Detection of Prion Protein mRNA in Normal and Scrapie-infected Tissues and Cell Lines

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SUMMARY

Prion protein (PrP) forms the fibrils or prion rods isolated from scrapie-infected brain and has been proposed as the major component of the infectious agent of this slowly progressive spongiform encephalopathy. In previous Northern blot analyses PrP-specific mRNAs have been found in both normal and scrapie-infected brains but not in spleen, an organ which harbours large titres of infectivity. In the present study, mouse PrP DNA was used to probe for PrP mRNA in assorted tissues and cells. A re-examination of mouse and hamster spleens revealed that they contained low levels of PrP mRNA (approx. 0.8% of that in brain mRNA). No consistent differences were observed between normal and scrapie-infected tissues. Also positive for PrP mRNA under stringent hybridization conditions were mouse epithelial, neuroblastoma, erythroid, B-lymphocytic and embryo fibroblast tissue culture cell lines, a hamster ovary cell line, a rat glioma cell line, and human T lymphocytic and neuroblastoma cell lines. In contrast, no PrP mRNA was detected in two mouse myeloid cell lines and one T cell lymphoma. These results provide evidence that PrP is a protein common to numerous, but not all, cell types besides those of the brain.

Scrapie, transmissible mink encephalopathy, kuru and Creutzfeldt-Jakob disease form a closely related group of infectious degenerative neuropathies in animals and man (Hadlow, 1959; Klatzo et al., 1959; Prusiner & Hadlow, 1979). The causative agents of these diseases have yet to be definitively identified. Recent attempts to purify the scrapie agent from brain tissue have yielded fractions which contain as the major component a proteinase K-resistant form of prion protein (PrP) called PrP 27-30 (Bolton et al., 1982; Prusiner et al., 1982; McKinley et al., 1983) or scrapie-associated fibril protein (Diringer et al., 1983; Braig & Diringer, 1985). PrP 27-30 appears to be a modified form of normal endogenous PrP (Oesch et al., 1985; Chesebro et al., 1985; Cho, 1986; Rubenstein et al., 1986; Hope et al., 1986; Meyer et al., 1986). There is no evidence to suggest that the normal and scrapie-associated forms of PrP are encoded by separate genes. It has been proposed that either the scrapie-associated PrP or the fibrils are forms of the infectious scrapie agent (Merz et al., 1981, 1987; Bolton et al., 1982; Prusiner et al., 1983; Diringer et al., 1983; Manuelidis et al., 1985; Meyer et al., 1986; Rubenstein et al., 1986; Hope et al., 1986). No conclusive tests of these proposals have been reported however. It remains plausible that the scrapie agent is a minor and as yet undetected component of fibril-containing fractions of brain and that fibril formation is a pathological by-product of the scrapie disease (Rohwer, 1984; Braig & Diringer, 1985; Czub et al., 1986b).

If endogenous PrP is altered to create the scrapie agent, then PrP synthesis may establish the potential of a cell to replicate the scrapie agent. Thus it is relevant to determine whether, in an animal with scrapie, PrP follows a distribution similar to that of the scrapie agent. In scrapie-infected mice, the infectious agent is found primarily in the brain, spinal cord, spleen and lymph nodes (Eklund et al., 1967). Forms of the PrP protein have been detected in brain fractions on
polyacrylamide gels or Western blots (Bolton et al., 1982; Diringer et al., 1983; Cho, 1986; Rubenstein et al., 1986; Hope et al., 1986; Meyer et al., 1986; Kascsak et al., 1986) and in spinal cord and spleen by Western blot analyses (Rubenstein et al., 1986). PrP has also been found in mouse epithelial cells by metabolic labelling and immunoprecipitation (Caughey et al., 1988).

