A Family of Secreted Mucins from the Parasitic Nematode *Toxocara canis* Bears Diverse Mucin Domains but Shares Similar Flanking Six-cysteine Repeat Motifs*

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Eukaryotic parasites encode numerous homologs of host genes of physiological or immunological importance (1–5). Where such homologs are secreted by parasites into their environment in the mammalian host, it seems likely that they play an important role in mimicking or interfering with host molecules, to ensure the long term survival of the parasite. Many of these homologs are derived from ancestral gene families shared between host and parasite, while others may have evolved convergently as adaptations to the parasitic mode of life.

One parasite with remarkable longevity in vertebrate tissues is the nematode *Toxocara canis*. Although the life cycle is completed only in the definitive canine host, larval stages are capable of infecting a vast range of organisms, including humans in which it causes visceral and ocular larva migrans (6). The immune system is singularly ineffective against the larvae, due in part to the parasite’s ability to shed its surface coat when under attack from antibodies and/or leukocytes (7–10). In addition, larvae release prodigious quantities of heavily glycosylated *Toxocara* excretory/secretory (TES)1 antigens, which can be collected by *in vitro* cultivation (11–14).

Prominent among these antigenic components are the TES-120 glycoproteins (15), a closely migrating set of *O*-glycosylated proteins, which are major constituents of both the larval surface coat and the TES antigens (10, 15, 16). The structures of the predominant *O*-glycans on TES glycoproteins have been determined as two closely related novel trisaccharides, 2-O-Me-Fuc(a1–2)4-O-Me-Gal(β1–3)-GalNAc and 2-O-Me-Fuc(a1–2) Gal(β1–3)-GalNAc (17, 18). The cloning of one member of the TES-120 family revealed a serine-rich mucin-like sequence designated *Tc-muc-1* (19). The protein product of *Tc-muc-1* encodes a small protein with a signal peptide but no trans-membrane domain, indicating that it may fall to the sea anemone potassium channel-blocking toxin BgK, forming three disulfide bonds within each subunit.

**K-value**

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* This work was supported by the Medical Research Council and the Wellcome Trust. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF167707 (Tc-muc-2), AF167708 (Tc-muc-3), and AF167709 (Tc-muc-4). ‡ Present address: London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom. § Present address: PPL Therapeutics, Roslin, Edinburgh EH25 9PP, United Kingdom. ¶ Present address: ICAPB, University of Edinburgh, Edinburgh EH25 9PP, United Kingdom. §§ Present address: London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom. §§§ Present address: ICAPB, University of Edinburgh, Edinburgh EH25 9PP, United Kingdom. ¶¶ Present address: ICAPB, University of Edinburgh, Edinburgh EH25 9PP, United Kingdom. ** To whom correspondence should be addressed. Fax: 44-131-650-5450; E-mail: rick.maizels@ed.ac.uk.

1 The abbreviations used are: TES, Toxocara excretory/secretory; MALDI-TOF, matrix-assisted laser desorption ionization/time of flight; MS, mass spectrometry; EST, expressed sequence tag; RP, reverse phase; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; SAX, strong anion exchange.

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nel blocking toxins (25, 26) and metalloproteinases (27). As a result, the term SXC (six-cysteine) domain was introduced (28).

The discovery of a mucin-like surface coat/secreted product of tissue-dwelling nematodes provides intriguing parallels with transformed mammalian cells, which may express aberrant mucin forms and gain metastatic potential as a result (29–31). For this reason, we have searched for and identified novel secreted mucin glycoproteins from *T. canis*. Although there is no sequence homology between nematode and vertebrate mucins, both groups combine diverse repetitive Ser/Thr-rich tracts with flanking cysteine-rich domains (21, 32), indicating that convergent routes have led these phylogenetically distant taxa to adopt similar designs for glycoconjugates that function in the mammalian environment.

### EXPERIMENTAL PROCEDURES

**Parasites and Parasite Extracts** — Live adult *T. canis* were recovered from naturally infected dogs, and female worms were maintained in RPMI 1640 containing penicillin (100 units ml⁻¹), streptomycin (100 µg ml⁻¹), gentamicin (25 µg ml⁻¹), and amphotericin B (2.5 µg ml⁻¹) for 5 days. Culture media were replaced daily, and eggs liberated into the media were collected by centrifugation at 1000 × g. Eggs were hatched and infective larvae were maintained in vitro in serum-free RPMI 1640, as described previously (33). Spent tissue culture medium containing material released by live parasites was collected and concentrated using an Amicon 10 ultraconcentrator (Amicon) and stored at -80 °C.

