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Cloning of a Gene Whose Expression Is Increased in Scrapie and in Senile Plaques in Human Brain

Abstract. *A complementary DNA library was constructed from messenger RNA's extracted from the brains of mice infected with the scrapie agent. The library was differentially screened with the objectives of finding clones that might be used as markers of infection and finding clones of genes whose increased expression might be correlated with the pathological changes common to scrapie and Alzheimer's disease. A gene was identified whose expression is increased in scrapie. The complementary DNA corresponding to this gene hybridized preferentially and focally to cells in the brains of scrapie-infected animals. The cloned DNA also hybridized to the neuritic plaques found with increased frequency in brains of patients with Alzheimer's disease.*

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Scrapie is a naturally occurring slow infection of sheep caused by a class of unconventional infectious agents that have unusual properties. These properties, and the co-purification of a 27- to 30-kilodalton (kD) protein and infectivity, have led to speculation that the enigmatic informational molecule in scrapie is not a nucleic acid (*1–5*). It is now clear, however, that the 27- to 30-kD "prion" protein is the product of a normal cellular gene that is expressed to the same extent in infected and uninfected animals (*6, 7*). The distinctive pathological changes of scrapie are neuronal vacuolation and the formation of abnormal fibrils (*8–12*), but in some animal models of scrapie and cognate diseases (kuru, Creutzfeldt-Jakob disease) there are other pathological alterations that resemble those seen in senile dementia of the Alzheimer type (*13–15*).

We reasoned that whatever the nature of the infectious moiety in scrapie might be, (i) it would use existing cellular biochemical pathways for replication; and (ii) it might induce expression of genes involved in pathological changes common to Alzheimer's disease and scrapie. We therefore looked for messenger RNA's (mRNA's) whose expression was increased in scrapie, and perhaps in Alzheimer's disease, by preparing a complementary DNA (cDNA) library from mRNA's of scrapie-infected mice and then screening the library differentially. Total RNA was isolated by homogenizing (in guanidium isothiocyanate) brains from nine RML Swiss mice infected 5 months earlier with a 10^{-2} dilution of a 10 percent homogenate of brain obtained from mice infected with scrapie (Chandler-adapted strain) (*16*). At the time the animals were killed, they were symptomatic and had titers 10^8 times the median lethal dose per gram of brain. Polyadenylated [poly(A)$^+$] RNA was selected by two cycles of chromatography on oligo(dT)-cellulose (*17*). The cDNA was prepared, as described (*18*), by reverse transcription followed by second strand synthesis with DNA polymerase I. The double-stranded DNA product was digested with S1 nuclease, treated with Eco RI methylase, and ligated to Eco RI dodecamer linkers. After cleavage with Eco RI, DNA larger than 0.6 kilobase was isolated by gel filtration (Sephacryl S1000). The scrapie cDNA (20 ng) was ligated with 1 μg of λgt10 (*19*) cut with Eco RI. The phage was packaged and plated on a lawn of BNN104 (C600r−M$^+$hflA150). Phage λgt10 is lysogenic in hfl (high-frequency lysogeny) strains, and insertion of DNA into the single Eco RI site of the cI λ repressor gene yields recombinants that form clear plaques on BNN104 (*19*).

About 7 percent of the packaged phage were recombinants, and more than 99 percent of 365,000 independent recombinants contained inserts. The library was amplified in BNN104, and 5000 recombinant phage were picked and transferred to fifteen 15-cm plates for screening. Replicas were made from each plate on nylon filters (Pall Biodyne) in accordance with the manufacturer's protocols. The replicate filters were acetylated (*20*) and prehybridized. For differential screening, one set of filters was hybridized to ^{32}P-labeled cDNA that was reverse-transcribed from poly(A)$^+$ RNA of scrapie-infected brain, with oligo(dT) used as primer. The other set of filters

