De Novo Biosynthetic Profiling of High Abundance Proteins in Cystic Fibrosis Lung Epithelial Cells

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In previous studies with cystic fibrosis (CF) IB3-1 lung epithelial cells in culture, we identified 194 unique high abundance proteins by conventional two-dimensional gel electrophoresis and mass spectrometry (Pollard, H. B., Ji, X.-D., Jozwik, C. J., and Jacobowitz, D. M. (2005) High abundance protein profiling of cystic fibrosis lung epithelial cells. Proteomics 5, 2210–2226). In the present work we compared the IB3-1 cells with IB3-1/S9 daughter cells repaired by gene transfer with AAV-(wild type)CFTR. We report that gene transfer resulted in significant changes in silver stain intensity of only 20 of the 194 proteins. However, simultaneous measurement of de novo biosynthetic rates with [35S]methionine of all 194 proteins in both cell types resulted in the identification of an additional 31 CF-specific proteins. Of the 51 proteins identified by this hybrid approach, only six proteins changed similarly in both the mass and kinetics categories. This kinetic portion of the high abundance CF proteome, hidden from direct analysis of abundance, included proteins from transcription and signaling pathways such as NFκB, chaperones such as HSC70, cytoskeletal proteins, and others. Connectivity analysis indicated that ~30% of the 51-member hybrid high abundance CF proteome interacts with the NFκB signaling pathway. In conclusion, measurement of biosynthetic rates on a global scale can be used to identify disease-specific differences within the high abundance cystic fibrosis proteome. Most of these kinetically defined proteins are unaffected in expression level when using conventional silver stain analysis. We anticipate that this novel hybrid approach to discovery of the high abundance CF proteome will find general application to other proteomic problems in biology and medicine. Molecular & Cellular Proteomics 5: 1628–1637, 2006.

Cystic fibrosis is a common lethal genetic disease that is manifest by intrinsic hyperactivation of proinflammatory signaling pathways in the lung (1). The CF airway is massively populated by inflammatory mediators such as IL-81 that initially come from the lung epithelial cells (2) as well as tumor necrosis factor α, leukotrienes, and others (3, 4). Although it has been long known that CF is due to mutations in the CFTR gene (5–7), the mechanism linking the CFTR mutation to inflammatory disease is still not known. Recent pharmacogenomic and pharmacoproteomic studies have implicated defects in both the TNFα/NFκB signaling pathway (8–11) and the HSP70 system (12). However, parallels between changes in mRNA and cognate proteins are infrequent in eukaryotic cells principally because of many overlapping layers of post-transcriptional and post-translational regulation.

We have therefore embarked on a global approach to the CF proteome in cultured CF lung epithelial cells and in primary cultures of bronchial lung epithelium obtained by brush biopsy of CF patients. Using an approach of 2-D gel electrophoresis (2DGE) and mass spectrometry, we recently identified 194 unique high abundance proteins in the CF lung epithelial IB3-1 cell system (13). Preliminary comparisons between the IB3-1 cells and IB3-1/S9 cells, a daughter cell line repaired by gene transfer with wild type CFTR, revealed that relatively few changes in silver-stained features could be detected for any of the 194 high abundance proteins. We therefore used [35S]methionine to measure the de novo biosyn-

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1 The abbreviations used are: IL-8, interleukin 8; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; 2-D, two-dimensional; 2DGE, 2-D gel electrophoresis; AAV, adenovirus-associated virus; GO, gene ontology; KRT, keratin; HMGCS1, hydroxymethylglutaryl-CoA synthase 1; TCTP, translationally controlled tumor protein; UCHL1, ubiquitin carboxyl-terminal hydrolase 1; IKK, IkB kinase.
thetic rates of each of the 194 proteins we had previously identified on the 2-D gel format. The advantage of radiolabeling is that an entire proteome can be visualized at once from a phosphorimaging or autoradiograph, and experimental differences can be immediately annotated. We report here that there is significant proteomic information that can be obtained by simultaneously measuring the individual mass levels and de novo biosynthetic rates for all identified proteins. We conclude that de novo biosynthetic labeling can be used in combination with conventional mass labeling to identify disease-specific differences within the high abundance cystic fibrosis proteome. These differences can be used to generate a hybrid high abundance proteomic signature for cystic fibrosis.

