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Inner ear proteomics of mouse models for deafness, a discovery strategy[☆]

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Abstract

Inner ear dysfunction is often associated with defective hair cells. Therefore, hair cells are the focus of study in many of the mouse mutants showing auditory and vestibular deficits. However, harvesting sufficient numbers of hair cells from the tiny bony mouse inner ear for proteomic analysis is challenging. New approaches that would take advantage of mouse mutants and avoid processing steps, such as decalcification or microdissection, would be more suitable for proteomic analysis. Here, we propose a novel approach called SSUMM—Subtractive Strategy Using Mouse Mutants. SSUMM takes advantage of the differences between control and affected or mutant samples. We predict that SSUMM would be a useful method in proteomics, especially in those cases in which the investigator must work with small numbers of diverse cell types from a tiny organ. Here, we discuss the potential utility of SSUMM to unravel the protein expression profiles of hair cells using the *Pou4f3* mouse mutant as an example. *Pou4f3* mutant mice exhibit a total loss of inner and outer hair cells, but supporting cells remain relatively intact in the cochlea, thus providing an excellent model for identifying proteins and transcripts that are specific to the hair cell at all life stages. SSUMM would maximize the sensitivity of the analyses while obviating the need for tedious sessions of microdissection and collection of hair cells. By comparing the mutant to control ears at specific time points, it is possible to identify direct targets of a gene product of interest. Further, SSUMM could be used to identify and analyze inner ear development markers and other known genes/proteins that are coexpressed in the ear. In this short technical report, we also discuss protein-profiling approaches suitable for SSUMM and briefly discuss other approaches used in the field of proteomics.

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Keywords

Proteomics; Mass spectrometry; Gel electrophoresis; Otology

1. Introduction

The mouse models available for human hereditary hearing disorders offer extraordinary tools for molecular pathway studies and drug discovery (<http://www.sanger.ac.uk/PostGenomics/mousemutants/deaf/>). These single mutation mouse models having well-characterized phenotypes on genetically defined backgrounds enable us to use gene and protein expression profiling to identify molecular pathways involved in inner ear structure and function. Proteomic approaches have become increasingly successful for the study of complex biological problems relevant to the auditory system. The majority of previous investigations were performed on other species including humans (Henzl et al., 1997, 1998, 2001; Hurle et al., 2003; Ornitz et al., 1998; Sakaguchi et al., 1998; Thalmann, 2001; Thalmann et al., 1980, 1986, 1987, 1990, 1994, 1995a,b, 1997a,b, 2001a,b, 2003; Thalmann and Thalmann, 1987, 1999; Wang et al., 1998). Only a few protein-profiling studies have been carried out in mouse inner ears (Henzl et al., 1997; Hurle et al., 2003; Ornitz et al., 1998; Sakaguchi et al., 1998; Wang et al., 1998). One reason for the lack of such studies is the difficulty of accessing the small number of diverse cell types within the hard temporal bone that encases the inner ear. As the technology of proteomics moves from theory to practical reality, auditory scientists will have to determine the most appropriate strategies for this technology in order to overcome difficulties particular to this research, such as the limited sample amounts and heterogeneous cellular composition of the tiny mouse inner ears. In this short technical report, we describe a subtractive strategy which overcomes these difficulties, taking advantage of mouse mutant and genomic information resources accumulated in the last decade. For a more comprehensive review of the application of proteomics to auditory research, the reader is referred to Thalmann (this issue) and McGuire and Casado (2004).

1.1. Genetics of deafness

Hearing loss affects more than 28 million individuals in all age groups in the United States (NIDCD Health Information home-page, <http://www.nidcd.nih.gov/health/statistics/hearing.asp>). Approximately one-half of these cases are thought to be of hereditary origin. Noise-induced and age-related hearing loss (presbycusis) may have genetic components as predisposing factors as well. The fundamental processes involved in the development and physiology of hearing are controlled by hundreds of genes. To date, nearly 150 deafness-related loci have been mapped, and more than 50 genes have been identified (www.uia.ac.be/dnlab/hhh/). Gene identification is only the first step towards finding a cure for human deafness. The next critical step for understanding the pathophysiology of hearing disorders is to profile the expression and regulation of deafness genes at the proteome and transcriptome level. Experimental animal models such as the mouse can play a key role in these critical steps.

