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An abundantly expressed mucin-like protein from *Toxocara canis* infective larvae: The precursor of the larval surface coat glycoproteins

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ABSTRACT **Evasion of host immunity by *Toxocara canis* infective larvae is mediated by the nematode surface coat, which is shed in response to binding by host antibody molecules or effector cells. The major constituent of the coat is the TES-120 glycoprotein series. We have isolated a 730-bp cDNA from the gene encoding the apoprotein precursor of TES-120. The mRNA is absent from *T. canis* adults but hyperabundant in larvae, making up approximately 10% of total mRNA, and is trans-spliced with the nematode 5′ leader sequence SL1. It encodes a 15.8-kDa protein (after signal peptide removal) containing a typical mucin domain: 86 amino acid residues, 72.1% of which are Ser or Thr, organized into an array of heptameric repeats, interspersed with proline residues. At the C-terminal end of the putative protein are two 36-amino acid repeats containing six Cys residues, in a motif that can also be identified in several genes in *Caenorhabditis elegans*. Although TES-120 displays size and charge heterogeneity, there is a single copy gene and a homogeneous size of mRNA. The association of overexpression of some membrane-associated mucins with immunosuppression and tumor metastasis suggests a possible model for the role of the surface coat in immune evasion by parasitic nematodes.**

Toxocara canis is an ascarid nematode parasite of dogs (1). Eggs voided in the feces of infected dogs mature in the soil to an infective stage that hatches in the stomach of human and other paratenic hosts. Larvae released in this way penetrate the tissues, causing muscular weakness, eosinophilia, hepatosplenomegaly, and bronchospasm (2–4); blindness can also occur when migrating larvae enter the eye. This larval stage is in a state of arrested development and can survive *in vivo* for many years (5). Larvae in the arrested state may be cultured indefinitely in serum-free medium (6) and release a set of glycoproteins termed *Toxocara* excretory/secretory (TES) antigens at around 1% of their body weight per day (7). The TES antigens include components found in the cuticle and the protective surface coat of *T. canis* larvae (8, 9), which forms a barrier between the parasite and the host immune system. The bulk of TES is composed of two abundant antigens, TES-32 and TES-120 (10, 11).

TES-120 has been identified as the major component of the surface coat by a combination of radioiodination and electron microscopy (9). TES-120 migrates as a triplet on one-dimensional SDS/PAGE gels and displays charge heterogeneity by two-dimensional gel analysis. Metabolic labeling has shown it to be the major protein synthesized by larvae, with a particularly high incorporation of Ser and Thr (11). TES-120 binds to *Helix pomatia* lectin (which recognizes terminal *N*-acetylgalactosamine) but is unaffected either by digestion with *N*-glycanase (7) or by the presence of tunicamycin during its biosynthesis (11). Alkali degradation (which cleaves O-linked sugars) releases a predominant 15-kDa product from TES (11), indicating that TES-120 is heavily O-glycosylated.

In this study we describe the structure of the apomucin-like protein encoded by a hyperabundant stage-specific trans-spliced cDNA *nmuc1* and propose that this is the precursor of TES-120. The 22-nt 5′ trans-spliced leader sequence SL1, first observed in the nematode *Caenorhabditis elegans* (12), is apparently ubiquitous among nematode species (13). It is present on >80% of mRNAs in *Ascaris lumbricoides*, as may also be the case in *C. elegans* (14). In this report, we exploit this property to amplify and isolate full-length trans-spliced mRNA from *Toxocara* and describe a single transcript that is unusually highly represented within this population.§

