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Characterization of The *Drosophila melanogaster* Ribosomal Proteome

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Abstract: We have combined high-resolution two-dimensional (2-D) gel electrophoresis with mass spectrometry to identifying proteins represented in a 2-D gel database of *Drosophila melanogaster* ribosomes. First, we purified ribosomes from third instar *Drosophila* larvae and constructed a high-resolution 2-D gel database containing 58 Coomassie blue stained polypeptides. Next, we carried out preparative 2-D PAGE to isolate some of the polypeptides and characterize them by MALDI-TOF. Using this strategy we identified 52 ribosomal spots in the database, and in each case confirmed their identity by MALDI-TOF/TOF. The database can be used to analyze *Minute* mutants of *Drosophila*.

Keywords: *Drosophila* • ribosome • proteomics • 2D gels • MALDI-TOF

1. Introduction

Over the last 15 years, the process of cataloging the proteins of single-celled microorganisms, cells, biological fluids, tissues, and whole organisms has progressed at a rapid pace in parallel with advances in protein and peptide separation, detection, and identification. This progress is evident from the numerous databases that are available (see for example the directory of 2D databases on the ExPASy server at http://us.expasy.org/ch2d/). There has also been constant innovation in 2D gel electrophoresis technology and in methods of identification, and the availability of complete genome sequences.

These techniques, however, are not free of limitations, at least for complex eukaryotes where the number of proteins in a single cell is estimated to approach 10 000.[1] This means that proteome analysis on whole cell extracts tends to miss low-abundance proteins. One solution to this problem is to take advantage of the compartmentalization of the eukaryotic cell, and to analyze subcellular organelles. These can be isolated in good yield and their protein complexity is lower than that of whole cells. Classical cell subfractionation yields four major fractions: nucleus, mitochondria, microsomes and cytosol.

Drosophila is a well-established model organism in cellular and developmental biology, which we have studied by proteomic techniques for several years. For the reasons adduced above, as well as the need for markers of cellular organelles, we have initiated a project to analyze *Drosophila* subproteomes. On this ocassion, we have focused on the ribosome. *Drosophila* ribosomes, and their subunits, are typical of those of eukaryotes. They consists of two subunits, one large (60S) and one small (40S), each containing only protein and RNA. Two sites on the ribosomal large subunit are involved in translation, namely the aminoacyl site (A site) and the peptidyl site (*P* site). In addition to synthesizing proteins, ribosomes are responsible for directing polypeptides to their correct cellular location. Some of the *Drosophila* ribosomal proteins have been biochemically and genetically characterized.[2−4]

Aside from their merely descriptive interest, the building and analysis of the *Drosophila* ribosomal proteome has importance, for two main reasons. First, mutations affecting ribosomal protein genes have a characteristic phenotype long familiar to *Drosophila* biologist as *Minutes*.[5] *Minutes* are a common class of haplo-insuficient mutations of *Drosophila* characterized by developmental delay and short, usually thin, bristles. Body size is also reduced in some but not all *Minutes*. There is molecular evidence that some (perhaps all) *Minute* loci are genes coding for ribosomal proteins. Second, the number of *Drosophila* ribosomal proteins known to be involved in other processes than protein synthesis is increasing. For example, *Drosophila* ribosomal protein S3 cleaves DNA containing 8-oxoguanine residues and also contains an associated apurinic/apyrimidinic endonuclease activitie.[6] Similarly, mutants in *Drosophila* ribosomal protein S6 display larval melanotic tumors characteristic of mutations affecting the insect cellular immune system, and the affected animals develop grossly hypertrophied hematopoietic organs.[7] These results, and others, suggest that ribosomal proteins are involved in regulatory processes that may be important in normal development.

We have compiled a catalog of the ribosomal proteome using highly purified ribosomes from *Drosophila* third instar larvae. This provides a basis for identifying proteins localized to the ribosome and can be used as a reference to examine the effects of specific mutations on the ribosomal proteome.

2. Materials and Methods

2.1. Fly Strains and Cell Culture. Laboratory stocks of wild-type *D. melanogaster* [strain Vallecas (Spain)] were used. The *Minute* mutant used, y[1]w[*]; P[w[+mC] = lacW]RpL14[1]/TM2 was obtained from FlyBase (Bloomington stock center).

