On Marathons and Sprints: An Integrated Quantitative Proteomics and Transcriptomics Analysis of Differences Between Slow and Fast Muscle Fibers*

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Skeletal muscle tissue contains slow as well as fast twitch muscle fibers that possess different metabolic and contractile properties. Although the distribution of individual proteins in fast and slow fibers has been investigated extensively, a comprehensive proteomic analysis, which is key for any systems biology approach to muscle tissues, is missing. Here, we compared the global protein levels and gene expression profiles of the predominantly slow soleus and fast extensor digitorum longus muscles using the principle of in vivo stable isotope labeling with amino acids based on a fully lysine-6 labeled SILAC-mouse. We identified 551 proteins with significant quantitative differences between slow soleus and fast extensor digitorum longus fibers out of >2000 quantified proteins, which greatly extends the repertoire of proteins differentially regulated between both muscle types. Most of the differentially regulated proteins mediate cellular contraction, ion homeostasis, glycolysis, and oxidation, which reflect the major functional differences between both muscle types. Comparison of proteomics and transcriptomics data uncovered the existence of fiber-type specific posttranscriptional regulatory mechanisms resulting in differential accumulation of Myosin-8 and α-protein kinase 3 proteins and mRNAs among others. Phosphoproteome analysis of soleus and extensor digitorum longus muscles identified 2573 phosphosites on 973 proteins including 1040 novel phosphosites. The in vivo stable isotope labeling with amino acids-mouse approach used in our study provides a comprehensive view into the protein networks that direct fiber-type specific functions and allows a detailed dissection of the molecular composition of slow and fast muscle tissues with unprecedented resolution. Molecular & Cellular Proteomics 11: 10.1074/mcp.M111.010801, 1–16, 2012.

Skeletal muscles contain different types of fibers, which are responsible for specific biological properties and functions of individual muscles. Muscle fibers have been classified into slow type I and fast type II fibers mainly based on myofibrillar ATP staining and immunohistochemistry using specific antibodies (1).

Slow type I fibers show a red tint, contain high numbers of mitochondria, and their energy supply is mainly based on oxidative metabolism. These features enable slow fibers to execute long lasting contractions, which are essential for the maintenance of body posture. The primary function of type II fibers is the rapid contraction of muscles. Fast fibers are divided into three additional subclasses: Type IIb and IIx (also known as IIId) are glycolytic fibers, whereas type IIa fibers are more comparable to oxidative type I fibers (2). Type II fibers, which mainly derive their energy from glycolysis, are thus more susceptible to fatigue compared with Type I fibers.

Muscle fibers have also been classified based on the expression of different isoforms of myosin heavy chain (MyHC) proteins. Myosins are the major contractile proteins and their activation by ATP and Ca2+ ions results in shortening of muscle fibers. For example, slow type I fibers express MyHCⅠβ and the three fast fiber types express MyHCⅡa, MyHCⅡb, and MyHCⅡx (3), respectively. Although the MyHC-based classification is used most often, several other marker proteins for slow and fast muscles have been described. For example cardiac troponin C (Tnc1), a regulatory Ca2+ binding protein, is expressed in slow and cardiac muscle tissue, whereas Troponin C/STNC (Tnnc2) is predominant in fast type II fibers (4). Likewise, the calcium-ATPase pumps SERCA1 and 2, which are required for re-uptake of calcium into the sarcoplasmatic reticulum after contraction and for subsequent muscle relaxation (5) are also expressed differentially in slow and fast twitch fibers. SERCA1 is more abundant in fast...
twitch fibers, whereas SERCA2 is overrepresented in slow muscle fibers (6). Another example of an unequal protein distribution between fast and slow fibers is the catalytic A-subunit of the calcium-dependent serine-threonine phosphatase calcineurin, which shows predominant expression in fast fibers (7). Activation of calcineurin-dependent pathways plays an important role for muscle hypertrophy and fiber transition (8) in response to physical exercise, aging, or metabolic diseases such as diabetes (9, 10).

Our current knowledge of fiber type specific protein expression has been established primarily by traditional biochemical and immunohistochemical techniques. More recently, several cDNA-microarray based studies providing a more systematic and unbiased characterization of muscle tissues has helped to improve our understanding of muscle physiology and the mechanisms of fiber transition (11, 12). However, transcriptional profiles do not necessarily correlate with the steady-state levels of corresponding proteins and fail to give insight into signaling pathways such as e.g. by protein kinase cascades.

So far, most studies aiming at in-depth characterization of the skeletal or heart muscle proteomes have chosen an approach based on two-dimensional gel electrophoresis (13, 14, 15), which yielded a limited number of reliably identified proteins. Recent advances in mass spectrometry based proteomics, however, offer new options allowing identification and quantification of thousands of proteins (16, 17, 18). These new techniques have already been exploited in a few studies to characterize differences in protein abundance between different muscle tissues, including limb and extraocular muscles using one dimensional-gel or isoelectric focusing fractionation in combination with high mass accuracy liquid chromatography-tandem MS (LC-MS/MS) analysis (19, 20).

Here we report a large-scale quantitative analysis to compare the proteomes and transcriptomes of slow soleus and fast extensor digitorum longus (EDL)1 muscles of the mouse. Our analysis is based on the use of 13C6-lysine-labeled mice (‘SILAC-mouse’), which provides an internal protein standard. We quantified >2100 proteins in slow and fast muscles, of which ~25% showed a differential expression pattern. Comparison of proteomics data to mRNA expression profiles obtained from the same samples revealed the existence of specific post-transcriptional regulatory mechanisms in slow and fast muscle fibers although the majority of proteins and mRNA molecules showed a similar distribution. Furthermore, we identified 1040 novel class 1 phosphorylation sites indicating a widespread phosphorylation of sarcomeric proteins in skeletal muscle cells. All data were implemented into the newly developed Quantumus database and are readily accessible.

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1 The abbreviations used are: MyHC, myosin heavy chain; GO, Gene Ontology.

EXPERIMENTAL PROCEDURES

Materials and Reagents—Mouse diet substituted for 13C6-lysine was obtained from Silantes (Martinsried, Germany). Mice were generated in house from C57Bl/6 colony. All chemicals used for tissue extraction, digests, and liquid chromatography were purchased from Sigma-Aldrich and Carl Roth GmbH (Karlsruhe, Germany), except for lysyl endopeptidase (R), which was obtained from WAKO GmbH (Neuss, Germany).

