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REVIEW

Functional genes and proteins of *Trichinella* spp.

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Abstract Research of *Trichinella* proteins has been conducted with emphasis on excretory–secretory (E–S) products of muscle larvae because of two reasons. The first is that it has prominent and narrow specific antigenicity, and the second is that it may play some role in nurse cell formation after being secreted into host muscle cells. Proteomic analysis of E–S proteins was further advanced by the aid of new analytical methods such as gene cloning, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry, and expressed sequence tags database analysis. As the research progressed, the interest of researchers moved to identification of function of E–S products, which has shed further light on the intriguing relationships between parasites and hosts. Major constituents of the E–S products include 43-, 53-, and 45-kDa glycoprotein derived from the stichosome. Many proteins were discovered in E–S products after the 43-, 53-, and 45-kDa proteins although the relationships among them remain unclear. Some of the new proteins were partially defined in terms of their function including nuclear antigens, MyoD-like protein, TsJ5 protein, etc. There are better-characterized proteins based on the gene molecular method, which allow easier identification of the function of proteins of interest. Such examples were demonstrated by proteinases, proteinase inhibitors, heat shock proteins, glycosidases, etc.

Introduction

Trichinella is a genus of nematode that infects a wide variety of vertebrate hosts. Larvae at a muscle stage are an infective form of *Trichinella* spp. In the host stomach, larvae are released with the aid of host gastric juice and develop into adult worms in the host intestine, and the female begins to produce the second generation of larvae, which migrate through the whole body of the host. Infection causes satellite cell proliferation (Matsuo et al. 2000; Wu et al. 2001) and transformation of muscle cell to the nurse cell in the capsule (known as cyst; Jasmer 1990; Despommier et al. 1990). Two clades in genus *Trichinella* have been identified: the encapsulated clade and the nonencapsulated clade (Zarlenga et al. 2006). Muscle larvae in encapsulated species develop a thick collagen capsule, and the nonencapsulated species develop only a very thin collagen capsule (Xu et al. 1997).

Muscle cell transformation is likely initiated by excretory–secretory (E–S) products released from the larvae (Ko et al. 1994). *Trichinella* spp. produces a variety of biologically active proteins, which may or may not be a part of E–S products. Proteins in E–S products likely affect host cells and tissues for respective purposes, and non-E–S proteins likely are engaged in internal reactions within the parasites. Among them, E–S products of *Trichinella* spp. have received a great deal of attention not only from an immunological point of view but also from the point of host–parasite interaction because of assumption that the intracellular parasite secretes some functional proteins and alters the host cell in such a way that *Trichinella* spp. can establish parasitism and survive for a longer period of time.

The 43-, 53-, and 45-kDa glycoproteins in larval E–S products contain tyvelose-containing antigen (Wisnewski et al. 1993; Romarís et al. 2002a; Arasu et al. 1994), which is the major antigen recognized by the host during infection (Appleton et al. 1991). Furthermore, these glycoproteins have been characterized at the molecular level, and it has been shown that these proteins are important for muscle cell transformation, capsule formation, and continuation of

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parasitism due to *Trichinella* spp. However, it is completely obscure how *Trichinella* spp. changes terminally differentiated muscle cells into nurse cell, whose architecture has never been designed in the host genome.

Protein engineering is an extremely hot topic of recent biological studies. Functional proteins have been produced for many purposes including therapeutic usage. Parasite proteins are not an exception. Efforts seeking for such an important protein responsible for host cell transformation in E–S products resulted in the discovery of many functional proteins owing to recent progress in molecular technology. In addition to the 43-, 53,- and 45-kDa glycoproteins, the E–S proteins of *Trichinella* spp. contain some functional proteins such as heat shock proteins, endonucleases, proteinases, protein kinases, proteinase inhibitors, superoxide dismutases, glycosidases, etc. This article will review the characterization of *Trichinella* proteins with emphasis on E–S products, including analytic information by means of classical wet laboratory techniques as well as molecular techniques.

Proteomic analysis of *Trichinella* proteins

To better understand the muscle cell transformation leading to capsule formation, it is critical to examine the nature of *Trichinella* E–S products that affect the infected muscle cells after being released to the cell cytoplasm. In this section, some landmark papers on the characterization of *Trichinella* proteins will be reviewed in chronological order.

Early investigators used classical laboratory techniques such as electrophoresis or gel filtration to investigate the nature of E–S products. Despommier and Laccetti (1981) characterized antigenic proteins derived from a large-particle fraction of muscle larvae of *Trichinella spiralis* in relation to their molecular weights, isoelectric points, carbohydrate contents, antigenicity, and their ability to induce protection in mice. Gel filtration yielded five major peaks of material, while sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) revealed a minimum of 28 proteins ranging in molecular weight from 11 to 200 kDa. Analytical isoelectric focusing on SDS-PAGE yielded 37 bands of protein, while the periodic acid-Schiff reaction performed on a similar gel revealed 22 glycoproteins. Most proteins were within a pI range of 4.0–7.0, while all of the glycoproteins had pI ranging from 4.0 to 6.5. Secreted proteins of muscle larvae, adult males, and newborn larvae of *T. spiralis* were relatively few in number and were different for each stage, whereas somatic proteins demonstrated an unresolved smear in all cases (Parkhouse and Clark 1983).

T. spiralis and *Trichinella pseudospiralis* are independent species in the genus *Trichinella*. These two species are similar but different in terms of host responses. By high-power resolution (two-dimensional Western blot analysis), Wu et al. (1999) showed that antigenic peptides of *T. spiralis* consisted of about 100 peptide spots, and the molecular weight of these peptides ranged from 22 to 80 kDa, and pI ranged from 4 to 7. Antigenic peptides of *T. pseudospiralis* consisted of about 20 to 30 peptide spots, and the molecular weights ranged from 25 to 80 kDa, and pI ranged from 4 to 7. The E–S products of *T. spiralis* and *T. pseudospiralis* were highly cross-reactive with each other.

Recent investigators adapted new molecular techniques. Robinson et al. (2005) have used matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry for identification of the peptide spots of muscle larvae E–S products. In addition, MALDI-TOF mass spectrometry and laser chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) were used to identify the peptide spots, and these data were analyzed by specific *Trichinella* expressed sequence tag (EST) databases (Robinson and Connolly 2005). They identified 43 out of 52 protein spots analyzed as the major secreted glycoproteins. The 43 protein spots represented only 13 different proteins indicating that there are multiple proteins isoforms present in the E–S products. Also, two major groups of *T. spiralis*-specific proteins and several *T. pseudospiralis*-specific proteins were identified using two-dimensional gel electrophoresis and tandem mass spectrometry (Robinson et al. 2007a). Liu et al. (2007) cloned six newborn larva stage-specific and four adult worm stage-specific genes of *T. spiralis* using suppression subtractive hybridization technique.

A dataset for expressed *Trichinella* genes may support proteomic methods to identify parasite proteins involved in specific interactions. In addition, genome information provides comparative analysis among life cycle stages relative to biological similarities and differences, some of which may involve host cell interactions. Comparisons with other sequences can also identify putative proteins that are restricted to nematodes or nematode groups, thus providing insight that may be related to parasitism. Mitreva et al. (2004) analyzed the transcripts of *Trichinella* spp. using ESTs that are produced from cDNA libraries for immature L1, mature muscle larvae, and adult stages of *T. spiralis* and showed that 10,130 ESTs were grouped into 3,454 gene clusters.