1.2. Mouse models as tools to unravel the genetic basis for human deafness

Mice have many well-known advantages over other species for the study of human disease. The mouse ear is remarkably similar in structure and function to the human ear, and both species have many similar hearing disorders (Alagramam et al., 2001a,b, 2005; Donahue et al., 2003; Ikeda et al., 1999, 2002; Johnson et al., 1997, 1998, 2000, 2001, 2003; Johnson and Zheng, 2002; Letts et al., 2000; Lorenz-Depiereux et al., 2004; Munroe et al., 2000; Noben-Trauth et al., 1997, 2003; Staecker et al., 2001; Zheng et al., 1998, 1999, 2004; Zheng and Johnson, 2001). Because mouse mutations can be maintained in a controlled genetic

background, it is possible to analyze the effects of a mutant gene for same-sex littermates that differ only in a single mutated gene out of the whole genome (Silver, 1995). To date, nearly 50 human hearing disorders have been identified that have parallel mouse disorders due to mutations in orthologous genes (www.jax.org). Mouse models as tools have been very useful for unraveling the genetic basis of human deafness. These mouse mutants also provide extremely useful tools to elucidate genomic regulatory networks and represent a powerful dynamic proteomic model system to study human deafness. To our knowledge, only a few proteomic studies of the mouse inner ear have been conducted on mutant mice, such as tilted (*tilt*). *tilt* is characterized by vestibular dysfunction associated with specific otoconial agenesis. However, proteomic analysis of mouse mutants that are deaf due to defects in the neuroepithelia have yet to be undertaken (Hurle et al., 2003; Ornitz et al., 1998). The small structures of the inner ear are separated by relatively large fluid spaces within a hard bony shell, and these factors pose difficult challenges to ear researchers. For example, because the total number of hair cells (inner+outer) per mouse ear is only about 3300 (Ding et al., 2001), more than 300 ears would be required to obtain the 1,000,000 hair cells necessary to extract microgram quantities of protein. Because hair cells must be isolated from a large number of ears and because of their enclosure by bone, neither laser capture microdissection (Lee et al., 2003) nor fluorescence-activated cell sorting (FACS) (Yang et al., 2004) would be a simple approach to collect a sufficient number of cells without damage due to processing, including in the case of laser capture fixation, sectioning, and microdissection. An easier approach that takes advantage of mouse mutants lacking hair cells would be an important step to circumvent the problems discussed earlier.

1.3. Gene expression at the mRNA level and why we need to study gene expression using a proteomic approach

In the past few years, the development of array-based methods for the analysis of genome-wide expression at the mRNA level has helped to generate a more integrated view of the relationship between the genome, gene expression, and phenotypes (Cui and Churchill, 2003). Recently, differential gene expression microarray analysis has contributed significantly to the identification of a downstream gene that is controlled by the *Pou4f3* transcription factor (TF) in the ear (Hertzano et al., 2004). The array-based experiments permit the measurement, simultaneously and semiquantitatively, of changes in gene expression between two different biological states. However, interpretation of the connection between changes in the mRNA expression level and phenotype is often complicated for several reasons: (1) relative differences between mRNA and protein turnover, i.e., a protein can still be abundant while the mRNA is no longer detectable because its synthesis has stopped; (2) variation of function of translational machinery and translation efficiencies between different cells at different stages; (3) post-translational modifications, such as removal of signal peptides and phosphorylation, of proteins; and (4) complex interactions with other proteins. These complicated processes cannot be deduced from cDNA microarray or other nucleic acid-based methodologies. Genetic information is only indicative of the cell's potential and does not reflect the actual state in a given cell at a given time. The concept of proteome characterization has emerged to provide complementary and critical information by revealing the regulation, activities, quantities, and interaction of every protein in the cell and how these quantities respond to developmental and environmental signals. Furthermore, the majority of clinically effective drugs are targeted to proteins and in particular to receptors. The study of the proteome in relation to the genome can potentially lead to drug discovery for the cure of diseases. Understanding the proteome at different disease stages can help us use the correct treatment at the right time. New hypotheses can be generated based on the results of this approach.

