*Journal of Life Science* **2005**, Vol. 15. No. 3. 427~433 ©JLS

# Proteome Analysis of *Drosophila melanogaster* Used 2-DE and MALDI-TOF-MS

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Received April 28, 2005 / Accepted May 27, 2005

With the completely discovery of the *Drosophila* genome sequence, the next great challenge is to extract its biological information by systematic expression and to perform functional analysis of the gene. Here we reported a proteome analysis of *D. melanogaster* with two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS). The cell extracts of *D. melanogaster*, 200 μg were resolved to more than 400 silver-stained spots by 2-DE. The most abundant protein spots were ranged from 4.0~7.5 of p*I* and from 15~90 kDa of molecular weight. The excised spots were destained and in-gel digested by trypsin. The masses of the resulting peptide mixtures were measured by MALDI-TOF-MS. Identified proteins were compared with measured peptide mass and a dynamic peptide searching database which is accessible via the internet. The results revealed that identified proteins were produced by 59 genes derived from 65 protein spots.

**Key words** – *Drosophila melanogaster*, Proteomics, Two-dimensional electrophoresis, MALDI-TOF-MS

*Drosophila melanogaster* is an outstanding model system for the molecular genetics and developmental biology in higher eukaryotes[2]. Recently, full genomic sequence of the *D. melanogaster* was reported[1]. Since the genomic sequence was completely discovered, the future we will try to precise definition of the biological functions of the predicted open reading frames deduced from genomic sequence.

The proteome is the entire proteins to be expressed by the genome in a particular cell, tissue, or organism at a given time under a specific set of environmental conditions. Proteome analysis is the large-scale study of gene function at the protein level. Proteomics is to study proteome by methods of protein separation by 2-DE and protein identification by mass spectrometry (MS), such as MALDI-TOF-MS, electrospray ionization (ESI) and quadrupole time-of-flight mass spectrometer (QTOF-MS). Proteomics is powerful technique for identification of protein and their post-transitional modification, comparison of differential expression protein in various conditions and study of protein-protein interaction. The word proteomics has been traditionally included in displaying a large number of proteins through two-dimensional polyacrylamide gels from a given cell line or organism[23]. In the 1990s, biological mass spectrometry had been emerged as an analytical method that removed most of the limitations of protein analysis. This development was coupled with the availability of the entire genomic sequence in public databases[16]. The individual components resolved a complex mixture of proteins through 2-DE could be analyzed by mass spectrometry. The technological progress leading to the development of soft methods for ionization such as MALDI and ESI have made it possible to obtain even the primary sequence information of peptides and proteins. In particular, MALDI-TOF has been extensively used for protein identification in large-scale proteome projects by comparing theoretical tryptic digests derived from sequence databases with experimental digests[17].

*D. melanogaster* has previously reported many results of proteome research such as 2-DE database of *D. melanogaster* [5], protein interaction map[6], ventral furrow formation[7], larval hemolymph proteins[20] and hemolymph[21]. However, analysis of total proteome in adult *D. melanogaster* have not reported with 2-DE and MALDI-TOF-MS until now. In this study, adult *D. melanogaster* proteome was separated by 2-DE used 3~10 IPG (immobilized pH gradient) strip, and abundant protein spots were excised, destained, and then followed by in-gel digestion with trypsin. The masses of peptide mixtures were measured by MALDI-TOF-MS and measured mass data applied to search the protein sequence database for DROSOPHILA MELNOGASTER using the ProteinProspector package program (UCSF). The result of

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proteome analysis exhibited that *D. melanogaster* proteins were identified by 59 genes expressed to 65 protein spots, including transport proteins, enzymes and structural proteins.

## Materials and Methods

### Experimental conditions used and materials

Wild type *D. melanogaster* and medium was purchased with sangmulnara (http//www.biozoa.co.kr). Flies were reared on culture tube in a 12 h/12 h light/dark photoperiod at 25℃ and 50% humidity. Each adult *D. melanogaster* was collected in a conical tube and stored at -70℃ until use.