2.2. Purification of Ribosomes. We adapted a method for isolating yeast ribosomes.[8] In summary, *Drosophila* third instar larvae were homogenized in Buffer 1 (80 mM ClK, 10 mM Tris pH 7.4, 12.5 mM Cl_2Mg, and 5 mM β-mercaptoethanol) by four strokes with a Potter-Elvehjem homogeneizer. The homogenate was filtered through sterile gauze and centrifuged at 10 000 × *g* for 15 min. The supernatant was decanted and centrifuged at 166 000 × *g* for 120 min. The microsomal pellet was then resuspended in Buffer 2 (20 mM Tris pH 7.4, 100 mM Cl_2Mg,

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Table 1. Identification by MALDI-TOF and MALDI-TOF/TOF−MS of Acidic Polypeptides (lef) Recorded in the *Drosophila* Ribosome Database

SSP no.	KDa/p*I*	*N*/%[a]	sequence determined by MS/MS	protein identified	fly base ID FBgn
3433	34.2/6.48	10/41.0	1222.63 VVELFDEFPK 1278.64 GHLENNPQLEK 1339.72 TSFFQALSIPTK 1519.83 NLLAIAATTEVEFK 1822.03 GTIEIINDVPILKPGDK 2588.44 APARPGAIAPLHVIIPAQNTGLGPEK	**ACIDIC RIBOSOMAL PROTEIN LP0**	0000100
6250	9.3/5.32	3/40	1937.95 DHASVQLSIVDVDPETGR	**Rp S21**	0015521
6520	30.2/4.76	15/51	1094.54 YTDTTPIAGR 1656.86 SAHSIGLMWWLLAR 1694.85 FTPGAFTNQIQPAFR 1755.89 SVEWPVVVDLFFYR 2734.34 MLVATTHLGSENVNFQMEQYVYK	**Rp 40**	0003517
7306	11.7/4.65	9/64	1373.72 ILSSVGVEVDAER 1640.91 MRYVAAYLLAVLGGK 2384.20 LSSMPVGGGGAVAAADAAPAAAAGGDKK	**ACIDIC RIBOSOMAL PROTEIN LP2**	0003274
8348	11.5/4.33	2/42	1790.91 AANVEVEPYWPGLFAK 2718.44 DLITNIGSGVGAAPAGGAAPAAAAAAPAAESK	**ACIDIC RIBOSOMAL PROTEIN LP1**	0002593

[a] *N* = Number of matched peptides. % = Coverage of full length protein by tryptic peptides.

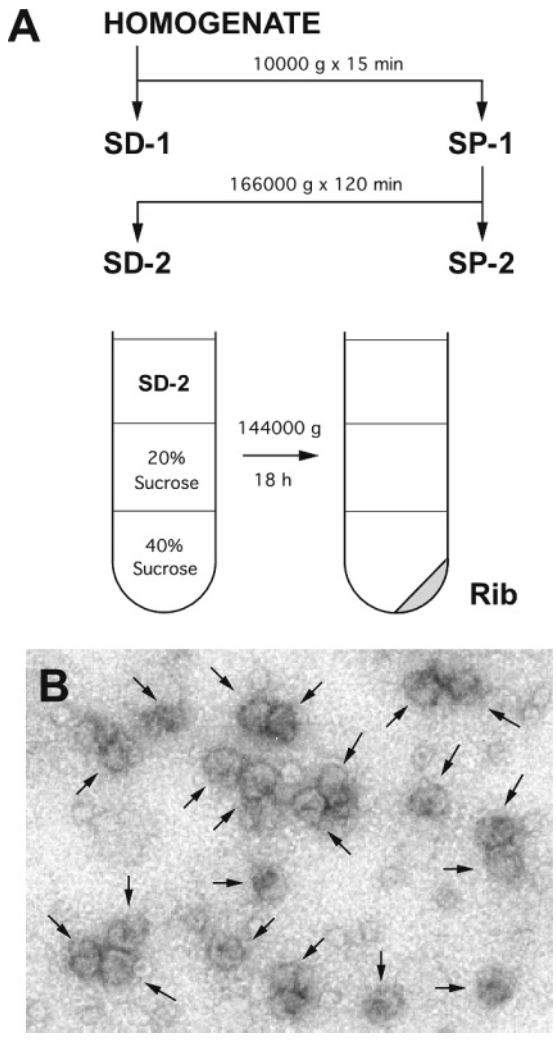

Figure 1. (A) Schematic of the hybrid Percoll/metrizamide discontinuous density gradient used for the isolation of ribosomes from *Drosophila melanogaster* third instar larvae. (B) Morphology of the purified ribosomes. The preparation was deposited on a grid (Niquel, 200 mesh) coated with a specimen support film of carbon-colodion. After 3 min the ribosomes were stained with 2% uranyl acetate in water for 45 s.

500 mM ammonium acetate and 5 mM β-mercaptoethanol) and centrifuged over two layers of 20% and 40% sucrose at