Major *Trichinella* E–S proteins with unrevealed function

The 43-, 53-, and 45-kDa glycoproteins, which are three major antigenic proteins, have received more attention than other E–S proteins because these three proteins are candidates of immunodiagnostic antigens for trichinellosis and are present in much greater amounts in the E–S products, but the function of the 43-, 53-, or 45-kDa protein remains unknown.

It should be emphasized that the 43-, 53-, and 45-kDa glycoproteins share a unique so-called TSL-1 antigen

(Wisnewski et al. 1993; Romarís et al. 2002a; Arasu et al. 1994). TSL-1 antigen is one of the most intriguing glycoprotein antigens, which induce a powerful antibody response in parasitized animals and can be used for immunodiagnostic purposes (Appleton et al. 1991). The unique and critical sugar of TSL-1 antigen is 3,6-dideoxyarabinohexose (tyvelose; Wisnewski et al.1993).

The 43-kDa glycoprotein

In this section, the 43-kDa glycoprotein will be reviewed in terms of its biochemical properties and possible functions.

The early authors Gold et al. (1990) isolated 43-kDa glycoprotein from E–S products of *T. spiralis* muscle larvae by biochemical methods and provided convincing characterization with respect to their biochemical and immunological properties. This was followed by molecular characterization by Su et al. (1991) who cloned and expressed the 43-kDa glycoprotein (they called the 49-kDa antigen) in *Escherichia coli*. The predicted 344 amino acid of the 43-kDa glycoprotein have an N-terminally located signal peptide and a potential helix–loop–helix motif (HLH) that resembles HLH domains critical in the function of muscle differentiation factors such as MyoD and myogenin in the main body of the protein (Vassilatis et al. 1992).

The 43-kDa glycoprotein may be responsible for capsule formation that occurs immediately after the entrance of muscle cells by the newborn larvae because the gene encoding a 43-kDa glycoprotein is expressed by either precapsule or postcapsule muscle larvae but not expressed by adult worms (Wu et al. 2002). And the diverse isoforms of the *43-kDa* gene appear to be expressed by immature L1 (Mitreva et al. 2004).

T. spiralis and *T. pseudospiralis* are similar but different in terms of host response, including morphology of the capsule, immunological responses, and expression of genes and E–S proteins. Infection causes muscle cell degeneration, which is restricted around the worm in the case of *T. spiralis* infection, but the affected area spreads over the entire length of muscle cell in the case of *T. pseudospiralis* infection (Matsuo et al. 2000; Wu et al. 2001). These different pathological changes in muscle cell could be attributed to the differences of the E–S products released to the host cells. The homolog of the 43-kDa glycoprotein of *T. spiralis* exists in the E–S products of *T. pseudospiralis* (Vassilatis et al. 1996a). Comparison of the amino acids sequence of the *T. pseudospiralis* glycoprotein with the *T. spiralis* 43-kDa glycoprotein indicated that the two proteins are very similar (about 84% homology), but the molecular weight of the homologous glycoprotein from *T. pseudospiralis* was 38 kDa (Nagano et al. 2004).

The 43-kDa glycoprotein of *T. spiralis* is secreted in the host muscle cell and may influence and alter the host cells that may lead to the nurse cell formation. Therefore, the location of the 43-kDa protein in the host muscle cell is critical. Vassilatis et al. (1992) reported that the antibodies against the 43-kDa recombinant protein strongly reacted with the nurse cell nuclei and the nematode itself. However, Jasmer et al. (1994) reported that antibodies which specifically recognized 43-kDa glycoprotein failed to bind detectably with in situ and isolated host nuclei and nuclear extracts. In addition, immunodominant epitopes of specific 43-kDa glycoprotein of *T. spiralis* could not be detected in hypertrophic nuclei of injected muscles (Ko et al. 1994). The protein immunologically related to the 43-kDa glycoprotein but not to the 43-kDa glycoprotein itself was detected in the nuclei of mature nurse cells (Vassilatis et al. 1996b).

Recently, it has been reported that the 43-kDa glycoprotein may have deoxyribonuclease IIα (DNase IIα) activity. DNase IIα, which is an acidic endonuclease, was found in lysosomes and nuclei, and it is also secreted. Its *Caenorhabditis elegans* homolog is required for digesting DNA of apoptotic cell corpses and dietary DNA. The homologs of DNase IIα with differences at the purported active site histidine residue were detected in the 43-kDa glycoprotein of *T. spiralis* and *T. pseudospiralis* (MacLea et al. 2003). This study was expanded by Jasmer and Kwak (2006) who investigated the ability of a 43-kDa glycoprotein to interfere with mammalian skeletal muscle gene expression. They constructed a plasmid containing the gene of 43-kDa glycoprotein, and the gene was expressed as a recombinant protein in C2C12 myoblasts. They showed that the effects of transfection of the *43-kDa* gene to the cell lines resulted from similarities of the 43-kDa glycoprotein to DNase IIα.

The 53-kDa glycoprotein

In this section, the 53-kDa glycoprotein will be reviewed in terms of its biochemical properties and possible functions.

Zarlenga and Gamble (1990, 1995) first identified genes encoding the 53-kDa glycoprotein of *T. spiralis* and elucidated the complete sequence and characteristic of the 53-kDa gene. The homolog of the *T. spiralis* 53-kDa glycoprotein is present in *T. pseudospiralis* E–S products (Wu et al. 1998), and the complete sequences and characteristics of the genes encoding the 53-kDa glycoprotein of *T. pseudospiralis* have been determined (Nagano et al. 2004). The amino acid sequence of the *T. pseudospiralis* 53-kDa glycoprotein shows a low homology (about 68%) to that of the *T. spiralis* 53-kDa glycoprotein.

The gene encoding the 53-kDa glycoprotein of *T. spiralis* is expressed by postcapsule larvae and adult worms but not by precapsule larvae and newborn larvae (Wu et al. 2002). And the 53-kDa glycoprotein is present in the β-granules but not in the α-granules (Romarís et al.2002a).

Type I stichocyte granules, which are present within adult stichocytes, resemble β-granules of muscle larvae, and the two granules share antigenicity (Takahashi et al. 1992). Therefore, the 53-kDa glycoprotein, which is present within β-granules, could be expressed in adult worms. These data indicate that the 53-kDa glycoprotein is not responsible for muscle cell transformation nor capsule formation, but the protein plays some role in the continuation of parasitism and modulation of host immune response.