EXPERIMENTAL PROCEDURES

Cultured Cells and Reagents—The CF lung epithelial cells IB3-1 and AAV-(wild type)CFTR-repaired IB3-1/S9 have been described previously (Ref. 8, 9, and 14; see supplemental materials, SM.1). Both IB3-1 and IB3-1/S9 cells were grown in serum-free LHC-8 medium (Biofluids, Bethesda, MD; see supplemental materials, SM.2). The high IL-8 secretion phenotype was routinely monitored by ELISA (R&D Systems, Boston, MA). To avoid potential problems with clonal drift, new batches of frozen cell stocks were thawed at least every 3 months (after ~12 passages) during the course of the experiment.

The proteomic radiolabeling protocol for cells was as follows. Briefly CF cells ("IB3-1" or explants from bronchial biopsies) and repaired cells ("IB3-S9") were grown in T75 flasks to 80% confluence. To begin the labeling process, cultures were first incubated for 30 min in methionine-free, gentamicin-free LHC-8 medium. Cultures were then incubated in the same medium supplemented with 200 μCi/ml [35S]methionine (PerkinElmer Life Sciences) for an additional 30 min.

PRELIMINARY STUDIES OVER LONGER TIME COURSES HAD INDICATED THAT THE 30-MIN TIME POINT WAS IN THE LINEAR PORTION OF THE BIOSYNTHETIC PROCESS FOR HIGH ABUNDANCE PROTEINS. THE CELLS WERE THEN WASHED, AND TOTAL PROTEINS WERE PREPARED FOR 2-D GEL ELECTROPHORESIS. THE ENTIRE ANALYSIS WAS PERFORMED ON A TOTAL OF 30 INDEPENDENTLY PERFORMED EXPERIMENTS, IN TRIPlicate, FEATURING BOTH IB3-1 AND IB3-1/S9 CELLS IN PARALLEL. ADDITIONAL RADIOLABELING INFORMATION IS GIVEN IN THE SUPPLEMENTAL MATERIALS (SM.3).

Human Subjects—CF and non-CF individuals who were undergoing a bronchoscopy for a clinical indication were eligible. Patients (or parents on behalf of a person <18 years of age) signed informed consent for an Institutional Review Board- and General Clinical Research Center-approved protocol to obtain bronchial specimens. An additional assent form was signed by adolescent participants. Bronchoscopy was performed under general anesthesia. Bronchial brushings were obtained from second or third generation airways under direct visualization and immediately immersed in ice-cold gentamicin-supplemented LHC-8 medium. Bronchoalveolar lavage was performed according to the Cystic Fibrosis Therapeutics Development Network standard operating procedure. There were no significant or serious adverse events associated with specimen collection.