### Preparation of cell extract of D. *melanogaster*

Adult *D. melanogaster* (1 g) was suspended in 5 ml of lysis buffer (9.5 M urea, 4% CHAPS, 35 mM Tris, 65 mM DTT, 1 mM EDTA, 0.01% SDS, 10 mg/ml DNase, 2.5 mg/ml RNase A, 0.5% ampholite (3~10) and ground for 20 min with Ultra Homogenizer (IKA, Germany). Homogenized samples were clarified by centrifugation at 13,000 rpm for 10 min at 4℃. The resulting supernatant was transferred to new eppendorf tube and stored at -70℃ until use.

### Protein quantitation

Protein concentrations were determined by the Bradford method[4], using protein assay reagent (Bio-Rad) diluted five-fold in distilled water. Reagent (990 μl) was mixed with 10 μl protein sample. The absorbance at 595 nm was monitored. Bovine serum albumin (Sigma) was used as the standard concentration.

### Two-dimensional electrophoresis

Isoelectric focusing (IEF) was performed using IPG strips [22]. Strips were rehydrated under the passive condition; 0 V, 20℃, 14~16 h reaction in the protein IEF Cell (Bio-Rad, USA). Each solubilized protein samples (200 μg) was applied to the strips (17 cm, Bio-Rad, USA) in the pH ranges of 3. 0~10.0. IEF for the treated gel according to preset method of the protein IEF Cell was performed by slightly modified conditions composed of the conditioning step, voltage ramping, and final focusing. The first step was to remove salt ions and charged contaminants at 10,000 V for 3 h. When the conditioning step was completely performed, the linear voltage ramping step was followed for 2 h and the current was limited not to exceed 50 μA/strip. The final focusing step was programmed to finish the focusing process once the maximum voltage was reached terms of 80,000 volt-hours. The current limit was designed not to exceed 50 μA/strip as well. After completely focusing, the strips were equilibrated by 0.375 M Tris buffer (pH 8.8) containing 6 M urea, 2% SDS, 20% glycerol, 2% DTT and 0.01% bromophenol blue, and followed by the addition of the same buffer supplemented with 2.5% iodoacetamide[8]. SDS-PAGE was done by the method of Laemmli[11] using a 12% separating polyacrylamide gel (17×16 cm) without any stacking gel. The second-dimensional separation was carried out overnight at 20 mA/gel and was stopped when the bromophenol blue dye was arrived at the bottom of the gel. The separated proteins were visualized by silver staining[9].

### Image analysis

A gel image was obtained by scanning the silver stained gels by the Model GS-710 Calibrated Imaging Densitometer (Bio-Rad, USA) and documented by the PDQUEST.

### In-gel digestion of protein spots

An in-gel digestion was carried out by the method described by O'Connell and Stults[14]. Gel pieces containing protein spots were rehydrated by adding a digestion buffer on ice. The buffer containing 12.5 ng trypsin per ml was added enough to cover the pieces and incubated for 45 min on ice. The enzyme solution was removed and replaced with 20 μl of the buffer without enzyme, keeping the gel pieces wet during enzymatic cleavage overnight at 37℃, and the gel pieces were subjected to a vigorous vortexing for 30 min. Twenty μl of the digested solution was transferred into a clean eppendorf tube, dried in a vacuum, and the resulting samples were dissolved in 1 μl of 0.1% trifluoroacetic acid (TFA).

### Peptide mass fingerprinting

A matrix solution composed of α-cyano-4-hydroxy cinnamic acid (40 mg/ml) in 50% acetonitrile and 0.1% TFA was prepared for peptide mass fingerprinting. Two μl of the matrix solution and sample solution were mixed, applied onto the target well, rapidly dried, and washed using cold deionized water. The mixture solution was dried for 10 min at room temperature and subjected to a MALDI-TOF-MS operation using the Voyager Biospetrometry Workstation (PE Biosystem, USA) using the following parameter: 20 kV accelerating voltage, 75% grid voltage, 0.02% guide wire, 150 ns delay time and a mass gate was from 800 to 3,000.