1.4. Current stage of proteomics in auditory developmental and disease processes

Pioneering proteome studies have been carried out by Isolde and Ruediger Thalmann (see Washington University Inner Ear Protein Database <http://oto.wustl.edu/thc/innerear2d.htm>) using standard 2DE and mass spectrometry (2DE/MS). However, since most of these studies were carried out on guinea pigs for which few deafness models are available, these studies have not spurred the exponential growth of proteomic studies seen in other organs. Briefly, these investigators have found (1) two highly abundant proteins, OCP1 and OCP2, present in the epithelial support complex of the organ of Corti (OC); (2) oncomodulin, a novel calcium-binding protein, exclusively expressed in the outer hair cells of the OC; (3) two proteins, ApoJ and ApoD, highly concentrated in endolymph and perilymph, a finding that has been applied to ear pathology, specifically perilymph fistula; and (4) otoconin-90, the major matrix protein of the otoconia of the vestibular gravity receptor organs. In bovine ear experiments, Ikezono et al. demonstrated that the protein product of the *Coch* gene, which underlies the human deafness syndrome DFNA9 (Robertson et al., 1998), is abundant in the ear (Ikezono et al., 2004). 2DE has been applied in several other studies but with varying success (Boulassel et al., 2000). Other studies include localizations of individual proteins and functional determinations (Royaux et al., 2003), as well as correlation of expression of the actin filament-bundling protein espin with stereociliary bundle formation in the developing ear (Li et al., 2004).

2. Proteomics technologies

Several proteomics technologies including 2D-PAGE, 2D-DIGE, ICAT, SELDI-TOF (high-resolution surface-enhanced laser desorption and ionization-time of flight), MudPIT (multidimensional protein identification technology), and protein arrays have played valuable roles in drug discovery or elucidation of physiologic or pathologic processes in other organ systems such as heart (Anderson, 2005), liver (Girard et al., 2005), brain (Bildl et al., 2004), and tumors (Alaiya et al., 2005). Shotgun proteomics is a gel-free approach based on multidimensional liquid chromatography separation of complex peptide mixtures coupled to mass spectrometry (MS). For shotgun sequencing of a whole genome, DNA fragments are sequenced and computationally reassembled to determine the genome of an organism. Similarly, in shotgun proteomics, complex protein mixtures are digested into peptides, analyzed by tandem mass spectrometry, and the peptides are mapped by computer algorithms onto proteins to determine the original content of the mixture (Yates, 1998). Most of the above proteomics technologies are not suitable for the study of mouse inner ears. For example, Shin et al. had to switch to chicken from mouse and needed more than 400 chickens for their proteomic profiling of hair bundle proteins (Shin et al., 2006). Here, we describe a novel subtractive strategy with 2D-DIGE or ICAT proteomics technologies that only require a few mouse ears for profiling protein expression in hair cells.

3. Quantitative proteomics: 2D difference gel electrophoresis (2D-DIGE)

The rapidly expanding field of proteomics relies heavily upon two-dimensional electrophoresis (2DE) as one of the best high-throughput methods for measuring parallel changes in the expression levels of thousands of individual proteins, to offer a global view of the state of a proteome (Klein and Thongboonkerd, 2004). Proteins are first separated across a gel according to their isoelectric point and then separated in a perpendicular direction on the basis of their molecular weights. After 2D separation, the two or more gels are stained with silver, SYPRO Ruby or other dyes, the gels are scanned and images are compared using software packages to match the proteins found in the different gels. Despite the advances provided by commercially available precast gels for SDS-PAGE, precast strips with immobilized gradients for isoelectric focusing, and improved software, the difficulty of matching spots between different gels and

the limited dynamic range of some colorimetric stains have hampered quantitation of proteins found in post-stained gels (Alban et al., 2003).