The immunogenicity of the 53-kDa glycoprotein using monoclonal antibodies (mAbs) was consecutively studied by Romarís and her colleagues. The native 53-kDa glycoprotein in larval E–S products of *T. spiralis* contains tyvelose-containing antigen and shows a marked heterogeneity in glycosylation (Romarís et al. 2002a). Some of the epitopes recognized by mAbs are differentially expressed in *Trichinella* spp. The epitope recognized by mAb US5 on the 53-kDa glycoprotein (another O-glycan/peptide epitope) is present only in *T. spiralis*, whereas those recognized by mAbs US8 and US9 (peptide epitopes) are present in encapsulated *Trichinella* species (Romarís et al. 2002a). The 53-kDa glycoproteins from the species *Trichinella britovi*, *Trichinella murrelli*, and genotype T8 have higher molecular weight (60 kDa) than the 53-kDa glycoprotein from *T. spiralis*, *Trichinella nelsoni*, and genotype T6 and from *Trichinella nativa* (55 kDa; Romarís et al. 2003). The variation in molecular weight of the 53-kDa glycoprotein is due to small differences in amino acid sequence, leading to change in the number of glycosylation sites. The 53-kDa glycoprotein from *T. spiralis* bears species-specific epitopes that induce antibody formation during infection. The antigenicity of the 53-kDa glycoprotein is mainly due to protein epitopes, and the antibody response against glycan epitopes is less important (Romarís et al. 2003). The epitope recognized by mAb US9 which is present in all encapsulated *Trichinella* species is a linear peptide of eight residues and located in the amino-terminal region, while the corresponding epitope recognized by mAb US5 which is present only in *T. spiralis* is a 47-amino-acid sequence containing two alpha-helix regions flanked by random coils (Perteguer et al. 2004).

The 45-kDa glycoprotein

A novel 45-kDa protein as well as the 43-kDa glycoprotein secreted from *T. spiralis* was biochemically isolated (Gold et al. 1990). Molecular analysis by Arasu et al. (1994) revealed that the secreted 45-kDa protein is a tyvelose-bearing glycoprotein and is present in the β- and γ-stichocytes of the secretory organs of muscle larvae. The gene encoding this protein belongs to a multicopy gene family present on a single DNA and encodes several larval proteins in the 40-50 kDa range.

The homolog of the 45-kDa protein of *T. spiralis* exists in the E–S products of *T. pseudospiralis*, and the 45-kDa protein is composed of two distinct subgroups, tyvelosylated and untyvelosylated (Robinson et al. 2007a). Bioinformatics analysis identified that the secreted 45-kDa protein is a family of trypsin-like serine proteases (Robinson et al. 2007a).

Minor *Trichinella* proteins with unrevealed function

In E–S products or not E–S products, there are many interesting proteins which have been partially characterized. These minor proteins will be briefly reviewed in the following section.

Nuclear antigens

The so-called nuclear antigens are parasite proteins (approximately 71, 79, 86, and 97 kDa) detected in host cell nuclei by means of immunostaining with antibodies against *T. spiralis* proteins (Jasmer et al. 1994; Yao and Jasmer 1998). These nuclear antigens can be depleted from the host nuclei of muscle cells by mebendazole treatment, which further strengthens the hypothesis that these nuclear antigens are secreted by parasites (Yao et al. 1998). Nuclear antigens may play a role in regulating the infected cell phenotype, but the functions of these nuclear antigens are unresolved.

Myogenic regulatory factor, MyoD-like protein

A gene encoding a *T. spiralis* helix–loop–helix protein with homology to the *MyoD*-like gene was cloned by Connolly et al. (1996). This gene is expressed constitutively during the muscle larval and adult stages. A purified recombinant MyoD-like protein binds to a high-affinity mouse MyoD: DNA-binding site in vitro.

TsJ5 protein

The *tsJ5* gene was cloned from the muscle larvae of *T. spiralis* (Lindh et al. 1998). The TsJ5 protein is not a helix–loop–helix protein but represents a novel protein with properties in common with some myogenic repressors. The *tsJ5* gene is expressed at higher levels in encapsulating species than in nonencapsulating species, and the tsJ5 recombinant protein affects the formation of MyoD:DNA complexes in vitro (Kuratli et al. 1999). The TsJ5 protein is found in E–S products, on the cuticular surface and in the body wall muscle of *T. spiralis* and *T. pseudospiralis* muscle larvae (Kuratli et al. 2001).

T. pseudospiralis-specific 15-kDa protein

Chung and Ko (1999) reported that the protein with a molecular weight of 15 kDa is present in E–S products of

T. pseudospiralis. This protein exists only in the stichocytes of *T. pseudospiralis* and not in *T. spiralis*. However, the functions of this protein are unknown.

DNA-binding protein

A DNA-binding protein (approximately 30 kDa) is present in the E–S products of the infective-stage larvae of *T. pseudospiralis* (Mak and Ko 2001).

The 21- and 28-kDa proteins of T. pseudospiralis and T. spiralis

The proteins with a molecular weight of 21 or 28 kDa are present in E–S products of *T. pseudospiralis* and *T. spiralis*. Any related proteins with a significant homology have not been identified by database searches. The expression of these genes is restricted largely to the 30 days postinfection muscle larvae (Nagano et al. 2001a, 2002).

Newborn-larvae-specific protein

The glutamic acid-rich protein is transcribed specifically in the newborn larvae stage of *T. spiralis* and is present in the periphery of the developing stichocytes within newborn larvae (Zarlenga et al. 2002).

Nematode-specific cysteine–glycine domain proteins

Nematode-specific proteins of *T. spiralis* have a similarity to a predicted secreted or extracellular *C. elegans* protein. The region of similarity includes a conserved cysteine–glycine (CCG) domain, which is nematode specific. Two cysteine–glycine domains (Ts-CCG-1 and Ts-CCG-2) are present in the predicted *T. spiralis* protein, and particularly Ts-CCG-2 protein is present in E–S products (Gare et al. 2004). The *Ts-CCG-1* gene is constitutively expressed, and *Ts-CCG-2* gene expression is restricted to the muscle larvae.

The proteins with FYVE zinc-finger domain

The FYVE finger domain is a cysteine-rich zinc-finger-like motif that coordinates two zinc atoms. The function of this domain is to target signal-transducing proteins to cell membranes through binding to the membrane lipid phosphatidylinositol-3-phosphate with high specificity. A protein with zinc-finger domain in *T. spiralis* (approximately 55 kDa) is present in the crude extracts of muscle larvae, adult worms, and newborn larvae (Fu et al. 2005). Another protein with zinc-finger motifs is present only in the adult worms of *T. spiralis*, and this protein contains putative DNA-binding motifs (Hu et al. 2005).

Rcd1-like protein

Required cell differentiation 1 (Rcd1) was initially identified as a factor essential for the commitment to nitrogen starvation-invoked differentiation in fission yeast. The murine Rcd1 protein was identified as a cofactor of the *c-myb* proto-oncogene product, and the c-Myb and Rcd1 proteins physically interact with each other and that the c-myb-specific *mim-1* promoter is downregulated by Rcd1 (Haas et al. 2004). The *Rcd1*-like gene was cloned from muscle larvae of *T. pseudospiralis* (Nagano et al. 2006). The Rcd1-like protein is mainly synthesized in the stichocytes, secreted into the host cell.

Trichinella proteins with defined function

The functions of some proteins of *Trichinella* spp. have been revealed. Such information is not only useful for cell biological understanding of host–parasite relationships but it also raises the possibility of application to therapeutic treatment. Defining protein function used to be difficult, but recent molecular techniques allow easier identification by homology searching between specimen genes and DNA sequences posted to the GeneBank database.

Proteinases

Among functional proteins, proteinase is one of most well investigated. Proteinases secreted by parasitic organisms may be involved in a wide variety of adaptive functions such as tissue penetration, larval migration, immunoevasion, retardation of blood coagulation, digestion, molting, and degradation of cellular matrix. These proteinases can also serve as immunodominant antigens, stimulating a protective immune response, or as potential targets for chemotherapy (Todorova and Stoyanov 2000).

