Proteomic Profiling of β-cells Using a Classical Approach – Two-dimensional Gel Electrophoresis

Introduction

Pancreatic β-cells produce the insulin hormone and store it within secretory granules. Elevated glucose levels trigger the fusion of secretory granules with the plasma membrane and the extracellular release of insulin. This, in turn, lowers the glucose levels in the blood by promoting glucose uptake into the cells. Impaired insulin secretion and diminished response to insulin signaling cause type 2 diabetes, while the complete lack of insulin resulting from the autoimmune destruction of β-cells is responsible for the onset of type 1 diabetes. Elucidating the mechanisms presiding insulin production and secretion is therefore critical for the understanding and treatment of this common disease. To this aim, many laboratories have been investigating how the exposure of β-cells to glucose and various hormones and cytokines changes their gene expression profile.

Genomics vs. proteomics: advantages and limitations

cDNA microarrays are widely used for large screens of gene expression profiling in many cells and tissues. Hence, numerous of such studies have been performed in pancreatic islets, β-cells and insulinoma cells using different conditions and cDNA chips (Kaestner et al., 2003; Mazzarelli et al., 2006; Scearce et al., 2002; Schuit et al., 2002; Van Lommel et al., 2006). Despite the wealth of information generated through these studies and the increasing affordability and reliability of microarrays, this approach alone cannot provide a complete picture, since the levels of an mRNA and the corresponding protein may vary significantly (Gygi et al., 1999). Divergences between gene and proteomic profiling can also result from different detection sensitivities, experimental design and sample preparation, post-transcriptional and translational regulation, alternative splicing, post-translational modifications and different stabilities of an mRNA and the corresponding product (Sparre et al., 2005). Proteomic studies are therefore necessary to yield complementary information to gene expression.

Key words
- beta cells
- diabetes
- autoimmunity
- proteomics
- glucose regulation
- two-dimensional differential gel electrophoresis
data and gain insight about the adaptive changes of complex systems such as living cells.

2-DE and MS for proteomic studies

The proteome of a mammalian eukaryotic cell consists of ~10,000–30,000 proteins, with the number of expressed polypeptides exceeding that of genes due to alternative splicing and co- and post-translational modifications. A powerful and commonly used approach to study proteomic expression is two-dimensional gel electrophoresis (2-DE), and especially difference gel electrophoresis (DIGE), followed by mass spectrometry (MS) for protein identification (Gorg et al., 2004). The basic technology for 2-DE was illustrated by O’Farrell in 1975 (O’Farrell, 1975) and it is based on the separation of proteins according to their charge (isoelectric point, pI) and size (molecular weight) in the first (isoelectric focussing, IEF) and second dimension (SDS-PAGE), respectively. Routinely, ~2,000 proteins can be simultaneously resolved in a single 2-DE, depending on the chosen pH gradient and gel size (Gorg et al., 2004). To achieve an even greater resolution, several overlapping narrow-range pH gradients can be used (Westbrook et al., 2001), but even this approach does not allow the detection of low abundance proteins (Gygi et al., 2000), at least without restoring to immunoblotting. To overcome this limit, samples can be pre-fractioned by RP-HPLC to decrease the complexity of the protein mixture (Van den Bergh et al., 2003b). Another major drawback of 2-DE is the resolution of membrane and large hydrophobic proteins, since most of them are not soluble in solution containing a single non-ionic or zwitterionic detergent (Luche et al., 2003) and thus do not enter the gel during the first dimension (Luche et al., 2003; Van den Bergh et al., 2003b). The overall consequence is that only a relative small percentage of the whole proteome can be effectively examined by 2-DE followed by mass spectrometry. Despite these limitations, 2-DE remains a very powerful technique and certainly the most commonly used for proteomic studies. A schematic overview of the approaches used for 2-DE and 2-D DIGE followed by MS is shown in Fig. 1.