### Identification of the proteins

The peptide mass fingerprints were analyzed using the program MS-Fit of ProteinProspector developed by UCSF Mass Spectrometry Faculty[10]. The DROSOPHILA MELNOGASTER of the Swiss-prot database was searched for protein identification. Monoisotopic peptide masses were used search the database, allowing a molecular weight and p*I* range of observed on 2-DE gel ±20,000 Da and ±1.5, a peptide mass accuracy of 50 ppm, and one partial cleavage.

## Results and Discussion

### 2-DE profiles of the whole cell proteins of adult *D. melanogaster*

Proteome profiling employed the measurement of global mRNA and protein expression patterns, respectively, for the identification of individual genes/proteins and clusters of genes/proteins that mediate particular aspects in a physiological and pathophysiological process. The proteome analysis technique has been developed by the wide availability of a number of prokaryotes and eukaryotes[12].

We performed proteome analysis of adult *D. melanogaster*. The whole cell proteins solution of the strain was loaded onto precast IPG strips (17 cm) of pH gradient ranging from 3.0 to 10.0 for the first-dimensional separation. The strips were loaded onto a 12% acrylamide gel of 17×16 cm for the second dimensional electrophoresis. After running SDS-PAGE, the separated spots were visualized by silver staining. The whole cell proteins solution showed more than 400 silver-stained spots in the p*I* regions of 3.0~10.0, as shown in Fig. 1. The most abundant protein spots were enriched in p*I* regions of 4.5~7.0 and their molecular masses were between 15 and 90 kDa.

### Peptide fingerprinting and protein identification

As shown in Fig. 1, the silver-stained spots generated by 2-DE using the IPG strip of pH 3.0~10.0 and SDS-PAGE were numbered, excised and destained, followed by in-gel digestion using trypsin for peptide fingerprinting. The mass of resulting peptide mixtures was measured by MALDI-TOF-MS and MS-Fit of ProteinProspector. Among the 200 protein spots processed, 65 proteins were identified as shown in Table 1. The identified spots produced to be expressed from 59 genes. Information of identified proteins was obtained from NCBI (Genbank, Protein, Pubmed), EXPASY (SWISS-PROT, Flybase, PROSITE or pfam) database.

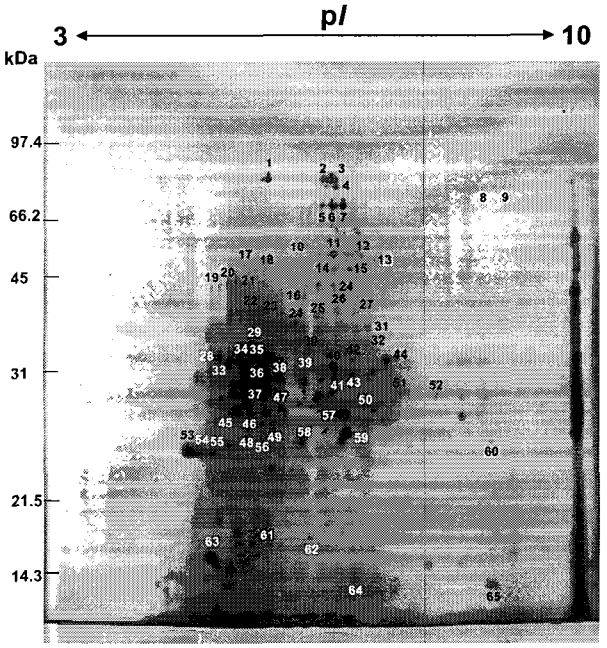


Fig. 1. Two-dimensional gel electrophoresis map of adult *D. melanogaster* proteome. Proteins (200 μg) were separated by IEF using 17 cm IPG strips (pH 3~10) and subsequently on 12% SDS-PAGE gel; detection by silver staining. The original gel size was 18×20×0.12 cm. X-axis indicates p*I* values of 3~10, and Y-axis marks protein molecular weight by kDa. Numerals in gel image mean arbitrarily marked protein spots.

