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The PACAP-Regulated Gene Selenoprotein T Is Highly Induced in Nervous, Endocrine, and Metabolic Tissues during Ontogenetic and Regenerative Processes

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Selenoproteins contain the essential trace element selenium whose deficiency leads to major disorders including cancer, male reproductive system failure, or autoimmune thyroid disease. Up to now, 25 selenoprotein-encoding genes were identified in mammals, but the spatiotemporal distribution, regulation, and function of some of these selenium-containing proteins remain poorly documented. Here, we found that selenoprotein T (SelT), a new thioredoxin-like protein, is regulated by the trophic neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) in differentiating but not mature adrenomedullary cells. In fact, our analysis revealed that, in rat, SelT is highly expressed in most embryonic structures, and then its levels decreased progressively as these organs develop, to vanish in most adult tissues. In the brain, SelT was abundantly expressed in neural progenitors in various regions such as the cortex and cerebellum but was undetectable in adult nervous cells except rostral migratory-stream astrocytes and Bergmann cells. In contrast, SelT expression was maintained in several adult endocrine tissues such as pituitary, thyroid, or testis. In the pituitary gland, SelT was found in secretory cells of the anterior lobe, whereas in the testis, the selenoprotein was present only in spermatogenic and Leydig cells. Finally, we found that SelT expression is strongly stimulated in liver cells during the regenerative process that occurs after partial hepatectomy. Taken together, these data show that SelT induction is associated with ontogenesis, tissue maturation, and regenerative mechanisms, indicating that this PACAP-regulated selenoprotein may play a crucial role in cell growth and activity in nervous, endocrine, and metabolic tissues. **(*Endocrinology* 152: 4322–4335, 2011)**

Selenium (Se) is considered as one of the most important micronutrients owing to its numerous benefits for human health, from the first stages of embryonic development to later times of aging (1, 2). Se deficiency leads to multiple disorders affecting many organs. For instance, Se is of the utmost importance for the development and function of the central nervous system (CNS) because its deficiency leads to several important deficits including mental disorders (3) or the generation of intractable epileptic seizures (4). Se is also crucial for the male reproduc-

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Abbreviations: BrdU, Bromodeoxyuridine; CgA, chromogranin A; CNS, central nervous system; CP, cortical plate; DCX, doublecortin; E20, embryonic d 20; EGL, external germinative layer; ER, endoplasmic reticulum; GFAP, glial fibrillary acidic protein; GPx, glutathione peroxidase; GRP78, glucose-related protein 78; IZ, intermediate zone; P1, postnatal d 1; PACAP, pituitary adenylate cyclase-activating polypeptide; QPCR, quantitative PCR; RMS, rostral migratory stream; ROS, reactive oxygen species; RT, reverse transcription; Sec, selenocysteine; SelT, selenoprotein T; SelW, selenoprotein W; SVZ, subventricular zone; VAMP, vesicle-associated monoamine protein.

tive system because it was shown that Se-deficient male rats exhibit reduced fertility (5, 6). Many studies also showed that Se deficiency plays a key role in the pathogenesis of endemic myxedematous cretinism (7), associated with thyroid atrophy (8). In fact, the majority of Se beneficial effects are actually mediated by the biological activity of proteins in which it is incorporated, the selenoproteins, where the micronutrient is inserted as a selenocysteine (Sec), the 21st natural amino acid (9, 10). In mammals, 25 selenoprotein-encoding genes have been identified (11) whose entire invalidation through Sec tRNA gene knockout leads to early fetal lethality in rodents (12). In agreement, selenoproteins are key effectors involved in major cellular processes, including cell survival and homeostasis. During noxious conditions, many selenoproteins, such as the glutathione peroxidases (GPx), act to protect cells from oxidative stress. For instance, GPx1 (9), selenoprotein W (SelW) (13), and selenoprotein H (14) possess the ability to neutralize reactive oxygen species (ROS) through a redox mechanism mediated by Sec. In addition, diverse essential functions were attributed to other selenoproteins, such as their implication in signal transduction and transcription regulation pathways, *e.g.* thioredoxin reductases (15), or in thyroid hormone activation and deactivation, *e.g.* deiodinases I, II, and III, which catalyze iodothyronine deiodination (16). Selenoproteins display specific tissue and subcellular expression patterns (17), although these characteristics remain undocumented for some of them. Each selenoprotein is selectively addressed to specific subcellular sites where it exerts important functions. For instance, endoplasmic reticulum (ER)-resident selenoproteins contribute to many essential functions such as the regulation of Ca^{2+} homeostasis, *e.g.* selenoprotein T (SelT) (18), SelN (19), and SelM (20), and protein folding quality control and the unfolded-protein response, *e.g.* 15 kDa selenoprotein and SelS (21).

Among the ER-located selenoproteins, little is known about the distribution and function of the recently characterized SelT in nervous, endocrine, and metabolic tissues. We have previously shown that SelT gene expression is up-regulated during neuroendocrine differentiation induced by the neurotrophic factor pituitary adenylate cyclase-activating polypeptide (PACAP) (22). In pheochromocytoma PC12 cells, this neuropeptide induces cell cycle arrest along with the extension of neurites and the establishment of a neuroendocrine phenotype sustained by modulation of the expression of several genes including *SelT* (23, 24). Confocal imaging revealed that SelT is primarily localized in the ER and the Golgi apparatus (18, 25). Additional experiments carried out in PACAP-differentiated PC12 cells demonstrated that SelT is involved in Ca^{2+} homeostasis and is essential for the secretory activity (18). In fact, SelT contains a conserved motif called thioredoxin-like fold (25), C-X-X-U, characterized by the presence of a cysteine residue (C) at the vicinity of Sec (U), separated by two other amino acids (X), which holds the biological activity of the protein. Indeed, this molecular feature is also found in the mammalian SelW, -V, and -H and confers an important redox activity that may underlie the cellular and physiological functions of these proteins (26). It has been recently shown that SelT could also be involved in cell adhesion and redox equilibrium status (27). Together, these observations indicate that SelT could play important homeostatic and regulatory roles in different tissues. However, its spatiotemporal distribution and regulation remain largely unknown. In the present study, we sought to determine the patterns of SelT expression, particularly in nervous, endocrine, and metabolic organs, at different stages of life and during differentiation and regeneration. Accordingly, we performed a precise mapping of its distribution and subcellular localization in various developing and adult nervous and endocrine tissues, including the brain, the cerebellum, and the testis. Furthermore, we investigated SelT regulation during the regenerative process occurring after partial hepatectomy.

Materials and Methods

Animals

Wistar rats were used throughout this study and were obtained from Charles River Laboratories (L'Arbresle, France). Animals were housed under standard and constant conditions, with controlled lighting (12-h light, 12-h dark cycle) and temperature (22 ± 2 C), and allowed access to food and water *ad libitum*, in agreement with the Regional Veterinary Services guidelines (agreement no. B-76-451-04). Embryos were surgically removed from time-mated pregnant Wistar rats. All experiments were conducted in accordance with the European Committee Council Directives and approved by the Normandy Ethics Committee on Animal Experiments (agreement no. N/01-11-07/09/12-10). Involved investigators are trained and authorized to conduct experiments on laboratory animals.

Partial hepatectomy experiments

Hepatectomy was performed by resection of half of the liver left lateral lobe (28). The resected part of the hepatic lobe was used as a control for Western blotting analyses. For immunofluorescence, a 4% paraformaldehyde perfused liver was used for controls. To visualize cell proliferation in the regenerating liver, we performed bromodeoxyuridine (BrdU) injections (50 mg/kg; Sigma-Aldrich, Saint-Quentin Fallavier, France) 4 and 2 h before euthanasia.

Cell culture and treatment

Rat pheochromocytoma PC12 cells were maintained in DMEM (Sigma-Aldrich) supplemented with 10% horse serum (Invitrogen, Cergy Pontoise, France), 5% fetal bovine serum

(Sigma-Aldrich), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) at 37 C in 5% CO_2. Twenty four hours after plating, cells were treated with 100 nM PACAP38 for 16 h. This peptide was synthesized by the solid-phase methodology (29).

Primary cultures of bovine chromaffin cells were obtained after retrograde perfusion of bovine adrenal glands with collagenase as previously described (30). Cells were then plated in the same medium at a density of 10^6 cells/ml in poly-L-lysine-coated 12-well plates and treated overnight with 100 nM PACAP38.

For neuroblast culture, dorsolateral cerebral cortices were mechanically dissociated in the Neurobasal culture medium (Invitrogen) containing 50 U/ml penicillin (Sigma-Aldrich), 50 μg/ml streptomycin (Sigma-Aldrich), 1% BSA, 2 mM glutamine (Invitrogen), and 2% B27 serum-free supplement (Invitrogen). Cells were plated on 0.1 mg/ml poly-D-lysine and 5 μg/ml laminin-coated coverslips previously placed in culture dishes and incubated at 37 C in 5% CO_2.

Reverse transcription (RT) and quantitative PCR (QPCR)

Total RNA was isolated using the Tri-Reagent (Sigma-Aldrich) and reverse-transcribed using the ImProm-II Reverse Transcription System (Promega, Charbonnières, France). Relative expression of the SelT gene was quantified by real-time PCR with appropriate primers (forward, 5′-ATGCAACAGCTGGT TCAAATTCT-3′; reverse, 5′-TGATCGATGGTGTGGGAT TG-3′). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and chromogranin A (CgA) were used as internal controls (forward, 5′-TCCGGACGCACCCTCAT-3′, and reverse, 5′-CGGT TGACCTCCAGGAAATC-3′, for GAPDH; and forward, 5′-GACAGCCACAGGTCATTTCAA-3′, and reverse, 5′-GCCG GGCTTCGTCTTCA-3′, for CgA) (30). PCR was carried out using SYBR Green PCR Master Mix (Applied Biosystems, Courtaboeuf, France), with 300 nM primers, in an ABI Prism 7500 (Applied Biosystems). Relative SelT and CgA amplification efficiencies were assessed, and the amounts of SelT mRNA were determined using the comparative cycle threshold method (Applied Biosystems).

