Integrated view and comparative analysis of baseline protein expression in mouse and rat tissues

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Abstract

The increasingly large amount of public proteomics data enables, among other applications, the combined analyses of datasets to create comparative protein expression maps covering different organisms and different biological conditions. Here we have reanalysed public proteomics datasets from mouse and rat tissues (14 and 9 datasets, respectively), to assess baseline protein abundance. Overall, the aggregated dataset contains 23 individual datasets, which have a total of 211 samples coming from 34 different tissues across 14 organs, comprising 9 mouse and 3 rat strains, respectively.

We compared protein expression between the different organs, including the distribution of proteins across them. We also performed gene ontology and pathway enrichment analyses to identify organ-specific enriched biological processes and pathways. As a key point we carried out a comparative analysis of protein expression between mouse, rat and human tissues. We observed a high level of correlation of protein expression among orthologs between all three species in brain, kidney, heart and liver samples. Finally, it should be noted that protein expression results have been integrated into the resource Expression Atlas for widespread dissemination.

Author summary

We have reanalysed 23 baseline public proteomics datasets stored in the PRIDE database belonging to various mouse and rat tissues and organs. Overall, the aggregated dataset contained 211 samples, coming from 34 different tissues across 14 organs, comprising 9 mouse and 3 rat strains, respectively. We analysed the distribution of protein expression across organs in both species. Then we performed gene ontology and pathway enrichment analyses to identify organ-specific enriched biological processes and pathways. We also carried out a comparative analysis of baseline protein expression between mouse, rat and human tissues, observing a high level of expression correlation among orthologs in all three species, in brain, kidney, heart and liver samples. To disseminate these findings, we have integrated the protein expression results into the resource Expression Atlas.

1. Introduction

High-throughput mass spectrometry (MS)-based proteomics approaches have matured significantly in recent years, becoming an essential tool in biological research [1]. This has been the consequence of very significant technical improvements in MS instrumentation, chromatography, automation in sample preparation and computational analyses, among other areas. The most used MS-based experimental approach is Data Dependent Acquisition (DDA) bottom-up proteomics. Among the main quantitative proteomics DDA techniques, label-free approaches remain very popular, although labelled-approaches, especially those techniques based on the isotopic labelling of peptides (MS² labelling), such as iTRAQ (Isobaric tag for relative and absolute quantitation) and TMT (Tandem Mass Tagging), are increasingly popular as well.

Following the steps initiated by genomics and transcriptomics, open data practices in the field have become embedded and commonplace in proteomics in recent years. In this context, datasets are now commonly available in the public domain to support the claims published in the corresponding manuscripts. The PRIDE database [2], located at the European Bioinformatics Institute (EBI), is currently the largest resource worldwide for public proteomics data deposition. PRIDE is also one of the founding members of the global ProteomeXchange consortium [3], involving five other resources, namely PeptideAtlas, MassIVE, iProX, jPOST and PanoramaPublic. ProteomeXchange has standardised data submission and dissemination of public proteomics data worldwide.

As a consequence, there is an unprecedented availability of data in the public domain, which is triggering multiple applications [4], including the joint reanalysis of datasets (so-called

meta-analysis studies) [5-7]. Indeed, public proteomics datasets can be systematically reanalysed and integrated e.g., to confirm the results reported in the original publications, potentially in a more robust manner since evidence can be strengthened if it is found consistently across different datasets. Potentially, new insights different to the aims of the original studies can also be obtained by re-analysing the datasets using different strategies. It is the so-called re-purposing of public datasets [8], including for instance proteogenomics studies, for genome annotation purposes [9-12].

In this context of reuse of public proteomics data, PRIDE has started to work on developing data dissemination and integration pipelines into popular added-value resources at the EBI. This is perceived as a more sustainable approach in the medium-long term than setting up new bioinformatics resources. One of them is Expression Atlas [13], a resource that has enabled over the years easy access to gene expression data across species, tissues, cells, experimental conditions and diseases. Only recently, protein expression information coming from reanalysed datasets has been integrated in the ‘bulk’ section of Expression Atlas. As a result, proteomics expression data can be integrated with transcriptomics information, mostly coming from RNA-Seq experiments. So far, we have performed two main meta-analysis studies involving the reanalysis and integration of: (i) 11 public quantitative datasets coming from cell lines and human tumour samples [14]; and (ii) 25 human baseline datasets coming from 32 different organs [15].

The next logical step is to perform an analogous study of baseline protein expression in two of the main model organisms: *Mus musculus* and *Rattus norvegicus*. To date, there are only a small number of bioinformatics resources providing access to reanalysed MS-based quantitative proteomics datasets, and even fewer if one considers only mouse and rat data. In

this context, at the end of 2020, ProteomicsDB [16] released a first version of the mouse proteome, based on the re-analysis of five datasets. To the best of our knowledge, there is no such public resource for rat data yet anywhere. Additionally, although antibody-based human protein expression information is provided *via* the Human Protein Atlas [17] , their efforts are focused on human protein expression. Here, we report the reanalysis and integration of 23 public mouse (14 datasets) and rat (9 datasets) label-free datasets, and the incorporation of the results into the resource Expression Atlas as baseline studies. Additionally, we report a comparative analysis of protein expression across mouse, rat and human (in this case using the results reported at [15] using the same methodology).