The two-dimensional difference gel electrophoresis (2-D DIGE) was first developed by Unlü and colleagues (Unlu et al., 1997). In this particular application, the protein mixtures are labeled prior to 2-DE by amino reactive fluorescent dyes (Cy3 and Cy5), which are size- and charge-matched and possess distinctive fluores-
cence spectra. Therefore, this method facilitates a direct and reproducible comparison between protein samples and it overcomes the disadvantage of gel-to-gel variations (Van den Bergh et al., 2003a). Additionally, an internal pooled standard labeled with Cy2 is used for normalization across multiple gels and further reduces experimental variation, thereby increasing the accuracy of measurements and the statistical confidence concerning differences in the protein expression (Alban et al., 2003; Marouga et al., 2005; Van den Bergh and Arckens, 2004). The reliability of the quantitative comparisons is also strengthened by the linearity, sensitivity and wide dynamic range of the CyDyes, which are superior relative to those of silver staining (Marouga et al., 2005). The main drawback of the DIGE technique is that proteins are labeled with different efficiencies depending on their lysine content. Accordingly, an abundant protein that is easily visualized by Coomassie Blue or silver staining may be barely detectable with the DIGE labeling because of its low lysine content (Zhou et al., 2002).

Other proteomics procedures

Additional methods for proteomics exists that can complement 2-DE, but which are also time-consuming and costly, even more than 2-DE. In this regard, two main additional proteomics technologies can be distinguished: MS-based techniques and array-based proteomics (de Hoog and Mann, 2004). MS-based proteomics such as liquid-chromatography electrospray ionization (ESI)-MS (LC-MS) or LC-MS/MS, that are preferred for the analysis of complex protein mixtures (Aebersold and Mann, 2003). For quantitative proteomics, LC-MS/MS can be combined with (1) stable isotope labeling by amino acids in cell culture (SILAC), which consists in the differential labeling of proteins in cultured cells through the incorporation of a “light” or “heavy” form of a given amino acid (Aebersold and Mann, 2003; Ong and Mann, 2005); or with (2) amine-reactive isobaric tagging reagents using a chemical labeling strategy (also referred to as iTRAQ). The special advantage of this technology is to allow the parallel quantitative proteomic analysis of four different samples (Aggarwal et al., 2006; Ross et al., 2004).

The antibody array-based proteomics delivers another tool for quantitative assessments. Although it requires capture antibodies immobilized to a surface and labeling of the cellular proteins, this has become an important approach for complementing MS-based proteomic analysis of specific proteins (Haab, 2006; Lv and Liu, 2007).

Proteomics of pancreatic islets and insulinoma cell lines: general considerations

To gain insight into how exposure of β-cells to conditions associated with the pathogenesis of diabetes, such as chronic hyperglycemia and inflammation, affect their protein expression, proteomic studies have mainly focused on changes induced upon long-term incubation of β-cell models with high glucose or cytokines. Alternatively, 2-DE reference maps were generated to provide useful information for future studies of the disease pathogenesis. Table 1 shows an overview about some recent proteomic studies performed in β-cells. Analyses of the islet proteome were mostly conducted using the broad pH 3–10 range, while the availability of larger samples in the case of insulinoma cell lines facilitated the choice of the narrower and more analytic ranges between pH 4–7 and pH 6–9. The output of a screen is influenced not only by the chosen pH gradient, and especially its length for separation, but also by the threshold-settings and the sensitivity of the detection method. Depending on the starting material and the chosen parameters the number of detected spots in these studies varied between ~300 and ~3000 (Table 1). Similar in all studies was the yield of proteins identified by MS, which usually ranged between 60% and 80%, whereas the failure in the identification was mainly due to insufficient protein amount or limitations of the queried databases.

