Comparative Analysis of Selenocysteine Machinery and Selenoproteome Gene Expression in Mouse Brain Identifies Neurons as Key Functional Sites of Selenium in Mammals

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Although dietary selenium (Se) deficiency results in phenotypes associated with selenoprotein depletion in various organs, the brain is protected from Se loss. To address the basis for the critical role of Se in brain function, we carried out comparative gene expression analyses for the complete selenoproteome and associated biosynthetic factors. Using the Allen Brain Atlas, we evaluated 159 regions of adult mouse brain and provided experimental analyses of selected selenoproteins. All 24 selenoprotein mRNAs were expressed in the mouse brain. Most strikingly, neurons in olfactory bulb, hippocampus, cerebral cortex, and cerebellar cortex were exceptionally rich in selenoprotein gene expression, in particular in GPx4, SelK, SelM, SelW, and Sep15. Over half of the selenoprotein genes were also expressed in the choroid plexus. A unique expression pattern was observed for one of the highly expressed selenoprotein genes, SelP, which we suggest to provide neurons with Se. Cluster analysis of the expression data linked certain selenoproteins and selenocysteine machinery genes and suggested functional linkages among selenoproteins, such as that between SelM and Sep15. Overall, this study suggests that the main functions of selenium in mammals are confined to certain neurons in the brain.

Selenium (Se) is an essential micronutrient that occurs in proteins in the form of the 21st amino acid, selenocysteine.

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ISH, in situ hybridization; MOB, main olfactory bulb; CA1–CA3, Ammon’s horn; DG, dentate gyrus; ER, endoplasmic reticulum; APOE2, apolipoprotein E receptor 2; PBS, phosphate-buffered saline; BisTris, 2-(bis[2-hydroxyethyl]amino)-2-(hydroxymethyl)propane-1,3-diol.
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for more than 21,000 genes in adult mouse brain (26). The data-set provides both mRNA in situ hybridization (ISH) data and a detailed reference atlas. This resource represents a comprehensive platform for exploring gene expression in the brain and can be used to examine and compare expression of all selenoprotein genes. A bioinformatics algorithm has also been developed that allows semi-automatic searches for genes showing similar hybridization patterns (27).

In this work, we extracted expression information for various brain regions based on the ISH image data provided by ABA for all mammalian selenoprotein genes as well as for the Sec machinery genes (i.e. the genes involved in Sec biosynthesis and incorporation). We normalized and quantified the original gene expression data and clustered them by hierarchical cluster analysis to identify linkages among selenoproteins and between selenoproteins and the Sec machinery. Our analysis identified neurons in hippocampus, olfactory area, cerebellar cortex, and isocortex as the sites with increased selenoprotein gene expression. Cluster analysis allowed us to identify functional links among selenoproteins and their biosynthetic machinery. The results of this study open new opportunities for research on Se, an essential trace element, and are important for understanding the relationship between Se and brain function.

EXPERIMENTAL PROCEDURES

The ABA Resources and Query Proteins—The ABA provides an automated platform for high throughput ISH that supports systematic analysis of gene expression in young adult (8-week-old) mouse brain (26). We also used a subsequent software tool, Brain Explorer version 1.3 (downloaded from the ABA website), for viewing ABA gene expression data in the framework of the Allen Reference Atlas (ARA) in three dimensions, comparing expression data for multiple genes and navigating two-dimensional ISH images from the ABA. Both coronal and sagittal datasets were analyzed for each gene of interest if available.

We analyzed expression data for 24 known mouse selenoprotein genes and for genes coding for known Sec machinery components. Except for SelH, ISH data were available for all examined genes (each was manually selected and viewed through the publicly accessible ABA application) as follows: (i) selenoproteins (total of 23) Gpx1, Gpx2, Gpx3, Gpx4, Tr1 (Tnxrd1), Tgr (Tnxrd3), Tr3 (Tnxrd2), Dio1, Dio2, Dio3, Sel1 (D5Wsu178e), Sek, SelM, SelN (Sepn1), SelO (1300018B18Rik), SelP (Sep1l), SelR (or MsrB1, Sepx1), SelS (H47), SelT (2B10407C02Rik), SelV (BC089491), SelW (Sepw1), Sp52 (Sephs2), and Sep15; and (ii) Sec machinery (total of 5): Sec synthase (DS4ertd135e), PSTK, Secp43 (Trspp1), SBP2 (Secisp2), and EF-Sec (Efssec). It should be noted that Sp52 is both a Sec-containing protein and a component of the Sec insertion machinery. In addition, we also included a recently identified SelP receptor, ApoER2 (28). Several housekeeping genes were also examined, including β-actin (Actb), α-tubulin (Tubal1), hypoxanthine phosphoribosyltransferase (Hprt), ribosomal protein L11 (Rpl11), dynene cytoplasm 1 heavy chain 1 (Dyn1h1), glyceraldehyde-3-phosphate dehydrogenase (Gapdh), and cytochrome c (Cyc1). These genes served as controls, and for quantification purposes (see below), Hprt was used.