2. Result

2.1. Baseline proteomics datasets

Overall, we quantified protein expression from 34 healthy tissues in 14 organs coming from 23 datasets. The analyses covered a total of 1,173 MS runs from 211 samples that were annotated as healthy/control/non-treated samples thus representing baseline protein expression. Non-control/disease samples associated with these datasets were also analysed but are not discussed here. Normalised protein abundances values (as ppb, parts per billion) from both control/healthy/non-treated and disease/treated tissue samples are available to view as heatmaps in Expression Atlas. The protein abundances along with sample annotations, sample quality assessment summary and experimental parameter inputs for MaxQuant can be downloaded from Expression Atlas as text files. Data selection and reanalysis protocol is shown in Fig. 1, the total number of proteins and peptides identified in these datasets are shown in Table 1.

Figure 1. An overview of the study design and reanalysis pipeline. QA: Quality assessment.

Expression Atlas accession numbers	PRIDE dataset identifiers	Tissues	Organs	Species	Strain	Number of MS runs	Number of samples	Number of protein groups [†]	Number of peptides [†]	Number of unique peptides [†]	Number of unique genes mapped [†]
E-PROT-7 [§]	PXD000867 ^[18]	Liver	Liver	<i>Mus musculus</i>	C57BL/6J	24	4	12,792	246,738	167,725	9,715
E-PROT-10 [§]	PXD000288 ^[19]	Triceps muscles	Triceps Muscles	<i>Mus musculus</i>	C57BL/6	36	3	10,870	189,553	126,670	6,421
E-PROT-16	PXD003155 ^[20]	Cerebellum, Liver	Brain, Liver	<i>Mus musculus</i>	C57BL/6	24	12	4,508	59,696	45,728	3,797
E-PROT-74	PXD004612 ^[21]	Achilles and Plantaris tendon	Tendon	<i>Mus musculus</i>	C57BL/6	8	8	457	6,643	3,271	273
E-PROT-75	PXD005230 ^[22]	Hippocampus, Cerebellum, Cortex	Brain	<i>Mus musculus</i>	C57BL/10J	72	36	7,663	63,479	41,683	6,037
E-PROT-76	PXD009909 ^[23]	Retina	Eye	<i>Mus musculus</i>	ND4 Swiss Webster	12	1	5,002	29,454	24,961	3,686
E-PROT-77	PXD012307 ^[24]	Lung	Lung	<i>Mus musculus</i>	C57BL/6	32	2	6,809	106,391	73,950	5,795
E-PROT-78	PXD009639 ^[25]	Lens	Eye	<i>Mus musculus</i>	CD1	10	1	4,519	20,779	18,006	3,064
E-PROT-79	PXD019394 ^[26]	Heart, Kidney, Liver, Lung, Brain, Spleen, Testis, Pancreas	Heart, Kidney, Liver, Lung, Brain, Spleen, Testis, Pancreas	<i>Mus musculus</i>	Swiss-Webster	96	8	9,853	141,506	105,701	8,185
E-PROT-81	PXD012636 ^[27]	Left atrium, Left ventricle, Right atrium, Right ventricle	Heart	<i>Mus musculus</i>	C57BL/6	120	4	7,772	146,966	99,577	6,435
E-PROT-82	PXD019431 ^[28]	Articular cartilage	Articular cartilage	<i>Mus musculus</i>	BALB\ c	72	6	1,815	17,695	15,191	1,518
E-PROT-83	PXD022614 ^[29]	Brain	Brain	<i>Mus musculus</i>	C57BL/6J: Rj	120	6	6,645	97,443	69,884	5,673

					C57BL/6JR ccHsd						
E-PROT-84	PXD004496 ^[30]	Hippocampus	Brain	<i>Mus musculus</i>	C57BL/6J	204	17	4,192	37,363	30,100	3,424
E-PROT-85	PXD008736 ^[31]	Right atrium, Sinus node	Heart	<i>Mus musculus</i>	C57BL/6J	143	6	7,906	144,926	94,379	6,554
E-PROT-86 [§]	PXD012677 ^[32]	Amygdala	Brain	<i>Rattus norvegicus</i>	Sprague Dawley	3	3	1,872	15,326	12,367	1,382
E-PROT-87 [§]	PXD006692 ^[33]	Lung	Lung	<i>Rattus norvegicus</i>	Sprague Dawley	10	10	2,079	14,440	11,696	1,398
E-PROT-88 [§]	PXD016793 ^[34]	Liver	Liver	<i>Rattus norvegicus</i>	Sprague Dawley	8	8	4,787	57,998	46,411	3,743
E-PROT-89 [§]	PXD004364 ^[35]	Testis	Testis	<i>Rattus norvegicus</i>	Sprague Dawley	3	3	2,351	15,880	13,674	1,601
E-PROT-91	PXD001839 ^[36]	Left ventricle	Heart	<i>Rattus norvegicus</i>	F344/BN	12	12	1,345	10,310	8,804	925
E-PROT-92 [§]	PXD013543 ^[37]	Left ventricle	Heart	<i>Rattus norvegicus</i>	Wistar	8	8	1,858	17,303	13,622	1,340
E-PROT-93	PXD016958 ^[38]	First segment of proximal tubule, second segment of proximal tubule, third segment of proximal tubule, medullary thick ascending limb, cortical thick ascending limb, distal convoluted tubule, connecting tubule, cortical collecting duct, outer medullary collecting duct, inner medullary collecting duct	Kidney	<i>Rattus norvegicus</i>	Sprague Dawley	132	32	7,846	103,886	83,662	6,130

E-PROT-94	PXD003375 ^[39]	Caudal and rostral segments of spinal cord	Spinal cord	<i>Rattus norvegicus</i>	Wistar	21	18	2,477	29,213	22,025	1,926
E-PROT-95 [§]	PXD015928 ^[40]	Tendon	Tendon	<i>Rattus norvegicus</i>	Wistar	3	3	199	1,253	1,063	101
TOTAL	23 datasets (Mouse: 14, Rat: 9)	34 tissues (Mouse: 21, Rat: 18)	14 organs (Mouse: 12, Rat: 8)			1,173 MS runs (Mouse: 973, Rat: 200)	211 samples (Mouse: 114, Rat: 97)				

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144 **Table 1.** List of mouse and rat proteomics datasets that were re-analysed. [§]Only normal/untreated samples within this dataset are reported in this

145 study. However, results from both normal and disease samples are available in Expression Atlas. [†] Numbers after post-processing.