A recurring concern of proteomic analyses on purified islets relates to their heterogeneous composition in regard to endocrine cells and to presence of other cell types, including acinar and duct cells as well as blood vessel cells. Albeit attenuated, the problem of these “contaminations” persists even when using dissociated primary β-cells. Moreover, the purity of islets and primary β-cells varies among different preparations. Similar considerations clearly apply also to DNA microarray studies (Van Lommel et al., 2006). Comparative genomic and proteomic studies on human islets should be handled even more cautiously due to the variability introduced by individual changes and by the less controlled conditions that can be applied for the procurement of tissues from humans relative to laboratory animals. To partially obviate these limits proteomic studies have also been performed in pancreatic β-cell lines, especially the mouse insulinoma MIN-6 cells and different rat insulinoma INS-1 cell clones. These lines are often used as surrogates for primary β-cells because they are more homogeneous and abundant and their preparation is less time consuming and expensive. However, as already shown by studies on gene expression (Van Lommel et al., 2006), their proteomic profile can be significantly different from that of primary β-cells.

Proteomic studies in pancreatic islets

In 2001, Sanchez and colleagues began to create the first 2-DE reference map of mouse pancreatic islets (Sanchez et al., 2001) for the SWISS-2D PAGE of the ExPASy (Expert Protein Analysis System) proteomics server (Gasteiger et al., 2003), which also allows easy access to other groups. They identified 63 spots corresponding to 44 proteins from mouse C57Bl/6j islets (Sanchez et al., 2001). This reference map was further used to analyze proteomic changes in islets between obese C57Bl/6j lep/lep mice and lean littermates (Sanchez et al., 2002). Six proteins were found to be up-regulated and three to be down-regulated. Treatment with rosiglitazone, an insulin-sensitizer drug, significantly modulated the levels of four of these proteins in lep/lep mice towards the levels of lean mice, including the actin-binding proteins tropomyosin isoform 1, profilin and profilin fragment, which may affect insulin granule trafficking and secretion. Additionally, rosiglitazone increased carboxypeptidase B precursor in both lep/lep and normal mice, which could participate in the processing of insulin (Sanchez et al., 2002). Next, Nicolls et al. identified 88 proteins of normal adult mouse islets, including some with chaperone activity or in common with neurons, which were not previously known to be expressed in islets (Nicolls et al., 2003). The Bergsten laboratory has reported the proteomic changes induced by stimulation of mouse islets with high glucose for 24h. In that study, 77 regulated proteins were identified, 28 of which could be added to the list of previously
<table>
<thead>
<tr>
<th>β-cell type</th>
<th>Experimental design</th>
<th>Detection method</th>
<th>pH gradient</th>
<th>No. of detected spots</th>
<th>Threshold</th>
<th>No. of regulated spots increased</th>
<th>No. of regulated spots decreased</th>
<th>No. of identified spots/unique proteins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse islets (C57BL/6j)</td>
<td>total</td>
<td>silver/coomassie staining</td>
<td>pH 3.5–10 NL, 18 cm</td>
<td>9–16%</td>
<td>2528</td>
