biguous with respect to the length of the poly(C)/(G) stretches (supporting online text and figs. S5 and S6). Such variable homopolymeric C or G stretches, generated in the course of replication because of slipped-strand mispairing, have been reported in other species to be involved in phase variation, an adaptation strategy to generate phenotypic variation (*18*) (supporting online text). Although showing no similarity to database entries, PPA1880 and PPA2127 both contain characteristic multiple repeats of the dipeptide proline-threonine [PT repetitive protein (PTRP)]. Such PT repeats have been detected in antigenic proteins of *Mycobacterium tuberculosis* (*19*). Additional PTRPs (PPA1715, PPA2210, and PPA2270) were found in *P. acnes* with characteristics of surface proteins, which may represent further host-interacting factors (Table 1 and supporting online text).

Several HSPs have been identified as major targets of the immune response in bacterial pathogens. Homologs of GroEL and DnaK were found and characterized in *P. acnes* (*6*). The genome sequence contains several more HSPs, such as DnaJ (PPA916 and PPA2038), GrpE (PPA2039), and an 18-kD protein (PPA737), the homologs of which in mycobacteria are major immune reactive proteins (*20*). Further proteins show similarities to known bacterial immunogenic factors, such as PPA765, to antigen 84 of *M. tuberculosis* and *M. leprae*, which is a highly immunogenic protein involved in the symptoms of multibacillary leprosy (*21*). Porphyrins are produced by *P. acnes* in high amounts, and these are also thought to be involved in inflammation (*1*) (supporting online text). In the presence of increasing oxygen tension, the interaction of molecular oxygen with released porphyrins generates toxic, reduced oxygen species, which can damage keratinocytes and lead to cytokine release (fig. S7).

In summary, the genome sequence clearly reveals many proteins involved in the ability of *P. acnes* to colonize and reside in human skin sites as well as a pronounced potential to survive a spectrum of environments. This capacity helps to explain the ubiquity of *P. acnes* and also its potential hazards, for example, the public health problems associated with Blood Bank contaminations, or the contamination of the human genome sequence database with *P. acnes* sequence. The GenBank entry AAH14236.1, a proposed human protein, is in fact a *P. acnes* protein (PPA1069).

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S7
Table S1
References and Notes

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Synthetic Mammalian Prions

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Recombinant mouse prion protein (recMoPrP) produced in *Escherichia coli* was polymerized into amyloid fibrils that represent a subset of β sheet–rich structures. Fibrils consisting of recMoPrP(89–230) were inoculated intracerebrally into transgenic (Tg) mice expressing MoPrP(89–231). The mice developed neurologic dysfunction between 380 and 660 days after inoculation. Brain extracts showed protease-resistant PrP by Western blotting; these extracts transmitted disease to wild-type FVB mice and Tg mice overexpressing PrP, with incubation times of 150 and 90 days, respectively. Neuropathological findings suggest that a novel prion strain was created. Our results provide compelling evidence that prions are infectious proteins.

Prion diseases are responsible for some devastating neurological diseases, including Creutzfeldt-Jakob disease in humans, and present as infectious, genetic, and sporadic illnesses (*1*). The production of a new prion strain in Tg mice expressing an artificial, chimeric PrP transgene (*2*) encouraged us to renew our effort to produce synthetic wild-type prions. Earlier, we and others were unable to produce prion infectivity with the use of recombinant wild-type PrP refolded into β sheet–rich isoforms (*3*, *4*); hence, we turned to mutant PrPs.

Mice expressing high levels of MoPrP-(P101L), which harbors a mutation (Pro^{101} → Leu) analogous to the human prion protein mutation that causes Gerstmann-Sträussler-Scheinker syndrome, develop neurodegeneration spontaneously at an early age. Brain extracts prepared from these Tg mice transmit prion disease to Tg mice expressing low levels of MoPrP(P101L), designated Tg196 mice (*5*). About 30% of Tg196 mice develop spontaneous illness at ~550 days of age. We synthesized a 55–amino acid peptide composed of MoPrP residues 89 to 143 with the P101L mutation and folded it into a β-rich conformation. The aggregated peptide produced neurologic dysfunction in Tg196 mice within ~1 year after inoculation, whereas the non–β-rich form did not (*6*). The incubation time for these mutant prions did not change upon serial passage (*7*). Even though the disease-causing $MoPrP^{Sc}$(P101L) readily formed amyloid in the brains of Tg196 mice, resistance of the protein to limited proteolysis could be demonstrated only under "mild" digestion conditions.