Western blot

Tissues were homogenized in a lysis buffer [50 mm Tris-HCl (pH 7.4), 2 mM β-mercaptoethanol, 2 mM phenylmethanesulfonyl fluoride, 1% Triton X-100, and an additional mix of protease inhibitors (Roche, Mannheim, Germany)], and proteins were quantified by the Bradford method (Bio-Rad, Marnes-la-Coquette, France). Fifty micrograms of proteins from each sample were separated on a 13% SDS-PAGE and immunoblotted onto a nitrocellulose membrane (Amersham Pharmacia Biotech, Les Ulis, France), as previously described (31) using SelT (1:2000) (18), α-tubulin (1:2500), and β-actin (1:1000) specific antibodies.

Immunohistochemistry

A saturation step was performed with 1% goat or donkey normal serum, diluted in a solution containing 1% BSA and 0.3% Triton X-100 in PBS for 45 min at room temperature before incubation overnight with the SelT antibody (1:400) at 4 C. SelT polyclonal antibody was generated in rabbits against the SelT-derived peptide WSKLESGHLPSMQ, and its specificity was previously established through an immunocytochemistry experiment performed on SelT-deficient PC12 cells (18). This first antibody was revealed with a secondary antirabbit IgG antibody coupled to fluorescein isothiocyanate (1:200) (Invitrogen). Several co-stainings were carried out with antibodies that are listed in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Slides were counterstained with 1 μg/ml 4′,6-diamino-2-phenylindole (Sigma-Aldrich) in PBS for 90 sec. Negative controls were performed using rabbit preimmune serum or preincubation of SelT antibody with the antigenic peptide (10^{-5} M). In both cases, no immunoreaction was observed. Tissue sections were examined with a Leica SP2 upright confocal laser scanning microscope (DMRAX-UV) equipped with the Acousto-Optico Beam Splitter system (Leica). For immunohistochemical experiments using 3,3′-diaminobenzidine, SelT primary antibodies were revealed with a biotinylated goat antirabbit antiserum (Vector Laboratories, Burlingame, CA) and an avidin-biotin peroxidase complex (ABC kit; Vector Laboratories). Tissue sections were finally counterstained with thionine (Sigma).

Results

SelT expression and regulation in undifferentiated and differentiated chromaffin cells

To determine the regulation of SelT gene expression in undifferentiated and differentiated cells, we compared the effect of PACAP on its mRNA levels in PC12 cells and in bovine chromaffin cells (Fig. 1). After overnight incubation in the presence or absence of 100 nM PACAP38, we observed that the neuropeptide differentially affects SelT gene expression in the two cell types. Indeed, PACAP induced an approximately 3.5-fold increase in SelT mRNA levels in PC12 cells but had no effect in mature adrenochromaffin cells (Fig. 1A). Time-course (6–120 h) and dose-response (10^{-10}–10^{-4} M) studies were also performed in bovine chromaffin cells to confirm the lack of PACAP effect on SelT gene expression in mature cells (data not shown). Interestingly, in basal conditions, we noticed that SelT mRNA levels are significantly higher in proliferating PC12 cells than in differentiated chromaffin cells (data not shown). Moreover, a Western blot analysis of SelT in the adrenal gland revealed that this protein is particularly abundant during the first postnatal days but is undetectable in the adult tissue (Fig. 1B). Taken together, these results indicate that SelT may exert its function during proliferation and differentiation processes, as suggested by its higher mRNA levels in proliferating compared with mature cells, and the stimulatory effect of PACAP on its gene expression in differentiating cells.

SelT expression and distribution during embryogenesis and brain ontogenesis

We subsequently assessed SelT abundance and tissue distribution during embryogenesis (Fig. 2). A RT-QPCR

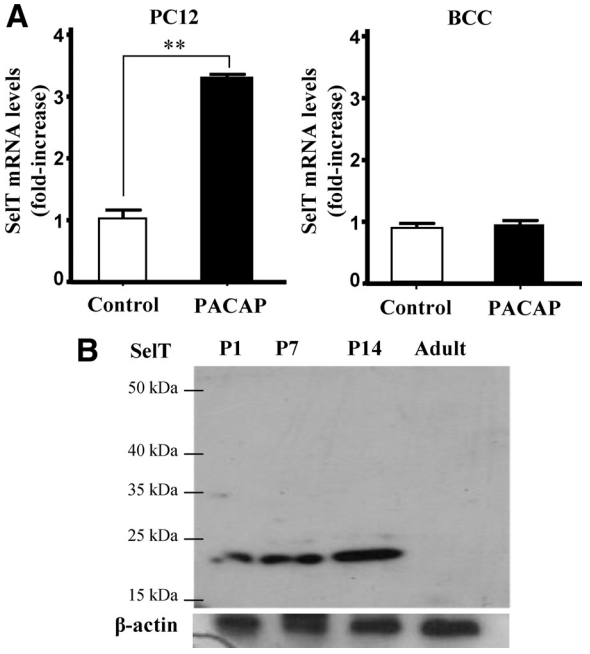

FIG. 1. SelT expression in proliferative and differentiated chromaffin cells. A, Effect of PACAP treatment on SelT gene expression in cultured pheochromocytoma PC12 cells and adult bovine chromaffin cells (BCC). Both cell types were incubated overnight in control conditions or with 100 nM PACAP. SelT mRNA levels, determined by quantitative PCR, are expressed as fold increase over corresponding control value and represent means ± SEM of four independent experiments (one-way ANOVA; **, $P < 0.01$ *vs.* control). B, Western blot analysis of SelT levels in adrenal glands collected from 1-, 7-, and 14-d-old pups, and adult rats. Fifty micrograms of proteins were loaded on a 13% SDS-PAGE, and β-actin was used as an internal loading control. The autoradiography shown is representative of three independent experiments. Molecular weight markers are indicated.

(Fig. 2A) and a Western blot (Fig. 2B) screening showed that SelT is expressed at high levels in each tissue of the embryonic d 20 (E20) embryos analyzed. Immunohistochemical experiments confirmed that SelT is widely distributed in a range of E15 and E20 growing embryonic tissues (Fig. 2C). In E15 embryos, SelT was homogeneously expressed in all developing organs, although its levels were particularly elevated in the immature CNS. Indeed, we noticed that SelT is particularly concentrated in the forebrain (neocortex and thalamus), the midbrain, and the hindbrain. A similar pattern was observed in E20 embryos (Fig. 2C), where SelT was also expressed at high levels in many organs, such as heart, lung, or liver (Fig. 2, D and E). Moreover, we also found that SelT is particularly abundant in developing endocrine organs, such as thyroid, adrenal gland, or testis (Fig. 2, F–H). SelT was also highly expressed in immature nervous cells from every part of the brain (Fig. 2, I–M). For instance, an important immunopositive signal was observed in the olfactory bulb (Fig. 2J), particularly in the mitral cell layer (Fig. 2K), in the cerebral periventricular cells (Fig. 2L), or in the cerebral neocortex (Fig. 2M). Using immunofluorescence, we investigated the pattern of SelT expression and its subcellular localization in forebrain during development (Fig. 3). We found an intense SelT signal in the embryonic cortex, which gradually decreased after birth and totally disappeared in adult cortex (Fig. 3A). Then, we explored SelT cell-type distribution in the developing forebrain. In particular, we studied SelT colocalization with nestin and doublecortin (DCX), which are specifically expressed in proliferating and in differentiating neural progenitors, respectively. In E15 cortex, SelT was found in nestin-positive cells in the subventricular zone (SVZ) and intermediate zone (IZ), whereas it colocalized with DCX in the cortical plate (CP) (Fig. 3B). At postnatal d 1 (P1), SelT was still present in the nestin-positive cell population of the SVZ, which, at this stage, is labeled by glial fibrillary acidic protein (GFAP) antibody, because these precursor cells also give rise to astrocytes (Fig. 3B). SelT was still found in DCX-positive cells of the CP at P1 but at P14, SelT expression declined and no longer colocalized with the remaining DCX-positive cells. However, SelT was still detectable at low levels in SVZ-emerging astrocytes (Fig. 3B). Given that SelT was found in cortical precursors in the embryo, we employed primary cultured cortical neurons to establish SelT intracellular localization in developing cells (Fig. 3C). SelT was mainly detected in the ER because it colocalized with the ER-resident glucose-related protein 78 (GRP78), but not with the vesicle-associated membrane protein (VAMP) present in neuritic extents. Thus, in the developing forebrain, SelT is found in the ER of immature and early differentiating neural cells.

We also investigated SelT tissue distribution in the cerebellum, a hindbrain structure that, interestingly, develops postnatally in rodents and contains various neural cell populations (Fig. 4). Western blot analysis showed the occurrence of SelT expression in the cerebellum at all the postnatal stages examined, including adult (Fig. 4A). In the developing cerebellar cortex, we observed that SelT is expressed in neurons of the external germinative layer (EGL) which contains DCX-negative proliferating cells present in its most external part and DCX-positive differentiating cells present in its internal part (Fig. 4, B–D). During the first postnatal weeks, EGL cells begin radial migration along β-S-100-positive Bergmann astrocyte fibers (Fig. 4, B–D) to generate the internal granule cell layer, which is devoid of SelT labeling. Once granule cell migration is achieved, Bergmann cells labeled by β-S-100 antibody became SelT positive (Fig. 4E). In the Purkinje cell layer, we also observed an intense SelT immunoreactivity in the calbindin-positive immature Purkinje neurons (Fig. 4F, P1–14). SelT expression declined at P14 and was undetectable in the adult differentiated Purkinje cells (Fig. 4F, adult). These observations confirmed the high expres-