2.2. Protein coverage across organs and datasets

One of the main aims of this study was to study protein expression across various organs. To enable simplicity of comparison [15] we first grouped 34 different tissues into 14 distinct organs, as discussed in ‘Methods’. We defined ‘tissue’ as a distinct functional or structural region within an ‘organ’. We estimated the number of ‘canonical proteins’ identified across organs by first mapping all members of each protein group to their respective parent genes. We defined the parent gene as equivalent to the UniProt ‘canonical protein’ and we will denote the term ‘protein abundance’ to mean ‘canonical protein abundance’ from here on in the manuscript.

2.2.1. Mouse proteome

A total of 21,274 protein groups were identified from mouse datasets, among which 8,176 protein groups (38.4%) were uniquely present in only one organ and 70 protein groups (0.3%) were ubiquitously observed (see the full list in Supplementary file 2). Mouse protein groups were mapped to 12,570 genes (canonical proteins) (Supplementary file 3). We detected the largest number of canonical proteins in samples from liver (9,920, 78.9% of the total) and the lowest numbers in samples from tendon (273, 2.2%) and articular cartilage (1,519, 12.1%) (Fig. 2A). The lower number of proteins identified in tendon could be attributed to the smallest sample size (only one sample out of 114, 0.9%). Also, tendon is a relatively hypocellular tissue, which has a low protein turnover rate. Dataset PXD000867, containing mouse liver samples, had the highest number of canonical proteins detected (9,715, 77.3%), while the least number of proteins were detected in dataset PXD004612 (tendon, 273, 2.2%), as highlighted above (Fig. 2B).

We studied the normalised protein abundance distribution in organs (Fig. 2C) and found that all organs, except tendon, had similar median abundances. However, one cannot attribute biological meaning to these observations, since the method of normalisation by definition fixes each sample to have the same “total abundance”, which then gets shared out amongst all proteins. The normalised protein abundance distribution in datasets indicated a higher than median abundances detected in datasets PXD004612 (tendon) and PXD003164 (testis) (Fig. 2D). A linear relation was observed with the number of canonical proteins detected in datasets and organs, when compared to the relative amount of their spectral data (Fig. 2E). We found a large number of proteins uniquely present in one organ (Fig. 2F).

Figure 2. (A) Number of canonical proteins identified across different mouse organs. The number within the parenthesis indicates the number of samples. (B) Canonical proteins identified across different datasets. The number within the parenthesis indicate the number of unique tissues in the dataset. (C) Range of normalised iBAQ protein abundances across different organs. The number within the parenthesis indicates the number of samples. (D) Range of normalised iBAQ protein abundances across different datasets. The number within parenthesis indicate the number of unique tissues in the dataset. (E) Comparison of total spectral data with the number of canonical proteins identified in each dataset and organ. (F) Distribution of canonical proteins identified across organs.

2.2.2. Rat proteome

A total of 7,769 protein groups were identified across 8 different rat organs among which 3,649 (46.9%) protein groups were unique to one specific organ while 13 (0.16%) protein groups were present among all organs (see full list in Supplementary file 2). The protein groups were mapped to 7,116 genes (canonical proteins) (Supplementary file 3). The highest number of canonical proteins (6,106, 85.1%) was found in rat kidney samples. The lowest number of canonical proteins (101, 1.4%) was found in samples from tendon, as shown in Fig. 3A. The largest number of canonical proteins identified in kidney is because of the relatively large number of samples (32 samples), when compared to other organs. However, it is interesting to note that large number of canonical proteins were detected in liver samples, which relatively has fewer number of samples, when compared to number of samples in heart or spinal cord.

Datasets PXD016958 and PXD016793 consisted entirely of kidney and liver samples, respectively, and as mentioned above had the largest number of canonical proteins identified

(Fig. 3B). The normalised protein abundances were similar among the various organs and datasets (Fig. 3C, D). We also observed a linear relation between the number of canonical proteins identified and the MS spectra identified (Fig. 3E). As seen in mouse datasets, we also observed a large number of proteins uniquely present in one organ (Fig. 3F).

Figure 3. (A) Number of canonical proteins identified across different rat organs. The number within the parenthesis indicates the number of samples. (B) Canonical proteins identified across different datasets. The number within the parenthesis indicate the number of unique tissues in the dataset. (C) Range of normalised iBAQ protein abundances across

different organs. The number within the parenthesis indicates the number of samples. (D)
Range of normalised iBAQ protein abundances across different datasets. The number within
parenthesis indicate the number of unique tissues in the dataset. (E) Comparison of total
spectral data with the number of canonical proteins identified in each dataset and organ. (F)
Distribution of canonical proteins identified across organs.

2.3. Protein abundance comparison across organs

Next, we studied how protein abundances compared across different datasets and organs. The
presence of batch effects between datasets makes this type of comparisons challenging. To
aid comparison of protein abundances between datasets we transformed the normalised iBAQ
intensities into ranked bins as explained in ‘Methods’, i.e., proteins in bin 5 are highly
abundant while proteins in bin 1 are expressed in lower abundances.