The remaining unidentified spot appeared with no spectrum or weak spectrum with mass spectrometry or good spectrum but with no matching protein in database.

Among them we identified proteins to relate with transport protein, enzyme, structure protein and others. Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a nonexchangeable site on the alpha-chain. Microtubules is affect of cell motility, division, and secretion, as well as intercellular transport[19]. Porin protein is different ionic selectivity and the sensitivity to voltage gradients across the outer mitochondrial membrane. Function of porin protein is sieving gates for polar metabolites[13]. Guanine nucleotide-binding proteins (G proteins) are involved as modulators or transducers in various transmembrane signaling systems. Talluri *et. al.* identified another *Drosophila* G protein α subunit, dGqα-3[18]. Soluble NSF (N-ethylemaleimide-sensitive factor) attachment protein is located at cytoplasmic peripheral membrane and required for vesicular transport between the endoplasmic reticulum and the golgi apparatus[15].

Table 1. List of protein identified in adult *Drosophila melanogaster*

| Spot No | Protein name | Family | Gene name | SWISS-PROT | NCBI entry | pI | MW (kDa) |
|---|---|---|---|---|---|---|---|
| 1 | DNA mismatch repair protein spellchcker 1 | Muts | SPEL1 or CG4215 or BG:DS01068.9 | P43248 | 33860181 | 5.6 | 103.3 |
| 2 | cGMP-dependent protein kinase 1 | Ser/Thr protein kinase | PKG21D or DG1 or CG3324 | Q03042 | 17380465 | 6.0 | 86.8 |
| 3 | Molybdenum cofactor synthesis protein cinnamon | moaB/mog | CIN or CG2945 or EG:BACR37P7.3 | P39205 | 17380356 | 6.5 | 74.6 |
| 4 | Putative glycogen [starch] synthase | Mammalian/fungal glucogen synthase | CG6904 | Q9VFC8 | 12230129 | 6.1 | 79.2 |
| 5 | Putative actin interacting protein 1 | WD-repeat AIP1 | CG10724 | Q9VU68 | 12230779 | 6.3 | 66.6 |
| 6 | Enolase | Enolase | ENO | P15007 | 119351 | 6.1 | 46.6 |
| 7 | Enolase | Enolase | ENO | P15007 | 119351 | 6.1 | 46.6 |
| 8 | Cytochrome P450 6a8 (CYPVIA8) | Cytochrome P450 | CYP6A8 or CG10248 | Q27593 | 12644425 | 8.8 | 58.1 |
| 9 | Cytochrome P450 6a13 (CYPVIA13) | Cytochrome P450 | CYP6A13 or CG2397 | Q9V4U9 | 11386695 | 8.8 | 58.1 |
| 10 | Probable nuclear hormone receptor HR3 (dHR3) | Nuclear hormone receptor | HR46 or HR3 or NR1F4 or CG11823 | P31396 | 399369 | 6.3 | 55.5 |
| 11 | Ornithine decarboxylase 1 (ODC) | Orn/Lys/Arg decarboxylase class II | ODC1 or CG8721 | P40807 | 12643278 | 5.7 | 44.2 |