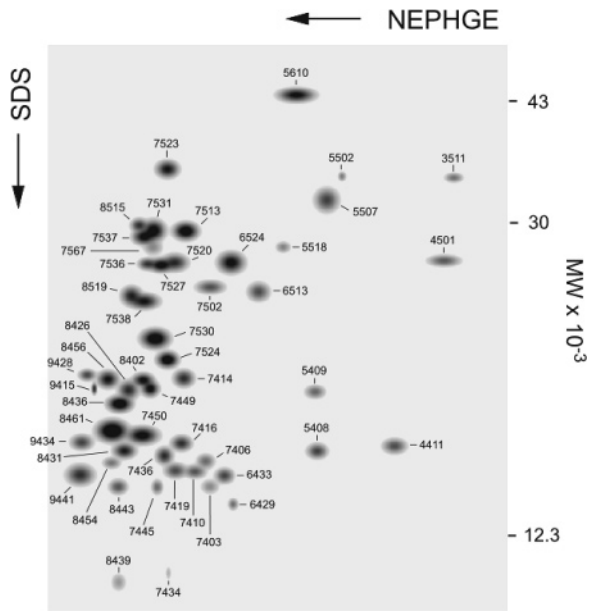

Figure 2. Two-dimensional gel of basic ribosomal polypeptides (NEPHGE). A total of 100 μg was applied per gel and 51 polypeptides were separated and recorded in the database. All the SSP numbers catalogued are indicated in the figure. The pH ranges from 9.0 (left) to 7.5 (right).

144 000 × *g* for 18 h to give a translucent pellet of purified ribosomes. The structural integrity of the ribosomes was assessed by electron microscopy as follows: the pellet was resuspended in Buffer 1 and a small drop deposited on a grid (Niquel, 200 mesh) coated with a specimen support film of carbon-colodion. After 3 min, the ribosomes were stained with 2% uranyl acetate in water for 45 s. The sample was air-dried and observed with a JEOL-1010 microscope operating at 80 kV.

2.3. Two-Dimensional Gel Electrophoresis. Two-dimensional gel electrophoresis was performed as described by O'Farrell[9,10] with some modifications.[11] Briefly, the first dimension for resolving acidic proteins (IEF) was performed on 230 × 2.3 mm 4% (w/v) polyacrylamide gels containing 2% ampholytes (1.6% pH 5−7; 0.4%, pH 3.5−10) at 1200 V for 20 h. Basic proteins (NEPHGE) were resolved on 150 × 2.3 mm 4% (w/v) polyacrylamide gels containing 2% ampholytes (1% pH 7−9; 1% pH 8−9.5) at 400 V for 4.5 h. The second dimension employed a 15% polyacrylamide gel(24 × 24 cm) run overnight

Table 2. Identification by MALDI-TOF and MALDI-TOF/TOF−MS of Basic Polypeptides (Nephge) Recorded in the *Drosophila* Ribosome Database