Parallel Silver Staining and Radiolabeling of CF IB3-1 and Repaired IB3-1/S9 Cells—As shown in Table I and Fig. 1, high abundance proteins from CF lung epithelial IB3-1 cells (17) and their AAV-(wild type)CFTR-repaired daughter cell line IB3-1/S9 (18) can be compared by simultaneous imaging with either silver stain or incorporation of [35S]methionine. Fig. 1, a and b, shows a comparison of silver-stained images of CFTR-repaired and parental CF cells, respectively. There were substantial similarities, as might be anticipated, as well as significant but fewer optical density differences. Below each silver-stained gel, Fig. 1, c and d, shows the same gels, respectively, but imaged according to incorporation with [35S]methionine. There appear to be many more radio-labeled features than silver-stained features. The numbers
| PROTEIN                                                                 | SwissProt | HUPO#  | SILVER | 35(S)M |
|------------------------------------------------------------------------|-----------|--------|--------|--------|
| **GO: cytoskeleton**                                                   |           |        |        |        |
| Keratin 18, Type I                                                    | P05783    | KRT18  | ▼      | ▼      |
| calcium binding protein BDR-1                                         | P37235    | HPCAL1 | ▼      | ▼      |
| Annexin V (5)                                                         | P08758    | ANXA5  | ▼      | ▼      |
| Tropomyosin-α-4                                                      | P07226    | TMP4   | ▼      | ▼      |
| CAP Z-β                                                               | P47756    | CAPZB  | ▼      | ▼      |
| Chloride Intracellular Channel Protein 1                               | O00299    | CLIC1  | ▼      | ▼      |
| tubulin-β-1-chain (OK.SW-cl.56)                                        | P05218    | TUBB   | ▼      | ▼      |
| kinesin-like protein 8                                                | Q9NSKO    | KNSL8  | ▼      | ▼      |
| **GO: Metabolism**                                                    |           |        |        |        |
| Adenosylhomocysteinase                                                | P23526    | AHCY   | ▼      | ▼      |
| Hydroxymethylglutaryl-CoA-synthase 1                                   | Q01581    | HMGCS1 | ▼      | ▼      |
| Acetyl-CoA-Acetyltransferase 2                                         | Q9BWD1    | ACAT2  | ▼      | ▼      |
| Hypoxanthine-Guanine PhosphoribosylTransferase                        | P00942    | TPI1   | ▼      | ▼      |
| galactokinase                                                          | P51570    | GALK1  | ▼      | ▼      |
| Sialic Acid Synthase                                                  | Q9NR45    | NANS   | ▼      | ▼      |
| Ribose-Phosphate pyrophosphokinase II                                  | P11908    | PRPS2  | ▼      | ▼      |
| **GO: "Other"**                                                       |           |        |        |        |
| Tumor Metastatic Process-Associated Protein                           | P15531    | NME1   | ▼      | ▼      |
| KIAA0193 (Secernin1)                                                  | Q12765    | SCRN1  | ▼      | ▼      |
| Immunoglobulin E Binding Factor                                        | P06734    | FCER2  | ▼      | ▼      |
| Copine I                                                              | Q99829    | CPNE1  | ▼      | ▼      |
| FLJ12983 (ATP/GTP binding protein-like 3)                              | Q8NEM8    | AGBL3  | ▼      | ▼      |
| MHC Class 1b antigen                                                  | P17693    | HLA-G  | ▼      | ▼      |
| Thioredoxin peroxidase (Peroxiredoxin 2)                              | P32119    | PDRX2  | ▼      | ▼      |
| glyoxylase 1                                                          | Q04760    | GLO1   | ▼      | ▼      |
| Peroxiredoxin 6 (Antioxidant protein 2)                               | P30041    | PDRX6  | ▼      | ▼      |
| Platelet activating Factor acetylhydrolase1Bγ                         | Q15102    | PAFAH1B3| ▼    | ▼      |
| **GO: Proteasome**                                                    |           |        |        |        |
| Ubiquitin Carboxy Terminal Hydrolase (PGP 9.5)                         | P09936    | UCHL1  | ▼      | ▼      |
| Ubiquitin-Conjugating Enzyme E2N                                       | Q16781    | UBE2N  | ▼      | ▼      |
| Proteasome subunit -α, type 6                                         | P34062    | PSMA3  | ▼      | ▼      |
| Proteasome subunit -β, type 3                                         | P25788    | PSME2  | ▼      | ▼      |
| **GO: Signal Transduction**                                           |           |        |        |        |
| Translationally Controlled Tumor Protein (TCTP)                        | P13693    | TPT1   | ▼      | ▼      |
| Guanyl Cyclase Activating Protein 3                                   | O95843    | GUCA1C | ▼      | ▼      |
| MAP Kinase Interacting Serine/Threonine Kinase 2                      | Q9HBH9    | MKNK2  | ▼      | ▼      |
| Rab GDP Dissociation Inhibitor-β                                      | P50395    | GDI2   | ▼      | ▼      |
| Serine/threonine-protein kinase 3                                     | Q13188    | STK3   | ▼      | ▼      |
and intensities of radiolabeled spots increased with exposure time. Most silver-stained spots had some associated radiolabel, whereas many radiolabeled features were devoid of silver stain. Apparently many of the low abundance proteins, otherwise hidden by the relative insensitivity of the silver stain paradigm, can be detected by highly sensitive de novo biosynthesis.