2.3.1. Mouse proteome

We found that 1,086 (8.6%) proteins were found with their highest expression in at least 3
organs, with a median bin value greater than 4 (Supplementary file 3). On the other end of the
scale, 138 (1.1%) canonical proteins were found with their lowest expression in at least 3
organs, with a median bin value less than 2. The bin transformed abundances in all organs are
provided in Supplementary file 3.

To compare protein expression across all organs in mouse, we calculated pairwise Pearson
correlation coefficients (rp) across 117 samples (Fig. 4A). We observed a good correlation of
protein expression within the brain (median rp = 0.55) and heart (median rp = 0.82) samples.
We performed PCA on all samples from mouse datasets for testing the effectiveness of bin
transformation method in reducing batch effects. Fig. 4B shows the clustering of samples

from various organs of mouse. We observed samples from the same organ clustered together. For example, we can observe that brain samples all clustered together in one group, although they come from different datasets (Fig. 4C). However, we also observed that samples from other organs such as liver, did not cluster according to their organ types but clustered together with the dataset they were part of, indicating the presence of residual batch effects.

Figure 4. (A) Heatmap of pairwise Pearson correlation coefficients across all mouse samples. The colour represents the correlation coefficient and was calculated using the bin transformed

iBAQ values. The samples were hierarchically clustered on columns and rows using Euclidean distances. (B) PCA of all samples coloured by the organ types. (C) PCA of all samples coloured by their respective dataset identifiers. The numbers in parenthesis indicate the number of datasets for each organ. Binned values of canonical proteins quantified in at least 50% of the samples were used to perform the PCA.

2.3.2. Rat proteome

Similarly, in rat, on one hand, 311 (4.3%) proteins were found with highest expression in at least 3 organs with a median bin value greater than 4. On the other hand, 27 (0.37%) canonical proteins were found with their lowest expression in at least 3 organs, with a median bin value of less than 2. The bin transformed abundances in all organs are provided in Supplementary file 3.

The samples from rat datasets were also better correlated among each other (Fig. 5A) than in the case of mouse. We observed a good correlation of protein expression within samples from liver (median Pearson's correlation $r_p = 0.92$), lung (median $r_p = 0.84$), spinal cord (median $r_p = 0.80$), heart (median $r_p = 0.84$) and brain (median $r_p = 0.93$). We also observed a good clustering in the PCA of samples coming from the same organ type (Fig. 5B). Kidney, lung, spinal cord and heart samples all clustered together according to their organ type. Fig. 5C shows the samples based on the dataset they are part of. However, most organ samples are part of individual datasets except samples from heart, which came from two datasets (PXD001839 and PXD013543). Fig. 5C shows that the heart samples clustered into two groups, wherein each cluster included samples from a different dataset.

Figure 5. (A) Heatmap of pairwise Pearson correlation coefficients across all rat samples. The colour represents the correlation coefficient and was calculated using the bin transformed iBAQ values. The samples were hierarchically clustered on columns and rows using Euclidean distances. (B) PCA of all samples coloured by the organ types. (C) PCA of all samples coloured by their respective dataset identifiers. The numbers in parenthesis indicate the number of datasets for each organ. Binned values of canonical proteins quantified in at least 50% of the samples were used to perform the PCA.

2.4. The organ elevated proteome and the over-representative biological processes

Based on their expression, canonical proteins were classified into three different groups based on their organ specificity: “mixed”, “group-enriched” and “organ-enriched” (see Supplementary file 4). We considered over-expressed canonical proteins in each organ as those which were in “group-enriched” and “organ-enriched”. The analysis showed that on average, 20.8% and 26.0% of the total elevated canonical proteins were organ group-specific in mouse and rat, respectively (Fig. 6). In addition, 4.3% and 14.2% were unique organ-enriched in mouse and rat, respectively. The highest ratio of organ-enriched in mouse was found in liver (13.6%), whereas it was found in kidney in rat (39.8%).

We then performed a gene ontology (GO) enrichment analysis of those proteins that were 'organ-enriched' and group-enriched' using GO terms associated with biological processes. We found 1,036 GO terms to be statistically significant in all organs, as seen in Supplementary file 5. The most significant GO terms for each organ are shown in Table 2.

Figure 6. Organ specificity of canonical proteins in (A) mouse and (B) rat.

Organ	Species	GO ID	Description	adjusted p-value
Articular cartilage	<i>Mus musculus</i>	GO:0030198	Extracellular matrix organization	8.94×10^{-38}
		GO:0043062	Extracellular structure organization	8.94×10^{-38}
		GO:0045229	External encapsulating structure organization	8.94×10^{-38}
Brain	<i>Mus musculus</i>	GO:0050804	Modulation of chemical synaptic transmission	7.03×10^{-65}
		GO:0099177	Regulation of trans-synaptic signalling	7.03×10^{-65}
		GO:0050808	Synapse organization	1.41×10^{-48}