SSP no.	KDa/p*I*	*N*/%	sequence determined by MS/MS	protein identified	fly base ID FBgn
3511	34.2/6.48	13/48	1222.63 VVELFDEFPK 1519.83 NLLAIAATTEVEFK 1822.02 GTIEIINDVPILKPGDK 2588.45 APARPGAIAPLHVIIPAQNTGLGPEK	**ACIDIC RIBOSOMAL PROTEIN LP0**	0000100
4501	25.4/8.89	10/26	900.47 YLPHSAGR 1140.65 RQAVDVSPLR	**Rp S5a**	0002590
5408	17.8/9.79	9/55	1625.89 HPELESIPNLHVIK 1693.94 SYLHLPPEIVPSTLK 1985.92 HYYWYLTNEGIEELR	**Rp S10 b**	0031035
5409	17.5/9.21	2/20	1612.83 HSGNIGFEDILAIAR 2099.99 HPHDVIDELNEGSIEVPAE	**Rp L12**	0034968
5502	34.0/9.79	20/40	788.35 TDYYAR 945.45 SFNADVHR 1155.57 RFPGYSAETK 1560.73 AHIFGQHVADYMR	**Rp L5**	0064225
5507	27.4/9.43	17/60	870.42 FNFETGR 1092.59 AELNEFLTR 1272.62 IYETPETEYK 1606.90 IPPPSKPLDDLSEAK	**Rp S3**	0002622
5610	44.9/11.48	27/59	1093.56 LNDLFGTWK 1326.67 FVIWTESAFAR 1460.76 GPLVVYDKDEGLR 2306.08 QAYAVSELAGHQTSAESWGTGR	**Rp L4**	0003279
6429	14.6/9.84	8/56	871.54 IVVNLTGR 1100.58 WTNNLLPSR 1189.60 FDVPINDIEK 1686.77 HGYIGEFEIVDDHR	**Rp S15 Aa**	0010198
6433	15.1/9.94	10/58	1027.56 LLDFHNIR 1233.63 IAGYVTHLMGR 1559.78 GLQLTQPNTNNFGR 2405.19 DNYVPAVSALEQDIIEVDADTK	**Rp S17**	0005533
6513	21.4/9.71	10/46	1046.53 GVTFGFQYK 1170.60 FLDGLYVSEK 1298.69 KFLDGLYVSEK 1521.72 HLALDMYMPDKR	**Rp L9**	0015756
6524	28.8/10.16	14/57	1370.68 TYAYLTPDLWK 1567.84 SLEEIYLYSLPIK 2501.21 EMPLGSTPYQAYSDFLSKPTPR	**Rp S2**	0004867
7403	12.4/9.52	4/28	1267.77 LVLIASNTPALR 1333.63 SEIEYYAMLAK 1460.72 KSEIEYYAMLAK	**Rp L30**	0015745
7410	16.2/10.35	6/44	1054.58 TPGPGAQSALR 1381.77 EEVQVQLGPQVR 1429.74 IEDVTPIPSDSTR 1736.98 VQKEEVQVQLGPQVR	**Rp S14a**	0004403
7414	21.1/10.04	7/38	988.50 SGTVGFQHR 1036.56 YDGIILNTK 1573.86 VLEQLTGQQPVFSK 2410.15 ENFSSTGNFGFGIQEHIDLGIK	**Rp L11**	0013325
7416	17.1/10.12	12/54	760.43 LSSIGQR 932.55 IANQIVFK 1008.56 ALQALEHAR 1670.78 ELAPYDPDWFYVR	**Rp S19a**	0010412
7419	14.5/10.20	3/25	970.55 RVHNIGFK 988.54 SAINEVVTR 1680.88 LYTYVTYVPVSTFK	**Rp L31**	0025286
7434	9.5/9.5	2/27	1261.67 DLLHPLPAEEK 1593.77 LVQHPNSYFMDVK	**Rp S27**	0039300
7436	16.9/10.10	14/67	1021.53 DILVQYDR 1229.66 EPVQAVQVFGR 1379.76 VNGRPLEQIEPK 1385.76 REPVQAVQVFGR 1427.77 VSGGGHVAQIYAIR	**Rp S16**	0034743
7449	18.4/10.55	11/50	817.46 GQWVSLK 1289.64 HGVIPLSTYMR 1638.90 IFNVTQHAVGVIVNK 1914.05 KLEEPIALAPIPYEFIA	**Rp L21**	0032987
7450	17.1/10.3 0	9/52	1091.58 GISQSALPYR 1971.02 SVGLKPDIPEDLYHMIK	**Rp S13**	0010265
7502	23.7/10.49	8/45	1331.72 NSIVVIDATPFR 1479.70 VEQALEDQFTSGR 1607.77 LETGNFAWASEGVAR 1676.85 IADVVYNASNNELVR	**Rp S8**	0039713
7513	29.1/10.19	18/58	756.37 TGEFFR 1117.53 DSQGHVFATR 1200.74 ESLPLLIFLR 2139.07 YPDPLIHANDSVQVDIASGK	**Rp S4**	0011284
7520	25.5/9.88	6/22	815.41 DQFHIR 1188.64 GAFGKPQGTVAR 1553.79 YRPEHGPIAAWEK	**Rp L10**	0024733
7523	32.2/10.10	1/3	1376.68 VNNLGNNVTFER	**Rp L22**	0015288

Table 2 (Continued)

