Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4309–4313, May 1993
Neurobiology

Localization of the mRNA for a chicken prion protein by *in situ* hybridization

(acetylcholine/brain)

DAVID A. HARRIS*†, PRITI LELE‡, AND WILLIAM D. SNIDER‡

Departments of *Cell Biology and Physiology and ‡Neurology, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Gerald D. Fischbach, January 6, 1993 (received for review October 8, 1992)

ABSTRACT **The infectious agent (prion) responsible for transmissible spongiform encephalopathies in humans and animals is composed primarily of a 33- to 35-kDa glycoprotein called PrP^{Sc} (scrapie isoform of prion protein), which is a posttranslationally modified form of the normal cell-surface protein PrP^{C}. Little is known about the function of PrP^{C}. Interestingly, chPrP, the chicken homologue of PrP^{C}, copurifies with a factor from brain that stimulates synthesis of acetylcholine receptors on skeletal muscle cells. Using *in situ* hybridization, we report here that chPrP mRNA is widely distributed in cholinergic and noncholinergic neurons throughout the adult central nervous system, including those in the telencephalic striata, thalamus and hypothalamus, optic tectum, medulla, cerebellum, and spinal cord. The mRNA is present in the brain and spinal cord as early as embryonic day 6 and is also found in dorsal root ganglia, retina, intestine, and heart. Our data suggest that if chPrP serves to regulate acetylcholine receptor number on postsynaptic targets, this is not its only function. It is likely that the protein plays a more widespread role in the central nervous system and perhaps elsewhere, possibly one related to intercellular communication, adhesion, or recognition. The chicken embryo represents an attractive experimental system in which to investigate the normal developmental function of PrP^{C}.**

The spongiform encephalopathies are a group of transmissible, degenerative diseases of the central nervous system including Creutzfeldt–Jakob disease, Gerstmann–Sträussler syndrome, and kuru in humans, as well as scrapie and bovine spongiform encephalopathy in animals (1). The infectious agent, or prion, thought to be responsible for these diseases is composed primarily, if not exclusively, of a glycoprotein of molecular mass 33–35 kDa, referred to as PrP^{Sc} (scrapie isoform of prion protein), which is a posttranslationally modified form of the normal cellular protein PrP^{C} (2–4).

The function of PrP^{C} remains unknown, but the protein is likely to play an important role since it has been highly conserved during evolution (5) and is abundantly expressed in the brain as well as in some peripheral tissues (6–8). Mice in which the PrP gene has been deleted by homologous recombination are normal by several criteria, suggesting that loss of this protein may have very subtle effects, or that other proteins can substitute for PrP in these mice (9).

Several possible functions for PrP^{C} have been suggested. Since it is a cell-surface protein (10), it may play a role in cell-to-cell recognition, adhesion, or signaling. In this regard, it has been proposed that the protein participates in lymphocyte activation (7). PrP mRNA appears in the neural tube of the mouse early in embryonic development (11), and its level in hamster brain is regulated by nerve growth factor (12). These results suggest a function for the protein in neural development and differentiation. Since PrP mRNA and protein are also present in some nonneural cells, a more general cellular role during embryogenesis and in the adult is possible (11).

We have been studying a chicken PrP (chPrP) that is the avian homologue of PrP^{C} from mammals (referred to as ch-PrLP in ref. 13). chPrP is identical to mammalian PrP at 33% of its amino acid positions, including an uninterrupted stretch of 24 identical residues, and it displays the same structural domains. Moreover, the major biochemical and cellular properties of the avian and mammalian proteins are similar (14), as are the intron–exon structures of the genes that encode them (unpublished data).

chPrP was originally identified because it copurified with an activity (ARIA; acetylcholine receptor-inducing activity) from chicken brain that stimulates synthesis of nicotinic acetylcholine receptors on skeletal muscle cells (13). Available biochemical data are inconclusive about whether chPrP and ARIA are identical molecules or whether they are distinct proteins that copurify. Nevertheless, we have been interested in the possibility that chPrP may play a role in regulation of neurotransmitter receptors on muscle and possibly in the central nervous system as well.

We report here on the anatomical localization of the mRNA for chPrP in adult and embryonic chickens. We find that this messenger is widely distributed throughout the brain and spinal cord, where it is found in both cholinergic and noncholinergic neurons. It is also present in several nonneural tissues. Our results suggest that chPrP subserves a widespread cellular function that begins early in embryonic development. Although the protein copurifies with an ARIA, regulation of nicotinic receptors is unlikely to be its only function. The extensive information available on chicken development and the ease of manipulating embryos experimentally should make it possible to further explore the normal function of PrP^{C} in this species.