Generation of 13C6-Lysine Labeled Mice—Mice fully labeled with 13C6-Lysine were generated as described (21), except that food pellets containing the heavy lysine were purchased from a commercial source (Silantes, Martinsried, Germany). Labeling efficiency was >96% in the F2 generation of mice maintained on a heavy lysine substituted diet as described previously. Ten-week-old individuals of the F2 generation were used for all experiments. The incorporation rate and labeling efficiency of heavy SILAC labeled mice was monitored in each experiment by assessing the average SILAC ratio on a subset of proteins from muscle tissue.

Sample Preparation—Mice were sacrificed by ketanest injection. The thorax was opened immediately after respiratory arrest before the heart was perfused with PBS via the left ventricle and the right ventricle was cut open for drainage. Successful perfusion and blood cell removal was assessed by observing the color change from red to grayish-white of blood rich organs such as lung and liver. Soleus and Extensor digitorum muscles were dissected from the hindlimbs of the animals, washed in PBS, and snap frozen in liquid nitrogen (frozen in liquid nitrogen (frozen in liquid nitrogen (frozen in liquid nitrogen (!heavy = 2, !light = 2). Muscles from heavy and light SILAC-labeled animals (5–13 mg wet weight) were mechanically homogenized in 250 μl of ice cold modified RIPA buffer containing 1% Nonidet P-40, 0.1% sodium deoxycholate, 50 mM Tris-HCl pH 7.5, 150 mM NaCl, and 1 mM EDTA supplemented with complete protease inhibitor mixture (Roche) using an Ultraturrax disperser (IKA, Staufen, Germany) and incubated for 5 min on ice to extract proteins. Alternatively, we used SDS lysis buffer (4% SDS, 100 mM Tris/HCl pH 7.6) to extract muscle fibers. To remove debris lysates were centrifuged at 19,000 x g for 10 min. Supernatants were collected and protein content was determined using detergent compatible Lowry protein assay (Bio-Rad DC). Fifty to 80 μg of a 1:1 mixture of heavy and light muscle RIPA extracts were then separated by gel electrophoresis on precast 4–12% NuPAGE gradient gels (Invitrogen, Carlsbad, CA) and stained with the Colloidal Blue Staining Kit (Invitrogen). Each lane was cut into 15 evenly sized gel pieces and processed for GeLC-MS/MS. Briefly, proteins within gel pieces were subjected to reduction and alkylation, followed by endopeptidase Lys C cleavage. Peptides were then extracted as described (22), desalted, and concentrated by Stage Tips (23). SDS lysates were subjected to the FASP protocol followed by Offgel separation as described in (24). For phosphopeptide enrichment we used a combination of (FASP) and strong-cation chromatography followed by titanium dioxide enrichment (25).

LC-MS/MS Analysis—Each sample, representing the peptide content of one gel piece was analyzed by nano-Reverse Phase Chromatography using an Agilent 1100 nanoflow system that was online coupled via in house packed fused silica capillary column emitters (length 15 cm; ID 75 μm; resin ReproSil-Pur C18-AQ, 3 μm) and a nanoelectrospray source (Proxeon) to an LTQ Orbitrap XL mass spectrometer (Thermo Scientific). Peptides were eluted from the C18 column by applying a linear gradient from 5–35% buffer B (80% acetonitrile, 0.5% acetic acid) over 150 min. The mass spectrometer was operated in the data-dependent mode, collecting collision induced MS/MS spectra from the five most intense peaks in the MS (LTQ-FT full scans from m/z 300 to m/z 1800; resolution r = 60,000; LTQ isolation and fragmentation at a target value of 10000). For the identification of phosphopeptides, an LTQ-Orbitrap Velos mass spectrometer was used and MS/MS spectra were generated by higher
RESULTS

Large-scale Quantification of Proteins in Slow and Fast Muscles Using SILAC-assisted Mass Spectrometry—For accurate protein quantification we used the stable isotope labeling of amino acid in cell culture approach (SILAC), which is based on the metabolic incorporation of non-radioactive amino acids in which carbon and/or nitrogen atoms are substituted by heavy isotopes (i.e. $^{13}$C$_6$-lysine) (28). Recently, we extended this technique to in vivo conditions in which a mouse is labeled with the $^{13}$C$_6$-lysine isotope (21). By feeding mice over two generations with a $^{13}$C$_6$ lysine diet, virtually all natural $^{12}$C$_6$ containing lysine amino acids are replaced by the heavy amino acid.

To identify and quantify proteins that are either predominantly found in fast and slow twitching muscle fibers or common to both muscle types we isolated fast (EDL muscle) and slow (soleus muscle) fibers from the lower leg of the mouse hind limb. In contrast to rats, where the soleus muscle consists mainly of type I fibers, soleus muscles in mice contain an equal amount of type I and type IIa/IIx fibers (29). Despite these restrictions we choose the soleus muscle as the slow muscle reference for our proteomic analysis because it contains the highest amount of type I fibers and the remaining type II fibers are mostly oxidative. Based on peak intensities (see methods), equal amounts of labeled soleus proteins were mixed with unlabeled soleus and EDL muscle extracts, respectively. As a control we performed a crossover experiment by mixing labeled EDL with unlabeled EDL and Soleus extracts (Fig. 1). Forward and crossover experiments were performed with two biological replicates each. To decrease sample complexity, proteins were separated by one-dimensional SDS-PAGE. Each lane was separated into 15 slices following Coomassie-blue staining and subjected to in-gel digest with LysC to obtain lysine only containing peptides. We used the filter aided sample preparation (FASP) protocol in combina-
tion with isoelectric focusing of peptides (Offgel, Agilent) to increase our identification rate for proteins with reduced solubility. In total, we analyzed 123 fractions by LC-MS/MS using an LTQ-Orbitrap XL or an LTQ-Orbitrap XL Velos (Thermo-Fisher Scientific) mass spectrometer. Using a false discovery rate <1% we identified 537,282 MS/MS spectra, which resulted in the identification of 28,924 peptides corresponding to 3447 proteins with at least one unique peptide (supplemental Table S1). Quantifications (e.g. SILAC ratios) were obtained for 2163 proteins between Soleus and EDL muscle tissue.

