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Spontaneous generation of distinct prion variants with recombinant prion protein from a baculovirus-insect cell expression system

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ABSTRACT

Prion diseases are transmissible and progressive neurodegenerative disorders characterized by abnormal prion protein (PrP^{Sc}) accumulation in the central nervous system. Generation of synthetic PrP^{Sc} in a cell-free conversion system and examination of its transmissibility to animals would facilitate testing of the protein-only hypothesis and the understanding of the molecular basis of sporadic prion diseases.

In this study, we used recombinant prion protein from a baculovirus-insect cell expression system (Bac-rPrP) and insect cell-derived cofactors to determine whether Bac-rPrP^{Sc} is spontaneously produced in intermittent ultrasonic reactions. No spontaneous generation of Bac-rPrP^{Sc} was observed at 37 °C, but when the reaction temperature was increased to 45 °C, Bac-rPrP^{Sc} was generated in all trials. Some Bac-rPrP^{Sc} variants were transmissible to mice, but when the reaction was repeated for 40 rounds, the transmissibility was lost. Notably, a variety of Bac-rPrP^{Sc} variants, including non-transmissible ones, differing in resistance to proteinase K and cofactor dependence during amplification, was generated under the same experimental conditions, including the same sonication settings and cofactors. However, their characteristics also disappeared after 40 reaction rounds and the variety converged onto a single variant. These results indicate that various Bac-rPrP^{Sc} variants with different transmissibility to mice and structural properties are generated, which compete with each other and gradually converge onto a variant with a slightly faster amplification rate.

1. Introduction

Transmissible spongiform encephalopathies, or prion diseases, are transmissible and fatal neurodegenerative disorders that include Creutzfeldt-Jakob disease in humans and scrapie, bovine spongiform encephalopathy (BSE), and chronic wasting disease in animals [1]. In vitro conversion reaction systems have been developed to understand the molecular process of conversion of normal cellular form of PrP (PrP^{C}) into PrP^{Sc}. Subsequently, in vitro conversion reaction systems have developed into two types of systems. One type of system is called protein misfolding cyclic amplification (PMCA) [2], and ultrasound is used to accelerate the reaction. The other system is called real time quaking-induced conversion (RT-QuIC), in which the reaction is accelerated by agitation [3].

In such a cell-free conversion reaction system, synthetic PrP^{Sc} can be spontaneously generated without seeding when the reaction is repeated. The first propagation of the generated synthetic PrP^{Sc} to animals has been shown to vary. The differences in the biochemical and structural properties of in vivo-derived PrP^{Sc} and synthetic PrP^{Sc} generated in unseeded reactions, as well as the molecular mechanisms that determine their transmissibility in animals, have not yet been fully elucidated.

In this study, we used recombinant PrP (rPrP) derived from a

Abbreviations: Bac-rPrP, baculovirus-derived recombinant prion protein; BH, brain homogenate; BSE, Bovine spongiform encephalopathy; cPMCA, conventional protein misfolding cyclic amplification; Ec-rPrP, recombinant prion protein from *Escherichia coli*; GPI, glycosylphosphatidylinositol; iPMCA, insect cell protein misfolding cyclic amplification; PA, polyadenylic acid; PE, phosphatidylethanolamine; PK, Proteinase K; PMCA, protein misfolding cyclic amplification; PrP^{C}, normal cellular form of prion protein; PrPres, Proteinase K-resistant prion protein; PrP^{Sc}, abnormal prion protein; rPrP, recombinant prion protein; RT-QuIC, real time quaking-induced conversion; WB, Western blotting.

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baculovirus-insect cell expression system (Bac-rPrP) as a substrate to investigate the molecular basis of the spontaneous generation of PrP^{Sc}. The primary advantage is that, unlike rPrP from *Escherichia coli* (Ec-rPrP), the Bac-rPrP has N-glycosylation and glycosylphosphatidylinositol (GPI)-anchored modifications. An additional advantage is that it is easier to prepare than brain-derived PrP^{C}. Furthermore, Bac-rPrP can be propagated while retaining its strain-specific nature in prion-seeded reactions [4]. Here, we investigated whether Bac-rPrP^{Sc} is spontaneously generated in intermittent sonication reactions (insect cell PMCA, iPMCA) in the presence of insect cell-derived cofactors and whether the spontaneous Bac-rPrP^{Sc} (spBac-rPrP^{Sc}) is transmissible to wild-type mice.

