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How Does Trichinella spiralis Make Itself at Home?

The nerve cell-parasite complex of Trichinella spiralis is unlike anything else in Nature. It is derived from a normal portion of striated skeletal muscle cell and develops in a matter of 15 to 20 days after the larva invades that cell type. What are the molecular mechanisms at work that result in this unique relationship? Here, Dickson Despommier presents a hypothesis to account for its formation, in which secreted tyvelosylated proteins of the larva play a central role. These proteins are always present in the intracellular niche of the larva from Day 7 after infection and may be responsible for redirecting host genomic expression, leading to nerve cell formation.

The list of parasitic strategies infecting humans is long and rich in diversity. Within each of us, numerous fundamental, niches are occupied. While striped skeletal muscle tissue ranks as one of the most abundant, only a handful of protozoans and helminths have been successful in colonizing this niche. For example, among the numerous species of protozoa, only a few (eg. Trypanosoma cruzi, Toxoplasma gondii, Trichinella spiralis, Sarcocystis sp. and Hepatoplasma sp.) have succeeded. A smaller number of helminth species, mostly larval stages of cestodes, and even fewer species of larval nematodes, have found a home there. Nematodes in the genus Trichinella are the remarkable exception, with four recognized species (Trichinella spiralis, T. nativa, T. britovi and T. pseudospiralis), and more likely to achieve species status, that not only live and thrive there, but have in all likelihood evolved complex strategies for remodel- ing that niche into one that they can occupy for many months to years. Unlike the majority of intracellular parasites, Trichinella occupies the host cell without killing it, and thus it is considered one of the most successful of all parasitic symbions, because it is this strategy that enables it to travel world-wide and extend its range into all parts of the earth in which the scavenging of carrion occurs. By what mechanism(s) does this nematode accomplish its goal of long-term survival? As alluded to, one plausible hypothesis is that the parasite is responsible for remodeling the muscle cell, and does so by secretting a variety of proteins into its intracellular niche, resulting in a reprogramming of host genomic expression. There are several lines of indirect evidence in support of this view, in addition to

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the fact that no other skeletal muscle cell myopathy remotely resembles the complexity of permanent changes associated with those encountered during
nurse cell formation.

The hypothesis predicts that after the larva enters
the muscle cell it assumes the role of both architect
and construction foreman, informing the host via its
peptides how to go about changing its new surround-
ings. The result is the nurse cell\(^1\), a dramatically
altered portion of infected myocyte devoid of muscle-
specific proteins (Fig. 1a) that is multinucleated (Fig.
1b), and whose presumed function is to support the
growth, development and maintenance of the para-
site relationship in which the parasite remains
metabolically active\(^4\) are nutrient acquisition and
parasite relationship in which the parasite remains
maintenance

Capsule collagen synthesis

The nurse cell–parasite complex is surrounded by
a collagen capsule\(^6\) and consists predominantly of
two collagen types, IV and VI (Fig. 1d and e), both of
which are synthesized by the nurse cell\(^7\). Parasite
secretion of proteins within the matrix of the infected
host cell begins on Day 7 after infection\(^2\) (Fig. 1h).
The onset of host collagen type IV and type VI mRNA
synthesis is between Days 7 and 8. By Day 8, parasitic
peptides localize to the nucleoplasm of all enlarged
nurse cell nuclei\(^7,8\). Hence, upregulation of these two
genes is temporally coincident with peptide
secretion. Throughout the period of collagen synthe-
sis, all enlarged nuclei remain transcriptionally active,
resulting in the overexpression of these two collagen
proteins. Collagen type IV synthesis then ceases on
about Day 26, while synthesis of type VI collagen
continues throughout the infection at a low level\(^6\).
Thus, each of these two host genes appears to be
under separate regulatory control mechanisms.