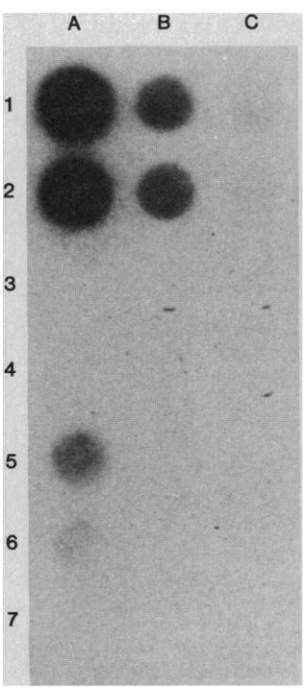

Fig. 1. Dot-blot hybridizations of RNA with Scr-1 DNA probe. Lanes A, B, and C contain, respectively, 1, 0.2, and 0.05 μg of poly(A)$^+$ RNA, or five times as much total RNA. RNA was transferred to nitrocellulose and hybridized with ^{32}P-labeled Scr-1 DNA as described (*23*). (1) Poly(A)$^+$ RNA from brains of scrapie-infected mice; (2) total RNA from infected mice; (3) wheat germ RNA; (4) calf thymus RNA; (5) poly(A)$^+$ RNA from brains of uninfected mice; and (6 and 7) total RNA from brains of uninfected animals from two sources.

was hybridized to ^{32}P-labeled cDNA synthesized from poly(A)$^+$ RNA obtained from uninfected brains of age- and sex-matched mice.

In the initial screening of 5000 clones, one clone consistently hybridized preferentially to cDNA from scrapie-infected brains; this low frequency of scrapie-specific clones suggests that relatively small changes in gene expression accompany infection. DNA from this recombinant phage, Scr-1, was isolated, cleaved with Eco RI, and subjected to electrophoresis in a 1 percent agarose gel. The 1-kb insert was concentrated by electrophoresis into DEAE paper. After elution and chromatography over DE52, the DNA was labeled by nick translation with ^{32}P- or ^{125}I-deoxycytosine triphosphate (specific activities, 10^9 dpm/μg and 3×10^8 to 8×10^8 dpm/μg, respectively).

In RNA dot-blot experiments, the ^{32}P-labeled Scr-1 insert hybridized to total and poly(A)$^+$ RNA from both scrapie-infected and uninfected mice, but hybridization to infected brain was about 20 times that to uninfected brain (Fig. 1).

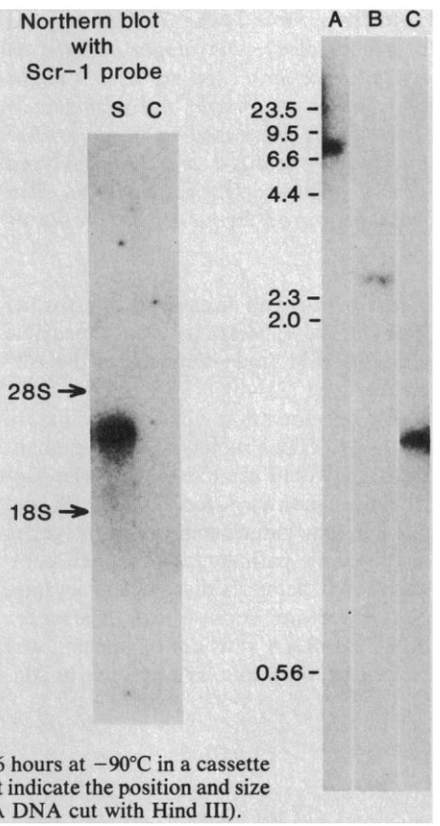

Fig. 2 (left). Northern blot of mouse RNA's with ^{32}P-labeled Scr-1 cDNA. Glyoxylated total RNA (6 μg) from scrapie-infected (S) or uninfected brain (C) was subjected to electrophoresis (3.5 V/cm) in a 1 percent agarose gel. After transfer to nitrocellulose (23), Scr-1 RNA was identified by hybridization to ^{32}P-labeled Scr-1 cDNA as described in the legend to Fig. 3. The size of Scr-1 RNA was estimated from markers (ribosomal RNA) electrophoresed in parallel. The position of the 18*S* and 28*S* markers is indicated in the margin of the figure. Fig. 3 (right). Southern blot of mouse genomic DNA with ^{32}P-labeled Scr-1 DNA. Mouse DNA (6 μg) from uninfected mice was cleaved according to the manufacturer's instructions with Bam HI (lane A) or Taq I (lane B). The products were separated electrophoretically (0.6 V/cm, 16 hours, 0.7 percent agarose), transferred to Pall Biodyne filters, and hybridized with ^{32}P-labeled Scr-1 in 50 percent formamide, 5× standard saline citrate (SSC), 5× Denhardt's solution, salmon sperm DNA (250 μg/ml), and 50 m*M* sodium phosphate buffer (*p*H 6.8) at 43°C for 16 hours. ^{32}P-labeled Scr-1 (2×10^7 cpm/ml) was used as the probe. Filters were washed according to the manufacturer's instructions, except that the final wash was in 0.1× SSC and 0.1 percent sodium dodecyl sulfate at 65°C. Lane C is an internal calibration control of one genome equivalent of Scr-1 DNA. The blots were exposed to x-ray film for 6 hours at −90°C in a cassette with amplifying screens. Bars and numbers at left indicate the position and size of molecular weight markers in kilobase pairs (λ DNA cut with Hind III).