2. Materials and methods

2.1. Animals

Experiments were performed using ICR and C57BL/6J mice. Female mice were used for all experiments. Experiments were approved by the Committee for Animal Experiments of the National Institute of Animal Health (approval ID: 11-008, 13-005) and by the Animal Care and Use Committee of the University of Miyazaki (approval ID: 2019-010) and were performed in accordance with the Guideline for Animal Experiments at the Ministry of Agriculture, Forestry, and Fisheries of Japan and the University of Miyazaki.

2.2. Insect cell PMCA (iPMCA)

iPMCA was carried out using an automatic cross-ultrasonic protein activating apparatus (ELESTEIN 070-GOT, Elekon Science Corp., Chiba, Japan), as reported previously [5]. Amplification was performed via 32 cycles of sonication (pulse oscillation for 3 s repeated five times at intervals of 0.1 s), followed by incubation at 37 °C or 45 °C for 30 min with gentle agitation. The reaction mixture was prepared by combining approximately 250 ng of immobilized-metal affinity chromatography-purified mouse baculovirus-derived recombinant PrP (Bac-rPrP) and 10 μL of Proteinase K (PK) and heat-treated HighFive cell (Life technologies, Carlsbad, CA, USA) lysate with 85 μL of phosphate buffered saline (PBS), 0.25% (v/v) Triton X-100, 4 mM EDTA, and 5 μg synthetic polyA (Sigma, St. Louis, MO, USA). A 1-mm-diameter zirconia bead (ZB-10, TOMY, Tokyo, Japan) was placed in each tube. The products obtained after the first round of amplification were diluted 1:10 with a new iPMCA reaction mixture, and a second round of amplification was performed. This process was repeated when necessary.

2.3. Western blotting

The reaction products were digested with 50 μg/mL PK at 37 °C for 1 h. An equal volume of 2 × SDS sample buffer was added to the samples, which were then boiled for 5 min. The samples were separated by SDS-polyacrylamide gel electrophoresis using 12% Tris-glycine gels or NuPAGE 12% Bis-Tris gels (Thermo Fisher Scientific) and transferred onto polyvinylidene difluoride membranes. An anti-PrP T2-horseradish peroxidase (HRP) antibody [6] was used to detect PrP unless otherwise noted. The blotted membrane was developed with SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL, USA), and chemiluminescence signals were detected using Chemiimager (Alpha InnoTec, San Leandro, CA, USA).

2.4. Bioassays

The products of each spBac-PrP^{Sc} amplified in rounds 3, 10, 20, and 40 were diluted 1:10 with PBS, and 20 μL of each diluted sample was injected intracerebrally into 3-week-old female C57BL/6J mice under sevoflurane anesthesia. Animals that had any neurological symptoms were observed and sacrificed, and their brains were removed. The right hemisphere of the brain was fixed in formalin for histopathological analysis, and the left hemisphere was stored at −80 °C for biochemical analysis.

2.5. Histopathological studies

The right brain hemisphere was fixed in 10% buffered formalin solution. Coronal slices of the brain were cut, immersed in 98% formic acid to reduce infectivity, and then embedded in paraffin wax. Sections with a thickness of 4 μm were cut and stained with hematoxylin and eosin or analyzed by immunohistochemistry. For the neuropathological analysis, the lesion profile was determined from the hematoxylin and eosin-stained sections by scoring the vacuolar changes in nine standard gray matter areas, as described previously [4]. For the immunohistochemistry analysis, PrP^{Sc} was detected in brain sections by the hydrated autoclaving method using the anti-PrP monoclonal antibody 31C6 [7]. Immunoreactions were developed using an anti-mouse universal immuno-peroxidase polymer (Nichirei Histofine Simple Stain MAX-PO (M), Nichirei, Tokyo, Japan) as the secondary antibody and 3-3' diaminobenzidine tetrachloride as the chromogen.