Angiogenesis in nurse cell formation and
maintenance

Two essential requirements of any long-term host-
parasite relationship in which the parasite remains
metabolically active\(^1\) are nutrient acquisition and
waste disposal. It is likely that \(T. \) spiralis accomplishes
these two tasks in one operation; namely by attracting
a highly permeable set of blood vessels (ie. the circu-
laratory rete) to the surface of the outer collagen cap-
sule (Fig. 2)\(^9–11\). In this way, the larva could assure a
constant source of small molecular weight metab-
olites for itself, while ridding its living space of meta-
abolic byproducts. The mechanism(s) by which the worm accomplishes this is by induction of the angi-
genic program\(^12\). This may involve an initial hypoxic
event\(^15\) early on within the nurse cell. Hypoxia in many
situations (eg. wound healing and tumorigenesis) leads
to upregulation of vascular endothelial growth factor
(VEGF), which in turn elicits the construction of new
vessels. We detected VEGF mRNA by \(in situ\) hybrid-
ization in the cytoplasm of the developing nurse cell
beginning on Day 7 (Fig. 1h), up to eight months after initial
infection of the muscle cell\(^14\). The presence of
VEGF peptide was observed shortly thereafter, begin-
ning on Day 9 (Fig. 1g) using immunohistochemical
methods, and was demonstrable within the nurse cell
from that point on. Thus, the VEGF gene remains
upregulated throughout the infection period, while
the mRNA signal appears to be strongest at Day 15. A
constant, low level of production of VEGF peptide
(also known as vascular permeability factor) after cir-
culatory rete formation is complete implies a perma-
nently heightened state of vascular permeability, and
would present obvious advantages to the parasite for
maintaining itself within the host for long periods of
time.

The vessels of the circulatory rete are now known
to be derived from adjacent venules, not arterioles as
was thought previously\(^2\). This may involve a
rapid exchange of nutrients and wastes, but offers less
than optimal conditions for the efficient exchange of gasses
between the nurse cell and the red blood cells that
circulate past it. These observations are consistent
with data collected from a variety of experimental ap-
proaches indicating that larval and nurse cell (Fig. 1c)
energy metabolism are anaerobic\(^15\). This metabolic
strategy explains how the parasite remains infectious
for another host (ie. scavenger) from days up to
weeks after the death of the infected host (depending
upon the ambient temperature) in its decaying muscle
tissue – the ultimate in anaerobic environments. This
phenomenon is also seen under laboratory conditions\(^26\).

Information exchange

The comparison between building a house and
constructing a nurse cell is an especially attractive
one, because at the heart of the relationships between
the host and the parasite and a new home buyer and
their contractor is the requirement that they commu-
nicate with one another. Without the exchange of
information the possibilities for long-term relation-
ships are greatly reduced, provided that the organism
in question remains metabolically active (ie. not
encysted or dormant, as is the case for larval \(T. \) taeniis
sp. and pseudocysts of \(T. \) gondii, or even latent viruses).

Intravital microscopy has revealed that \(T. \) spiralis
constantly moves about within its nurse cell\(^1\), slowly
rocking back and forth and probing its immediate
environment with its anterior end, expending energy
as it does so. The worm is anything but quiescent.
Thus, \(T. \) spiralis's ability to construct and especially
to maintain its nurse cell almost certainly depends
upon a common communication system.

Mammalian intercellular communication systems
depend upon a wide range of secreted signaling mol-
ecules\(^17,18\) (ie. cytokines), which direct specific cellular
behavior. Presumably, \(T. \) spiralis uses similar mol-
ecules to carry out its own developmental programs\(^3,9\).
In addition, however, it must instruct the host, most
probably using its secreted signaling molecules (Fig.
3), which 1 call 'parakines'\(^25\). The host cell then re-
sponds to those signaling molecules, enabling the
nurse cell to form. While the existence of parakines is
predicted based on the complex interactions that
occur between the mammalian cell and the worm
during nurse cell formation\(^19\), the precise function and
characterization have so far eluded molecular para-
sitologists. In fact, none of the sequences of any