Although PrP amyloid deposition in brain is pathognomonic of prion disease, it is a nonobligatory feature (*8*). Moreover, full-length PrP^{Sc} does not polymerize into amyloid, whereas N-terminally truncated PrP^{Sc} (designated PrP 27-30) assembles into amyloid fibrils (*9*). On the basis of the foregoing findings, we reasoned that

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PrP amyloid represents a limited subset of β-rich PrPs, one or more of which might be infectious. Two protocols were used to produce the fibrils from recMoPrP(89–230) expressed in *E. coli* (*10*) (fig. S1); one used monomeric recMoPrP(89–230) to produce the amyloid fibrils (denoted "unseeded"), and the other used some of the unseeded fibrils as a seed for the production of nascent fibrils (denoted "seeded") (*11*).

The seeded and unseeded amyloid fibrils composed of recMoPrP(89–230) were inoculated intracerebrally into Tg(MoPrP,△23–88)9949/*Prnp*$^{0/0}$ mice (henceforth Tg9949 mice) expressing MoPrP(89–231) at a level 16 times that of normal PrP^{C} in Syrian hamster (SHa) brain. In Tg9949 mice, we do not know whether the glycolipid anchor of MoPrP(89–231) is attached to Ser^{230} or Ser^{231}. The two amyloid preparations as well as control phosphate-buffered saline (PBS) were prepared and injected on the same day. If contamination had been a problem, then the PBS-inoculated mice should have developed disease (table S1) unless the amyloids were selectively tainted.

Mice receiving recMoPrP(89–230) amyloid developed neurologic disease between 380 and 660 days after inoculation (Fig. 1A) (table S1). In earlier studies, uninoculated Tg9949 mice lived for more than 500 days without any signs of neurologic dysfunction (*12*). In the study reported here, Tg9949 mice were healthy at ~670 days of age and failed to show any signs of disease at 620 days after inoculation with PBS or 525 days with SHa Sc237 prions (table S1).

The shortest incubation times for Tg9949 mice inoculated with seeded amyloid and unseeded amyloid were 382 days and 474 days, respectively. Western blot analysis of brain homogenates of these two mice revealed that the Tg9949 mouse inoculated with seeded amyloid had more protease-resistant PrP^{Sc} than the brain of the unseeded amyloid-inoculated mouse (Fig. 1B). Whether the different incubation times and diverse biochemical profiles reflect higher levels of PrP^{Sc} in the seeded amyloid relative to the unseeded amyloid, or whether these findings signify the creation of two different prion strains, remains to be established. No protease-resistant PrP was found by Western blotting in the brain of a Tg9949 mouse inoculated with PBS and killed at 530 days after inoculation; by this time, five of seven Tg9949 mice inoculated with seeded recMoPrP(89–230) amyloid had succumbed to disease (Fig. 1B). Consistent with the view that the amyloid fibrils contain low levels of MoPrP^{Sc}(89–230), we were unable to detect this protein in fibril preparations by immunoblotting, and we observed incubation times of ~500 days on first passage followed by shortening to ~250 days on second passage (table S1). For all strains of prions studied to date, low titer inocula produce prolonged incubation times that shorten upon subsequent passage (*13*). On first passage, mice do not become ill until the prion titers in their brains reach maximal levels. Whether the amyloid fibrils protected the small amounts of PrP^{Sc} found within them or modified the retention of PrP^{Sc} in brain after inoculation remains to be determined.

Residual MoPrP^{C}(89–231) after proteinase K (PK) digestion of brain homogenate from a PBS-inoculated Tg9949 mouse reflects the high level of PrP^{C} expression (Fig. 1B, lane 5), a problem encountered with some other Tg mice. Fortunately, the level of residual MoPrP^{C}(89–231) after PK digestion is sufficiently low as to not present problems with the interpretation of the data.

