Commentary

Bacterial menageries inside insects

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Bacteria display a wide diversity of specialized interactions with eukaryotic hosts. A common way to classify bacterial–eukaryote associations is along an axis from pathogenic to mutualistic: that is, how much does the infection help or hurt the host relative to noninfected individuals? From the host perspective, this distinction is central. But for the bacteria themselves, pathogenesis and symbiosis present similar obstacles. Primary among these is the invasion of host cells or tissues in the face of generalized cellular and chemical defenses. Remarkably, studies during the past decade of a number of phylogenetically diverse pathogens of plants and animals have shown that host invasion often depends on a set of evolutionarily homologous genes that have been transferred among distinct bacterial lineages (1–3). This group of loci encodes the Type III secretion system, a complex of about 20 proteins that together form a membrane-spanning structure that functions in transporting proteins outside of the bacterial cell (1–3). Transport is, at least sometimes, induced by contact with host cells, and the effectors are delivered to the host cytoplasm and linked to suppression of host defenses and/or invasion of host cells.

A new study by Dale et al. (4), reported in this issue of PNAS, shows that Sodalis glossinidius, an intracellular symbiotic bacterium restricted to tsetse flies (Glossinia spp.), possesses a Type III secretion system and that this system is essential for entry of the bacteria into cultured insect cells and for infection of intact hosts. Sodalis is one of a large set of bacteria that are maternally transmitted in invertebrate lineages and that are mutualistic or not obviously pathogenic. This is the first instance in which an animal symbiont has been shown to use a secretory system associated with pathogenesis for host infection. Clearly, we still have incomplete knowledge of the taxonomic distribution of Type III systems and

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of the complete range of ecological interactions mediated by these genes. The discovery by Dale et al. extends the role of this particular transport apparatus to a broader range of interactions between bacteria and animal hosts.

The bacteria harboring Type III systems are phylogenetically diverse and include members of several major bacterial divisions, with a concentration of known cases in the Enterobacteriaceae, the group containing Escherichia coli and close relatives (1–3). Among animal pathogens, Type III secretion systems have been found in Yersinia pestis (the organism causing plague), Salmonella enterica (the organism causing typhus), Shigella spp. (the organism causing dysentery), and the more distantly related genus Chlamydia (the organism causing sexually transmitted and other diseases). In plants, gene clusters encoding Type III systems occur in various Erwinia species, Pseudomonas syringae, and other disease-causing bacteria, and also in symbiotic Rhizobium species that function in nitrogen fixation. These gene complexes are typically encoded within pathogenicity islands, that is, segments of chromosomal DNA that are present in pathogens but absent from related nonpathogens and that are required for virulence (5). In many cases, pathogenicity islands are known to have been acquired through horizontal transfer of DNA; this is evident from sequence characteristics such as distinctive GC content, flanking regions with insertion sequences, or an association with known sites of phage integration.

The findings on the Type III system in Sodalis reflect a history of horizontal transfer of this gene cluster among hosts; the very low divergences between genes from Sodalis and those from Salmonella and Shigella suggest that the common ancestor of these genes occurred more recently than divergence of the bacteria

themselves. Although all fall within the Enterobacteriaceae, phylogenies and sequence divergences based on 16S rDNA sequences do not indicate an extremely close relationship between Sodalis and the other two genera. Because 16S rDNA genes are thought not to be horizontally transferred among bacteria, this incongruence implies the transfer of the Type III gene cluster. Although Type III systems have long been known to be acquired by bacterial pathogens, Sodalis is transmitted primarily through vertical passage from mother to developing progeny and is possibly beneficial or at least not obviously harmful to hosts. Thus, the capacity for intimate symbiosis may have evolved within a pathogenic lineage possessing these genes, or it may have been acquired when a bacterium more loosely associated with insect hosts obtained this gene cluster from some other bacterium, most likely a pathogen.