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sary enzyme, tyvelose epimerase, to apparently does not possess the necessary enzyme. The genital primordium nematodes found only in the order Trichurata. The genital primordium is a unique structure among highly specialized organ, the stichosome. The stichosome consists of about 50 stichocytes35. Each of the cell types – five have been identified based on electron microscopy studies on the morphology of their granules – synthesize secretory granules of a single variety, while each granule type contains many novel peptides29. Some of these peptides are secreted during the muscle phase35,36, while others are stored and then secreted during the early intestinal phase37,38. As mentioned, it is not certain which peptides function in either phase of the life cycle, as only a few have been studied. The adult worm version of the stichosome is completely different from that of the larva in that each of its 50 stichocytes contain secretory granules that have no morphological equivalent to the larva. For example, none of the stichocyte-specific secreted peptides of the adult parasite are tyvelosylated29. Therefore, it is unlikely that the larva uses its tyvelosylated secreted proteins to gain entrance into its intracellular niche in the small intestine after being swallowed by the next host, as some have suggested40, because the adult can locate there without loss of fecundity when transferred from one animal to another through oral passage by syringe41. Perhaps the larva is merely getting rid of its larval stichocyte contents in the small intestine as a precocious behavior in anticipation of its rapid (ie. 28 h) development to adulthood.

secreted peptide molecule from the larva corresponds to any known host cytokine or intracellular messenger21–28. Furthermore, the timing of synthesis and subsequent release of host–parasite signals and the activities that they result in are not known.

The larva of Trichinella spiralis can secrete some 40 different proteins26–30 (Fig. 3), most of which are glycosylated31 with an unusual, highly antigenic sugar moiety, tyvelose (3,6-dideoxy arabinohexose)32. In fact, this specific configuration of tyvelose is produced only by the L1 of Trichinella spiralis. Furthermore, all tyvelosylated peptides emanate from the larva’s highly specialized organ, the stichosome, a unique structure among nematodes found only in the order Trichurata. The genital primordium (ie. the posterior half of the worm) apparently does not possess the necessary enzyme, tyvelose epimerase, to synthesize this sugar. The stichosome comprises about 50 stichocyte cells35. Each of the cell types – five have been identified based on electron microscopy studies on the morphology of their granules – synthesize secretory granules of a single variety, while each granule type contains many novel peptides29. Some of these peptides are secreted during the muscle phase35,36, while others are stored and then secreted during the early intestinal phase37,38. As mentioned, it is not certain which peptides function in either phase of the life cycle, as only a few have been studied. The adult worm version of the stichosome is completely different from that of the larva in that each of its 50 stichocytes contain secretory granules that have no morphological equivalent to the larva. For example, none of the stichocyte-specific secreted peptides of the adult parasite are tyvelosylated29. Therefore, it is unlikely that the larva uses its tyvelosylated secreted proteins to gain entrance into its intracellular niche in the small intestine after being swallowed by the next host, as some have suggested40, because the adult can locate there without loss of fecundity when transferred from one animal to another through oral passage by syringe41. Perhaps the larva is merely getting rid of its larval stichocyte contents in the small intestine as a precocious behavior in anticipation of its rapid (ie. 28 h) development to adulthood.

