rat liver pattern identifications, according to the method described in the text. Twenty-nine proteins are identified.

$$pI_{RAT\,PLASMA} = f_{RAT\,LIVER\ X\text{-}pI}\left(f_{RAT\,PLASMA\text{-}LIVER\ X\text{-}RAT\,LIVER\ X}\left(f_{RAT\,PLASMA\ X\text{-}RAT\,PLASMA+LIVER\ X}\left(X_{RAT\,PLASMA}\right)\right)\right) \quad (8)$$

This unified approach, in which one well-populated 2-D pattern is used to standardize a family of other patterns, has the additional advantage that the resulting p*I* and M_r scales are directly compatible. Hence one can compare the relative p*I*'s of mouse and rat versions of a sequenced protein in a consistent p*I* measurement system, and select likely inter-species analogs based on positional relationships on common scales. Adoption of immobilized pH gradient (IPG) technology [4–7] will result in substantial improvements in p*I* positional reproducibility for standard 2-D maps such as those presented here; however, we believe that our approach will continue to be useful in establishing the empirical pH gradient actually achieved by such gels under given experimental conditions (temperature, urea concentration, *etc.*), in relating patterns run on different IPG ranges and using different lots of IPG gels (between which some variation will persist). Development of rodent organ maps is a continuing effort in our laboratories [8–10], and results in regular additions of identified proteins. Those who wish to receive current rodent liver maps, with color annotations, should send a stamped self-addressed envelope to the first author.

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