Exploitation of the Intestinal Microflora by the Parasitic Nematode Trichuris muris

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ponds, and thus provides some indication of stability among different community states. However, these data alone cannot be used to definitively test stability, and experimental perturbations of species densities would be required to unequivocally discern multiple stable equilibria (*25*).

The results from this long-term experiment show that the deterministic processes inherent in niche-based theories of community assembly predominate in lower-productivity systems, whereas stochastic processes due to differential colonization history and priority effects predominate in higher-productivity systems. The key mechanism underlying this relationship was that the realized pool of species that could potentially live in lower-productivity sites was more or less nested within the pool of species that could potentially live in higher-productivity sites. Although the generality of this specific mechanism in other ecosystems remains to be elucidated, I would argue that it is likely to be a rather general phenomenon. Species that are tolerant of harsh conditions such as low productivity can often be found on occasion in adjacent, more benign habitat types, and indeed can flourish in those habitats in the absence of interspecific interactions (*26*). For example, a large number of experiments have manipulated productivity in grassland ecosystems, and these experiments often show that lower-productivity treatments have a smaller, nested, pool of plant species that can potentially exist in those treatments relative to higher-productivity treatments that have a larger species pool (*17*). Similar patterns of nestedness along a productivity gradient seem to be evident in large-scale surveys of freshwater fish in lakes (*10*).

Some of the best evidence for stochastic processes underlying community assembly comes from relatively productive environments such as tropical rainforests and coral reefs (*13*, *18*, *27*, *28*), whereas deterministic processes may play a stronger role in less productive temperate forests (*29*, *30*), grasslands (*31*, *32*), and marine intertidal habitats (*33*). As such, the results from this experimental study are likely to be general, suggesting that the relative importance of stochasticity increases with increasing productivity, and providing a likely mechanism for the positive relationship between increasing productivity (and lower latitude) and increasing biodiversity at large spatial scales, even in the absence of systematic variation in habitat heterogeneity or biogeographic constraints.

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Exploitation of the Intestinal Microflora by the Parasitic Nematode *Trichuris muris*

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The inhabitants of the mammalian gut are not always relatively benign commensal bacteria but may also include larger and more parasitic organisms, such as worms and protozoa. At some level, all these organisms are capable of interacting with each other. We found that successful establishment of the chronically infecting parasitic nematode *Trichuris muris* in the large intestine of mice is dependent on microflora and coincident with modulation of the host immune response. By reducing the number of bacteria in the host animal, we significantly reduced the number of hatched *T. muris* eggs. Critical interactions between bacteria (microflora) and parasites (macrofauna) introduced a new dynamic to the intestinal niche, which has fundamental implications for our current concepts of intestinal homeostasis and regulation of immunity.

The mammalian gut contains around 10^{13} bacteria (*1*), the majority of which belong to the phyla Bacteroidetes or Firmicutes (*1*, *2*). Coevolution with these microbes has driven the functional morphology and immune function of the gastrointestinal tract (*3*–*5*). Without microbes, aberrant physiology develops together with problems in host defense. Both can be rectified upon reintroduction of bacteria (*6*). Additionally, childhood exposure to microbes can direct the maturing immune system to develop a tolerance to environmental antigens, the so-called "hygiene hypothesis" (*7*). More recently this concept has been extended to include "macrofauna" of the gut, such as helminth parasites (*8*). A helminth-driven T_H2 and regulatory helper T cell response has evolved to counter infection and repair the damage that these parasites cause (*9*). Dysregulation of these immune responses leads to prolonged infection and disease. Indeed, helminths have been found to be a major force underlying the evolution and selection of interleukin genes (*10*). Thus, gut commensal bacteria and gastrointestinal-dwelling helminths have lived in close association throughout evolution. Relationships between bacteria and metazoa have already been documented, such as filarial worms and the endosymbiont *Wolbachia* (*11*); however, a functional nonendosymbiotic relationship between prokaryotes and parasitic metazoa within the infected