Several authors have reported proteinases in the E–S products of the infective-stage larvae or adult worms of *Trichinella* spp. Serine proteinases and metalloproteinases in whole crude extracts and E–S products from *T. spiralis* muscle larvae were identified by Criado-Fornelio et al. (1992). The proteinases in crude extracts had molecular weights of 48, 54, and 62 kDa, and the proteinases in E–S products had molecular weights of 33, 62, and 230 kDa. de Armas-Serra et al. (1995) reported a class-undetermined proteinase from E–S products of *T. spiralis* muscle larvae, which was a single polypeptide with an estimated molecular weight of 35 kDa and an isoelectric point of 6.2.

Todorova et al. (1995) showed that the proteinases secreted from adult worms of *T. spiralis* degraded fibrinogen and plasminogen, and degradation was susceptible to the action of serine, cysteine, and aspartyl proteinase inhibitors. Serine proteinases in adult worms were present in E–S

products, and the purified enzymes (approximately 18, 40, and 50 kDa) displayed enzymatic activity (Todorova and Stoyanov 2000).

A *T. spiralis* muscle larvae stage-specific antigenic serine proteinase was cloned and characterized as a secretory tyvelose-bearing glycoprotein using molecular techniques (Romarís et al. 2002b). Nagano et al. (2003) also identified the serine proteinase from *T. spiralis* muscle larvae and confirmed the proteinase activity of the recombinant protein. They showed that the serine proteinase gene is mainly expressed in muscle larvae, but the proteinase is not present in E–S products. Whereas, Robinson et al. (2005) showed by proteomic analysis that this serine proteinase is present in the E–S products. Recently, a putative serine proteinase composed of two proteinase domains was identified (Trap et al. 2006). This proteinase is expressed at various developmental stages of *T. spiralis* but not found in E–S products.

These works mentioned above mainly dealt with serine proteinase. A cysteine proteinase secreted from the muscle larvae of *T. spiralis* was reported by Moczon and Wranicz (1999), and a metalloproteinase in E–S product of *T. spiralis* was reported by Lun et al. (2003).

Proteinase inhibitors

Serine proteinase inhibitor (serpin) inactivates proteinases by forming complexes with serine proteinase. Possible functions of serpin have been postulated in a number of other systems including modulation and inhibition of host immune responses (Macen et al. 1993). A *serpin* gene of *T. spiralis* muscle larvae was cloned by Nagano et al. (2001b). The recombinant protein of the serpin inhibited 82% of the activity of the serine proteinase. The *serpin* gene was restricted largely to the newborn larvae and muscle larvae, and serpin is found within the stichocytes of muscle larvae in the early stage of infection.

Cystatins comprise a diverse group of cysteine protease inhibitors and important immunomodulatory factors when secreted by parasitic nematodes. A novel cystatin-like protein (46 kDa) secreted from *T. spiralis* is identified as a new member of the noninhibitory cystatin-related proteins by Robinson et al. (2007b). Cystatin-like protein gene expression is largely restricted to intracellular stages, predominantly in the adult worms. Interestingly, this protein is not present in the E–S products of *T. pseudospiralis*. This may explain why inflammatory response in *T. pseudospiralis* infections is less than in *T. spiralis* infection because the cystatin-like protein of *T. spiralis* acts as a proinflammatory factor. Sugane and Matsuura (1990) reported DNA sequences of a 46-kDa protein from muscle larvae of *T. spiralis* without referring its function. But this 46-kDa protein seems to be the cystatin-like protein because the sequences of this protein are completely identical to that of the cystatin-like protein published by Robinson et al. (2007b).

Heat shock proteins

Heat shock proteins (Hsps) may protect parasites against stress or injury and may play an important role in tissue invasion and intracellular survival. They have been documented in a large variety of species.

The Hsps in *Trichinella* spp. were documented for the first time in both crude extracts and E–S products of the heat-shocked infective-stage larvae using SDS-PAGE (Ko and Fan 1996). The major Hsps in crude extracts of *T. spiralis* are 20, 47, 50, 70, 80, and 86 kDa, and the major Hsps in the E–S products are 11, 45, 53, and 64 kDa. In *T. pseudospiralis*, the major Hsps in the crude extracts are 20, 26, 31, 50, 53, 70, 80, and 86 kDa, and in the E–S products 11, 35, 37, 41, and 64 kDa. The 70-kDa Hsp of *T. spiralis* was detected by Western blotting using mAbs (Martinez et al. 2000). The 70-kDa Hsp of *T. britovi* was detected in the nuclei of the muscle larvae but not in adult worm nuclei (Vayssier et al. 1999).

Martinez et al. (1999) compared three stimuli, elevated temperature, hydrogen peroxide, and mebendazole for their ability to induce heat shock responses in *T. spiralis* muscle larvae and observed that the exposure to hydrogen peroxide resulted in the induction of constitutive and higher Hsps. The expression levels of the 50-kDa Hsp from infective-stage larvae of *T. spiralis* immediately and persistently increased after oxidative and cold shock (Martinez et al. 2002). They investigated the relationship between infectivity and the expression levels of Hsp 70 and Hsp 60. Oxidative stress caused a significant increase in Hsp levels and total loss of infectivity, but cold oxidative stress caused no alterations in either Hsp levels or infectivity (Martinez and Rodriguez-Caabeiro 2005).

Three heat-induced genes in *T. spiralis* were identified using the suppression subtractive hybridization technique (Mak et al. 2001). These genes are homologous to *histone H3* gene, *histone H2B* gene, and translationally controlled tumor protein (*TCTP*) gene. The TCTP is a stress- and growth-related protein with antiapoptotic and immunomodulatory activities. After heat shock treatment, the expression levels of histone H3, histone H2B, and TCTP increased, but the RNA level of *TCTP* did not (Mak et al. 2007). The expression of TCTP may be upregulated at the translational level rather than at transcriptional level during early stage of stress adaptation.

Hsp 60 is a family of ubiquitous molecular chaperonins that regulate posttranslational folding, assembly, and the targeting of proteins. Some chaperonins are known to be expressed as a response to stress whereas others are constitutively expressed. However, the thermal, cold,

acidic, and oxidative treatment did not elicit significant changes in the expression of mitochondrial Hsp 60 of the *T. spiralis* muscle larvae (Wong et al. 2002). A small heat shock gene of *T. spiralis*, which had a high sequence identity in alpha crystallin domain, was cloned by Wu et al. (2007b). This small heat shock protein is expressed at various developmental stages of *T. spiralis* and possesses chaperone activity to suppress the thermally induced aggregation of citrate synthase.

Glycosidases

In extracts of *T. spiralis* adult worms and muscle larvae, the exoglycosidases, β-*N*-acetyl-D-glucosaminidase, β-*N*-acetyl-D-galactosaminidase, alpha-1-fucosidase, alpha-D-glucosidase, and alpha-D-mannosidase, are present at high levels (Rhoads 1985). These glycosidases are detected also in culture fluids of both muscle larvae and adult worms.

The β-*N*-acetyl-D-hexosaminidase has been detected in the E–S products of various parasitic organisms. Its functional roles have been postulated to be linked to various events including host cell invasion, modification and/or remodeling of cell surfaces, or carbohydrate breakdown for nutritional purposes. The β-*N*-acetyl-D-hexosaminidase is present in the muscle larvae of *T. spiralis*, and the enzyme is a glycoprotein with an estimated molecular weight of 100 kDa and is localized on cell membranes and the epicuticle (Rhoads 1988). The secreted glycosidase from *T. spiralis* with significant activity is only an exo-β-hexosaminidase (Bruce and Gounaris 2006). This enzyme is glycosylated with an apparent molecular weight of 50 kDa, and the glycan is decorated with tyvelose.