MATERIALS AND METHODS

Preparation of cDNA. RNA was isolated by using an acid guanidinium thiocyanate extraction method based on Chomczynski and Sacchi (15), adapted by A. Scott (44). Live adult worms were snap-frozen and stored at −70°C before use. Larvae were hatched and cultured as described (6, 10) and maintained at 37°C for at least 3 days before use. One adult male or 1000 larvae were ground to a powder in liquid nitrogen and mixed with 1 ml of solution D (4 M Guanidinium thiocyanate/0.5% sarcosyl/0.1 M 2-mercaptoethanol/25 mM sodium citrate, pH 7.0). After inversion, 100 μl of 2.0 M sodium acetate (pH 4.0), 1 ml of water-saturated phenol, and 200 μl of chloroform were added. This was vortex-mixed and left at 4°C for 15 min. After centrifugation at 10,000 × *g* for 20 min, RNA was precipitated from the supernatant by addition of 1 vol of isopropanol and incubation at −20°C for 1 h. This was pelleted at 14,000 × *g* for 10 min at 4°C, and the vacuum-dried pellet was resuspended in solution D and precipitated again. Pellets were resuspended in 20 μl of H_2O and stored at −20°C; 2 μl of RNA solution was used in subsequent cDNA preparations, without purification of mRNA. cDNA was prepared with a Perkin–Elmer/Cetus GeneAmp RNA-PCR kit, on a Hybaid HB-TR1 thermal reactor. Reverse transcription was carried out by using the 3′ poly(A)$^+$ tail complementary primer DGDT [5′-AATTCGGATCCCCCG-GG$(T)_{18}$-3′], which contains 5′ *Bam*HI and *Sma* I restriction sites. For PCR, three 5′ primers were designed, DGSL1, MBSL1-2A, and DGSL3, to place the resulting cDNAs in all three reading frames relative to the frame of the *lacZ* gene of pBluescript II SK$^+$. DGSL1 (5′-GGGCGGCCGCGGTTCA-ATTACCCAAGTTGGAG-3′) was used for the construction

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Abbreviation: TES, *Toxocara* excretory/secretory.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U39815).

of pTcSL1 described here. The sequences of DGSL1 and MBSL1-2A deviate from that of SL1 by removing termination codons and contain 5′ *Not* I restriction sites. In the PCR (35 cycles of 94°C for 1 min, 50°C for 3 min, and 75°C for 15 min) *Pfu* DNA polymerase (Stratagene) was used.

Cloning the cDNA TcSL1. The TcSL1 band was gel-purified from a 0.8% agarose gel (Fig. 1*A*) and reamplified by PCR. This DNA was purified and digested with *Not* I and *Bam*HI and directionally cloned into pBluescriptII SK+.

Southern Blot Analysis. DNAs were electrophoresed on 0.8% agarose gels and transferred to Hybond-N membrane (Amersham). DNA probes were labeled with digoxygenin by random priming, and detected after hybridization using a digoxygenin DNA labeling and detection kit (Boehringer Mannheim). Hybridization and washing were carried out at high stringency by prehybridizing for 1 h at 65°C in 5× SSC (0.75 M NaCl/0.075 M sodium citrate) containing 1% blocking reagent (Boehringer Mannheim, catalog no. 1093657), 0.1% *N*-lauroylsarcosine (sodium salt), 0.02% SDS, and denatured sonicated salmon sperm DNA (100 μg/ml), followed by hybridization solution containing freshly denatured probe DNA and denatured salmon sperm DNA (100 μg/ml). Filters were incubated overnight at 65°C and then washed for two 5-min periods at 65°C in 2× SSC/0.1% SDS and for two 15-min periods at 65°C in 0.1× SSC/0.1% SDS. The chemiluminescent substrate Lumigen PPD (Boehringer Mannheim 1357 328) was used as a substrate for the anti-digoxygenin-hapten-conjugated alkaline phosphatase. Hybridization and washing were carried out at high stringency as described, with autoradiography, in the Boehringer Mannheim kit protocol.

DNA Sequencing. cDNA nucleotide sequences were determined by the Sanger dideoxynucleotide chain-termination method with double-stranded DNA (16), ^{35}S-labeled dATP, and a T7 sequencing kit (Pharmacia). The sequences of both strands were determined of two copies of TcSL1: one PCR product and one phagemid pnmuc1.2. Both were identical.

