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Dangerous Liaisons between a Microbe and the Prion Protein

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Aren't bugs a source of continuous amazement? Consider, for example, how cunningly bacteria conspire to shanghai the molecular machines of their mammalian hosts for their own goals. Besides serving the bugs, this evil intelligence is exploitable for studying cellular physiology, and the bewildering affinity of bacterial toxins for crucial host cell proteins has taught us many a thing on how cells work.

Perhaps *Brucella* may help teach us the function of the normal prion protein (1). *Brucella* species are Gram-negative facultative intracellular pathogens. They invade, resist intracellular killing, and replicate in phagocytic and nonphagocytic cells (2). But how does *Brucella* initiate replication in macrophages? The contact with the bug instructs the macrophage to internalize it; the mode of internalization (Fcγ and complement receptors vs. uptake of nonopsonized bugs) determines the fate of the bug (2).

Brucella actively modulates its own engulfment. It induces peculiar membrane ruffles at its site of contact with the macrophage and slow "swimming internalization" into a macropinosome. Then, *Brucella* takes full control of the macropinosome. It inhibits its maturation into a degradative lysosome (3) and reprograms it to acquire endoplasmic reticulum markers and mature into a "replicative phagosome" where bacteria start multiplying (Fig. 1; reference 2).

For playing these tricks, Brucella relies on a set of virulence factors, including a bacterial injection organelle termed VirB or type IV secretion system (4–8). When exposed to macrophages in vitro, *virB*-deficient bugs cannot modulate phagocytosis and are degraded in the lysosome. But what does the VirB system exactly do in this context? According to Watarai et al. (1), it may be needed for transporting the bacterial heat shock protein Hsp60 onto the surface of the bug. Unexpectedly, the chaperonin Hsp60, which normally hangs out in the cytoplasm and deals with unfolded proteins (9), turns out to reside on the surface of wild-type *Brucella abortus* but not *virB* mutants.

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On the macrophage surface, *Brucella* attaches to cholesterol-rich microdomains called lipid "rafts" (10–12). There, Hsp60 appears to snatch at an unlikely friend, the cellular prion protein (PrP^C), which is encoded by the *Prnp* gene and resides preferentially in rafts. PrP^C is apparently recruited to the macrophage's membrane protrusions, which engulf the bacteria, and subsequently to the early macropinosome. In $Prnp^{o/o}$ macrophages, *B. abortus* does not modulate phagocytosis nor phagosome maturation. This is strikingly similar to the behavior of *Brucella virB* mutants and suggests that the Hsp60–PrP^C interaction is instrumental for these actions.

The latter presumption is backed up by animal studies. Wild-type *Brucella* replicates in wild-type mice, whereas *virB* mutants do not (13). In Watarai's (1) experiments, both wild-type and VirB-deficient bugs are avirulent in PrP^C-deficient mice. Hence, the Hsp60–PrP^C connection is important in real infections (Fig. 1).

So, what can *Brucella* teach us about PrP^C? The prion is the infectious agent causing transmissible spongiform encephalopathies (14). Its only known constituent is PrP^{Sc}, a conformational isoform of PrP^C, which is expressed at various levels in most mammalian cells. The only established function of PrP^C in vivo is to enable transmissible spongiform encephalopathies. Ablation of *Prnp* abrogates prion replication (15) and pathogenesis (16). However, the physiological function of PrP^C has remained mysterious. $Prnp^{o/o}$ mice show no obvious developmental defect and live long, happy lives (17). Subtle changes in circadian rhythms (18) and alterations of hippocampal function (19–22) have been described in $Prnp^{o/o}$ mice. However, some of these phenotypes were not reproduced by others (23) and none were clarified in molecular terms.

Biochemically, PrP^C was reported to do almost everything, including the opposite of everything. For example, PrP^C binds copper (24) and was suggested to be a cuproenzyme, but others hold this finding for a red herring (25). PrP^C may have antiapoptotic properties (26–29), but others find that it sensitizes neurons to apoptosis (30). PrP^C peptides might be neurotoxic (31), or maybe not (32). Prion toxicity might be due to retrotranslocation of PrP^C from the endoplasmic reticulum to the cytoplasm (33), but maybe retrotranslocation does not occur after all (34).

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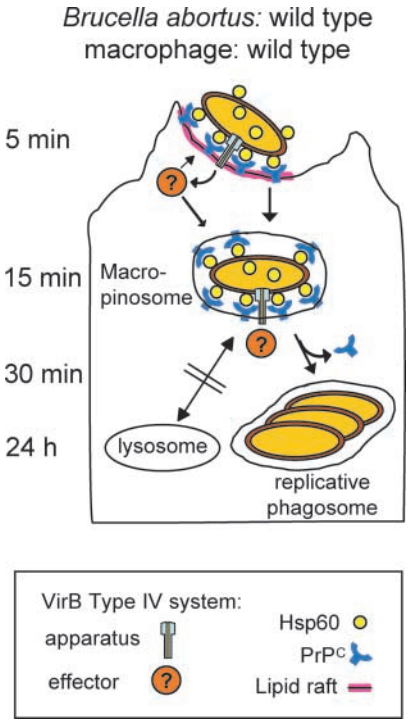

Figure 1. Macrophage manipulation by *B. abortus*: A role for the Hsp60–PrP^C interaction. *B. abortus* transports Hsp60 via the VirB type IV system onto its surface. Upon encounter with a macrophage, Hsp60 binds to PrP^C, which is embedded in lipid rafts on the macrophage surface. This is thought to modulate phagocytosis (swimming internalization), mediate macropinosome formation, inhibit lysosome fusion, and steer the macropinosome to the formation of the replicative phagosome. Other, hitherto unknown effector proteins traveling via the VirB system are also involved (see text; based on the findings by Watarai et al. [reference 1]).