Protein kinases

Arden et al. (1997) showed that serine–threonine protein kinases activity is present in E–S products of *T. spiralis* infective-stage larvae and identified two serine–threonine protein kinases of 70 and 135 kDa in E–S products using autophosphorylation. The major phosphorylated proteins (50- and 55-kDa proteins) contain mainly phosphoserine and appear to represent differentially glycosylated variants of a 35-kDa polypeptide.

Endonucleases

Double-stranded endonuclease activity is present in the E–S products of the infective-stage larvae of *T. spiralis* and is present in much smaller amounts in the E–S products of *T. pseudospiralis*. The endonuclease activity is associated with at least three molecular forms, designated approximately as 25, 30, and 58 kDa, respectively (Mak and Ko 1999). Unlike the double-stranded endonuclease, the single-stranded molecule is divalent cation independent and is present in both *T. spiralis* and *T. pseudospiralis* E–S products (Mak et al. 2000). The single- and double-stranded endonucleases are likely to be encoded by different proteins and may have different functions.

Thymidylate synthase

Thymidylate-synthase-specific activity is present at a high and constant level in crude extracts from muscle larvae and adult worms of *T. spiralis* and *T. pseudospiralis* (Rode et al. 2000). Dabrowska et al. (2004) cloned *thymidylate synthase* gene from the cDNA of *T. spiralis* and confirmed enzymatic activity of recombinant thymidylate synthase. The expression level of thymidylate synthase was similar in muscle larvae, adult worms, and newborn larvae.

Macrophage migration inhibitory factor

Migration inhibitory factor (MIF) was first identified as a soluble protein secreted by sensitized lymphocytes, which inhibited the migration of macrophages. Recently, it has been discovered that MIF not only plays a critical role in inflammation but also has endocrine and enzymatic function. Parasites usually possess the ability to escape from host immune attack. Some parasites secrete a homolog of host MIF that has the capability of modifying the activity of human monocytes–macrophages.

The MIF of *T. spiralis* was detected in E–S products of muscle larvae (Tan et al. 2001). The MIF recombinant protein inhibited migration of human peripheral blood mononuclear cells but had no effect on anti-CD3-stimulated murine T cell proliferation. The homolog of MIF from *T. spiralis* is present in *T. pseudospiralis* E–S products (Wu et al. 2003). The *MIF* gene is expressed in various developmental stages, including in adult worms, newborn larvae, and muscle larvae. The MIF exists in the muscle cells of the body wall and some stichocytes of larvae. The MIF recombinant protein profoundly inhibited the macrophage accumulation around the Sephadex beads transplanted in mice.

Nucleotide-metabolizing enzymes

Tissue damage results in a variety of molecular signals that activate elements of the immune system. Recently, it has been shown that the key regulators of these events are extracellular nucleotides, which signal through purinergic receptors (Gounaris and Selkirk 2005).

Nucleoside diphosphate kinases (NDPKs) play a key role in the maintenance of intracellular pools of deoxynucleoside triphosphates and nucleoside triphosphates (NTPs) via the transfer of phosphate from an NTP donor

to an NDP acceptor. *T. spiralis* secretes an NDPK with molecular weight of 17 kDa, and a possible function of NDPK might lie in the regulation of host cell proliferation and differentiation (Gounaris et al. 2001).

Exoenzymes: apyrase, 5'-nucleotidase, and adenosine deaminase are secreted by *T. spiralis* (Gounaris 2002). These proteins constitute an enzymatic cascade which catalyzes the degradation of extracellular nucleotides, with a potential physiological role in the regulation of purinergic signaling.

A nucleotidase secreted from *T. spiralis* catalyzes the hydrolysis of nucleoside 5'-diphosphates and 5'-monophosphates but not 5'-triphosphates (Gounaris et al. 2004). The sequence of the nucleotidase is homologous to 5'-nucleotidases from a wide variety of organisms but contains no sequences specifically conserved in apyrases, suggesting that it is a representative of a new class of secreted nucleotidase.

Prosaposin and GM2 activator protein

The lysosomal degradation of glycosphingolipids with small carbohydrate chains requires the presence of nonenzymatic cofactor like prosaposin and ganglioside GM2 activator protein (GM2AP).

Infective-stage larvae and adult worms of *T. spiralis* secrete a protein homologous to prosaposin (Selkirk et al. 2004). The protein is secreted in an unprocessed form with an estimated molecular weight of 56 kDa and contains the TSL-1 antigens. The protein is localized to membrane-bound vesicles and more complex multilamellar organelles in diverse tissues including the hypodermis, intestine, and stichocytes. The prosaposin facilitates invasion of intestinal epithelial cells and subsequent migration through this cell layer. GM2AP secreted from *T. spiralis* was reported by Bruce et al. (2006). This protein does not facilitate degradation of GM2 ganglioside by *N*-acetyl-β-hexosaminidase A because of the absence of a domain implicated in binding to hexosaminidase.

Enolase

Enolase is an enzyme which catalyzes 2-phospho-D-glyceric acid (2PGA) to phosphoenolpyruvate (PEP) in the glycolytic pathway where ATP is synthesized in the process of metabolizing glucose to pyruvic acid. Enolase of parasites enhances the activation of plasminogen, and plasminogen mediated by enolase contributes to tissue migration of the parasites (Bernal et al. 2004).

The enolase from *T. spiralis* muscle larvae was identified by Nakada et al. (2005). The recombinant full-length enolase of *T. spiralis* had no activity in the conversion of 2PGA to PEP but gained enolase activity after cutting off the signal peptide from the full-length protein. The enolase is in crude extracts of muscle larvae but is not present in the E–S products.

Superoxide dismutases

Superoxide dismutase (SOD) is an important antioxidant and catalyzes the conversion of superoxide anion into hydrogen peroxide and molecular oxygen. It exists commonly in most organisms to protect against endogenous oxidative stress.

Copper–zinc SOD activity is present in both crude extracts and E–S products of *T. pseudospiralis*, and the expression of cytosolic SOD is substantially higher in infective-stage larvae than in adult worms (Wu et al. 2006). The manganese SOD of *T. pseudospiralis* was reported by Wu et al. (2007a). This manganese SOD is a mitochondrial enzyme because of the presence of mitochondrial transit peptides and maturation cleavage site in this protein. The expression level of manganese SOD is lower than that of copper–zinc SOD in infective-stage larvae. The manganese SOD is highly expressed in the infective-stage larvae but not in adult worms.

Caveolin

Caveolins are integral membrane proteins which play a role in cholesterol homeostasis and transport, endocytosis mechanisms, and regulation of signal transduction in differentiated cells. The caveolin of *T. spiralis* is an adult stage-specific protein and gradually accumulates on the surface of oocytes and embryos, suggesting that the caveolin of *T. spiralis* plays some role in oocyte maturation and embryogenesis during development (Hernandez-Bello et al. 2007).

Prolactin

The prolactin-like hormone (23 kDa) of *T. spiralis* was identified in E–S products and the stichocytes within the muscle larvae (Quintanar et al. 2007).