3. Results

3.1. Spontaneous generation of Bac-rPrP^{Sc} by increasing the reaction temperature to 45 °C

We first examined whether Bac-rPrP could be converted into an abnormal form with proteinase K (PK) resistance (Bac-rPrP^{Sc}) in the presence of insect cell-derived cofactors by intermittent sonication without seeding with brain-derived PrP^{Sc}. Unexpectedly, formation of spBac-rPrP^{Sc} was not observed after repeating the reaction eight to 30 times at 37 °C (Fig. 1A). Therefore, we determined whether increasing the reaction temperature to 45 °C increased the probability of spBac-rPrP^{Sc} formation. The results showed that Bac-rPrP^{Sc} was generated in all cases at 45 °C after two to 15 rounds of the reaction (Fig. 1A and B). In comparison, we performed up to 20 rounds of unseeded conventional PMCA (cPMCA) at 37 °C or 45 °C with mouse brain homogenate (BH), but no PrP^{Sc} was spontaneously generated (Fig. 1A).

3.2. Transmissibility of the five spBac-rPrP^{Sc} variants to wild-type mice

Next, we examined whether the generated spBac-rPrP^{Sc} was transmissible to wild-type (C57BL/6J) mice. The five spBac-rPrP^{Sc} variants used to inoculate mice were named B1, B2, A1, C1, and C3. Although none of the mice inoculated with B2, A1, or C3 developed neurological symptoms or showed accumulation of PrP^{Sc} in the brain, some mice inoculated with B1 after reaction round three (B1/R3), C1/R3, C1/R10, or C1/R20 were symptomatic, showed accumulation of PrP^{Sc}, or both (Table 1). However, none of the mice developed symptoms or exhibited PrP^{Sc} accumulation after being inoculated with variants subjected to 40 reaction rounds, even among those inoculated with the C1. Moreover, semi-quantitative RT-QuIC analysis revealed that PrP^{Sc}-positive mice inoculated with spBac-rPrP^{Sc} (B1/R3 or C1/R3) also had a seeding activity of 5–6 times the log of the 50% seeding dose (SD_{50})/mg of brain tissue (Table S1). In contrast, the seeding activities of PrP^{Sc}-negative mice were below the detection limit of RT-QuIC (Table S1). These results indicate that detection of PrP^{Sc} in the brain corresponds to the presence of seeding activity.