Brain Regions of ARA—ARA provides an anatomic framework for brain regions. When selecting an ISH image pane, the relevant ARA automatically updates to the nearest corresponding reference atlas section. In our work, we selected 159 regions, including major parts of the brain: cerebral cortex (25 regions), cerebral nuclei (14 regions), cerebellar cortex (18 regions), cerebellar nuclei (3 regions), interbrain (including thalamus and hypothalamus, 22 and 18 regions, respectively), midbrain (23 regions), hindbrain (33 regions), and non-neuron regions (3 regions). A complete list of these regions is shown in supplemental Table S1.

Definition of Gene Expression Signals in Different Regions of Mouse Brain—To extract the expression information for each gene (including controls) and for different brain regions from the original ISH images, we used both the ABA on-line tool and Brain Explorer to visually examine expression signals based on the relative measurement of gene expression provided by ABA (hereafter RMABA). RMABA is labeled on a discrete eight-color scale of increasing level of expression (from low to high: blue-aqua-turquoise-bright green-yellow-gold-light orange-orange), as the ISH process is not strongly quantitative in the sense of measuring transcript copy number (26). In addition, the range of expression (Rex, percentage of expressing area in a given region normalized by the whole area) was considered. We used the following tags to manually define the gene expression signal in each region: (i) −, no signal observed in the area; (ii) +, RMABA is blue, and Rex < 5%; (iii) +, RMABA is blue to aqua, and Rex < 25%; (iv) +, RMABA is aqua to turquoise, and Rex ≥ 25%; (v) +, RMABA is green or yellow, and Rex ≥ 50%; (vi) +, RMABA is yellow to gold, and Rex ≥ 50%; (vii) +, RMABA is light orange or orange, and Rex ≥ 50%; (viii) +, RMABA is orange, and Rex ≥ 75%.

A series of increasing integers from 0 to 7 were then assigned to the above tags, and the initial gene expression signal profiles were obtained for each gene. We repeated this procedure twice for both the ABA on-line tool and Brain Explorer software. Finally, four independent datasets (or replicates) were obtained.

Gene Expression Signal Normalization and Quantification—The gene expression signals defined above could not be directly used to measure the abundance of selenoprotein mRNAs. Instead, we utilized experimental mRNA levels for different selenoprotein genes and Sec machinery genes in the whole mouse brain, which were determined using real time PCR (29), to quantify gene expression. The reported mRNA levels of different selenoproteins and Sec machinery in whole mouse brain were normalized to the Hprt gene, whose levels were most consistent, among housekeeping genes, when different tissues and animals were considered (29). This gene was also used to normalize the ABA expression signals within brain regions. Given the observed expression signal Xg,i of a query gene g and Xh,i of a control gene h in region i, the normalized expression signal could be calculated as shown in Equation 1,

\[
E_i(g,i) = \frac{X_{g,i} + q_g}{X_{h,i} + q_g} \tag{1}
\]

where qg is an “artificial” value (or pseudocount) to avoid zero-
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probability expression when no signal of a particular gene was observed in certain region. In this study, we defined \( q_g = 0.1 \).

The average expression signal \( E_i(g) \) among 159 brain regions was then calculated as shown in Equation 2,

\[
E_i(g) = \frac{\sum E_i(g,i)}{N_i}
\]  

(Eq. 2)

where \( N_i \) is the number of brain regions (159 in this study). Here, \( E_i(g) \) was regarded as measure of the mRNA levels for selenoproteins and Sec machinery in the whole mouse brain (designated \( E(g) \)). Based on the correlation between \( E_i(g) \) and \( E(g) \), the abundance of mRNA (normalized by \( Hprt \)) in a given brain region \( E(g,i) \) could then be inferred as indicated in Equation 3,

\[
E(g,i) = E_i(g,i) \frac{E(g)}{E_i(g)}
\]  

(Eq. 3)

The final \( E(g,i) \) was calculated as the average of replicates.

**Gene Expression Profile Clustering**—To investigate the relationship among expression patterns of different genes, normalized expression values were further quantified as shown in Equation 4,

\[
M_g,j = \frac{\sum E(g,j,i)}{t_i} \frac{E(g,0,i)}{t_i}
\]  

(Eq. 4)

where \( t_i \) is the number of available replicates for region \( i \), and \( E(g,0,i) \) is the average expression value of gene \( g \) in all regions in the \( t_i \)-th replicate. If the expression value was not available, Equation 5 was used,

\[
M_g,j = \frac{\sum E(g,j,i)}{t_i} \frac{E(g,0,i)}{t_i}
\]  

(Eq. 5)

where \( E(g,0,i) \) is the average expression signal of gene \( g \) in all regions in the \( t_i \)-th replicate.

Hierarchical cluster analysis was performed with CLUSTER software (30). We chose the complete linkage clustering algorithm in the software for gene clustering, and the final results were represented graphically using the Java TreeView tool (31). Cells with log ratios of 0 (unchanged compared with the average level) were colored black, increasingly positive log ratios with red of increasing intensity, and increasingly negative log ratios with green of increasing intensity, respectively.