Northern blot analysis of preparations of poly(A) RNA is a sensitive method for screening tissues and cells for gene expression. Using cloned PrP cDNAs as hybridization probes in Northern blot analyses, PrP mRNA was detected in the brains of normal and scrapie-infected hamsters and mice (Oesch et al., 1985; Chesebro et al., 1985; Robakis et al., 1986) and in hearts and lungs of normal animals (Oesch et al., 1985; Robakis et al., 1986). No difference in PrP mRNA expression was observed in the brains of normal and scrapie-infected animals. Two studies failed to detect PrP mRNA in spleens of either normal or scrapie-infected mice or hamsters by Northern blot analyses (Chesebro et al., 1985; Robakis et al., 1986). In another study, Oesch et al. (1985) report the detection of PrP mRNA in spleen by a slot blot, a technique more prone to false positive artefacts than the Northern blot procedure. The issue of whether or not the spleen synthesizes PrP mRNA is of interest because of the spleen’s high titre of scrapie agent in infected animals (Eklund et al., 1967). A lack of PrP mRNA would indicate that the spleen does not synthesize PrP. This would suggest that either the PrP is not a necessary component of the scrapie infectious agent (Czub et al., 1986a) or the agent found in the spleen is deposited there after replication in another tissue(s) which can synthesize PrP.

In the present study, we examined spleen and a variety of other tissues and tissue culture cell lines for the expression of PrP mRNA. Poly(A) RNA preparations were probed with mouse PrP cDNA cloned from scrapie-infected mouse brain (Fig. 1). The PrP probe hybridized under stringent conditions to a single approx. 2·3 kb mRNA band of both scrapie-infected and normal brain with no quantitative or molecular weight difference observed between the two. In addition, extended autoradiography also revealed low levels of PrP mRNA in poly(A) RNA preparations from mouse and hamster spleen and liver which were not detected in our previous studies (Chesebro et al., 1985). In all cases similar levels of PrP mRNA were found in both normal and scrapie-infected tissues.

The distribution of PrP mRNA in tissue culture cell lines not containing infectious scrapie agent was also investigated. Single PrP mRNA bands were observed in poly(A) RNA preparations from mouse epithelial (C127), neuroblastoma (CCL147), erythroid (AA60), embryo fibroblast (B6-3T3) and B lymphocytic (1593) tissue culture cell lines, a hamster ovary cell line (CCL61), human T lymphocytic (A3.01), astrocytoma (HTB14) and neuroblastoma (HTB10) cell lines, and a rat glioma cell line (C6Bu3). The PrP mRNA from the human cells electrophoresed slightly more slowly than PrP mRNA from mouse, hamster or rat cells on the gels. This is consistent with previous reports that brain PrP mRNAs from mouse and hamster migrate as 2·1 to 2·5 kb molecules (Chesebro et al., 1985; Oesch et al., 1985), whereas the human form migrates at 3·0 kb (Robakis et al., 1986). The finding that mouse PrP cDNA hybridizes to hamster and human PrP mRNA under stringent conditions is consistent with the high degree of homology between the reported sequences of the mouse, hamster and human brain PrP cDNAs (Locht et al., 1986; Oesch et al., 1985; Robakis et al., 1986; Liao et al., 1986). The presence of PrP mRNA in these tissue culture cells suggests that PrP is expressed not only in the brain but also in a wide assortment of other mouse, hamster, human and rat cells. In contrast, no PrP mRNA was detected in either a differentiated (5402) or an undifferentiated (7320) mouse myeloid cell line or in a mouse T cell lymphoma line (MBL-2) (Fig. 2), indicating that PrP gene expression is not universal.