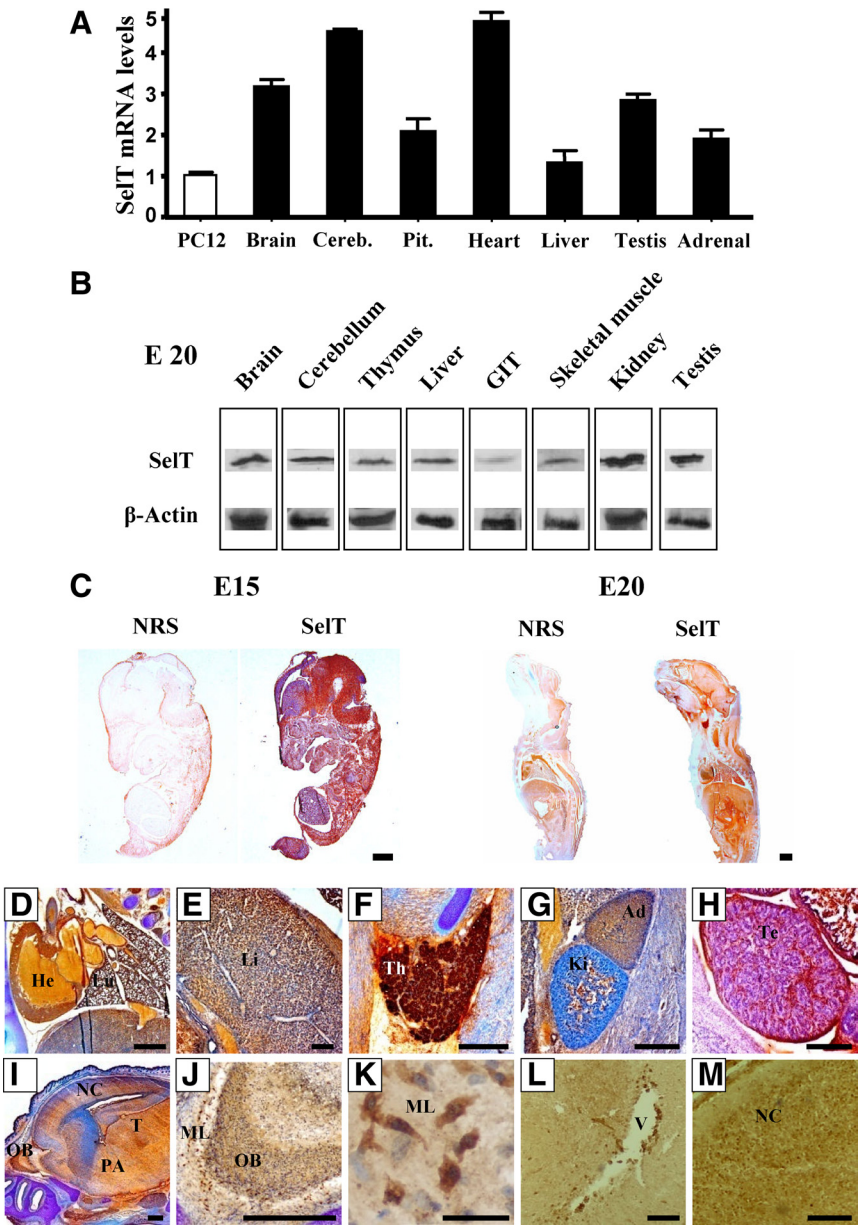

FIG. 2. SelT expression during rat embryogenesis. SelT expression patterns during embryonic development were investigated using RT-QPCR, immunoblotting, and immunohistochemistry experiments. A, RT-QPCR quantification of SelT mRNA levels in several E20 embryonic tissues (Cereb., cerebellum; Pit., pituitary). SelT mRNA relative abundance in embryonic tissues was compared with that in PC12 cells, and the results are expressed as means ± SEM of three independent experiments. B, Western blot analysis of SelT in a broad range of tissues from E20 Wistar rat embryos (50 μg proteins loaded on 13% SDS-PAGE; GIT, gastrointestinal tract). β-Actin was used as an internal loading control. C, Immunohistochemical localization of SelT in 12-μm-thick sections from rat E15 and E20. Control staining was performed with rabbit nonimmune serum (1:200). The immunoreaction was revealed by incubation with 3.3′-diaminobenzidine. *Scale bars*, 1 mm. D–F, Magnified views of SelT labeling in E20 embryos, with thionine counterstaining. SelT was detected in heart (He) and lung (Lu) (D); liver (Li) (E); thyroid (th) (F); kidney (Ki) and adrenal gland (Ad) (G); and testis (Te) (H). *Scale bars*, 500 μm. L and M, SelT immunolocalization in E20 CNS (I–K are counterstained with thionin). I, Brain sagittal section exhibiting an intense SelT signal in several nervous structures (OB, olfactory bulb; NC, neocortex; T, thalamus; PA, preoptic area). *Scale bar*, 500 μm. J, Olfactory bulb, where SelT was strongly detected in the mitral cell layer (ML) and in the olfactory bulb differentiating field (OB). *Scale bar*, 500 μm. K, Mitral cell layer (ML). *Scale bar*, 20 μm. L, Cerebral ventricle (V). *Scale bar*, 50 μm. M, Cerebral neocortex (Nc). *Scale bar*, 50 μm.

sion of SelT in proliferating and differentiating cells of the nervous system during development and revealed that this selenoprotein is expressed in Bergmann astrocytes in the adult brain.

SelT expression and tissue distribution in adult

Although our previous data indicated that SelT is mainly expressed in embryonic but not adult tissues such as the adrenal gland or most parts of the adult brain, we nevertheless investigated its expression in different other adult tissues to foster our observations. Interestingly, in a 3-month-old rat, we found that SelT mRNA levels are relatively low (Fig. 5A) compared with those observed in embryonic tissues (see Fig. 2A), excepted in the pituitary gland where SelT expression was high (Fig. 5A). In accordance, high SelT protein levels were also detected in several endocrine tissues such as the pituitary gland, thyroid, and testis but also the thymus (Fig. 5B). In addition, SelT was detected to a limited extent, in the whole-brain extract (Fig. 5B) but was not detected in liver, gastrointestinal tract, muscle, adrenal, kidney (Fig. 5B), heart, lung, and spleen (data not shown).

Because SelT was detectable in the adult brain and was mainly expressed in neural progenitors during embryogenesis, we hypothesized that SelT could be found in adult brain areas involved in neurogenesis, such as the cortical SVZ, the hippocampal dentate gyrus, and the olfactory bulb. Therefore, we examined these cerebral structures by immunohistochemistry but found no SelT-immunopositive cells either in the dentate gyrus or in the fourth ventricle SVZ (data not shown). In contrast, we detected a fair amount of SelT in the olfactory bulb, which was comparable to that found in the cerebellum (Fig. 6A). To determine the distribution of SelT in the olfactory bulb, we examined sagittal sections that cover the entire process of neurogenesis, from the fourth ventricle area where cells are

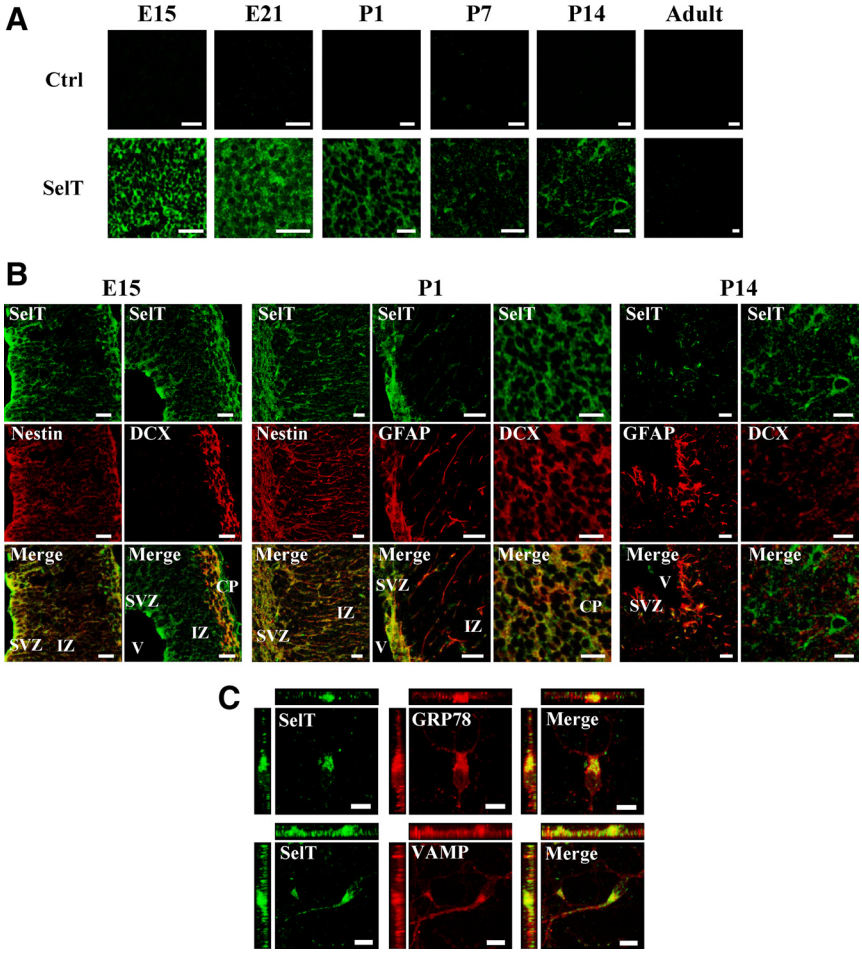

FIG. 3. SelT expression during ontogenesis of the rat CNS. A, Immunostaining of SelT in the developing and adult neocortex. Control (Ctrl) experiments were performed by preincubating SelT antibodies with the immunogenic peptide (10^{-5} M). Immunostaining was carried out on several sections from three different embryos and four adult rats, and a representative image is shown for each developmental stage. *Scale bars*, 20 μm. B, Colocalization of SelT with cellular markers for immature (nestin) and differentiating neuroblasts (DCX), and astrocytes (GFAP) in E15, P1, and P14 neocortex. In E15 and P1 cortex, SelT signal was strongly detected in the nestin-positive cells located in the SVZ and to a lesser extent the IZ as well as in the DCX-positive cells from the CP. However, at P1, the emerging nestin-positive cells give rise to the GFAP-positive astrocyte lineage, in which SelT expression is maintained. These two cortical layers are still immature at these early stages, unlike at P14, when neurogenesis is completed and SelT expression declined and did not colocalize with DCX but remained detectable in the SVZ-located immature astrocytes. *Scale bars*, 20 μm. C, Analysis of the subcellular colocalization of SelT with ER and secretory granule markers in immature neurons. Cultured rat cortical neurons from E15 were processed for immunocytochemistry using the specific antibodies anti-GRP78 for ER and anti-VAMP for dense-core vesicles. A tridimensional analysis of neuronal precursors performed with confocal microscope showed that SelT colocalizes with GRP78 but not with VAMP. *Scale bars*, 10 μm.

generated to the olfactory bulb where these neurons settle down (Fig. 6B). We observed that SelT immunoreactivity is concentrated along the rostral migratory stream (RMS) (Fig. 6, B and C). In this structure, SelT colocalized with GFAP but not with two markers of immature neurons, DCX and nestin (Fig. 6C). Interestingly, SelT labeling revealed the existence of two distinct astrocyte subpopulations, which are located in the glial tube or in its neighborhood (Fig. 6D). Indeed, SelT immunoreactivity was detected in large fibrous astrocytes present in the neuroblast migratory stream but not in thin and star-shaped astrocytes that literally surrounded the migratory stream (Fig. 6, E–G). Thus, it appears that SelT is specifically expressed in the glial subpopulation contacting the migrating neuroblasts of the olfactory bulb in the adult brain (Fig. 6F).