SSP no.	KDa/p*I*	*N*/%	sequence determined by MS/MS	protein identified	fly base ID FBgn
7524	22.6/10.61	18/66	920.53 IIGEYGLR 1031.60 LFQGNALLR 1195.65 MKLDYVLGLK 1257.72 QVVNIPSFVVR 1488.77 ELLTLDEKDEKR	**Rp S9**	0010408
7527	28.2/10.86	16/47	755.49 VLQLFR 1001.52 LNTPTGGWR 1166.58 NQFYVPAEAK 1302.70 VPITDNFVIER 1420.63 ANHYVNGGDFGNR	**Rp L7**	0005593
7530	23.6/11.04	8/23	1393.70 VFDGIPSPYDKR 1710.85 LSHEVGWHYQDVIK	**Rp L13A**	0037351
7531	29.1/10.19	16/54	780.36 TNFNER 1606.85 VPPPIHQFSQTLDK 1848.03 LKVPPPIHQFSQTLDK	**Rp L7A**	0014026
7536	29.7/10.72	15/42	1360.65 VPEHLNDAYFR 1437.87 NLTPGTVLILLAGR 2007.94 YLQNMFALHSSQYPHR	**Rp L6**	0039857
7537	27.8/11.15	3/13	1003.53 AMVGIVAGGGR 1066.62 GAPLAVVHFR 1719.82 TSGNYATVIAHNQDTK	**Rp L8**	0024939
7538	24.8/10.98	11/38	1019.52 TWFNQPAR 1188.61 NEQPAVVEFR 1468.67 GNNMIPNQHYHK 1893.05 GPVLPIKNEQPAVVEFR	**Rp L13**	0011272
7567	28.4/10.75	3/14	1038.67 LITPVVLQR 1298.69 DIPGLTDTTIPR 1705.80 MGQVVEADILGDEWK	**Rp S6**	0004922
8402	21.0/10.62	11/39	905.50 NFGIWLR 1000.62 IKFPLVQR 1050.53 QVYETSPVK 1031.60 LFQGNALLR 1195.65 MKLDYVLGLK 1257.72 QVVNIPSFVVR 1488.77 ELLTLDEKDEKR	**Rp L18**	0035753
7527	28.2/10.86	16/47	755.49 VLQLFR 1001.52 LNTPTGGWR 1166.58 NQFYVPAEAK 1302.70 VPITDNFVIER 1420.63 ANHYVNGGDFGNR	**Rp L7**	0005593
7530	23.6/11.04	8/23	1393.70 VFDGIPSPYDKR 1710.85 LSHEVGWHYQDVIK	**Rp L13A**	0037351
7531	29.1/10.19	16/54	780.36 TNFNER 1606.85 VPPPIHQFSQTLDK 1848.03 LKVPPPIHQFSQTLDK	**Rp L7A**	0014026
7536	29.7/10.72	15/42	1360.65 VPEHLNDAYFR 1437.87 NLTPGTVLILLAGR 2007.94 YLQNMFALHSSQYPHR	**Rp L6**	0039857
7537	27.8/11.15	3/13	1003.53 AMVGIVAGGGR 1066.62 GAPLAVVHFR 1719.82 TSGNYATVIAHNQDTK	**Rp L8**	0024939
7538	24.8/10.98	11/38	1019.52 TWFNQPAR 1188.61 NEQPAVVEFR 1468.67 GNNMIPNQHYHK 1893.05 GPVLPIKNEQPAVVEFR	**Rp L13**	0011272
7567	28.4/10.75	3/14	1038.67 LITPVVLQR 1298.69 DIPGLTDTTIPR 1705.80 MGQVVEADILGDEWK	**Rp S6**	0004922
8402	21.0/10.62	11/39	905.50 NFGIWLR 1000.62 IKFPLVQR 1050.53 QVYETSPVK 1174.61 SRFWYFLR	**Rp L18**	0035753
8426	17.2/10.94	5/32	735.42 VVQAYR 973.50 DDEVQVIR 1021.50 QNPFVSSSR 1092.57 HFQAPSHIR 1699.83 ENANGTNVYVGIHPSK	**Rp L26**	0036825
8431	16.0/10.59	11/57	1056.51 ANPFGGASHAK 1077.57 GHAVGDIPGVR 1190.70 VANVSLLALYK 1205.66 KGHAVGDIPGVR 2197.05 DGSLNYIEENDEVLVAGFGR	**Rp S23**	0033912
8436	19.2/11.07	7/41	802.45 VSPWSVK 1133.69 LLTIAFNTLK 1222.71 VLVDGPLTGVPR 1354.76 LVAIVDVIDQNR	**Rp L14**	0017579
8439	8.2/10.29	2/28	1066.62 QSLPPGLQVK 1225.67 FLYTLVVQDK	**Rp L38**	0040007
8443	9.2/9.94	4/41	1090.58 LNNQVLFDK 1100.62 LITPSVVSER 1594.82 QVVQHHSQVIYTR	**Rp S25**	0010413
8454	15.0/11.23	2/20	1354.72 VTPDVVFAFGFR 1661.83 STGFALIYDTLDFAK	**Rp S24**	0034751

Table 2 (Continued)

SSP no.	KDa/p*I*	*N*/%[a]	sequence determined by MS/MS	protein identified	fly base ID FBgn
8456	17.6/11.12	10/43	924.51 IHGNTGAVR 1105.55 NLPGHAMGHR 1194.64 NQHENQAILK	**Rp L35A**	0037328
8461	17.0/10.71	10/46	968.46 YHPGYFGK 1002.56 LWSLVGAEK 1131.61 FRPEINLDK	**Rp L27A**	0010410
8515	29.4/10.95	18/52	773.41 TNVHFR 1140.55 YPLTTEAAMK 1238.72 VNVLIRPDGQK 1684.89 IEDNNTLVFLTHLR 1812.99 KIEDNNTLVFLTHLR	**Rp L23A**	0026372
8519	24.0/10.78	13/43	1048.54 VLMEYIHK 1235.58 HLYHDKYMK 1894.96 VWLDPNEINEIANTNSR	**Rp L19**	0002607
9415	17.5/11.07	2/13	1037.58 VTWTVLYR 1255.76 AIVGASLAEILAK	**Rp L24**	0032518
9428	18.4/11.52	3/14	753.39 LSYNTR 977.53 AFLIEEQK 1352.73 GITPSRPSERPR	**Rp L34b**	0037686
9434	14.4/11.18	3/21	733.40 SVFPQR 904.52 YKPLDLR 1570.86 QLDELKNELLSLR	**Rp L35**	0029785
9441	13.5/11.38	7/43	1128.57 EVVGHAPYEK 1315.72 EELSNILTQLR	**Rp L36**	0002579

[a] N = Number of matched peptides. % = Coverage of full length protein by tryptic peptides.

at 18 °C. The gels were stained with Coomassie Brilliant Blue and silver nitrate. The silver stained gels were digitized at 176 × 176 μm resolution with a pdi scanning densitometer, and the resulting 2-D images analyzed with PDQUEST software (Version 5.1).