2D fluorescence difference in gel electrophoresis (2D-DIGE) is a relatively new technique that uses fluorescent dyes to label protein samples prior to 2D-PAGE (polyacrylamide gel electrophoresis) (Hoffert et al., 2004). The DIGE technique allows multiple samples to be coseparated and visualized on one 2D gel. (The method is outlined in Fig. 1). Up to three protein extracts [for example, one control (CT), one mutant (MT), and a pooled internal standard (PIS) with equal aliquots of all controls and mutants, i.e., CT+MT] can be labeled with different fluorescent cyanine dyes (CyTM3, Cy5, and/or Cy2), and combined and separated by 2D-PAGE. Up to three images of the gel are captured using the Cy3, Cy5, and/or Cy2 excitation wavelengths. Using image analysis software, the differences in protein expression between the labeled protein samples can be determined. The dyes have a linear response to over five orders of magnitude of protein concentration, offer subnanogram sensitivity, and are compatible with MS analysis.

Advantages of 2D-DIGE are (1) it significantly reduces gel-to-gel variations; (2) it can be used to detect 1.2-fold differences between experimental and control samples; (3) it allows quantification of protein ratios, similar to silver staining, from 0.25–0.95 ng of a 50-kDa protein (Tonge et al., 2001) but has a better dynamic range of quantitation; (4) up to 5000 spots can be quantified per gel (Zhou et al., 2002), but typically 1000–2000 spots are resolved; and (5) the required amount of total protein is only 50 µg per sample for the minimal dye method and 5 µg per sample for the scarce sample method (DIGE System User Manual, 18-1137-17AB, GE Healthcare). 2D-DIGE and MS have been used successfully in several representative proteomic studies. Kleno et al. have identified several proteins as biomarkers of induced liver toxicity (Kleno et al., 2004); Friedman et al. have identified 52 unique proteins that changed in abundance in colon cancer cells (Friedman et al., 2004); Gade et al. proved that CyDyeTM labeling allows detection of almost as many protein spots as staining with silver or SYPRO Ruby (Gade et al., 2003); and Somiari et al. demonstrated quantitative and qualitative differences in protein abundance among different breast cancer stages (Somiari et al., 2003).

3.1. A subtractive strategy

Hair cells are essential elements of the sensory apparatus of the inner ear, and inner ear dysfunctions are frequently associated with defective hair cells. Therefore, hair cells represent the focus of many studies of mouse mutants with auditory and/or vestibular deficits. However, as mentioned earlier, harvesting a sufficient number of hair cells for protein analysis from the tiny inner ear of the mouse is challenging. New approaches that take advantage of mouse mutants and avoid processing steps, such as decalcification or microdissection, would be more suitable for proteomic analysis. Here, we report a novel approach—the subtractive strategy—based on the use of mouse mutants (SSUMM), such as *Pou4f3* mutant mice, characterized by a loss of a particular cell population or organ.

Pou4f3 is a class IV POU domain transcription factor which plays a critical role in the maturation and survival of hair cells in the mouse auditory and vestibular sensory epithelia (Xiang et al., 1998). The consequences of targeted deletion of *Pou4f3* in mice on a B6-129 mixed background are defects in balance and hearing caused by loss of auditory and vestibular hair cells during late gestation and early postnatal life. The overall architecture of the inner ear is unaffected (Xiang et al., 1997), and mutant mice survive into adulthood. Mutant mice exhibit a total loss of inner and outer hair cells, whereas supporting cells remain relatively intact. This provides an excellent model of the subtractive strategy for identification of proteins and transcripts specific to the hair cell at all life stages, thereby maximizing analytical sensitivity while minimizing potential dissection artifacts. In addition, we can identify direct targets of *Pou4f3* protein, and we can also identify early developmental markers and other protein

markers coexpressed in the ear. Genes associated in this way with Pou4f3 include but are not limited to *Notch1~4*, *gata3*, *p27^{Kip1}*, *Eya1*, *S100a1*, *Tbx3*, *Shh*, *Fgfr3*, *Bmp5*, *Jag2*, and *Fkh10*. Other genes that are clustered in the same category with any of the above genes can be further analyzed. By means of the subtractive strategy, proteins expressed in hair cells can be profiled at later developmental stages because the entire complement of hair cells is lost in the mutants.