**In Situ Hybridization of SelM and SelH**—The expression of SelM and SelH genes at different developmental stages and adult rat brain was studied by in situ hybridization. For this purpose, three brains per time point were dissected and frozen in the gaseous phase of liquid nitrogen. Frontal and horizontal sections (10–15 \( \mu \)m) were fixed in 4% paraformaldehyde (w/v), washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4), and dehydrated. For in situ hybridization, the following antisense (and corresponding sense) oligonucleotides were used: 5’-gag ctt ctc tgg agg gcc ctt ctt aat acc aat cca gag ttc aac ac-3’ (SelH, GenBankTM accession number EST235143_R), and 5’-gga ggt gtc tca tca cca ggt tgt ggt aca gtt gaa tgt cct gag tga ca-3’ (SelM, GenBankTM accession number XM_223554). The chosen oligonucleotides showed no significant cross-matches with other nonredundant and EST nucleotide sequences by BLAST analyses.

**Immunofluorescence Confocal Microscopy Analysis of SelM**—Whole mouse brains were dissected from adult mice immediately after decapitation and fixed in 4% paraformaldehyde in PBS for 2 h (all procedures were carried out at room temperature). After three washes in PBS (15 min each), the samples were dehydrated through an ethanol series and embedded in paraffin. Paraffin sections of the brain samples (~5 \( \mu \)m thick) were processed using standard de-paraffin and re-hydration methods, blocked in 3% bovine serum albumin in PBS for 1 h, and incubated for 2 h in 1% bovine serum albumin in PBS containing 0.05% Tween 20 (PBS-T) and rabbit anti-SelM antibodies (1:100 dilution). Brain sections were then washed three times (15 min each) in PBS-T and incubated in 1% bovine serum albumin in PBS-T containing Cy5-conjugated donkey anti-rabbit IgGs (Jackson ImmunoResearch, 1:100) for 1 h. After three rinses in PBS-T, the samples were stained with 4’,6-diamidino-2-phenylindole, mounted, and examined with an Olympus FV500 confocal system.

**Western Blot Analyses**—Different brain parts, including the olfactory bulb, hippocampus, hypothalamus, cerebral cortex and cerebellar cortex, were isolated from freshly dissected mouse brains. These brain parts, along with the other tissues from the same mouse (liver and testis), were snap-frozen in liquid nitrogen and then stored at \(-80^\circ\text{C}\). Samples were homogenized in PBS containing complete protease inhibitors (Roche Applied Science), sonicated, and centrifuged at 14,000 \( \times g \) for 15 min. Supernatants were collected, and the lysates were normalized with regard to protein concentration. 10% BisTris NOVEX gels (Invitrogen) were used, and each well was loaded with 25 \( \mu \)g of indicated tissue homogenate. The proteins were separated by SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Invitrogen), and probed with specific antibodies described previously by our laboratories (also shown in the Supplemental Material). Secondary horse-radish peroxidase-conjugated antibodies were from Amersham Biosciences, and chemiluminescent peroxidase substrate was from Sigma.

**RESULTS**

**Global Analysis of Selenoprotein and Sec Machinery Gene Expression in Mouse Brain**—Original ISH image data could be identified in the ABA dataset for 23 selenoprotein genes and 5 Sec machinery genes. The remaining mouse selenoprotein gene (SelH), was not represented in the ABA dataset, but it was detected in mouse brain in previous studies (29), and we experimentally verified its expression during development in rat brain. Thus, all mouse (rodent) selenoprotein genes appear to be expressed in the brain. Whether a full selenoproteome is also expressed in other organs is not known. We further analyzed expression profiles of all detected selenoprotein genes across 159 brain regions. The corresponding expression levels for each selenoprotein gene were then calculated using absolute mRNA levels for whole mouse brains (based on real time PCR data).
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![Diagram showing the percentage of brain regions with different expression levels for selenoprotein genes.](http://www.jbc.org/)

**FIGURE 1.** General features of expression of selenoprotein and Se machinery genes in mouse brain. The actual expression level of each indicated gene in each brain region in the ABA dataset was calculated based on the experimental expression level in whole mouse brain (29). Using hprt as a reference gene, expression levels of each examined gene were divided into the following four groups: undetectable (0.05–0.5), low expression (0.5–2), medium expression (2), and high expression (>2). Proportions of brain regions with indicated expression levels are represented in the form of a histogram. These data were used to classify selenoproteins into highly expressed selenoprotein genes (at least a medium expression level in more than half, and a high expression level in one-third of the brain regions) and selenoprotein genes expressed at low or undetectable levels in most regions of mouse brain.