The detection of PrP mRNA in spleen prevents us from separating the presence of scrapie agent in spleen after infection from the ability of spleen cells to synthesize PrP. If endogenous PrP is the substrate for the formation of scrapie agent, then the different titres of scrapie agent achieved in the spleen and brain during the course of the disease might be the result of differing amounts of PrP gene expression in these tissues. A comparison of the relative levels of scrapie infectivity and PrP mRNA in the spleens and brains of mice and hamsters is shown in Table 1. Unfortunately, due to the inherent inaccuracy of the assay for scrapie agent, the ratio of scrapie titre in spleen and brain ranges across approximately two orders of magnitude making it
Fig. 1. Northern blot analyses of poly(A) RNA (6 μg/lane) from cell lines and tissues of normal and clinically affected scrapie mice (a), hamsters (b, lanes 1 to 7), humans (h, lanes 8 to 10) and rats (b, lane 11). Poly(A) RNA was isolated from tissue culture cells and tissues, electrophoresed in 6% formaldehyde-1% agarose gels, blotted onto Amersham Hybond-N sheets and baked as described (Chesebro et al., 1985) except for the substitution of Hybond sheets for nitrocellulose. The blots were prehybridized for 3 h at 60 to 63 °C in 3 x SSPE, 0.1% SDS, 1 x Denhardt’s solution and 0.05 mg/ml sheared and freshly boiled salmon sperm DNA and hybridized at 60 to 63 °C for 18 to 60 h in the same buffer containing labelled PrP cDNA insert (sp. act. 6.6 × 10^7 d.p.m./μg). After rinsing with 2 x SSPE and 0.1% SDS the blots were washed in the same buffer for 60 min at 68 °C and then in 0.1 x SSPE and 0.1% SDS for 30 min at 68 °C. The spleen, liver and ovary lanes were autoradiographed at -70 °C for 24 days (mouse) and 27 days (hamster) and all others for 3-5 days using a DuPont Lightning Plus intensifying screen. The 1.3 kb PrP insert was isolated from 8 μg of clone 7 (Locht et al., 1986) by EcoRI digestion followed by agarose gel electrophoresis and excision of the appropriate fragment. After purification on an Elutip ion exchange column (Schleicher and Schüll), the insert was labelled with 32P using a New England Nuclear nick translation kit, separated from unincorporated label by gel filtration (Sephadex G-50) chromatography, boiled 5 min and used at a concentration of 14 ng/ml in the hybridization buffer. (a) Lane 1, mouse epithelial cell line (C127; Lowy et al., 1978). Lane 2, mouse neuroblastoma cells, a clone (Neuro 2A) of DK neuroblastoma cells (Race et al., 1987) supplied by Dr D. Kingsbury (George Washington University Medical Center, Washington D.C., U.S.A.). Lane 3, erythroid line (AA60) derived from an (B10.A × A ) F1 mouse inoculated with Friend virus complex (Britt et al., 1984). Lane 4, B6-3T3 cells, a 3T3-like embryo fibroblast line derived from C57BL/6J mice, kindly provided by Dr R. Buller (these laboratories). Lane 5, B cell lymphoma line (1593) from a C57BL/10 × BALB.b F1 mouse inoculated with Friend murine leukaemia helper virus (F-MuLV; Chesebro et al., 1983). Lane 6, normal brain tissue. Lane 7, scrapie-infected brain tissue. Lane 8, normal spleen. Lane 9, scrapie-infected spleen. Lane 10, normal liver. Lane 11, scrapie-infected liver. (b) Lane 1, hamster ovary-derived cells (CCL61) supplied by the American Tissue Culture Collection (ATCC, Rockville, Md., U.S.A.). Lane 2, hamster normal brain. Lane 3, hamster scrapie-infected brain. Lane 4, hamster normal liver. Lane 5, hamster scrapie-infected liver. Lane 6, hamster normal spleen. Lane 7, hamster scrapie-infected spleen. Lane 8, human T lymphocyte cell line (A3.01; Folks et al., 1985) obtained from Dr T. Folks (NIH, Bethesda, Md., U.S.A.). Lane 9, human astrocytoma (HTB14) cells (ATCC). Lane 10, human neuroblastoma (HTB10) cells (ATCC). Lane 11, rat glioma-derived (C6Bu3) cells supplied by Dr M. Nirenberg (NIH).
Fig. 2. Northern blot of poly(A) RNA from mouse tissue culture lines. The RNA preparations (6 μg/lane) were blotted and probed with the 32P-labelled PrP insert (1.5 x 10^6 d.p.m./μg) (a) as described in Fig. 1. Neuroblastoma poly(A) RNA (lane 1) was the same as that analysed in Fig. 1 and was included as a PrP-positive control. By comparing the maximum autoradiographic exposure time to the minimum required to detect mouse brain and spleen PrP mRNA (not shown) it was estimated that the maximum level of PrP mRNA that would not be detected by this procedure was < 0.1% of the PrP mRNA in mouse brain. As a control to show that ample intact RNA from the PrP-negative cell lines was transferred to the filter, another cloned cDNA (23I) was used to reprobe the blot (b). The 23I cDNA clone was obtained from a hamster brain library and was uncharacterized except that it hybridized to filters blotted with mRNA from both hamster and mouse brain and spleen. Probe 23I (4.1 x 10^6 d.p.m./μg) gave a similar signal with both the PrP-positive neuroblastoma and the PrP-negative poly(A) RNAs showing that RNA from all four samples was bound to the filter. There was no indication, such as band tailing, that degradation of the poly(A) RNAs had occurred. The autoradiographic exposures (−70 °C) were for 1 week (a) and 6 h (b) with an intensifying screen. A myeloid leukaemia line (7320) was cultured from a (B10 × IRW)F1 mouse inoculated with F-MuLV (lane 3) and appears well differentiated with granules and multilobed nuclei (Chesebro et al., 1983). The derivation of the 5402 myeloid leukaemia cell line (lane 2) was similar to that of the 7320 line, however the cells appear undifferentiated (unpublished data). The MBL-2 T cell line (lane 4) was derived from Moloney virus-infected C57BL/6 mice (Glynn et al., 1968).