2.4. In-Gel Digestion and Mass Spectrometry Analysis. Coomassie- or silver-stained protein spots were excised, digested with trypsin (Promega, Madison, WI, CA) and processed for mass analysis as previously described.[12] Peptides were analyzed with the Applied Biosystems 4700 proteomics analyzer (Applied Biosystems, Framingham, MA) with TOF/TOF ion optics. MS spectra were obtained in reflectron mode using an acceleration voltage of 1 kV. Desorption and ionization was performed with an Nd:YAG laser operating at 355 nm, and the final mass spectrum was produced by averaging 3600 laser shots. The products of trypsin autodigestion were used for internal calibration. All MS/MS sequencing analysis were performed using the same equipment.

2.5. Protein Identification Using the Drosophila Database. The first step in protein identification consisted of mass fingerprinting. The mono-isotopic peptide mass fingerprinting data obtained from MALDI-TOF were then used to search for candidates in the SWISS-PROT/TrEMBL no redundant protein database.

3. Results

3.1. Isolation of Ribosomes from *Drosophila* Third Instar Larvae. Figure 1 summarizes the method used to the purify ribosomes from third instar larvae of *Drosophila melanogaster*. Two differential centrifugations followed by centrifugation through a discontinuous density layer produced a pellet of purified ribosomes. We obtained a yield of 900 μg of ribosomal protein from 450 third instar larvae. The ratio of the absorbances at 260 and 280 nm was 1.92, very close to the canonical value of 2 indicative of functional integrity. The structural integrity of the purified ribosomes was confirmed by electron microscopy. Figure 1B shows a small representative field in which one can see a high concentration of intact ribosomes free of cellular contaminants.

3.2. General Pattern of Ribosomal Proteins of *Drosophila*. Four hundred micrograms of ribosomal protein obtained as described above were lyophilized, resuspended in lysis buffer, and analyzed by 2D gel electrophoresis. Figure 2 shows a representative Coomassie-stained 2-D gel of the basic polypeptides of the ribosomes of mature larvae. The gels were analyzed with the PDQUEST system, and each polypeptide was assigned a number in the database. In this way we compiled a database of 58 polypeptides (7 acidic, IEF and 51 basic, NEPHGE). This constitutes our reference ribosomal database.

3.3. Isolation of Proteins for MALDI-TOF. After calibration with Coomassie blue-stained 2-D gels, we ran preparative gels with precise amounts of the purified ribosomes from third instar larvae. After staining, we picked these spots and subjected them to in-gel digestion with trypsin followed by MALDI-TOF analysis. In this way we were able to identify 52 of the spots (5 acidic and 47 basic) in our database. In all the cases analyzed, we were able to confirm the information obtained from the peptide mass fingerprinting by tandem mass spectrometry. A total of 175 MS/MS spectra were analyzed. All the ribosomal proteins identified in this study are listed in Tables 1 and 2 in order of SSP number. Their molecular weights, isoelectric points, names, identified sequence tags, and Flybase accession numbers are also included. As an example, Figure 3A shows the MALDI-TOF spectrum of the tryptic digest of basic polypeptide SSP 8431 identified as ribosomal protein S23, and Figure 3B reproduces a detail of the fragmentation spectrum of peak 1190.70, corresponding to the polypeptide VANVSLLALYK.

3.4. Pattern of Ribosomal Proteins of *Minute* Mutants of *Drosophila*. We have analyzed in detail with the PDQUEST system the patterns of ribosomal proteins of one *Minute* mutant of Drosophila affecting protein RpL14 respectively, and compared them with the wild type. Three different concentrations of ribosomal proteins were analyzed in each case and the analysis was performed three times. Figure 4 shows a small region of the NEPHGE gel containing the protein of interest. Ribosomes from the y[1]w[*]; P[w[+mC] = lacW]RpL14[1]/TM2 mutant, that affects protein Rp L14 (SSP 8436), have a 45% less of this protein than the other sample analyzed.

By contrast the levels of the proteins not affected by the mutation (SSP 7524 or SSP 7449 in Figure 4 for example) were practically identical in the two samples.