Distribution and general features of selenoprotein gene expression are shown in Fig. 1. Six (GPx4, SelK, SelM, SelP, SelW, and Sep15) of 23 selenoprotein genes showed at least a medium expression level in more than half and a high expression level in one-third of brain regions. These genes were designated as highly expressed genes. Of these six genes, GPx4, SelP, and SelW genes were expressed at a high level in over 90% of brain regions. These observations are consistent with previous studies that found high SelP and SelW gene expression in rodent brain (15, 20, 21, 23, 29, 32). On the other hand, many selenoprotein genes, including those coding for essential proteins, e.g. cytosolic and mitochondrial TRs, and those expressed at high levels in certain organs, e.g. GPx2, GPx3, and TGR, were expressed at low levels or had undetectable mRNA in most brain regions. Moreover, GPx1 and TR1, the best characterized and most abundant selenoproteins in liver and many other organs, were not among the highly expressed selenoprotein genes. It is also interesting that the three Dio genes (Dio1–3), which were previously reported to be significantly expressed in the developing brain of mammals (33, 34) and frog (35), were undetectable in more than 85% of the regions of adult mouse brain. However, it has been reported that Dio2 may serve specific roles through regulated expression in specific hypothalamic cells (36–38).

Similar analyses were also carried out for Sec machinery genes. All five examined Sec machinery genes were detected in different regions of mouse brain. We further limited our analysis to SBP2 and Secp43, as real time mRNA levels in the whole brain were not available for other Sec machinery components (29). Although SBP2 was expressed at low levels in most brain areas, it clearly mimicked expression patterns of five highly expressed selenoprotein genes (GPx4, SelK, SelM, SelW, and Sep15), suggesting that increased levels of SBP2 were needed to support expression of some or all of these selenoproteins.

Regionally Enriched Selenoprotein Gene Expression—Several selenoprotein genes showed common, yet highly complex expression patterns, whereas some had unique patterns. To identify brain regions with elevated selenoprotein gene expression, we examined each of the 159 brain regions for sets of expressed selenoprotein genes. Representative subdivisions were extracted from these regions based on brain anatomy, function, transmitter systems, and neuron populations affected by major neurodegenerative disorders. Supplemental Table S2 summarizes the data for these regions by showing normalized selenoprotein gene expression levels. A complete expression profile of selenoproteins in 159 brain regions is included in supplemental Table S1.

Main olfactory bulb (MOB, 21 selenoprotein genes), Ammon’s horn (CA1–CA3, 20 selenoprotein genes), piriform area (19 selenoprotein genes), anterior olfactory nucleus (19 selenoprotein genes), cortical amygdalar area (19 selenoprotein genes), accessory olfactory bulb (19 selenoprotein genes), isocortex (or neocortex, e.g. somatosensory areas, 19 selenoprotein genes), and all folia of vermis and hemispheres of cerebellar cortex (19 selenoprotein genes) were the top regions with regard to the number of expressed selenoprotein genes (supplemental Table S2). Except for a small number of brain stem nuclei, we found that selenoprotein-enriched areas were located in the following four basic brain regions: hippocampus, olfactory area, cerebellar cortex, and isocortex (the part of cerebral cortex with uniform six layers). Olfactory cortex, piriform area, and hippocampus, all belonging to allocortex, showed unique selenoprotein expression patterns. An illustration of this general pattern is given in supplemental Fig. S1 that features elevated SelM gene expression in cerebellum, MOB, CA, and dentate gyrus (DG) structures of hippocampus. We further refer to these four regions as the regions with high selenoprotein gene expression. Except for SelP in hippocampus, expression levels of all highly expressed selenoprotein genes were at least medium, and of GPx4, SelW, and Sep15 genes were high in...
these four regions. Other selenoprotein genes, which were expressed at low or undetectable levels in most brain areas, were also found to be expressed at increased levels in these four regions.

In contrast to the above regions, major parts of the midbrain exhibited a much lower expression of selenoprotein genes. Supplemental Table S2 also summarizes the regions in which the lowest numbers of selenoprotein genes were detected, including oculomotor nucleus, Edinger-Westphal nucleus, nucleus Raphe pontis, anteroventral periventricular nucleus and dorsal premammillary nucleus. Only highly expressed selenoproteins were detected in most of these brain regions. In addition, lower expression of selenoprotein genes was observed in the largest white matter structure in the brain, corpus callosum. The low levels of selenoprotein mRNAs in these structures suggest lower dependence of these structures on selenoproteins and Se.

**Cellular Localization of Selenoprotein Expression**—Many brain regions show a multilayered neural architecture, a pattern that is also evident in the analysis of selenoprotein gene expression. In contrast, the distribution of glial cells is generally more uniform, except in large fiber tracts devoid of neuronal cell bodies. Analysis of the ABA dataset suggests that certain cell types/layers rather than a distinct anatomical localization are associated with specific expression of selenoprotein genes. To investigate the cytoarchitectonic features of selenoprotein gene expression, we manually examined selenoprotein expression patterns in different cell types and layers that were characterized by significant selenoprotein gene expression (supplemental Table S3). We used expression signal tags to reflect the expression patterns instead of the expression level per se to better represent changes in expression between brain regions. However, it should be noted that the same signal tag may represent different mRNA expression levels for different selenoprotein genes, e.g. “/H11001/H11001” corresponds to low expression level for SelS, medium expression level for SelM, and high expression level for GPx4 (see “Experimental Procedures”).

