Supporting Information

Edgeworth *et al.* 10.1073/pnas.0813342106

SI Materials and Methods

Culturing and Storage of N2aPK1 Cells. Vials containing 3 million N2aPK1 cells in Opti-MEM 10% FCS and 1% penicillin/streptomycin (OFCS), 6% DMSO were stored in liquid nitrogen. Cells were thawed and expanded for no more than 4 passages before use in the scrapie cell endpoint assay (SCEPA). Prolonged subculturing may lead to reduced susceptibility to RML prion infection.

Preparation of Homogenates. Noninfected mouse brain homogenates were prepared from *Prnp*$^{0/0}$ mice [42] (backcrossed onto an FVB-N background) as 20% wt/vol in PBS by passing through 21- to 23.5-gauge needles successively. Homogenate was stored as 20-μl aliquots at −80 °C. RML prion-infected brain homogenates were prepared from terminally sick wild-type CD-1 mice.

Preparation of Steel Wires. Steelex monofilament wires, USP 4/0 (B. Braun) were cut to 2.5 cm lengths. Batches of 100 wires were washed in 20 ml 2% vol/vol Triton X-100 in deionized water for 2 h and then for 5 × 15 min in 50 ml deionized water. Wires were sterilized in 70% vol/vol ethanol in deionized water for 10 min and air-dried in a class 2 biological safety cabinet.

Coating of Wires and Exposure to Cells (Wire Assay). Wires were incubated with 5 ml RML prion-infected brain homogenate serially diluted into 10^{-4} *Prnp*$^{0/0}$ brain homogenate in OFCS for 3 h at room temperature and then washed in PBS containing 1% penicillin/streptomycin for 5 × 15 min on a rotary wheel. They were air-dried, placed at maximum of 20 wires per well of a 6-well tissue culture cluster plate (Corning), and covered with a suspension of 3×10^5 N2aPK1 cells in 5 ml OFCS. After 3 d at 37 °C and 5% CO_2, the wires with adherent cells were transferred into fresh wells containing 1 ml OFCS. The cells were dislodged from the wires by vigorous pipetting, collected in a separate tube, and counted. One thousand cells suspended in 300 μl were seeded into wells of 96-well plates (Corning) and after 3 d split at a ratio of 1:3. After reaching confluence the cells were twice split 1:3 and 3 times 1:8. Once cells were confluent following the second and third 1:8 split, 25,000 cells were seeded onto Elispot plates (MultiScreen Immobilon-P 96-well filtration plates [Millipore] activated with 70% ethanol) and washed twice with 160 μl PBS by suction. Plates were dried at 50 °C for 2 h and, if necessary, stored at 4 °C.

Elispot Assay. The cells dried onto the membranes of Elispot plates were treated with proteinase K (PK; 1.32×10^{-4} units; Roche) in 60 μl/well of lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.5% wt/vol sodium deoxycholate, 0.5% vol/vol Triton X-100) and incubated at 37 °C for 30 min. The lysis solution was removed by suction, and the plates were washed twice with 160 μl PBS/well, then incubated with 2 mM PMSF for 10 min. Plates were dried by suction and incubated with 120 μl of 3 M guanidinium thiocyanate, 10 mM Tris-HCl (pH 8.0) per well for 10 min at room temperature. Plates were washed 6 times with 160 μl PBS/well and then incubated with 120 μl/well of Superblock (Pierce) for 1 h. The plates were dried by suction and 60 μl of the anti-PrP antibody ICSM18 (0.6 μg/ml in 1% wt/vol nonfat milk powder in TBST [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% vol/vol Tween 20] per well was added and incubated for 1 h at 20 °C. Plates were washed 6 times with 160 μl TBST/well, and 60 μl of alkaline phosphatase-conjugated anti-IgG1 (Southern Biotechnology Associates; 0.4 μg/ml in 1% nonfat milk/TBST) per well was added. After 1 h at 20 °C the plates were washed 6 times with 160 μl TBST/well, the underdrains were removed, and the plates were air-dried. Alkaline phosphatase-conjugated substrate (Bio-Rad) was prepared as detailed by manufacturer, and 58 μl/well was added for 20 min at 20 °C. After washing twice with 160 μl deionized water per well, the plates were air-dried and stored at −20 °C. PrP^{Sc}-containing cells were counted using a Zeiss KS Elispot system (Stemi 2000-C stereo microscope equipped with a Hitachi HV-C20A color camera and a KL 1500 CD scanner and WellScan software from Imaging Associates). Settings were optimized for PrP^{Sc}-containing cells by adjusting parameters relative to negative controls to give the highest signal-to-noise ratio. This was achieved by using the training feature of the software following the manufacturer's guidelines. The upper detection limit of the system is ≈1,000 spots.