To facilitate data analysis and to provide public access to our skeletal muscle data set, an online proteomic database called QuantiMus was developed. This database contains all information obtained by using the MaxQuant software tool, including the number of identified peptides, unique peptides, Silac-ratios, PTMs and Mascot-scores (27). Both unregulated and regulated proteins are grouped into ratio bins. Data base searches create graphical overviews, which display localization of all identified peptides within a protein sequence (http://quantimus.mpi-bn.mpg.de). For the comparison of soleus and EDL muscles we selected only proteins that were quantified comparing Sol(h)/Sol(l) experiments against the EDL sample such as extracts of total muscle. To test whether the peak intensity of detected SILAC-pairs would influence the overall ratio distribution and thus accuracy of our quantitative determinations we plotted the sum of the light and heavy peak intensities against the corresponding log2 ratios. As shown in (Fig. 2A), the ratio distribution of labeled versus non-labeled soleus sample was close to 1, indicating accurate quantification within the whole range of peak intensities. Of note, the overall ratio distribution is more scattered between soleus and EDL compared with the control soleus and soleus ratio (Fig. 2A) illustrating quantitative differences in the proteome composition between both muscle fiber types. A similar observation is made when binned log ratios of all quantified proteins are displayed in a frequency distribution plot: The distributions centered more closely on a ratio of 1 for the comparison between light and heavy muscle fibers of the same type, although they are clearly more dispersed when different fiber types were compared (Fig. 2C). The reproducibility of our quantitative measurements appeared very high as indicated by the Pearson correlation coefficient between both experiments (0.86) that was obtained by plotting all log ratios obtained from the soleus (h)/EDL (l) experiment against the respective crossover ratios (EDL (h)/soleus (l)) (Fig. 2B).

Because extracts from the SILAC-labeled mouse served as an internal protein standard in our experiments we were able to calculate protein ratios between unlabeled soleus and unlabeled EDL simply by dividing ratio 2 through ratio 1 (see Fig. 1), which yields quantitative differences between both muscle types. Most detected proteins showed a ratio close to 1:1, which indicates equal amounts of proteins in both muscles. Myopodin, e.g., was detected with a Sol(h)/Sol(l) ratio of 1 and Sol(h)/EDL(l) ratio of 0.8, which finally resulted in a Sol(l)/EDL(l) ratio of 1.2. The crossover experiment showed for Myopodin also an EDL(l)/Sol(l) 1 ratio (Fig. 2D). Some proteins, such as the ATP citrate lyase were measured with a ratio of 4.3 between Sol(h)/Sol(l). However, a similar ratio of 4.9 was also determined comparing Sol(h)/EDL(l), resulting finally in a direct Sol(l)/EDL(l) ratio of 1.2. Similarly, Glutathione peroxidase 3 was detected with a Sol(h)/Sol(l) ratio of 0.4 and Sol(h)/EDL(l) ratio of 0.4, respectively, which again resulted in a SOL(l)/EDL(l) ratio of 1:1.1 (Fig. 2D). To estimate the total number of proteins that were differentially regulated between slow and fast muscle we calculated the geometric mean between the forward and crossover experiments and set the cutoff value to 1.5 or 2 (30, 31). Using this definition we identified 252 proteins that were enriched in the soleus and 299 proteins enriched in the EDL muscle (supplemental Table S1).

SILAC-assisted Mass Spectrometry Greatly Increases the Number of Known Slow and Fast Muscle Enriched Proteins—To confirm the overall quality of our in vivo-SILAC approach we first focused on proteins that are well described components of either slow or fast muscle fibers, for example, the sarcoplasmic/endoplasmic reticulum calcium associated ATPases 1 and 2 (6), which are important for the regulation of Ca\(^{2+}\) homeostasis. SERCA1 has been shown to be more abundant in fast twitch fibers, whereas SERCA2 is the predominant form in slow muscle fibers. Our analysis yielded very similar results indicating the validity of the mass spectrometry approach. Fig. 3 depicts mass spectra of SILAC-peptide pairs for SERCA1 (A) and 2 (B) as representative examples. The corresponding ratios of both proteins are listed in (Table IA, B). The identification of specific isoforms of myosin heavy chains (MyHC) by mass spectrometry presents a more challenging problem because extensive sequence homologies exist between different MyHC proteins (32). We took a conservative approach and used only unique peptides for protein quantification to avoid any misinterpretation of our data set (Fig. 4A), which reduced the number of quantifiable peptides considerably. For example, MyHCs-Iβ, -lα, -lβ, Myosin-8, and Myosin-13 share the peptide HADSVAELGEQIDLNQRKV (Fig. 4C), which therefore was not used for quantification. Only
24 unique peptides out of ~90 peptides which were measured from the MyHC-β protein group were used for quantification because of sequence identities. The measured ratio of 11:1 between Soleus versus EDL indicated greatly increased MyHC-β (gene: myh7) levels in the slow soleus compared with the fast EDL muscle (Fig. 4B, upper left two panels). We also identified increased levels of MyHC-IIa in soleus muscle compared with EDL (Fig. 4B, lower left two panels). In contrast, MyHC-IIb and MyHC-13 were more abundant in the EDL muscle (Fig. 4B, right hand panels), which is consistent with previous reports about the distribution of MyHC proteins (33). Similar results were obtained for several slow myosin light
chains (MyLC). In conclusion, the in vivo SILAC approach was able to generate a rapid and accurate estimation of the distribution of proteins in slow and fast muscle fibers even for molecules that are relatively difficult to distinguish by traditional biochemical means.

Pronounced quantitative differences between slow and fast muscle fibers were also found for several other proteins located in or near the Z-disc: the ankyrin repeat domain containing protein 2 (also known as mArpp) (34), which was described as a stretch sensor specifically expressed in type I muscle fibers, showed a top-ranking SILAC-ratio of 39:1 between soleus and EDL. FATZ-1 (also known as calsarcin-2/Myozemin-1) and FATZ-3 (calsarcin-3/Myozemin-3) (35) showed a strong enrichment in fast fibers whereas FATZ-2 (calsarcin-1/Myozemin-2) was more abundant in the soleus muscle corroborating previous reports. Differential accumulation in slow versus fast muscles was also found for M-band proteins that are required for the regular packing and organization of thick filaments. So far, three Myomesins, namely Myomesin-1 (also known as Skelemor or EH-myozemin) (36), Myomesin-2 (also known as M-protein) (37), and Myomesin-3 (38) have been localized to the M-band. Our results supported previous findings that Myomesin-2 is predominantly located in fast (EDL) and Myomesin-3 in slow muscles (soleus). In contrast, increased levels of Myomesin-1 were detected in the EDL, which corrects the view that Myomesin-1 is uniformly distributed in all skeletal muscle tissues.

So far, ~60 proteins have been described to be localized within sarcomeric structures mostly based on immunohistochemistry and two dimensional gel electrophoresis experiments (39, 40). In our single large-scale study we determined the relative distribution between slow and fast muscles for virtually all known sarcomeric proteins. Moreover, we were able to measure quantitative differences of a large group of additional muscle proteins in slow and fast muscles (Fig. 5A), which might help to resolve the molecular composition of the contractile apparatus and its specific properties in different muscle types.