MATERIALS AND METHODS

Antisense RNA probes were transcribed from the plasmid p65-21 (13) after linearization with *Pvu* II using T3 polymerase and uridine 5′-[α-[^{35}S]thio]triphosphate according to the manufacturer's directions (Stratagene). Sense-strand probes were transcribed from the plasmid pGEM-AS after linearization with *Bam*HI using SP6 polymerase. pGEM-AS was constructed by cloning an *Eco*RV/*Pvu* II fragment from p65-21 into the *Hinc*II site of pGEM-1 (Promega). The antisense and sense-strand probes span the identical regions of the chPrP cDNA (nucleotides 1474–2017; figure 1 of ref. 13). Probes were hydrolyzed in 100 mM $NaHCO_3$ (pH 10.2) at 60°C for 45 min to reduce their size to an average of 150

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PrP, prion protein; PrP^{c}, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; chPrP, chicken prion protein; ARIA, acetylcholine receptor-inducing activity.

†To whom reprint requests should be addressed.

nucleotides. Probes had a specific activity of 1.4×10^9 dpm/μg and were used at a final concentration of 200 ng/ml.

Embryonic and adult chickens were perfusion-fixed in formalin, tissue was embedded in paraffin, and 8-μm sections were cut on a microtome and mounted on glass slides derivatized with 3-aminopropyltriethoxysilane. Deparaffinized sections were hybridized, washed, and prepared for emulsion autoradiography as described by Wanaka *et al.* (15), except that RNase was used at 20 μg/ml, and hybridization was carried out at either 45°C or 55°C. Sections were exposed at 4°C for 7–14 days and after development were counterstained with hematoxylin and eosin and viewed in a Nikon Microphot FXA microscope with bright-field and dark-field condensers. The stereotaxic atlas of Kuenzel and Masson (16) was used for anatomic identification.

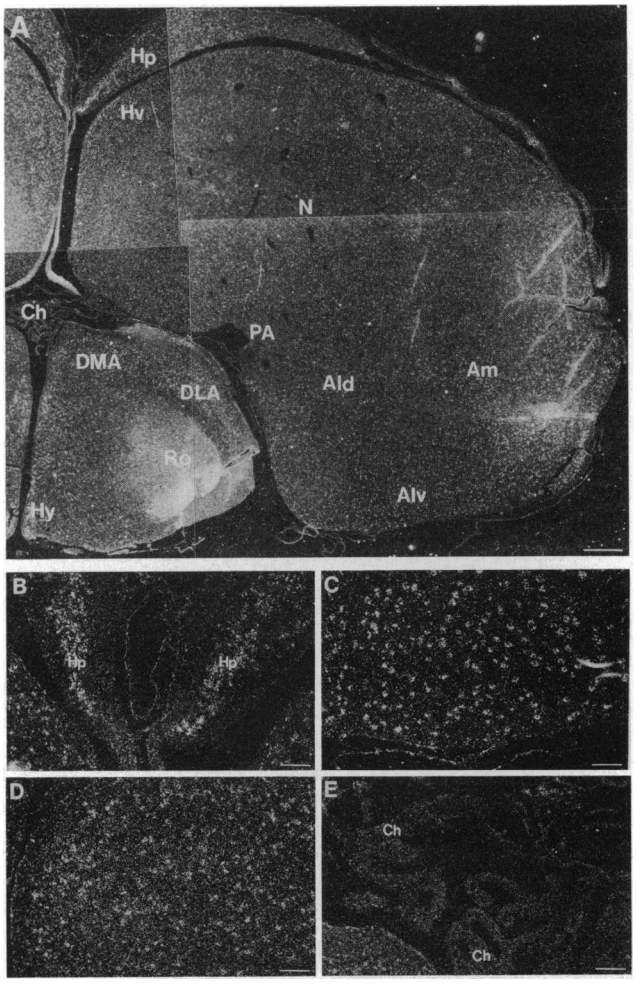

FIG. 1. Localization of chPrP mRNA in the telencephalon and diencephalon of adult chicken. Sections were hybridized with a ^{35}S-labeled antisense RNA probe. (*A*) Transverse section showing widespread distribution of chPrP mRNA. AId, archistriatum intermedium, pars dorsalis; AIv, archistriatum intermedium, pars ventralis; Am, archistriatum mediale; Ch, choroid plexus of the lateral ventricle; DMA, nucleus dorsomedialis anterior of the thalamus; DLA, nucleus dorsolateralis anterior of the thalamus; Hp, hippocampus; Hy, hypothalamus; N, neostriatum; PA, paleostriatum augmentatum; Ro, nucleus rotundus. The bright appearance of the nucleus rotundus is an optical artifact; this region does not contain more silver grains than other areas of the thalamus. (*B*) Higher magnification view of the paired hippocampi, which are among the most intensely hybridized regions of the telencephalon. (*C*) Densely labeled neurons in the AIv. (*D*) DMA nucleus of the thalamus. (*E*) Choroid plexus of the lateral ventricle, which is not hybridized above background levels. (Bars: *A*, 1000 μm; *B–E*, 100 μm.)

