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How Does *Trichinella spiralis* Make Itself at Home?

D.D. Despommier

The nurse 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 nurse cell formation.*

The list of parasites infecting humans is long and rich in species diversity. Within each of us, numerous fundamental niches tempt the uninvited. While striated skeletal muscle tissue ranks as one of the most abundant[1], only a handful of protozoans and helminths have been successful in colonizing this niche[2]. For example, among the numerous species of protozoa, only a few (eg. *Trypanosoma cruzi*, *Toxoplasma gondii*, *Trachipleistophora hominis*, *Sarcocystis* sp. and *Hepatazoa* 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 five recognized species (*Trichinella spiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* and *T. pseudospiralis*)[3], and more likely to achieve species status, that not only live and thrive there, but have in all likelihood evolved complex strategies for remodeling that niche[4] 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 symbionts, 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[4] states that the parasite is responsible for remodeling the muscle cell, and does so by secreting 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 surroundings. The result is the nurse cell[4], 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 parasite throughout its life in that essential niche. Figures 1 and 2 summarize some of the modifications that the host cell undergoes over the 15–20 day period during which the nurse cell developmental program is upregulated. Each graphic represents a summary of data gathered from a variety of laboratories.

Capsule collagen synthesis

The nurse cell–parasite complex is surrounded by a collagen capsule[5] and consists predominantly of two collagen types, IV and VI (Fig. 1d and e), both of which are synthesized by the nurse cell[6]. Parasite secretion of proteins within the matrix of the infected host cell begins on Day 7 after infection[7] (Fig. 1h). The onset of host collagen type IV and type VI mRNA synthesis is between Days 7 and 8. By Day 8, parasite peptides localize to the nucleoplasm of all enlarged nurse cell nuclei[7,8]. Hence, upregulation of these two host genes is temporally coincident with peptide secretion. Throughout the period of collagen synthesis, 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[4] 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 circulatory rete) to the surface of the outer collagen capsule (Fig. 2)[9–11]. In this way, the larva could assure a constant source of small molecular weight metabolites for itself, while ridding its living space of metabolic byproducts. The mechanism(s) by which the worm accomplishes this is by initiation of the angiogenic program[12]. This may involve an initial hypoxic event[13] 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* hybridization in the cytoplasm of the developing nurse cell beginning on Day 7 (Fig. 1f), up to eight months after initial infection of the muscle cell[14]. The presence of VEGF peptide was observed shortly thereafter, beginning 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 circulatory rete formation is complete implies a permanently 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[4], and they have the diameter of sinusoids, thus facilitating the rapid flow of formed elements through them. The large diameter of the vessels, compared with capillaries, also favors 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 approaches 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. scavengers) 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[16].

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 communicate with one another. Without the exchange of information the possibilities for long-term relationships are greatly reduced, provided that the organism in question remains metabolically active (ie. not encysted or dormant, as is the case for larval *Taenia* sp. and pseudocysts of *T. gondii*, or even latent viruses). Intravital microscopy has revealed that *Trichinella* constantly moves about within its nurse cell[4], 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, *Trichinella*'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 molecules[17,18] (ie. cytokines), which direct specific cellular behavior. Presumably, *T. spiralis* uses similar molecules to carry out its own developmental programs[19]. In addition, however, it must instruct the host, most probably using its secreted signaling molecules (Fig. 3), which I call 'parakines'[20]. The host cell then responds 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, their identification and characterization have so far eluded molecular parasitologists. In fact, none of the sequences of any

