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Hijacking the Cell: Parasites in the Driver's Seat

Minireview

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Protozoan parasites have evolved a remarkable ability to survive in their hosts, often for long periods of time and sometimes with minimal pathogenicity. The mechanisms employed are as diverse as the evolutionary origins of this group, which can be appreciated by examining any of the recently developed "trees of life" based on slowly-evolving molecules such as ribosomal RNAs (Sogin et al., 1989). Protozoan parasites emerge from the most ancient eukaryotic lineages, with branches much deeper than those observed among the younger metazoan kingdoms of fungi, plants, and animals. Reflecting their antiquity, the study of protozoans has yielded abundant examples of molecular novelty, including new organelles (hydrogenosomes in trichomonads, glycosomes and kinetoplast DNA networks in trypanosomatids, rhoptries and micronemes in apicomplexans) and molecular phenomena (bent DNA, glycosylphosphatidyl inositol (GPI) protein anchors, *trans*-splicing, RNA editing, programmed DNA rearrangements). Especially for the latter, their discovery in protozoa often has led the way to appreciation of their importance in "higher" creatures.

Most parasitic lineages derive from non-parasitic ancestors, and during the course of evolution each has solved the survival puzzle in a different manner. Traditionally, studies have focused upon how parasites resist host defenses, by antigenic variation, resistance to complement and immunological attack, and/or escape to "safe" compartments such as the host cell cytoplasm. What we are now learning is the extent to which intracellular parasites have become masters at manipulating the structure and pathways of the host cell for their own nefarious purposes, in order to create a more hospitable environment. One strategy commonly taken is a major remodeling of host cell compartments, thereby radically changing the cell's architecture and function. Another is a sophisticated manipulation of host cell signaling pathways, inhibiting some in order to preclude hostile responses or, even more remarkably, activating others in order to exploit them for invasion and/or survival. How the parasites accomplish these feats is for the most part not well understood, and the emergence of genetic methods in these organisms over the last few years promises an exciting future in answering these questions.

Remodeling and/or Construction of Parasite-Friendly Compartments

Members of the protozoan phylum Apicomplexa, such as Toxoplasma gondii and the malaria parasite Plasmodium falciparum, display a remarkable ability to invade different types of host cells. Toxoplasma invades by an active actin-dependent mechanism (Dobrowolski and Sibley, 1996), and after entry establishes a parasitophorous vacuole with the assistance of products secreted by the parasite's apical rhoptry and microneme organelles. However, instead of undergoing normal vacuolar acidification brought about by combination with host cell lysosomes, the parasite intervenes to prevent membrane fusion. Simultaneously, it sets about gaining access to cellular nutrients by forming small pores in the vacuolar membrane (Schwab et al., 1994). In this manner it enjoys a rich and comfortable life-style at the host cell's expense.

Even more spectacular is the process conducted by malaria while entering the host's red blood cells, normally completely lacking intracellular membrane trafficking and endocytic pathways. Here, the parasite must force its way into the host cell, again with the aid of the products of its apical organelles, but now creating a parasitophorous vacuole completely de novo. Once inside, malaria then erects a new secretory pathway *outside* of itself, leading from the parasite and its parasitophorous vacuole, through the infected cell all the way to the erythrocyte surface, and complete with a variety of membranous components undoubtedly involved in trafficking (Haldar and Holder, 1993). Enzymes normally associated with the synthesis of the internal secretory pathway occur in the extracellular secretory pathway as well, suggesting that it may be synthesized at least partially in situ (Elmendorf and Haldar, 1994). The extracellular secretory pathway allows the parasite to radically modify and reshape the host cell. Proteins such as those encoded by the P. falciparum *var* gene family are deposited on the erythrocyte surface, and mediate sequestration of infected cells in the peripheral vasculature away from the protective clearing action of the spleen (Smith et al., 1995). This is a fantastic system in which the genesis of protein trafficking systems can be studied, and the use of the Green Fluorescent Protein in transfected parasites (Ha et al., 1996; Figure 1 shows an example for Leishmania) should facilitate this. The novelty of the extracellular pathway offers an attractive target for those inevitably seeking to treat this dreaded disease (Lauer et al., 1995).

Manipulation of Host Cell Signaling Pathways

Like Toxoplasma, the kinetoplastid protozoan Trypanosoma cruzi is able to invade a number of mammalian cell types. In doing so, the parasite must successfully interact with two different signaling systems. The first is the TGFβ pathway, which is essential for parasite invasion (Ming et al., 1995). The mechanism by which this pathway contributes to survival is unknown, and it raises the possibility that T. cruzi possesses intrinsic TGFβ activity of its own, the nature and origins of which promise to be of great interest. Reliance upon TGFβ-like signaling may represent an example of subversion by capture or mimicry of the hosts' own signaling stimuli, as observed in other microbial pathogens. The second

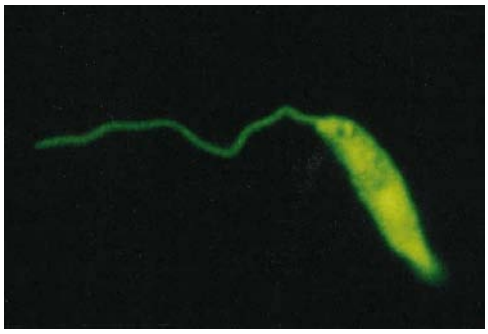

Figure 1. Leishmania major Expressing the Aequorea Green Fluorescent Protein

Reprinted from Ha et al., 1996, with kind permission from Elsevier Science-NL, Sara Burgerhaststraat 25, 1055 KV Amsterdam, The Netherlands. See Ha et al. (1996) for details and examples of fusion proteins targeted to specific cellular compartments.

pathway involves recruitment of host cell lysosomes to the site of entry of the parasite into the host cell, which are thought to play a role in invasion as well as subsequent differentiation (Burleigh and Andrews, 1995). This process is probably independent of the TGFβ pathway, and is triggered by a parasite-derived molecule activating a calcium/phosphoinositide-dependent host cell pathway. Once transiently ensconced in the entry vacuole, T. cruzi escapes into the protected environment of the cytoplasm with the aid of a pore-forming lysin, a strategy also taken by prokaryotic pathogens such as Listeria.