RESULTS

Tissue sections were hybridized *in situ* with a ^{35}S-labeled antisense RNA probe complementary to 544 nucleotides from the 3′ noncoding region of chPrP mRNA. Hybridization was also performed with a sense-strand probe derived from the same region to confirm the specificity of the observed signals (see Figs. 3 *E* and *F* and 4*B*). An antisense probe derived from the coding region gave results similar to those obtained with the antisense probe from the noncoding region (data not shown). Probe specificity has been demonstrated by Northern blot analysis (13).

Adult Animals. Expression of chPrP mRNA was widespread at all levels of the neuroaxis. In the telencephalon, cells in all major gray-matter areas hybridized to the antisense probe, including those in the archistriatum, paleostriatum, neostriatum, and hyperstriatum (Fig. 1*A*). Cells in the hippocampus, an area of the mammalian brain rich in PrP

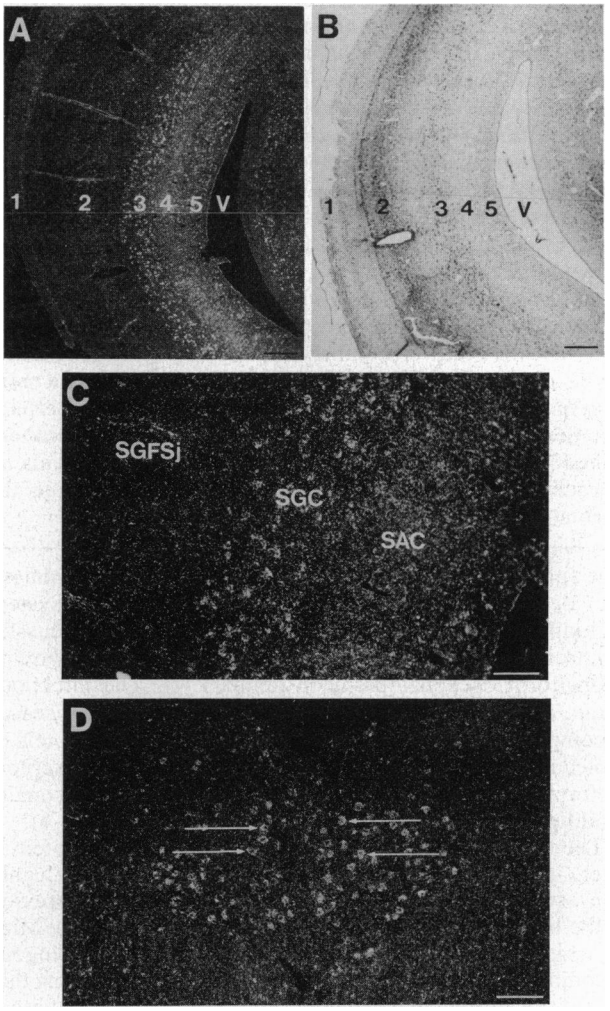

FIG. 2. Localization of chPrP mRNA in the optic tectum of adult chicken. (*A*) Transverse section hybridized with a ^{35}S-labeled antisense RNA probe. (*B*) Adjacent section stained with cresyl violet, shown in bright field. Cytoarchitectonic layers (17) are indicated by numbers as follows: 1, stratum opticum; 2, layer i of the stratum griseum et fibrosum superficiale (SGFS); 3, stratum griseum centrale (SGC); 4, stratum album centrale (SAC); 5, stratum griseum periventriculare and stratum fibrosum periventriculare. Neurons in the SGC stain intensely, while neurons in the densely populated SGFSi hybridize only weakly. (*C*) Higher magnification of the tectal lamina. Note that the SAC, which is a fiber layer, does not hybridize but appears bright under dark-field illumination. (*D*) Neurons (arrows) of the Edinger–Westphal nucleus hybridize strongly. (Bars: *A* and *B*, 300 μm; *C* and *D*, 100 μm.)

mRNA and protein, were heavily labeled (Fig. 1*B*). Discrete collections of grains over individual neurons were easily resolved in most regions, as shown in Fig. 1*C* (archistriatum intermedium). Cells throughout the thalamus (Fig. 1*D*), hypothalamus, as well as other regions of the diencephalon also hybridized. The choroid plexus was negative (Fig. 1*E*).