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References

Appleton JA, Bell RGM, Homan W, van Knapen F (1991) Consensus on *Trichinella spiralis* antigens and antibody. Parasitol Today 7:190–192

Arasu P, Ellis LA, Iglesias R, Ubeira FM, Appleton JA (1994) Molecular analysis of antigens targeted by protective antibodies in rapid expulsion of *Trichinella spiralis*. Mol Biochem Parasitol 65:201–211

Arden SR, Smith AM, Booth MJ, Tweedie S, Gounaris K, Selkirk ME (1997) Identification of serine/threonine protein kinases secreted by *Trichinella spiralis* infective larvae. Mol Biochem Parasitol 90:111–119

Bernal D, de la Rubia JE, Carrasco-Abad AM, Toledo R, Mas-Coma S, Marcilla A (2004) Identification of enolase as a plasminogen-binding protein in excretory–secretory products of *Fasciola hepatica*. FEBS Lett 563:203–206

Bruce AF, Gounaris K (2006) Characterisation of a secreted N-acetyl-beta-hexosaminidase from *Trichinella spiralis*. Mol Biochem Parasitol 145:87–93

Bruce AF, Gares MP, Selkirk ME, Gounaris K (2006) Functional characterisation of a nematode secreted GM2-activator protein. Mol Biochem Parasitol 147:224–229

Chung YY, Ko RC (1999) A novel cDNA clone encoding a specific excretory/secretory antigen of larval *Trichinella pseudospiralis*. Parasitol Res 85:685–691

Connolly B, Trenholme K, Smith DF (1996) Molecular cloning of a myoD-like gene from the parasitic nematode, *Trichinella spiralis*. Mol Biochem Parasitol 81:137–149

Criado-Fornelio A, de Armas-Serra C, Giménez-Pardo C, Casado-Escribano N, Jimenez-Gonzalez A, Rodríguez-Caabeiro F (1992) Proteolytic enzymes from *Trichinella spiralis* larvae. Vet Parasitol 45:133–140

Dabrowska M, Jagielska E, Ciesla J, Plucienniczak A, Kwiatowski J, Wrannicz M, Boireau P, Rode W (2004) *Trichinella spiralis* thymidylate synthase: cDNA cloning and sequencing, and developmental pattern of mRNA expression. Parasitology 128:209–221

de Armas-Serra C, Giménez-Pardo C, Jimenez-Gonzalez A, Bernadina WE, Rodríguez-Caabeiro F (1995) Purification and preliminary characterization of a protease from the excretion–secretion products of *Trichinella spiralis* muscle-stage larvae. Vet Parasitol 59:157–168

Despommier DD, Laccetti A (1981) *Trichinella spiralis*: proteins and antigens isolated from a large-particle fraction derived from the muscle larva. Exp Parasitol 51:279–295

Despommier DD, Gold AM, Buck SW, Capo V, Silberstern D (1990) *Trichinella spiralis*: secreted antigen of the infective L1 larva localizes to the cytoplasm and nucleoplasm of infected host cells. Exp Parasitol 71:27–38

Fu BQ, Liu MY, Kapel CM, Meng XP, Lu Q, Wu XP, Chen QJ, Boireau P (2005) Cloning and analysis of a novel cDNA from *Trichinella spiralis* encoding a protein with an FYVE zinc finger domain. Vet Parasitol 132:27–30

Gare D, Boyd J, Connolly B (2004) Developmental regulation and secretion of nematode-specific cysteine–glycine domain proteins in *Trichinella spiralis*. Mol Biochem Parasitol 134:257–266

Gold AM, Despommier DD, Buck SW (1990) Partial characterization of two antigens secreted by L1 larvae of *Trichinella spiralis*. Mol Biochem Parasitol 41:187–196

Gounaris K (2002) Nucleotidase cascades are catalyzed by secreted proteins of the parasitic nematode *Trichinella spiralis*. Infect Immun 70:4917–4924

Gounaris K, Selkirk ME (2005) Parasite nucleotide-metabolizing enzymes and host purinergic signalling. Trends Parasitol 21:17–21

Gounaris K, Thomas S, Najarro P, Selkirk ME (2001) Secreted variant of nucleoside diphosphate kinase from the intracellular parasitic nematode *Trichinella spiralis*. Infect Immun 69:3658–3662

Gounaris K, Selkirk ME, Sadeghi SJ (2004) A nucleotidase with unique catalytic properties is secreted by *Trichinella spiralis*. Mol Biochem Parasitol 136:257–264

Haas M, Siegert M, Schurmann A, Sodeik B, Wolfes H (2004) c-Myb protein interacts with Rcd-1, a component of the CCR4 transcription mediator complex. Biochemistry 43:8152–8159

Hernandez-Bello R, Bermudez-Cruz RM, Fonseca-Linan R, Garcia-Reyna P, Le Guerhier F, Boireau P, Ortega-Pierres G (2007) Identification, molecular characterisation and differential expression of caveolin-1 in *Trichinella spiralis* maturing oocytes and embryos. Int J Parasitol 38:191–202

Hu ZP, Garcia-Reyna P, Fu BQ, Yang J, Li CV, Yang YP, Liu MY, Ortega-Pierres G, Boireau P (2005) A stage-specific open reading frame from three-day old adult worms of *Trichinella spiralis* encodes zinc-finger motifs. Parasite 12:151–157

Jasmer DP (1990) *Trichinella spiralis*: altered expression of muscle proteins in trichinosis. Exp Parasitol 70:452–465

Jasmer DP, Kwak D (2006) Fusion and differentiation of murine C2C12 skeletal muscle cells that express *Trichinella spiralis* p43 protein. Exp Parasitol 112:67–75

Jasmer DP, Yao S, Vassilatis DK, Despommier D, Neary SM (1994) Failure to detect *Trichinella spiralis* p43 in isolated host nuclei and in irradiated larvae of infected muscle cells which express the infected cell phenotype. Mol Biochem Parasitol 67:225–234

Ko RC, Fan L (1996) Heat shock response of *Trichinella spiralis* and *T. pseudospiralis*. Parasitology 96:85–95

Ko RC, Fan L, Lee DL, Compton H (1994) Changes in host muscles induced by excretory/secretory products of larval *Trichinella spiralis* and *Trichinella pseudospiralis*. Parasitology 108:195–205

Kuratli S, Lindh JG, Gottstein B, Smith DF, Connolly B (1999) *Trichinella* spp.: differential expression of two genes in the muscle larva of encapsulating and nonencapsulating species. Exp Parasitol 93:153–159

Kuratli S, Hemphill A, Lindh JG, Smith DF, Connolly B (2001) Secretion of the novel *Trichinella* protein TSJ5 by *T. spiralis* and *T. pseudospiralis* muscle larvae. Mol Biochem Parasitol 115:199–208

Lindh JG, Connolly B, McGhie DL, Smith DF (1998) Identification of a developmentally regulated *Trichinella spiralis* protein that inhibits MyoD-specific protein: DNA complexes in vitro. Mol Biochem Parasitol 92:163–175

Liu MY, Wang XL, Fu BQ, Li CY, Wu XP, Le Rhun D, Chen QJ, Boireau P (2007) Identification of stage-specifically expressed genes of *Trichinella spiralis* by suppression subtractive hybridization. Parasitology 134:1443–1455