Heart	<i>Mus musculus</i>	GO:0060047 GO:0008016 GO:0060537	Heart contraction Regulation of heart contraction Muscle tissue development	7.10×10^{-11} 4.43×10^{-10} 6.16×10^{-10}
Kidney	<i>Mus musculus</i>	GO:0015711 GO:0044282 GO:0016054	Organic anion transport Small molecule catabolic process Organic acid catabolic process	4.59×10^{-19} 4.91×10^{-15} 6.25×10^{-15}
Eye	<i>Mus musculus</i>	GO:0007601 GO:0001654 GO:0099504	Visual perception Eye development Synaptic vesicle cycle	7.54×10^{-50} 5.31×10^{-31} 8.36×10^{-18}
Liver	<i>Mus musculus</i>	GO:0016569 GO:0016570 GO:0019369	Covalent chromatin modification Histone modification Arachidonic acid metabolic process	6.26×10^{-10} 1.71×10^{-08} 1.71×10^{-08}
Lung	<i>Mus musculus</i>	GO:0120031 GO:0030031 GO:0044782	Plasma membrane bounded cell projection assembly Cell projection assembly Cilium organization	3.61×10^{-14} 3.61×10^{-14} 9.83×10^{-14}
Pancreas	<i>Mus musculus</i>	GO:0007586 GO:0032328	Digestion Alanine transport	0.005 0.018
Spleen	<i>Mus musculus</i>	GO:0046649 GO:0050776 GO:0045087	Lymphocyte activation Regulation of immune response Innate immune response	4.12×10^{-22} 2.00×10^{-20} 2.23×10^{-20}
Tendon	<i>Mus musculus</i>	GO:0003012 GO:0050879 GO:0050881	Muscle system process Multicellular organismal movement Musculoskeletal movement	1.46×10^{-25} 3.14×10^{-19} 1.46×10^{-25}
Testis	<i>Mus musculus</i>	GO:0048232 GO:0003341 GO:0044782	Male gamete generation Cilium movement Cilium organization	8.75×10^{-49} 3.04×10^{-38} 6.78×10^{-37}
Triceps muscles	<i>Mus musculus</i>	GO:0061061 GO:0055002 GO:0003009	Muscle structure development Striated muscle cell development Skeletal muscle contraction	1.56×10^{-14} 2.41×10^{-14} 3.53×10^{-14}
Brain	<i>Rattus norvegicus</i>	GO:0099537 GO:0007268 GO:0098916	Trans-synaptic signalling Chemical synaptic transmission Anterograde trans-synaptic signalling	1.79×10^{-60} 1.79×10^{-60} 1.79×10^{-60}
Heart	<i>Rattus norvegicus</i>	GO:0061061 GO:0003012 GO:0055001	Muscle structure development Muscle system process Muscle cell development	2.94×10^{-17} 6.30×10^{-16} 4.00×10^{-15}
Kidney	<i>Rattus norvegicus</i>	GO:0006396 GO:0045944 GO:0006260	RNA processing positive regulation of transcription by RNA polymerase II DNA replication	6.19×10^{-13} 7.29×10^{-06} 1.74×10^{-05}
Liver	<i>Rattus norvegicus</i>	GO:0008202 GO:0016054 GO:0032787	Steroid metabolic process Organic acid catabolic process Monocarboxylic acid metabolic process	2.74×10^{-10} 1.61×10^{-09} 1.64×10^{-09}
Lung	<i>Rattus norvegicus</i>	GO:0031589 GO:0009617 GO:0030036	Cell-substrate adhesion Response to bacterium Actin cytoskeleton organization	7.62×10^{-08} 7.62×10^{-08} 1.40×10^{-07}
Spinal cord	<i>Rattus norvegicus</i>	GO:0061564 GO:0099537 GO:0007268	Axon development Trans-synaptic signalling Chemical synaptic transmission	4.26×10^{-18} 5.93×10^{-16} 5.93×10^{-16}
Tendon	<i>Rattus norvegicus</i>	GO:0030199 GO:0061448 GO:0001501	Collagen fibril organization Connective tissue development Skeletal system development	1.23×10^{-13} 2.31×10^{-09} 3.39×10^{-09}

Testis	<i>Rattus norvegicus</i>	GO:0019953 GO:0051704 GO:0007018	Sexual reproduction Multi-organism process Microtubule-based movement	3.98×10^{-24} 1.61×10^{-18} 4.00×10^{-12}
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Table 2. Analysis of the top three GO terms for each organ in mouse and rat using the elevated organ-specific canonical proteins and group-specific as described in the ‘Methods’ section.

2.5. Protein abundances across orthologs in three species

We had previously analysed 25 label-free proteomics datasets from healthy human samples to assess baseline protein abundances in 14 organs following the same analytical methodology [15]. We compared the expression of canonical proteins identified in all three organisms (rat, mouse and human). Overall, 13,248 detected human genes (corresponding to the canonical proteins) were compared with 12,570 genes detected in mouse and 7,116 genes detected in rat. The number of orthologous mappings (i.e., “one-to-one” mappings, see ‘Methods’) between rat, mouse and human genes are listed in table 3. We only considered one-to-one mapped orthologues for the comparison of protein abundances.

Species	Identified genes	Orthologs of human genes identified in [15]	Percentage of genes with different mapping against identified human genes				
			one-to-one	one-to-many	many-to-many	many-to-one	not mapped
<i>Mus musculus</i>	12,570	10,601	80.4%	1.9%	0.56%	1.46%	15.7%
<i>Rattus norvegicus</i>	7,116	6,058	82.0%	2.2%	0.70%	0.25%	14.9%

Table 3. Homologs identified in mouse and rat datasets when compared with the background list of genes identified in human datasets (Supplementary file 2 in [15]).