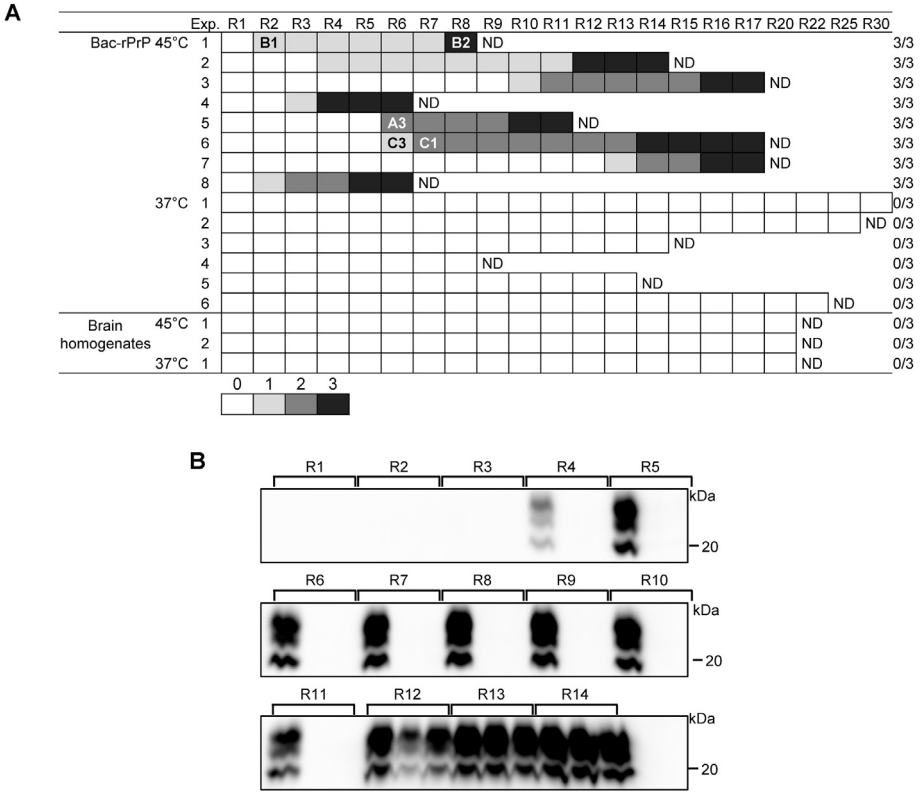

Fig. 1. Spontaneous generation of Bac-rPrP^{Sc} in serial unseeded reactions at 45 °C. (A) The emergence of spontaneous Bac-rPrP^{Sc} (spBac-rPrP^{Sc}) for each round of insect cell protein misfolding cyclic amplification (iPMCA) is represented graphically. Unseeded serial iPMCA was performed by adding one-tenth of the volume of the previous reaction solution to the next reaction solution. Serial amplification at 45 °C or 37 °C was independently repeated at least eight or six times, respectively, and triplicate samples were assayed in each amplification experiment. Conventional PMCA (cPMCA) using normal mouse brain homogenate was performed at 45 °C or 37 °C for comparison. The letters and numbers indicate each spBac-rPrP^{Sc} that was used in further experiments. (B) The results of the third serial amplification experiment at 45 °C are shown as an example.

Table 1
Bioassay results in the first inoculation.

Inoculum		Transmission rate (%) (WB-positive/total mice)	The days of post inoculation	
Variant	Round		WB-positive	WB-negative
B1	R3	40% (2/5)	454, 746[a]	577, 746, 746
	R10	0% (0/8)		567, 708, 708, 708, 708, 708, 728, 728
	R40	0% (0/7)		615, 684, 706, 706, 706, 725, 726
B2	R3	0% (0/8)		421, 620, 656, 712, 712, 712, 728, 728
	R10	0% (0/7)		658, 676, 712, 712, 712, 728, 728
	R20	0% (0/7)		407, 711, 711, 711, 711, 711, 728
	R40	0% (0/6)		543, 592, 610, 704, 704, 704
A3	R3	0% (0/5)		201, 355, 672, 748, 748
	R10	0% (0/7)		294, 593, 699, 707, 728, 728, 728
	R40	0% (0/2)		690, 701
C1	R3	71.4% (5/7)	515, 565, 617, 636, 760	215, 515
	R10	83.3% (5/6)	477, 503, 505, 531[a], 559[a]	316
	R20	14.3% (1/7)	571	494, 501, 581, 582, 649, 701
	R40	0% (0/6)		561, 662, 686, 686, 813, 831
C3	R3	0% (0/7)		530, 705, 714, 714, 714, 729, 729
	R10	0% (0/7)		253, 309, 426, 610, 662, 683, 700
	R20	0% (0/7)		372, 662, 699, 704, 711, 722, 722
	R40	0% (0/7)		627, 704, 704, 704, 704, 722, 722

[a] Clinically positive mice.

3.3. Comparison of PK resistance levels and the conversion efficiency of the five spBac-rPrP^{Sc} variants

We compared the band patterns of PK-resistant PrP (PrPres) induced by the five spBac-rPrP^{Sc} variants (B1, B2, A3, C1, and C3) and Bac-rPrP^{Sc} produced by seeding with any of the three mouse-adapted prion strains (mBSE, ME7, Chandler) by Western blotting (WB). Among the five spBac-rPrP^{Sc} variants produced after R10, A3/R10 showed distinctly different PrPres characteristics compared with other variants and was weakly recognized by T2-horseradish peroxidase (HRP) (Fig. 2A) and M-20 antibodies (Fig. S1A). However, the WB signal intensity when SAF84 (epitope: 160–170) was used was high after signal adjustment (Fig. 2A). Furthermore, epitope mapping showed that A3/R10 was hardly recognized by the