In the optic tectum, large neurons in the stratus griseum centrale, which are the major efferent neurons of the tectum, hybridized strongly (Fig. 2 *A–C*). Less intense hybridization was seen in the thick stratum griseum et fibrosum superficiale, particularly layer i, which is densely populated with both large and medium-sized neurons. The stratum opticum and the stratum album centrale, which contain fiber tracts and no perikarya, were not labeled above background. Ependymal cells lining the tectal ventricle were also unlabeled. Strong hybridization was seen in cells of the Edinger–Westphal nucleus (Fig. 2*D*).

Neurons throughout the rhombencephalon were labeled above background. In the medulla, neurons in the glossopharyngeal nucleus and dorsal motor nucleus of the vagus hybridized especially intensely (Fig. 3*D*).

In the cerebellum, Purkinje cells were heavily labeled, and cells in the granular layer were also clearly hybridized above background levels (Fig. 3 *A–C*). Cells in the molecular layer and the white matter were unlabeled.

In cross-sections of adult spinal cord, silver grains were highly concentrated in large neuronal cell bodies in the ventral horn (Fig. 4 *A* and *C*). Based on their position and size, these cells are likely to be motor neurons. Less intense hybridization is also observed over smaller neurons in the dorsal horn and intermediate zone. Little or no specific signal was seen in the white matter.

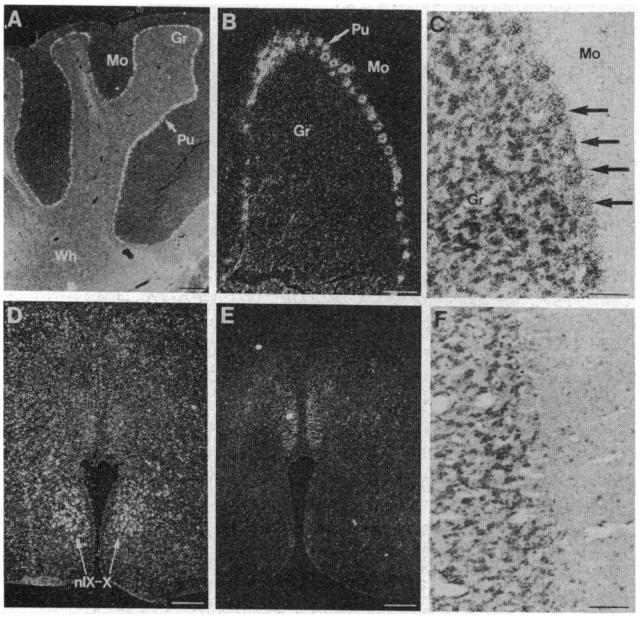

FIG. 3. Localization of chPrP mRNA in adult cerebellum and medulla. Sections were hybridized with ^{35}S-labeled antisense (*A–D*) or sense (*E* and *F*) RNA probes. (*A*) Sagittal section through cerebellar folia of an adult chicken showing intense labeling of the Purkinje cell layer (Pu) and less intense labeling of the granular layer (Gr). Molecular layer (Mo) was unlabeled. White matter (Wh) does not hybridize but appears bright under dark-field illumination. (*B*) Higher magnification of a cerebellar folium. (*C*) Bright-field image showing labeling of Purkinje cells (arrows) and cells in the granular layer. An adjacent section hybridized with a sense-strand probe shows very few silver grains (*F*). (*D*) Transverse section through the medulla showing intense hybridization of neurons in the glossopharyngeal nucleus (nIX) and the dorsal motor nucleus of the vagus (nX) (arrows). Cells throughout other regions of the medulla are also labeled. Very few grains are seen over an adjacent section hybridized with a sense-strand probe (*E*). (Bars: *A*, *D*, and *E*, 200 μm; *B*, 100 μm; *C* and *F*, 50 μm.)