This material is referred to as TES product.

**cDNA Library and EST Data Set** — A cDNA library made from mRNA of *T. canis* infective larvae cloned into the λ-phage vector Uni-ZAP XR (Stratagene) was a generous gift from Dr. C. Tripp and Dr. R. Grieve (Heska Corp., Fort Collins, CO). From this library, 261 expressed sequence tags (ESTs) were generated in our laboratory (34) and cDNAs for *Tc-muc-1* (19) and an additional three mucin genes were identified. Plasmids were generated from each of these clones, and the inserts were sequenced in full using a combination of vector-derived and genespecific primers. All of the ESTs encoding the mucins were truncated at the 5’ end. True 5’ ends of each cDNA were therefore isolated from the cDNA library by polymerase chain reaction using a combination of gene-specific and nematode spliced leader 1 (SL-1) (22, 35) primers. The 5’ amplicons were cloned into the T-ended vector pGEM-T (Promega), and each plasmid was sequenced. Sequencing was performed using the

![Fig. 1. Schematic representation of four *T. canis* mucin predicted proteins.](image)

Domain organization of four mucins are presented based on the deduced amino acid sequences of *Tc*-MUC-1 to -4. Signal peptides (green) are based on predictions made by the SignalP program yielding mean S values of 0.887–0.948. Six-cysteine domains (SXC, yellow) containing 36 amino acids conform with the motif described by Gems et al. (22). Mucin-like domains are shown in red, with the respective repeat motifs given beneath each protein. Numbering indicates amino acid positions. Domains expressed in bacteria for antibody production are indicated.

![Fig. 2. Alignment of mucin sequences.](image)

A, protein alignment of the serine/threonine-rich regions of *Tc*-MUC-1–4. Gaps have been inserted to maximize identity. Residues identical in four or more of the sequences are contained in black boxes, while conservative changes are denoted by gray boxes. B, protein alignment showing the degree of similarity between the paired SXC domains of *Tc*-MUC-1, -2, and -4, and to a lesser extent, *Tc*-MUC-3. The six cysteine residues (yellow) of each SXC domain are numbered. Blue boxes denote peptides derived from aspartyl endoproteinase digestion (N series), and red boxes those produced from trypsin treatment (T series), as summarized in Table II. The dashed blue box represents peptide cleaved during purification of 5.2.N.2 and 5.3.N.4 (see Table II legend). Two key substitutions providing discriminating molecular masses for MALDI-TOF MS are arrowed. Asterisks denote C-terminal stop codons.
AmpliTaq DyeDeoxy Terminator cycle sequencing system (Applied Biosystems) and an automated ABI Prism 377 DNA sequencer (Applied Biosystems). Analyses of nucleotide and deduced amino acid sequences were performed using the MacVector 6.0 software program (Oxford Molecular). Data base searches were performed using the BLAST server to search a nonredundant set of data bases (36). Sequence alignments were performed using the GCG PILEUP program (Genetics Computer Group) and Boxshade server. The translated protein sequence was analyzed using the SignalP program in order to identify a putative signal peptide and corresponding cleavage point (37).

Expression of Recombinant Proteins—Primers were designed to clone regions encoding the SXC domains 1–2 of Tc-MUC-1 and SXC domains 1–2 of Tc-MUC-3, as these segments showed most divergence in protein sequence. The resulting amplicons were cloned into the T-ended expression vector pET-29T (Novagen), and the ligated products were transformed into competent Escherichia coli strain XL1-blue, which lacks the T7 RNA polymerase gene; plasmids from cells carrying the insert were then transformed into E. coli strain BL21 carrying the DE3 episome, which possesses T7 RNA polymerase. Both recombinant proteins were soluble only in the presence of 6 M urea and were purified using a metal chelation resin (His Bind Resin; Novagen) under denaturing conditions according to the manufacturer’s instructions. Urea was removed by stepwise dialysis against Tris-buffered saline.

Immunization of Mice—BALB/c mice were immunized with either rTc-MUC-1 or rTc-MUC-3 (10 μg) emulsified in Freund’s complete adjuvant followed by two boosts at days 28 and 42 with 10 μg of protein in Freund’s incomplete adjuvant, administered via the subcutaneous route. Sera were collected at day 49.