One of the most obvious regions that showed patches of elevated expression of selenoprotein genes was the hippocampus. However, the selenoprotein expression patterns in this formation were not uniform (Fig. 2A and supplemental Fig. S2). A number of selenoprotein genes were strongly expressed in the pyramidal cells of the CA1–3 and granule cells of DG (Fig. 2A and supplemental Table S3), but the expression levels of many selenoprotein genes were different among CA1, CA2, and CA3 regions. For example, SelM showed highest expression in CA1/CA2, GPx4 in CA2/CA3, and SelS in CA1. On the other hand, Sep15 and SelW showed uniform expression levels in all CA regions. Moreover, the expression pattern of TR1 was a notable exception with the higher expression signal in the DG than in the CA areas. Expression of SelP was more homogeneous in the hippocampus. In contrast to other highly expressed selenoprotein genes, we did not observe SelP gene expression in the major neurons of CA and DG structures, suggesting an oligodendroglial or astrocytic expression pattern of this protein. In contrast, selenoprotein biosynthetic machinery genes, e.g. SBP2, were generally expressed at the same levels in CA1–3 and DG and
correlated with expression levels of GPx4, SelM, SelW, but not SelP genes. The resolution of the data was not sufficient to quantify selenoprotein expression in hippocampal interneurons located in, for example, the strata oriens or radiatum, but signals with a distribution reminiscent of interneurons were observed (e.g. for SelM).

Olfactory bulb was another region with significant selenoprotein gene expression, but as in the hippocampus, the observed expression pattern was not uniform. Selenoprotein genes and Sec machinery genes in MOB were mainly detected in the glomerular, mitral, and granule cell layers (Fig. 2B, gl, mi, and gr, and supplemental Fig. S3), with the highest levels in the mitral layer. Again, the SelP expression pattern suggested glial expression in white matter. As in CA and DG structures in the hippocampus, SelP expression appeared to be excluded from the areas with elevated selenoprotein expression. However, it is interesting that the highest expression of the SelP gene was found within the olfactory nerve layer of MOB, which is located on the surface of the bulb. Expression of other selenoprotein genes was not detected in this area.

In the cerebellar cortex, which contains nearly 50% of neurons in the brain, almost all detected selenoprotein genes were expressed in the Purkinje cell layer (Fig. 2C and supplemental Fig. S4, and supplemental Table S3). SelM had the most significant expression signals covering Purkinje cells, granule cells, and deep cerebellar nuclei. A similar expression pattern was observed for GPx4, TR1, MsrB1 (supplemental Fig. S4), and the Sec machinery gene SBP2, although at a lower signal level. Expression of SelS, SelW, and Sep15 was largely restricted to Purkinje cells. Thus, Purkinje cells apparently express virtually all selenoproteins. SelP gene expression was detected in both the monolayer associated with Purkinje cell layer and deep white matter. The monolayer expression was not significantly elevated for SelP gene (as was observed for other highly expressed selenoprotein genes), and SelP-expressing cells were spaced and had highly variable SelP expression levels. In contrast, other selenoprotein genes, such as SelS, SelW, and SelT, showed uniform expression in Purkinje cells. It is thus possible that SelP expression is confined to radial glia (or Bergmann glia), whose cell bodies are located next to the soma of Purkinje cells and the signal may sometimes be misinterpreted as arising from Purkinje cells. Further experiments would be needed to test this possibility, but it fits the idea of separation of high SelP and high selenoprotein expression in different cell types.

We also sampled expression of selenoprotein genes in different layers of the isocortex. For the purpose of presentation, we selected the primary somatosensory area overlying the dorsal hippocampal formation. Interestingly, some selenoprotein genes showed layer-specific expression (Fig. 2D and supplemental Fig. S5). For example, the SelW gene was highly expressed in layer II compared with other layers, and GPx4 and Sep15 gene expressing cells were enriched in layers II and IV. On the other hand, SelM gene was enriched in layer V. Analogous patterns were also observed for Sec machinery genes. In contrast, SelP showed a uniform distribution in different layers.

Besides the four major selenoprotein-enriched regions, certain brain stem nuclei were detected to express multiple selenoprotein genes. As an example for such nuclei, we selected the facial motor nucleus (VII) that is entirely composed of cholinergic motor neurons. Thirteen selenoprotein genes were detected in this nucleus. Most selenoprotein genes and Sec machinery genes, such as GPx4, SelK, SelM, SelW, and SBP2, were expressed in a pattern resembling the distribution of motoneurons (Fig. 3).

In addition to neurons and glial cells in various brain regions, we observed significant expression of selenoprotein genes in the choroid plexus of both the lateral ventricle and the fourth ventricle. The choroid plexus is a highly vascularized structure located in the ventricles that produces cerebrospinal fluid and is a key part of the blood-brain barrier. Here, expression of 14 selenoprotein genes was detected. GPx4 and Sep15 genes were expressed in particularly high levels (Fig. 4). SelP was detected previously in human cerebrospinal fluid (24). Here, SelP expression was also detected at elevated levels, suggesting secretion of SelP into mouse cerebrospinal fluid.