Tg9949 mice inoculated with seeded amyloid exhibited extensive vacuolation with gliosis in the cerebellum, hippocampus, brainstem, and white matter (fig. S2A). The distribution, density, and morphology of the vacuoles were different for unseeded and seeded amyloid, raising the possibility that they represent two different prion strains (fig. S2A). Vacuolation, astrocytic gliosis, and PrP^{Sc} accumulation were more widely dispersed in gray matter regions in the brains of mice inoculated with unseeded amyloid relative to seeded amyloid (Fig. 2, A to D). The neuroanatomic distributions of vacuoles associated with unseeded and seeded amyloid were different from those found with mouse RML prions (compare fig. S2A with fig. S2B). With unseeded amyloid, the majority of vacuoles measured 20 to 50 μm in diameter (Fig. 2A), whereas most vacuoles from RML prions were 10 to 30 μm in diameter (Fig. 2E). From the seeded amyloid inoculum, smaller (10 to 20 μm) and larger (20 to 50 μm) vacuoles were evenly represented (Fig. 2C). With both unseeded and seeded amyloid, PrP^{Sc} was deposited in gray matter as relatively large solitary masses 5 to 20 μm in diameter and at the perimeter of many vacuoles (Fig. 2, B and D). In contrast, RML-infected mice exhibited fine granular accumulations of PrP^{Sc} (Fig. 2F).

Prions in the brains of Tg9949 mice inoculated with seeded amyloid were designated MoSP1 (mouse synthetic prion strain 1). Serial transmission of MoSP1 prions from Tg9949 mice to wild-type FVB mice and to Tg(MoPrP-A)4053 mice (henceforth Tg4053 mice) gave mean incubation times of 154 and 90 days, respectively (Fig. 3) (table S1). Tg4053 mice express MoPrP-A at a level 8 times that of SHaPrP^{C} in hamster brain (*14*). Protease-resistant PrP^{Sc} was found in the brains of both wild-type FVB and Tg4053 mice inoculated with MoSP1 prions (Fig. 1C).

Well-defined PrP amyloid plaques as well as numerous, densely packed, finely granular PrP^{Sc} deposits were identified in the brains of

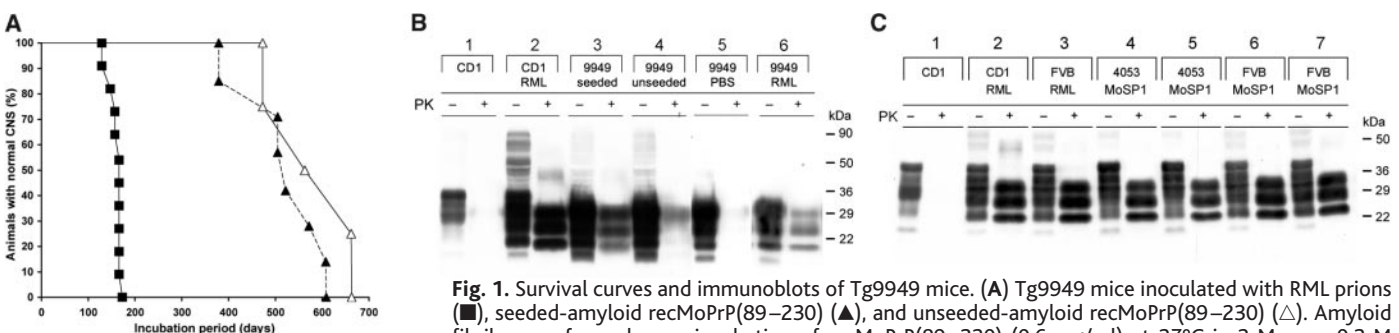

Fig. 1. Survival curves and immunoblots of Tg9949 mice. (**A**) Tg9949 mice inoculated with RML prions (■), seeded-amyloid recMoPrP(89–230) (▲), and unseeded-amyloid recMoPrP(89–230) (△). Amyloid fibrils were formed upon incubation of recMoPrP(89–230) (0.6 mg/ml) at 37°C in 3 M urea, 0.2 M NaCl, 50 mM sodium acetate buffer, pH 5.0, as described (*11*). The kinetics of fibril formation were monitored with a thioflavin T binding assay (*24*). Inocula were prepared by dialysis of the fibrils in PBS buffer, pH 7.2, for 2 days. Concentration of recMoPrP(89–230) in the inocula was ~0.5 mg/ml. (**B**) Immunoblot of PrP^{Sc} in brains of Tg9949 mice. Paired-samples lanes: 1, uninoculated normal CD1 mouse; 2, RML-inoculated CD1 mouse; 3, Tg9949 mouse inoculated with seeded amyloid; 4, Tg9949 mouse inoculated with unseeded amyloid; 5, Tg9949 mouse inoculated with PBS and killed at 580 days of age; 6, Tg9949 mouse inoculated with RML prions. (**C**) Immunoblot of PrP^{Sc} in brains of wild-type CD1, wild-type FVB, and Tg4053 mice. Paired-samples lanes: 1, uninoculated normal CD1 mouse; 2, RML-inoculated CD1 mouse; 3, RML-inoculated FVB mouse; 4 and 5, Tg4053 mouse inoculated with MoSP1 (brain homogenate from Tg9949 mice inoculated with seeded recMoPrP amyloid); 6 and 7, FVB mouse inoculated with MoSP1 (brain homogenate from Tg9949 mice inoculated with seeded recMoPrP amyloid). Symbols in (B) and (C): –, undigested control sample; +, samples subjected to limited PK digestion. Apparent molecular weights based on migration of protein standards are indicated.