<td>–</td>
<td>–</td>
<td>63/44</td>
<td>(Sanchez et al., 2001)</td>
</tr>
<tr>
<td>mouse islets (C57BL/6j)</td>
<td>CS7B/6j vs. C57B/6j lep/lep</td>
<td>silver/coomassie staining</td>
<td>pH 3.5–10 NL, 18 cm</td>
<td>9–16%</td>
<td>&gt;2500 (2528)</td>
<td>&gt;2</td>
<td>3</td>
<td>6/9</td>
<td>(Sanchez et al., 2002)</td>
</tr>
<tr>
<td>mouse islets (BALB/c)</td>
<td>total</td>
<td>coomassie staining</td>
<td>pH 3–10</td>
<td>10%</td>
<td>n.s.</td>
<td>–</td>
<td>–</td>
<td>up to 150 excised / 88</td>
<td>(Nicoll et al., 2003)</td>
</tr>
<tr>
<td>mouse islets (C57BL/6j)</td>
<td>freshly isolated vs. 24 h 11 mM Glc</td>
<td>SYPRO ruby/silver staining</td>
<td>pH 3–10 NL, 11 cm</td>
<td>8–16%</td>
<td>1074 vs. 1254</td>
<td>&gt;2</td>
<td>187</td>
<td>192</td>
<td>124 / 77</td>
</tr>
<tr>
<td>mouse islets (Kunming)</td>
<td>7 days with or w/o MLD-STZ</td>
<td>silver/coomassie staining</td>
<td>pH 3–10, 17 cm</td>
<td>12%</td>
<td>~600</td>
<td>≥2</td>
<td>4</td>
<td>3</td>
<td>7 / 7</td>
</tr>
<tr>
<td>mouse pancreas (C57BL/6j)</td>
<td>diet-induced obesity/diabetes vs. normal</td>
<td>SYPRO Orange</td>
<td>pH 3–10, 17 cm</td>
<td>15%</td>
<td>~450</td>
<td>≥2</td>
<td>3</td>
<td>1</td>
<td>4 / 3</td>
</tr>
<tr>
<td>rat islets (WF)</td>
<td>24 h IL–1β</td>
<td>35 S-methionine labeling</td>
<td>pH 3.5–7; pH 6.5–10.5; 17.5 cm</td>
<td>10%</td>
<td>52</td>
<td>≥2</td>
<td>47</td>
<td>60 / 57</td>
<td>(Andersen et al., 1997; Larsen et al., 2001)</td>
</tr>
<tr>
<td>rat islets (BB-DP/WF)</td>
<td>24 h IL–1β</td>
<td>35 S-methionine labeling</td>
<td>pH 3.5–7; pH 6.5–10.5; 17.5 cm</td>
<td>12.5%</td>
<td>1815</td>
<td>–</td>
<td>22</td>
<td>60</td>
<td>51 / 45</td>
</tr>
<tr>
<td>rat islets (Wistar)</td>
<td>18–20 h with or w/o BL1282</td>
<td>silver staining</td>
<td>pH 3–10, NL</td>
<td>12%</td>
<td>~600</td>
<td>&gt;1.5</td>
<td>30</td>
<td>23</td>
<td>22 / 22</td>
</tr>
<tr>
<td>human islets</td>
<td>total</td>
<td>coomassie staining</td>
<td>pH 3–10, NL</td>
<td>8–16%</td>
<td>774</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>130 / 66</td>
</tr>
<tr>
<td>human islets</td>
<td>26h 5 mM Glc vs. 5mM Glc + 2 mM insulin</td>
<td>DIGE</td>
<td>pH 3–10, 24 cm</td>
<td>15%</td>
<td>&gt;2000</td>
<td>&gt;2</td>
<td>4%</td>
<td>9%</td>
<td>n.s. / 36</td>
</tr>
<tr>
<td>human islets</td>
<td>4 days with or w/o prolactin</td>
<td>coomassie staining</td>
<td>pH 3–10, 11 cm</td>
<td>12.5%</td>
<td>269 ± 8 vs. 283 ± 8</td>
<td>≥2</td>
<td>14</td>
<td>–</td>
<td>19 / 16</td>
</tr>
<tr>
<td>mouse MN–6 cells</td>
<td>Glc responsive vs. non-responsive</td>
<td>DIGE</td>
<td>pH 4–7, 24 cm</td>
<td>12.5%</td>
<td>3351</td>
<td>≥1.2</td>
<td>59</td>
<td>152</td>
<td>n.s. / 35</td>
</tr>
<tr>
<td>rat INS–1E cells</td>
<td>control vs. 4h and 24 h IL–1β + INF-γ</td>
<td>DIGE</td>
<td>pH 4–7, 24 cm</td>
<td>12.5%</td>
<td>2214 ± 164</td>
<td>≥1.1</td>
<td>1.1</td>
<td>total: 159</td>
<td>141 / 92 (both pH ranges)</td>
</tr>
<tr>
<td>rat INS–1E cells</td>
<td>control vs. 4h and 24 h IL–1β + INF-γ</td>
<td>DIGE</td>
<td>pH 6–9, 24 cm</td>
<td>12.5%</td>
<td>1641 ± 73</td>
<td>≥1.1</td>
<td>1.1</td>
<td>total: 40</td>
<td>141 / 92 (both pH ranges)</td>
</tr>
<tr>
<td>rat INS–1E cells</td>
<td>control vs. 4h and 24 h IL–1β + INF-γ</td>
<td>RuBPS</td>
<td>pH 4–7, 24 cm</td>
<td>12.5%</td>
<td>800</td>
<td>≥1.1</td>
<td>total: 305</td>
<td>86 / 75</td>
<td>(Fernandez et al., 2008)</td>
</tr>
</tbody>
</table>

