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Developmental expression of PrP in the post-implantation embryo

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Abstract

Since prion protein (PrP) mRNA and PrP^C expression levels in transgenic (Tg) mice using the CosSHa.tet vector correlate well with the PrP transgene copy, we constructed *Prnp-LacZ* Tg animals expressing β -galactosidase that was inserted into the CosSHa.tet vector. The CosSHa.tet vector was created from a large PrP cosmid clone in which the PrP open reading frame was deleted. In the developing nervous system, the β -galactosidase marker was not expressed in the neural progenitors of the mitotically active ventricular zone. It is first expressed in cells that have ceased proliferating, migrated radially from the ventricular zone, and differentiated into neurons in the intermediate layer. At E11.5 p.c., motor neurons in the ventral neural tube clearly express the marker transgene. Expression in dorsal neural tube neurons is observed at later stages, after their differentiation. These results indicate that *Prnp* gene expression in the nervous system begins in post-mitotic neural cells that have undergone neuronal differentiation. This pattern of *Prnp* expression in the nervous system appears to persist throughout the adult life of mammals.

Keywords

Prion; *Prnp*; expression regulation; transgenic mice; β -galactosidase

INTRODUCTION

The prion diseases include Creutzfeldt-Jakob disease (CJD) of humans, scrapie of sheep, and bovine spongiform encephalopathy (BSE or “mad cow” disease). Recently, prion diseases have attained world-wide prominence due to the identification of variant CJD (vCJD) that is apparently linked to the ingestion of prion-contaminated beef products. Epidemiologists predict that as many as 100,000 cases of vCJD may occur during the next several decades, depending on incubation time [1,7,37].

Prion diseases present sporadically in >85% of cases, are dominantly inherited in 10%, and acquired by infection in <1% [27]. While this triad of disease manifestations cannot be

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explained by a viral etiology, causation by prions readily accommodates the sporadic, genetic and infectious presentations.

The disease-causing prion [26] is composed solely of an alternatively folded isoform, designated PrP^{Sc}. Limited proteinase K digestion of PrP^{Sc} results in N-terminal truncation of the protein to form PrP 27–30 consisting of ~140 amino acid residues. Through a poorly understood process, PrP^{Sc} is formed from the normal, cellular precursor denoted PrP^C, which is expressed at high levels in the brain and many other organs. Recently, synthetic prions have been produced from recombinant (rec) mouse (Mo) PrP(89–230) that was produced in *E. coli* and polymerized into amyloid [16,17].

In the CNS, PrP^C is expressed primarily in neurons and astrocytes and at low levels in oligodendrocytes [15,22]. PrP^C is synthesized on the endoplasmic reticulum before being trafficked through the Golgi where it is glycosylated and acquires a glycosylphosphatidyl inositol (GPI) moiety that anchors the protein to the outer leaflet of the plasma membrane. The structure of PrP^C obtained by nuclear magnetic resonance (NMR) spectroscopy indicates that its carboxy-terminal half forms a globular domain around three α -helices while its amino-terminus appears flexible and relatively unstructured [6,12,28]. In contrast to the high α -helical content of PrP^C, PrP^{Sc} has a high β -sheet content [25]. From EM crystallography studies, PrP^{Sc} is thought to contain a large β -helix [8].

Recent studies suggest the PrP^C functions in signal transduction. PrP^C binds to NCAM resulting in the phosphorylation of fyn, a cytoplasmic tyrosine kinase [23,30,32]. Such a process is thought to function in the neurite differentiation [3,13]. Full-length recPrP was found to stimulate both dendritic and axonal outgrowth in cultured hippocampal neurons. Toward increasing our understanding of the function of PrP^C, we undertook a study of the developmental expression pattern of PrP^C. Immunoblotting studies on the brain of the mouse embryo at 16 days post-coitum (E16 p.c.) [21] as well as a more discriminating *in situ* hybridization analysis detected PrP mRNA within the developing brain and spinal cord at E13.5 p.c. [19]. In these studies, evaluation of prion protein gene (*Prnp*) expression during early embryogenesis was not possible because of the low resolution of the techniques used.

In the work reported here, we examined expression of *Prnp* during early development using a β -galactosidase marker under the control of *Prnp* gene promoter elements in transgenic (Tg) mice. We found clear evidence for previously unrecognized *Prnp* expression in the developing embryo starting at E7.5 p.c. Sites of early expression include the developing head, brain, neural tube, the primitive streak region, skin, peripheral nervous system, perineural mesenchyme, and peripheral organs. The *Prnp* expression patterns obtained correlated well with immunoblot and immunocytochemical detection of PrP^C.