both FVB (Fig. 4F) and Tg4053 mice inoculated with MoSP1 prions (*15*). The vacuolation scores (the percentage of an area occupied by vacuoles) were greater in the brains of Tg4053 mice than in those of FVB mice (fig. S3A). Additionally, the density of vacuoles (number per area) seen with MoSP1 prions was greater than that seen with RML prions in Tg4053 mice (fig. S3B). RML prions failed to cause vacuolation in the caudate nucleus, septal nuclei, and cerebellar white matter in Tg4053 mice. These findings argue that the MoSP1 strain remained stable during passage from Tg9949 to Tg4053 mice but seemed less stable upon passage in FVB mice, consistent with the pattern of PrP immunostaining (Fig. 4F).

The specificity of the response in Tg9949 mice to recMoPrP(89–230) polymerized into amyloid was established by the absence of disease after inoculation with PBS and Sc237 prions (table S1). When the healthy Tg9949 mice inoculated with Sc237 prions were killed at 525 days after inoculation, five of seven Tg9949 mice inoculated with the seeded amyloid were already ill.

Many attempts to solubilize PrP^{Sc} under nondenaturing conditions and to renature PrP 27-30 have been unsuccessful (*16*, *17*); hence, progress in elucidating the structure of PrP^{Sc} has been slow (*18*). In purified fractions, PrP^{Sc} forms amorphous aggregates, whereas PrP 27-30 spontaneously assembles into amyloid polymers in the presence of detergent (*19*). Some investigators have argued that prions are infectious amyloids, ignoring findings that full-length PrP^{Sc} does not form amyloid (*20*, *21*). Notably, the fibrillar morphology of prion rods composed of PrP 27-30 disappeared after exposure to the organic solvent 1,1,1-trifluoro-2-propanol (TFIP) while prion infectivity was retained (*22*). Also of interest is a short peptide composed of 27 amino acids corresponding to PrP residues 100 to 126 that readily forms amyloid (*23*). On this background, we pursued investigations directed at producing synthetic prions using PrP amyloid as a surrogate marker for the folding of MoPrP(89–230) into a biologically active conformation. The rapidity and ease of measuring thioflavin T binding that reflects amyloid formation (*24*) permitted us to determine conditions under which recMoPrP(89–230) would polymerize.

Our finding that amyloid fibrils harbor detectable levels of prion infectivity allows us to draw some conclusions about mammalian prions. First, PrP is both necessary and sufficient for infectivity. Second, neither the Asn-linked oligosaccharides nor the glycosylphosphatidylinositol anchor are required for prion infectivity, because recMoPrP(89–230) contains neither of these posttranslational modifications (*25–28*). Whether the Asn-linked oligosaccharides or the glycosylphosphatidylinositol anchor increase the efficiency of PrP^{Sc} formation for particular prion strains is unknown (*29*). Third, variations in PrP glycosylation are not required for prion diversity, and the biological information carried by distinct strains of prions resides in PrP^{Sc}, as previously suggested (*2*, *30*, *31*). Fourth, spontaneous formation of prions, which is thought to be responsible for sporadic forms of prion disease in livestock and humans, can occur in any mammal expressing PrP^{C} (*32*).