| 12 | Alkaline phosphatase 4 precursor | Alkaline phosphatase | APH-4 or CG1462 | Q24238 | 29427685 | 5.9 | 65.3 |
| 13 | Lamin (CPG-IF) | Intermediated filament | LAMC | Q03427 | 729915 | 6.4 | 69.9 |
| 14 | Eukaryotic initiation factor 4A (elf-4A) | DEAD box helicase | EIF-4A or EIF4A or L(2L)162 | Q02748 | 12644381 | 5.4 | 45.9 |
| 15 | Probable cytochrome P450 303a1 | Cytochrome P450 | CYP303A1 or L(2)35FB or CG4163 | Q9V399 | 11386647 | 6.3 | 57.9 |
| 16 | Ribonucleoside-diphosphate reductase M2 chain | Ribonucleoside diphosphate reductase small chain | RNRS or CG8975 | P48592 | 12644287 | 5.4 | 45.1 |
| 17 | Alpha-amylase-related protein precursor | Glycosyl hydrolases | AMYREL or CG8221 | O18408 | 17865655 | 4.9 | 55.4 |
| 18 | Hypothetical protein CG 7816 | ZIP transporter | CG7816 | Q9VAF0 | 12585532 | 5.2 | 38.9 |
| 19 | Tubulin alph-3 chain | Tubulin | ALPHA-TUB84D or TUBA84D | P06605 | 135416 | 5.0 | 49.9 |
| 20 | ATP synthase beta chain mitochondrial precursor | ATPase alpha/beta chains | ATPSYN-BETA | Q05825 | 584809 | 5.2 | 54.1 |
| 21 | ATP synthase beta chain mitochondrial precursor | ATPase alpha/beta chains | ATPSYN-BETA | Q05825 | 584809 | 5.2 | 54.1 |
| 22 | ATP synthase beta chain mitochondrial precursor | ATPase alpha/beta chains | ATPSYN-BETA | Q05825 | 584809 | 5.2 | 54.1 |
| 23 | ATP synthase beta chain mitochondrial precursor | ATPase alpha/beta chains | ATPSYN-BETA | Q05825 | 584809 | 5.2 | 54.1 |
| 24 | Hexokinase type 2 | Hexokinase | HEX-T2 or HEX or 32849/CG5443 | Q9NFT7 | 29337238 | 6.5 | 49.7 |
| 25 | Serine/threonine protein phosphatase beta isoform (Flap wing protein) | PPP phosphatase | FLW or PP1-9C or PP1-BETA-9C or CG2096 | P48462 | 1346761 | 5.8 | 37.7 |
| 26 | Zipper protein precursor | Not known | UZIP or ZIP or CG3533 | P10379 | 31340518 | 5.8 | 37.7 |
| 27 | Probable cytochrome P450 303a1 | Cytochrome P450 | CYP303A1 or L(2)35FB | Q9V399 | 11386647 | 6.3 | 57.9 |
| 28 | Tubulin alpha-3 chain | Tubulin | ALPHA-TUB84D or TUBA84D or CG2512 | P06605 | 135416 | 5.0 | 49.9 |
| 29 | Transcription factor dp | E2F/DP | DP | Q24318 | 3122917 | 5.8 | 42.7 |
| 30 | Putative mitogen-activated protein kinase kinase kinase 7 | Ser/Thr protein kinase | TAKL1 | P83104 | 17368362 | 6.5 | 45.2 |
| 31 | 26S proteosome non-ATPase regulatory subunit 6 | 1 PCI domain | RPN7 or CG5378 | Q9V3G7 | 20978559 | 6.1 | 45.4 |
| 32 | Probable deoxyhypusine synthase (DHS) | Deoxyhypusine synthase | CG8005 | Q9VSF4 | 8928108 | 6.1 | 46.0 |