**Bioinformatical Analysis Reveals Differential Enrichment of GO-terms in Slow and Fast Muscles**—To obtain a better insight into differences between slow and fast muscles, which are reflected by different protein concentrations we performed a comprehensive search for specific GO-terms, which were overrepresented among proteins differentially expressed in soleus and EDL. First, we used a GO-term analysis to identify enriched terms for “biological process,” “cellular localization,” and “molecular function” in soleus and EDL tissue. The soleus muscle showed a strong overrepresentation of categories for oxidative metabolism, fatty acid metabolic processes, and mitochondrial proteins, which mirrors its dependence on oxidative phosphorylation required for efficient generation of ATP (Fig. 5B). Furthermore, we detected several marker proteins typical for high oxygen consumption, such as Myoglobin (41) and carbonic anhydrase III (42). In contrast, analysis of EDL proteins revealed a clear overrepresentation of GO-terms for carbohydrate metabolic processes, epitomized by the detection of almost all glycolytic enzymes. Over-representation of sarcoplasmatic structures (GO: 0016529) emphasized the need of enhanced Ca\(^{2+}\) release and re-uptake in EDL muscles.

Although the differential distribution of the above-mentioned processes were not surprising and in line with previous reports we also detected a number of unexpected differences between both fiber types. Interestingly, several proteins of the extracellular matrix (ECM), including bigly-
can, a secreted proteoglycan, asporin, and periostin-2, which are secreted by muscle fibers, were all increased in the soleus muscle. Other proteoglycans, such as keratocan, and Glypican, were enriched in the EDL. Several other ECM proteins such as lumican fibromodulin, fibronectin, decorin, and several collagens did not show any significant changes.

Another class of enzymes that was markedly different between both muscle types were Glutathione S-transferases (GSTs), which are detoxifying enzymes (43) also involved in cell signaling (44). Four different GST enzymes, including Mu-7, Omega-1, kappa-1, and the microsomal GST-1 were enriched in the soleus muscle, emphasizing their role in the prevention of oxidative damage. In contrast, GST Mu1, 2 and 5 showed increased levels in EDL. The GST-Mu2 isoform was described as a regulator of the ryanodine receptor (RyR) (45). Similar protein concentrations in soleus and EDL muscles were determined for other GSTs including A4, P1, and theta1.

The presence of specific subsets of GST enzymes in slow and fast muscle fibers reflects potential differences in detoxification and GST-mediated signaling processes that may impinge on the overall physiology of both fiber types. We also observed enriched GO-terms for amino acid metabolism in EDL samples. The enrichment of 9 amino acid tRNA synthetases in fast muscle tissue might indicate a higher metabolic activity in the fast EDL muscle because of the increased mechanical “wear and tear” and a demand for higher protein turnover in these fibers.

Slow and Fast Muscles Show Different Protein Kinase and Phosphatase Expression Levels—Muscle contraction and relaxation requires phosphorylation and dephosphorylation of numerous proteins. Therefore, we were particularly interested to determine potential differences in fiber type specific expression of protein kinases and phosphatases. In total, we identified and quantified more than 122 kinases and 86 phosphatases. Apart from nearly all glycolytic kinases, we found a
10-fold higher expression of the calcium/calmodulin-dependent protein kinase type IIα (CaM) in the EDL compared with the soleus muscle supporting the importance of Ca\(^{2+}\) signaling for fast muscle fibers. The 5′-AMP-activated protein kinase also showed increased levels in the EDL. This enzyme is one of the immediate downstream targets of CaM kinase, suggesting its role in the regulation of muscle fiber types.
which acts as a metabolic master switch and regulates several metabolic pathways including glucose uptake and glycolysis. Similarly, phosphorylase kinase, an important regulator of glycogen homeostasis (46), was also detected at higher levels in the EDL underscoring the requirement of sufficient glucose supply in the EDL. Furthermore, we detected enriched levels of the ERK activator kinase 1, 2, 4, and 6 in the EDL, which belong to mitogen-activated protein kinases (MAPK). An important substrate of the MAPK signaling is the 90 kDa ribosomal S6 kinase, which was also enriched in the EDL muscle. The S6 kinase phosphorylates the ribosomal protein S6 and acts as an activator of protein biosynthesis indicating increased protein turnover in fast muscles.

Five dual specific phosphatases (DUSP), enzymes that are able to dephosphorylate both tyrosine and serine/threonine residues, were detected in our samples. Three of them were enriched in the EDL including DUSPD1, dual specificity phosphatase 3 (Dusp3), and dual specificity protein phosphatase 23 (Dusp23). In contrast, the protein phosphatase regulatory subunit 1A (Ppp1r1a), the phosphatase 1B (Ppm1b), the Inositol polyphosphate 4-phosphatase type II (Inpp4b) and the catalytic subunit of the Myo-inositol monophosphatase A2 (Impa2) were more abundant in the soleus muscle.

The distinct expression pattern of kinases and phosphatases, which distinguishes soleus from EDL, suggests significant differences of corresponding target proteins and the kinetics of phosphorylation and dephosphorylation. It seems reasonable to assume that such differential phosphorylation events are instrumental for different physiological processes in fast and slow muscles.

**Toward a Skeletal Muscle Phosphoproteome**—As a first step to establish a comprehensive skeletal muscle phosphoproteome we isolated phosphopeptides from EDL and soleus muscles by cation exchange chromatography, further enriched the phosphopeptides by TiO2 affinity purification, and subjected them to mass spectrometry. In total, we identified 2573 phosphorylation sites on 973 proteins with high accuracy and a localization probability > 0.75 (median localization probability 0.99) (supplemental Table S2). Comparison with entries from other phosphopeptide databases, such as www.Phosida.org (~25,000 phosphopeptides), L6 rat cell culture line (2230 phosphopeptides) (47), mouse atlas (48), and www.PhoosphoSitePlus.org (~94,000 phosphopeptides) revealed that 1040 candidate phosphorylation sites were novel. For example, we identified unknown phosphosites for several sarcomeric proteins, including sites for tropomyosins, for muscle creatine kinase, and for myosin heavy chain proteins. Interestingly, the overlap to other phospho-databases that feature different tissues was rather low suggesting the existence of tissue specific phosphorylation patterns. We found that the overall distribution of serine (~78%) and threonine (~18%) phosphorylation sites was similar to datasets obtained in other tissues (25, 47) (supplemental Fig. S1A). However, we monitored a clear increase of tyrosine phosphorylation sites of ~4% compared with 1–2% usually found in other cells and tissues (25, 47). For example, 42 novel tyrosine phosphorylation sites were detected on the giant protein titin. Mapping of these sites revealed a preferential accumulation of newly mapped tyrosine phosphorylation sites to functional domains, including the fibronectin type 3 like domain (FN3-like) and the PEVK domain (supplemental Fig. S2). Analysis of phosphorylation motifs by MaxQuant indicated that proline-dependent CK1/2 and PKA motifs were more abundant compared with other motifs (supplemental Fig. S1B). We would like to emphasize that the repertoire of phosphorylation sites obtained in this study represents a profound repository and starting point for future studies focusing on the regulation of cell signaling events in skeletal muscle. All identified phosphorylation sites were implemented in the Quantimus database.