Among human and mouse orthologues we observed relatively high levels of correlation of protein abundances in brain ($R^2 = 0.61$), heart ($R^2 = 0.65$) and liver ($R^2 = 0.56$) (Fig. 7A). Human and rat orthologs showed also relatively high levels of correlation in brain ($R^2 = 0.62$), kidney ($R^2 = 0.53$) and liver ($R^2 = 0.56$), but almost no correlation in lung ($R^2 = 0.12$) and testis ($R^2 = 0.18$) (Fig. 7B). Between mouse and rat orthologs, the correlation of protein abundances was higher in liver ($R^2 = 0.65$), kidney ($R^2 = 0.54$) and brain ($R^2 = 0.57$) samples, when compared to the samples coming from the rest of the organs (Fig. 7C). Fig. 7D shows an illustration of some example comparisons of individual orthologs using binned protein abundances.

For the same corresponding subsets, we also investigated the correlation of protein expression between various organs within each organism. We observed that in general the correlation of protein expression was slightly lower between organs within the same species, when compared to a higher correlation, which was observed among orthologs (Supplementary file 6). The found lower correlation of protein expression between different organs was more apparent in mouse and rat.

Among the orthologs expressed in all organs in all three species, 747 (12.3%) orthologs were detected with a median bin expression value of more than 4, i.e., proteins that appear to have conserved high expression in all organs and all tissues. Additionally, 13 (0.2%) orthologs were found with a median bin expression value less than 2 in all organs, although, it is harder to detect consistently proteins with low abundances across all organs. A full list of the binned protein abundances of orthologs is available in Supplementary file 7. The illustration of all binned protein abundances across the three species is shown in Supplementary file 8.

Figure 7. Comparison of protein abundances (in ppb) between one-to-one mapped orthologs of mouse, rat and human in various organs. (A) Pairwise correlation using normalised protein abundances of human and mouse orthologues. (B) Human and rat orthologs. (C) Mouse and rat orthologs. (D) As an example, the comparisons of binned protein expression of ten randomly sampled orthologs are shown. All data corresponding to all cases (as reported in panel D) are available in Supplementary file 7 and their corresponding illustration of binned values is available in Supplementary file 8. Orthologs in (D) are shown using their human Gene Symbol.

2.6. Pathway enrichment analysis

Based on the ortholog protein expression analysis described above, we mapped canonical proteins from mouse and rat to the corresponding ortholog human proteins, which were

subsequently subjected to pathway-enrichment analysis using Reactome (Fig. 8). After filtering out the disease and statistically insignificant pathways, there were 2,990 pathways found in all the organs of mouse and 2,162 pathways in all the organs of rat. In mouse samples, the largest number of pathways (367) were found in articular cartilage, and the lowest number of pathways was from liver (44). We also observed that Neuronal System-related pathways are predominantly present in the brain and eye, which is consistent with expectations. In rat samples, brain included the largest number of pathways (387), while the lowest number of pathways was found in tendon, with 117.

Figure 8. Pathway analysis of the canonical proteins, showing the statistically significant representative pathways (p-value < 0.05) in (A) mouse and (B) rat organs.

3. Discussion

We have previously reported two meta-analysis studies involving the reanalysis and integration in Expression Atlas of public quantitative datasets coming from cell lines and human tumour samples [14], and from human baseline tissues [15], respectively. In this study, we reanalysed mouse and rat baseline proteomics datasets representing protein expression across 34 healthy tissues and 14 organs. We have used the same methodology as in the study involving baseline human tissues, which enabled a comparison of protein expression levels across the three species. Our main overall aim was to provide a system-wide baseline protein expression catalogue across various tissues and organs of mouse and rat and to offer a reference for future related studies.

We analysed each dataset separately using the same software (MaxQuant) and the same search protein sequence database. The disadvantage of this approach is that the False Discovery Rate (FDR) statistical thresholds are applied at a dataset level and not to all datasets together as a whole. However, as reported before [15], using a dataset per dataset analysis approach is in our view the only sustainable manner to reanalyse and integrate quantitative proteomics datasets, at least at present. As also reported in previous studies, one of the major bottlenecks was the curation of dataset metadata, consisting of mapping files to samples and biological conditions. Very recently, the MAGE-TAB-Proteomics format has been developed and formalised to enable the reporting of the experimental design in proteomics experience, including the relationship between samples and raw files, which is recorded in the SDRF-Proteomics section of the file [41]. Submission of the SDRF-Proteomics files to PRIDE is now supported. The more well-annotated datasets in the public domain, the easier data re-use activities will become.

The generated baseline protein expression data can be used with different purposes such as the generation of protein co-expression networks and/or the inference of protein complexes. For the latter application, expression data can be alone or potentially refining predictions obtained using different methods such as the recently developed AlphaFold-based protein complexes predictions [42]. Mouse and rat are widely used species in the context of drug discovery, the latter especially, to undertake regulatory pre-clinical safety studies. Therefore, it is important to know quantitative protein expression distribution in these species in different tissues [43] to assist in the selection of species for such studies and also for the interpretation of the final results.

In addition to the analyses reported, it would also be possible to perform correlation studies between gene and protein expression information. However, we did not find any relevant public datasets in the context of this manuscript where the same samples were analysed by both techniques, which is the optimal way to perform these studies. Future directions in analogous studies will involve: (i) additional baseline protein expression studies of other species, including other model organisms or other species of economic importance; (ii) the inclusion of differential datasets (e.g. using TMT and/or iTRAQ); and (iii) include relevant proteomics expression data coming from the reanalysis of Data Independent Acquisition (DIA) datasets [44].