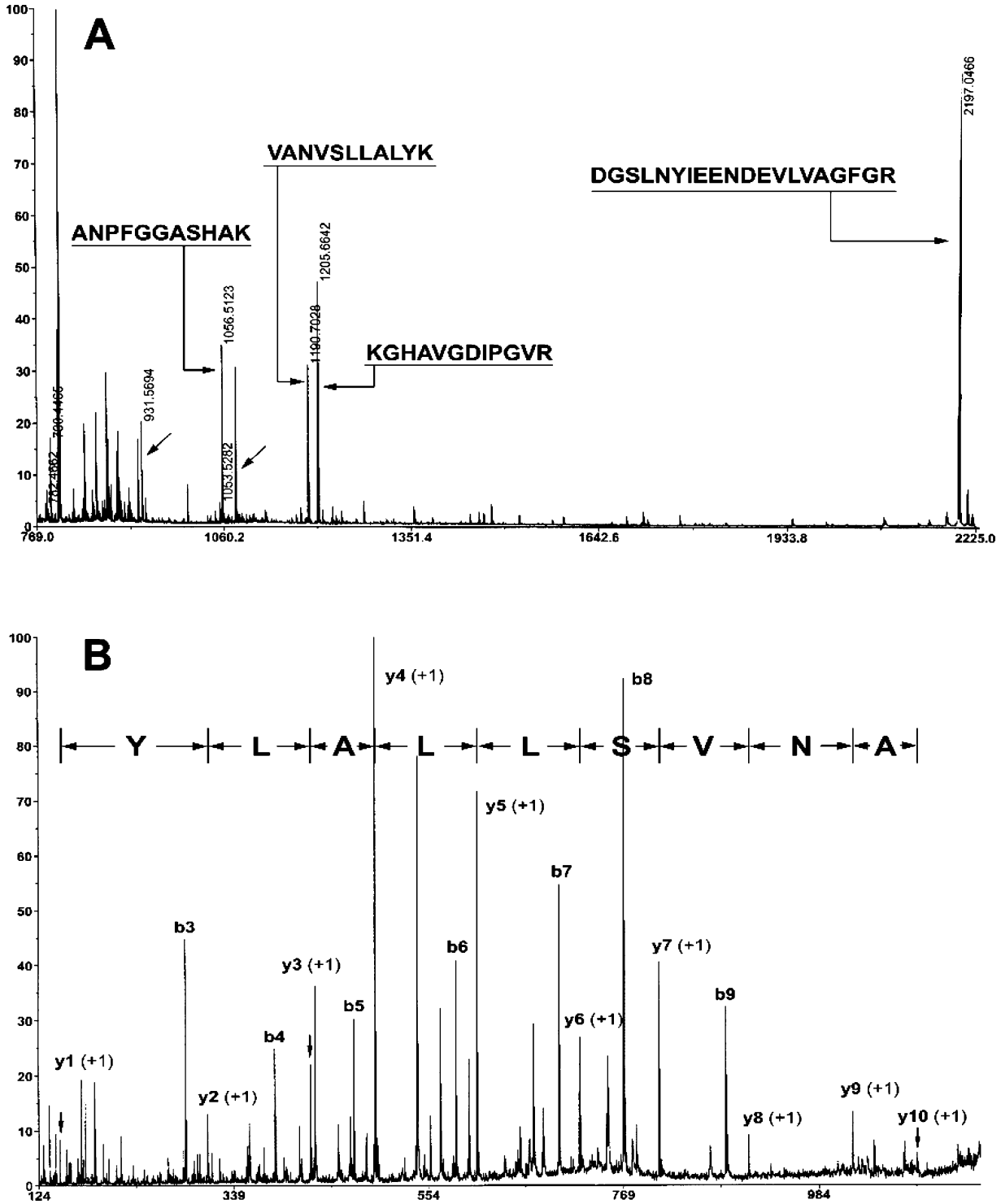

Figure 3. (A) MALDI-TOF−MS spectrum of the tryptic digest of basic polypeptide SSP 8431. The labeled peaks represent 7 of the 11 peptides matched to *Drosophila melanogaster* ribosomal protein S23. Some of the peptides analyzed by MALDI-TOF/TOF−MS are also indicated, with their sequences underline. (B) A detail of the MS/MS spectrum of the 1190.70 Da peptide, matched to the sequence VANVSLLALYK.

4. Discussion

Peptide mass fingerprinting by MALDI-TOF−MS is an excellent technique for identifying proteins in 2-D gels, especially in the case of well-defined genomes such as that of *Drosophila*. The present experimental approach has enabled us to identify 52 spots by peptide mass fingerprinting, and to identify them from their MS/MS spectra in the *Drosophila* ribosomal proteome. Tables 1 and 2 list the polypeptides identified in the database and include the corresponding accession numbers in the *Drosophila* genome database (http//flybase.bio.indiana.edu), which provides extensive genetic and molecular information about each polypeptide. Equally fundamental for this study is the Ribosomal Protein Gene Database (http://ribosome.miyazaki-med.ac.jp/table.html) that contains detailed

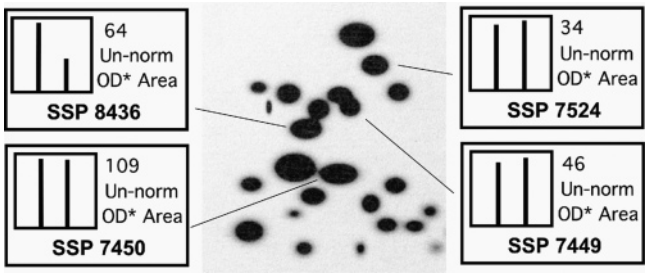

Figure 4. Two-dimensional gel of basic ribosomal polypeptides (NEPHGE) of Drosophila *Minute* mutant. Insert, each bar represents the quantification of the polypeptide in wild type (left) and y[1]w[*]; P[w[+mC] = lacW]RpL14[1]/TM2 that affects protein Rp L14 (right). The numbers at the upper right of the histograms are the values of the highest bar in the graphs. The other bars are drawn in proportion to the highest bar. Only a small area of the gel is shown.

information about ribosomal proteins, including their sequences (genomic, cDNA, and amino acid), intron/exon structures, genomic locations, and information about orthologs. In addition, it is possible to compare the structures of the corresponding gene in different organisms and make multiple amino acid sequence alignments.