Table 1. continued

| Spot No | Protein name | Family | Gene name | SWISS-PROT | NCBI entry | pI | MW (kDa) |
|---|---|---|---|---|---|---|---|
| 33 | Tubulin alpha-3 chain | Tubulin | ALPHA-TUB84D or TUBA84D or CG2512 | P06605 | 135416 | 5.0 | 49.9 |
| 34 | Probable ribonuclease HI large subunit | RNase HII | CG13690 | Q9VPP5 | 11387117 | 5.0 | 38.8 |
| 35 | Guanine nucleotide-binding protein G(9) alpha subunit | G-alpha | G-ALPHA-49B or G-A49B or DGQ | P23625 | 417034 | 5.2 | 41.3 |
| 36 | Probable vacular ATP synthase subunit d2 | V-ATPase | CG4624 | Q9VCQ3 | 12585514 | 5.4 | 40.3 |
| 37 | Probable vacular ATP synthase subunit d2 | V-ATPase | CG4624 | Q9VCQ3 | 12585514 | 5.4 | 40.3 |
| 38 | Ornithine decarboxylase 2 (ODC) | Orn/Lys/Arg decarboxylase class-II | ODC2 | P40808 | 729302 | 5.5 | 44.1 |
| 39 | Putative neutral sphingo myelinase | Neutral sphingomyelinase | CG12034 | Q9VZS6 | 32172461 | 5.7 | 45.5 |
| 40 | Glutathione S-transferase D7 | GST | GSTD7 or GSTD26 or CG4371 | Q9VG93 | 12643919 | 5.6 | 25.4 |
| 41 | Neutral/ectodermal development factor IMP-L2 precursor | 2 immunoglobulin-like C2-type | IMPL2 or CG15009 | Q09024 | 25453447 | 5.7 | 29.4 |
| 42 | Fat body protein P6 | SDR | FBP2 | P54398 | 1706769 | 6.4 | 30.6 |
| 43 | Fat body protein P6 | SDR | FBP2 | P54398 | 1706769 | 6.4 | 30.6 |
| 44 | Vortage-dependent anion selective channel | Eukaryotic mitochondrial porin | PORIN or VDAC or POR-1 or CG6647 | Q94920 | 6174942 | 6.4 | 30.6 |
| 45 | Adapter molecule CrK | 1 SH2 | CRK or CG1587 | Q9XYM0 | 13124035 | 5.1 | 31.2 |
| 46 | Probable prefoldin subunit 2 | Prefoldin beta subunit | L(3)01239 or CG6302 | Q9VTE5 | 12230497 | 5.5 | 16.2 |
| 47 | Basic helix-loop-helix transcription factor Roi | 1 basic helix-loop-helix | AMOS or ROI or ROLO or CG10393 | Q9Y0A7 | 20137578 | 5.6 | 22.6 |
| 48 | Ubquitin-conjugation enzyme E2-22kDa (Ubiquitin-protein ligase) | Ubiquitin-conjugating enzyme | UBCD4 | P52486 | 1717856 | 5.4 | 223 |
| 49 | Ubquitin-conjugation enzyme E2-22kDa (Ubiquitin-protein ligase) | Ubiquitin-conjugating enzyme | UBCD4 | P52486 | 1717856 | 5.4 | 223 |
| 50 | Vortage-dependent anion selective chanel | Eukaryotic mitochondrial porin | PORIN or VDAC or POR-1 or CG6647 | Q94920 | 6174942 | 6.4 | 30.6 |
| 51 | Alcohol dehydrogenase | SDR | ADH or CG3481 or BG:DS01486.8 | P00334 | 113424 | 7.7 | 27.8 |
| 52 | Protein CG11722 | UPF0240 | CG11722 or BCDNA:AT14909 | Q9VH39 | 30913424 | 8.9 | 23.8 |
| 53 | Soluble NSF attachment protein | SNAP | SNAP or CG6625 | Q23983 | 18202518 | 5.3 | 33.0 |
| 54 | Soluble NSF attachment protein | SNAP | SNAP or CG6625 | Q23983 | 18202518 | 5.3 | 33.0 |
| 55 | Soluble NSF attachment protein | SNAP | SNAP or CG6625 | Q23983 | 18202518 | 5.3 | 33.0 |
| 56 | Ras-related protein Rab-3 | Small GTPase | RAB3 or CG7576 | P25228 | 131800 | 5.0 | 24.9 |
| 57 | ATP synthase D chain, mitochondrial | Not known | ATPSYN-D or CG6030 | Q24251 | 14286117 | 6.1 | 20.2 |
| 58 | Lathal (2) essential for life protein | Small heat shock protein | L(2)EFL or CG4533 | P82147 | 13124197 | 5.8 | 21.3 |
| 59 | Mitochondrial import inner membrane translocase subunit Tim9B | Tim8/Tim10 | TIM9B or TIM9 or CG17767 | Q9Y0V3 | 12230186 | 5.9 | 13.5 |
| 60 | Probable glucosamine 6-phosphate N-acetyltransferase | Acetyltransferase | CG1969 | Q9VAI0 | 9296991 | 7.6 | 24.5 |
| 61 | Eukaryotic translation initiation factor 5A | Elf-5A | EIF-5A or CG3186 | Q9GU68 | 25090601 | 5.0 | 17.6 |
| 62 | Pheromone-binding protein related protein 6 precursor (PBPRP-6) | PBP/GOBP | OS-E or PBPRP6 or CG11422 | Q23970 | 2494874 | 5.7 | 16.1 |
| 63 | Proponin C isoform 2 | Troponin C | TPNC47D or TNC47D | P47948 | 1351268 | 4.0 | 17.9 |
| 64 | Protein spitz precursor | 1 EGF-like | SPI or CG10334 | Q01083 | 1174423 | 7.5 | 26.0 |
| 65 | Ejaculatory bublb specific protein II precursor (PEB-mell) | Not known | PEBII or CG2665 | Q23982 | 29427684 | 8.0 | 6.3 |