**Comparison of RNA and Protein Expression Levels in Slow and Fast Muscles Indicates Multiple Levels of Regulation**—cDNA hybridization to microarrays is an important technology to obtain global views of transcript levels within cells or tissues. To investigate the correlation between mRNA transcript levels and protein abundances we performed hybridizations with Affymetrix Chip sets (mouse gene 1.0 ST Array; n = 3 for each muscle type) using RNA isolated from soleus and EDL muscles. To allow comparison of microarray probes to proteins we matched international protein database. Comparison of RNA and protein expression levels in slow and fast muscles indicates multiple levels of regulation. Within cells or tissues, to investigate the correlation between mRNA transcript levels and protein abundances, we performed cDNA hybridizations with Affymetrix Chip sets (mouse gene 1.0 ST Array; n = 3 for each muscle type) using RNA isolated from soleus and EDL muscles. To allow comparison of microarray probes to proteins, we matched international protein database.
genes (21%) showed changes in the same direction generating a Pearson correlation of 0.78 (Fig. 6D).

To assess the biological function of transcriptionally regulated genes a GO-term analysis was performed in a similar manner to the analysis of the proteomics data. Using a 1.5-fold change as cutoff we observed an enrichment for GO-terms for muscle contraction (GOBP: 0006936), for lipid metabolic process (GOBP: 0006629) and for fatty acid metabolic process (GOBP: 0006631) in the soleus. GO-analysis of EDL samples showed a similar enrichment of GO-terms as in our proteomics dataset including terms for carbohydrate metabolism and fast muscle contraction. Taken together the enrichment for similar GO-terms in our proteomics and mRNA expression analysis reflects similar regulations of mRNA and protein expression.
protein abundances for most of the analyzed genes (supplementary Fig. S4).

Interestingly, we found striking differences in protein and mRNA expression levels for several molecules indicating that the identity of slow and fast muscles is in part achieved by post-transcriptional regulation. For example, 10 genes showed higher mRNA levels in Soleus (five genes) and EDL (five genes), which was not matched by changes at the protein level (supplemental Table S5). In particular, we noted increased mRNA levels for two complement proteins in the Soleus, which was surprising given the fact that these proteins are secreted serum proteins. Similarly, α-protein kinase 3 also known as Myocytic induction/differentiation originator (Midori) (51) was enriched at the mRNA level in soleus but showed no concentration differences at the protein level.

Another group of genes showed elevated protein levels in either soleus or EDL muscles but had similar mRNA levels in both fiber types (fold change <1.5, p value >0.05). Most of the proteins with increased levels in the soleus but identical mRNA levels were localized to mitochondria (16 proteins) or within the membrane fraction (eight proteins). In addition, Myosin-8 protein was increased in the soleus but showed matching mRNA concentrations in either muscle (supplemental Fig. S5). Forty-three proteins were enriched in the EDL but did not show significant changes on the mRNA level. For example, five tRNAsynthetases, four secreted proteins, three kinases, and four membrane proteins including the EGF-receptor showed similar mRNA expression level but increased concentrations at the protein level. Furthermore, the M-band protein Myomesin-1 (Skelemin) and the extracocular myosin heavy chain EO-MyHC (Myosin, heavy polypeptide 13) were more abundant in the EDL as proteins compared with their mRNAs suggesting fiber-specific regulatory mechanisms for these sarcomeric proteins (supplemental Fig. S6). Our findings reveal the existence of regulatory events at the posttranscriptional or posttranslational level in fast and slow muscles and emphasize the complex relationship between gene expression and protein abundances, which help to shape the distinct phenotype of different muscle fiber types.

**DISCUSSION**

Numerous studies have described the ultrastructure and molecular diversity of skeletal muscle tissues after the initial recognition of differences between white and red muscle tissue by Ranvier in the year 1873. The general structure of muscle fibers is rather similar despite the presence of different fiber types and the ability of skeletal muscle fibers to adapt to different physiological demands. So far, the composition of skeletal muscle or individual muscle fibers was mostly assessed in a qualitative manner Only limited numbers of proteins were quantified. Very few proteomics studies attempted to quantify proteins in muscle tissues (52–54) and to this end only one two-dimensional-gel electrophoresis-based compared soleus and EDL muscle in rats detecting a limited number of proteins (15). Most studies were devoted to rats, guinea pigs, and rabbits whereas considerably less is known for smaller mammalia such as mice (1, 39). The in vivo SILAC approach used in this study allowed a comprehensive assessment of proteins in slow and fast muscle fibers in mice. Comparison of protein and mRNA data extended the analysis and provided insights into the regulatory events that shape the identity of fast and slow muscles at the post-transcriptional level.

The SILAC method employs the metabolic incorporation of stable isotopes of essential amino acids into cellular proteomes and has been successfully applied for in vitro-cell culture experiments (28). Here, we used metabolic labeling in the SILAC-mouse, in which all tissues are completely labeled with the heavy 13C6 lysine isotope (21, 55) to allow global protein quantification. This method, which is based on mixing control and experimental samples with the heavy protein standard, avoids additional post-extraction modification steps of proteins or peptides and therefore significantly reduces potential methodological errors. Analysis of SILAC-data sets is further facilitated by the use of several specialized software tools such as MSQuant, MaxQuant and Proteome Discoverer (Thermo Scientific), which can be readily used together with LysC digests generating peptide pairs with 6 Da mass differences.