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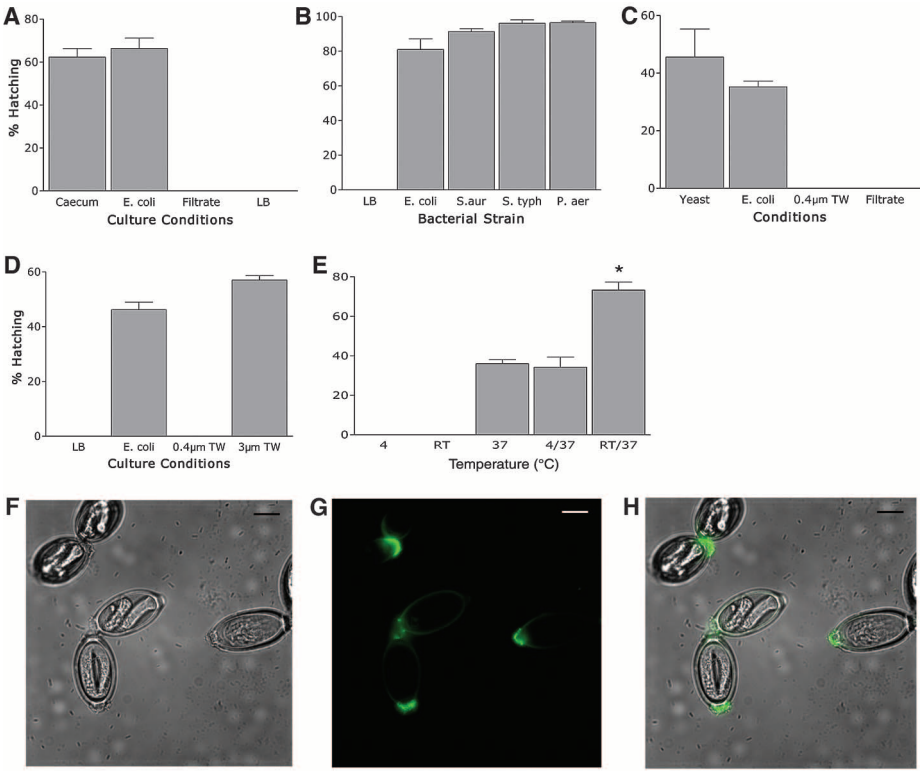
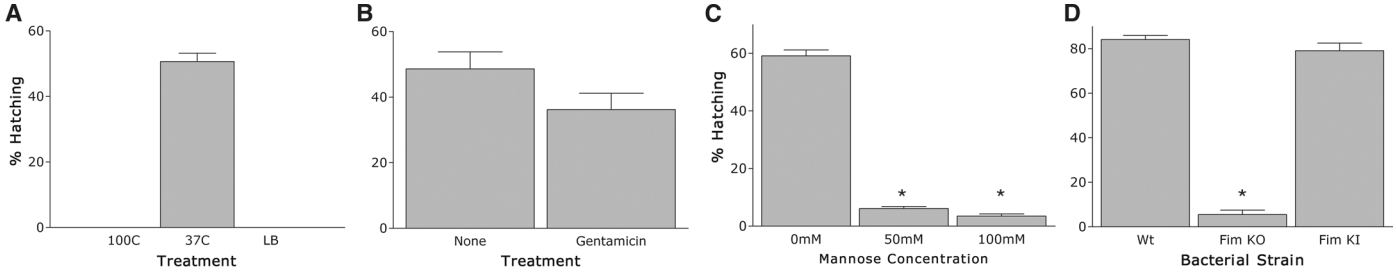


Fig. 1. *T. muris* eggs are induced to hatch in vitro by contact with bacteria. (**A**) Hatching of *T. muris* eggs for 2 hours at 37°C incubated with 5-cm sections of mouse cecum, *E. coli* bacterial suspension, or 0.22-μm filtered (overnight) bacterial suspension. Luria-Bertani (LB) broth was used as negative control. (**B**) *T. muris* eggs were cultured with four strains of bacteria (*E. coli*, *S. aureus*, *S. typhimurium*, or *P. aeruginosa*) for 2 hours at 37°C. (**C**) *T. muris* eggs incubated with *Saccharomyces cerevisiae*, with or without 0.4-μm transwell or 0.22-μm filtered *S. cerevisiae* cultures for 2 hours at 37°C. (**D**) Hatching of *T. muris* eggs in *E. coli* bacterial suspension or with 0.4-μm or 3-μm transwells. (**E**) *T. muris* eggs incubated for 2 hours with *E. coli* at 4°C, room temperature (RT), or 37°C. Eggs that did not hatch at 4°C or room temperature were then incubated for a further 2 hours at 37°C (4/37 and RT/37, respectively). (**F** to **H**) *T. muris* eggs were cultured at 37°C for 1 hour with GFP-expressing bacteria, then washed in phosphate-buffered saline before further incubation for 1 hour at 37°C. (F) Light-field image; (G) fluorescence image; (H) combined image. Scale bar, 10 μm. All figures show means ± SEM, *$P < 0.01$, using analysis of variance (ANOVA).