As mentioned above, we performed a comparative analysis of baseline protein expression across human, mouse and rat. It was possible to perform this analysis for six common organs (brain, heart, kidney, liver, lung and testis). Ortholog expression across species is useful to infer protein function across experimentally studied proteins. This is particularly useful as evolutionarily closely related species are likely to conserve protein function. We could not

find in the literature an analogous comparative study performed at the protein level. However, protein expression from closely related orthologs across tissues or organs has been compared at the transcriptomics level, providing a complete picture of gene expression. In this context, many studies have compared gene-expression in mouse, rat and human orthologues and found that orthologues had generally a highly correlated expression tissue distribution profile in baseline conditions [45-49]. Gene expression levels among orthologs were found to be highly similar in muscle and heart tissues, liver and nervous system and less similar in epithelial cells, reproductive systems, bone and endocrine organs [47]. Studies have also shown that variability of gene expression between homologous tissues/organs in closely related species can be much lower than the variability between unrelated tissues within the same organism [45, 46], in agreement with the results reported here at the protein level.

In conclusion we here present a meta-analysis study of public mouse and rat baseline proteomics datasets from PRIDE. We demonstrate its feasibility, perform a comparative analysis across the three species and show the main current challenges. Finally, the data is made available *via* Expression Atlas. Whereas there are several analogous studies performed at the gene expression level for mouse and rat tissues, this is the first of this kind at the protein expression level.

4. Materials and Methods

4.1. Datasets

As of May 2021, there were 2,060 mouse (*Mus musculus*) and 339 rat (*Rattus norvegicus*) MS proteomics datasets publicly available in the PRIDE database (<https://www.ebi.ac.uk/pride/>). Datasets were manually selected based on the selection criteria described previously [15]. Briefly, we selected datasets where baseline expression experiments were performed on (i) label-free samples from tissues not enriched for post-translational modifications; (ii) Thermo Fisher Scientific instruments such as LTQ Orbitrap, LTQ Orbitrap Elite, LTQ Orbitrap Velos, LTQ Orbitrap XL ETD, LTQ-Orbitrap XL ETD, Orbitrap Fusion and Q-Exactive, since they represent a large proportion of datasets in PRIDE and to avoid heterogeneity introduced by data from other vendor instruments; (iii) had suitable sample metadata available in the original publication or it was possible to obtain it by contacting the authors; and (iv) our previous experience in the team of some datasets deposited in PRIDE, which were discarded because they were not considered to be useful (data not shown). Overall, 14 mouse and 9 rat datasets were selected from all mouse and rat datasets for further analysis. Table 1 lists the selected datasets. The 23 datasets contained a total of 211 samples from 34 different tissues across 14 organs (meaning groups of related tissues, more details below), comprising 9 different mouse and 3 rat strains, respectively. The sample and experimental metadata were manually curated using the information provided in the respective publications or by contacting the original authors/submitters. Annotare [50] was used for annotating the metadata and stored using the Investigation Description Format (IDF) and Sample-Data Relationship Format (SDRF) file formats [41], which are required for integration in Expression Atlas. An overview of the experimental design including experimental factors, protocols, publication information and contact

information are present in the IDF file, and the SDRF includes sample metadata describing the relationship between the various sample characteristics and the data files contained in the dataset.

4.2. Proteomics raw data processing

All datasets were analysed with MaxQuant (version 1.6.3.4) [51, 52] on a Linux high-performance computing cluster for peptide/protein identification and protein quantification. Input parameters for each dataset, such as MS1 and MS2 tolerances, digesting enzymes, fixed and variable modifications, were set as described in their respective publications, with two missed cleavage sites. The FDR at the PSM (peptide spectrum match) and protein levels were set to 1%. The MaxQuant parameters were otherwise set to default values: the maximum number of modifications per peptide was 5, the minimum peptide length was 7, the maximum peptide mass was set to 4,600 Da, and for the matches between runs the minimum match time window was set to 0.7 seconds and the minimum retention time alignment window was set to 20 seconds. The MaxQuant parameter files are available for downloading from Expression Atlas. The *Mus musculus* UniProt Reference proteome release-2021_04 (including isoforms, 63,656 sequences) and *Rattus norvegicus* UniProt Reference proteome release-2021_04 (including isoforms, 31,562 sequences) were used as the target sequence databases for mouse and rat datasets, respectively. The built-in contaminant database within MaxQuant was used and a decoy database was generated by MaxQuant by reversing the input database sequences after the respective enzymatic digestion. The datasets were run separately in multi-threaded mode.

4.3. Post-processing

The post-processing of results from MaxQuant is explained in detail in [15]. In brief, the protein groups labelled as potential contaminants, decoys and those with less than 2 PSMs were removed. Protein intensities in each sample were normalised by scaling the iBAQ intensity values to the total amount of signal in each MS run and converted to parts per billion (ppb).

$$ppb_iBAQ_i = \left(iBAQ_i / \sum_{i=1}^n iBAQ_i \right) \times 1,000,000,000$$

The ‘majority protein identifiers’ within each protein group were mapped to their Ensembl gene identifiers/annotations using the Bioconductor package ‘mygene’. For downstream analysis only protein groups whose isoforms mapped to a single unique Ensembl gene ID were considered. Protein groups that mapped to more than one Ensembl gene ID are provided in Supplementary file 1. The protein intensity values from different protein groups with the same Ensembl gene ID were aggregated as median values. The parent genes to which the different protein groups were mapped to are equivalent to ‘canonical proteins’ in UniProt (https://www.uniprot.org/help/canonical_and_isoforms) and therefore the term protein abundance is used to describe the protein abundance of the canonical protein throughout the manuscript.

4.4. Integration into Expression Atlas

The calculated canonical protein abundances (mapped to genes), together with the validated SDRF files, summary files detailing the quality of post-processing and the input MaxQuant parameter files (mqpar.xml) were integrated into Expression Atlas (<https://www.ebi.ac.uk/gxa/home>) as proteomics baseline experiments (E-PROT identifiers are available in Table 1).