Analysis of DNA and Putative Protein Sequence. Nucleic acid sequences were analyzed with the MACMOLLY 3.0 program and on the SEQNET facility at Daresbury (United Kingdom), by using the University of Wisconsin sequence analysis (GCG) package. Data base searches were performed with the FASTA and TFASTA algorithms (17) or by using the BLAST e-mail server (18) to search a nonredundant set of data bases. The GCG PILEUP program was used to make sequence alignments.

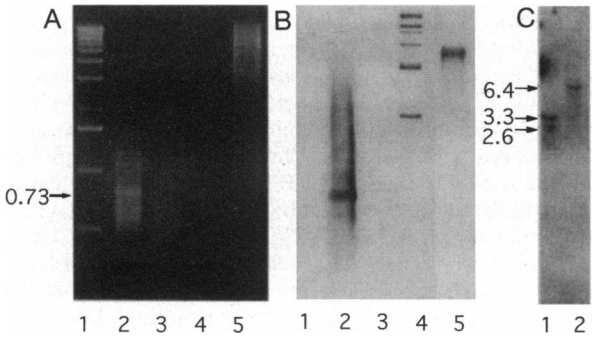

FIG. 1. RNA-PCR with *T. canis* mRNA reveals stage-specific expression of a 0.73-kb hyperabundant cDNA. (*A*) RNA-PCR of *T. canis* mRNA using primers complementary to 5′ spliced leader (SL1) and the 3′ poly(A)$^+$ tail, separated on a 1% agarose gel and stained with ethidium bromide. Lanes: 1, 1-kb ladder markers (BRL); 2, infective larval DNA; 3, adult DNA; 4, plasmid pTCSL11, which includes the cloned 730-bp cDNA; 5, *T. canis* chromosomal DNA digested with *Eco*RV. (*B*) Southern blot of the gel shown in *A*, probed with the 730-bp cDNA clone. The exposure of lane 5 was 5-fold longer than for the other four lanes. (*C*) Southern blot of genomic DNA of *T. canis* probed with the *nmuc1* insert. Lane 1 is *Hin*dIII-digested DNA; lane 2 is *Bam*HI.

Polyacrylamide Gel Electrophoresis. Metabolic labeling of *T. canis* larvae with [^{14}C]Ser was as described (11); [^{14}C]Lys labeling was carried out in identical fashion by using Lys-free medium. Two-dimensional nonequilibrium pH-gradient gel electrophoresis (NEPHGE) utilized ampholines from pH 3.5 to 10 (Pharmacia 1809-001) in the first dimension (19) and then SDS/5–25% polyacrylamide gel electrophoresis in the second dimension with an acrylamide/*N,N*′-methylenebisacrylamide ratio of 74:1.

RESULTS

A 0.73-kb cDNA Is Expressed in Larvae but Not Adults. Cloning of nematode genes is greatly facilitated if they are naturally trans-spliced at the 5′ end with the conserved SL1 22-nt sequence first reported in *C. elegans* (12). To ascertain if trans-splicing is a major feature of *T. canis* mRNA, we prepared first-strand cDNA from *T. canis* RNA (adult males and larvae) by using a primer complementary to the 3′ poly(A)$^+$ tail and then performed PCR with a sense SL1 primer and the oligo-(dT)-containing primer DGDT. The cDNAs generated ranged from 2 to 0.3 kb in adult and larval stages, but the larval profile was distinct in the presence of abundant products identified as visible bands (Fig. 1*A*). In particular, 0.73-kb and 1.1-kb bands were prominent in cDNA prepared from larvae but not adults. The most abundant 0.73-kb band (designated TcSL1) was gel-purified and cloned into the plasmid vector pBluescriptII SK$^+$ by using restriction sites present on the PCR primers. A Southern blot of SL cDNA from adult males and larvae was probed with TcSL1, and hybridization to larval but not adult cDNA was seen (Fig. 1*B*), indicating the absence of TcSL1 expression in adult males.