The Scr-1 probe hybridized to a 3.7-kb species of RNA from scrapie-infected mouse brain (Fig. 2). In Southern blots of genomic DNA from the livers of uninfected mice (Fig. 3, lanes A and B), the Scr-1 probe hybridized to a single Bam HI fragment, and the level of hybridization was identical to that of a probe for another single-copy gene (V_{K167}, a kappa variable-region probe). That the Scr-1 gene is present at one copy per haploid genome is further supported by the comparable levels of hybridization to the Bam HI fragment (Fig. 3, lane A), Taq I fragment (lane B), and an internal calibration control of one genome equivalent of Scr-1 (lane C).

Scrapie-infected tissues contain a 27- to 30-kD prion protein that is associated with infectivity (*1*, *12*). To assess the relation of the Scr-1 cDNA to the mRNA coding for this polypeptide, we used the known amino terminal sequence (*13*) to synthesize a mixed oligonucleotide probe for the prion protein. The prion probe identifies an mRNA of 2.1 to 2.4 kb present in equal amounts in infected and uninfected animals (*6*, *7*). The different size of Scr-1 mRNA and its increased expression in infected animals are evidence that this mRNA is unrelated to prion mRNA. Moreover, Scr-1 cDNA does not hybridize to the prion DNA clone isolated from a library of cDNA's obtained from scrapie-infected mouse brain.

To evaluate the cellular distribution of Scr-1 RNA, we hybridized brain sections in situ (*20*) with the Scr-1 probe labeled with ^{125}I. As expected, the Scr-1 probe hybridized to a greater extent to scrapie-infected tissues than to uninfected tissues, and in some areas there was a striking focal pattern to the increased hybridization (Fig. 4, a and b). The hy-

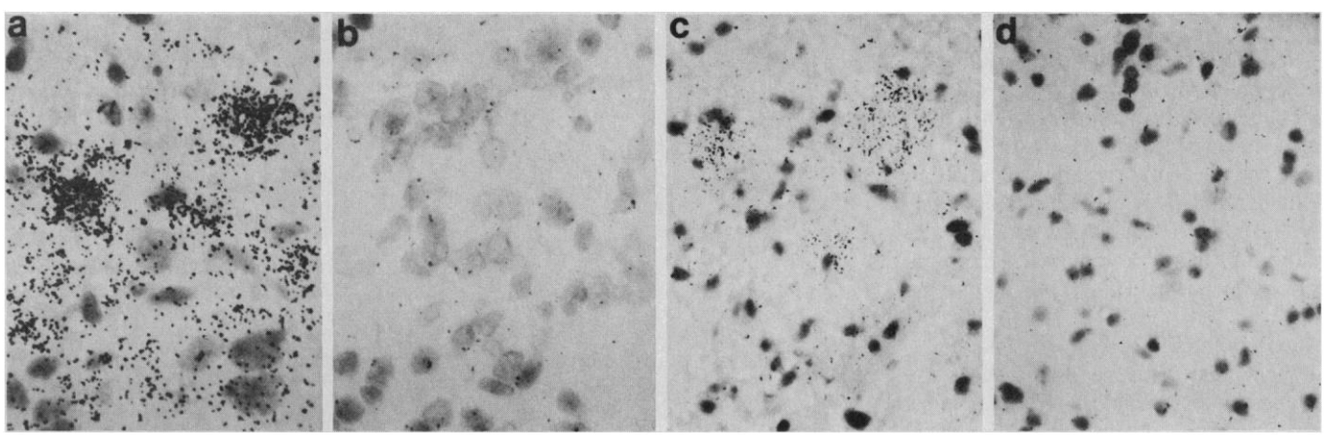

Fig. 4. In situ hybridization with ^{125}I-labeled Scr-1 DNA. Frozen sections (10 μm) were cut from brain and picked up on treated glass slides. After pretreatment to increase diffusion of the probe, the sections were hybridized in situ as described (*21*). After being washed, the slides were coated with NTB-2 emulsion, exposed for 2 days at 4°C, developed, and stained with hematoxylin and eosin. All magnifications were originally ×625. (a) Scrapie-infected hamster. (b) Control hamster. (c) Senile dementia of the Alzheimer type. (d) Schizophrenia.