Western Blots—Recombinant proteins (0.5 μg) and TES products (2.5 μg) were electrophoretically separated, transferred to nitrocellulose membrane, then probed with sera from mice immunized with the respective recombinant proteins and with normal mouse serum. Molecular mass marker positions are indicated (in kDa) on the left margin.

FIG. 3. Anti-MUC reactivity to TES-120 proteins. TES probed with antisera raised to recombinant SXC domains of T. canis mucins. Lane 1, anti-Tc-MUC-1; lane 2, anti-Tc-MUC-3; lane 3, normal mouse serum. Molecular mass marker positions are indicated (in kDa) on the left margin.

FIG. 4. SDS-PAGE analysis of TES products and fractions. A, total TES products stained with Stains-all reagent (note blue color obtained for TES-120 protein indicating extensive glycosylation) or carbamylated with 3H. SDS-PAGE was performed using 12% polyacrylamide gels in a Bio-Rad Miniprotean cell. B, SDS-PAGE analysis of 14C-labeled TES products separated by strong anion exchange chromatography. Fractions 4, 5, and 6 were retained and further separated by RP-HPLC. C, SDS-PAGE analysis of RP-HPLC fractions made from SAX samples 4, 5, and 6.

Amino Acid Composition Analysis and MALDI-TOF Mass Spectrometry—A total of seven fractions were analyzed for total amino acid composition, and for mass values by MALDI-TOF mass spectrometry. Three fractions contained sufficient material for proteolytic digestion with trypsin and Asp-N proteases. Peptides were purified on RP-HPLC and subjected to MALDI-TOF MS. Where quantities of peptide permitted, N-terminal protein sequencing was performed by Edman degradation.

Homology Modeling of Proteins—The cysteine-rich SXC domains of nematode proteins show similarity to the Tox-1 module present in potassium channel blocking toxins from cnidarians (24, 27). NMR-determined structures are available for the BgK toxin from Bunodosoma granulifera (26) and ShK toxin from Stichodactyla helianthus (25). We therefore constructed separate alignments of SXC domains -1 and -2 of Tc-MUC-1 with BgK (Protein Data Bank code 1BGK) and ShK (Protein Data Bank code 1ROO). The alignments were used as input for the homology modeling program MODELLER (38), and figures were produced using Swiss PdbViewer.
The RP-HPLC profiles of fractions 5.2 and 5.3 corresponded to related but distinct genes. All were truncated, so full-length clones were not available in some fractions (Table II). Moreover, several additional ESTs corresponded to related but distinct genes. All were truncated, so full-length clones were isolated from the cDNA library and fully sequenced. These novel apomucins were designated Tc-muc-2, Tc-muc-3, and Tc-muc-4, and their predicted protein sequences are schematically represented in Fig. 1.

Each cDNA is trans-spliced at the 5′ end with the nematode trans-spliced leader SL1, which facilitated isolation of full-length clones, and each predicted protein contains a typical signal sequence. The mature proteins are all combinations of two distinct modules, one being the mucin-like Ser/Thr-rich tract which acts as a locus for O-glycosylation, and the other being pairs of 36-amino acid SXC domains. All proteins contain two N-terminal units. The predominance of either Ser/Thr in the repeat regions varies between proteins: Tc-MUC-1 is Ser-rich, while the rest have varying repetitive units of Thr, Gly, Pro, and Ala, all with a predominance of Thr.

The cysteine-rich SXC domains show a high level of conservation, with the N-terminal Tc-MUC-3 sequence being the most diverse. The C-terminal domains of Tc-MUC-1, -2, and -4 (Fig. 2) makes it unlikely that a specific antisera could distinguish between these proteins. Although the Ser/Thr-rich repeat units of each protein are distinct, O-glycosylation of this tract in vivo may render antisera raised to recombinant constructs useless for recognizing the native proteins. However, the C-terminal SXC domain of Tc-MUC-3 bears sufficient differences from the other mucins to raise specific antibodies to this protein. At the same time, we generated antibody to the SXC domains of Tc-MUC-1.

TES proteins, secreted in vitro by live larval parasites, were then separated by SDS-PAGE for Western blot analysis. This showed that antisera raised to Tc-MUC-3 SXC domains 1–2 (rTc-MUC-3) recognized a higher molecular weight band of the TES-120 family (Fig. 3), confirming that Tc-MUC-3 is indeed a secreted glycoprotein. The antisera raised to recombinant Tc-MUC-1 SXC domains (rTc-MUC-1) was, as expected, less specific in its recognition of TES-120 proteins, binding to multiple bands most of which were of lower apparent molecular weight than the band recognized by anti-rTc-MUC-3 serum (Fig. 3).