n.s. = spot numbers were not specified in the study, NL = non-linear, WF = Wistar Furth rats, BB-DP = Bio-feeding diabetes-prone rats, MLD-STZ = multiple low-doses of streptozocin, RuBPS = Ruthenium bathophenanthroline disulfonate
known mouse islet proteins (Ahmed and Bergsten, 2005). The same group has recently produced the first 2-DE draft of the human islet proteome by identifying 130 spots corresponding to 66 proteins (Ahmed et al., 2005). The major classes of identified proteins corresponded to metabolic enzymes (25%), molecular chaperones (23%), structural and cytoskeletal proteins (17%) and proteins with nuclear functions and protein biosynthesis (10%).

The cytokine interleukin-1β (IL-1β) has been implicated in the death of β-cells in both type 1 (Mandrup-Poulsen, 1996) and type 2 diabetes (Donath et al., 2008). Several studies have therefore analyzed the influence of IL-1β on the expression and modification of islet proteins. In one study, islets of BioBreeding diabetes-prone (BB-DP) rats were exposed to 150 pg/ml IL-1β for 24 h, which induced changes in 82 out of 3185 spots (Christensen et al., 2000). For technical reasons, the same investigators re-identified the regulated spots in preparative gels of islets of Wistar Furth (WF) rats (Sparre et al., 2002), from which the BB-DP rat strain was originally derived. This approach allowed the identification of 45 proteins from 51 spots, which were then assigned to one of the following six functional groups: energy transduction and redox potential (17.8%); glycolysis and tricarboxylic acid cycle enzymes (13.3%); protein, DNA and RNA synthesis, chaperons, protein folding (31.1%); signal transduction, regulation, differentiation and apoptosis (13.3%); cellular defense (4.4%) and other functions (20%) (Table 1). In a previous study a similar exposure of WF islets to IL-1β allowed the separation of 105 regulated spots, 52 being up-regulated, 47 down-regulated and 6 de novo synthesized (Andersen et al., 1997). Sixty of these spots were later matched to 57 proteins, some of which generated several spots, possibly due to post-translational modifications (Larsen et al., 2001). While only 14 islet proteins were regulated in both BB-DP and WF rats by IL-1β, most of the other affected proteins exhibit similar functions (Larsen et al., 2001; Sparre et al., 2002). Of note, IL-1β increased the production of nitric oxide (NO) by inducing the expression of calcium-dependent inducible NO synthase (iNOS). Of the 105 IL-1β-regulated spots in WF islets, 23 changed following pharmacological inhibition of NO production, while 19 were affected by incubation with a NO-releasing compound. Thus, changes induced by IL-1β on islet protein expression are both NO-dependent and NO-independent, with the majority being NO-independent (John et al., 2000). Unlike IL-1β, insulin promotes β-cell survival and stimulates β-cell proliferation (Beith et al., 2008; Johnson et al., 2006). Following treatment of human islets with 2 nM insulin for 36 h, 13% of the detected spots changed, some being also regulated by stimulation with 25 mM glucose for 36 h. Insulin regulated proteins were involved in oxidative stress suppression and mitochondrial metabolism, cytoskeleton and apoptosis. Among the increased proteins was Bridge-1, a positive regulator and binding partner of Pdx1. This effect may contribute to the anti-apoptotic effect of insulin (Johnson et al., 2006). Another hormone with a beneficial effect on β-cell mass is prolactin (related to growth hormone), because it stimulates β-cell proliferation, insulin production and secretion as well as preventing β-cell death (Bordin et al., 2004; Fujinaka et al., 2007; Jensen et al., 2005; Nielsen et al., 2001). Stimulation of human islets with prolactin for 4 days up-regulated 14 proteins, of which 12 were identified and connected with metabolism (glycolysis and tricarboxylic acid cycle), protein synthesis, chaperone and ion channel function (Labriola et al., 2007). The study of these proteins may provide insights into insulin secretion and proliferation of adult β-cells.