Results

Supporting information (SI) Table S1 shows that under the conditions of our assay, wires are about 6× more efficient in binding and transferring prion infectivity to cells than disks.

Table S2 shows that increasing the number of wire-exposed cells per well of the SCEPA increases the m value (i.e., the average number of infectious units delivered to a well). Thus, in the case of wires coated with 10^{-4} RML, the m values increased from 0.12 to 0.97 and >3.1 as the cell number was increased from 100, to 500, to 1,000, respectively. The number of infectious units per 1,000 cells is about the same, within the limits of error—namely, 1.2, 1.94, and 3.1.

Table S2 also shows that by increasing the number of cells per well to 4,000, it is possible to detect infectivity at a 10^{-10} dilution of RML-infected brain.

Table S1. Comparing steel wires and disks for their ability to bind and transfer RML infectivity

Surface	Number cells/well	Proportion positive wells	m	% input infected
Wire + 1% w/v RML	10	47/48	3.87	38.7
Disks + 1% w/v RML	11	22/48	0.61	5.6
Wire + 0.01% w/v RML	500	18/24	1.39	0.28
Disks + 0.01% w/v RML	500	5/24	0.23	0.05

Steel disks (Goodfellow, 10 mm diameter x 0.5 mm) and monofilament wires (USP 4/0 cut to 2.5 cm length) were coated with RML -infected brain homogenate for 2 h, then washed thoroughly in PBS. N2aPK1 cells were grown in direct contact with metal surface for 3 d, harvested, seeded into 96-well plates and processed as detailed above. The m value refers to the mean number of infectious particles in a sample, calculated $P_{(0)} = e^{-m}$, as described in *Materials and Methods.* "% input infected" refers to the percentage of wire-adherent cells that gave rise to a PrP^{Sc}-positive well.

Table S2. Wire infectivity assay performed with varying number of cells per well at the SCEPA stage

Dilution RML	Cells seeded per well	$TCIU_w$ per well, m	$TCIU_w$ normalized to 1,000 cells (mean ± SEM)
10^{-2}	1	1.37	1,390
10^{-2}	2	0.46	235
10^{-2}	5	0.87	174
10^{-2}	10	1.55	155
10^{-3}	20	0.23	11.5
10^{-4}	100	0.12	1.2
10^{-4}	500	0.97	1.94
10^{-4}	1,000	>3.1	>3.1
10^{-5}	1,000	0.57	0.57 (± 0.05)
10^{-6}	1,000	0.28	0.28 (± 0.02)
10^{-7}	1,000	0.15	0.15 (± 0.02)
10^{-8}	2,000	0.24	0.12 (± 0.015)
10^{-9}	2,000	0.08	0.04 (± 0.01)
10^{-10}	4,000	0.116	0.0029 (± 0.002)

Wires were incubated in RML homogenate at the concentrations indicated, washed, and exposed to N2aPK1 cells. Adherent cells were collected and the SCEPA performed. The background of the assay was determined in a large-scale experiment by exposing PK1 cells to wires coated with uninfected homogenates and assaying them by SCEPA. Four of 960 wells, or 0.42%, were positive by our criteria; thus, a (negligible) background of 0.0042 × total wells (0.1 wells per 24 wells or 0.2 wells per 48 wells) was subtracted from the positive wells and m values ($TCIU_w$ per well) were calculated as explained in *Methods*. $TCIU_w$ well was divided by the number of cells seeded per well to give normalized $TCIU_w$ per well. Some of these values are repeated from Table 1.