RESULTS

Developmental expression of β -galactosidase driven by the *Prnp* promoter

PrP mRNA and PrP^C expression levels in transgenic animals using the CosSHa.tet vector correlate well with the PrP transgene copy [33,34,39]. Furthermore, the spatiotemporal distribution of expression in Tg mice is very similar to that in wild-type (wt) animals. The properties of this vector are reminiscent of those observed with other vectors containing locus control regions (LCR), controlling the local conformation of chromatin and the precise temporal and spatial regulation of transgene expression (reviewed by [10]).

When examined at E13.5 p.c., embryos from Tg(*Prnp-LacZ*)3755/*Prnp*^{+/+} and Tg(*Prnp-LacZ*)3762/*Prnp*^{+/+} showed a similar distribution of LacZ expression that included all the

previously documented anatomic sites of PrP expression [20]. The Tg(*Prnp-LacZ*)3755/*Prnp*^{+/+} line was chosen for further characterization because of higher expression levels.

Expression within the developing embryo was observed as early as E7.5 p.c. (Fig. 1A and B). LacZ activity was evident in the allantois and in extra-embryonic membranes, mostly in the deciduum but also weakly in amniotic membranes. Expression in cephalic folds became evident at E8.0 p.c. (Fig. 1C) and intensified at E8.5 p.c. in the region of presumptive mesencephalic and hindbrain regions as the folds deepened prior to their closure (Fig. 1D and E). At the hindbrain level, expression appeared as two horizontal bands, possibly corresponding to segmentally restricted rhombomeres (Fig. 1D). Caudally, strong expression was found in the caudal portion of the neural plate lateral to the late primitive streak (Fig. 1D). Expression in the amniotic membranes was also widespread at this stage (Fig. 1D).

From stages E8.5 to E9.5 p.c., intense LacZ expression was seen first in the region of the mesencephalon (Fig. 1D and E) followed by a shift to the region of the telencephalon as the anterior neuropore closed from E9 to E9.5 p.c. (Fig. 1F). Cross-sections through the mesencephalon and telencephalon (Fig. 1F, arrows 1 and 2) showed that there was no expression in the neuroectoderm; rather, the intense expression was located in the adjacent mesenchyme (Fig. 1H and I). Caudally, LacZ expression was found within the epithelium of the late primitive streak stage (Fig. 1G-3; see label 3 in Fig. 1D for orientation) and in the caudal-most neural tube epithelium (Fig. 1G, 1 and 2; see labels 1 and 2 in Fig. 1D for orientation). More rostral levels of the neural tube that have a mitotically active ventricular zone were devoid of *Prnp-LacZ* expression from E9.0 to E11 p.c. (Fig. 1D).

LacZ expression in the nervous system (both brain and neural tube) was evident at E11.5 p.c. as precursors from the mitotically active ventricular zone ceased to divide and migrated radially to settle and differentiate. LacZ expression was observed from hindbrain to lower spinal cord in the ventral part of the neural tube, where motor neurons formed in ventral horns (Fig. 2A and B). At later stages, LacZ expression was present in the dorsal neurons following the ventro-dorsal wave of neuronal differentiation within the neural tube. Dorsal roots, ventral roots, dorsal root ganglia, as well as peripheral nerves and the notochord all showed LacZ expression (Fig. 2C).

At E13.5 p.c., expression was detected widely within the nervous system (Fig. 2F). LacZ stain was strongest in the septum, cortical plate, dorsal thalamus, hypothalamus, mammillary body region, pons, cerebellar anlage, medulla, spinal cord, dorsal root ganglia, peripheral nerves, sympathetic ganglia and trunk. In addition, expression in developing peripheral organs was evident in the tongue, heart (also observed at E8.5 to E9.5 p.c.), lungs, duodenum, intestine, olfactory epithelium, palate, genital protuberance, adrenal glands, and limbs (Fig. 2D, F and G). Externally, expression was observed on the skin especially in areas undergoing complex morphogenetic specialization, such as the mammary elevation, external ear, nostrils, and the base of future vibrissae (Fig. 2E).