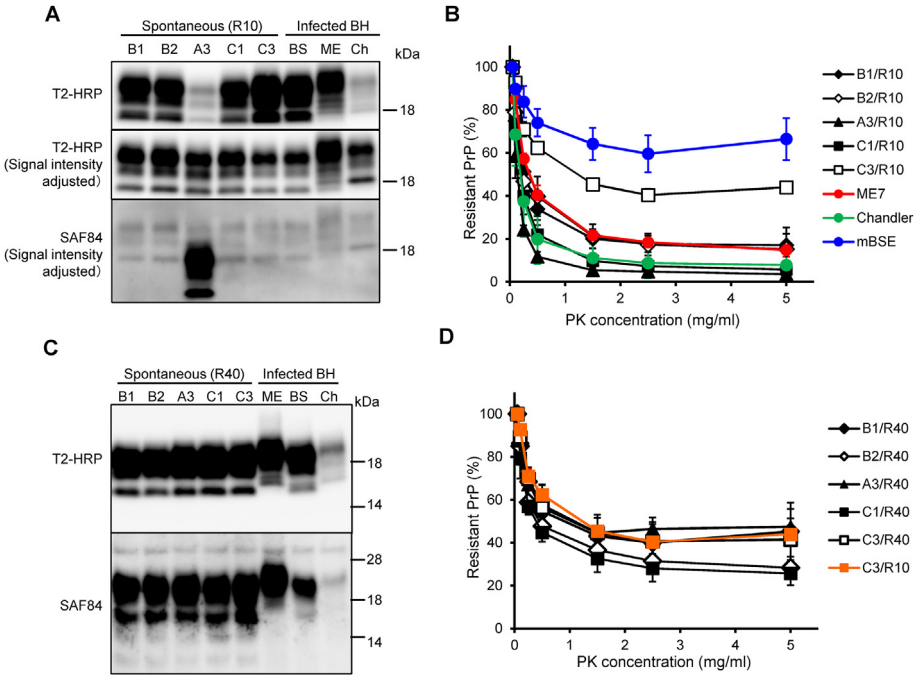

Fig. 2. Comparison of biochemical properties of five spBac-rPrP^{Sc} variants. (A) Western blot (WB) analysis was performed using equivalent volumes of spBac-rPrP^{Sc} variants at R10 and Bac-rPrP^{Sc} from R3 of mBSE (BS)-, ME7 (ME)- and Chandler (Ch)-seeded amplification (top row). To compare banding patterns, WB analysis was performed using T2-HRP (middle row) and SAF84 (bottom row) to match the signal intensity of each sample. (B) The PK resistance of each spBac-rPrP^{Sc} at R10 and Bac-rPrP^{Sc} seeded with three mouse-adapted prion strains at R3 was measured using a PK degradation assay. The graphs show the mean relative intensity and standard deviation (SD) of the Bac-rPrP^{Sc} signal at each test PK concentration. The data were analyzed using two-way ANOVA and Tukey's multiple comparisons test. There were significant differences among all spBac-rPrP^{Sc} variants except the B1 and B2. There were no significant differences among the B1, B2, and ME7 strains and between the C1 and Chandler. There were significant differences between the mBSE and others ($p < 0.0001$). (C) WB analysis of spBac-rPrP^{Sc} from R40 was performed using T2-HRP (upper row) and SAF84 (lower row) with an equal volume of each iPMCA product. (D) The PK resistances of the five spBac-rPrP^{Sc} variants from R40 and that of C3/R10 were measured using a PK degradation assay (mean ± SD). Each set of data was compared with the results obtained with C3/R10 Bac-rPrP^{Sc}. B1/R40, A3/R40, and C3/R40 Bac-rPrP^{Sc} did not exhibit significant differences, but B2/R40 and C1/R40 Bac-rPrP^{Sc} exhibited a significant difference ($p < 0.0001$).