Trichuris is a genus comprising more than 50 species of whipworm, an extremely prevalent and successful group of intestinal-dwelling nematode parasites infecting many diverse mammalian hosts, with *T. trichiura* estimated to infect almost 1 billion people (*13*). All *Trichuris* species inhabit the large intestine (cecum and colon). Infection proceeds upon ingestion of embryonated eggs from the external environment. Upon hatching, the larvae emerge from polar egg opercula and establish infection within the epithelium of the crypts of Lieberkühn of the cecum and colon. Following the characteristic four molts, dioecious adult parasites develop to patency (at a rate dependent on the host), mate, and release unembryonated eggs into the environment via the feces. Here, we investigated a bacterially driven mechanism of hatching of mouse whipworm, *T. muris*, that facilitates infection of the mammalian host and subsequent immune response.

T. muris eggs were induced to hatch in vitro when incubated for at least 30 min with explants of mouse cecum containing substantial numbers of bacteria at 37°C (Fig. 1A). To define the role of bacteria in this process, we incubated eggs in a culture of *Escherichia coli*, a common gut commensal. In the presence of *E. coli*, hatching was observed at a similar level to that seen with gut explants; 0.4-μm filtration to remove *E. coli* from cultures prevented hatching and showed that a structural component of the bacteria, not a secreted molecule, was responsible for the hatching (Fig. 1A). Further analysis confirmed that a variety of microorganisms (five strains of bacteria and one of yeast) could induce efficient hatching over 2 hours to levels comparable with that found with



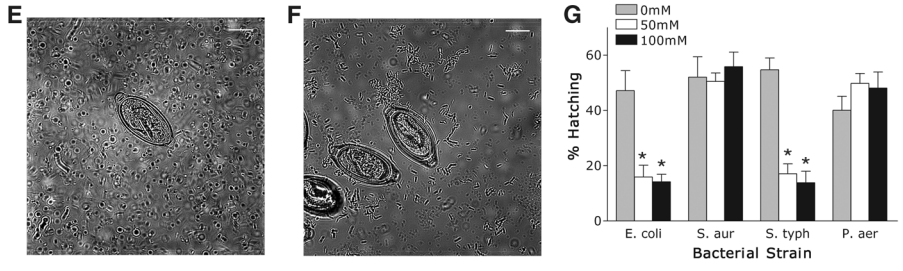


Fig. 2. *T. muris* eggs are induced to hatch in vitro after contact with type 1 fimbriae. (**A**) *T. muris* eggs were incubated for 2 hours at 37°C with *E. coli* preincubated for 10 min at 37° or 100°C. LB broth alone was used as a negative control. (**B**) *T. muris* eggs were incubated for 2 hours at 37°C with *E. coli* pretreated for 30 min with gentamicin (600 μg/ml) or untreated as a control. (**C**) *T. muris* eggs were incubated for 2 hours at 37°C with *E. coli* treated with 0, 50, or 100 mM mannose 30 min before and during hatching. (**D**) *T. muris* eggs were incubated for 2 hours at 37°C with wild-type *E. coli* (strain PK1162), FimKO *E. coli*, or FimKI *E. coli*. (**E** and **F**) *T. muris* eggs were incubated with FimKO (E) or FimKI (F) *E. coli* for 1 hour at 37°C. Scale bar, 10 μm. (**G**) *T. muris* eggs after 2 hours of incubation at 37°C with *E. coli*, *S. aureus*, *S. typhimurium*, or *P. aeruginosa* plus 0, 50, or 100 mM mannose before and during hatching. All figures show means ± SEM, *$P < 0.001$ using ANOVA.