Besides the changes induced by single cytokines and hormones, another interesting aspect, especially in relationship to the pathogenesis of type 1 diabetes, is how the islet proteome is affected by the induction of β-cell destruction in vivo. To this aim, comparisons were performed between mouse control islets and islets from mice which had been injected with multiple low-doses of streptozotocin (MLD-STZ) to kill β-cells and induce diabetes (Xie et al., 2008). Relative to control islets, seven proteins changed their expression levels in diabetic islets, three being down-regulated ( ATP synthase subunit beta, ATPB; peroxiredoxin-4, PRDX4; and ubiquinol-cytochrome-c reductase complex core protein 1, UQCR1) and four up-regulated (calreticulin, CRTC; lithostathine 1, LIT1; lithostathine 2, LIT2; and voltage-dependent anion-selective channel 1, VDAC1). VDAC1 was also increased in human islets exposed to prolactin (Labriola et al., 2007). Also relevant for β-cell destruction and type 1 diabetes is the comparison of proteomic changes associated with either allogeneic or autoimmune rejection of transplanted islets. A recent study in rats indicated that in the case of autoimmune insulitis 310 out of 2590 spots changed their expression, while only 53 spots were altered in the case of allogeneic rejection of transplanted islets. Notably only 4 spots (1%) were changed in both processes, suggesting the involvement of different cellular responses (Christensen et al., 2006). A better understanding of these findings will require the identification of the regulated proteins.

Type 2 diabetes and obesity are closely linked and are associated with impaired insulin secretion. One study, therefore, investigated a mouse model of diet-induced diabetes to analyze changes in the pancreatic proteome associated with the development of the disease. In comparison to non-diabetic pancreata, the 2-DE profile of diabetic pancreata differed significantly in 4 spots, 3 of which were up-regulated (2 spots of regenerating islet-derived 1, REG1; and 1 spot of REG2) and one down-regulated spot (glutathione peroxidase, GSHPX1). As these proteins are involved in proliferation of pancreatic β-cells and oxidative stress, their involvement in type 2 diabetes is plausible (Qiu et al., 2005). Another study analyzed the impact of BL11282 on the rat islet proteome. This imidazoline compound is a potential anti-diabetic drug, which increases glucose-induced insulin release. BL11282 induced the up-regulation of 30 spots and the down-regulation of 23 spots. The 22 regulated spots which were identified included proteins involved in protein folding, Ca2+ binding and housekeeping enzymes (glycolysis) or inhibitors of enzymes (Jagerbrink et al., 2007). Interestingly, one of the proteins, i.e. CRTC, was also increased in MLD-STZ induced diabetic mice (Xie et al., 2008), suggesting that some proteins and the related pathways may be affected in both type 1 and type 2 diabetes.