The assays of infectivity that were crucial to the Dale et al. study (4) were possible because of the prior development of methods for maintaining Sodalis in pure culture and in insect cell lines. In most cases, symbiotic, intracellular bacteria cannot be cultured, severely limiting experimental studies of how they infect hosts. Despite the inability to culture most of these organisms, there has been a recent dramatic increase in knowledge of the diversity and genomic composition of symbionts, especially those that are maternally transmitted and intracellular in insects (6–9). This increase is based on ability to obtain DNA sequences from noncultivable organisms and on phylogenetic analyses of these sequences. In many insects, including tsetse flies, other blood-feeding insects and many phloem sap-feeding insects, an individual host contains an obligate association with a "primary" symbiont as well as additional associations, sometimes facultative, with organisms sometimes referred to as "secondary" symbionts (10). The classification as

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primary or secondary symbiont types was established on the basis of early microscopy studies, with primary symbionts defined as obligate associates occupying specialized host cells and secondary symbionts encompassing a diverse set of associates occupying various tissues and often showing more erratic distribution among hosts (10).

The diversity of bacterial residents within insect bodies spans a variety of interaction types and evolutionary patterns. The organisms classified as primary symbionts often descend from ancient infections based on molecular phylogenetic studies of the last decade (e.g., refs. 6, 11, and 12). For example, *Buchnera aphidicola*, the primary symbiont of aphids, has been vertically transmitted for over 100 million years and shows various genomic changes, some reflecting mutualistic adaptations for provisioning hosts with amino acids lacking from their phloem sap diet (e.g., refs. 6, 8, and 13). Tsetse flies also contain primary symbionts, *Wigglesworthia glossinidia* (14), and although less is currently known about their genetics and role in hosts, they resemble *Buchnera* in aspects of their molecular evolution and show a long history of codiversification with their hosts (11). In contrast, phylogenetic studies on secondary symbionts such as *Sodalis* often indicate a history of horizontal infection in addition to the usual maternal route. The effect of such organisms on host fitness, that is whether they are mutualists or pathogens, is usually not as clear as in the case of primary symbionts. Another widespread group of symbionts, present in many (15) or even most (16) insects is *Wolbachia pipientis*, a maternally transmitted symbiont linked to reproductive abnormalities. Horizontal transfer of *Wolbachia* is well established based on phylogenetic and experimental studies (e.g., refs. 17 and 18).

Based on molecular phylogenetic analyses, the diversity of insect symbionts is particularly high within the gamma Proteobacteria and especially within the Enterobacteriaceae. Whereas *Buchnera* and *Wigglesworthia* fall just outside of the Enterobacteriaceae, a whole menagerie of insect symbionts, including *Sodalis*, are grouped into several independently derived clades within it (19–21). The heterogeneity of the insect hosts infected by each of these clades indicates that symbiont lineages are able to move among insect host species, including both closely related species within genera or families and, at least occasionally, members of different orders. For example, the closest relative of *Sodalis* is a symbiont of *Sitophilus* weevils feeding on stored grain. Another clade contains the symbiont of the bloodsucking bug *Triatoma infestans*, a symbiont causing reproductive abnormalities in parasitoid wasps, and several secondary symbionts of psyllids (20). Such observations indicate direct or indirect transfer of bacterial symbionts between these phylogenetically and ecologically divergent insect groups. Why have certain bacterial groups specialized as intimate associates of insects when these hosts are so heterogeneous?

Mutualistic symbionts and pathogens share similar problems in addition to that of establishing infections and overcoming defenses. In pathogens as well as mutualistic bacteria, limitation of damage to host cells is usually essential for persistence and subsequent spread to new hosts. Selection against virulence is higher in life cycles that require persistence within individual hosts. This pressure for decreasing damage and increasing benefits to hosts will be especially great if the infection is transmitted between successive host generations. Thus, pathogens sometimes may evolve into mutualists following the establishment of vertical transmission. However, the transition into effective mutualists requires that the bacterial genome possess the capacity for processes beneficial to hosts, such as provision of nutrients (22).

Virulence is expected even in maternally transmitted symbionts when there is both some incidence of horizontal infection and a correlation between virulence and infectivity. But experimental determination of whether or not a chronic infection by a bacterium has a net positive or negative effect is not simple. For example, *Wolbachia* often affects insect reproductive mechanisms, but it can be essentially benign and may sometimes even improve host fecundity (e.g., ref. 23). The single report on fitness effects of insect secondary symbionts from the Enterobacteriaceae yielded mixed results: infected aphids had lower survival and reproduction under normal conditions but showed improved performance under high temperatures (24).