In contrast to the parasites above, members of the genus Leishmania are relatively lazy and passively invade cells with well-developed phagocytic abilities such as macrophages. The trade-off here becomes the dangers faced inside these specialized anti-microbial cells. Remarkably, Leishmania does not escape from the phagolysosome, nor does it remodel it extensively to be more hospitable; the parasitophorous vacuole remains replete with hydrolytic enzyme activities, and at a typically lysosomal acidic pH. Instead, the parasite has adapted to these conditions, managing in some manner to "tough it out." Contributing to this may be an abundance of glycoconjugates synthesized by the parasite, both prior to and after invasion, many of which are anchored to the surface membrane by modified GPI-anchors similar to those of certain membrane proteins (McConville and Ferguson, 1993). These defenses, however, are not sufficient to resist the attack that can be brought to bear by an activated macrophage, such as generation of a strong NO response. Thus, a successful parasite must avoid or interfere with activation. Correspondingly, Leishmania invasion has a pervasive inhibitory influence on many host signaling pathways, including those involving protein kinase C, JAK/STATs, calcium, inositol phosphates, and those leading to activation of c-fos, IL-12, and apoptosis (Carrera et al., 1996; Moore and Matlashewski, 1994; Reiner, 1994). Yet at the same time, other pathways are left relatively unperturbed (cAMP-dependent) or are even induced (synthesis of a subset of chemokines). Thus, Leishmania has deftly chosen among the pathways of the host cell those it wishes to inhibit, ignore, or activate, presumably in service of its need to survive within the host.

Lastly, the apicomplexan Theileria parva takes manipulation of host pathways to another level. Not only does the parasite survive intracellularly, but its infection of T and B cells leads to cellular transformation, similar to that seen in tumor cells (Williams and Dobbelaere, 1993). Infected host cells show activation of well known pathways of transformation, such as those involving casein kinase. In this manner, Theileria guarantees itself a permanent and expanding residence within the host.

The examples above are just a few of what promises to be a flood of new data highlighting the importance of how parasites actively manipulate the processes of the host cell. Indeed, it is likely that in addition to tinkering in fundamental ways with the architecture of the cell, protozoan parasites promise to be sophisticated probes of host cell signaling pathways, as they have already proven to be in understanding the regulation of immune cell subsets.

The Search for Molecules Implicated in Parasite Virulence and Pathogenesis

As our knowledge of pathways affected by parasites increases, attention turns now to the process of identifying the parasite molecules that mediate the remarkable events transpiring in the host cell. This will be familiar to students of viral and bacterial pathogenesis as the search for parasite "virulence factors." Already, application of the methods of biochemistry and molecular biology has yielded numerous attractive molecules for model-builders searching for virulence genes. Examples of these include a multitude of surface molecules well positioned to interface with the host, such as the malaria circumsporozoite protein which mediates attachment of the invading sporozoite to hepatocytes (Cerami et al., 1992), or any of a number of genes up-regulated in infective stages of parasites. The initiation of several parasite genome projects promises to expand this roster considerably.

Accompanying this has been the development of critical genetic tools for use in parasites. In the groups where progress has been greatest (trypanosomes, Leishmania, and Toxoplasma), one can now readily express or knockout genes, and carry out functional genetic rescue of interesting mutants. A variety of "bells and whistles" such as regulatable expression and artificial chromosomes are also available. Recent successes with the introduction of DNA into Entamoeba histolytica, Giardia lamblia, and malaria will undoubtedly lead to similarly rapid deployments there.

In 1988, Stanley Falkow, in recognition of the ability of molecular biology to provide an abundance of genes for which roles in pathogenesis could be envisioned, proposed a set of "molecular Koch's postulates" to serve as a guide in testing and assessing their role (Falkow, 1988). These may be summarized as follows: first, the property under study should be reasonably associated with pathogenicity or infectivity; second, that specific inactivation of a candidate gene should lead to a significant loss in virulence; and third, that restoration of gene function should fully restore pathogenicity. As many workers hopeful of knockout phenotypes in yeast and mammalian cells can testify, this stringent criterion can both satisfy and disappoint.

Thus far, tests analogous to those proposed by Falkow have not been widely applied in parasitic systems due to the recency of the necessary methodology. One example is that elimination of the rhoptry protein encoded by Toxoplasma *ROP1* failed to affect invasion (Kim et al., 1993). Of course, this may reflect the existence of compensatory or redundant pathways. In contrast, Leishmania mutants defective in the synthesis of the surface glycolipid lipophosphoglycan (LPG) show an inability to survive in both the insect vector and while establishing infections in the macrophage. Here, the combined efforts of several labs have shown that restoration of the defective gene can restore survival. Other examples will undoubtedly be forthcoming. The application of Falkow's tests in parasite systems is particularly important, as culture and genetic manipulations can lead to changes in virulence unrelated to the planned genetic modifications.

The marriage of insights gained into the mechanisms and cell biology of parasitism, and the arrival of much needed genetic tools for identifying and dissecting the genes carrying out these processes, assures us that the next few years will be one of the most exciting periods in molecular parasitological research.

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