Chromatographic Separation of TES-120 Proteins—TES-120 components, which migrate in 5–25% gradient SDS-PAGE gels with an apparent molecular mass of 120 kDa, were then fractionated by sequential ion-exchange and RP-HPLC. Fig. 4A shows the protein profile of unfractionated TES products on SDS-PAGE, as revealed by Stains-all, which produces a distinct blue staining of TES-120, reflecting its high carbohydrate content. SAX chromatography was then used to separate TES-120 (fractions 4–6) from the remaining TES proteins (fractions 7 and 8) (Fig. 4B). Each of the TES-120 fractions were then separated using reverse-phase HPLC, resulting in samples containing proteins of distinct mobility on SDS-PAGE (Fig. 4C).

Identity of Mucins in TES-120—Each of the fractions shown in Fig. 4C was subjected, without proteolytic degradation, to mass spectrometric determination of molecular weight and amino acid compositional analysis. Table I summarizes these results, in which four distinct molecular masses are defined. In each case, the true mass is less than half the apparent molecular weight on SDS-PAGE, attributable to the high carbohydrate content. Fraction 4.2, which displays the largest band on SDS-PAGE, has the highest mass (53.7 kDa), and is threonine/alanine-rich with little serine. In contrast, fraction 5.2 contains the smallest protein (39.7 kDa) (Fig. 5) and is markedly serine-rich (23–25 mol %) and is histidine-free (data not shown). Two further species, of 47.8 and 45.0 kDa, are in fractions 5.3 and 6.1 (Fig. 5), respectively, but are similar with respect to threonine/alanine abundance.

Sufficient material was available in some fractions to perform proteolysis with trypsin and Asp-N followed by mass spectrometry and Edman degradation of N-terminal residues. From fraction 5.2, the major tryptic fragment was found to derive from a region with identical peptide sequence between Tc-MUC-1 and Tc-MUC-2, but two Asp-N peptides gave masses consistent with Tc-MUC-1 and not with Tc-MUC-2, in each case due to a single amino acid difference (Fig. 6). One of these residues (Thr-117 in Tc-MUC-1) was, as expected, less specific in its recognition of TES-120 proteins, binding to multiple bands most of which were of lower apparent molecular weight than the band recognized by anti-rTc-MUC-3 serum (Fig. 3).

Chromatographic Separation of TES-120 Proteins—...
sion of Tc-MUC-2 (3/261 cDNAs) is consistent with the predominance of secreted mucins among the total protein synthetic profile of T. canis larvae.

Tc-MUC-3 is assigned to the 45.0-kDa component, which migrates on SDS-PAGE with a higher apparent molecular weight than either Tc-MUC-1 or Tc-MUC-2 (Fig. 4) on the basis of selective antibody reactivity, and the presence of four proteolytic peptides with masses that match the deduced amino acid sequence (Table II). The difference in molecular weight between the precursor peptide and mature mucin is significantly lower for Tc-MUC-3 (Table III), indicating that this may be less heavily glycosylated than the other two secreted mucins.

The remaining mucin, Tc-MUC-4, could not be assigned to any of the purified fractions. This protein may either not be secreted or is expressed at a relatively low level. The latter may be the case as Tc-muc-4 was present as only a single clone in the EST data base (34). There also remains an unidentified secreted protein in the lowest mobility fraction (4.1), which matched none of the sequences described here.

**Conservation of SXC Domains**—A striking feature of Toxocara mucins is the presence of paired SXC domains. A homolog of Tc-MUC-2 (3/261 cDNAs) is consistent with the predominance of secreted mucins among the total protein synthetic profile of T. canis larvae.