Lun HM, Mak CH, Ko RC (2003) Characterization and cloning of metallo-proteinase in the excretory/secretory products of the infective-stage larva of *Trichinella spiralis*. Parasitol Res 90:27–37

Macen JL, Upton C, Nation N, McFadden G (1993) SERP1, a serine proteinase inhibitor encoded by myxoma virus, is a secreted glycoprotein that interferes with inflammation. Virology 195:348–363

MacLea KS, Krieser RJ, Eastman A (2003) A family history of deoxyribonuclease II: surprises from *Trichinella spiralis* and *Burkholderia pseudomallei*. Gene 305:1–12

Mak CH, Ko RC (1999) Characterization of endonuclease activity from excretory/secretory products of a parasitic nematode, *Trichinella spiralis*. Eur J Biochem 260:477–481

Mak CH, Ko RC (2001) DNA-binding activity in the excretory–secretory products of *Trichinella pseudospiralis* (Nematoda: Trichinelloidea). Parasitology 123:301–308

Mak CH, Chung YY, Ko RC (2000) Single-stranded endonuclease activity in the excretory–secretory products of *Trichinella spiralis* and *Trichinella pseudospiralis*. Parasitology 120:527–533

Mak CH, Su KW, Ko RC (2001) Identification of some heat-induced genes of *Trichinella spiralis*. Parasitology 123:293–300

Mak CH, Poon MW, Lun HM, Kwok PY, Ko RC (2007) Heat-inducible translationally controlled tumor protein of *Trichinella pseudospiralis*: cloning and regulation of gene expression. Parasitol Res 100:1105–1111

Martinez J, Rodriguez-Caabeiro F (2005) Relationship between heat shock protein levels and infectivity in *Trichinella spiralis* larvae exposed to different stressors. Parasitol Res 97:213–218

Martinez J, Perez-Serrano J, Bernadina WE, Rodriguez-Caabeiro F (1999) In vitro stress response to elevated temperature, hydrogen peroxide and mebendazole in *Trichinella spiralis* muscle larvae. Int J Parasitol 29:1457–1464

Martinez J, Perez-Serrano J, Bernadina WE, Rodriguez-Caabeiro F (2000) Detection of heat shock protein-70 from *Trichinella spiralis* larvae using a modification of the routine western blotting procedure. J Parasitol 86:637–639

Martinez J, Perez-Serrano J, Bernadina WE, Rodriguez-Caabeiro F (2002) Oxidative and cold shock cause enhanced induction of a 50 kDa stress protein in *Trichinella spiralis*. Parasitol Res 88:427–430

Matsuo A, Wu Z, Nagano I, Takahashi Y (2000) Five types of nuclei present in the capsule of *Trichinella spiralis*. Parasitology 121:203–210

Mitreva M, Jasmer DP, Appleton J, Martin J, Dante M, Wylie T, Clifton SW, Waterston RH, McCarter JP (2004) Gene discovery in the adenophorean nematode *Trichinella spiralis:* an analysis of transcription from three life cycle stages. Mol Biochem Parasitol 137:277–291

Moczon T, Wranicz M (1999) *Trichinella spiralis*: proteinases in the larvae. Parasitol Res 85:47–58

Nagano I, Wu Z, Nakada T, Matsuo A, Takahashi Y (2001a) Molecular cloning and characterization of a 21 kDa protein secreted from *Trichinella pseudospiralis*. J Helminthol 75:273–278

Nagano I, Wu Z, Nakada T, Matsuo A, Takahashi Y (2001b) Molecular cloning and characterization of a serine proteinase inhibitor from *Trichinella spiralis*. Parasitology 123:77–83

Nagano I, Wu Z, Nakada T, Boonmars T, Takahashi Y (2002) Molecular cloning and characterization of a novel protein of *Trichinella pseudospiralis* excretory–secretory products. J Helminthol 76:165–170

Nagano I, Wu Z, Nakada T, Boonmars T, Takahashi Y (2003) Molecular cloning and characterization of a serine proteinase gene of *Trichinella spiralis*. J Parasitol 89:92–98

Nagano I, Wu Z, Boonmars T, Takahashi Y (2004) Molecular cloning and characterisation of two kinds of proteins in excretory–secretory products of *Trichinella pseudospiralis*. Int J Parasitol 34:491–500

Nagano I, Wu Z, Takahashi Y (2006) Molecular cloning and characterization of an Rcd1-like protein in excretory–secretory products of *Trichinella pseudospiralis*. Parasitology 133:785–792

Nakada T, Nagano I, Wu Z, Takahashi Y (2005) Molecular cloning and functional expression of enolase from *Trichinella spiralis*. Parasitol Res 96:354–360

Parkhouse RM, Clark NW (1983) Stage specific secreted and somatic antigens of *Trichinella spiralis*. Mol Biochem Parasitol 9:319–327

Perteguer MJ, Rodríguez E, Romarís F, Escalante M, Bonay P, Ubeira FM, Gárate MT (2004) Minor interspecies variations in the sequence of the gp53 TSL-1 antigen of *Trichinella* define species-specific immunodominant epitopes. Mol Immunol 41:421–433

Quintanar JL, Salinas E, Guerrero R, Gomez R, Vidal S, Aranda J, Clapp C (2007) Prolactin-like hormone in the nematode *Trichinella spiralis* larvae. Exp Parasitol 116:137–141

Rhoads ML (1985) Glycosidases of *Trichinella spiralis*. Mol Biochem Parasitol 16:137–148

Rhoads ML (1988) Purification, characterization, and immunochemical studies of beta-*N*-acetyl-D-hexosaminidase from the parasitic nematode *Trichinella spiralis*. Mol Biochem Parasitol 31:57–69

Robinson MW, Connolly B (2005) Proteomic analysis of the excretory–secretory proteins of the *Trichinella spiralis* L1 larva, a nematode parasite of skeletal muscle. Proteomics 17:4525–4532

Robinson MW, Gare DC, Connolly B (2005) Profiling excretory/secretory proteins of *Trichinella spiralis* muscle larvae by two-dimensional gel electrophoresis and mass spectrometry. Vet Parasitol 132:37–41

Robinson MW, Greig R, Beattie KA, Lamont DJ, Connolly B (2007a) Comparative analysis of the excretory–secretory proteome of the muscle larva of *Trichinella pseudospiralis* and *Trichinella spiralis*. Int J Parasitol 37:139–148

Robinson MW, Massie DH, Connolly B (2007b) Secretion and processing of a novel multi-domain cystatin-like protein by intracellular stages of *Trichinella spiralis*. Mol Biochem Parasitol 151:9–17

Rode W, Dabrowska M, Zielinski Z, Golos B, Wranicz M, Felczak K, Kulikowski T (2000) *Trichinella spiralis* and *Trichinella pseudospiralis*: developmental patterns of enzymes involved in thymidylate biosynthesis and pyrimidine salvage. Parasitology 120:593–600

Romarís F, Escalante M, Lorenzo S, Bonay P, Gárate T, Leiro J, Ubeira FM (2002a) Monoclonal antibodies raised in Btk(xid) mice reveal new antigenic relationships and molecular interactions among gp53 and other *Trichinella* glycoproteins. Mol Biochem Parasitol 125:173–183