Interestingly, ApoER2 was observed to have elevated gene expression in the four selenoprotein-enriched areas as well as in the choroid plexus (Fig. 2 and Fig. 4). This finding is consistent with the idea that ApoER2 is important for SelP transport within the brain, especially in selenoprotein-enriched areas.
Verification of Selenoprotein Expression in the Brain—We wanted to extend the expression data of one of the most heavily expressed selenoproteins, SelM, to other rodents, and we thus performed ISH for this gene in the rat hippocampus at different developmental stages. Fig. 5A shows that this gene was highly expressed first around birth in hippocampal CA3 and later in all principal divisions of the hippocampus. In parallel, SelM protein was readily detected in mouse brain by immunohistochemistry, which was found to be most prominent in cerebellar Purkinje cells (Fig. 5B). In the cerebellar granule cell layer, some unidentified cells stained positive for SelM, possibly interneurons.

Because the mRNA expression data for SelH were not available in the ABA dataset, we performed ISH, using rat brain development as a model. Interestingly, SelH was highly expressed during development, but it fell after birth and was below detection limit in the adult brain (Fig. 6). Using reverse transcription-PCR, however, SelH gene expression was detected in adult mouse brain in a separate study (29). Therefore, all 24 selenoprotein genes were expressed in mouse (or rodent) brain.

Because mRNA expression does not always reflect protein levels, we analyzed expression levels for several selenoproteins in different regions of the mouse brain by Western blot assays (Fig. 7). Liver and testes were used as controls, and as expected, the liver showed high expression of GPx1 and MsrB1, whereas SelS and GPx4 were particularly abundant in mouse testes. We found that TR3 was expressed at similar levels in all analyzed samples, including liver, testes, and various brain regions. This is consistent with a previous observation that gene expression levels of TR3 in liver and brain are not significantly different (39). Consistent with SelM mRNAs levels in whole brain, this selenoprotein showed higher expression levels in brain than in other organs. Within brain, expression of several examined selenoproteins, including GPx4, SelM, MsrB1, SelW and SelS, was particularly high in cerebellar cortex. In contrast, selenoprotein expression was low in hippocampus and olfactory bulb. Here, signal intensity may simply be a consequence of low neuronal density, because neurons, not glial cells, are the major site of selenoprotein expression. Another possibility is that these regions store high levels of selenoprotein transcripts, which can be used for quick selenoprotein expression and subsequent degradation. Such a mechanism would be consistent with the regulation requiring fast protein turnover and may be particularly relevant to olfaction and memory.

Clustered Gene Expression—Information on selenoprotein gene expression in different regions of mouse brain provided us with an opportunity to identify possible functional linkages among selenoproteins and between specific selenoproteins and components of Sec insertion machinery by comparing their expression patterns. To this end, we subjected the selenoprotein and Sec insertion gene expression dataset for 159 selected brain regions to clustering with CLUSTER (30). Gene expressions were classified by hierarchical cluster analysis and displayed in a correlation map (supplemental Fig. S6). A fraction of the map corresponding to the regions with elevated selenoprotein gene expression is shown in Fig. 8. Consistent with significant selenoprotein gene expression in CA1–3 and DG regions of the hippocampus and in cell layers of the olfactory bulb, isocortex, and cerebellar cortex structures, genes for several Sec machinery components were significantly expressed in these regions. In contrast, although other structures that consist of discrete nuclei, such as the hypothalamus, pons, medulla, and midbrain, had locally enriched expression of certain selenoprotein genes, the expression level for most genes was similar to or even lower than the average level in the whole brain. Almost all selenoprotein genes, which were expressed at low levels in most brain regions, clustered in one branch, whereas most highly expressed selenoprotein genes clustered in another
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Finally, pairs of genes exhibiting a similar expression pattern were identified. For example, Sep15 and SelM clustered together. These are homologous proteins and are both targeted to the endoplasmic reticulum (40). Possibly, these proteins have similar functions in vivo. However, differences in expression patterns for these selenoprotein genes were also identified. For example, SelM gene expression was relatively low in several brain stem nuclei and choroid plexus, whereas Sep15 gene was highly expressed in these structures (supplemental Table S2). In addition, the SBP2 gene clustered within the group containing highly expressed selenoprotein genes, suggesting that SBP2 expression is elevated to support increased expression of a select group of selenoprotein genes in certain regions of mouse brain.

DISCUSSION

Our study represents, to date, the most complete analysis of selenoprotein gene expression in the mammalian brain. Expression of mRNA for selenoproteins and Sec machinery genes was analyzed in 159 regions of the adult mouse brain, and cluster analysis revealed coexpression patterns of potential functional significance. To complete the analysis, ISH and Western blot experiments were carried out. These data will aid in the design and interpretation of experiments aimed at elucidating the mechanisms of how Se, through selenoproteins, supports brain function.