**Table II**

Peptides 5.2.N.2 and 5.3.N.4 were generated by AspN cleavage but did not commence with D. These peptides are from homologous parts of Tc-MUC-1 and Tc-MUC-2, respectively. A shorter peptide, DCQLF, was also present in 5.3.N.4, which corresponds to Tc-MUC-2 118–122, indicating correct AspN cleavage followed by lability of the F-M bond during purification. A similar event is presumed to have occurred during Tc-MUC-1 proteolysis. All deduced peptides terminate with the correct amino acid for enzymatic cleavage.

| Peptide     | Sequence         | Mass       | Corresponds to        | Predicted mass |
|-------------|------------------|------------|-----------------------|----------------|
| Protein 5.2 | Tryptic peptide TcNIC | 3672.6     | Tc-MUC-1 136–166      | 3671.0         |
|             | 5.2.T.7          |            |                       |                |
| AspN peptide | TPLCF            | 3396.1     | Tc-MUC-2 142–172      | 3395.8         |
|             | 5.2.N.2          |            | Tc-MUC-1 117–143      |                |
| AspN peptide | DCANFV           | 3499.6     | Tc-MUC-1 148–176      | 3499.0         |
| Protein 5.3 | Tryptic peptide CALTCG(F/C) | 999.1 | Tc-MUC-2 175–182      | 991.3          |
|             | 5.3.T.7          |            |                       |                |
| AspN peptide | TCNICSC         | 3672.2     | Tc-MUC-1 136–166      | 3671.0         |
|             | 5.3.T.9          |            |                       |                |
| AspN peptide | MPLCFQYPYSRAIQGCR | 3426.7 | Tc-MUC-2 142–172      | 3425.9         |
|             | 5.3.N.4          |            | Tc-MUC-2 123–149      |                |
| AspN peptide | DCANFV           | 3473.7     | Tc-MUC-2 154–182      | 3472.9         |
| Protein 6.1 | Tryptic peptide Not determined | 1796.4 | Tc-MUC-3 80–94        | 1795.9         |
|             | 6.1.T.1          |            |                       |                |
| Tryptic peptide | Not determined | 1481.8 | Tc-MUC-3 208–219      | 1481.6         |
| Tryptic peptide | Not determined | 3652.4 | Tc-MUC-3 228–258      | 3651.9         |
| Tryptic peptide | Not determined | 1090.4 | Tc-MUC-3 261–269      | 1090.4         |

**Fig. 6.** RP-HPLC profiles of tryptic and AspN protease fragments. Digests of fractions 5.2 and 5.3 were made, separated by RP-HPLC (left-hand panels) and individual peptide fragments subject to MALDI-TOF MS analysis. Right-hand panels show MALDI-TOF MS analysis of homologous peptides from each protein (5.2.N.7 and 5.3.N.9). The mass difference between the two peptides corresponds to a Pro/Ala substitution between Tc-MUC-1 and Tc-MUC-2 (see Fig. 2).
has been identified in cnidarian (e.g. sea anemone) proteins, in which the CXXXCCXC motif is reproduced (24, 27). Remarkably, aligning SXC domains from \(T. \) canis and the cnidarian \(B. \) granulifera (26) shows similar positioning not only of cysteines, but also of a number of charged and hydroxyl residues (Fig. 7). This strengthens the notion that the SXC may be a protein module of ancient evolutionary origin.

**Predicted Structure of the SXC Domains**—Based on the known structure of the cnidarian toxins, a structural model of the SXC domains -1 and -2 of \(Tc\)-MUC-1 was made. Fig. 8 presents the model of SXC domain 1 from \(Tc\)-MUC-1 in comparison to BgK from \(B. \) granulifera, and a similar structure was derived for domain 2 (data not shown). Although the pairing of disulfide bonds is likely to be the same in both proteins, the second \(\alpha\) helix between the third and fourth cysteines of BgK is not reproduced in \(Tc\)-MUC-1, reflecting the insertion of three additional residues around Tyr-20.

**DISCUSSION**

Mucins fulfill many essential functions in mammalian tissues, providing viscosity and physical protection to epithelial surfaces and bearing a dense array of glycans critical for cell adhesive interactions and trafficking (20, 21, 39–41). It is therefore not unexpected that a parasite which survives with facility in the same environment may itself express and secrete mucin-like glycoproteins. This report, however, demonstrates some surprising features of the mucin gene family of \(T. \) canis. They are expressed and secreted at a high level, far above any mucin gene from the free-living nematode \(C. \) elegans, indicating a primary role in immune evasion by parasites. At a structural level, all the mucin-like proteins contain cysteine-rich domains, as has been noted for most vertebrate mucins (21, 42), but in the form of a distinct six-cysteine array, which is incorporated into a range of both mucin and non-mucin proteins.