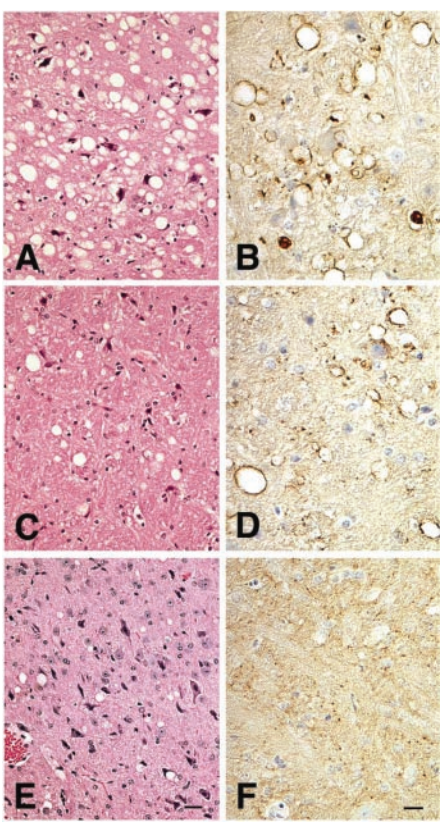

Fig. 2. The distinguishing neuropathological features of unseeded recPrP amyloid (**A** and **B**), seeded recPrP amyloid (**C** and **D**), and RML prions (**E** and **F**) in the pons of Tg9949 mice. Left column: hematoxylin and eosin (H&E) stain (scale bar, 50 μm); right column: immunohistochemistry of PrP^{Sc} by the hydrated autoclaving method using the PrP-specific HuM-R2 monoclonal antibody fragment (*43*) (scale bar, 25 μm).

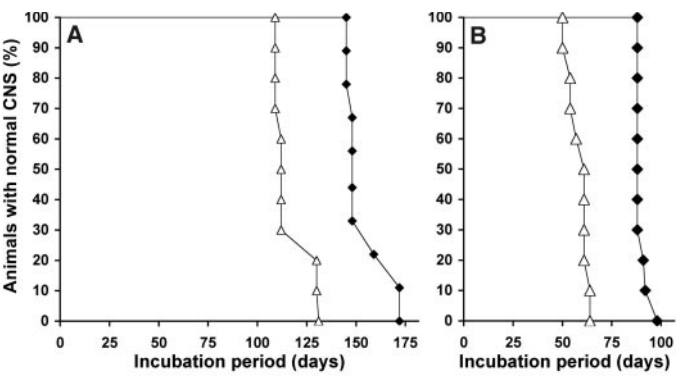

Fig. 3. (**A**) Survival curves of FVB mice inoculated with RML (△) and MoSP1 (◆) prions. (**B**) Survival curves of Tg4053 mice inoculated with RML (△) and MoSP1 (◆) prions.

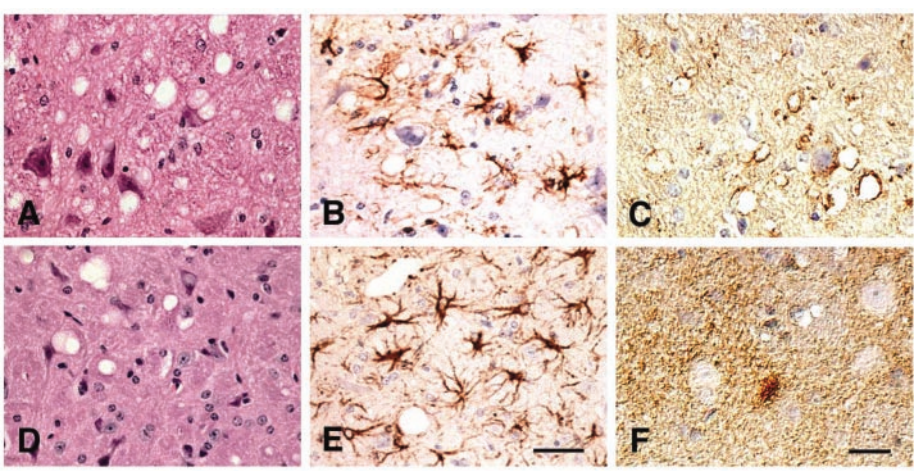

Fig. 4. Comparison of neuropathological changes in the pons associated with inoculation of seeded recPrP preparations into Tg9949 mice (**A** to **C**) and of MoSP1 (prions derived from clinically ill Tg9949 mice inoculated with seeded recPrP amyloid) inoculated into FVB mice (**D** to **F**). Both passages show the neurohistological characteristics of a prion disease: vacuoles (spongiform degeneration) shown by H&E staining (left); reactive astrocytic gliosis shown by glial fibrillary acidic protein immunohistochemistry (center); and accumulation of PrP^{Sc} visualized by hydrated autoclaving immunohistochemistry with HuM-R2 monoclonal antibody (right). Scale bars, 50 μm [(A), (B), (D), (E)], 25 μm [(C) and (F)].