Comparative analysis of PrP^C and LacZ expression in the brains of Tg(*Prnp-LacZ*) mice

Immunohistochemical localization of PrP^C was compared to sites of *Prnp-LacZ* expression in the adult brain using serial vibratome sections. PrP^C immunostaining colocalized with sites of LacZ expression particularly in nerve cell bodies (Fig. 3A–D). Occasional small cells in white matter, presumably astrocytes or oligodendrocytes, also expressed lower levels of LacZ, consistent with low expression of PrP^C in glial cells [15]. In the hippocampus, strong LacZ expression is seen in the pyramidal cells of Ammon's horn, the granule cells of the dentate gyrus, and nerve cell bodies in the hilus of the dentate (Fig. 3E and C). The same nerve cell bodies were also strongly immunopositive for PrP^C. In the cerebral cortex, nerve cell bodies in layers II to VI of the cerebral cortex showed both expression of LacZ and PrP^C.

immunopositivity (Fig. 3A and B). The large pyramidal neurons of layer V showed particularly high levels of expression of both *Prnp*-LacZ and PrP^C (Fig. 3A, B, and E). LacZ expression was also evident in the cerebellum in the Purkinje cell layer, the granule cell layer, and deep cerebellar nuclei (Fig. 3F–H). Small numbers of cells in both the granule cell layer, possibly the larger Golgi cells, and in the superficial molecular layer, possibly the outer stellate cells, showed strong LacZ expression. Expression was weak but detectable in the white matter tracts, where glia was labeled (Fig. 3C and H).

Developmental expression of PrP^C isoforms

We examined early embryonic expression of PrP^C by Western blot analysis using the PrP-specific RO73 polyclonal antiserum (Fig. 4A). For all preparations, wt FVB mouse embryos were surgically separated from extraembryonic membranes. Care was taken when removing those membranes because they contain high levels of PrP^C. Expression of PrP^C was found as early as E8.5 p.c. (Fig. 4A). Thereafter, expression steadily increased throughout development. From E8.5 to E11.5 p.c., only slowly migrating PrP^C isoforms (to 30–34 kDa) corresponding to fully glycosylated and monoglycosylated PrP^C molecules were detectable, whereas the more rapidly migrating isoforms of 25–28 kDa, corresponding to unglycosylated PrP^C, were observed starting at E12.5 p.c. This probably reflects quantitative differences in the expression levels of these various forms at different stages of development.

We also examined developmental expression of PrP^C in Syrian hamster (SHa) as *Prnp* genomic sequences from this species were used for the construction of the CosSHa.tet vector. SHa brains revealed a similar increase in expression between E12.5 p.c. and postnatal day 14 (P14), after which expression decreased slightly at P28 and more so in the adult brain (Fig. 1B). Expression of PrP^C glycoforms during early postnatal development but not in the adult animal was characteristic of some hamster tissues. Homogenates of SHa skin at P0 showed a PrP^C band migrating to ~30 kDa (Fig. 4B, P0.5) whereas no band was detected at 12 weeks (Fig. 4B, Adult). Between P0 and P28, there was a progressive shift in the representation of glycoforms, with 34 and 35 kDa isoforms becoming more prevalent. Four PrP^C glycoforms were identified, migrating to 35, 34, 32, and 30 kDa.

Tissue-specific distribution of PrP^C isoforms

Expression of PrP^C was detected in nearly all adult tissues tested, including brain, skeletal muscle, heart, lung, spleen, thymus, kidney, skin, intestine, stomach, testis, epididymis, seminal vesicles, ovaries, uterus and mammary glands (Fig. 4C). PrP^C was not detected in some organs, such as the liver (Fig. 4C). Heterogeneity in PrP glycoforms that was not found in brain was observed among different organs (Fig. 4C).

DISCUSSION

Using Northern blots, *Prnp* gene expression was investigated during the late stages of embryonic development and the neonatal period [21]. *In situ* hybridization analysis has also identified PrP transcripts as early as E6.5 p.c. in extra-embryonic membranes, while expression was detected in the nervous system (CNS and PNS) and in peripheral organs (tooth, kidney, extra-embryonic and amniotic membranes, intestine) in E13.5 p.c. embryos [20]. Here, using the LacZ reporter system driven by the PrP promoter, we were able to identify *Prnp* expression in the early postimplantation embryos (E7.5 to E8.5 p.c.).