Fig. 2 shows a detailed comparison of a portion of the gels from Fig. 1 as imaged by either silver or radiolabel. Silver stain at low resolution is shown in the upper left, and radiolabel of the same region at low resolution is shown in the upper right. The acutely truncated silver-stained peaks emphasize the narrow dynamic range of silver staining. Operationally these silver-stained features saturate out quickly as a function of mass. The radiolabeled features show graded peaks, consistent with the 5-log dynamic range characteristic of radioactivity. Radiolabeled peaks therefore saturate out slowly as a function of radiolabel. Also there are additional features that are otherwise not apparent in the companion silver-stained image. The lower images in Fig. 2 (a and b) are equivalent expanded views of the same regions that are either silver-stained or radiolabeled. The many additional radiolabeled features can be appreciated when compared with the companion expanded silver-stained image. Importantly most of the radiolabeled features that occurred in the absence of a companion silver-stained image lacked sufficient mass of protein to permit identification by conventional mass spectrometry.

Quantitative Comparison of Silver-stained and Radiolabeled Features between CF IB3-1 and Repaired IB3-1/S9 Cells—Thirty different experiments with IB3-1 and repaired IB3-1/S9 cells were performed over a 2-year period in which cells were pulsed with [35S]methionine and the proteins were separated on 2-D gels. The features visible by radiolabel were identified by superposition with known proteins as identified in the IB3-1/S9 proteomic atlas (13). Fig. 3 shows a comparison between individual protein masses (silver stain volumes) of 194 specific proteins in repaired IB3-1/S9 cells with the cognate proteins in the CF IB3-1 cells. Error bars for different proteins are based on analysis of all data. The filled red circles identify those proteins that differed between IB3-1 and IB3-1/S9 cells at the p < 0.05 level of significance. An equivalent comparison of radiolabeled features can be seen in Fig. 4. The distributions show that cognate radiolabeled proteins were distributed in similar fashion in both CF IB3-1 and repaired

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FIG. 1. Parallel analysis of CF lung epithelial IB3-1 and AAV-(wild type)CFTR-repaired IB3-1/S9 cells by silver stain and biosynthetic labeling with [35S]methionine. 

a, proteins from (wild type)CFTR-repaired IB3-1/S9 cells are separated on a pH 4–7 2DGE format. Proteins are imaged by silver staining.
b, proteins from CF IB3-1 cells are separated on a pH 4–7 2DGE format. Proteins are imaged by silver staining.
c, proteins from (wild type)CFTR-repaired IB3-1/S9 cells incubated for 30 min with [35S]methionine and separated on a pH 4–7 2DGE format. Proteins are imaged by autoradiography.
d, proteins from CF IB3-1 cells incubated for 30 min with [35S]methionine and separated on a pH 4–7 2DGE format. Proteins are imaged by autoradiography.

FIG. 2. Detail from three-dimensional rendering of 2DGE of IB3-1 cell proteome. Silver represents local features detected by silver stain. [35S]Met represents the same local region as detected by radiolabel. 

a, low magnification image of a region from a silver-stained 2-D gel. 
b, low magnification image of the same region as in a but imaged by 35S radiolabel. 
c, high magnification from the circled domain in a, silver stain. 
d, high magnification from the circled domain in b, radiolabel. The radiolabeled scan reveals ~5-fold more features than the silver-stained scan. The peaks of the radiolabeled features are graded compared with the chopped off forms of the silver-stained features.
IB3-1/S9 cells. Here again the filled red circles indicate proteins whose differences were significant at the \( p < 0.05 \) level. Importantly the significantly different silver-stained proteins were infrequently identical to significantly different radiolabeled proteins (see comparisons in Table I).

A comparison of silver-stained feature volumes and radiolabeled feature volumes is shown in Fig. 5. Although approximately linear, the \( R^2 \) value of this graph is visibly and analytically much greater than the individual comparisons of IB3-1 and IB3-1/S9 cells in Figs. 3 and 4. Clearly even for the IB3-1 cell alone, the data for silver-stained and radiolabeled proteins demonstrate substantial disparity.