Although we have used the polymerization of recMoPrP(89–230) into amyloid fibrils to generate prion infectivity, we hasten to add that other β-rich forms of recMoPrP(89–230) may also harbor infectivity. Preliminary results suggest that preparations of β-oligomers formed from recMoPrP(89–230) may also contain low levels of prion infectivity (*33*). Such findings emphasize the need to define optimal conditions for prion formation in vitro under which high levels of PrP^{Sc} can be generated. Moreover, previous difficulties in creating infectious prions in vitro from recPrPs enriched for β-structure may be due to the tendency of mammalian PrPs to fold into biologically irrelevant β-rich isoforms (*3*, *4*, *11*). In studies of fungal prions, the ease of assaying infectivity (*34*) and the ability to study millions of colonies made the creation of in vitro infectivity from recombinant proteins more tractable (*35*–*37*). Whereas yeast prions form within the cytoplasm (*38*), mammalian prions are thought to be produced on the cell surface in caveolae-like domains (*39*, *40*).

From Tg mouse studies, it is well established that templates improve the likelihood of forming an infectious β-rich isoform (*8*, *12*). In the studies reported here, we see evidence that seeded amyloid fibrils exhibit shorter incubation times than their unseeded progenitor (Fig. 1A). It remains to be determined whether this is due to the greater number of PrP^{Sc} molecules within seeded fibrils relative to unseeded fibrils, or whether this reflects strain differences.

Our results have important implications for human health. The formation of prions from recPrP demonstrates that PrP^{C} is sufficient for the spontaneous formation of prions; thus, no exogenous agent is required for prions to form in any mammal. Our findings provide an explanation for sporadic Creutzfeldt-Jakob disease for which the spontaneous formation of prions has been hypothesized. Understanding sporadic prion disease is particularly relevant to controlling the exposure of humans to bovine prions (*41*). That bovine prions are pathogenic for humans is well documented; more than 150 teenagers and young adults have already died from prion-tainted beef derived from cattle with bovine spongiform encephalopathy (*42*). Moreover, the sporadic forms of Alzheimer's and Parkinson's diseases as well as amyotrophic lateral sclerosis and the frontal temporal dementias are the most frequent forms of these age-dependent disorders, as is the case for the prion diseases. Important insights in the etiologic events that feature in these more common neurodegenerative disorders, all of which are caused by the aberrant processing of proteins in the nervous system, are likely to emerge as more is learned about the molecular pathogenesis of the sporadic prion diseases.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5684/673/DC1
Materials and Methods
Figs. S1 to S3
Table S1
References

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Host-to-Parasite Gene Transfer in Flowering Plants: Phylogenetic Evidence from Malpighiales

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Horizontal gene transfer (HGT) between sexually unrelated species has recently been documented for higher plants, but mechanistic explanations for HGTs have remained speculative. We show that a parasitic relationship may facilitate HGT between flowering plants. The endophytic parasites Rafflesiaceae are placed in the diverse order Malpighiales. Our multigene phylogenetic analyses of Malpighiales show that mitochrodrial (*matR*) and nuclear loci (18*S* ribosomal DNA and *PHYC*) place Rafflesiaceae in Malpighiales, perhaps near Ochnaceae/Clusiaceae. Mitochondrial *nad1B-C*, however, groups them within Vitaceae, near their obligate host *Tetrastigma*. These discordant phylogenetic hypotheses strongly suggest that part of the mitochondrial genome in Rafflesiaceae was acquired via HGT from their hosts.

Malpighiales are one of the most diverse clades of flowering plants uncovered in recent phylogenetic analyses. The order comprises 27 families (*1*) previously assigned to 13 different orders (*2*), including more than 16,000 species spanning tremendous morphological and ecological diversity (*3*). Recent surprising additions to Malpighiales are the endophytic holoparasites Rafflesiaceae (*4*), which lack leaves, stems, and roots, and rely entirely on their host plants, species of *Tetrastigma* (Vitaceae), for their nutrition. Despite their extreme vegetative reduction, they are unmistakable in flower, producing the largest flowers in the world, which mimic rotting flesh—an enticement to the carrion flies that pollinate them (*5*).

Barkman *et al*. (*4*) used mitochondrial (mt) *matR* sequences to place Rafflesiaceae firmly with Malpighiales [100%