TcSL1 Encodes an Apomucin-Like Protein. The PCR-derived clone was sequenced on both strands and used to isolate a clone from a *T. canis* L2 cDNA library (kindly provided by C. Tripp and R. B. Grieve). The two clones were identical except for a difference in the poly(A)$^+$ tail position relative to the putative ATAA polyadenylylation signal (Fig. 2). In both, the putative translation initiation codon was 11 nt on the 3′ side of the SL1 sequence, and the coding sequences were identical. TcSL1 contains one long open reading frame encoding a 177-amino acid residue protein with a predicted molecular weight of 17,565 (Fig. 2). An 18-residue N-terminal hydrophobic signal sequence was predicted, with a score of +6.18 on the SIGNALASE program (20).

The deduced protein is extremely rich in the hydroxy amino acids Ser and Thr. The putative mature protein (without the signal sequence) has a predicted molecular weight of 15,829 and a Ser/Thr content of 41.8%, with a Ser/Thr ratio of 2.4:1. The mature TcSL1 protein has two distinct regions: an N-terminal 86-residue Ser/Thr-rich region, designated the ST region, and a 72-residue C-terminal Cys-rich region. The ST region is slightly hydrophilic (Fig. 3) and contains 72.1% Ser and Thr residues organized into an array of 11 largely conserved heptamer repeats of the sequence STSSSSA with partial repeats on either side. Ser and Thr residues are potential targets for O-glycosylation, and tandemly repeated sequences rich in O-glycosylation sites are characteristic of mucins, as is the presence of interspersed Pro residues (21). Accordingly, the sequence was designated *nmuc1* (nemomucin 1).

The C-terminal region contains alternating hydrophobic and hydrophilic regions (Fig. 3) and 12 Cys residues out of a total of 72 residues. The sequence reveals two 36-amino acid repeats with identically spaced Cys residues and an overall 38.9% identity. This 36-amino acid motif is also found in genes from the free living nematode *C. elegans* (see below) and was therefore designated the NC6 [nematode (Cys)$_6$] motif.