Se is a trace element indispensable for mammals and is present in each organ and in body fluid. Numerous studies have examined the levels of Se in human and animal tissues and found an uneven distribution of this trace element (41–45). Se content of human brain (∼88 ng/mg wet weight) is much lower than that of kidney and liver (∼469 and ∼221 ng/mg wet weight, respectively (45). Similarly, Se content of mouse brain is...
lower than that of most other organs (15). However, during Se deficiency, the brain shows an ability to preserve this trace element, whereas other organs readily lose Se (13, 15, 46). In addition, several studies revealed that the regions of human brain enriched for gray matter tend to have higher Se levels, whereas white matter was found to have reduced Se, and Se appeared to concentrate in glandular parts of the brain (41, 44, 47). Similar patterns were observed for animals (48, 49). However, how this information can be translated at the level of selenoproteins, which mediate the biological effects of Se, is not known. Expression patterns and levels for most brain selenoproteins and Sec machinery factors have not been documented, although significant data exist for some selenoproteins, such as GPx1, a selenoenzyme family that removes hydroperoxides and is expressed in all cell types (50–52), and SelP, a selenium transport selenoprotein (16, 17, 22).

Previous studies found that some of the analyzed selenoprotein genes were expressed predominantly in neurons (such as GPx4), or in both neuronal and glia cells (such as GPx1 and TR). However, our study suggests that neurons are the primary sites of selenoprotein expression in the brain. This result nicely matches the data on neuron-specific deletion of the tRNA[Ser]Sec where the majority of analyzed selenoproteins were severely reduced or were undetectable in brain as analyzed by Western blot experiments. Thus, whatever the mechanism of neurological damage may be in mice with reduced brain Se content (16, 17, 54, 55), it likely results from impaired neuronal selenoprotein expression.

Another salient finding of this work is that there are four regions in the brain that express the following: (i) the most selenoprotein genes within the same cells, and (ii) the highest levels of selenoprotein mRNAs. These regions were olfactory bulb, cerebral cortex, hippocampus, and cerebellar cortex. Taking into account the equally strong expression of selenoprotein genes in some brain stem nuclei, as shown for the facial nucleus, we can rule out a methodological bias based on the size of the brain structure. Moreover, the striatum expresses only average levels of selenoprotein genes, although it is a comparably homogeneous structure. Thus, homogeneity of cell types also does not introduce a bias for interpretation of ISH data. This indicates that our findings are of biological significance, although we cannot, at present, correlate high selenoprotein gene expression with certain types of neuron (pyramidal versus

FIGURE 8. A fraction of the correlation map that shows elevated gene expression for most selenoproteins in the four selenoprotein-enriched areas. The dendrogram and the image were produced as described in the text; the color scale is from saturated green (significant negative change) to black (no significant change) and then to saturated red (significant positive change). Red and green colors represent increased and decreased expression, respectively, when compared with the average expression level for each gene. Each gene is represented by a single row of colored boxes; and each region is represented by a single column. Highly expressed selenoprotein genes are shown in red font, and selenoprotein genes expressed at a low or undetectable level in most brain regions in green font. The four selenoprotein-enriched areas are highlighted in red.

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granule) or with certain neurotransmitters (glutamate, γ-aminobutyric acid, or acetylcholine).

An additional significant result of this work is that hierarchical cluster analysis of gene expression data is shown to be a useful method to extract biological information from the ISH data base. To be able to correlate the ABA signals with actual gene expression levels, we took advantage of the data on experimental gene expression for selenoproteins and Sec machinery in the whole mouse brain (29). With this approach, normalized expression levels of selenoprotein genes in individual regions of mouse brain could be inferred, enhancing the primary data provided by the Atlas. Conclusions of biological significance based on the bioinformatics approach included the finding that, among Sec machinery genes, SBP2 expression most closely correlated with high selenoprotein gene expression, supporting the hypothesis that SBP2 is the limiting factor for selenoprotein translation. In addition, we have previously found that SelM and Sep15 are distantly related proteins of unknown function (40, 56). It is thus significant that these two selenoproteins clustered together in the expression data.

Which selenoprotein genes are most important to brain function? Our study identified six highly enriched selenoprotein genes, including GPx4, SelK, SelM, SelP, SelW, and SelP15. Particularly abundant were GPx4 and SelW genes, which were detected at high levels in more than 90% regions of mouse brain. Of the six selenoproteins with elevated gene expression levels, the functions of SelM, SelW, SelP15, and SelK are not known. It is interesting, however, that classical gene targeting of SelP, but not liver-specific inactivation, leads to neurological dysfunc-
tion and motor incoordination (16, 19, 54). Although SelP-deficient mice can be rescued phenotypically by increasing dietary Se intake, synaptic function and spatial learning have been reported altered in the knockouts (57). In addition, GPx4 has recently been shown to be essential for neuronal development and survival (25). Thus, high and clustered SelM, SelK, and SelW gene expression is also consistent with important physiological functions of these proteins in the brain. Unfortunately, little is known about their biochemical functions.