All the 52 identified spots corresponded to full-length polypeptides and represent 90% of the 58 polypeptides recorded in the database. In one case, a spot present in both the acidic and basic gels was found to contain the same polypeptide, identified as Acidic Ribosomal Protein LP0. There was also one case in which we identified two different polypeptides in the same spot (SSP 7450, Nephge). Peptide fingerprinting enabled us to assign one of these unambiguously to Ribosomal Protein S13, a polypeptide with a molecular weigh of 17.1 and an isolectric point of 10.3. Nevertheless, the data from MS/MS of two peaks obtained in the general spectrum also indicated the presence of ribosomal protein S18, with a molecular weigh of 17.6 and an isoelectric point of 10.4, values that would explain the comigration of the two polypeptides. RpS18 has an access number in Flybase of 0010411, map on chromosome 2 and belongs to the 40S ribosomal subunit. This means that the identified spots actually contain 52 different protein species.

The genes encoding the detected polypeptides mapped over three of the four *Drosophila* chromosomes in proportions consistent with the latters' sizes: 21 polypeptides on chromosome 2, 20 on chromosome 3, and 11 on the X chromosome. No polypeptides mapped to chromosome 4. Genes for both ribosomal subunits were represented on these chromosomes. Twenty-one 40S subunit and thirty-one 60S subunit polypeptides were mapped.

We detected two non ribosomal polypeptides in the gels that had not been included in the database. Both of them appeared in the acidic gels and corresponded to the two chains of ferritin, an intracellular protein that binds iron ions, thus preventing the accumulation of toxic levels of free iron ions. Ferritin is a spherical shell that consists of 24 identical subunitsfolded into ellipsoids with a combined molecular weight of 474 000, thus accounting for its presence as a contaminant during ribosome purification.

Severall of the ribosomal proteins identified here are involved in proceses additional to protein synthesis. For example, ribosomal protein S10b is thought to be involved in cytoskeleton organization[13] and ribosomal protein L30 is implicated in peripheral nervous system development.[14] Equally, some of the identified polypeptides are involved in interesting genetic interactions. For example RpL19, mutants of which result in a severe *Minute* phenotype, interacts genetically with Delta (Dl) a gene encoding a surface transmembrane protein with EGF repeats in its extracellular domain. It also interacts with the if[3] mutation of the PS2a (position specific) integrin gene. It has been proposed that the pleiotropic *Minute* syndrome can affect, probably indirectly, one or more steps of wing morphogenesis that involve surface adhesion of epithelial cells.[15]

Also worthy of mention is the interaction that seems to exist between the ribosomal gene S21, also known as *overgrown* hematopoietic organs, with ribosomal gene Rp40. Down-regulation of the *Drosophila* ribosomal protein S21 gene (rpS21) causes a dominant weak *Minute* phenotype and recessively produces massive overgrowth of the hematopoietic organs and particularly of the most anterior lobes, which become as big as the brain. Genetic studies reveal that P40 underexpression drastically enhances imaginal disc overgrowth in rpS21-deficient larvae, whereas viable combinations between rpS21 and rp40 affect the morphology of bristles, antennae, and aristae.[16]

The most fundamental, and ancient, activities of the ribosome are the same in all kingdoms of life,[17] although little is known about riboprotein mutations in mammals. Theoretically, the *Minute* phenotype should be found in organisms other than *Drosophila*. In fact, it has recently been reported that *Belly spot and tail* (*Bst*) is a mouse mutation that disrupts pigmentation, somitogenesis and retinal cell fate determination, and it has been characterized as a deletion within the *Rpl24* riboprotein gene.[18] *Bst* has been classified as a mouse *Minute*, and provided the first detailed characterization of a mammalian ribosomal protein mutation. Turner syndrome in humans has also been compared to the *Minute* phenotype and ascribed to haploinsufficiency for HS4.[19] This proposal has been disputed,[20] and several genes are likely to be involved in the syndrome.[21] We have shown that the database is sensitive enough to detect quantitative variations due to *Minute* mutations, at least in the case analyzed.

We expect that the *Drosophila* ribosomal database reported here will be useful for analyzing how it is modified under specific mutant and genetic conditions in *Drosophila* and that the results will be capable of extrapolation to other animal models.

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