***nmuc1* Corresponds to a Hyperabundant Trans-Spliced mRNA.** Although the TcSL1 band is highly prominent in

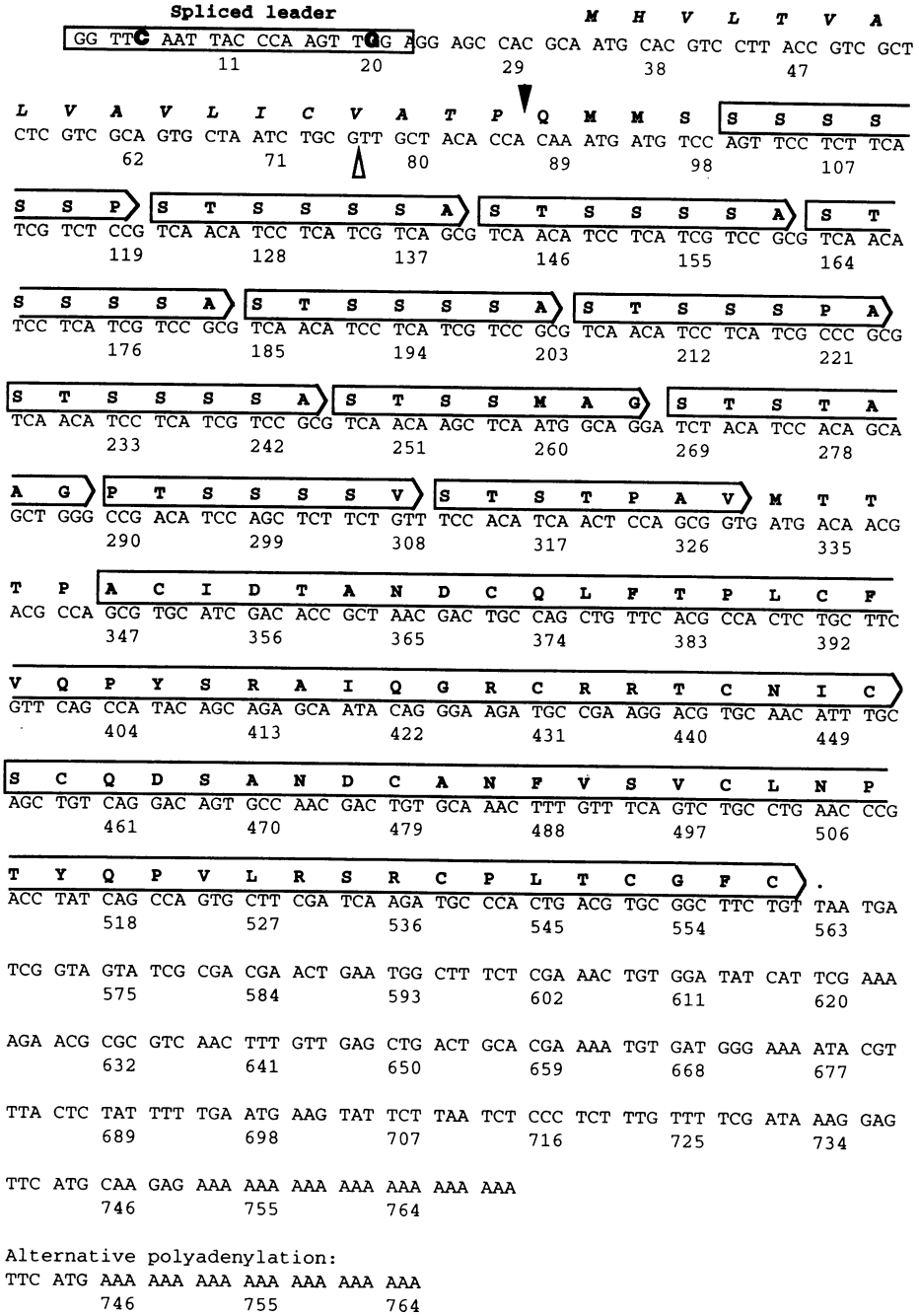

FIG. 2. Nucleotide and predicted amino acid sequence of the *nmuc1* gene. The spliced leader (SL1) 22-nt sequence is boxed. Two nucleotide changes were introduced in the primer to remove potential stop codons: T → C at nt 5 and T → G at nt 19 (shown in boldface type). Open arrowhead shows the location of the intron, and solid arrowhead shows the predicted signal peptidase cleavage site. The predicted signal peptide residues are in italic type. The Ser-rich heptameric repeats and the 36-residue NC6 motifs are highlighted with arrowed boxes. Both polyadenylylation products are shown.

PCR-amplified SL cDNA, this may reflect preferential amplification rather than true abundance. To resolve this issue, a *T. canis* larval cDNA library in λZapII (from C. Tripp and R. B. Grieve) was plated out and screened with *nmuc1* by hybridization at high stringency. *nmuc1* hybridized to 8.2–13.0% of plaques; thus, the level of transcription of TcSL1 is extraordinarily high relative to other genes. Eight clones were purified (λnmuc1.1–8) for phagemid isolation. PCR analysis showed four contained approximately full-length inserts (data not shown). The cDNA insert of one of these was amplifiable by using a primer complementary to the SL1 sequence. The 12 nt at the 5′ end of this clone, pnmuc1.2, were identical to the 3′ end of *C. elegans* trans-spliced SL RNA.