To apprehend the high expression of SelT also in certain endocrine tissues, we first examined its immunoreactivity in the pituitary (Fig. 7, A–D), a tissue where its gene expression is elevated. Interestingly, SelT labeling was exclusively detected in the anterior pituitary and was totally absent in the neurohypophysis (Fig. 7, A–C). Moreover, SelT labeling colocalized with those of the secretory proteins CgA and CgB (Fig. 7D), indicating that SelT is present in secretory cells of the anterior pituitary. SelT was also found in the testis, which hosts two major activities: the spermatogenic process within the seminiferous tubules and hormone production in Leydig cells (Fig. 7, E–G). We found that SelT is abundant at all the examined stages, particularly during the first postnatal weeks (Fig. 7, E and F). SelT was detected in all seminiferous tubule cells, including Sertoli cells and spermatogonia (Fig. 7F). Moreover, the steroidogenic Leydig cells were also strongly labeled (Fig. 7, F and G). In the adult, SelT immunostaining remained intense in these endocrine cells, whereas Sertoli cells were devoid of labeling. SelT immunoreactivity was also observed in germ cells, in particular spermatogonia, pachytene, spermatocytes I, spermatocytes II, and spermatids, present at the different stages of spermatogenesis (Fig. 7G). Thus, these data and those observed in the olfactory bulb indicate that SelT expression is maintained in tissues or cells undergoing maturation processes.

SelT induction during hepatic regeneration

To ascertain the association between SelT gene expression and cell maturation, including proliferation and differentiation, we analyzed its expression during hepatic regeneration. In the rat liver, very similarly to what

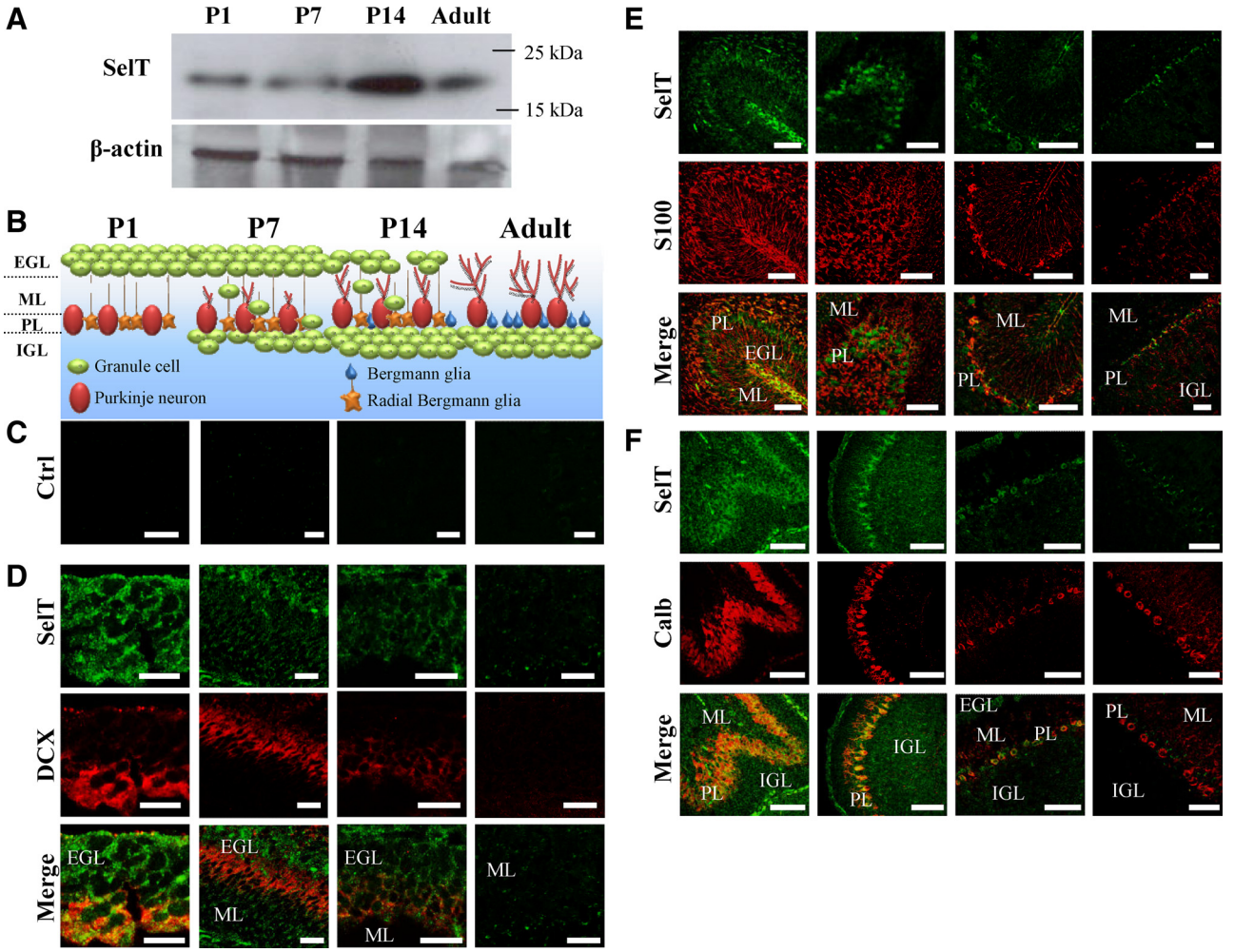

FIG. 4. SelT distribution during cerebellar ontogenesis in rat. A, Western blot analysis of SelT occurrence in the cerebellum was performed at various stages of postnatal development and in the adult. β-Actin was used as an internal loading control. B, Schematic representation showing the histological organization of cerebellar cortex during ontogenesis. Immature granule cells generated in the EGL migrate radially through the molecular layer (ML) and the Purkinje cell layer (PL) to constitute the internal granular cell layer (IGL). This migration is supported by Bergmann astrocytes, which are arranged as radial glia. C, A control (Ctrl) experiment was performed with a preincubation of the SelT antibodies with the immunogenic peptide at 10^{-5} M. *Scale bars*, 25 μm. D–F, Immunofluorescence experiments showing that SelT exhibits a layer-specific and stage-dependant pattern of expression. D, SelT distribution in the EGL. In postnatal cerebellum, SelT was found in the entire EGL and colocalized with DCX in the postmitotic cells located at the inner half of the EGL. In adult, DCX and SelT are both absent in EGL. *Scale bars*, 25 μm. E and F, SelT distribution in Purkinje cell layer. E, In P14 and adult rats, SelT is detected in S100-positive mature Bergmann astrocytes. F, In young rats, SelT colocalized with calbindin (Calb). *Scale bars*, 50 μm.

we observed in other organs, SelT was found at high levels during development and then decreased rapidly after birth (Fig. 8A). No SelT labeling was detected in adult rat liver (Fig. 8, B and C). However, after a partial hepatectomy, SelT expression was strongly induced in the injured liver (Fig. 8, B and C), concomitantly to hepatic cell proliferation, which was assessed by BrdU incorporation (Fig. 8C). To identify in which hepatic cell types SelT was induced under these conditions, we investigated its colocalization with β-catenin. Using this specific hepatocyte cell marker, hepatocytes appeared as large cells, with distinguishable round nuclei, organized in rows that do not express SelT. Between these hepatocytes are located small interstitial cells, corresponding to Kupffer and Ito cells, in which SelT was strongly induced after partial hepatectomy (Fig. 8D).

Discussion

Selenoproteins are increasingly recognized as essential for nervous, endocrine, and metabolic tissue development and function (32), and the alteration of their synthesis is associated with major disorders, including diabetes, thyroid disease, muscular dystrophy, and azoospermia (33, 34). SelT was initially identified through *in silico* analysis (35) and a pangenomic study of PACAP-induced genes during neuroendocrine cell differentiation (18, 22). Dur-

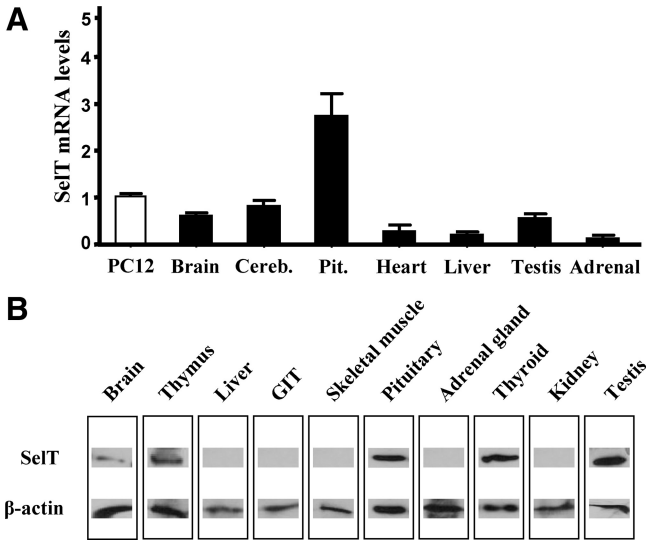

FIG. 5. SelT expression in adult rat tissues. A, SelT mRNA abundance in tissues collected from 3-month-old adult Wistar rats (Cereb., cerebellum; Pit., pituitary). SelT mRNA relative abundance in adult tissues was compared with that in PC12 cells, and the results are expressed as means ± SEM of three independent experiments. B, SelT expression pattern was determined by Western blot analysis in different adult rat tissues. SelT was detected in brain, thymus, pituitary, thyroid, and testis but not in liver, gastrointestinal tract (GIT), muscle, adrenal, or kidney. The immunoblot shown is representative of three independent experiments, and β-actin was used as an internal loading control.

ing this process, PACAP promotes cell cycle arrest, cell survival, and the secretory activity in PC12 cells (24), in a Ca^{2+}-dependent manner. Accordingly, SelT whose subcellular localization was mapped to ER membrane in PC12 cells, proved to play an essential role in Ca^{2+} homeostasis and hormone secretion during PACAP-induced PC12 cell differentiation (18). However, although PACAP acts as a neuroendocrine regulator of adrenal function and catecholamine release (36, 37), we found here that it was unable to stimulate SelT gene expression in differentiated chromaffin cells. In addition, SelT was expressed at intense levels during adrenal ontogenesis but not in the adult adrenal gland. These observations suggested that SelT may exert important roles during cell differentiation and tissue ontogenesis in which PACAP plays a major role (24) and thus were motivating arguments to study in depth its *in vivo* distribution during the ontogenesis of nervous, endocrine, and metabolic tissues.