The protein spot numbers are indicated the 2-DE map in Fig. 1. Protein name, family name, gene name, SWISS-PROT accession number, NCBI entry number, theoretical *pI* and molecular weight are indicated. WD-repeat: Trp-Asp repeat, UPF: Unclassfied protein family, SDR: Short-chain dehydrogenases/reductases, EGF: Epidermal growth factor.

Three membrane proteins, zipper protein precursor, hypothetical protein CG 7816 and lamin[3] were founded. Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.

The aim of this study was to create a protein map of adult *D. melanogaster* that can serve as a reference map for future studies on *Drosophila* proteins, such as development study through comparison with embryo, disease, response of insecticide or drug etc. Therefore, we will continuously study differential expression of proteins expressed during different development stages.

## References


1. Adams, M. D., S. E. Celniker, R. A. Holt, *et al.* 2000. The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185-2195.
2. Baggerman G., A. Cerstiaens, A. De Loof, and L. Schoofs. 2002. Peptidomics of the larval *Drosophila melanogaster* central nervous system. *J. Biol. Chem.* **277**, 40368-40374.
3. Bossie, C. A. and M. M. Sanders. 1993. A cDNA from *Drosophila melanogaster* encodes a lamin C-like intermediate filament protein. *J. Cell Sci.* **104**, 1263-1272.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
5. Ericsson, C., Z. Petho, and H. Mehlin. 1997. An on-line two-dimensional polyacrylamide gel electrophoresis protein database of adults *Drosophila melanogaster*. *Electrophoresis* **18**, 484-490.
6. Giot, L., J. S. Bader, C. Brouwer, *et al.* 2003. A protein interaction map of *Drosophila melanogaster*. *Science* **302**, 1727-1736.
7. Gong, L., M. Puri, M. Unlu, M. Young, K. Robertson, S. Viswanathan, A. Krishnaswamy, S. R. Dowd, and J. S. Minden. 2004. *Drosophila* ventral furrow morphogenesis: a proteomic analysis. *Development* **131**, 643-656.
8. Gorg, A., W. Postel, S. Gunther, J. Weser, J. R. Strahler, S. M. Hanash, L. Somerlot, and R. Kuick. 1988. Approach to stationary two-dimensional pattern: influence of focusing time and immobiline/carrier ampholytes concentrations. *Electrophoresis* **9**, 37-46.
9. Heukeshoven, J. and R. Dernick. 1988. Improved silver staining procedure for fast staining in PhastSystem Development Unit. I. Staining of sodium dodecyl sulfate gels. *Electrophoresis* **9**, 28-32.
10. http://www.proteinprospector.ucsf.edu
11. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.
12. Langen, H., C. Gray, D. Roder, J. F. Juranville, B. Takacs, and M. Fountoulakis. 1997. From genome to proteome: protein map of *Haemophilus influenzae*. *Electrophoresis* **18**, 1184-1192.