4.5. Protein abundance comparison across datasets

To compare protein abundances, the normalised protein abundances (in ppb) from each group of tissues in a dataset were converted into ranked bins. In this study, ‘tissue’ is defined as a distinct functional or structural region within an ‘organ’. For example, hippocampus, cerebellum and cortex are defined as ‘tissues’ that are part of the brain (organ) and similarly sinus node, left atria, left ventricle, right atria, right ventricle are defined as ‘tissues’ in heart (organ). Protein abundances were transformed into bins by first grouping MS runs from each tissue within a dataset as a batch. The normalised protein abundances (ppb) for each MS run within a batch were sorted from lowest to highest abundance and ranked into 5 bins. Proteins whose ppb abundances are ranked in the lowest bin (bin 1) represent lowest abundance and correspondingly proteins within bin 5 are of highest abundance in their respective tissue. When merging tissues into organs, median bin values were used.

Proteins that were detected in at least 50% of the samples were selected for PCA (Principal Component Analysis) and was performed using R (The R Stats package) [53] using binned abundance values. For generating heatmaps, a Pearson correlation coefficient (rp) for all samples was calculated on pairwise complete observations of bin transformed values. Missing values were marked as NA (not available). For each organ a median rp was calculated from all pairwise rp values of their respective samples. Samples were hierarchically clustered on columns and rows using Euclidean distances.

4.6. Organ-specific expression profile analysis

For comparison across organs, the tissues were aggregated into organs and their median bin values were considered. As described previously [15] the classification scheme done by Uhlén *et al.* [17] was modified to classify the proteins into one of the three categories: (1) “Organ-enriched”: present in one unique organ with bin values 2-fold higher than the mean bin value across all organs; (2) “Group enriched”: present in at least 7 organs in mouse or in

at least 4 organs in rat, with bin values 2-fold higher than the mean bin value across all organs; and (3) “Mixed”: the remaining canonical proteins that are not part of the above two categories.

Enriched gene ontology (GO) terms analysis was carried out through over-representation test described previously [15], it was combined with “Organ-enriched” and “Group enriched” mapped gene lists for each organ. In addition, Reactome [54] pathway analysis was performed using mapped gene lists and running pathway-topology and over-representation analysis, as reported previously [15].

4.7. Comparison of protein expression across species

The g:Orth Orthology search function in the g:Profiler suite of programs [55] was used for translating gene identifiers between organisms. Since a custom list of gene identifiers could not be used as the background search set, the mouse and rat genes were first mapped against the background Ensembl database. The resulting list of mouse and rat genes mapped to human orthologs were then filtered so that they only included parent gene identifiers of the protein groups from mouse and rat organs identified in this study and the parent genes of human organs described in our previous study (Supplementary file 2 in [15]), respectively.

The orthologs were grouped into various categories denoting the resulting mapping between identifiers: “one-to-one”, “one-to-many”, “many-to-one”, “many-to-many”, and “no mappings” between gene identifiers. Only “one-to-one” mapped ortholog identifiers were used to compare protein intensities between mouse, rat and human organs. The normalised ppb protein abundances of the one-to-one mapped orthologues in 6 organs (brain, heart, kidney, liver, lung and testis), that were studied across all three organisms were used to assess

the pairwise correlation of protein abundances. The linear regression was calculated using the linear fit ‘lm’ method in R.

Data availability

Expression Atlas E-PROT identifiers and PRIDE original dataset identifiers are included in Table 1.

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Authors’ contributions

SW, DGS, AP, DJK selected and curated the datasets. SW, AP, DGS performed analyses. AC and ARJ helped in the interpretation of results and designed approach for data normalisation. NG, SF, PM, and IP helped in the integration of the results into Expression Atlas. SW, AP, DGS, JAV wrote the manuscript. JAV, ARJ and IP obtained the funding for performing the study. All authors have read and approved the manuscript.

Supporting information

Supplementary file 1: Protein groups from all datasets that are mapped to more than one Ensembl Gene ID.

Supplementary file 2: Median protein abundances (in ppb) for each protein group across various tissue samples in each organ.

Supplementary file 3: Median binned protein abundances across various tissue samples in each organ of mouse and rat.

Supplementary file 4: Organ distribution of canonical proteins in mouse and rat.

Supplementary file 5: Gene Ontology enrichment analysis of ‘organ-enriched’ and ‘group-enriched’ proteins.

Supplementary file 6: Correlation of protein expression between organs within each organism.

Supplementary file 7: Binned protein abundances of one-to-one mapped orthologs across all organs studied.

Supplementary file 8: Figure illustrating binned protein abundances of all one-to-one mapped orthologs across six common organs in mouse, rat and human.

Abbreviations

DDA: Data Dependent Acquisition

DIA: Data Independent Acquisition

FOT: Fraction of Total

GO: Gene Ontology

iBAQ: intensity-based absolute quantification

iTRAQ: Isobaric tag for relative and absolute quantitation

IDF: Investigation Description Format

- 605 MS: Mass Spectrometry
- 606 ppb: Parts per billion
- 607 PCA: Principal Component Analysis
- 608 rp: Pearson correlation coefficient
- 609 SDRF: Sample and Data Relationship Format
- 610 TMT: Tandem Mass Tagging

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PRIDE database

Curation

Analysis

QA & Integration into EA

A **C** **B** **D** **E** **F**

A

B

C

A

Mouse

B

Rat

A

B