Hence, no unified view of PrP^C function in health and disease has emerged from these observations, apart from the fact that PrP^C is dispensable for life and normal development.

And yet reverse genetics shows that PrP^C must have some biological function and bind to one or more partners in a functionally meaningful way. Transgenic expression of amino proximally truncated PrP^C mutants causes cerebellar degeneration and early death (35). This phenotype is only observed in *Prnp*$^{o/o}$ mice and is fully reverted by substoichiometric coexpression of full-length PrP^C. It follows that truncated PrP interferes with a physiological function of PrP^C and that its effector domain lies in its amino proximal half. A similar phenotype is elicited by overexpression of the Dpl protein (36), which resembles truncated PrP^C and may therefore represent an endogenous PrP^C antagonist (37).

Population genetics provides further evidence that PrP^C is doing more than bestowing prion diseases on us. Protective variations in the human prion gene, which arose recently in evolution, have disseminated much more efficiently among human populations than nonprotective polymorphisms (38). This provides a compelling case for a role in evolutionary fitness, similarly to globin gene mutations that are protective against malaria. Selective pressure to maintain heterozygosity might have come from Kuru, a cannibalism-transmitted prion disease that was a prime cause of death in New Guinea tribes. One disturbing conclusion is that cannibalism was commonplace among our ancestors (38). Consequently prion diseases, now exceedingly rare, have probably ravaged human populations in the distant past. What is most mystifying, *Prnp* null alleles were not selected for, despite the presumptive evolutionary advantage of resistance to cannibalism-induced prion disease.

At face value, the resistance of PrP^C knockout animals to *B. abortus* infection provides further hints to the usefulness of PrP^C. Animals have probably dealt with *Brucella* infections for a long time and *Brucella*-resistant individuals with disrupted Hsp60 binding domains from PrP^C should have had an edge. However, this has not happened.

So, what are the elusive functions and partners of PrP^C? Several PrP^C binding factors have been described, including the laminin receptor precursor protein (39), heparan sulfate (40), N-CAM (41), and bcl-2 (42), yet none of these interactions were linked to biological functions. Could PrP^C serve as a general Hsp60 sensor? Hsp60 homologues are found in bacteria and all eukaryotic cells, and can induce inflammation and immune responses (43, 44). It will be exciting to test whether PrP^C is involved in an Hsp60-dependent common "danger sensing" mechanism for detection of destructed body cells and pathogenic microbes (43, 44).

Watarai's provocative report hints at novel, surprising aspects of bacterial and prion biology. Many of the following exciting ramifications deserve to be studied: (a) Hsp60 is the first *B. abortus* protein whose presence on the outer surface relies on the type IV secretion system, yet it does not seem to participate to the type IV secretion apparatus itself. How is Hsp60 recognized and transported by the VirB type IV system? And how is it retained on the bacterial surface? (b) What is the function of the Hsp60–PrP^C complex? Is it simply slowing down the initial steps of phagocytosis to allow sufficient time for injection via the VirB type IV system and the manipulation of the early macropinosome by other so far unidentified effector proteins? Or does it serve as an anchor for assembling a whole set of host cellular proteins on the macropinosome membrane? (c) The Hsp60–PrP^C interaction is clearly insufficient for proper macropinosome formation and maturation. Hence, the function of the type IV secretion system must go beyond surface exposure of Hsp60. Which effector proteins travel via this pathway and what is their function? (d) Bacteria often accomplish their deeds by disrupting specialized cellular functions. Does Brucella interfere with the function of PrP^C? If so, what does it get out of it? Answers to these questions may come from analyzing interactions between Hsp60 and amino terminally truncated versions of PrP^C.

Many microbial pathogens invade and replicate within host cells. All these bugs face death in the lysosome and have devised strategies to escape this fate. *Shigella* and *Listeria* lyse the vacuole membrane and dwell in the host cell cytoplasm. Others (including *Salmonella typhimurium*, *Legionella pneumophila*, mycobacteria, *Chlamydia trachomatis*, and certain *Escherichia coli* strains) manipulate the endo-

some/lysosome pathway and reside in some type of restructured vesicle. Interestingly, some of these bugs (mycobacteria [45], *C. trachomatis* [46], and certain *E. coli* strains [47]) need, like *Brucella*, intact lipid rafts on the host cell surface to reach this niche (48). It will be exciting to test whether the Hsp60–PrP^C connection is involved. Future work may test whether host cell manipulation via PrP^C will be an exception, or the rule.

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