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Perusal of parasitic nematode 'omics in the post-genomic era

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ABSTRACT

The advent of high-throughput, next-generation sequencing methods combined with advances in computational biology and bioinformatics have greatly accelerated discovery within biomedical research. This "post-genomics" era has ushered in powerful approaches allowing one to quantify RNA transcript and protein abundance for every gene in the genome – often for multiple conditions. Herein, we chronicle how the post-genomics era has advanced our overall understanding of parasitic nematodes through transcriptomics and proteomics and highlight some of the important advances made in each major nematode clade. We primarily focus on organisms relevant to human health, given that nematode infections significantly impact disability-adjusted life years (DALY) scores within the developing world, but we also discuss organisms of veterinary importance as well as those used as laboratory models. As such, we envision that this review will serve as a comprehensive resource for those seeking a better understanding of basic parasitic nematode biology as well as those interested in targets for vaccination and pharmacological intervention.

1. Introduction

Parasitic helminths presently infect well over one billion people worldwide, and the number of individuals harboring one or more species may increase in the absence of therapeutic intervention. Among helminth parasites, the nematodes include multiple organisms with significant impact on human and animal health—including roundworms, hookworms, filarial worms, whipworms, and others [1]. Phylum Nematoda, which includes both free-living and parasitic species, has been divided into five main clades [2,3] as well as 12 more specific clades [4]. Only clades containing nematodes that are parasites of vertebrate animals are discussed here (clades I, III, IV, and V), with a focus on species of medical and veterinary importance and their laboratory models. Within each clade, species are grouped and discussed by Family.

Over the past decade, advances in sequencing technology as well as bioinformatic capabilities have allowed an unprecedented number of helminth genomes to be sequenced [5]. While full genome sequences provide investigators with a "toolbox" for their organism of interest, the expression patterns and functions of these genes are often unknown. In recent years, investigators have begun leveraging genome sequences to interrogate the expression, localization, and function of every gene in the genome by utilizing whole transcriptome shotgun sequencing (RNAseq) and proteomics. RNAseq takes advantage of next-generation sequencing to quantify particular types of RNA transcripts, including polyadenylated RNA, small RNAs, or total RNA, which can be isolated from whole organisms, dissected tissues, or specific developmental stages. Advances in proteomic methods coupled with annotated genomes that can provide predicted protein sequences for every gene provide investigators with new capabilities to identify proteins in biological samples. The number of studies using these post-genomic 'omic methods to investigate nematode parasitism has greatly increased since their most recent review [6]. Thus, the overarching goal of this review is to provide a broad overview of the most recent data.

Resources to navigate this data-rich environment are of incredible importance. Both NEMBASE (http://www.nematodes.org/nembase4) [7] and Nematode.net (http://nematode.net) [8] have cataloged many datasets for nematode parasites. Recently, WormBase ParaSite (http://parasite.wormbase.org/) has brought many features of WormBase to the parasite community [9]. The utility of these databases will undoubtedly increase as they are expanded and brought to bear on problems specific to parasitism.

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2. CLADE I

The diverse nematodes found in clade I include parasites of vertebrates, invertebrates, and plants, as well as free-living species (Table 1). From a human health perspective, *Trichuris trichiura* is perhaps the most important with approximately 600–800 million people currently infected worldwide [10]. *Trichinella spiralis* is also an important clade I pathogen that is transmitted via undercooked meat and infects approximately 10,000 people throughout the world each year [11].

2.1. Trichuridae

The life cycle of *Trichuris* spp., including *T. trichiura* (human), *T. muris* (mouse and rat), and *T. suis* (swine), involves adult female and male worms that reside in the large intestine, with their anterior ends buried in the mucosal epithelium [12]. *Trichuris* ova passed in host feces embryonate in soil and hatch upon ingestion, releasing first-stage larvae (L1) in the small intestine through a mechanism reliant on host microbiota [13]. Adult worms develop after four molts and subsequently reside within an intestinal epithelial syncytium in the host [12].