3.2. Technical highlights

3.2.1. Sample preparation—Inner ear dissections and protein extractions: Mice are euthanized, and their crania flash frozen in a -20°C chamber for 1 min. The temporal bones are rapidly separated from the skull in ice-cold lysis buffer, dissected in a Petri dish containing ice cold buffer, and the soft tissues removed. The inner ears are immersed in 200 μl of 2-DE lysis buffer (7 M urea, 2 M thiourea, 30 mM Tris, 5 mM magnesium acetate, 4% CHAPS, 1% NP-40, pH 8.0–9.0). The samples are sliced into several pieces and incubated at room temperature for 30 min with vortexing at 10-min intervals. The homogenate is centrifuged at $14,000\times g$ for 10 min, and the clear supernatant was transferred to a clean and sterile microcentrifuge tube and stored at -80°C until needed. In our experience, 200 μg of total protein from either one adult or two P2 inner ears can be obtained by this method.

The protein samples are treated with a 2D Clean-Up kit (GE Healthcare). After treatment, each protein pellet is resuspended in 20 μl of DIGE lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris, pH 8.5). The pH is adjusted to between 8 and 9.

3.3. Saturation dyes method for 2D-DIGE

In the CyDyeTM DIGE fluors for the scarce samples method, the PIS sample is bulk labeled with CyDyeTM DIGE Fluor Cy3 saturation dye in sufficient quantity for inclusion as a standard on every gel. Proteins (5 μg) from each wild type and mutant sample can be labeled separately with CyDyeTM DIGE Fluor Cy5 saturation dye. For detailed methods, please see reference Shaw et al. (2003). Currently, two new CyDyeTM DIGE Fluor dyes (Cy3 and Cy5) are available commercially (from GE Healthcare) for *saturation* labeling in a 2D gel. This method was designed for samples that are difficult to prepare in sufficient quantity for the minimal dye method (see below) or traditional 2D gels.

3.4. Minimal dyes method for 2D-DIGE

With the CyDyeTM DIGE Fluor minimal dyes method, one lysine group is labeled per protein (Nordvarg et al., 2004). CyDyeTM DIGE Fluor minimal dyes have an NHS-ester reactive group and are designed to attach via an amide linkage to the epsilon amino group of lysine of proteins. The ratio of dye to protein is specifically designed to ensure that the dyes are limiting in the reaction. As a result, the dyes label approximately 1–2% of the available proteins and then only on a single lysine per protein, i.e., one dye per protein, or “minimal labeling.” The advantage of the minimal dyes method is that it allows accurate detection and quantitation of up to three prelabeled protein samples including PIS on the same 2D electrophoresis gel using the commercially available CyDyeTM DIGE Fluor dyes Cy2, Cy3, and Cy5 (from GE Healthcare). Moreover, it generates gel images of similar appearance and pattern to traditional post-staining gels (Fig. 2). This method is useful when samples are available in typical amounts (50 μg –150 μg) for 2D gels, and where a more complex experimental design is desired (e.g., multiple samples in one experiment, multiple conditions being compared, etc.).

Additional protein can be added to samples in one of the gels (designated as the Prep Gel) to increase the total protein concentration, eliminating the need for a separate preparative 2D-DIGE, which enables downstream processing and analysis of differentially expressed proteins by peptide mass fingerprinting. The first dimension was performed using Immobiline DryStrips

(18 cm pH 3–10 NL) on IPGphor II, and second dimension was performed using 12.5% SDS-PAGE gels on DALTsix. Typhoon 9400 is used to scan for all three dyes. The spot maps from all other gels can be matched to the Prep Gel in the image analysis software. The total amount of protein to be loaded on the Prep Gel should be in the range of 300–1000 mg.