Analysis of Silver-stained and Radiolabeled CF Proteomes Using a Hierarchical Clustering Algorithm—To investigate the informational basis of the substantial disparity between silver-stained and radiolabeled features in the IB3-1 cell system, we analyzed all 30 experiments using a hierarchical clustering algorithm. As shown in Fig. 6, this analytic approach clearly permitted the discrimination between IB3-1 and repaired IB3-1/S9 cells. Furthermore the analysis showed a clear discrimination between silver-stained and radiolabeled features. As with all displays of this sort, red marks are high values, and green marks are low values. The horizontal axis on the top of the image shows a primary dyad that separates silver from radiolabel and a secondary dyad that separates IB3-1 from IB3-1/S9 cells. On the vertical axis of identified proteins, the primary dyad distinguishes between an upper cluster of proteins with lower silver staining and higher \( ^{35}\text{S} \)methionine incorporation from a lower cluster of proteins with higher silver staining and lower \( ^{35}\text{S} \) incorporation. The upper cluster of low total mass proteins appears to be more biosynthetically active than the lower cluster of high mass proteins. Individual subdyads connecting various proteins identify those high abundance proteins whose expressions are coupled as a function of the CFTR mutation.

Further ImageMaster analysis of the proteins also allowed us to identify a subcategory of 51 CF-specific proteins whose expression of either total level or biosynthetic rate differed significantly (false discovery rate <10%) when comparing IB3-1 cells with (wild type)CFTR-repaired IB3-1/S9 cells. As shown in

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**FIG. 3.** Comparison of intensities of silver-stained features from CF lung epithelial IB3-1 with equivalent silver-stained spots on AAV-(wild type)CFTR-repaired IB3-1/S9 cells. Filled red circles denote proteins showing significant differences between cells at the \( p < 0.05 \) level.

**FIG. 4.** Comparison of intensities of radiolabeled features from CF lung epithelial IB3-1 with equivalent radiolabeled features on AAV-(wild type)CFTR-repaired IB3-1/S9 cells. Filled red circles denote proteins showing significant differences between cells at the \( p < 0.05 \) level.

**FIG. 5.** Comparison of intensities of silver-stained features from CF lung epithelial IB3-1 with equivalent radiolabeled features. Filled red circles denote proteins showing significant differences between cells at the \( p < 0.05 \) level.
Table I, we have distinguished the functional significances of these proteins in terms of their respective gene ontologies (GOs). The proteins are further color-discriminated in Table I by either an elevation (red) or a decrease (green) in either relative level of expression or biosynthetic rate. Of the 51 proteins, only six were similarly discriminated by both mass and biosynthetic rate. These are (i) keratin 18 (KRT18), (ii) hydroxymethylglutaryl-CoA synthase 1 (HMGCS1), (iii) ubiquitin carboxyl-terminal hydrolase 1 (UCHL1), (iv) translationally controlled tumor protein (TCTP), (v) guanyl cyclase-activating protein 3, and (vi) HSP27 (HSPB1). As will be seen later in the connectivity map (viz. Fig. 7), the literature indicates that two of these proteins (KRT18 and HSP27/HSPB1) actually interact with CFTR.

**DISCUSSION**

These data show that there is significant disease-specific kinetic information in the high abundance proteome of CF cells that can be retrieved by proteome-wide measurement of the individual rates of protein biosynthesis. We found that there were only six examples of overlap between the class of proteins whose total levels, defined by difference in silver stain, distinguish disease from control, and the class of proteins whose rates of biosynthesis also distinguish disease from control. These six proteins are KRT18 (Type 1), HMGCS1, UCHL1, TCTP, GUCA1C, and HSP27/HSPB1. Of the 51 high abundance proteins that significantly distinguished the CF from control proteome in the IB3-1 cell system, 15 were exclusively contributed by differential silver staining, and 30 were exclusively contributed by differential biosynthetic rate. An important methodological contribution from this novel approach to disease proteomics is the ability to simultaneously identify proteins whose abundance and rates of biosynthesis are modified by the CF mutation. These data cannot otherwise be captured using the conventional...
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Fig. 7. Connectivity map for high abundance CF proteome. A literature-based connectivity analysis of the 51 high abundance proteins indicates that 17 (33%) of them are linked to the inflammatory process either directly or through NFκB. IL-8 by ELISA assay is massively elevated in IB3-1 cells relative to CFTR-repaired IB3-1/S9 cells. IL-8, an 8-kDa protein, is too small to be detected on the 2-D gel. Proteins highlighted in green are elevated in IB3-1 cells (CF). Proteins highlighted in red are reduced in IB3-1 cells. Purple lines (connected by purple balls) indicate documented protein-protein interactions. Blue lines (connected by blue squares) indicate documented regulatory interactions. Some binding reactions (e.g. KRT18 and HSPA8 with CFTR) are also regulatory. The map is documented by 56 literature citations, which are actively accessible at 162.129.32.44. ANSA, N-acetylneuraminic acid synthase; TUBB, tubulin β polypeptide.