1E4, 8G8, and 6D11 antibodies but was recognized by the 4E10 and R20 antibodies (Fig. S1B). These results indicate that the PK cleavage site of PrPres in A3/R10 is located at the C-terminal side, compared to the other mutants. However, the features of A3/R10 were lost in A3/R40, and at R40, all five spBac-rPrP^{Sc} variants showed similar patterns on WB images after probing with T2-HRP, SAF84, or M-20 antibodies (Figs. 2C and S1A). C3/R10 was indistinguishable from the five spBac-rPrP^{Sc} variants at R40, indicating that they had converged onto the R40 type at this stage.

When PK resistance was compared at concentrations up to 5 mg/mL, C3/R10 showed greater PK resistance than the other four strains, and mBSE-seeded Bac-rPrP^{Sc} had even higher PK resistance than C3/R10 (Fig. 2B). When compared at R40, the four strains other than C3/R40 also showed similar PK resistance levels to that of C3/R10. There was little difference in C3 between R10 and R40 (Fig. 2D).

To measure the effect of cofactors, iPMCA was performed without insect cell-derived cofactors or with polyadenylic acid (PA) or phosphatidylethanolamine (PE) as a cofactor. In the absence of insect cell-derived cofactors, only C3/R3, C3/R10, and B1/R10 maintained PrPres after R6 of iPMCA (Figs. S2A and C). In the presence of PA, only C3/R3, B1/R10, C1/R10, and C3/R10 maintained PrPres after R6. In the presence of PE, PrPres was observed in C3/R10, and smaller amounts were observed in B1/R10 and B2/R10 (Fig. S2B). Of note, PrPres was not maintained during iPMCA when any of the three mouse-adapted strains (mBSE, ME7, Chandler) were used for seeding in the absence of insect cell-derived cofactors or when PA or PE was included (Figs. S2B and C). Taken together, these results reveal characteristic differences in the cofactor dependence of each of the five spBac-rPrP^{Sc} variants at the R10 stage.

3.4. Second and third passages of mice that were PrP^{Sc} positive after the first inoculation with spBac-rPrP^{Sc}

To determine whether the prion adaptation reaction occurs in proteins from PrP^{Sc}-positive mice inoculated with spBac-rPrP^{Sc}, second and third passages of BHs were performed. When mice were inoculated with B1/R3 (454 dpi), the survival times were reduced from 189 days on average for the second passage to 157 days for the third passage. However, when mice were inoculated with C1/R3 (515 dpi), the survival times were approximately 150 days for both the second and third passage from BHs (Table S2). The survival times were similar to those of mice inoculated with ME7, which was used as a control (Table S2). These results suggest that for B1/R3, further prion adaptation occurred from the second to the third passage, whereas for C1/R3, adaptation was already complete in the second passage. The log SD_{50}/mg brain tissue of mice inoculated with two passages of C1/R3 was 6.50 ± 0.35 (mean ± SD) (Table S3), which was significantly higher ($p = 0.012$ in an unpaired two-tailed *t*-test) than that of C1/R3-inoculated mice (5.5 ± 0.54) that became PrP^{Sc} positive after first inoculation.

Electrophoretic images of WBs of PrPres in brain tissue from mice inoculated with the second and third passages of BHs were similar to those from mice inoculated with the ME7 strain (Fig. 3A). A PK degradation assay also revealed that the PK resistance curve for PrPres from one mouse inoculated with the second passage of C1/R3 (C1/R3, 2nd) and an ME7-infected mouse were indistinguishable, whereas PrPres from mice inoculated with the first passage of C1/R3 (C1/R3, 1st #1, and #2) was slightly more sensitive to PK treatment (Fig. 3B).