Given the importance of finding novel therapeutic targets, Foth et al. focused on the anterior portion of the parasite (the stichosome) that directly interfaces with the host. They found an increased abundance of transcripts encoding many chymotrypsin A-like serine proteases, a gene family that is expanded in the *T. trichiura*, *T. muris*, and *T. suis* genomes [14,15], that are known to degrade host intestinal mucins, and that may also serve digestive or immunomodulatory functions [14,16]. Moreover, multiple DNase II-encoding transcripts with signal peptides were identified in the stichosome—indicating a possible function in degrading host DNA to dampen immune responses [14]. The mRNA and small RNAs expressed in the stichosome of *T. suis* encode a full complement of RNAi machinery as well as high levels of diverse peptidases and porins relative to adult worm posterior bodies. Curiously, 2/3 of miRNAs are most abundant in larval stages rather than in adults, with a significant decrease in the L3-L4 transition, suggestive of developmental regulation [15]. Consistent with evidence that trichurid nematodes may protect against allergic reactions, atopy, and autoimmune conditions [17], proteins such as fructose bisphosphate aldolase and heat shock protein 70 have been identified as potential immunomodulatory agents [18,19]. *Trichuris* spp. also inhibit neutrophil-mediated tissue remodeling via secreted chymotrypsin/elastase inhibitors [20] and release β-barrel toxins that perforate host cell membranes [14,15].

2.2. Trichinellidae

Trichinella spiralis can infect a variety of vertebrate animals, including humans, swine, mice, and rats. The adult female and male worms, which reside inside intestinal epithelial cells, reproduce sexually, with females laying motile L1. These intestinal L1 transverse the gut epithelium, access the circulatory system, and invade striated muscle cells that become nurse cells. These muscle larvae undergo developmental arrest as L1, which are the infective form. Following consumption of infected muscle tissue, host digestive enzymes release L1 that rapidly reach the intestine, where they develop into adult worms after four larval molts [12].

New insights into the biology of *T. spiralis* indicate RNA-mediated gene regulation, with up to 45% of genes being transcribed from both strands [21]. Additionally, *T. spiralis* pro-

Table 1
Selected post-genomic studies of clade I nematodes [2].

Species	Clade [4]	Host	Life stage(s)	Platform/Method	Accession	Reference
mRNAs						
Trichinella spiralis	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina Solexa	GEO: GSE39151	[21]
			newborn larvae, muscle larvae, adult	Illumina HiSeq2000	GEO: GSE39328	[22]
Trichuris muris	2A	Mouse	L2, L3, adult female, adult male, female and male tissues	Illumina HiSeq2000	AE: E-ERAD-125	[14]
Trichuris suis	2A	Swine	adult	Illumina GAII	n/a	[31]
			L1/L2, L3, L4, adult female, adult male, female and male tissues	Illumina HiSeq2000	ENA: PRJNA208415	[15]
Trichuris trichiura	2A	Human	adult	Ion Torrent Ion 318	ENA: PRJEB12315	[19]
Small RNAs						
Trichinella spiralis	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina Solexa	n/a	[24]
			L1 (muscle), adult	Illumina MiSeq	GEO: GSE56651	[23]
Trichuris suis	2A	Swine	L1/L2, L3, L4, adult female, adult male, adult female and male tissues	Illumina GAII	ENA: PRJNA208415	[15]
Methylation						
Trichinella spiralis	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina HiSeq2000	GEO: GSE39328	[22]
Proteins						
Trichinella spiralis	2A	Human, Swine	ESP from muscle larvae	PB MALDI-TOF-TOF, MS/MS		[27]
			ESP from muscle larvae	AB Sciex 4800 MALDI-TOF/TOF-MS		[25]
			surface protein from muscle larvae	AB Sciex 4800 MALDI-TOF MS		[28]
			adult somatic proteins	AB Sciex 4800 MALDI-TOF/TOF-MS		[29]
			surface protein from muscle and intestinal infective larvae	n/a		[30]
Trichuris trichiura	2A	Human	adult somatic extract	W Micromass Q-TOF micro MS		[18]

Abbreviations: Applied Biosystems (AB); European Bioinformatics Institute (EBI); EBI ArrayExpress (AE); EBI European Nucleotide Archive (ENA); excretory/secretory products (ESP); mass spectrometer (MS); matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF); National Center for Biotechnology Information (NCBI); NCBI Gene Expression Omnibus (GEO); PerSpective Biosystems (PB); Waters (W).

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