The temporal and spatial expression of the *Prnp*-LacZ transgene correlated well with the distribution of the gene product, PrP^C, as detected by immunocytochemistry in the adult brain and by immunoblot during embryogenesis (E8.5 p.c., Fig. 4). The anatomic sites of expression observed with this transgenic model overlap with those previously described by using *in situ*

hybridization in the developing tongue, palate, esophagus, intestine, olfactory epithelium [20]. Using the rapid and sensitive detection of the β -galactosidase reporter gene, we identified previously unrecognized sites of *Prnp* expression and were able to delineate the temporal sequence of *Prnp* expression during nervous system development.

In the developing nervous system, PrP is not expressed in the neural progenitors of the mitotically active ventricular zone. It is first expressed in cells that have ceased proliferating, migrated radially from the ventricular zone, and differentiated into neurons in the intermediate layer. At E11.5 p.c., motor neurons in the ventral neural tube clearly express the marker transgene (Fig. 2B). Expression in dorsal neural tube neurons is observed at later stages, after their differentiation (Fig. 2C). These results indicate that *Prnp* expression in the nervous system begins in post-mitotic neural cells that have undergone neuronal differentiation. This pattern of *Prnp* expression in the nervous system appears to persist throughout the adult life of mammals [15].

Neural crest cells are multipotent progenitor cells that contribute at both cranial and trunk levels to diverse cell lineages [5]. PrP promotor-driven LacZ expression labels neural crest-derived cells and structures. At cephalic levels, cranial neural crest cells contribute to the development of cranial nerve ganglia, as well as of skeletal elements of the skull and face [4,9]. Consistent with this role, strong LacZ activity is present in the cephalic mesenchyme surrounding the mesencephalon and telencephalon during early stages of head development. At the trunk level, neural crest cells contribute to the development of the peripheral nervous system and to many peripheral organs including the heart, thyroid, parathyroid and adrenal glands. Expression of *Prnp*-LacZ transgene labels many of these structures, including dorsal root ganglia, peripheral nerves, cranial ganglia, sympathetic trunk and ganglia, adrenals and heart.

PrP^C also appears to be expressed during embryonic development in the skin as previously reported [18,24]. LacZ detection indicates that this expression is initiated as early as day E12.5 p.c. This expression is also detectable by Western blot analysis at birth. Four distinct PrP^C bands were identified in the skin of neonatal Syrian hamsters. The proportion of each glycoform varied during the stages of postnatal development. Similar PrP^C glycoforms were observed during immunoblotting of other peripheral tissues (Fig. 4). Variable glycosylation of the two PrP^C asparagine-linked oligosaccharide sites resulted in three PrP^C bands, representing diglycosylated, monoglycosylated, and unglycosylated PrP glycoforms [29,38]. The presence of four or more PrP^C bands as found in this study suggests that differential proteolytic processing may also occur in some tissues during development. A similar observation has been reported for PrP^C in mature sperm, where it is subjected to N-terminal truncation [36].

While our *Prnp*-LacZ Tg model should facilitate studies aimed at defining the events linked to the modulation of PrP^C expression, it must be considered an imperfect system. Using gene knock-in technology is likely to eliminate any artifacts created by the integration of the CosSHa.Tet vector [31,40].

Our observations of *Prnp* expression in neural crest cells and post-mitotic neurons are consistent with a role for PrP^C in selective adhesion and migration processes. This conclusion is consistent with results from several recent studies arguing that PrP^C functions in signal transduction. PrP^C binds to NCAM resulting in the phosphorylation of fyn, a cytoplasmic tyrosine kinase [23,30,32]. Such a process is thought to function in the neurite differentiation [3,13]. Full-length recPrP was found to stimulate both dendritic and axonal outgrowth in cultured hippocampal neurons [13]. It seems likely that greater purchase on the function of PrP^C can be achieved by measuring the expression levels and localization of PrP, NCAM and fyn during development.

EXPERIMENTAL PROCEDURE

Production of transgenic mice

A cosmid-based expression vector (designated CosSHa.tet) was previously constructed from a 38 kbp genomic fragment isolated from the SHa *Prnp* locus [34]. We introduced a β -galactosidase marker gene, designated *LacZ*, into the CosSHa.tet vector.