Our approach allowed us to quantify more than 2100 proteins in fast and slow muscle fibers, which to the best of our knowledge, is the largest quantitative data set for proteins in skeletal muscle tissues in vivo. The identification of ~550 proteins, which we found to be differentially expressed in slow or fast muscles, greatly extends the number of fiber type-enriched proteins. Because virtually all already known fiber type-enriched proteins were contained in our data set we assume a fairly good coverage of the fast and slow skeletal proteomes. The Quantimus database, in which these data are stored, represents an easy and convenient tool to identify proteins of interest via a simple search function. Further links provide additional information such as associated peptides, which are described by their position in the protein, Mascot-scores, phosphorylation status, and SILAC ratios between soleus and EDL. The database will be updated continuously as new entries with corresponding parameters will become available in further experiments. Refined sample preparation protocols and improved resolution and sensitivity of mass spectrometric instrumentation might help to further extend the coverage of muscle proteomes.

One of the most common ways to discriminate different muscle fiber types is based on the content of different MyHC proteins. However, as already indicated earlier, the amino acid sequences of individual members of this protein family are rather similar and peptide assignments after mass spectrometric analysis is often complicated by high sequence homologies (56). For exact identification and quantification of fiber type specific MyHC isoforms it is essential to select only
peptides, which are unique for a given isoform. Using these criteria we were able to determine the abundance of MyHC’s in the soleus in the following order: MyHC-β > MyHC-IIa > MyHC-8, MyHC-10 in slow muscles. In contrast, we observed the following order for the EDL muscle: MyHC-IIb > MyHC-13, which is in good correlation to published datasets. Several studies confirmed the presence of the slow type II MyHC IIa in the soleus. Our finding reflects the mixed type I and type II fiber composition of the mouse soleus muscle, which is more heterogeneous compared with the soleus of other animals such as rats and rabbits (57). It has been suggested that the MyHC diversity of skeletal muscle fibers in different species depends mainly on their body size (3). “Light weight” animals such as mice might need only a small proportion of type I fibers to maintain their body posture and use the soleus mainly to support (fast) movements.

Two other important protein groups which have a strong impact on the phenotype and physiological properties of different muscle fiber types are kinases and phosphatases. We detected more than 100 kinases indicating good coverage of this protein class. Interestingly, marked differences in the expression profiles of several kinases were found. One of the kinases, which showed strong enrichment in fast compared with slow muscle was calcium/calmodulin dependent protein kinase II (CaMKII) (58). This kinase has been linked to pathological conditions including heart failure and hypertrophy but also to physiological processes such as mitochondrial biogenesis and fatty acid uptake (59). Although those processes are more prominent in slow muscles and cardiomyocytes the expression pattern of CaMKIIa discloses an important role in fast muscles. Several other kinases such as AMP kinase and several ERK-activator kinases were also selectively enriched in the EDL. Activation of the mitogen-activated protein kinases (MAPK) are involved in fiber type switching during exercise and regeneration (60). The cGKI dependent kinase α was one of the most abundant kinases in the soleus, which was surprising because cGKI dependent kinase has been mainly described in smooth muscles where it is involved in various different processes including muscle relaxation. In principle, it is possible that cGKI dependent kinase was derived from smooth muscle cells of arteries although the lack of enrichment of other smooth muscle proteins in soleus samples argues against such a possibility.

The main feature of muscle cells is the generation of contractile force mediated by myofibrillar proteins within the sarcomer. Force transmission occurs via connective tissue that is attached to tendons and bones. The extracellular matrix deposited by muscle fibers plays an important functional role in this process and has attracted some interest because of its involvement in several musculoskeletal diseases (61). Formation of the extracellular matrix is based mainly on secretion and deposition of collagen fibers and collagen associated proteins such as proteoglycans and fascilin proteins, which are the main components that determine mechanical properties of the ECM (62, 63). Our study revealed similar concentrations for some ECM proteins in fast and slow muscle including laminins, dystrophin, tenascin, and perlecan. Other proteins implicated in the formation of interstitial connective tissue and the basement membrane showed a differential distribution (Table 1A, B), which might in part account for distinct fiber strength between slow and fast muscles. The increased presence of fibrillin-1, periositin, and other ECM proteins in slow muscles seems to contribute to optimal mechanical stability of the connection between slow muscle fibers and associated tendons/bones. We propose that fast movements in the EDL require a more flexible ECM organization whereas the slow soleus muscle needs more stability and integrity.

The correlation of transcript and protein levels added another level of resolution to our study. No comprehensive comparison between protein concentrations and mRNA levels has been available for skeletal muscles preventing an unbiased view on posttranscriptional regulatory mechanisms. Although the bulk of proteins and mRNAs showed a good correlation in our study as indicated by a Pearson coefficient of 0.81, we identified a large group of proteins that was characterized by significant differences to corresponding mRNA levels. Because more molecules were differentially distributed between slow and fast muscles on the protein compared with the mRNA level we concluded that posttranscriptional mechanisms based on miRNAs and protein stability play an important role in establishing the specific molecular make-up of slow and fast muscle fibers. Although the correlation of mRNA and protein levels that we achieved is similar to other studies (64) one has to be aware of potential technical bias. For example, we were unable to measure large numbers of proteins expressed at low levels only in contrast to DNA microarray techniques that permit detection of low-abundance mRNA’s. Nevertheless, we believe that a combined analysis of microRNA expression profiles of soleus and EDL muscles together with the analysis of complementary sequences in 3’-untranslated regions of mRNAs of differentially regulated proteins will help to identify new targets for microRNA’s. A similar approach might be taken to identify processes that regulate the half-live of proteins in different muscles. Our results revealed that the number of proteins, which are present in different concentrations in slow and fast muscles, is higher than anticipated. The major extension of the catalogue of proteins with structural or regulatory functions in fast and slow muscles allows a more holistic view of the molecular networks responsible for distinct muscle functions.

**Data Availability**—All RAW files and sequence spectra can be downloaded from the Proteome Commons.org tranche (https://proteomecommons.org/tranche) server. Data set 1 (protein) contains all RAW data obtained from the Soleus/EDL SILAC quantification experiments. Data set 2 (phospho) includes all RAW files for the phosphopeptide identification. The
### Table I