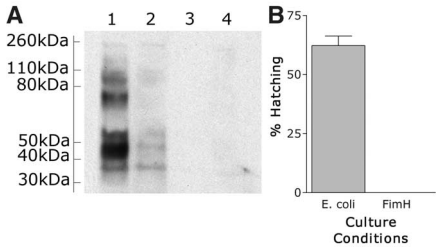


Fig. 3. (**A**) Far Western blot analysis of purified FimH binding to surface egg proteins. *T. muris* eggs are induced to hatch in vitro after contact with FimH adhesion on type 1 fimbriae. Egg proteins were subjected to SDS–polyacrylamide gel electrophoresis under reducing conditions and electrophoretically transferred onto nitrocellulose. The migration positions of protein standards are indicated on the left. Lane 1, purified FimH and secondary antibody; lane 2, purified FimH pre-incubated with mannose to specifically block binding; lane 3, purified FimH only (no secondary); lane 4, secondary only. (**B**) Purified FimH does not by itself cause hatching of *T. muris* eggs.

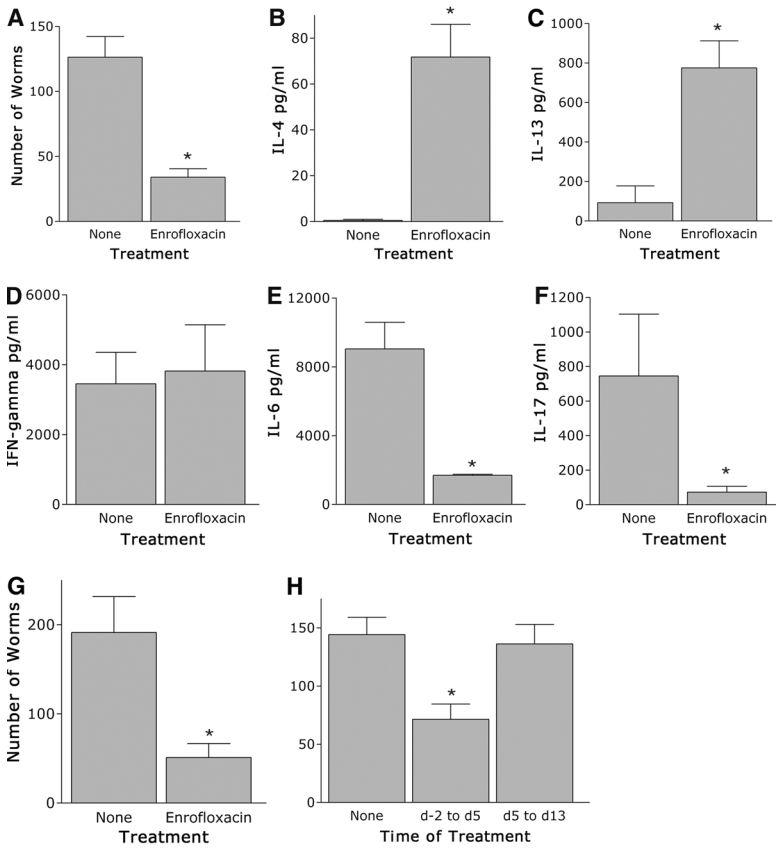


Fig. 4. *T. muris* eggs are induced to hatch in vivo by the presence of bacteria. (**A**) Worm burden at day 18 p.i. in AKR mice treated with enrofloxacin (Baytril) from day −2 p.i. to day 18 p.i. Untreated AKR mice were used as controls. (**B**) IL-4 secretion by antigen restimulated mesenteric lymph node cells from enrofloxacin-treated and untreated control AKR mice at day 18 p.i. assessed by cytokine bead array. (**C**) IL-13 secretion. (**D**) IFN-γ secretion. (**E**) IL-6 secretion. (**F**) IL-17 secretion. (**G**) Worm burden at day 18 p.i. in SCID mice treated with enrofloxacin from day −2 p.i. to day 18 p.i. Untreated SCID mice were used as controls. (**H**) Worm burden at day 14 p.i. in AKR mice treated with enrofloxacin at the beginning only (day −5 to day 5) or end only (day 5 to day 13) of infection. Untreated AKR mice used as controls. All figures show means ± SEM, *$P < 0.01$ by ANOVA.

gut explants over 18 hours (Fig. 1, B and C). By using transwells of different sizes, it was possible to confirm that direct contact between the bacteria and the eggs was required for hatching (Fig. 1, C and D). Bacterially promoted hatching only occurred at 37°C, suggesting that temperature is also a hatching cue (Fig. 1E), presumably to prevent hatching in the external environment where *T. muris* eggs embryonate. To locate the site of interaction between the bacteria and parasite eggs, we incubated the green fluorescent protein (GFP)–expressing *E. coli* strain PK1162 (*14*) with embryonated eggs. Bacteria clearly cluster around the opercula at the poles of the eggs (Fig. 1, F to H) where the worms emerge (movie S1).