The vacuolation profile and PrP^{Sc} accumulation in different

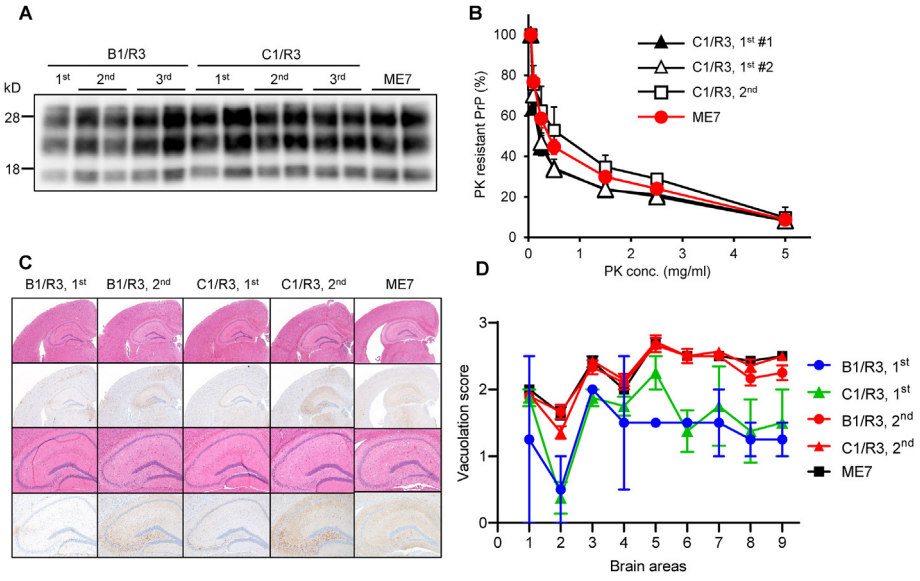

Fig. 3. Histopathological analysis of B1/R3 or C1/R3 variant inoculated first and second passaged mouse brain tissue. (A) WB analysis was performed on PrP^{Sc} accumulated in the brains of mice inoculated with the first, second, or third passage of B1/R3 or C1/R3 and mice inoculated with serial passages of ME7. Two mice were included in each group, except for mice inoculated with the first passage of B1/R3. (B) The PK resistance of PrP^{Sc} from two mice inoculated with the first passage of C1/R3 (C1/R3, 1st #1, and #2), one mouse inoculated with the second passage of C1/R3 (C1/R3, 2nd), and ME7-infected mice (ME7) was measured using a PK degradation assay. (C) Vacuolation (first and third rows) and PrP^{Sc} accumulation (second and fourth rows) in the brains of mice inoculated with the first and second passages of B1/R3, first and second passage of C1/R3, and ME7. (D) Vacuolation profiles in different brain areas of mice inoculated with the first and second passages of B1/R3 and C1/R3 and ME7. The brain regions are as follows: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septal nuclei of the paraterminal body; 8, cerebral cortex at the level of 4 and 5; and 9, cerebral cortex at the level of 7. The average lesion scores (5–10 animals/group) and standard error of the mean (SEM) are shown. The graph shows the mean and SD with two to seven mice per group. Significant differences among each group were evaluated using two-way ANOVA and Tukey's multiple comparisons test. There were no significant differences in the vacuolation scores of the B1/R3 and C1/R3 second passage and ME7 groups, but the differences were significant between the B1/R3 and C1/R3 first passage and ME7 groups ($p < 0.0001$) and between the first and second passage groups ($p < 0.0001$). There was no significant difference between the B1/R3 and C1/R3 first passage groups.

brain areas were compared among B1/R3- (mice receiving the first and second passage), C1/R3- (mice receiving the first and second passage), and ME7-infected mice (Fig. 3C and D). The vacuolation scores of the B1/R3 and C1/R3 second passage and ME7 inoculated groups were similar. However, there was a significant difference between these groups and the B1/R3 and C1/R3 first passage inoculation groups, with the latter generally having milder vacuolation (Fig. 3D).

4. Discussion

In this study, we found that the reaction temperature is an important factor for spontaneous generation of the abnormal form of PrP. It is generally known that when proteins partially unfold, they tend to aggregate and often form amyloid fibrils. When Ec-rPrP is intermittently sonicated [8] or agitated [9], amyloid fibrils are formed. Although sonication itself can cause partial unfolding of proteins to some extent, an increase in the reaction temperature from 37 °C to 45 °C likely increased the degree of partial unfolding of Bac-rPrP and promoted its spontaneous conversion into PrP^{Sc}.