Only a few genes encoding antigens secreted by the larva have been sequenced21–28 and only one of those, the 43 kDa polypeptide21,25, has a motif that is suggestive of a function that might be relevant to nurse cell formation. This tyvelosylated protein is synthesized by the alpha stichocytes of the larva, and after secretion locates exclusively to the nurse cell cytoplasm from Day 12 through Day 15 of nurse cell development28. The 43 kDa peptide contains a helix-loop-helix (HLH) motif, but lacks a preceding basic amino acid region. In contrast, a large family of
transcription factors possess both HLH and basic amino acid domains. Inhibitors of HLH transcription factors, such as Id and emc, contain HLH regions but lack a basic amino acid motif. In most cases, inhibitors of HLH transcription factors interact with their target molecules within the nucleoplasm, although in some cases, they can interact with them in the cytoplasm and then translocate to the nucleus as heterodimeric molecular complexes. Recently, a transcription factor inhibitor was described, IleB, that interacts with NF-kB in the cytoplasm and effectively prevents it from entering the nucleus. The 43 kDa peptide, although not similar in structure to IleB, contains an HLH domain. The 43 kDa peptide can be detected in the larva regardless of the age of the parasite, so 43 kDa peptide synthesis and secretion into the nurse cell cytoplasm is likely to be continuous, albeit below the level of detection after Day 15, when standard immunocytolocalization techniques at the light microscope level are applied to tissue sections. When the temporal aspect of its presence in the nurse cell is taken into account, speculation about its function would center around either late aspects of nurse cell formation or the maintenance phase. However, until its target molecule(s) is identified and functionally defined, the role of the 43 kDa peptide by the worm will remain unknown.

Other peptides, four of which contain the tyrosine and serine-rich signature, locate to the nucleoplasm of each enlarged nurse cell nucleus, beginning on Day 8 (Ref. 7) and remain there for the life of the parasite (up to eight months after intramuscular invasion in mice). None of these proteins has been isolated and characterized, but it is hard to imagine that they would play no role at all in nurse cell formation or maintenance considering their cellular location.

Thus, even with these few examples of secreted parasite proteins in hand, it is difficult not to conclude that the larva specifically directs at least some of them to precise subcellular compartments at prescribed times after it begins its life inside the host cell. If further research confirms these initial findings, then what is already known may represent a part of an overall molecular strategy for controlling host cell invasion and/or maintenance considering their cellular location.

Conclusions and prospects
Numerous issues remain unaddressed, and many more have yet to be posted regarding the biology of the nurse cell-parasite complexes. For example, more detail regarding the nature of the molecular and cellular changes that the host cell undergoes needs to be documented before we can begin to investigate fully the extent to which Trichinella influences host genomic expression. Knowing the amino acid sequence of each of the 43 kDa-secreted polypeptides of the L1 larva, and pinpointing their locations within the nurse cell throughout its development and cytoplasmic localization at the light and electron microscopy levels would be equivalent to being the first person to use the Rosetta Stone for understanding the Egyptian hieroglyphs. Hopefully, when Trichinella’s signaling molecules are fully translated, a more comprehensive overview of microbial pathogenesis will result, in which viral and intracellular bacterial and protozoan survival strategies can be integrated with those of this largest of all intracellular pathogens. Perhaps even more important, concepts of molecular control mechanisms of the differentiated state of mammalian cells will surely need to be revised once it is finally known how T. spiralis makes itself at home.

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References
The molecular epidemiology of pathogens remains future avenues to explore. Here, Michel Tibayrenc explains the main modern approaches to epidemiology of parasitic and other infectious diseases. In the past 20 years, genetic and molecular methods for the detection of pathogens have taken a major place in modern approaches to epidemiology of parasitic and other infectious diseases. Thus, Michel Tibayrenc explains the main concepts used in this field of research, with special emphasis on the approaches developed in his team, and suggests future avenues to explore.

The molecular epidemiology of pathogens remains a controversial field, viewed as a panacea by some people, but as a useless tool by others. According to the definition of the Centers for Disease Control (CDC) in Atlanta, molecular epidemiology means: ‘the various techniques derived from immunology, biochemistry, and genetics for typing or subtyping pathogens’. This definition has the merit of clarity; nevertheless, in my opinion, it is too restrictive, for it overly emphasizes the technical side, to the detriment of theoretical considerations. In this article, we will make four important points: (1) analysis of the genetic polymorphism of pathogens; (2) the various techniques derived from immunology, biochemistry, and genetics for typing or subtyping pathogens; (3) the medical questions raised by genetic polymorphism of pathogens; and (4) the future perspectives of this field.