

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

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

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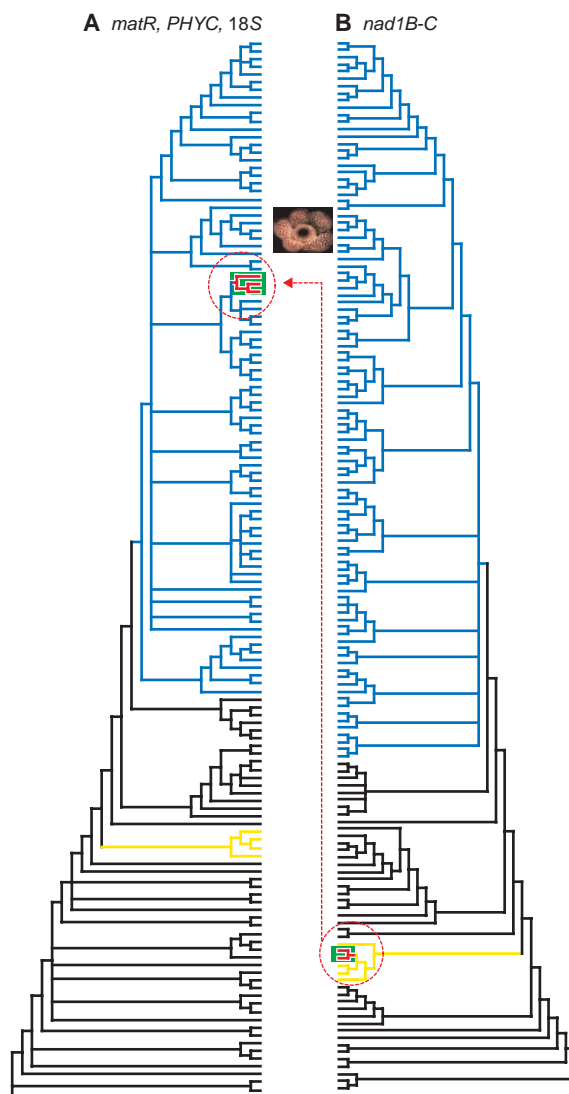
Horizontal gene transfer (HGT) between sexually unrelated species has recently been documented for higher plants, but mechanistic explanations for HGTs have remained speculative. We show that a parasitic relationship may facilitate HGT between flowering plants. The endophytic parasites Rafflesiaceae are placed in the diverse order Malpighiales. Our multigene phylogenetic analyses of Malpighiales show that mitochondrial (*matR*) and nuclear loci (18S 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%

Fig. 1. Two conflicting hypotheses about the phylogenetic placement of Rafflesiaceae. **(A)** The strict consensus of 136 angiosperms for combined mt *matR* and nuclear (*PHYC* and ribosomal 18S) data showing a well-supported (100% BP) Malpighiales clade (in blue), which includes all members of the order sensu APG II (1) plus Rafflesiaceae (in red; *Rafflesia*, *Rhizanthus*, and *Sapria*). **(B)** The strict consensus of 147 angiosperms for mt *nad1B-C* (the *nad1* intron 2 and part of the adjacent exons b and c) showing a well-supported (100% BP) Malpighiales clade, which includes all members of the order except Rafflesiaceae. Rafflesiaceae (*Rafflesia* and *Sapria*) are strongly placed (100% BP) in the basal eudicot family Vitaceae (in yellow) near their host genus, *Tetrastigma*. The dashed line is the hypothesized host/parasite HGT.



bootstrap percentage (BP)]. Their use of a single mt gene was appropriate in a family that has resisted placement with standard genetic loci. To further examine this placement, we obtained sequences representing all families of Malpighiales, all genera of Rafflesiaceae, and numerous basal eudicots for four loci from the mt and nuclear genomes (6). Low-copy nuclear genes are an underused resource for resolving the placement of problematic taxa, and *phytochrome C* (*PHYC*), as used here, has been useful for revealing relationships within Malpighiales (7).