On the other hand, expression levels of several well characterized selenoprotein genes were lower. Some of these, such as Dio1–3, are involved in fine-tuning of thyroid hormone signaling by local activation or inactivation of thyroid hormones. Although Dio2 has been implicated in hypothalamic orexigenic signaling (36), gene targeting of Dio2 yielded only mild effects on brain development and neurological function in mice (58). Genetic inactivation of Dio1 had no reported effect on the brain (59). Gene targeting of Dio3 disrupted normal thyroid hormone signaling in the hypothalamus, but is probably not required for neuronal function in general (60). The best studied selenoprotein in the brain is probably GPx1 (61). Genetic inactivation of GPx1 increased susceptibility of rodents to neurodegenerative disease, and overexpression of GPx1 mitigated resulting damage (19). However, no spontaneous neurological damage was reported in mice lacking GPx1.

An additional interesting finding of our investigation is the cell type-specific expression of selenoproteins within brain structures. Such a distribution suggests physiological associations between selenoprotein expression and specific functions exerted by these specific neurons. Thus, there is at present no apparent explanation as to why, e.g. within the Ammon’s horn, selenoprotein genes are differentially expressed between CA1, CA2, and CA3. In addition, layer-specific expression of selenoprotein genes has not been reported previously for cerebral cortex. What could be the functions of selenoproteins in the brain? With regard to GPxs, TRs, and MsrB1, the enzymes with known functions, it appears clear that redox regulation or protection from reactive oxygen species may be the main functions (54). It may well be that neuronal signaling or synaptic transmission are modulated by the (transient) redox potential around neurotransmitter receptors, and at least for one K+ channel regulation through reversible methionine oxidation has been demonstrated (62). Many selenoproteins with still unknown function have a common thioredoxin fold, suggesting catalysis of redox reactions. Several selenoproteins are associated with the endoplasmic reticulum (ER), including Sep15 and SelM, which may be involved in oxidative protein folding, and SelS, a component of the retrotranslocon. Thus, all these selenoproteins may be involved in protein quality control in the ER. SelN is also located in the ER, but mutations in humans lead to rigid spine muscular dystrophy or multiple minicore disease (63). Direct neurological defects have not been reported in these patients.

One highly expressed selenoprotein gene, SelP, had a unique expression pattern among selenoprotein and Sec machinery genes. It appeared that its expression and secretion mainly from astrocytes or oligodendrocytes supported selenoprotein synthesis in brain structures with high selenoprotein gene expression. In an unbiased approach, the original publication of the ABA classified SelP as marker for oligodendroglial cell or white matter tracts. Previously, it was found to be highly expressed in olfactory bulb and cerebellum (22, 23). SelP-like immunoreactivity was detected on neurons and some white matter tracts (24). Given its role as a Se carrier, its expression in glial cells would help store Se in the brain and also provide it to neurons for selenoprotein biosynthesis. Accordingly, many neurons express the SelP receptor, ApoER2 (28, 55), although ApoER2 is also a receptor component for Reelin, a secreted protein involved in brain development and synaptic transmission (53). Thus, we also examined the expression pattern of ApoER2 in 159 brain regions and found elevated expression in the four selenoprotein-enriched areas and in the choroid plexus (Fig. 2, Fig. 4, Fig. 8, and supplemental Fig. S6). This is consistent with the idea that ApoER2 is important for SelP-mediated Se transport into and within the brain. Considering its mRNA expression pattern, it might be that SelP immunoreactivity on neurons, e.g. Purkinje cells (24), arises from SelP binding to the ApoER2 on the cell surface rather than reflecting endogenous expression by the neurons. Nevertheless, we recently reported the presence of SelP in human cerebrospinal fluid and high immunoreactivity in ependymal cells lining the ventricles of the human brain (24), also supporting the above idea. Why other selenoproteins are also enriched in the choroid plexus, the source of cerebrospinal fluid, is not clear, but one may speculate that GPx4 and Sep15 may be
involved in the control of massive protein secretion by chro-
roid plexus cells.

Sec machinery genes had specific gene expression patterns in
different regions of mouse brain. The correlation of elevated
SBP2 expression with selenoprotein gene-enriched structures
has been noted above. EFsec mostly followed SBP2 expression.
Interestingly, both PSTK and SecP43 had rather specific expres-
sion in cortical layers II and IV/V, the two cortical layers that
had the highest selenoprotein expression (supplemental Fig.
S5).

In the future, the methods described here could be extended
to the entire ABA dataset and identify functional linkages
between selenoproteins and other proteins expressed in mouse
brain, e.g. other proteins involved in redox control, or could be
extended to developmental studies. More specifically, these
studies suggest candidate selenoproteins with presumed roles
in brain function. Therefore, gene expression profiles identified
in this study provide informative modality to investigate diver-
sity of selenoprotein functions in the brain.

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Selenoprotein Gene Expression in Mouse Brain

Comparative Analysis of Selenocysteine Machinery and Selenoproteome Gene Expression in Mouse Brain Identifies Neurons as Key Functional Sites of Selenium in Mammals

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