A full genome sequence for *Buchnera*, the mutualistic symbiont of aphids, is now available (8), and an obvious question is whether Type III or other pathogenicity associated gene complexes are present. Although this genome, which is highly reduced, does not possess a secretory system known to be linked with pathogenesis, it does contain a set of genes that are related but closer in sequence similarity to the flagellar export machinery of bacteria (designated as the *fli* genes). The function of these genes in *Buchnera* is not yet known, so it is not clear whether this obligate mutualism was originally dependent on the ancient acquisition of a gene cluster underlying a protein transport system.

This result adds to the general case that origins of intimate associations between bacteria and eukaryotes often depend on the acquisition of specialized genetic machinery. This proposal is supported also by the recent finding of a Type IV secretion system in *Wolbachia* (25). A large proportion of specialized bacterial–host interactions, involving manipulation of cellular responses and/or entry into host cells, may depend on a small number of evolutionary origins of complex secretory systems. These gene clusters were subsequently passed around among bacteria to produce a diversity of pathogenic and symbiotic interactions.

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1. Cheng, L. W. & Schneewind, O. (2000) *Trends Microbiol.* **8,** 214–220.
2. Galán, J. E. & Collmer, A. (2000) *Science* **284,** 1322–1328.
3. Hueck, C. J. (1998) *Microbiol. Mol. Biol. Rev.* **62,** 379–433.
4. Dale, C., Young, S. A., Haydon, D. T. & Welburn, S. C. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 1883–1888. (First Published January 30, 2001; 10.1073/pnas.021450998)
5. Groisman, E. A. & Ochman, H. (1996) *Cell* **87,** 791–794.
6. Moran, N. A. & Baumann, P. (2000) *Curr. Opin. Microbiol.* **3,** 270–275.
7. Baumann, P., Moran, N. A. & Baumann, L. (2000) in *The Prokaryotes*, ed. Dworkin, M. (Springer, New York).
8. Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. & Ishikawa, H. (2000) *Nature (London)* **407,** 81–86.
9. Fukatsu, T. & Nikoh, N. (2000) *Appl. Environ. Microbiol.* **66,** 643–650.
10. Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms* (Wiley, New York).
11. Chen, X., Li, S. & Aksoy, S. (1999) *J. Mol. Evol.* **48,** 49–58.
12. Thao, M. L., Moran, N. A., Abbot, P., Brennan, E. B., Burckhardt, D. H. & Baumann, P. (2000) *Appl. Environ. Microbiol.* **66,** 2898–2905.

13. Lai, C. Y., Baumann, L. & Baumann, P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3819–3823.
14. Aksoy, S. (1995) *Int. J. Syst. Bacteriol.* **45,** 848–851.
15. Werren, J. H. & Windsor, D. M. (2000) *Proc. R. Soc. London B* **267,** 1277–1285.
16. Jeyaprakash, A. & Hoy, M. A. (2000) *Insect Mol. Biol.* **9,** 393–405.
17. Huigens, M. E., Luck, R. F., Klaassen, R. H., Maas, M. F., Timmermans, M. J. & Stout

hamer, R. (2000) *Nature (London)* **405,** 178–179.
18. Vavre, F., Girin, C. & Bouletreau, M. (1999) *Insect. Mol. Biol.* **8,** 67–72.
19. Hypsa, V. & Aksoy, S. (1997) *Insect. Mol. Biol.* **6,** 301–304.
20. Thao, M. L., Clark, M. A., Baumann, L., Brennan, E. B., Moran, N. A. & Baumann, P. (2000) *Curr. Microbiol.* **41,** 300–304.
21. Sandström, J. P., Russell, J. A., White, J. P.

& Moran, N. A. (2001) *Mol. Ecol.* **10,** 217–228.
22. Moran, N. A. & Wernegreen, J. J. (2000) *Trends Ecol. Evol.* **15,** 321–326.
23. Vavre, F., Fleury, F., Lepetit, D., Fouillet, P. & Bouletreau, M. (1999) *Mol. Biol. Evol.* **16,** 1711–1723.
24. Chen, D. Q., Montllor, C. B. & Purcell, A. H. (2000) *Entomol. Exp. Appl.* **95,** 315–323.
25. Masui, S., Sasaki, T. & Ishikawa, H. (2000) *J. Bacteriol.* **182,** 6529–6531.