The *Prnp*- β -galactosidase reporter transgene was obtained by cloning a 3.7 kbp *HindIII*/*Bam*HI fragment from pSV- β -gal plasmid (Promega) into the *SalI*-digested CosSHa.tet cosmid expression vector using custom-made adapters. The ~40 kbp fragment used for microinjection was released using *NotI* and purified for microinjection. The DNA fragment was separated on a low melting agarose gel electrophoresis, purified using β -agarase, ethanol precipitated and dissolved in TE buffer (10 mM Tris-Cl, pH 7.5; 0.1 mM EDTA). Prior to microinjection, the DNA solution was dialyzed against TE buffer before diluting with TE to 2 ng/ μ l for microinjection. All Tg mice were obtained by microinjection of PrP-deficient (*Prnp*^{0/0}) zygotes as already described [11]. By breeding selected founders with FVB and/or *Prnp*^{0/0} mice, we established two lines of mice carrying a high and low copy number of the transgene, designated Tg(*Prnp-LacZ*)3755/*Prnp*^{+/+} and Tg(*Prnp-LacZ*)3762/*Prnp*^{+/+}, respectively. Screening of Tg (*Prnp-LacZ*) mice was performed using a probe from the 3' untranslated region from the cosmid vector.

For timed mating, embryos were staged counting the day the vaginal plug was detected as day 0.5 p.c. Embryos were fixed in a solution of 1% formaldehyde, 0.2% glutaraldehyde, 0.2% NP-40, 0.1% sodium deoxycholate, and phosphate-buffered saline (PBS) for 20 min, washed twice in PBS for 20 min, and stained for 4 h to overnight in a solution of 0.1% X-gal, 5 mM K₄Fe(CN)₆, 5 mM K₃Fe(CN)₆, 2 mM MgCl₂, 0.2% NP-40, and 0.1% sodium deoxycholate. Embryos were then rinsed in PBS and cleared in a series of 30, 50, and 80% glycerol/PBS solutions.

β -galactosidase staining and PrP immunohistochemistry on sections

Animals were perfused by intracardial puncture using 20 ml of PBS (pH 7.4) followed by 200 ml of 4% paraformaldehyde/PBS. Tissues/embryos were removed and embedded in a mix of gelatin, albumen and sucrose and sectioned at 50 μ m using a Leica VT1000S vibratome. Floating sections were stained using either the X-gal solution (as described above) for 30 min to 2 h or reacted with the α -PrP RO73 polyclonal antiserum at 1:1000 in Tris-buffered saline with 0.5% Tween-20 (TBST). Sections were incubated at 4°C for 16 h, washed with TBST and developed using a peroxidase-linked anti-rabbit antibody (Vector Laboratory, Burlingame, CA). All sections were transferred to slides and mounted under coverslips in Aquamount mounting medium (Polysciences Inc., Warrington, PA). Images were captured using a digital Polaroid DMC1 camera mounted on a Nikon eclipse E600 microscope. Observations were made on at least 3 independent specimens.

Western blot analysis

Protein concentration of tissue homogenates was assessed by the bicinchoninic acid assay (BCA: Pierce, Rockford, IL). Western blot analyses were performed as already described [2] using the RO73 polyclonal α -PrP antiserum [35] at 1:5000 dilution, the 3F4 monoclonal antibody [14] at 1:5000, the α -synaptophysin at 1:2000, α -actin at 1:2000 and revealed with the enhanced chemiluminescent detection system (Amersham Life Science, Arlington Heights, IL).

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Abbreviations

<i>Prnp</i>	gene encoding the prion protein
PrP	prion protein
PrP^C	normal, cellular prion protein
SHa	Syrian hamster
Tg	transgenic
Mo	mouse
CJD	Creutzfeldt-Jakob disease

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Figure 1.

Developmental expression of β -galactosidase driven by *Prnp* gene control elements in the post-implantation embryo. LacZ expression is shown between E7.5 to E13.5 p.c. by whole-mount staining. (A, B) At E7.5 p.c., expression is observed in the deciduum (de), allantois (al) and faintly in the amniotic membrane (am). (C) At E8.0 p.c., invaginating cephalic neural folds (nf) display LacZ expression. (D, E) At E8.5 p.c., expression is upregulated in the mesencephalic (me) and hindbrain (hb) levels. Ventrally, the heart (he) and caudally, the primitive streak region (ps), also show expression. (F) At E9.5 p.c., expression shifts rostrally toward the telencephalic (te) area, and decreases in the mesencephalic (me) area. (G) Transient LacZ expression in neuroectoderm prior to neural tube closure in the posterior neural tube at

E8.5 p.c. Sections 1, 2, and 3 correspond to levels 1–3 shown in panel D. **(H)** Rostral telencephalon (corresponding to level 1 in panel F) and **(I)** mesencephalon (corresponding to level 2 in panel F) at E9.5 p.c. The open anterior neuropore is visible. LacZ staining is confined to the cephalic mesenchyme (outside of the dotted lines). Abbreviations: am, amniotic membrane; de, deciduum; hb, hindbrain; he, heart; me, mesencephalon; nf, neural folds; nt, notochord; oc, optic cup; ov, otic vesicle; ps, primitive streak; sc, spinal cord; se, septum; sg, sympathetic ganglia; so, somites; te, telencephalon.