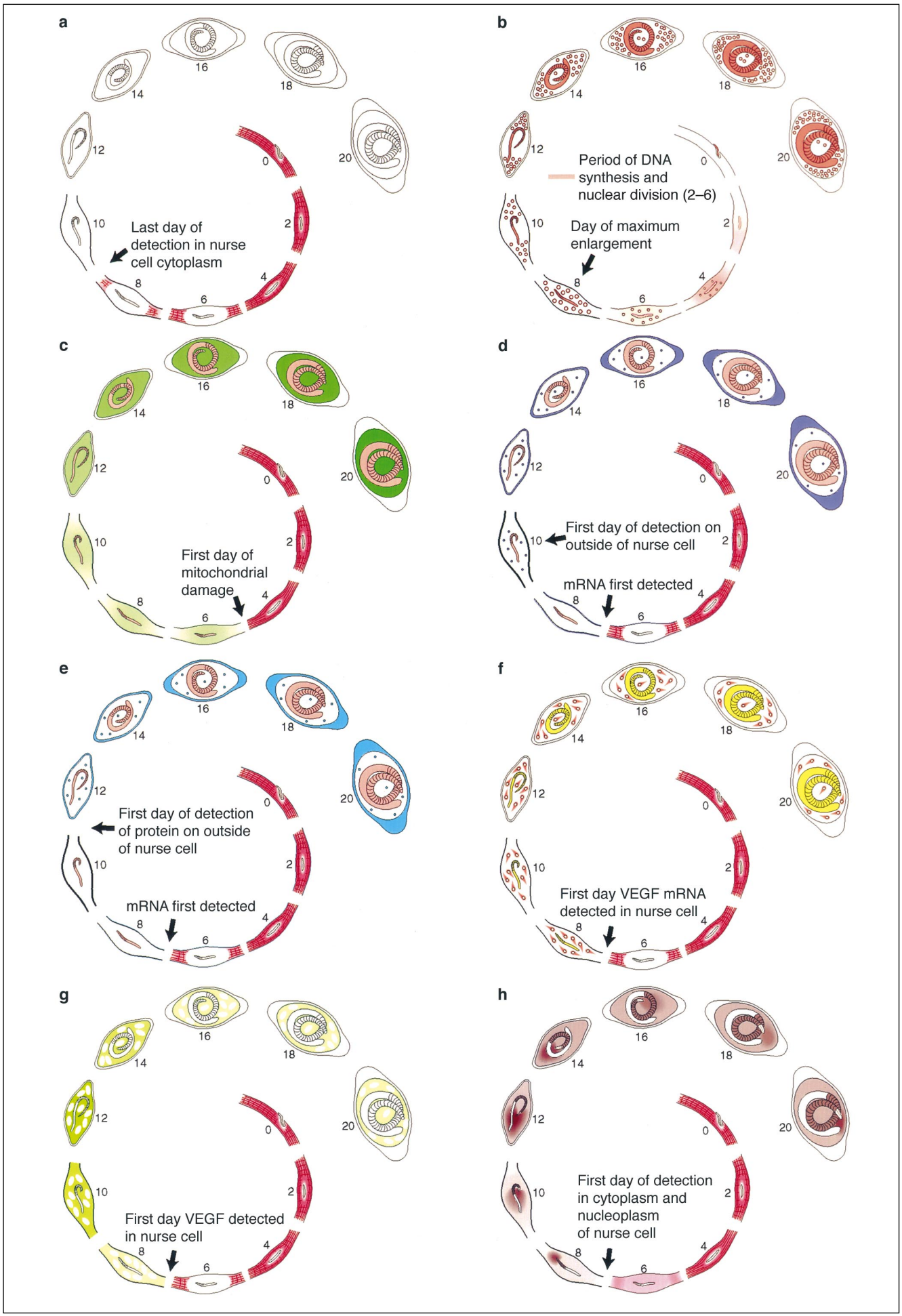
a
Last day of detection in nurse cell cytoplasm
b
Period of DNA synthesis and nuclear division (2–6)
Day of maximum enlargement
c
First day of mitochondrial damage
d
First day of detection on outside of nurse cell
mRNA first detected
e
First day of detection of protein on outside of nurse cell
mRNA first detected
f
First day VEGF mRNA detected in nurse cell
g
First day VEGF detected in nurse cell
h
First day of detection in cytoplasm and nucleoplasm of nurse cell

Fig. 1. (left) Changes during nurse cell formation. (a) Loss of muscle proteins (eg. actin, myosin and creatine kinase)[1,48]. No muscle contractile proteins can be detected beyond Day 8 after the parasite invades the muscle cell. (b) Enlargement and division of nurse cell nuclei during nurse cell formation[4,48]. Enlargement of nuclei occurs maximally on Day 8 after the larva invades the muscle cell and nuclei remain enlarged thereafter. Nuclear division and DNA replication occurs during the first 4–5 days after the larva invades the muscle cell, resulting in 4N DNA in each nucleus and approximately 40–60 nuclei per nurse cell. (c) Mitochondrial damage (ie. vacuolization of inner mitochondrial matrix)[14]. Mitochondrial damage can be detected throughout the infection period beginning on Day 5 after invasion of the muscle cell by the larva. (d) Collagen type IV synthesis[6]. Synthesis of mRNA begins on Day 7, while collagen protein is detected first on Day 11 after the larva invades the muscle cell. Synthesis of collagen protein ceases on Day 26. (e) Collagen type VI synthesis[6]. Synthesis of mRNA begins on Day 7 after the larva infects the muscle cell and continues at a low level throughout the infection period. (f) Vascular endothelial growth factor (VEGF) mRNA synthesis[14]. Synthesis begins on Day 7–8 after the larva invades the muscle cell and continues throughout the infection period. (g) VEGF peptide synthesis[14]. Synthesis begins on Day 8 after the larva invades the muscle cell and continues throughout the infection period. (h) Secretion of tyvelosylated proteins[7,47]. Secretion of tyvelosylated proteins begins on Day 7 after the larva invades the muscle cell and continues throughout the infection period.