Our phylogenetic analyses are summarized in Fig. 1 (8). The tree created from the

matR and nuclear loci firmly (100% BP) place Rafflesiaceae within Malpighiales. In contrast, the mt locus *nad1B-C* suggests that Rafflesiaceae are not members of Malpighiales but belong (100% BP) in Vitaceae near their host *Tetrastigma*. Each of these mutually exclusive hypotheses cannot be attributable to contamination (9), and each receives strong support from parsimony analyses and from alternative topology tests.

Which of these conflicting hypotheses reflect the true species affinities of Rafflesiaceae? Vitaceae possess several synapomorphies that are rare among angiosperms, including sieve-tube plastids with starch and protein inclusions, pearl glands, stamens opposite the petals, and seeds with a cordlike raphe. If Rafflesiaceae were embedded in Vitaceae, as suggested by *nad1B-C*, we would expect species to possess at least some of these characters, but they do not (2, 3). A definitive malpighialean sister group for Rafflesiaceae is unclear, given our data. However, the closest relatives suggested in the combined analysis (10),

Ochnaceae and Clusiaceae sensu lato, share tenuinucellate ovules (among mostly crassinucellate relatives) and staminal fusion with Rafflesiaceae (2, 3).

The position of Rafflesiaceae based on *nad1B-C* provides a new example of horizontal gene transfer. If *nad1B-C* were vertically transmitted, as we believe to be the case for the other loci, we would expect Rafflesiaceae to group with Malpighiales. Instead, phylogenetic evidence from *nad1B-C* suggests that part of the mt genome in Rafflesiaceae originated from their hosts, *Tetrastigma* (either stem or crown group members), and was horizontally transferred to these obligate parasites. A similar horizontal gene transfer (HGT) of *nad1B-C* was recently reported (11) in seed plants, involving a transfer from an asterid to *Gnetum*. And Bergthorsson *et al.* (12) have documented several instances of mt HGT between distantly related angiosperm groups.

The underlying mechanism for HGT between sexually unrelated plants, however, has been elusive. Various pathogens have been suggested as primary vector agents (11, 12). Our study documents a case in which there is no need to propose an intermediary vector for HGT. In these plants, the transfer appears to have been facilitated by the intimacy of the association between the host and the endophytic parasite, which lives its whole vegetative life as “an almost mycelial haustorial system,” “ramifying and anastomosing throughout the [tissues of the] host” (13). This pattern may be an important mechanism by which parasites assemble their genetic architecture, and additional cases of HGT should be sought among other endophytic parasites and their hosts. It will also not be surprising if reciprocal genetic transfers are found to have occurred, from parasite to host.

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only for Rafflesiaceae. Both approaches yielded congruent topologies, with the latter (shown here) being much better resolved. The spurious placement of Rafflesiaceae as sister to all angiosperms when 18S is used (4) may be attributable to high divergence in these small domains and suggests that 18S may not be as useless for placing problematic taxa as previously suggested (4).

9. We took several precautions to avoid and detect contamination. First, our results were independently corroborated in the laboratories of each author. Second, Rafflesiaceae data were acquired before starting any work on Vitaceae. Third, if our DNA were cross-contaminated we would not expect such strongly conflicting results regarding the placement of Rafflesiaceae, given the same DNA. Nor would we expect such a high degree of sequence divergence of *nad1B-C* between Rafflesiaceae and *Tetrastigma* (or other Vitaceae we sampled). If contamination occurred, we would expect sequences to be nearly

identical to those of other sampled Vitaceae, especially given the relatively low amount of sequence divergence between all accessions of Vitaceae in *nad1B-C* (0.51 to 0.95% sequence divergence). Not only is *nad1B-C* divergent within Rafflesiaceae (6.2%), but it is also highly divergent from other phylogenetically diverse Vitaceae (15) included here (2.5 to 3.3%).