Structural disruption of *E. coli* (boiling, 10 min) prevented hatching (Fig. 2A), although after bacteriostatic antibiotic treatment (gentamicin), *E. coli* were still able to induce hatching (Fig. 2B) but were not viable. Taken together, the data support a role for the intact bacterial surface as a critical component of the hatching process.

Type 1 fimbriae facilitate mannose-sensitive adherence of *E. coli* to cells and mucosal surfaces (*15*). They are encoded by the *fim* gene cluster and consist of a major structural subunit (FimA) and several minor components including the adhesin FimH located at the fimbrial tip that recognizes terminally located D-mannose moieties on cell-bound and secreted glycoproteins (*16*, *17*). To elucidate whether type 1 fimbriae played a role in hatching, we investigated the effect of exogenous mannose on hatching. The addition of mannose significantly inhibited hatching without affecting the numbers or viability of bacteria (Fig. 2C and fig. S1). Likewise, strain AAEC072A (FimKO), which lacks the *fim* gene cluster and does not express type 1 fimbriae (*18*) (fig. S2), did not promote hatching (Fig. 2D), although viability of these bacteria was comparable to that of wild-type Fim^+ *E. coli* strain PK1162 (fig. S3). In contrast, mannose-sensitive hatching was seen with strain AAEC072A harboring plasmid pLB254 (FimKI) encoding the cloned *fim* gene cluster (Fig. 2D and fig. S2). In contrast to the strains expressing type 1 fimbriae that associate with the opercula at the poles (Fig. 1, F to H, and Fig. 2, E and F), strain AAEC072A failed to adhere to the eggs (Fig. 2, E and F), confirming a role for type 1 fimbriae in mediating interactions between the parasite eggs and the bacterium. The observation that *Salmonella typhimurium* also exhibited mannose-sensitive hatching similar to that seen with *E. coli* (Fig. 2G) indicates that bacterially induced hatching is partly mediated by type 1 fimbriae. However, the Gram-negative bacterium *Pseudomonas aeruginosa* induced hatching in the presence of mannose and does not express type 1 fimbriae. Similarly, the ability of the Gram-positive bacterium *Staphylococcus aureus* to promote hatching indicates that other mechanisms also exist.

His-tagged FimH was purified (fig. S4) and used in a far Western blot analysis of solubilized surface egg proteins. Five to seven surface proteins were detected that bound to purified FimH, with the most intense signal generated from a protein of 45 kD (Fig. 3A). Mass spectrophotometry of these different bands and data mining of an expressed sequence tag database (NEMBASE) have identified putative proteins of *T. muris* and other parasitic nematodes, although further categorization will depend on sequencing of the *T. muris* genome. Purified FimH itself did not cause hatching of *T. muris* eggs (Fig. 3B), suggesting that some structural conformation, perhaps cross-linking by multiple FimH adhesins, is required. In addition, it is known that type 1 fimbriae mediate shear-dependent adhesion typified by weak binding under conditions of low flow that strengthens as the shear forces increase (*17*). Thus, it is perhaps not surprising that FimH on the bacterial surface behaves differently from the purified molecule. It is known that interaction between FimH and uroplakin molecules on the cell surface of uroepithelial umbrella cells or β_1 integrins on fibroblast cells is known to induce a signal transduction cascade (*19*). It is possible that the interaction between FimH and a mannosylated receptor on the surface of the *T. muris* eggs stimulates a

signal transduction cascade that leads to the emergence of the worm out of the egg.

Although the data clearly show that hatching in vitro requires an interaction between bacteria and the egg via type 1 fimbriae, it is unclear whether this is also important in vivo. Thus, we treated AKR mice with enrofloxacin from 2 days before infection throughout the course of infection to ascertain whether there was any effect on worm establishment. At day 21 post-infection (p.i.), the worm burden was significantly ($P < 0.001$) decreased in animals that had been treated with antibiotics (Fig. 4A). Interestingly, these animals made a stronger T_H2 response to infection, with increased levels of interleukin-4 (IL-4) (Fig. 4B) and IL-13 (Fig. 4C). Interferon-γ (IFN-γ) secretion was unchanged (Fig. 4D). Parasite-specific immunoglobulin G1 (IgG1) antibody production was increased in antibiotic-treated animals, reflecting the increased T_H2 response, whereas IFN-γ–controlled IgG2a levels were unaffected, reflecting the IFN-γ response (fig. S5). IL-17 has been found to be related to bacterial presence in the small intestine (*20*) and is significantly reduced in animals treated with antibiotics. Similarly, in our experiments, IL-17 expression was reduced in antibiotic-treated animals (Fig. 4F), as was IL-6, which drives IL-17 production (Fig. 4E).