In accordance with our previous data obtained by *in situ* hybridization showing a widespread expression of the SelT gene during embryogenesis (18), RT-QPCR, Western blot, and immunohistochemistry confirmed the abundance of this protein and its mRNA during various stages of rodent development. Indeed, SelT was detected in each tissue analyzed and was particularly abundant in the embryonic CNS, thyroid, and testis. These findings are in agreement with the data reported by Kryukov *et al.* (35) showing that the SelT transcript levels are particularly elevated in placenta and infant brain. In fact, several selenoproteins including SelN, -W, and -P and GPx have been shown to be highly expressed in embryos and to be involved in protection against oxidative stress (13, 38–41), in line with the finding that deletion of the Sec tRNA gene or some selenoprotein genes provokes early embryonic lethality, emphasizing the crucial role of selenoproteins during the first stages of development.

Because SelT is a PACAP-regulated gene and because this neuropeptide exerts potent trophic activities on cerebral and cerebellar cortex (42, 43), we investigated the expression of SelT in these developing brain areas. We observed a strong correlation of SelT temporal pattern of expression with that of neuronal precursor markers, in both growing cerebral and cerebellar cortex. Specifically, we studied SelT colocalization with nestin, a type VI intermediate filament protein expressed in proliferating neural progenitors (44), and DCX, a microtubule-associated protein whose expression is transient and limited to postmitotic immature neuronal cells (45). Contrary to these two distinct neurogenetic specific proteins, SelT was expressed in both proliferating and differentiating neuronal precursors such as those localized within the SVZ or the CP of embryonic growing neocortex and the EGL of the cerebellum. Moreover, at P1, SelT expression was maintained in SVZ-located neural progenitor cells that also expressed GFAP and that is known to give rise to glial cell population (46, 47). These data show that immature nervous cells display SelT expression during embryonic neurogenic and gliogenic processes. Such a pattern of expression is reminiscent of the effects of PACAP because this neuropeptide is involved, for instance, in granule and glial cell survival, proliferation, migration, and differentiation (48–50), thus suggesting that SelT may mediate some of these neurotrophic activities of PACAP. Although the role of SelT during CNS development is not known yet, it has been shown that other selenoproteins are also expressed at intense levels in developing CNS (51) and that their gene knockout in the brain yields severe phenotypes. Indeed, it was demonstrated that brain-targeted gene deletion of GPx4 provokes an intense neurodegeneration of hippocampal CA3 region at P13 (52), whereas that of thioredoxin reductase 1 evokes an important cerebellar hypoplasia due to a default in immature granule cell proliferation (53). SelT, which was also strongly expressed in the embryonic hippocampus and the cerebellum, may play an important role in nerve cell growth and differentiation.

The levels of SelT were also strikingly high in the fetal thyroid gland and testis. This intense expression likely reflects a role of the selenoprotein in these developing en-

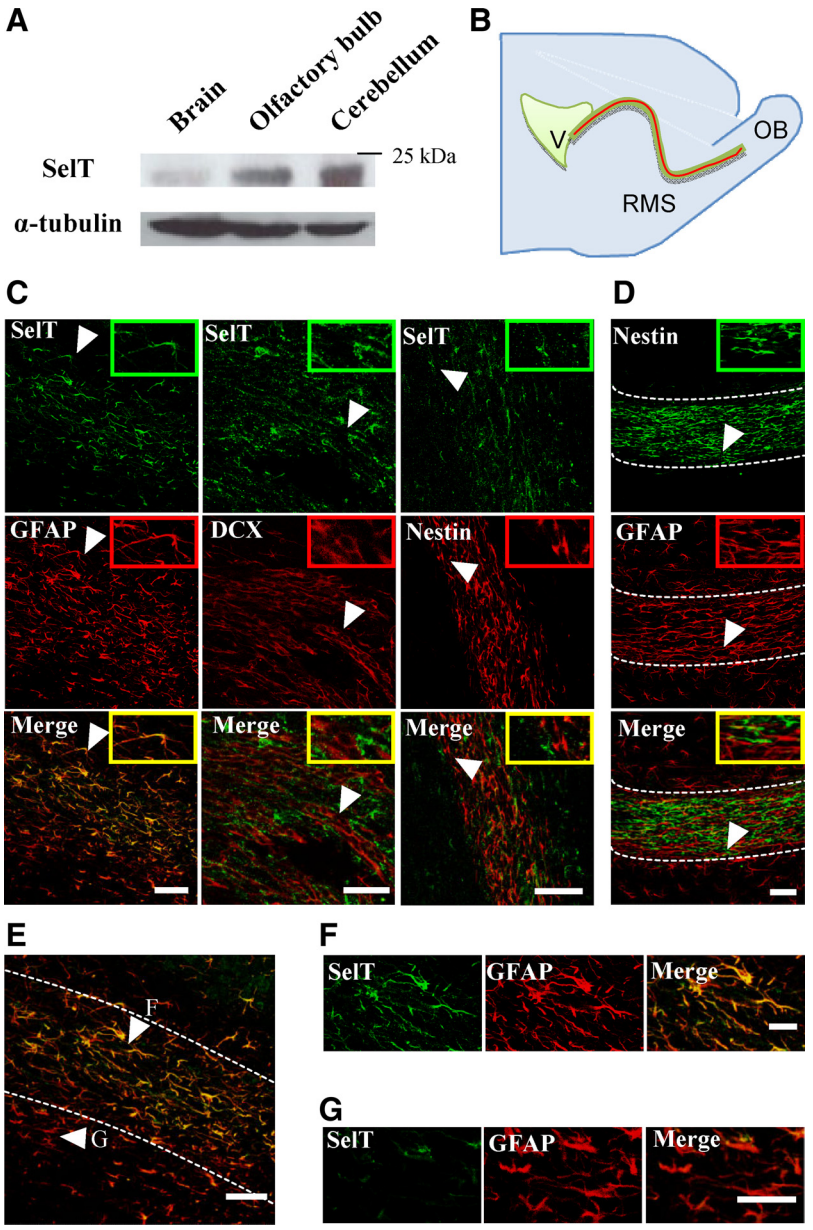

FIG. 6. Distribution of SelT in the adult brain. A, Western blot analysis showing a relatively low SelT expression in the whole adult brain, compared with cerebellum and the olfactory bulb. α-Tubulin was used as an internal loading control. B, Schematic representation showing a sagittal view of the forebrain with the newly formed neuroblasts (nestin and DCX-positive) emerging from the SVZ of the fourth ventricle (V) and migrating (*green line*) to the olfactory bulb (OB). The *red line* corresponds to astrocytes that along with the migrating neuroblasts form the RMS. C–G, Confocal microscopy imaging of the cellular distribution of SelT in the RMS, obtained from sagittal sections of the olfactory bulb. C, SelT colocalized with GFAP but not with nestin or DCX. *Scale bars*, 50 μm. D, GFAP and nestin double staining used to delineate the neuroblast migration flux, which is delimited by *dashed lines*, from the surrounding glial tube. *Scale bars*, 50 μm. *Insets* in top-right corner of each image (C and D) show a high magnification of representative cells that are indicated by *arrows* in the main images. E, Analysis of SelT and GFAP colocalization in RMS. SelT expression in GFAP-positive cells is more pronounced within the neuroblast migratory stream (delimited by *dashed lines*) than in the periphery of the RMS. *Scale bars*, 50 μm. F, Images showing SelT immunofluorescence in astrocytes located inside the RMS. G, Immunohistochemistry images showing the absence of SelT expression in astrocytes located outside the RMS. *Scale bars*, 25 μm.

docrine tissues akin to iodothyronine deiodinases and GPx, which are also expressed early during development to exert major regulatory activities in thyroid and testis, respectively (54, 55). In particular, SelT could be part of the mechanisms activated by PACAP during the growth and differentiation of testis cells, where the neuropeptide occurs during development (56, 57).

SelT is far less abundant in adult than in fetal tissues because many organs that express the selenoprotein during their ontogenesis became devoid of SelT in the adult. For instance, such a trend was observed in heart, lung, adrenal, liver, muscle, or cerebral cortex. In the adult brain, although neurons were devoid of SelT labeling, we noticed an important residual SelT immunoreactivity in two different astrocyte subpopulations, namely the cerebellar Bergmann and the rostral migratory stream glial cells. Bergmann glia is a cellular population whose exact function is still debated, although recent studies suggest it may serve as a stem cell pool in the cerebellum (58, 59). The RMS contains migrating neuroblasts that emerge from the SVZ of the lateral cerebral ventricle, surrounded by specialized glial cells that form a glial scaffold supporting neuroblast migration (60). We found that cells making the glial tube contain a large amount of SelT, whereas the neighboring star-shaped astrocytes remained SelT negative. This is the first report to our knowledge showing the presence of a selenoprotein in these specialized astrocytes, whose function within the RMS is essential for neuroblast migration (61). Interestingly, this neurogenic process is described as the fastest (70–80 μm/h) occurring in growing or adult brain (62), suggesting that the glial cells of the RMS may exhibit an intense metabolic activity that involves SelT. Finally, and contrary to what we observed during brain development, this selenoprotein was not detected during adult neurogenesis, which suggests that this process is regulated dif-