Romarís F, North SJ, Gagliardo LF, Butcher BA, Ghosh K, Beiting DP, Panico M, Arasu P, Dell A, Morris HR, Appleton JA (2002b) A putative serine protease among the excretory–secretory glycoproteins of L1 *Trichinella spiralis*. Mol Biochem Parasitol 122:149–160

Romarís F, Dea-Ayuela MA, Bolás F, Martinez-Fernandez AR, Sanmartin ML, Ubeira FM (2003) Heterogeneity and immunogenicity of the *Trichinella* TSL-1 antigen gp53. Parasite Immunol 25:297–305

Selkirk ME, Hussein AS, Chambers AE, Goulding D, Gares MP, Vásquez-Lopez C, Gárate T, Parkhouse RM, Gounaris K (2004) *Trichinella spiralis* secretes a homologue of prosaposin. Mol Biochem Parasitol 135:49–56

Su XZ, Prestwood AK, McGraw RA (1991) Cloning and expression of complementary DNA encoding an antigen of *Trichinella spiralis*. Mol Biochem Parasitol 45:331–336

Sugane K, Matsuura T (1990) Molecular analysis of the gene encoding an antigenic polypeptide of *Trichinella spiralis* infective larvae. J Helminthol 64:1–8

Takahashi Y, Mizuno N, Shimazu K, Araki T (1992) Ultrastructure, antigenicity, and histochemistry of stichocyte granules of adult *Trichinella spiralis*. J Parasitol 78:518–523

Tan TH, Edgerton SA, Kumari R, McAlister MS, Roe SM, Nagl S, Pearl LH, Selkirk ME, Bianco AE, Totty NF, Engwerda C, Gray CA, Meyer DJ (2001) Macrophage migration inhibitory factor of the parasitic nematode *Trichinella spiralis*. Biochem J 357:373–383

Todorova VK, Stoyanov DI (2000) Partial characterization of serine proteinases secreted by adult *Trichinella spiralis*. Parasitol Res 86:684–687

Todorova VK, Knox DP, Kennedy MW (1995) Proteinases in the excretory/secretory products (ES) of adult *Trichinella spiralis*. Parasitology 111:201–208

Trap C, Fu B, Le Guerhier F, Liu M, Le Rhun D, Romand T, Perret C, Blaga R, Boireau P (2006) Cloning and analysis of a cDNA encoding a putative serine protease comprising two trypsin-like domains of *Trichinella spiralis*. Parasitol Res 98:288–294

Vassilatis DK, Despommier D, Misek DE, Polvere RI, Gold AM, Van der Ploeg LHT (1992) Analysis of a 43-kDa glycoprotein from the intracellular parasitic nematode *Trichinella spiralis*. J Biol Chem 267:18459–18465

Vassilatis DK, Despommier DD, Polvere RI, Gold AM, Van der Ploeg LHT (1996a) *Trichinella pseudospiralis* secretes a protein related to the *Trichinella spiralis* 43-kDa glycoprotein. Mol Biochem Parasitol 78:25–31

Vassilatis DK, Polvere RI, Despommier DD, Gold AM, Van der Ploeg LHT (1996b) Developmental expression of a 43-kDa secreted glycoprotein from *Trichinella spiralis*. Mol Biochem Parasitol 78:13–23

Vayssier M, Le Guerhier F, Fabien JF, Philippe H, Vallet C, Ortega-Pierres G, Soule C, Perret C, Liu M, Vega-Lopez M, Boireau P (1999) Cloning and analysis of a *Trichinella britovi* gene encoding a cytoplasmic heat shock protein of 72 kDa. Parasitology 119:81–93

Wisnewski N, McNeil M, Grieve RB, Wassom DL (1993) Characterization of novel fucosyl- and tyvelosyl-containing glycoconjugates from *Trichinella spiralis* muscle stage larvae. Mol Biochem Parasitol 61:25–35

Wong CS, Mak CH, Ko RC (2002) Cloning and characterization of the mitochondrial heat-shock protein 60 gene of *Trichinella spiralis*. Parasitol Res 93:461–467

Wu Z, Nagano I, Takahashi Y (1998) Differences and similarities between *Trichinella spiralis* and *T. pseudospiralis* in morphology of stichocyte granules, peptide maps of excretory and secretory (E–S) products and messenger RNA of stichosomal glycoproteins. Parasitology 116:61–66

Wu Z, Nagano I, Takahashi Y (1999) A panel of antigens of muscle larvae of *Trichinella spiralis* and *T. pseudospiralis* as revealed by two-dimensional western blot and immunoelectron microscopy. Parasitology 118:615–622

Wu Z, Matsuo A, Nakada T, Nagano I, Takahashi Y (2001) Different response of satellite cells in the kinetics of myogenic regulatory factors and ultrastructural pathology after *Trichinella spiralis* and *T. pseudospiralis* infection. Parasitology 123:85–94

Wu Z, Nagano I, Nakada T, Takahashi Y (2002) Expression of excretory and secretory protein genes of *Trichinella* at muscle stage differs before and after cyst formation. Parasitol Int 51:155–161

Wu Z, Boonmars T, Nagano I, Nakada T, Takahashi Y (2003) Molecular expression and characterization of a homologue of host cytokine macrophage migration inhibitory factor from *Trichinella* spp. J Parasitol 89:507–515

Wu WK, Mak CH, Ko RC (2006) Cloning and characterization of the Cu/Zn superoxide dismutase of *Trichinella pseudospiralis*. Parasitol Res 98:281–287

Wu WK, Mak CH, Ko RC (2007a) Cloning and differential expression of manganese superoxide dismutase (Mn-SOD) of *Trichinella pseudospiralis*. Parasitol Res 98:251–258

Wu Z, Nagano I, Boonmars T, Takahashi Y (2007b) Thermally induced and developmentally regulated expression of a small heat shock protein in *Trichinella spiralis*. Parasitol Res 101:201–212

Xu D, Wu Z, Nagano I, Takahashi Y (1997) A muscle larva of *Trichinella pseudospiralis* is intracellular, but does not form a typical cyst wall. Parasitol Int 46:1–5

Yao C, Jasmer DP (1998) Nuclear antigens in *Trichinella spiralis* infected muscle cells: nuclear extraction, compartmentalization and complex formation. Mol Biochem Parasitol 92:207–218

Yao C, Bohnet S, Jasmer DP (1998) Host nuclear abnormalities and depletion of nuclear antigens induced in *Trichinella spiralis*-infected muscle cells by the anthelmintic mebendazole. Mol Biochem Parasitol 96:1–13

Zarlenga DS, Gamble HR (1990) Molecular cloning and expression of an immunodominant 53-kDa excretory–secretory antigen from *Trichinella spirali*s muscle larvae. Mol Biochem Parasitol 42:165–174

Zarlenga DS, Gamble HR (1995) Molecular cloning and expression of an immunodominant 53-kDa excretory–secretory antigen from *Trichinella spirali*s muscle larvae. Mol Biochem Parasitol 72:253

Zarlenga DS, Boyd P, Lichtenfels JR, Hill D, Gamble HR (2002) Identification and characterisation of a cDNA sequence encoding a glutamic acid-rich protein specifically transcribed in *Trichinella spiralis* newborn larvae and recognised by infected swine serum. Int J Parasitol 32:1361–1370

Zarlenga DS, Rosenthal BM, La Rosa G, Pozio E, Hoberg EP (2006) Post-Miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*. Proc Natl Acad Sci U S A 103:7354–7359