Proteomic Comparison of the IB3-1 Cell Line with Other CF Model Systems—Only a few attempts to analyze the proteomic signatures of CF model systems have been reported (19–21). In one recent report, HeLa cells were transfected with wild type or ΔF508-CFTR, and high abundance mass differences were evaluated (19). Two proteins, KRT8 and KRT18, were detected as being reduced in expression in the ΔF508-CFTR-transfected HeLa cells. In the present study of the IB3-1 cell system, we observed no change in KRT8. However, we did observe a significant reduction in KRT18 in the IB3-1 cell relative to the repaired IB3-1/S9 cell in both relative mass and relative biosynthetic rate.

In a recent pharmacoproteomic study in IB3-1 cells of the effects of 4-phenylbutyrate, a candidate CF drug, we reported 85 differentially expressed high abundance proteins (20). Two of the drug-dependent changes, KRT18 (reduced) and HSP70 (elevated), paralleled statistically significant changes reported here to be caused by gene transfer of (wild type)CFTR in the IB3-1/S9 cell line. Thus a reduction in KRT18 by either gene transfer or candidate drug seems to be a common theme in an admittedly limited sample.

At the level of the cfr−/− knock-out mouse, proteomic analysis of colonic crypt tissue and whole lung tissue has been described recently (21). The only significant difference detected was an elevated calcium-activated chloride channel, mOICAA3. The CF-relevant pathology of the cfr−/− knock-out mouse is said to be limited principally because of the expression of a mouse-specific alternative calcium-activated chloride channel in lung and elsewhere. By contrast, the human lung lacks a human equivalent of the mouse calcium-activated chloride channel and therefore suffers from CF-related lung pathology. An overlap with human CF cells would be unexpected and was not found. A recent study has also been reported that was limited to genomic changes in whole mouse lung tissue in response to either wild type or mutant human CFTR (22). Again no parallels occurred with the proteomic changes reported here in human lung CF epithelial cells.

Advantages of Radiolabels over Mass Labels for Global Analysis of Protein Kinetics—Previous attempts to label whole proteomes isotopically have principally been with mass labels (23–26). Mass labels have yielded specific information on relative levels of expression using mass spectrometry. However, the cumbersome technologies needed for this analysis and the relatively low signal-to-noise ratio have compromised the usefulness of mass labels for global analysis of biosynthetic rates. By contrast, radiolabels have the intrinsic property of being immediately available for global analysis on 2-D gels. However, the application of radiolabels to proteomics has been difficult because of the problem of identifying the labeled feature. The most efficiently radiolabeled features on 2-D gels have tended to be among the low abundance proteins, and therefore are virtually inaccessible to identification by mass spectrometry (27–30). In fact, from our analysis of the recent literature, only 32 radiolabeled proteomic features on 2-D gels have ever been specifically identified. Consequently global quantitation has not been an option. However, by limiting our investigation to the 194 known high abundance proteins in CF cells, we were able to work within this limitation and increase the number of identified, radiolabeled proteins by 5-fold. The limitation of our approach is that the analysis is biased toward the high abundance proteome. Yet we now know the identities of so many of the high abundance proteome features in CF lung epithelial cells that the study clearly edges into the realm of discovery science.