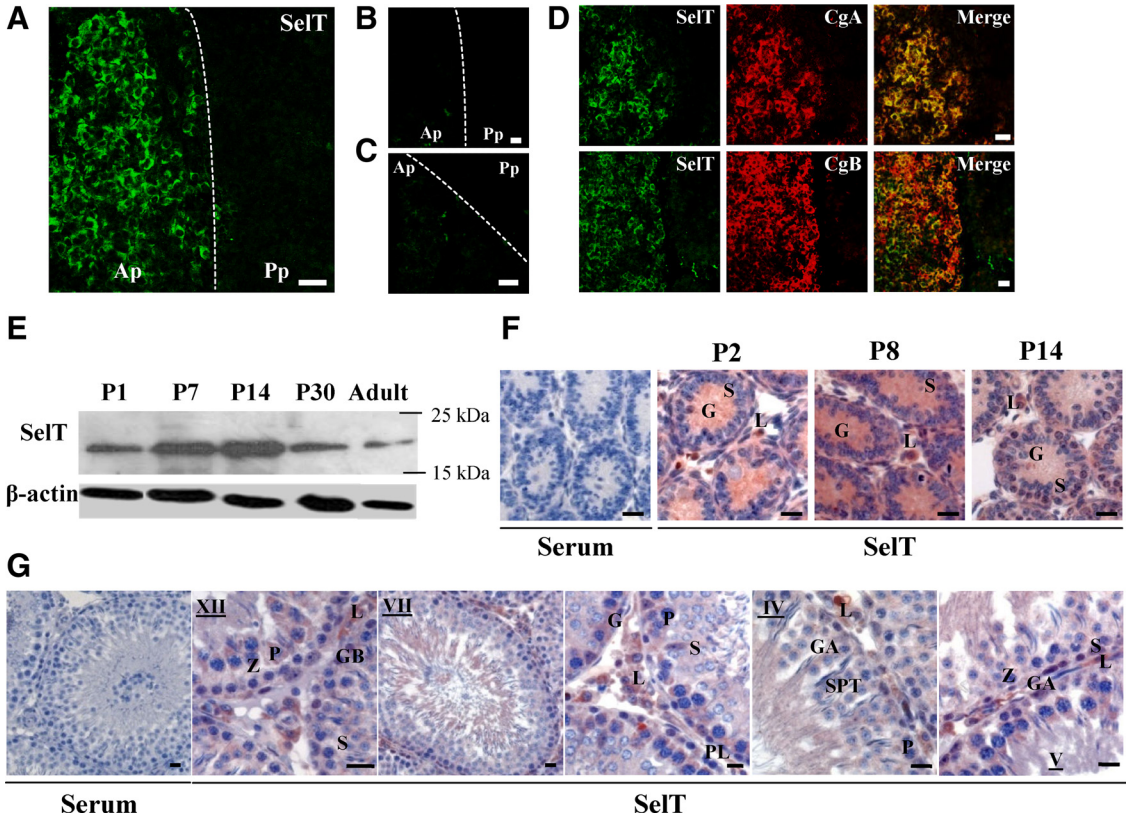

FIG. 7. SelT expression in rat pituitary and testis. Panels A–C, Confocal microscopy images showing SelT staining (panel A) and controls performed, respectively, with nonimmune rabbit serum (1:200) (panel B) and by preincubating SelT antibodies with the immunogenic peptide (10^{-5} M) (panel C) in both anterior (Ap) and posterior pituitary (Pp), which were separated with *dashed lines*. Panel D, Colocalization of SelT with CgA and CgB. *Scale bars*, 25 μm (panels A–D). Panel E, Western blot analysis of SelT expression in P1-30 and adult rat testis. β-Actin was used as an internal loading control. Panel F, Immunohistological localization of SelT distribution in growing seminiferous tubules at P2-14 (G, spermatogonia; S, Sertoli cell; L, Leydig cell). Panel G, SelT expression in adult seminiferous tubules and Leydig cells (L). SelT was detected in most spermatogenic cells, at all stages (indicated in roman numerals), such as type A (GA) and B (GB) spermatogonia (G), preleptotenes (PL), pachytenes (P), spermatids (SPT) but not in leptotenes (L) and zygotenes (Z). SelT was not detected in mature Sertoli cells (S). *Scale bars*, 50 μm.

ferently from that occurring during developmental neurogenesis, at least from the standpoint of SelT expression. Nevertheless, SelT may participate in adult neurogenesis in the olfactory bulb through its activation in neighboring astrocytes.

A striking finding of this study is the persistence of SelT expression in some endocrine tissues such as thyroid, pituitary, or testis. Interestingly, the pituitary gland, which is one of the major SelT-expressing tissues, contains higher amounts of Se than those found in brain (63), indicating that Se and selenoproteins, in particular SelT, may play a major role in the endocrine cells of the pituitary. Indeed, we found that SelT colocalized with CgA and CgB, two secretory proteins that are essential for the establishment and maintenance of the secretory activity of the pituitary gland (31, 64, 65). Although the function of selenoprotein family members remain poorly characterized in this endocrine gland, SelT appears as a new factor probably involved in the endocrine function of this tissue. This is in line with the broad-range stimulatory effect of PACAP on the different hormone-secreting cells of the adenohypophysis (66). In the testis, SelT was expressed at high levels in the proliferating and differentiating spermatogenic cells but also in Leydig cells. These testosterone-producing endocrine cells are highly sensitive to various stressful conditions to which they are continually exposed. Interestingly, it was previously reported that Se supplementation protects Leydig cells from the harm of diverse toxic agents (67). In addition, it has been shown that Leydig cells express phospholipid-hydroperoxide GPx (68), a selenoprotein involved in protection against oxidative stress. Therefore, SelT may participate in protection of testicular tissue against toxic agents including ROS. Similarly, thyroid in which SelT is abundantly expressed is the organ that contains the highest levels of Se in mammals and is known to express a large panel of selenoproteins (69). This particular phenotype could be linked to the important oxidative burst generated during thyroid hormone biosynthesis. Indeed, thyrocytes are continually exposed to potentially toxic ROS concentrations and therefore also express some detoxifying selenoenzymes, such as GPx1,

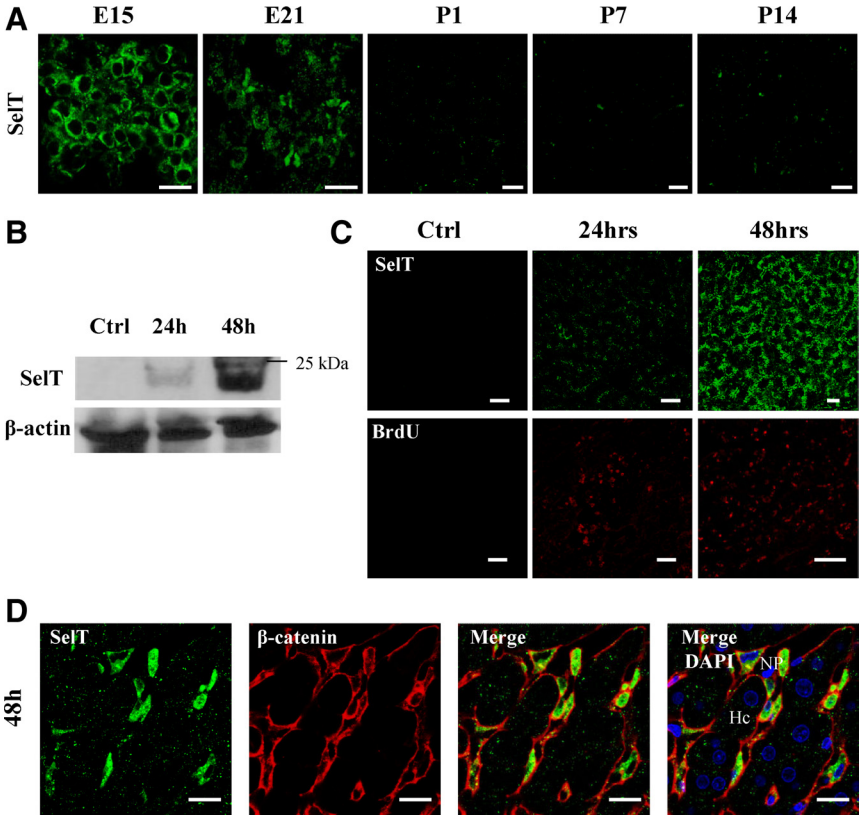

FIG. 8. SelT expression is reinduced during liver regeneration. A, SelT occurrence in liver during embryogenesis (E15-21) and after birth (P1-14). *Scale bars*, 20 μm. B and C, Effect of partial hepatectomy on SelT expression. B, Western blot analysis of SelT levels 24 and 48 h after partial hepatectomy in adult rat. β-Actin was used as an internal loading control (Ctrl). C, Immunostaining of SelT and BrdU, 24 and 48 h after hepatectomy. *Scale bars*, 50 μm. D, SelT and β-catenin double labeling of liver sections performed 48 h after hepatectomy. Hepatocytes (Hc), with a distinct round nuclei, are separated by SelT-positive nonparenchymal cells (NP). *Scale bars*, 20 μm.

-3, and -4 as a self-defense mechanism (70) that may include SelT.

Because SelT was mainly expressed in proliferating and differentiating cells in a broad range of fetal organs and some adult tissues, we sought to investigate SelT expression during tissue regeneration after a partial hepatectomy to further validate this association between SelT expression and cell proliferation and differentiation. During liver regeneration, hepatocytes, which constitute 80% of the liver parenchyma, begin to proliferate 24 h after resection to regenerate the missing tissue (71, 72). In parallel, sinusoidal cells, including Ito and Kupffer cells, and endothelial cells proliferate, respectively, 48 and 72 h after the injury (72). During the acute phase of liver regeneration, we showed that SelT is strongly induced at 48 h in cells located in the vicinity of β-catenin-expressing hepatocytes (73) but not in hepatocytes themselves. This overexpression occurred when the peak of DNA synthesis is usually detected, *i.e.* at 48 h, in Kupffer cells (74). These cells contribute actively to liver regeneration by modulating hepatocyte proliferation via paracrine secretion and action of several cytokines like TNF-α, IL-6, or TGF-β1 (75). Interestingly, the induction of cell proliferation during liver regeneration also relies upon the induction of several fetal genes (76), including the selenoenzyme deiodinase III, which locally inhibits thyroid hormone pro-differentiating activities (77). Indeed, during liver regeneration, this deiodinase is produced to convert T_4 and T_3, respectively in rT_3 and in 3,3′-diiodothyronine, which have no endocrine activities. The role of SelT in the regenerating liver remains to be identified.