Figure 2.

Expression of β -galactosidase driven from *Prnp* gene control elements in the nervous system, limbs, and skin of the developing embryo. Expression from Tg(*Prnp*-LacZ)E3755/*Prnp*^{+/+} is shown between stages E11.5 to E13.5 p.c. by whole-mount staining followed by dissection or sectioning to reveal expression. At E11.5 p.c., expression is evident in the ventral region of the (A) hindbrain and (B) neural tube at the trunk level that corresponds to motor neuron (mn) columns. Expression in the ectoderm (ec) and in surrounding mesenchyme (ms) is also evident. (C) At E12.5 p.c., expression extends to the dorsal neural tube. Expression includes dorsal root ganglia (dg), dorsal and ventral roots (dr, vr) as well as ectoderm and notochord (nt). At E13.5 p.c., LacZ activity is evident in the (D) developing limbs and (E) skin. (F) At E13.5 p.c., expression is detected in the CNS, PNS and in various developing peripheral organs. (G) Externally, expression is observed in various areas of the skin. Abbreviations: ad, adrenal; al, allantois; am, amniotic membrane; ar, apical ectodermal ridges; ce, cerebellar anlage; cp, cortical plate; dg, dorsal root ganglia; dt, dorsal thalamus; ee, external ear; fl, forelimb; gp, genital protuberance; hb, hindbrain; he, heart; hl, hindlimb; hy, hypothalamus; lu, lung; ma, mamillary area; md, medulla; me, mesencephalon; mg, mamillary gland elevation; mn, motor neuron column; nf, neural folds; nt, notochord; oc, optic cup; oe, olfactory epithelium; ov, otic vesicle; po, pons; pn, peripheral nerves; ps, primitive streak; sc, spinal cord; se, septum; sg,

sympathetic ganglia; so, somites; st, sympathetic trunk; te, telencephalon; to, tongue; vi, vibrissae.; vr, ventral root; vz, ventricular zone

Figure 3.

Expression of the *Prnp*-LacZ transgene in the adult brain coincides with endogenous PrP^C expression. Expression of the LacZ marker is shown in higher-magnification images of the neocortex (A–B), hippocampal dentate gyrus (C), cerebellar cortex and deep cerebellar nuclei (H). Expression of the LacZ marker is presented in coronal sections of the brain at the levels of the hippocampus (E) and cerebellum (F). Immunohistochemistry using RO73 polyclonal antiserum is shown for comparison in the neocortex (B) and hippocampus (D). Arrows in both (C) and (H) indicate scattered glial cells expressing LacZ. Abbreviations: bs, brainstem; cc, corpus callosum; cx, neocortex; dc, deep cerebellar nuclei; dg, dentate gyrus; ec, entorhinal cortex; gl, granule cell layer; hl, hilus of dentate gyrus; hp, hippocampus; ml, molecular layer;

pl, Purkinje cell layer; th, thalamus; wm, white matter tracts; Roman numerals I–VI in (**A**) refer to the layers of the neocortex.

Figure 4.

Expression of PrP^C in FVB mice and Syrian hamsters. Western blot analysis detects (A, C) mouse PrP^C using the RO73 polyclonal antiserum and (B) hamster PrP^C with the 3F4 monoclonal antibody. (A) Expression in entire embryos or in isolated heads and bodies (as indicated) from E8.5 p.c. to adulthood. (B) PrP^C expression in developing embryonic or neonatal Syrian hamster brains and skin. (C) PrP^C in adult mouse organs. Antisera against actin (bottom of panel A) and synaptophysin (bottom of panel B) were used as controls. Forty µg of homogenate were used per lane except for the brain homogenate from wt mouse ((A) and (C)), for which 5 µg were used. Abbreviations: E, embryonic day; Ep, epididymis; SHa, Syrian hamster; P, postnatal day.