### Proteomic studies in insulinoma cells

Dowling and colleagues compared glucose-responsive (low passage) vs. glucose non-responsive (high passage) MIN-6 cells to analyze possible mechanisms involved in the progressive loss of glucose-regulated insulin secretion. Out of 3351 spots detected in the pH range 4–7, 59 spots were increased and 152 spots were decreased using a very low threshold of ±1.2 fold difference. Thirty-five differentially expressed proteins were identified. Most
Interestingly, a major group of proteins associated with the endoplasmic reticulum (ER) was decreased in high passage MIN-6 cells. These proteins, namely ER protein 29 (ERP29), 78-kDa glucose-regulated protein (GRP78), 94-kDa glucose-regulated protein (GRP94) and protein disulfide isomerase (PDI), are implicated in protein folding and secretion in the secretory pathway, and thus their reduced expression may contribute to the decreased glucose responsiveness of high-passage MIN-6 cells (Dowling et al., 2006). Another study compared the expression profile of INS-1 832/13 cells exposed to 2.8 mM glucose vs. 16.7 mM glucose for 48 h to investigate potential changes induced by glucotoxicity. On average, 800 spots were detected, of which 305 (38.1%) varied significantly. Approximately 60% of the differentially expressed spots were identified, but only 86 of them contained a single protein, thus resulting in the identification of 75 regulated proteins. Only 11 of the regulated proteins were involved in metabolism, including the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which surprisingly was down-regulated (Fernandez et al., 2008).

INS-1E cells have been analyzed in regard to proteomic changes induced by the cytokines IL-1β and interferon-γ (INF-γ). Although similar studies had already been performed in isolated islets (Andersen et al., 1997; Christensen et al., 2000; John et al., 2000; Larsen et al., 2001; Sparre et al., 2002), the use of cultured insulinoma cells has allowed D’Hertog and coworkers to perform more complex investigations, perhaps with a greater reproducibility. Specifically, they compared the proteomic profiles of INS-1E cells exposed to cytokines IL-1β and INF-γ, either separately or in combination, for different length of times (1, 4 and 24 h) and in two different pH ranges (pH 4–7 and pH 6–9). Only minor proteomic alterations were detected upon short exposures (1 and 4 h) to a single cytokine, whereas drastic changes were observed after long exposure (24 h) of cells to both cytokines. In total 199 spots were regulated by cytokines, 70.9% of which were identified, corresponding to 92 distinct proteins implicated in multiple functions, including metabolism, insulin secretion, cytoskeleton organization, RNA synthesis and turnover and oxidative stress (D’Hertog et al., 2007).

All the aforementioned studies focused mostly on proteomic changes induced in pancreatic islets and insulinoma cells following their long-term (≥ 24 h) exposure to glucose or other agents. However, increased levels of insulin and other proteins of the insulin secretory granules can already be detected following a short (≤ 2 h) stimulation of β-cells with glucose and cAMP-inducing agents, such as IBMX (Alarcon et al., 1993; Guest et al., 1991; Guest et al., 1989). This rapid up-regulation of insulin granule biogenesis is mostly due to the activation of post-transcriptional mechanisms (Hinke et al., 2004; Itoh and Okamoto, 1980; Suckale and Solimena, in print; Welsh et al., 1985) including increased stability and translation of mRNA encoding SG components (Greenman et al., 2005; Wicksteed et al., 2003; Wicksteed et al., 2007). An important factor for these processes is the recruitment of polypyrimidine tract-binding protein 1, an mRNA binding protein, which stabilizes and increases the translation of mRNAs encoding insulin granule components (Knoch et al., 2004; Knoch et al., 2006; Tillmar et al., 2002). Our laboratory has therefore used 2-D DIGE and MS to analyze the rapid proteomic changes elicited by the stimulation of INS-1 cells with glucose and IBMX for 2 h (Süß et al., submitted). We have found that mRNA binding proteins represent the major class of molecules that are rapidly affected by these stimuli. These findings support the idea that mRNA binding proteins play a relevant role in the rapid adaptation of β-cells to glucose and other stimuli.