Furthermore, we showed that a variety of Bac-$rPrP^{Sc}$ variants, including non-transmissible ones, can be generated under the same sonication and cofactor conditions, resulting in differences in PK resistance and cofactor dependence for passages within 10 rounds. Prion strains or variants are assumed to be structural isomers of PrP^{Sc}, but the mechanism of their spontaneous generation remains unclear. There are two probable mechanisms for the generation of prion strains. One involves a prion strain being transmitted to and adapting to a different animal species, such as the mBSE strain, which is a mouse-adapted strain of classical BSE found in cattle [10]. Even if the amino acid sequence of PrP differs among species, the structural characteristics of the original strain may be maintained, and the strain could become a new strain of that species. The other case occurs when multiple prion strains or variants with the same PrP sequence occur spontaneously in one species. Different buffers and agitation conditions resulted in the formation of structurally different Ec-rPrP amyloid subtypes, giving rise to different disease types in transgenic mice overexpressing mouse PrP [11]. In addition, at least two types of PrP amyloid fibrils or PrP^{Sc} variants with different structural properties were formed under the same experimental conditions using Ec-rPrP [12]. These previous studies, together with our findings, provide corroboration that prion strains can be generated spontaneously. However, the mechanism by which different PrP^{Sc} variants are formed under the same experimental conditions is unknown. First, even if the experimental conditions are macroscopically the same, the local solution conditions are likely to be temporarily different, especially when manipulations such as sonication are performed. Amyloid fibril formation begins with nucleation, which is a rate-limiting step with a large activation barrier. Once the nucleus is formed stochastically, it continuously takes up monomeric proteins in solution and undergoes a conversion reaction, resulting in growth. During nucleation, small differences in the partial unfolding of monomeric proteins can lead to different nuclear structures. However, the fibril elongation reaction occurs at a much higher rate than nucleation because of its lower activation barrier. Therefore, once the first nucleus A is formed, it will predominate in one tube before a second, differently structured nucleus B is formed and grows. In such a case, the majority of amyloid fibrils in the tube will have the same structure as the fibrils derived from nucleus A. In another tube, nucleus B may have formed first by chance and become dominant. Such a mechanism may lead to the formation of multiple PrP^{Sc} variants even under macroscopically identical conditions.

However, most of the differences between these Bac-rPrP^{Sc} variants were lost after 40 reaction rounds, and the variants converged onto a single strain. Similar phenomena have been observed in studies using PMCA [13] and RT-QuIC [14] using the original prion strain as a seed. This phenomenon suggests that multiple variants are spontaneously generated in the same tube, competition is constantly occurring among them, and the variant with a slightly higher growth rate becomes dominant after successive reaction rounds.

In this study, transmissibility in the first inoculation of normal mice was observed only for some spBac-rPrP^{Sc} variants, but after the second and third passages, all inoculated mice developed the disease, and the incubation period decreased to approximately 150 days. Caution should be exercised in interpreting transmissibility of synthesized PrP^{Sc} results in general because the transmissibility and incubation period have been shown to be greatly influenced by factors such as the host PrP sequence, expression level, and inoculation route, in addition to the titer of the inoculum. Synthetic PrP^{Sc} generated by cell-free conversion systems such as PMCA and RT-QuIC often shows a prolonged incubation period and/or reduced [15–17] or absent infectivity titer [14,18] compared with in vivo-derived PrP^{Sc}. However, when samples from brains that tested positive for PrP^{Sc} in the initial inoculation of experimental animals were further passaged, as in this study, they reached levels comparable to those of in vivo-derived PrP^{Sc}. In other words, PrP^{Sc} with a more transmissible structure is selected for growth in the animal host.

Bac-rPrP is derived from insect cells and has N-glycosylations and GPI-anchored modifications, although its molecular structure is different from that of mammals [4]. However, the transmissibility and prion strain characteristics of iPMCA products seeded with in vivo-derived PrP^{Sc} were maintained at a level similar to those of cPMCA products obtained from BHs [5]. Some Ec-rPrP^{Sc} [19] and PrP^{Sc} from GPI-anchor-deficient PrP-expressing mice [20] were reported to be highly transmissible. Those studies and the present results indicate that the presence of post-translational modifications is not necessarily associated with transmissibility.

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Declaration of competing interest

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.04.137.

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