13. Messina, A., M. Neri, F. Perosa, C. Caggese, M. Marino, R. Caizzi, and V. De Pinto. 1996. Cloning and chromosomal localization of a cDNA encoding a mitochondrial porin from *Drosophila melanogaster*. *FEBS Letter.* **384**, 9-13.
14. O'Connell, K. L. and J. T. Stults. 1997. Identification of mouse liver proteins on two-dimensional electrophoresis gels by matrix-assisted laser desorption/ionization mass spectrometry of in situ enzymatic digests. *Electrophoresis* **18**, 349-359.
15. Ordway, R. W., L. Pallanck, and B. Ganetzky. 1994. Neurally expressed *Drosophila* encoding homologs for the NSF and SNAP secretory proteins. *Proc. Natl. Acad. Sci. USA.* **91**, 5715-5719.
16. Quadroni, M. and P. James. 1999. Proteomics and automation. *Electrophoresis* **20**, 664-677.
17. Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Anal. Chem.* **68**, 850-858.
18. Talluri, S., A. Bhatt, and D. P. Smith. 1995. Identification of a *Drosophila* G protein α subunit (dGqα-3) expressed in chemosensory cells and central neurons. *Proc. Natl. Acad. Sci. USA.* **92**, 11475-11479.
19. Theurkauf, W. E., H. Baum, J. Bo, and P. C. Wensink. 1986. Tissue-specific and constitutive alpha-tubulin genes of *Drosophila melanogaster* code for structurally distinct proteins. *Proc. Natl. Acad. Sci. USA.* **83**, 8477-8481.
20. Vierstraete, E., A. Cerstiaens, G. Baggerman, G. Van den Bergh, A. De Loof, and L. Schoofs. 2003. Proteomics in *Drosophila melanogaster*: first 2D database of larval hemolymph proteins. *Biochem. Biophys. Res. Commun.* **304**, 831-838.
21. Vierstraete, E., P. Verleyen, G. Baggerman, W. D'Hertog, G. Van den Bergh, L. Arckens, A. De Loof, and L. Schoofs. 2004. A proteomic approach for the analysis of instantly released wound and immune proteins in *Drosophila melanogaster* hemolymph. *Proc. Natl. Acad. Sci. USA.* **101**, 470-475.
22. Weekes, J., C. H. Wheeler, J. X. Yan, J. Weil, T. Eschenhagen, G. Scholtysik, and M. J. Dunn. 1999. Bovine dilated cardiomyopathy: proteomic analysis of an animal model of human dilated cardiomyopathy *Electrophoresis* **20**, 898-906.
23. Wilkins, M. R., C. Pasquali, R. D. Appel, K. Ou, O. Golaz, J. C. Sanchez, J. X. Yan, A. A. Gooley, G. Hughes, I. Humphery-Smith, K. L. Williams, and D. F. Hochstrasser. 1996. From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology* **14**, 61-65.

## 초록 : 이차원 전기영동과 펩타이드 지문 검색법을 이용한 초파리의 프로테옴 분석

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초파리는 유전학, 발달 생물학, 행동 유전학, 노화 연구에 이르기까지 수많은 연구에 이용 되어져 왔다. 최근 초파리 전체 유전자의 염기서열이 보고되었으며, 유전자의 기능 분석과 발현 단백질에 대한 연구가 진행되고 있다. 본 연구에서는 야생형 초파리에서 추출한 단백질을 이차원 전기영동을 통해 400 여개 이상의 단백질 스폿으로 분리하였으며, 각 스폿을 적출하여 트립신으로 처리하여 얻어진 펩타이드 단편을 MALDI-TOF-MS를 이용한 펩타이드 지문 검색으로 질량을 측정하였다. 측정된 질량을 초파리 데이터 베이스를 이용하여 분리한 단백질을 동정함으로써 59개의 유전자에서 발현되는 65개의 단백질 스폿을 동정하였다. 이러한 결과는 향후의 발생단계, 외부 자극, 노화 등에 관련되는 특이적 단백질 연구의 기초 자료로 활용될 수 있을 것이다.