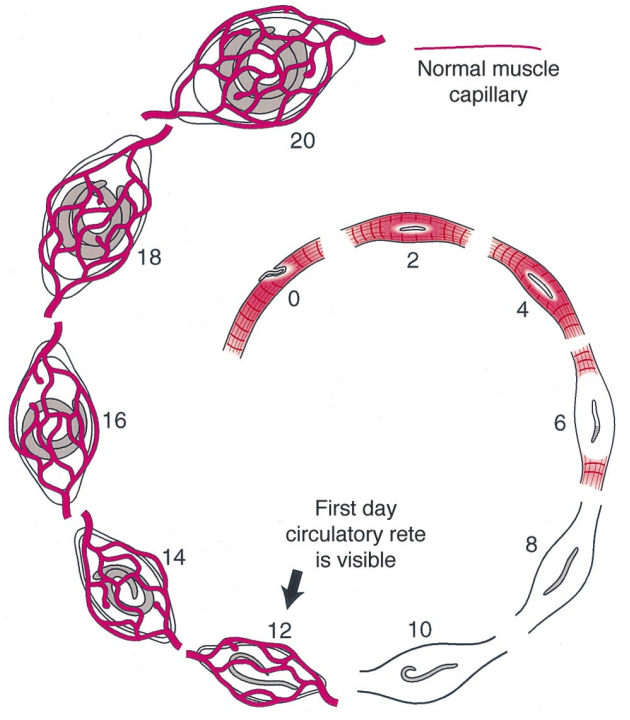

Fig. 2. Angiogenesis and nurse cell formation. Angiogenesis begins on about Day 12 after the larva invades the muscle cell and ceases by Day 26.

secreted peptide molecule from the larva corresponds to any known host cytokine or intracellular messenger[21–28]. 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 *T. spiralis* can secrete some 40 different proteins[29,30] (Fig. 3), most of which are glycosylated[31] 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*. 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 cells[33]. Each of the cell types – five have been identified based on electron microscopy studies on the morphology of their granules[34] – synthesize secretory granules of a single variety, while each granule type contains many novel peptides[29]. Some of these peptides are secreted during the muscle phase[35,36], while others are stored and then secreted during the early intestinal phase[37,38]. 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 tyvelosylated[39]. Therefore, it is unlikely that the larva uses its tyvelosylated secreted proteins to gain entrance into its intramulticellular niche in the small intestine after being swallowed by the next host, as some have suggested[40], because the adult can locate there without loss of fecundity when transferred from one animal to another through oral passage by syringe[41]. 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 sequenced[21,24–27] and only one of those, the 43 kDa polypeptide[21,25], 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 development[28]. 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

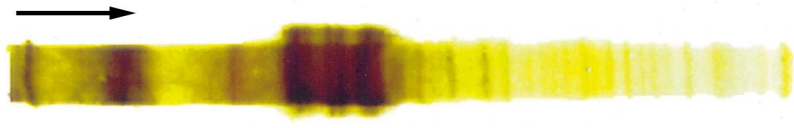

Fig. 3. Western blot of secreted proteins (ie. ES products) from the L1 larva of *Trichinella spiralis*. A minimum of 40 bands can be counted by means of one-dimensional SDS–PAGE followed by western blot analysis. The antiserum used was rabbit IgG and it was directed against the tyvelose sugar moiety only.

transcription factors possess both HLH and basic amino acid domains[42–44]. Inhibitors of HLH transcription factors, such as Id and emc, contain HLH regions but lack a basic amino acid motif[45]. 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[46], IκBε, that interacts with NF-κB in the cytoplasm and effectively prevents it from entering the nucleus. The 43 kDa peptide, although not similar in structure to IκBε, could function in a similar manner by interacting with newly synthesized host HLH transcription factors before their translocation to the nucleus. The mRNA encoding the 43 kDa peptide can be detected in the larva regardless of the age of the parasite[21], 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 use of the 43 kDa peptide by the worm will remain unknown.

Other peptides, four of which contain the tyvelose antigenic signature[28], locate to the nucleoplasm of each enlarged nurse cell nucleus[7,47], beginning on Day 8 (Ref. 7), and remain there for the life of the parasite (ie. 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 functions, making the genus *Trichinella* truly unique among helminths. In that regard, this remarkable symbiont seems more virus-like[8] than worm-like.

Conclusions and prospects

Numerous issues remain unaddressed, and many more have yet to be posited regarding the biology of the nurse cell–parasite complex. For example, more detail regarding the nature of the molecular and cellular changes[48] 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 all, not just a few, of the stichocyte-specific secreted polypeptides of the L1 larva, and pinpointing their locations within the nurse cell throughout its development by immunocytolocalization 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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Beyond Strain Typing and Molecular Epidemiology:
Integrated Genetic Epidemiology of Infectious Diseases

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In the past 20 years, genetic and molecular methods for characterizing pathogen strains have taken a major place in modern approaches to epidemiology of parasitic and other infectious diseases. Here, 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[1], 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, I will make four important points: (1) analysis of the genetic polymorphism of pathogens reveals much more than strain typing for epidemiological follow-up; (2) the medical questions raised by genetic polymorphism are very similar, whatever kind of pathogen is considered, be it a parasitic protozoan, a fungus, a bacterium or even a virus or a helminth[2–4]; (3) the methods and concepts of evolutionary genetics (population genetics and phylogenetic analysis) are essential for understanding the origin and predictable properties of the genetic polymorphism of pathogens[2–4]; and (4) the future

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