Analysis of the *nmuc1* Gene. PCR was carried out with primers complementary to the 5′ and 3′ ends of the *nmuc1* open reading frame and with either *T. canis* genomic DNA or *nmuc1* cDNA as template. The PCR product from *T. canis* genomic DNA was approximately 0.5 kb larger than that from *nmuc1* cDNA. No such increase in size was observed when a 5′ primer complementary to the open reading frame immediately 3′ of the signal peptide region was used. This suggested the presence of a 0.5-kb intron close to the 5′ end of the open reading frame. Sequencing of the PCR product containing the intron showed it to be located between nt 75 and 76 in a Val codon at position −4 relative to the signal peptide cleavage site (Fig. 2).

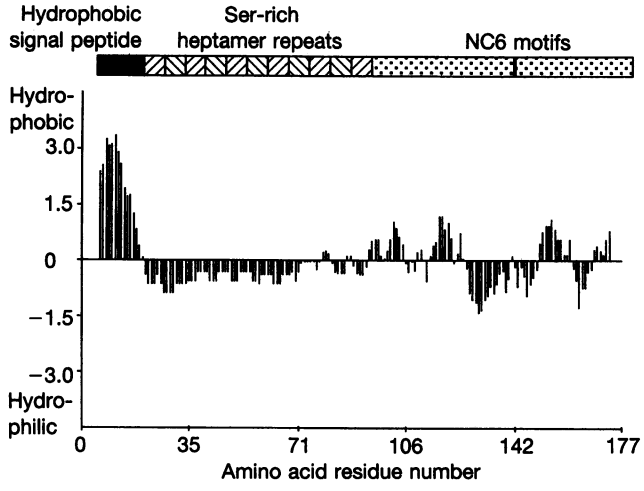

FIG. 3. (*Upper*) Schematic representation of domain structure. (*Lower*) Hydropathy plot for the deduced sequence of TES-120/*nmuc1*.

A Southern blot of *T. canis* genomic DNA was probed with *nmuc1*. This probe hybridized to a single 6.4-kb fragment in *Bam*HI-digested DNA but to two bands, of 3.3 kb and 2.6 kb, after *Hin*dIII restriction (Fig. 1*C*). Since the *nmuc1* cDNA sequence contains no *Hin*dIII sites, this indicated the presence of a *Hin*dIII site within the intron. Subsequent sequence analysis identified two *Hin*dIII sites in the intron (data not shown). Based upon the melting temperature of probe and the stringency of hybridization conditions, these data exclude the possibility of additional homologous genes with greater than 95% sequence identity.

Evidence That *nmuc1* Corresponds to the Major Surface Coat Glycoproteins TES-120. The deduced amino acid sequence for nmuc1 protein sequence is highly unusual in the absence of Lys residues. We therefore tested whether the abundant TES-120 corresponds to the *nmuc1* gene product by metabolic labeling with [^{14}C]Lys, in comparison to [^{14}C]Ser, which is strongly taken up (11). Two-dimensional NEPHGE/SDS/PAGE separation of protein extracts of labeled larvae showed extremely heavy incorporation of [^{14}C]Ser into the TES-120 bands but no incorporation of [^{14}C]Lys (Fig. 4).

Attempts to use mouse anti-TES sera against cDNA expression clones were not successful, perhaps because the high degree of glycosylation typical of mucins may obscure protein epitopes on native TES-120 from binding to antibodies. However, a short internal peptide sequence has been obtained from a single sample of gel-purified TES-120, of PLXFVQ, under conditions in which Cys residues were not detected. This sequence corresponds to residues 118–123 (PLCFVQ) of the deduced sequence. No other proteins containing this sequence were found in a BLAST data base search. Our data so far do not allow us to determine whether this sequence is derived from one or all of the TES-120 glycoproteins.