3.5. Mass spectrometry and protein identification

For MS analysis, preparative gels are run (when needed) using a pool containing each of the wild type and mutant lysates. 300–1000 µg protein is needed. The preparative gel image is automatically matched to the master gel image in the analytical gel match set using appropriate software. A spot-picking list is generated from the software and exported to a spot picker. The gel spots are excised as 1.4-mm-diameter plugs and delivered into 96-well micro-plates. In-gel digestion and peptide-mass fingerprinting are performed.

The matrix-assisted laser desorption/ionization (MALDI) technique-generated mass spectra are internally calibrated using trypsin peaks. The peptide masses are searched against the National Center for Biotechnology Information nonredundant mammalian database <http://www.ncbi.nlm.nih.gov/usingProFound>TM http://129.85.19.192/profound_bin/WebProFound.exe, and confirmed using a MascotTM search from Matrixscience http://www.matrixscience.com/search_form_select.html and the SwissProtTM database from MS-Fit <http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm>. One missed cleavage per peptide is allowed, and an initial mass tolerance of 100 ppm is used in all searches. Partial oxidation for methionine is assumed. Additional matches are searched by further analysis via liquid chromatography with tandem mass spectrometry detection (LC/MS/MS) methods when needed.

4. Alternative strategies to 2D/DIGE MALDI-TOF MS

There are methods for the quantitative study of protein expression that do not use 2D gels, e.g., exploiting isotopic labeling of samples. Approaches include labeling proteins after extraction with a chemical tag that can be supplied in several stable isotopic forms, for example, isotope-coded affinity tags (ICAT) (Alban et al., 2003), and a stable isotope that can be differentially incorporated during the growth of an organism (Krijgsveld et al., 2003). These methods can be viewed largely as complementary techniques to modern 2D gel experiments, having the potential to provide information on sets of proteins such as integral membrane proteins that are poorly represented on 2D gels.

1. If high-abundance proteins overshadow most other proteins in 2D/DIGE results, we need to eliminate the largest changes and capture the window of low-abundance proteins. Therefore, a multidimensional fractionation approach is needed to remove the most abundant proteins (Issaq et al., 2002). For example, the Agilent Multiple Affinity Removal System (Chromy et al., 2004) can remove six targeted, high-abundant proteins: albumin, IgG, anti-trypsin, IgA, transferrin and haptoglobin, and is especially useful for serum and organs with significant circulatory flow. Other methods include biochemical (cellular) fractionation. These approaches can significantly improve the results of protein separation and expand the dynamic range of the 2D/DIGE analytical methods. However, these procedures require much larger quantities of protein than the techniques described above.
2. If most membrane proteins are absent in 2D/DIGE, nongel isotope-coded affinity tagging (ICAT)-liquid chromatography coupled to mass spectrometry (LC/MS) analysis profiles the relative amounts of cysteine-containing peptides derived from tryptic digests of protein extracts as detailed by Tian et al. (2004) (Fig. 3). LC/MS can facilitate the identification of large numbers of membrane proteins and modifications and has the potential to provide insights into protein topology and

orientation in membranes. Cysteine content varies greatly between different organisms and needs to be defined for the mouse. In general, about 5% of most proteins do not contain cysteine and cannot be detected by sulfide labeling techniques like ICAT. ICAT-LC/MS has a clear bias for high-molecular weight membrane (Mr) proteins and can be complemented by the 2-DE-DIGE/MS method that has a preference for low-Mr proteins and can also identify cysteine-free proteins that are transparent to the ICAT-LC/MS method (Schmidt et al., 2004).

3. Immunohistochemistry, Western blots, RT-PCR, and/or RNA in situ hybridization approaches can be used to profile and validate protein and mRNA expression patterns of genes that are identified in the above experiments.