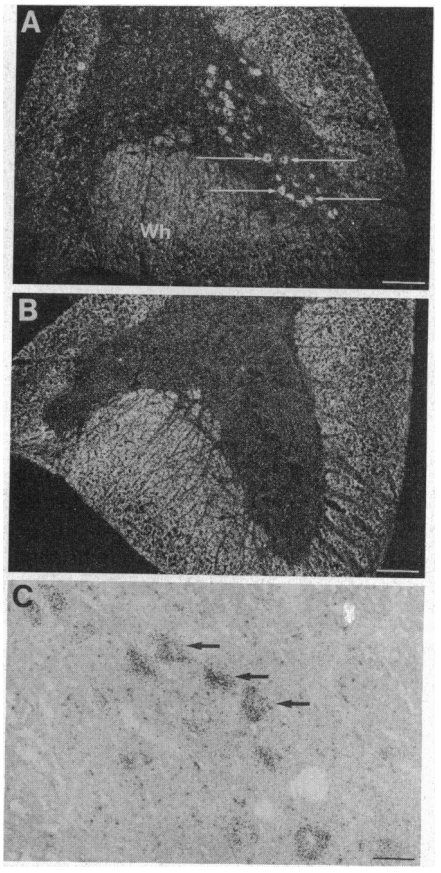

FIG. 4. Localization of chPrP mRNA in transverse sections of the adult spinal cord. Sections were hybridized with antisense (*A* and *C*) or sense (*B*) probes. (*A*) Motor neurons in the ventral gray matter hybridize intensely (arrows). Cells in the dorsal and intermediate gray zones hybridize more weakly. An adjacent section hybridized with a sense-strand probe shows very few silver grains (*B*). Note that the white matter (Wh) is not labeled but appears bright under dark-field illumination. (*C*) Bright-field image showing ventral motor neurons (arrows) heavily labeled with silver grains. (Bars: *A* and *B*, 200 μm; *C*, 50 μm.)

Embryos. At embryonic day 11, expression of chPrP mRNA is prominent in the central nervous system, as well as in several nonneural tissues (Fig. 5*A*). Silver grains are abundant in the gray matter of the spinal cord (Fig. 5*B*) as well as in the telencephalon, diencephalon, optic lobe, and rhombencephalon. Dorsal root ganglia were intensely labeled (Fig. 5*C*). In the retina, the ganglion cell layer hybridized strongly, and the inner nuclear layer hybridized more weakly (Fig. 5*E*). Small foci of silver grains were also observed in several peripheral tissues, including the aorta, gizzard, intestine (Fig. 5*F*), and heart (Fig. 5*G*). Whether these regions correspond to autonomic ganglia, intrinsic nerve plexuses, or to other structures remains to be determined. Nonspecific labeling of the liver, mesonephros, and pigment epithelium of the retina was seen with both sense and antisense probes.

At embryonic day 6, the earliest stage examined, specific labeling can be seen in the gray matter of the spinal cord (Fig. 5*D*) and throughout the thickness of the walls of the developing brain at all levels (data not shown).

DISCUSSION

The results presented here demonstrate that the mRNA for chPrP, an avian homologue of mammalian PrP^C, is widely