Table 1. Comparison of scrapie titres and PrP mRNA in mouse and hamster spleen and brain

<table>
<thead>
<tr>
<th>Animal</th>
<th>Infectivity titre*</th>
<th>Ratio of infectivity</th>
<th>Ratio of PrP mRNA†</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Brain</td>
<td>(spleen/brain)</td>
</tr>
<tr>
<td>Mouse</td>
<td>10^{8-2}-10^{10-2}</td>
<td>10^{6-6}-10^{7-4}</td>
<td>0.6-40%</td>
</tr>
<tr>
<td>Hamster</td>
<td>10^{5-5}-10^{6-0}</td>
<td>10^{7-2}-10^{8-9}</td>
<td>0.04-6%</td>
</tr>
</tbody>
</table>

* Mouse infectivity titres were obtained from the results of Eklund et al. (1967) and are expressed as LD_{50} intracerebral (i.c.) units per 0.03 g tissue. Hamster infectivity titres were obtained from the results of Kimberlin & Walker (1977) and represent LD_{50} i.c. units per 0.05 g tissue.

† To estimate the relative proportions of PrP mRNA present in spleen and brain it was assumed that the amount of PrP mRNA present in a band on the Northern blot was proportional to the amount of radioactive probe bound to the band. The latter was calculated using the ratio of the lengths of autoradiographic exposures required to detect PrP mRNA bands of similar weak densities combined with the exponential radioactive decay function. Test exposures indicated that the relative proportions estimated by this method are accurate to within approximately threefold.
impossible to compare accurately scrapie titre and PrP mRNA (Table 1). Nevertheless, in both mice and hamsters, the ratios of PrP mRNA in spleen compared to brain fall within the broad range of ratios of scrapie titre in these organs. Thus, although these data are not inconsistent with a correlation between PrP mRNA levels and scrapie titres achieved in brain and spleen, they do not allow us to conclude that a proportional relationship would remain if more accurate assays of scrapie agent and PrP mRNA were available. Regardless of the situation in spleen, the mouse lung is a tissue which expresses levels of PrP mRNA near that of the brain (Robakis et al., 1986) but has a scrapie titre about 10000-fold lower than in brain in the infected mouse (Eklund et al., 1967). Thus there does not appear to be a quantitative correlation between PrP mRNA synthesis and the ability of tissues to harbour scrapie agent. However, other factors besides PrP mRNA levels may govern the amount of PrP in various tissues. Scrapie-infected brain, for instance, appears to have at least ten times the normal level of PrP (Meyer et al., 1986) despite having similar amounts of PrP mRNA (Chesebro et al., 1985; Oesch et al., 1985). It remains to be determined whether PrP synthesis constitutes a minimum requirement for scrapie agent replication. Whether or not PrP is an integral component of the scrapie agent, it may nevertheless play a role in the pathology of the disease. However, despite the broad dissemination of PrP mRNA, the pathology of scrapie appears to be restricted almost exclusively to the central nervous system (CNS). If PrP is involved in such tissue-specific pathology, this might be explained by unusual qualitative or quantitative aspects of PrP structure or function in CNS tissues.

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REFERENCES


Short communication


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