(A) List of selected proteins enriched in the Soleus. The table is divided into different cellular compartments and fold changes are shown for the forward (soleus/EDL, S/E) and crossover (EDL/soleus, E/S) experiments. The p values of the mRNA fold changes are indicated in green (p value < 0.001), in yellow (p value 0.05–0.001), and in red (p value > 0.05). (B) List of selected proteins enriched in the EDL muscle

| TABLE 1A: | **Swiss** | **myosin & tropomysin** | **S/E** | **E/S** | **mRNA** | **p** | **Swiss** | **membrane** | **proteins** | **S/E** | **E/S** | **mRNA** | **p** |
|------------|-------------------------|-------------------------|---------|---------|----------|------|-------------------------|-------------------------|-------------------------|---------|---------|----------|------|
| **Q21283** | Myosin heavy chain 7, Myh7-beta | 10.5 | 0.1 | 33.2 | Q2VDN2 | Na(+)/K(+) ATPase alpha-1 | 2.7 | 0.3 | 2.9 |
| **Q11698** | Myosin heavy chain 9a | 14.5 | 0.1 | 3.1 | P14694 | NaK-transient ATPase | 2.0 | 0.4 | 2.0 |
| **Q29079** | Myosin heavy chain 10, non-muscle | 1.9 | 0.5 | 3.4 | Q5DS17 | 1-ADP transcytrosis | 1.8 | 0.4 | 1.9 |
| **P13542** | Myosin-B | 2.7 | n.d. | 1.1 | Q6R8M4 | Podocalyxin-like protein 1 | 2.4 | 0.4 | 1.7 |
| **P05542** | Myosin light chain 1, slow muscle | 17.1 | 0.01 | 52.6 | P34814 | Epoxide hydrolase 2 | 3.7 | 0.2 | 3.4 |
| **Q60434** | Myosin light chain 6B | 10.7 | 0.04 | 5.1 | Q415E3 | Transmembrane protein 65 | 1.8 | 0.4 | 1.3 |
| **Q37942** | Myosin regulatory light chain 12B | 1.7 | 0.6 | 1.3 | Q6V4K0 | Protein | 1.7 | 0.5 | 1.9 |
| **P15867** | Myosin regulatory light chain 2 | 8.4 | 0.02 | 23.3 | Q08867 | Platelet glycoprotein 4 | 2.6 | 0.3 | 2.2 |
| **Q65665** | Myosin binding protein C, slow | 3.9 | 0.3 | 1.1 | P53886 | Monocarboxylate transporter 1 | 2.3 | 0.4 | 2.2 |
| **Q68346** | Troponin T, slow skeletal muscle | 20.2 | 0.06 | 27.9 | Q8JH2U | Palmitoylin | 1.6 | n.d. | 2.5 |
| **Q68025** | Troponin I, slow skeletal muscle | 20.0 | 0.34 | 28.4 | Q7M5Y3 | Plicin | 2.4 | 0.3 | 1.3 |
| **P19103** | Troponin C, slow skeletal | 5.2 | 0.02 | 29.1 | | | | | |

| TABLE 1B: | **secreted & extracellular proteins** | **Swiss** | **Lipoprotein lipase** | 3.6 | n.d. | 2.7 |
|------------|-------------------------------|-----------------|-------------------|------|--------|--------|------|
| **Q1U343** | | | | | | | |
| **Q9EPR2** | | | | | | | |
| **Q9MRQ4** | | | | | | | |
| **Q6D4Y7** | | | | | | | |
| **Q6R2F4** | | | | | | | |
| **Q6R4K6** | | | | | | | |
| **P20627** | | | | | | | |
| **Q9CBW3** | | | | | | | |
| **Q9C159** | | | | | | | |
| **Q36818** | | | | | | | |
| **P11440** | | | | | | | |
| **P18125** | | | | | | | |
| **Q0W7T1** | | | | | | | |
| **Q6J6V6** | | | | | | | |
| **Q6T51B** | | | | | | | |
| **Q6R9P9** | | | | | | | |
| **Q7P8B8** | | | | | | | |
| **Q6R814** | | | | | | | |
| **Q6R8K5** | | | | | | | |
| **Q6T51B** | | | | | | | |

| **mitochondrial** | **Swiss** | **A12683** | BOH | 28.7 | 0.2 | 10.5 |
|-------------------|-----------------|-----------------|------|--------|--------|------|
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |

| **others** | **Swiss** | **A12683** | BOH | 28.7 | 0.2 | 10.5 |
|------------|-----------------|-----------------|------|--------|--------|------|
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |

| **ER & Ca2+ signaling** | **Swiss** | **Q6K771** | BOH | 28.7 | 0.2 | 10.5 |
|-------------------------|-----------------|-----------------|------|--------|--------|------|
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |

| **kinases & phosphatases** | **Swiss** | **Q6K771** | BOH | 28.7 | 0.2 | 10.5 |
|---------------------------|-----------------|-----------------|------|--------|--------|------|
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |

| **nuclear proteins** | **Swiss** | **Q6K771** | BOH | 28.7 | 0.2 | 10.5 |
|----------------------|-----------------|-----------------|------|--------|--------|------|
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |
| **Q6K771** | | | | | | | |

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**Molecular & Cellular Proteomics 11.6**

10.1074/mcp.M111.010801-13
| Swiss          | myosin & tropinin | S/E | E/S | mRNA | p  | Swiss | membrane proteins | S/E | E/S | mRNA | p  |
|---------------|-------------------|-----|-----|------|----|-------|-------------------|-----|-----|------|----|
| Q5X59         | Myosin heavy chain 2b | 0.5 | 5.9 | 4.7  |   | Q8R3Q9 | Tropinin protein (Tetraspanin 8) | 0.1 | 24.0 | 13.8 |   |
| B1AR99        | Myosin, heavy polypeptide 13 | 0.3 | 3.1 | 1.3  |   | Q8C7E7 | Starch-bdg. domain-cont protein 1 | n.d. | 8.0  | 1.6  |   |
| P09977        | Myosin light chain 1 | 0.3 | 2.7 | 1.4  |   | Q0DPM2 | Mdp41 | n.d. | 21.3 | 2.7  |   |
| P09974        | Myosin light chain 3 | 0.2 | 7.1 | 1.4  |   | Q5V8Z3 | Cleft lip and palate membr. protein 1 | 0.5 | 1.7  | 0.9  |   |
| P74577        | Myosin reg. light chain 2 | 0.3 | 2.9 | 1.8  |   | Q5E7T8 | Junctophilin-1 | 0.2 | 4.0  | 2.0  |   |
| B2R2E2        | Micr 2 kinase 2, skeletal muscle | 0.2 | 5.4 | 3.0  |   | Q5E7T8 | Junctophilin-2 | 0.2 | 4.0  | 2.4  |   |
| Q0X5E0        | Myosin-binding protein C, fast | 0.1 | 9.5 | 6.6  |   | Q6PA06 | Adjalin-2 | 0.2 | 3.7  | 2.0  |   |
| P70402        | Myosin-binding protein H | n.d. | 3.9 | 4.4  |   | Q6ZQ33 | Maladin | 0.2 | 4.8  | 2.6  |   |
| P13412        | Tropinin I, fast skeletal muscle | 0.3 | 3.5 | 1.3  |   | Q0Y1Y0 | Prot-Tyr. phosphatase-like A | 0.2 | 4.0  | 1.9  |   |
| P02601        | Tropinin C, skeletal muscle/STNC | 0.3 | 3.2 | 1.3  |   | Q5VW55 | VAP-33 | 0.4 | 2.4  | 1.4  |   |
| P56771        | Tropomyosin alpha 1-chain | 0.3 | 3.8 | 1.2  |   | P79732 | Sodium channel subunit beta-1 | 0.2 | 4.1  | 1.7  |   |
| P56774        | Tropomyosin beta chain | 0.4 | 2.4 | 0.9  |   | Q502Q4 | Adipocyte-specific protein 4 | 0.4 | 2.3  | 1.2  |   |