Altogether, these findings suggest that SelT may play a larger role than initially proposed (18). Indeed, SelT was defined as a thioredoxin-like fold-containing selenoprotein that includes the Sec-containing active motif, thus allowing its classification among the recently identified thioredoxin-like selenoprotein family (25). As a matter of fact, its localization in the ER suggested that SelT could be involved, through its thioredoxin-like domain, in other functions, including cell protection mechanisms. Indeed, many biological processes involving the ER, such as Ca^{2+} and redox homeostasis, protein folding control and the unfolded-protein response, are known to be essential for cell survival and function. In fact, it has recently been shown that reduction of SelT gene expression in fibroblasts results in the up-regulation of redox genes (27), suggesting that SelT, like other ER-located selenoenzymes such as Sep15 or SelS, -N, or -M (19–21) could be involved in redox mechanisms. In support of this hypothesis, SelT is highly induced in metabolically active proliferating and differentiating cells of the CNS and at the periphery during ontogenesis or in adult cells endowed with some plasticity such as glial cells during neurogenesis or germ cells during spermatogenesis. SelT may participate in the salutary activities of selenoproteins during these critical periods to cope with oxidative stress bursts. Moreover, the persistence of SelT expression in adult endocrine cells may be related either to the protection of these cells that face permanent demand of hormone synthesis or to the catalysis of posttranslational modifications such as disulfide bridge formation in hormones and related proteins, which occur in the ER. Additional studies will be required to uncover the precise

role of SelT in these vital mechanisms. As indicated by its induction during liver regeneration or after brain hypoxia (78), SelT activity is probably also required for protection and regeneration after tissue injury. However, it should be noted that SelT induction in liver occurred only in supporting cells but not in hepatocytes, suggesting that the SelT gene may be permanently silenced in the majority of cells after development, with only few exceptions like endocrine cells or some supporting cells such as glial cells and Kupffer cells. Therefore, in the adult, SelT may also exert an important role after induction in supporting cells after stress. SelT, which is a PACAP-regulated gene, is likely to be enrolled in the various protective effects of this trophic peptide, particularly against oxidative damage during development and stress (79, 80) in nervous, endocrine, and metabolic tissues.

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References

1. **Rayman MP** 2000 The importance of selenium to human health. Lancet 356:233–241
2. **Rayman MP, Rayman MP** 2002 The argument for increasing selenium intake. Proc Nutr Soc 61:203–215
3. **Hawkes WC, Hornbostel L** 1996 Effects of dietary selenium on mood in healthy men living in a metabolic research unit. Biol Psychiatry 39:121–128
4. **Weber GF, Maertens P, Meng XZ, Pippenger CE** 1991 Glutathione peroxidase deficiency and childhood seizures. Lancet 337: 1443–1444
5. **Wu AS, Oldfield JE, Shull LR, Cheeke PR** 1979 Specific effect of selenium deficiency on rat sperm. Biol Reprod 20:793–798
6. **Rosenfeld I, Beath OA** 1954 Effect of selenium on reproduction in rats. Proc Soc Exp Biol Med 87:295–297
7. **Dumont JE, Corvilain B, Contempre B** 1994 The biochemistry of endemic cretinism: roles of iodine and selenium deficiency and goitrogens. Mol Cell Endocrinol 100:163–166
8. **Contempre B, Dumont JE, Denef JF, Many MC** 1995 Effects of selenium deficiency on thyroid necrosis, fibrosis and proliferation: a possible role in myxoedematous cretinism. Eur J Endocrinol 133: 99–109
9. **Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG** 1973 Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588–590
10. **Driscoll DM, Copeland PR** 2003 Mechanism and regulation of selenoprotein synthesis. Annu Rev Nutr 23:17–40
11. **Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigó R, Gladyshev VN** 2003 Characterization of mammalian selenoproteomes. Science 300:1439–1443
12. **Bösl MR, Takaku K, Oshima M, Nishimura S, Taketo MM** 1997 Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). Proc Natl Acad Sci USA 94:5531–5534
13. **Chung YW, Jeong D, Noh OJ, Park YH, Kang SI, Lee MG, Lee TH, Yim MB, Kim IY** 2009 Antioxidative role of selenoprotein W in oxidant-induced mouse embryonic neuronal cell death. Mol Cells 27:609–613
14. **Novoselov SV, Kryukov GV, Xu XM, Carlson BA, Hatfield DL, Gladyshev VN** 2007 Selenoprotein H is a nucleolar thioredoxin-like protein with a unique expression pattern. J Biol Chem 282: 11960–11968
15. **Holmgren A, Lu J** 2010 Thioredoxin and thioredoxin reductase: current research with special reference to human disease. Biochem Biophys Res Commun 396:120–124
16. **Köhrle J** 2000 The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell Mol Life Sci 57: 1853–1863
17. **Köhrle J, Jakob F, Contempré B, Dumont JE** 2005 Selenium, the thyroid, and the endocrine system. Endocr Rev 26:944–984
18. **Grumolato L, Ghzili H, Montero-Hadjadje M, Gasman S, Lesage J, Tanguy Y, Galas L, Ait-Ali D, Leprince J, Guérineau NC, Elkahloun AG, Fournier A, Vieau D, Vaudry H, Anouar Y** 2008 Selenoprotein T is a PACAP-regulated gene involved in intracellular Ca2+ mobilization and neuroendocrine secretion. FASEB J 22:1756–1768
19. **Jurynec MJ, Xia R, Mackrill JJ, Gunther D, Crawford T, Flanigan KM, Abramson JJ, Howard MT, Grunwald DJ** 2008 Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle. Proc Natl Acad Sci USA 105:12485–12490
20. **Reeves MA, Bellinger FP, Berry MJ** 2010 The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation. Antioxid Redox Signal 12:809–818
21. **Shchedrina VA, Zhang Y, Labunskyy VM, Hatfield DL, Gladyshev VN** 2010 Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. Antioxid Redox Signal 12:839–849
22. **Grumolato L, Elkahloun AG, Ghzili H, Alexandre D, Coulouarn C, Yon L, Salier JP, Eiden LE, Fournier A, Vaudry H, Anouar Y** 2003 Microarray and suppression subtractive hybridization analyses of gene expression in pheochromocytoma cells reveal pleiotropic effects of pituitary adenylate cyclase-activating polypeptide on cell proliferation, survival, and adhesion. Endocrinology 144:2368–2379
23. **Deutsch PJ, Sun Y** 1992 The 38-amino acid form of pituitary adenylate cyclase-activating polypeptide stimulates dual signaling cascades in PC12 cells and promotes neurite outgrowth. J Biol Chem 267:5108–5113
24. **Ghzili H, Grumolato L, Thouënnon E, Tanguy Y, Turquier V, Vaudry H, Anouar Y** 2008 Role of PACAP in the physiology and pathology of the sympathoadrenal system. Front Neuroendocrinol 29:128–141
25. **Dikiy A, Novoselov SV, Fomenko DE, Sengupta A, Carlson BA, Cerny RL, Ginalski K, Grishin NV, Hatfield DL, Gladyshev VN** 2007 SelT, SelW, SelH, and Rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family. Biochemistry 46:6871–6882