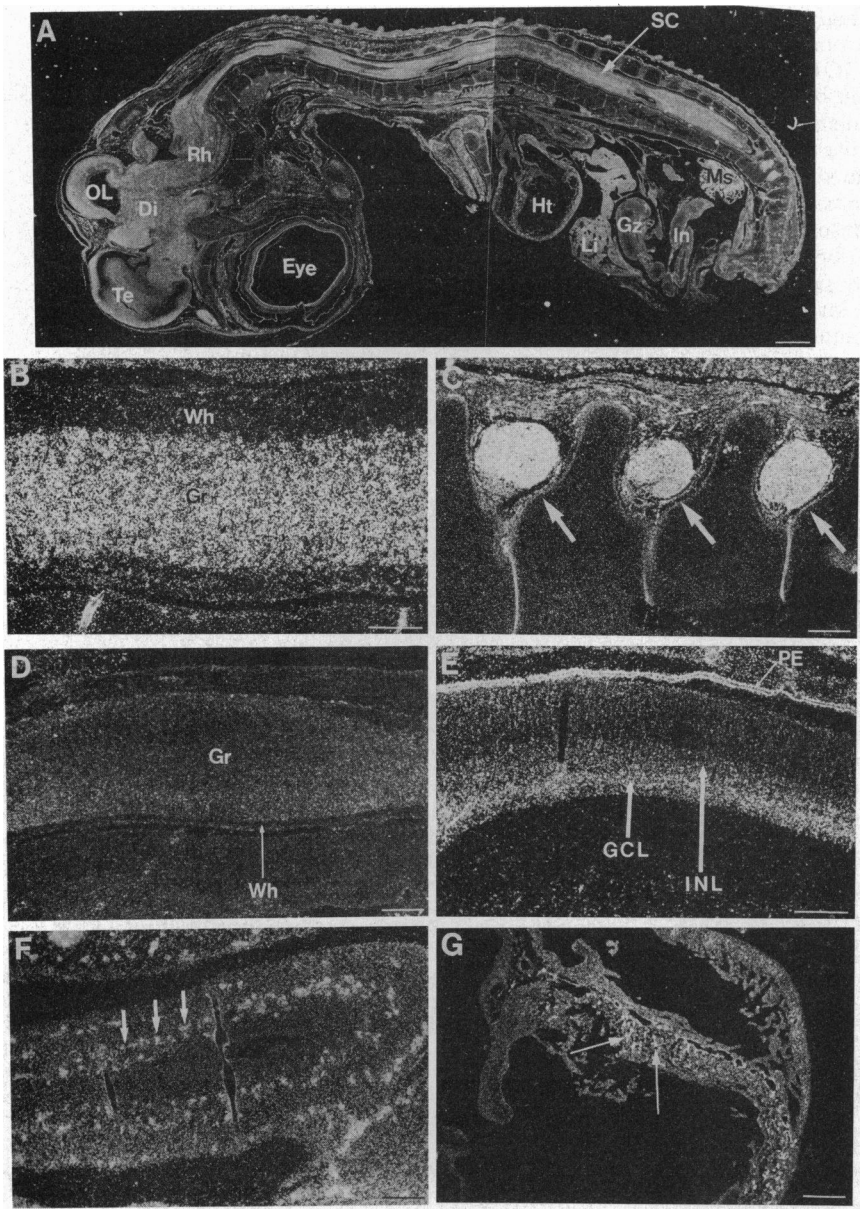

FIG. 5. Localization of chPrP mRNA in chicken embryos. Sections were hybridized with an antisense probe. (*A–C* and *E–G*) Day 11 embryos. (*D*) Day 6 embryo. (*A*) Sagittal section showing widespread distribution of chPrP mRNA throughout the central nervous system, as well as in several nonneural tissues. Te, telencephalon; Di, diencephalon; OL, optic lobe; Rh, rhombencephalon; SC, spinal cord; Ht, heart; Li, liver; Gz, gizzard; In, intestine; Ms, mesonephros. Liver, mesonephros, and pigment epithelium of the retina hybridize nonspecifically with both sense and antisense probes. (*B*) Spinal cord, showing intense hybridization in the gray matter (Gr) and no hybridization in the white matter (Wh). (*C*) Dorsal root ganglia (arrows) are heavily labeled with silver grains. (*D*) Sagittal section of the spinal cord of a day 6 embryo showing hybridization in the gray matter. White matter, which is unlabeled, is a very narrow zone at this stage. (*E*) Cross-section through the eye, showing heavy labeling of the retinal ganglion cell layer (GCL) and less intense labeling of the inner nuclear layer (INL). Pigment epithelium (PE) hybridizes nonspecifically with both sense and antisense probes. (*F*) Cross-section through the intestine, showing collections of silver grains (arrows) arranged circumferentially around the lumen. (*G*) Foci of grains (arrows) are visible in the walls of the heart. (Bars: *A*, 1000 μm; *B* and *D–G*, 100 μm; *C*, 200 μm.)

distributed in the central nervous system of adult and embryonic chickens. These results are consistent with previous Northern blot analyses, which demonstrate that chPrP mRNA is present in brain and spinal cord as early as embryonic day 6, increasing to highest levels in the adult (13). In those studies, the mRNA was also found to be present in several peripheral tissues, particularly in the embryo; these included muscle, heart, gizzard, liver, lung, intestine, spleen, and kidney.

We have localized chPrP mRNA by *in situ* hybridization to neurons at all levels of the neuroaxis from telencephalon to spinal cord and have also detected it in dorsal root ganglia and retina. Not all neuronal cell types seemed to contain equivalent amounts of chPrP mRNA. Spinal motor neurons and cerebellar Purkinje cells, for example, were intensely labeled, while cerebellar granule cells and neurons in the dorsal horn of the spinal cord hybridized more weakly. This variation was not directly correlated with cell size, since some small cells in the adult telencephalon displayed grain densities that were as high as those over spinal motor neurons and Purkinje cells. We cannot be certain whether there are any neurons that completely lack chPrP mRNA.

Nonneuronal cells in the central nervous system expressed low or undetectable levels of chPrP mRNA. White matter areas (in the spinal cord, optic tectum, and cerebellum, for example) were not labeled above background, suggesting that glial cells do not express this messenger. Ependymal cells lining the ventricles and choroidal epithelial cells were also negative. *In situ* hybridization studies of rodent brain have reached conflicting conclusions on whether PrP mRNA is expressed by these cell types (11, 18, 19). However, neither these studies nor our own rule out the presence of a low level of PrP mRNA in nonneuronal cells that would be difficult to distinguish from background.