The NC6 Motif Is Found in Other Nematode Genes. The *nmuc1* sequence was compared to DNA and protein sequence data bases (18). This identified a number of Ser-rich sequences, e.g., the *Dictyostelium discoideum* spore coat glycoprotein SP96 (22). However, many of the same sequences were identified in a search using the sequence $(Ser)_{50}$. In contrast, searches with the NC6 motif identified several homologous sequences. Two *C. elegans*-expressed sequence tags from a mixed stage library (23) contained paired NC6-like motifs. In both of these sequences, clones CEMSE03 (chromosome V) and CEMSD85 (unmapped), the NC6 motifs were located at the C-terminal end of the protein, as in TES-120. The N-terminal domains are unrelated to the ST domain of TES-120. Another *C. elegans* gene, zk643.6, was found to contain five

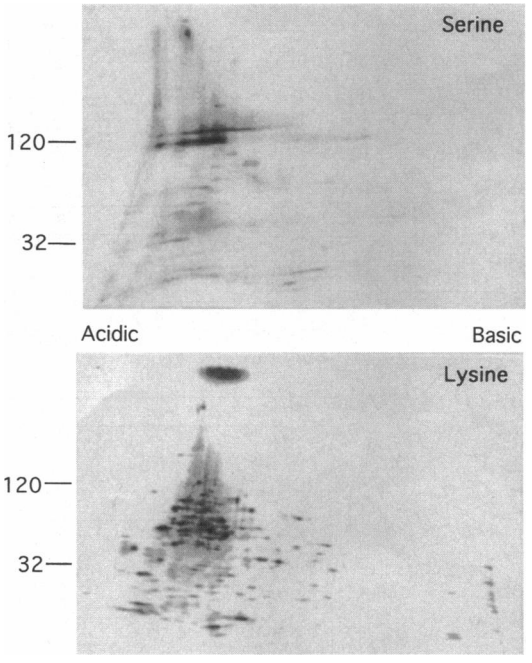

FIG. 4. Two-dimensional NEPHGE/SDS/PAGE separation of protein extracts of *T. canis* infective larvae metabolically labeled with [^{14}C]Ser (*A*) and [^{14}C]Lys (*B*). The positions of [^{14}C]Ser-labeled TES-120 and TES-32 are indicated. The TES-120 complex of bands is absent from the equivalent position in the [^{14}C]Lys-labeled gel.

tandem NC6 motifs (Fig. 5). zk643.6 is a putative open reading frame of unknown function identified in the *C. elegans* genome project on chromosome III (24).

Paired NC6 motifs were also found in the sequence derived from the 1.1-kb SL-PCR product from larval cDNA observed in Fig. 1. In this sequence, which corresponds to a 26-kDa secreted protein TES-26 (25), the motifs are at the N-terminal end of the protein (Fig. 5). The first NC6 motifs of TES-120 and TES-26 are more similar to each other than are either to the second motif, suggesting that a duplication of these domains preceded the evolution of the TES-120 and TES-26 genes. Sequence alignment of the 15 NC6 motifs now identified defines a consensus sequence $XCXDX_{4\text{-}6}CX_{4\text{-}8}CX_{12}CX_2TCX_2C$ (Fig. 5).

DISCUSSION

The *Toxocara* surface coat contains the O-linked TES-120 glycoproteins (9), which migrate as three distinct bands on SDS/PAGE gels. These are the most abundantly synthesized larval proteins, which preferentially incorporate [^{14}C]Ser and [^{14}C]Thr (11). Several lines of evidence suggest that *nmuc1* encodes a common precursor for this set of antigens: (*i*) the hyperabundance of the *nmuc1* transcript corresponding to the abundance of TES-120; (*ii*) the size of the mature putative protein (15.8 kDa) matching that of the 15- to 17-kDa core protein released by alkaline digestion of TES (11); (*iii*) the large proportion of Ser and Thr in *nmuc1* corresponding to the intense labeling of TES-120 with [^{14}C]Thr and [^{14}C]Ser; (*iv*) the absence of Lys in the *nmuc1* sequence corresponding to its absence from TES-120, as demonstrated by metabolic labeling experiments; (*v*) the peptide sequence obtained for residues 118–123. Further data consonant with this conclusion include the staining of the surface coat with the mucopolysaccharide stain ruthenium red (9), the concordance between the thickness of the surface coat and the predicted dimensions of TES-120 (see below), and *in vitro* translation studies of larval mRNA showing the most abundant product to be 15–17 kDa (26).