**Table 1B:** An Integrated Quantitative Proteomics and Transcriptomics Analysis

An integrated study comparing the proteomic and transcriptomic profiles of various cell types. The table below presents the SwissProt identifiers, expression levels (S/E), expression ratios (E/S), and mRNA levels for a selection of proteins and genes.

**Table 1—continued**

| Gene          | Function          | Expression | mRNA | p  |
|---------------|-------------------|------------|------|----|
| Q6J37        | Myosin-1          | 0.4 | 2.8 | 2.2  |   |
| Q8R4E4       | Myosin-3          | 0.2 | 4.5 | 2.7  |   |
| Q62324       | Myosin-1          | 0.5 | 2.1 | 0.9  |   |
| C55124       | M-protein         | 0.1 | 11.0| 2.2  |   |
| Q89900       | Alpha-actinin-3   | 0.1 | 10.9| 6.2  |   |
| P32484       | Parvalbumin α     | 0.1 | 21.0| 5.5  |   |
| Q67209       | PDZ and LIM domain protein 3 | 0.2 | 4.1 | 1.5  |   |
| Q6T7D7       | PDZ and LIM domain protein 7 | 0.1 | 8.9 | 1.9  |   |
| Q6V1D3       | AMP deaminase-1   | 0.1 | 6.7 | 2.2  |   |
| Q6Z0U4       | Nitric oxide synthase, brain | n.d. | 3.6 | 2.5  |   |
| Q70F96       | Cardiomyopathy-associated prot. 5 | 0.2 | 3.0 | 1.2  |   |
| P66833       | Actin, alpha cardiac muscle 1 | 0.1 | 7.8 | 5.0  |   |
| Q6J2U2       | Profilin-2, Profilin II | 0.6 | 1.5 | 1.3  |   |
| Q6J234       | Alpha-1-syntrophin | 0.6 | 1.7 | 1.0  |   |
| Q6D2N4       | Dystrobrevin alpha | 0.4 | 2.3 | 1.2  |   |
| Q6E9D7       | Dyferlin          | 0.5 | 1.9 | 1.3  |   |
| Q6U2Z1       | Caspase-interacting protein | 0.6 | 1.6 | 1.4  |   |
| Q6C1U2       | Smoothelin-like protein 2 | 0.1 | 2.6 | 3.3  |   |
| P18826       | Phosphoinositide kinase u M | 0.2 | 5.9 | 3.4  |   |
| Q89AMO       | AMPK subunit beta-2 | 0.3 | 3.6 | 6.1  |   |
| P07310       | Creatine kinase M-type | 0.3 | 3.2 | 1.2  |   |
| Q51938       | MAP kinase kinase | 0.7 | 1.6 | 1.3  |   |
| P07236       | MAP kinase kinase | 0.7 | 1.7 | 1.4  |   |
| Q6E5T4       | Serine/threonine protein kinase Nek7 | 0.4 | 1.8 | 1.3  |   |
| Q14BU2       | Serine/threonine protein kinase Nr6 | 0.6 | 1.5 | 1.5  |   |
| Q6A074       | APEX | 0.6 | 1.4 | 1.0  |   |
| Q6T1N1       | Car2u2 | n.d. | 10.5| 3.0  |   |
| Q6R0Y5       | Adenylyl kinase isoenzyme 1 | 0.6 | 1.8 | 1.3  |   |
| Q6A9H3       | Protein kinase C. u | 0.8 | 1.9 | 2.2  |   |
| Q6M796       | Dual spec. Phosphatase 23 | 0.4 | 4.4 | 2.2  |   |
| Q6Y2M0       | Protein phosphatase 1, 3A | 0.5 | 2.7 | 1.8  |   |
| Q6X910       | Calcium sensor 1 | 0.3 | 2.9 | 1.5  |   |
| P6D328       | Ser/Thr-protein phosphatase 2B | 0.2 | 3.0 | 1.9  |   |
| Q8R8N8       | Cardiac/myelin isoform 2 | 0.2 | 5.7 | 1.5  |   |
| Q8R4Z3       | SERCA1 | 0.2 | 5.6 | 1.2  |   |
| Q6M0K9       | Calmodulin 1 | 0.3 | 2.9 | 1.7  |   |
| Q6O1E9       | Calmodulin-1 | 0.2 | 4.1 | 1.8  |   |
| P70272       | Skeletal muscle ryanodine receptor | 0.2 | 3.8 | 1.3  |   |
| Q7T048       | Sarcolipin | 0.5 | 1.8 | 1.2  |   |
| Q7U1P3       | Calciumycin | 0.6 | 1.5 | 1.1  |   |
| Q8R9V2       | Calpain-1 | 0.4 | 2.2 | 1.5  |   |
| Q7O2K2       | Retinoic-2 | 0.3 | 2.9 | 1.5  |   |
| Q0D1G3       | Hedgehog acyltransferase-like | 0.2 | 4.1 | 1.7  |   |

**Table 1B (continued):**

**Table 1B:** An Integrated Quantitative Proteomics and Transcriptomics Analysis

A comprehensive analysis comparing the proteomic and transcriptomic profiles of various cell types. The table presents the SwissProt identifiers, expression levels (S/E), expression ratios (E/S), and mRNA levels for a selection of proteins and genes.
PNG documents “Phosphopeptides” contain MS/MS spectra of identified phosphopeptides in this study.

Data sets are available via the following hash code: zoHoIjcZuXHijCP6p+iylGL0ioePvPGcy2JFUQyHEHeBO
eW3mldBaeRhPCxYTMWHLQEMDgzS3DRhpyOPPKNAT1
C4AAAAAAACGA==. The content of the data sets are described in the preceding paragraph.

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[This article contains supplemental Figs. and Tables.]

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