Our results on the localization of chPrP mRNA correlate well with immunocytochemical studies of chPrP protein in which a monoclonal antibody raised to a peptide corresponding to residues 213–224 of the amino acid sequence was used (ref. 20; F. A. Johnson and G. D. Fischbach, personal communication). The protein was found at high concentrations in spinal motor neurons, neurons of the Edinger–Westphal nucleus, and cerebellar Purkinje cells. Weaker staining was seen in dorsal and medial gray matter of the spinal cord. There was little staining in white matter areas of the central nervous system.

Our studies also correlate well with several studies demonstrating a widespread distribution of PrP^C mRNA and

protein in embryonic and adult rodents. The mRNA for mouse PrP is detectable as early as embryonic day 13.5, a stage roughly equivalent to embryonic day 6 in the chicken (11). The messenger is found throughout the brain and spinal cord, as well as in peripheral ganglia and nerves, neurons of the olfactory epithelium and retina, and nonneural tissues such as intestine, kidney, dental lamina, and extraembryonic membranes. The amount of PrP mRNA increases postnatally in rodents, and in the adult brain it is found to be present in large neocortical neurons, cerebellar Purkinje cells, and hippocampal neurons (18, 21–23). DeArmond *et al.* (24) found that PrP was concentrated in neocortical pyramidal cells, cerebellar Purkinje cells, as well as in neurons of the hippocampus, septal nuclei, thalamus, and caudate. Little staining was observed in the granule cell layer of the cerebellum or in glial cells. Bendheim *et al.* (8) have recently reported a similar localization of the protein in brain, but they fail to find detectable levels in cerebellar Purkinje cells and in spinal cord, in contrast to our observations on chPrP.

The results reported here invite speculation about the normal function of chPrP. The protein was originally identified because it copurified with ARIA, a factor from chicken brain that stimulates the synthesis of nicotinic acetylcholine receptors on skeletal muscle cells (13). To date, biochemical data are inconclusive as to whether chPrP and ARIA are identical molecules or whether they are unrelated proteins that copurify (ref. 13; unpublished data).

Several neuronal cell types in which we have localized chPrP mRNA are cholinergic, consistent with a role for the protein in regulating acetylcholine receptors on postsynaptic targets. The mRNA is concentrated in spinal motor neurons, which innervate skeletal muscle, as well as in two classes of preganglionic cholinergic neurons, those in the Edinger–Westphal nucleus and in the dorsal motor nucleus of the vagus. In this regard, it is noteworthy that choline acetyltransferase and PrP mRNA increase in parallel in the basal forebrain of mice during development and after nerve growth factor administration (12). Nevertheless, our results make it clear that chPrP mRNA is not in any way restricted to cholinergic neurons. The mRNA is highly concentrated in cerebellar Purkinje cells, which are GABAergic, and in cells in the stratum griseum centrale layer of the optic tectum, which do not stain positively for choline acetyltransferase (25). In addition, the widespread distribution of chPrP mRNA throughout the neuroaxis, and in several peripheral tissues, makes such a neurotransmitter-restricted localization unlikely.

Even if chPrP plays a role in the regulation of acetylcholine receptors on postsynaptic targets, this is unlikely to be its only role. It is probable that the protein subserves a more general and widespread function, one that begins early in embryonic development and continues into adulthood. This function may be most important in neurons of the central nervous system, where chPrP mRNA is most abundant, but a role in other cell types is also consistent with our localization data. chPrP is attached to the cell surface by a glycosylphosphatidylinositol anchor, and specific fragments of the molecule are released into the extracellular medium (13, 14). These biochemical properties suggest a role in cell attachment, recognition, or intercellular signaling. Whether chPrP functions as a cell-surface receptor or, alternatively, as a soluble or membrane-bound ligand remains to be determined.

The chicken may represent a suitable species to further investigate the normal function of PrP^C. A great deal is known about the neuroembryology of the chicken, and the embryo is accessible to experimental manipulation (26, 27). Since considerable evidence indicates that the biochemical properties of chPrP and mammalian PrP are similar, it is likely that information gained about the function of the avian protein will be applicable to its mammalian counterpart.

Note Added in Proof. ARIA, the acetylcholine receptor-inducing protein, has been shown to be a ligand for the Neu tyrosine kinase receptor and is apparently unrelated to chPrP (28).