Functional Connectivity between Elements of the CF Proteome—The 51 CF-specific high abundance proteins in the
IB3-1 cell system that are most significantly affected by gene therapy with (wild type)CFTR can be analyzed by either gene ontology (GO, Table I) or by literature-based connectivity. The gene ontology analysis indicated that 32% of the proteins are associated with signal transmission and cytoskeletal components. The cytoskeleton has long been appreciated for its extensive history of regulatory interactions with CFTR (31–34) and signal transmission, especially focusing on intrinsic activation of NFXb signaling and IL-8 expression (8, 35). Proteins associated with stress, chaperone, and proteasome activities make up another 28% of the 51 CF-specific proteome elements. Possibly these activities are associated with the well known trafficking defect of (∆F508)CFTR directly or indirectly (36) or with proteasome-dependent activation of NFXb signaling (9). Thus ∼60% of the high abundance CF proteome, both in the IB3-1 cell system and in explants from CF lung epithelium, fall into these functionally relevant categories.

Quite another approach to interpreting these CF proteomical components is to consider connectivity analysis based on the literature database (15, 37). As shown in Fig. 7, we found that of the 51 CF-specific high abundance proteins in the IB3-1 cell system 17 (33%) of them are linked to the inflammatory process, as defined by high IL-8 expression, either directly or indirectly through the NFXb signaling pathway. Connectivity analysis can be used quantitatively by asking whether there is relative enrichment in a given dataset compared with the entire database. For example, the database has 49,731 proteins of which 3,261 have citation links to NFkB. Thus NFXb links to 6.6% of the database. By contrast, 11 of the 51 CF-specific high abundance proteins (20%) are linked by connectivity analysis to NFXb. This distribution is significantly different from what would be expected in a randomly generated list of proteins ($\chi^2$, $p < 0.001$).

The NFXb connection is therefore extremely significant, and the data show that RelB, a member of the NFXb family, is significantly reduced in expression in the CF lung epithelial cells. RelB has some remarkable biological properties that clearly could have relevance for CF pathophysiology. For example, in non-lymphoid cells such as fibroblasts in the mouse, RelB plays an essential role in the regulation of expression of proinflammatory mediators (38, 39). Consistently in RelB−/− fibroblasts, lipopolysaccharide induces a dramatic and persistent overexpression of seven chemokines, including MIP-2 and KC (keratinocyte chemoattractant), which are the IL-8 equivalents in the mouse (38). RelB is also regulated differently from RelA (viz. NFXb and p65) in that it is stimulated by the alternative IKKα pathway rather than by the canonical IKKβ and IKKγ pathway (40). If a biological parallel were to exist in human CF lung epithelial cells, the low expression of RelB would have the morbid consequence of sustaining proinflammatory signaling.

With specific respect to CFTR, the connectivity map shows that six elevated proteins are directly connected to each other by binding reactions and by this connectivity chain to CFTR. Some of these binding reactions are also regulatory. For example, it has been reported that KRT18 binds to CFTR, and that lowering the KRT18 level increases the delivery of mutant CFTR to the plasma membrane (19). Conceivably the low level of KRT18 in CF cells could be a compensatory mechanism to promote trafficking of mutant CFTR. The chaperone pair Hdj-2/Hsc70 (also known as HSPA8) interacts with and facilitates early steps in CFTR biogenesis (12, 40–42). However, elevated HSPA8, by arresting mutant CFTR in the endoplasmic reticulum, might just be making the trafficking problem worse. All of the connectors in the connectivity diagram can be clicked to access the relevant literature citations (see Fig. 7). The entire map is documented by 56 literature citations.

Conclusions—We conclude that de novo biosynthetic labeling on a global scale can be used to identify disease-specific kinetic differences within the high abundance cystic fibrosis proteome that would otherwise have been obscured from conventional analysis. We anticipate that this novel approach to discovery of the hidden CF proteome will find general application to other proteomic problems in biology and medicine.

* This work was supported by National Institutes of Health Grants RO-1-DK 53051-07 (to H. B. P.) and NO1-HV 28187 (to H. B. P.) and the Cystic Fibrosis Foundation (to H. B. P.) and in part by the Intramural Research Program of the National Institute of Mental Health (Grant ZO-1-MH-00388-29 LCS to D. M. J.J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ The on-line version of this article (available at http://www.mcponline.org) contains supplemental material.

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