bridization was species independent; Scr-1 cDNA from the mouse library was hybridized to sections of brain from an infected hamster (Fig. 4a). Most of the labeled cells appeared to be neurons, as judged by their morphology and anatomic distribution. The Scr-1 probe also hybridized preferentially to tissue sections of brain from three of three patients with neuropathologically confirmed Alzheimer's disease. In Alzheimer's disease, hybridization again was focal—in the cerebral cortex (Fig. 4c); but, in contrast to scrapie, the Scr-1 probe was localized in cellular processes rather than in cell bodies. By staining sections with thioflavine S after in situ hybridization to locate neuritic plaques and tangles, we found that the foci in cerebral cortex correspond to neuritic plaques that occur in the central nervous system of aging humans and with increased frequency in those with Alzheimer's disease [reviewed in (*20*)]. There was no increase in annealing of the Scr-1 probe to the neurofibrillary tangles of Alzheimer's disease or to tissues lacking senile plaques from three control patients (Fig. 4d). The Scr-1 probe also hybridized to relatively rare senile plaques in the brain tissues of three patients with multi-infarct dementia, Pick's dementia, and multiple sclerosis. The annealing of Scr-1 to tissues was specific, since pretreatment with ribonuclease reduced hybridization by 75 to 90 percent; and heterologous probes, as controls for sequence specificity (to measles virus, herpes simplex virus, and cytomegalovirus) hybridized at levels comparable to those of uninfected hamster and schizophrenia controls.

These findings provide evidence that the scrapie agent induces increased expression of at least one mRNA that has a number of intriguing properties as a possible marker for infection. This mRNA is expressed in brain neurons of two rodent species and is increased in some brain areas with the focal pattern of gene expression observed in other slow and persistent infections (*21*). Reduced hybridization after ribonuclease treatment was not complete. This partial resistance to ribonucleases is unusual; it has not been observed in conventional virus infections (*21*) but is a property of interferon mRNA in situ (*22*) and may prove to be of interest in view of the nuclease resistance of scrapie infectivity (*1*). These observations suggest further that increased expression of some cellular RNA's may be common to infection by unconventional agents and to degenerative changes in the aging human brain best exemplified by Alzheimer's disease. The experimental strategy described in this report will be useful in obtaining markers for degenerative human diseases and in selecting probes to investigate the pathogenesis of these conditions.

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Plasticity of Hippocampal Circuitry in Alzheimer's Disease

Abstract. *Two markers of neuronal plasticity were used to compare the response of the human central nervous system to neuronal loss resulting from Alzheimer's disease with the response of rats to a similar neuronal loss induced by lesions. In rats that had received lesions of the entorhinal cortex, axon sprouting of commissural and associational fibers into the denervated molecular layer of the dentate gyrus was paralleled by a spread in the distribution of tritiated kainic acid–binding sites. A similar expansion of kainic acid receptor distribution was observed in hippocampal samples obtained postmortem from patients with Alzheimer's disease. An enhancement of acetylcholinesterase activity in the dentate gyrus molecular layer, indicative of septal afferent sprouting, was also observed in those patients with a minimal loss of cholinergic neurons. These results are evidence that the central nervous system is capable of a plastic response in Alzheimer's disease. Adaptive growth responses occur along with the degenerative events.*

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Several neurological disorders, including Parkinson's, Huntington's, and Alzheimer's diseases, are associated with neuronal loss in specific brain regions (*1*). From animal studies it is well known that the central nervous system (CNS) can modify its neuronal circuitry in response to injury-induced denervation. When one set of axons is lost, the remaining afferents can sprout and replace the lost connections to maintain synaptic density (*2*). It is uncertain, however, whether plastic responses occur in the human brain after neuronal loss induced by aging, injury, or disease.

Recently, severe loss of cells was observed in the entorhinal cortex and subiculum of patients with Alzheimer's disease (AD); this cell loss was suggested to underlie some of the memory deficits in the disease (*3*). The perforant path, arising from the entorhinal cortex, is the major cortical input to the hippocampus, terminating on the granule cell dendrites in the outer two-thirds of the dentate gyrus molecular layer and on the distal portions of pyramidal cell dendrites of the hippocampus and subiculum (*4*). In response to the loss of entorhinal input in rodents, the dentate molecular layer un-