Another important question concerns the proteome of β-cell granules, as these organelles are responsible for insulin storage and secretion. To address this question, the composition of MIN-6 subcellular fractions enriched either in granules or in ER and Golgi complex were compared by 2-DE and MS. As expected, the latter fraction displayed typical ER proteins, while in the granule fraction only few proteins could be identified, including ion pumps and motor proteins (Rutter et al., 2006). This result is not entirely surprising, since most granule proteins are poorly resolved by 2-DE due to their high hydrophobicity. An alternative approach using nano-dimensional SDS-PAGE in combination with nano-LC-ESI-MS/MS allowed the identification of 130 proteins in an INS-1E subcellular fraction enriched in granules. Most of the proteins were classified into two groups: (1) intravesicular proteins and (2) membrane proteins (Brunner et al., 2007). Interestingly, 110 of these proteins had not been previously shown to be associated with granules. On the other hand, even with this approach some known components of the granules, such as prohormone convertase 1 (PC1/3) and islet cell autoantigen 512 (ICA512/IA-2), could not be detected.

The need for standardization and data accessibility

It is clear that the different studies described here are hardly comparable due to the large discrepancies in the 2-DE procedures that have been applied, are illustrated in Table 1. However, this limit does not apply only to analysis by 2-DE, but to proteomics investigations in general. Since 2001, the Human Proteome Organization (HUPO) spent significant effort in evaluating different procedures, proteomics methods and data analysis. Several pilot studies by HUPO Brain Proteome Project, in particular, have shown that similar but not completely identical protocols produce datasets that overlap only marginally due to differences in the conditions for sample preparation and handling, in the instrumentation, in the separation methods and in the search engines (Hamacher et al., 2008b). Hence, it is apparent that further advancement in the field will require the adoption of standard operation procedures and common rules addressing each of these potential sources for variability. In the case of human samples, due to the large variability among different individuals, the number of independent experiments should be especially large (Hamacher et al., 2008a; Hamacher et al., 2008b).

Also critical is the accessibility of proteomic datasets to the research community. PRIDE (Proteomics IDENTifications, <www.ebi.ac.uk/pride>) (Jones et al., 2008) and ProteomeCommons.org (<www.proteomecommons.org>), which was recently updated by addition of a FASTA file archive (Falkner et al., 2008), are two freely accessible repositories for MS-based proteomics data. A major specialized database for 2-DE data is the SWISS-2D PAGE (Hoogland et al., 2004), that contains several reference maps, such as those mentioned above. Notably, the 2-DE technology has generated an immense diversity of datasets and common formats are largely missing. Recently, the AGML (Annotated Gel Markup Language) format was developed and upgraded as a mean to provide a standardized format for 2-DE based proteomics (www.agml.org). A variety of raw data formats can be uploaded and converted into the AGML format, protocols can be

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interpolated, a connection to spot identification data by MS can be established and the results are accessible through the analysis page (Stanislaus et al., 2008).

Conclusions

The combined use of 2-DE and MS is a powerful strategy to analyze proteomic changes associated with different physiological and pathological conditions. Its major advantage relative to gene profiling screens is the possibility to detect post-translational modifications, which have profound influence on protein function. The major drawback is the ability to resolve only a limited fraction of the proteome, with the exclusion of membrane, large hydrophobic and low-abundant proteins. This approach is also labor-intensive and expensive. In the field of diabetes research 2-DE and MS have been used to investigate the proteomic changes elicited by exposure of islets and insulinoma cells to glucose, cytokines and other substances. Some islet proteins, and especially glycolytic enzymes, were most consistently found to change their expression in different studies. Overall, however, the results of these analyses are difficult to compare as each screening applied a different protocol. Thus, a major goal in this field is the development and adoption of standardized procedures for the resolution of the β-cell proteome.

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