10. Taxa in the combined analysis of 18S, *PHYC*, and *matR* were included if they were sampled for *matR* plus at least one of the two nuclear loci. We believe this analysis to represent the best estimate of Malpighiales phylogeny. Similar approaches combining multiple genes have provided powerful insights into angiosperm phylogeny where single gene studies have failed (16).
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KIF1A Alternately Uses Two Loops to Bind Microtubules

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The motor protein kinesin moves along microtubules, driven by adenosine triphosphate (ATP) hydrolysis. However, it remains unclear how kinesin converts the chemical energy into mechanical movement. We report crystal structures of monomeric kinesin KIF1A with three transition-state analogs: adenylyl imidodiphosphate (AMP-PNP), adenosine diphosphate (ADP)-vanadate, and ADP-ALFx (aluminum-fluoride complexes). These structures, together with known structures of the ADP-bound state and the adenylyl-(β,γ -methylene) diphosphate (AMP-PCP)-bound state, show that kinesin uses two microtubule-binding loops in an alternating manner to change its interaction with microtubules during the ATP hydrolysis cycle; loop L11 is extended in the AMP-PNP structure, whereas loop L12 is extended in the ADP structure. ADP-vanadate displays an intermediate structure in which a conformational change in two switch regions causes both loops to be raised from the microtubule, thus actively detaching kinesin.

To move along microtubules, kinesins (1) must alternate rapidly between tightly bound and detached states. How both dimeric (2, 3) and monomeric (4, 5) kinesins achieve this remains unclear. Because the binding energy in the strong-binding state [10 to $20 k_B T$ (3, 4), where k_B is the Boltzmann constant and T is absolute temperature] is too large for rapid spontaneous release, the energy for fast detachment of kinesin from the microtubule must come from a step of the ATP hydrolysis cycle. Large change(s) in free energy are expected to occur during four steps: ATP binding, hydrolysis, phosphate release, and ADP release. Both conventional kinesin and KIF1A bind tightly to microtubules in the nucleotide-free state and in the ATP-bound

state. In the ADP-bound state, conventional kinesin is detached from microtubules, whereas KIF1A is partially detached and diffuses freely along the microtubule. This is because loose binding of ADP-bound KIF1A is supported by the KIF1 family-specific K-loop at loop L12. A mutant KIF1A that lacks the K-loop detaches from the microtubule in the ADP-bound state, and the dissociation

constant markedly varies depending on the type of bound nucleotide, as is true for conventional kinesin (4). For historical reasons, the tightly bound state is called the strong-binding state, and the fully or partially detached state is called the weak-binding state. Recent work detected the phosphate release from a mutant kinesin, which stalls before the detached state (6, 7). This means that detachment occurs just at or after the phosphate release. Thus, the active process to detach kinesin from the microtubule should occur at the transition from the strong-binding state to the weak-binding state.

The active detachment process can be detected in KIF1A because of its property of binding to the microtubule during adenosine triphosphatase (ATPase) cycling. The apparent dissociation constant of KIF1A in the presence of ATP is the weighted average of the equilibrium dissociation constant of various intermediate states during the ATPase turnover. Because the dissociation constant is not significantly different between two major intermediate states, the AMP-PNP-bound and ADP-bound states (Table 1) (fig. S1) (8), the apparent dissociation constant during the ATPase turnover was not expected to be fundamentally different from these values. However, the apparent dissociation constant in the presence of 2 mM ATP was twice the expected

Table 1. (Apparent) equilibrium dissociation constants (K_d) for microtubules. K_d values are reported as means \pm SEM of at least three independent experiments. Conditions: 2 mM nucleotide or its analog, 50 mM imidazole, 5 mM Mg-acetate, 1 mM EGTA, and 50 mM K-acetate, pH 7.4 at 27°C (nd, not determined).

Nucleotide	K_d (nM)			
	Wild type	L12 [†]	L11 [‡]	L8 [§]
AMP-PNP	4.2 \pm 1.3	6.0 \pm 1.4	20.2 \pm 4.0	25.0 \pm 6.0
ADP	6.8 \pm 2.5	23.5 \pm 8.4	12.3 \pm 4.0	26.5 \pm 5.0
ATP*	10.8 \pm 1.8	40.5 \pm 11.8	nd	nd
ADP-ALFx	5.9 \pm 1.5	7.1 \pm 1.7	nd	nd
ADP-Vi	21.4 \pm 4.3	167 \pm 66	nd	nd

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*ATP regeneration system was used to maintain ATP/ADP level. [†]L12: CK1 (4). [‡]L11: K261A/R264A/K266A. [§]L8: K161A/R167A/R169A/K183A.