We thank Drs. Joel Price and Thomas Woolsey for the use of their photomicroscopy equipment and Li Zhang for help in preparing the figures. This work was supported by grants to D.A.H. from the National Institutes of Health (Clinical Investigator Development Award and Grant NS30137) and the Esther A. and Joseph Klingenstein Fund and to W.D.S. from the National Institutes of Health (Program Project NS17763).

1. Gajdusek, D. C. (1990) in *Virology*, eds. Fields, B. N. & Knipe, D. M. (Raven, New York), 2nd Ed., pp. 2289–2324.
2. Prusiner, S. B. (1982) *Science* **216,** 136–144.
3. Prusiner, S. B. (1989) *Annu. Rev. Microbiol.* **43,** 345–374.
4. Prusiner, S. B. (1991) *Science* **252,** 1515–1522.
5. Gabriel, J. M., Oesch, B., Kretzschmar, H., Scott, M. & Prusiner, S. B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9097–9101.
6. Oesch, B., Westaway, D., Wälchli, M., McKinley, M. P., Kent, S. B. H., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L., Prusiner, S. B. & Weissmann, C. (1985) *Cell* **40,** 735–746.
7. Cashman, N. R., Loertscher, R., Nalbantoglu, J., Shaw, I., Kascsak, R. J., Bolton, D. C. & Bendheim, P. E. (1990) *Cell* **61,** 185–192.
8. Bendheim, P. E., Brown, H. R., Rudelli, R. D., Scala, L. J., Goller, N. L., Wen, G. Y., Kascsak, R. J., Cashman, N. R. & Bolton, D. C. (1992) *Neurology* **42,** 149–156.
9. Büeler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H. P., DeArmond, S. J., Prusiner, S. B., Aguet, M. & Weissmann, C. (1992) *Nature (London)* **356,** 577–582.
10. Borchelt, D. R., Scott, M., Taraboulos, A., Stahl, N. & Prusiner, S. B. (1990) *J. Cell Biol.* **110,** 743–752.
11. Manson, J., West, J. D., Thomson, V., McBride, P., Kaufman, M. H. & Hope, J. (1992) *Development* **115,** 117–122.
12. Mobley, W. C., Neve, R. L., Prusiner, S. B. & McKinley, M. P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9811–9815.
13. Harris, D. A., Falls, D. L., Johnson, F. A. & Fischbach, G. D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7664–7668.
14. Harris, D. A., Huber, M. T., van Dijken, P., Shyng, S.-L., Chait, B. T. & Wang, R. (1993) *Biochemistry* **32,** 1009–1016.
15. Wanaka, A., Johnson, E. M. & Milbrandt, J. (1990) *Neuron* **5,** 267–281.
16. Kuenzel, W. J. & Masson, M. (1988) *A Stereotaxic Atlas of the Brain of the Chick (Gallus domesticus)* (Johns Hopkins Univ. Press, Baltimore).
17. LaVail, J. H. & Cowan, W. M. (1971) *Brain Res.* **28,** 391–419.
18. Kretzschmar, H. A., Prusiner, S. B., Stowring, L. E. & DeArmond, S. J. (1986) *Am. J. Pathol.* **122,** 1–5.
19. Brown, H. R., Goller, N. L., Rudelli, R. D., Merz, G. S., Wolfe, G. C., Wisniewski, H. M. & Robakis, N. K. (1990) *Acta Neuropathol.* **80,** 1–6.
20. Johnson, F. A. & Fischbach, G. D. (1991) *Soc. Neurosci. Abstr.* **17,** 1501.
21. Lieberburg, I. (1987) *Brain Res.* **417,** 363–366.
22. McKinley, M. P., Hay, B., Lingappa, V. R., Lieberburg, I. & Prusiner, S. B. (1987) *Dev. Biol.* **121,** 105–110.
23. Lazarini, F., Deslys, J. P. & Dormont, D. (1991) *Mol. Brain Res.* **10,** 343–346.
24. DeArmond, S. J., Mobley, W. C., DeMott, B. A., Barry, R. A., Beckstead, J. H. & Prusiner, S. B. (1987) *Neurology* **37,** 1271–1280.
25. Sorenson, E. M., Parkinson, D., Dahl, J. L. & Chiappinelli, V. A. (1989) *J. Comp. Neurol.* **281,** 641–657.
26. Hamburger, V. (1947) *A Manual of Experimental Embryology* (Univ. of Chicago Press, Chicago).
27. Hamilton, H. L. (1952) *Lillie's Development of the Chick: An Introduction to Embryology* (Holt, New York), 3rd Ed.
28. Falls, D. L., Rosen, K. M., Corfas, G., Lane, W. S. & Fischbach, G. D. (1993) *Cell* **72,** 801–815.