Like parasites, malignant tumors are able to circumvent immune surveillance, and the growth of epithelial cancers has been associated with overexpression or aberrant expression of mucin carbohydrate moieties (29–31, 43). Because parasite mucins are immunologically foreign to the host, secreted parasite mucins might simply create an immunological smoke-screen by generating antigen-antibody complexes away from the parasite (44). However, more targeted mechanisms are also possible. Overexpression of rat sialomucin, a membrane-bound mucin homologous to human \(MUC4\), both masks tumor antigens on transformed cells and blocks cytotoxic lymphocyte attack (45), while human epithelial cell mucin DF3/MUC1 has been reported to interfere with both eosinophil (46) and T cell function (47). Studies with protozoan parasites reveal that the glycosylphosphatidylinositol-anchored membrane mucin AgC10 from \(Trypanosoma cruzi\) binds to macrophages and blocks availability of \(\alpha\)-selectin, an essential component of the inflammatory process (48). The expression of many hundreds of such mucin genes by \(T. \) cruzi (49, 50) emphasizes the potential for parasite mucins to play a key role in immune evasion. We now plan to test purified \(T. \) canis mucins for their ability to interfere with similar immune mechanisms.

The \(T. \) canis MUC proteins each combine two evolutionarily distinct modules, the mucin and SXC domains. Many vertebrate mucins contain cysteine-rich motifs adjacent to Ser/Thr repeat regions (21, 51), but there is no similarity between SXC and, for example, the von Willebrand factor-like D domains shared by some human, porcine, and \(Xenopus\) mucins, or the epidermal growth factor-like repeat of human \(MUC3\) and \(MUC4\). In the vertebrate molecules, cysteine-rich flanking regions permit multimerization to produce the gel-like assemblies characteristic of epithelial surfaces (21, 51). It is therefore pertinent to analyze the structure of the SXC regions and predict whether they could be involved in higher order assembly.

The SXC motif acts as a cassette that is fused with a diverse range of protein domains, such as a phosphatidylethanolamine-binding protein (22) and a venom allergen homolog in \(T. \) canis (23), as well as tyrosinases, zinc metalloproteases, and some mucin-like proteins in \(C. \) elegans (28). Recently, SXC homologs from the phylum Cnidaria have been identified (24). Among these is BgK, a potassium channel-blocking toxin from the sea anemone \(B. \) granulifera that consists of a single SXC domain with three intramolecular disulfide bonds (26). The model that

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**TABLE III**

| Mucin gene | Mass | Predicted peptide mol wt | HPLC fraction no. | aa composition | Peptide sequence | Peptide mass | Antibody confirmation |
|------------|------|---------------------------|-----------------|----------------|----------------|-------------|---------------------|
| \(Tc\)-muc-1 | 39.7 | 15,747                    | 5.2             | 33            | 13  10         | Yes         | Yes                 |
| \(Tc\)-muc-2 | 47.8 | 16,151                    | 5.3             | 3             | 29  23         | Yes         | No                  |
| \(Tc\)-muc-3 | 45.0 | 26,012                    | 6.1             | 7             | 25  15         | No          | Yes                 |
| \(Tc\)-muc-4 | ND*  | 16,920                    | ND              | 2             | 39  18         | No          | No                  |

* ND, not determined.

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**FIG. 7. Conservation of SXC sequence.** Alignment of 10 SXC domains encoded by four mucin genes of \(T. \) canis, and the single SXC-like domain of BgK from \(B. \) granulifera. The conserved cysteine residues are in yellow on a black background. Hydroxyl residues (Ser/Thr) are shaded in green, acidic residues (Asp/Glu) in red, and basic (Arg/Gln/His/Lys) in blue.

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2 J. Murray, R. M. Maizels, and L. X. Liu, unpublished observations.
fide bonds are shown in that interferes with formation of a second ageal gland and the median secretory cell (15). If, as we sug-
etgest, \textit{T. canis} has evolved to successful parasitism by adapting pre-existing mucin-like genes from a free-living ancestor, the comparison between parasitic species and \textit{C. elegans} will hold many more instructive parallels and contrasts for the future.

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**Fig. 8. Structural model of SXC domain.** SXC domain 1 from \textit{Tc-MUC-1} was modeled based on the known NMR structure of BgK from \textit{Bunodosoma granulifera} (26). Cysteine residues that form disulfide bonds are shown in red. Residues conserved in all \textit{T. canis} SXC domains are shown in green, and the large aromatic residue (Tyr-20) that interferes with formation of a second \textalpha{} helix in \textit{Tc-MUC-1} is shown in magenta.
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