To rule out a major role for acquired immunity in worm reduction, we infected severe combined immunodeficient (SCID) mice and treated them with enrofloxacin throughout. At day 18 p.i., worm burdens were reduced in antibiotic-treated mice (Fig. 4G), demonstrating that it is not the increased T_H2 response that is solely responsible for the worm expulsion observed. A comparison of antibiotic treatment regimes in susceptible mice (treatment from day –5 to day +5 p.i. or day +7 to day 13 p.i.) clearly showed that depleting the microflora affected the establishment and not the subsequent survival of the worms within the intestine at day 14 p.i. (Fig. 4H). All animals treated with antibiotics had decreased aerobic and anaerobic flora as measured by growth on LB agar (fig. S6).

The paradigm that the adaptive immune system has evolved to control microbes has been modified to include the concept that the immune system is in fact controlled by microorganisms. Our results constitute evidence to suggest that we must extend this paradigm again to include "macrofauna," the metazoan parasites such as *Trichuris*. We can clearly see a critical relationship between bacteria and *T. muris* that has a marked effect on the host immune response to this parasite. We propose that temperature, together with an increase in bacterial load at these sites, provides optimum conditions for efficient hatching of *Trichuris* species eggs. Additionally, controlled damage of the intestinal epithelium provides an enhanced potential route of access for microflora to the adaptive immune system. This has subsequent beneficial consequences for the host, in that antibacterial T_H17 responses and T_H1 responses are generated that are known to control pathogenic bacterial pathogens (*20*, *21*). The induction of T_H17 and T_H1 responses in the gut is closely associated with the induction of regulatory T cell subsets (*22*). These are known to have beneficial effects for survival of long-lived chronic helminth infections in the absence of overt pathology and indeed are central to the tenet of the modified hygiene hypothesis (*8*). It is clear that in nature, coevolution of microflora of the gut and host should not be considered in the absence of the influence of macrofauna such as gastrointestinal-dwelling nematode parasites.

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Supporting Online Material

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Defective Cross-Presentation of Viral Antigens in GILT-Free Mice

Reshma Singh and Peter Cresswell*

Gamma-interferon–inducible lysosomal thiolreductase (GILT) promotes major histocompatibility complex (MHC) class II–restricted presentation of exogenous antigens containing disulfide bonds. Here, we show that GILT also facilitates MHC class I–restricted recognition of such antigens by $CD8^+$ T cells, or cross-presentation. GILT is essential for cross-presentation of a $CD8^+$ T cell epitope of glycoprotein B (gB) from herpes simplex virus 1 (HSV-1) but not for its presentation by infected cells. Initiation of the gB-specific $CD8^+$ T cell response during HSV-1 infection, or cross-priming, is highly GILT-dependent, as is initiation of the response to the envelope glycoproteins of influenza A virus. Efficient cross-presentation of disulfide-rich antigens requires a complex pathway involving GILT-mediated reduction, unfolding, and partial proteolysis, followed by translocation into the cytosol for proteasomal processing.

Cross-priming (*1*) is important for the development of specific $CD8^+$ T cell responses to viruses that do not directly infect antigen-presenting cells (APCs) (*2*). The critical APCs for cross-presentation are dendritic cells (DCs), which acquire antigens by phagocytosis of apoptotic and necrotic infected cells and migrate to secondary lymphoid organs to activate resident naïve $CD8^+$ T cells (*3*). Transfer of antigen from migratory DCs to resident $CD8\alpha^+$ DCs may be required (*4*, *5*). The pathways that generate complexes of MHC class I molecules with peptides derived from internalized antigens are not well understood. Occasionally the peptides are generated in the endocytic pathway and bind to recycling MHC class I molecules (*6*). However, the dominant mechanism involves translocation of the antigens into the cytosol, where proteasomal degradation generates peptides that

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