26. **Holmgren A** 2000 Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal 2:811–820
27. **Sengupta A, Carlson BA, Labunskyy VM, Gladyshev VN, Hatfield DL** 2009 Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells. Biochem Cell Biol 87:953–961
28. **Daveau M, Benard M, Scotte M, Schouft MT, Hiron M, Francois A, Salier JP, Fontaine M** 2004 Expression of a functional C5a receptor in regenerating hepatocytes and its involvement in a proliferative signaling pathway in rat. J Immunol 173:3418–3424
29. **Grumolato L, Louiset E, Alexandre D, Aït-Ali D, Turquier V, Fournier A, Fasolo A, Vaudry H, Anouar Y** 2003 PACAP and NGF regulate common and distinct traits of the sympathoadrenal lineage: effects on electrical properties, gene markers and transcription factors in differentiating PC12 cells. Eur J Neurosci 17:71–82
30. **Ait-Ali D, Turquier V, Grumolato L, Yon L, Jourdain M, Alexandre D, Eiden LE, Vaudry H, Anouar Y** 2004 The proinflammatory cytokines tumor necrosis factor-α and interleukin-1 stimulate neuropeptide gene transcription and secretion in adrenochromaffin cells via activation of extracellularly regulated kinase 1/2 and p38 protein kinases, and activator protein-1 transcription factors. Mol Endocrinol 18:1721–1739
31. **Montero-Hadjadje M, Elias S, Chevalier L, Benard M, Tanguy Y, Turquier V, Galas L, Yon L, Malagon MM, Driouich A, Gasman S, Anouar Y** 2009 Chromogranin A promotes peptide hormone sorting to mobile granules in constitutively and regulated secreting cells: role of conserved N- and C-terminal peptides. J Biol Chem 284:12420–12431
32. **Conrad M** 2009 Transgenic mouse models for the vital selenoenzymes cytosolic thioredoxin reductase, mitochondrial thioredoxin reductase and glutathione peroxidase 4. Biochim Biophys Acta 1790:1575–1585
33. **Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R** 2011 Selenium in human health and disease. Antioxid Redox Signal 14:1337–1383
34. **Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, Padidela R, Ceron-Gutierrez L, Doffinger R, Prevosto C, Luan J, Montano S, Lu J, Castanet M, Clemons N, Groeneveld M, Castets P, Karbaschi M, Aitken S, Dixon A, Williams J, Campi I, Blount M, Burton H, Muntoni F, O'Donovan D, Dean A, Warren A, Brierley C, Baguley D, Guicheney P, Fitzgerald R, Coles A, Gaston H, Todd P, Holmgren A, Khanna KK, Cooke M, Semple R, Halsall D, Wareham N, Schwabe J, Grasso L, Beck-Peccoz P, Ogunko A, Dattani M, Gurnell M, Chatterjee K** 2010 Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. J Clin Invest 120:4220–4235
35. **Kryukov GV, Kryukov VM, Gladyshev VN** 1999 New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. J Biol Chem 274:33888–33897
36. **Hamelink C, Tjurmina O, Damadzic R, Young WS, Weihe E, Lee HW, Eiden LE** 2002 Pituitary adenylate cyclase-activating polypeptide is a sympathoadrenal neurotransmitter involved in catecholamine regulation and glucohomeostasis. Proc Natl Acad Sci USA 99:461–466
37. **Turquier V, Yon L, Grumolato L, Alexandre D, Fournier A, Vaudry H, Anouar Y** 2001 Pituitary adenylate cyclase-activating polypeptide stimulates secretoneurin release and secretogranin II gene transcription in bovine adrenochromaffin cells through multiple signaling pathways and increased binding of pre-existing activator protein-1-like transcription factors. Mol Pharmacol 60:42–52
38. **Petit N, Lescure A, Rederstorff M, Krol A, Moghadaszadeh B, Wewer UM, Guicheney P** 2003 Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. Hum Mol Genet 12:1045–1053
39. **Castets P, Maugenre S, Gartioux C, Rederstorff M, Krol A, Lescure A, Tajbakhsh S, Allamand V, Guicheney P** 2009 Selenoprotein N is dynamically expressed during mouse development and detected early in muscle precursors. BMC Dev Biol 9:46
40. **Lee SR, Yon JM, Baek IJ, Kim MR, Park CG, Lee BJ, Yun YW, Nam SY** 2008 Spatiotemporal expression of the selenoprotein P gene in postimplantational mouse embryos. Int J Dev Biol 52:1005–1011
41. **Baek IJ, Yon JM, Lee BJ, Yun YW, Yu WJ, Hong JT, Ahn B, Kim YB, Kim DJ, Kang JK, Nam SY** 2005 Expression pattern of cytosolic glutathione peroxidase (cGPx) mRNA during mouse embryogenesis. Anat Embryol (Berl) 209:315–321
42. **Sanchez A, Chiriva-Internati M, Grammas P** 2008 Transduction of PACAP38 protects primary cortical neurons from neurotoxic injury. Neurosci Lett 448:52–55
43. **Falluel-Morel A, Vaudry D, Aubert N, Galas L, Benard M, Basille M, Fontaine M, Fournier A, Vaudry H, Gonzalez BJ** 2005 Pituitary adenylate cyclase-activating polypeptide prevents the effects of ceramides on migration, neurite outgrowth, and cytoskeleton remodeling. Proc Natl Acad Sci USA 102:2637–2642
44. **Hockfield S, McKay RD** 1985 Identification of major cell classes in the developing mammalian nervous system. J Neurosci 5:3310–3328
45. **Friocourt G, Koulakoff A, Chafey P, Boucher D, Fauchereau F, Chelly J, Francis F** 2003 Doublecortin functions at the extremities of growing neuronal processes. Cereb Cortex 13:620–626
46. **Kawase-Koga Y, Otaegi G, Sun T** 2009 Different timings of Dicer deletion affect neurogenesis and gliogenesis in the developing mouse central nervous system. Dev Dyn 238:2800–2812
47. **Richardson WD, Kessaris N, Pringle N** 2006 Oligodendrocyte wars. Nat Rev Neurosci 7:11–18
48. **Falluel-Morel A, Tascau LI, Sokolowski K, Brabet P, DiCicco-Bloom E** 2008 Granule cell survival is deficient in PAC1$^{-/-}$ mutant cerebellum. J Mol Neurosci 36:38–44
49. **Vaudry D, Gonzalez BJ, Basille M, Fournier A, Vaudry H** 1999 Neurotrophic activity of pituitary adenylate cyclase-activating polypeptide on rat cerebellar cortex during development. Proc Natl Acad Sci USA 96:9415–9420
50. **Masmoudi-Kouki O, Douiri S, Hamdi Y, Kaddour H, Bahdoudi S, Vaudry D, Basille M, Leprince J, Fournier A, Vaudry H, Tonon MC, Amri M** 2011 Pituitary adenylate cyclase-activating polypeptide protects astroglial cells against oxidative stress-induced apoptosis. J Neurochem 117:403–411
51. **Savaskan NE, Borchert A, Bräuer AU, Kuhn H** 2007 Role for glutathione peroxidase-4 in brain development and neuronal apoptosis: specific induction of enzyme expression in reactive astrocytes following brain injury. Free Radic Biol Med 43:191–201
52. **Seiler A, Schneider M, Förster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Rådmark O, Wurst W, Bornkamm GW, Schweizer U, Conrad M** 2008 Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. Cell Metab 8:237–248
53. **Soerensen J, Jakupoglu C, Beck H, Förster H, Schmidt J, Schmahl W, Schweizer U, Conrad M, Brielmeier M** 2008 The role of thioredoxin reductases in brain development. PLoS One 3:e1813
54. **Schomburg L, Riese C, Michaelis M, Griebert E, Klein MO, Sapin R, Schweizer U, Köhrle J** 2006 Synthesis and metabolism of thyroid hormones is preferentially maintained in selenium-deficient transgenic mice. Endocrinology 147:1306–1313
55. **Behne D, Weiler H, Kyriakopoulos A** 1996 Effects of selenium deficiency on testicular morphology and function in rats. J Reprod Fertil 106:291–297
56. **Koh PO, Noh HS, Kim YS, Cheon EW, Kim HJ, Kang SS, Cho GJ, Choi WS** 2003 Cellular localization of pituitary adenylate cyclase-activating polypeptide in the rat testis. Mol Cells 15:271–276
57. **Lacombe A, Lelievre V, Roselli CE, Salameh W, Lue YH, Lawson G, Muller JM, Waschek JA, Vilain E** 2006 Delayed testicular aging in pituitary adenylate cyclase-activating peptide (PACAP) null mice. Proc Natl Acad Sci USA 103:3793–3798

58. **Alcock J, Sottile V** 2009 Dynamic distribution and stem cell characteristics of Sox1-expressing cells in the cerebellar cortex. Cell Res 19:1324–1333
59. **Sottile V, Li M, Scotting PJ** 2006 Stem cell marker expression in the Bergmann glia population of the adult mouse brain. Brain Res 1099: 8–17
60. **Sun W, Kim H, Moon Y** 2010 Control of neuronal migration through rostral migration stream in mice. Anat Cell Biol 43:269–279
61. **Soria JM, Taglialatela P, Gil-Perotin S, Galli R, Gritti A, Verdugo JM, Bertuzzi S** 2004 Defective postnatal neurogenesis and disorganization of the rostral migratory stream in absence of the Vax1 homeobox gene. J Neurosci 24:11171–11181
62. **Nam SC, Kim Y, Dryanovski D, Walker A, Goings G, Woolfrey K, Kang SS, Chu C, Chenn A, Erdelyi F, Szabo G, Hockberger P, Szele FG** 2007 Dynamic features of postnatal subventricular zone cell motility: a two-photon time-lapse study. J Comp Neurol 505:190–208
63. **Thorlacius-Ussing O, Jensen FT** 1988 Selenium in the anterior pituitary of the rat after a single injection of 75Se sodium selenite. Biol Trace Elem Res 15:277–287
64. **Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y** 2008 Chromogranins A and B and secretogranin II: evolutionary and functional aspects. Acta Physiol (Oxf) 192:309–324
65. **Fischer-Colbrie R, Schmid KW, Mahata SK, Mahata M, Laslop A, Bauer JW** 1992 Sex-related differences in chromogranin A, chromogranin B and secretogranin II gene expression in rat pituitary. J Neuroendocrinol 4:125–130
66. **Rawlings SR, Hezareh M** 1996 Pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP/vasoactive intestinal polypeptide receptors: actions on the anterior pituitary gland. Endocr Rev 17:4–29
67. **Erkekoglu P, Rachidi W, Yuzugullu OG, Giray B, Favier A, Ozturk M, Hincal F** 2010 Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. Toxicol Appl Pharmacol 248:52–62
68. **Nayernia K, Diaconu M, Aumüller G, Wennemuth G, Schwandt I, Kleene K, Kuehn H, Engel W** 2004 Phospholipid hydroperoxide glutathione peroxidase: expression pattern during testicular development in mouse and evolutionary conservation in spermatozoa. Mol Reprod Dev 67:458–464
69. **Dickson RC, Tomlinson RH** 1967 Selenium in blood and human tissues. Clin Chim Acta 16:311–321
70. **Beckett GJ, Arthur JR** 2005 Selenium and endocrine systems. J Endocrinol 184:455–465
71. **Markiewski MM, DeAngelis RA, Lambris JD** 2006 Liver inflammation and regeneration: two distinct biological phenomena or parallel pathophysiologic processes? Mol Immunol 43:45–56
72. **Michalopoulos GK, DeFrances MC** 1997 Liver regeneration. Science 276:60–66
73. **Ashida K, Terada T, Kitamura Y, Kaibara N** 1998 Expression of E-cadherin, α-catenin, β-catenin, and CD44 (standard and variant isoforms) in human cholangiocarcinoma: an immunohistochemical study. Hepatology 27:974–982
74. **Widmann JJ, Fahimi HD** 1975 Proliferation of mononuclear phagocytes (Kupffer cells) and endothelial cells in regenerating rat liver. A light and electron microscopic cytochemical study. Am J Pathol 80: 349–366
75. **Malik R, Selden C, Hodgson H** 2002 The role of non-parenchymal cells in liver growth. Semin Cell Dev Biol 13:425–431
76. **Tanimizu N, Miyajima A** 2007 Molecular mechanism of liver development and regeneration. Int Rev Cytol 259:1–48
77. **Kester MH, Toussaint MJ, Punt CA, Matondo R, Aarnio AM, Darras VM, Everts ME, de Bruin A, Visser TJ** 2009 Large induction of type III deiodinase expression after partial hepatectomy in the regenerating mouse and rat liver. Endocrinology 150:540–545
78. **Ikematsu K, Tsuda R, Tsuruya S, Nakasono I** 2007 Identification of novel genes expressed in hypoxic brain condition by fluorescence differential display. Forensic Sci Int 169:168–172
79. **Shioda S, Ohtaki H, Nakamachi T, Dohi K, Watanabe J, Nakajo S, Arata S, Kitamura S, Okuda H, Takenoya F, Kitamura Y** 2006 Pleiotropic functions of PACAP in the CNS: neuroprotection and neurodevelopment. Ann NY Acad Sci 1070:550–560
80. **Chen Y, Samal B, Hamelink CR, Xiang CC, Chen Y, Chen M, Vaudry D, Brownstein MJ, Hallenbeck JM, Eiden LE** 2006 Neuroprotection by endogenous and exogenous PACAP following stroke. Regul Pept 137:4–19