4.1. Considerations for research design

Six biological replicates of RNA for microarray experiments are taken from the right ears of the same experimental mice from which left ears are used for 2D-DIGE/MS analysis, as described above. Data from the microarray experiments are not discussed here.

5. Data analyses and database

5.1. Combined statistical analyses of proteome and transcriptome experiments

The digitized raw data from the 2D-DIGE/MS and microarray results can be correlated and analyzed. Unique gene/protein identification numbers can be merged to ensure for cognate matching of each individual gene/protein of interest. The data can be analyzed using the ANOVA package. Particular transcripts can be matched with their protein counterparts by nucleotide sequence and amino acid sequence comparisons. Genes and proteins can be statistically analyzed and classified according to many different criteria, including GO terms (Gene Ontology, GO database: Molecular Function, Biological Process, or Cellular Component). High expression levels for cytoplasmic proteins, low levels for nuclear and membrane proteins, and high expression fluctuations for secreted proteins have been noted (Drawid et al., 2000). Changes in the relative level of protein expression may be detected that are as little as 1.2-fold for large volume spots on a 2D-DIGE gel (Tonge et al., 2001). Because detection is based on fluorescence, 2D-DIGE has a large dynamic range of about 10,000, which permits differential expression analysis of proteins at high and modest abundance (Tonge et al., 2001). The limitation of detection of 2D-DIGE for quantifying protein expression ratios is between 0.25 and 0.95 ng protein, which is similar to that for silver staining (Tonge et al., 2001). In a recent study (Zhou et al., 2002), the relative level of expression of ~1050 protein spots was compared in 250,000 laser dissected normal versus esophageal carcinoma cells (Johnson et al., 1998).

5.2. Correlation analysis of protein and mRNA expression

Ratios (mutant/control) for both protein and mRNA can each be converted into log₁₀ ratios. Statistical correlation analysis of protein and mRNA expression data can be used with the cosine correlation metric of comparison.

If changes for mRNAs and their cognate proteins are in the same direction, they may be correlated. If changes for mRNAs and their cognate proteins are in the opposite direction, they may be anti-correlated. Some of the reports in the literature show significant discordance between gene expression at the protein and transcript levels (Royaux et al., 2003). However, these analyses examined only one aspect of a biological system, i.e., the steady-state levels of mRNAs and proteins. Another important aspect that concerns the kinetic process of perturbation and how the correlation of mRNA and protein evolves during this process was not addressed.

5.3. Clustering

Global analysis of gene expression can reveal genes with clustered expression profiles that are likely to be functionally related. To establish such groups, self-organizing maps (SOMs) can be employed. SOMs allow easy visualization of changing gene expression profiles through time, and they place genes with similar profiles in adjacent groups. This produces a matrix with progressive, gradual transitions through the rows and columns. The genes that display a >1.6-fold change at any time point can be clustered.

5.4. Conclusion

Proteomic approaches have become increasingly successful for the study of complex biological problems. However, few protein-profiling studies of mouse auditory organs have been carried out. One reason for the lack of such studies is the difficulty of accessing the small numbers of diverse cells within the hard temporal bone that encases the inner ear. In this report, we describe a novel subtractive strategy and other technical highlights that are geared towards differential expression profiling of samples with small amounts of protein, in this case, using mouse mutants such as *Pou4f3* mutant mice, with a particular cell population loss or organ loss. The strategies and methods described in this article are well suited for transcriptome and proteome analysis of mouse inner ears.

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Fig. 1.
An outline of 2D fluorescence difference gel electrophoresis (2D-DIGE) working flow.

Fig. 2.

Cy3 image of proteins from normal inner ears of an adult mouse. 50 μ g of protein extracted from 2 mouse inner ears was resolved in 2D format, and approximately 1700 spots per gel were detected, which is nearly 8 times more than the 230 spots that we detected with the traditional 2DE gel from two similar mouse ears.

Fig. 3.
A work flow of ICAT/MS-based protein profiling.