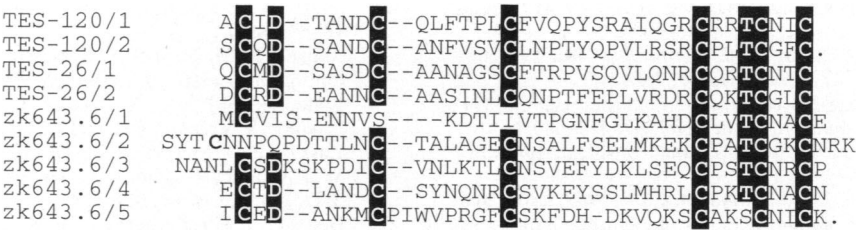

FIG. 5. Alignment of nine NC6 motifs from TES-120, TcSL-2, and zk643.6 from *C. elegans*. TES-120 sequence represents amino acids 105–177 reported in Fig. 2; TcSL-2 represents amino acids 22–94 as reported (24). The complete zk643.6 open reading frame consists of the five domains shown, from zk643.6/1 at the N terminus to zk643.6/5 at the C terminus, except for amino acids HNGTNPENKTGGNGGTGTQ, which are found between zk643.6/3 and zk643.6/4 (25). The homologous NC6 motif regions are boxed around the consensus residues of $XCXDX_{4\text{-}6}CX_{4\text{-}8}CX_{12}CX_2TCX_2C$.

The size and charge heterogeneity of TES-120 could result from the existence of a multigene family, from alternative mRNA splicing, or from extensive posttranslational modification. A single gene is indicated by the Southern blot hybridization pattern (Fig. 1*C*), while only the 730-bp transcript was evident when whole trans-spliced cDNA was probed with the *nmuc1* insert (Fig. 1*B*). The possibility of a tandem array of *nmuc1*-like genes, in which downstream copies would not receive the SL1 leader sequence (27) is not supported by the outcome of genomic PCR using various primers for the *nmuc1* sequence. However, in pulse–chase experiments, a single short-lived precursor has been observed at 58 kDa (11), indicating that size heterogeneity may indeed result from late post-translational modifications to a single TES-120 precursor.

The tandem repeat structure in the ST region of TES-120 and the heavy O-glycosylation are typical of mucins (21). Vertebrate mucins fall into two categories: secreted mucins, such as MUC-2 and porcine submaxillary mucin, and cell-membrane-associated mucins, such as sialomucin (CD34), leukosialin (CD43), and MUC-1, previously described as episialin, pancreatic tumor, and mammary gland mucin, respectively (28). Secreted mucins typically contain >500 amino acid residues, with a predominance of Thr over Ser. By these two criteria TES-120 more closely resembles a membrane mucin in being smaller in size (177 amino acid residues) and relatively rich in Ser, with a Ser/Thr ratio of 2.4.

Extensively O-glycosylated polypeptides take on an extended rod-like structure. Extensions of mucins have been measured at 0.25 nm per amino acid residue for porcine and ovine submaxillary gland mucins (28) and 0.20 nm per amino acid residue for CD43 (29). If extrapolated to TES-120, the predicted length for the 83-amino acid residue ST domain would be 17–20 nm. Given its 10- to 20-nm thickness (9), this suggests that the surface coat of *T. canis* larvae is composed of a monomolecular layer of TES-120.

TES-120 lacks a conventional transmembrane sequence, and its mode of attachment to the parasite surface remains to be defined. However, Tcn-3 antibody binds specifically to TES-32 (30) and coprecipitates TES-120 from undenatured TES (8). TES-32 localizes at the outer edge of the epicuticle (8), suggesting that it may serve as an anchor protein for the mucin, offering an explanation of the mechanism of surface coat morphogenesis. TES-120 is secreted in internal excretory glands, and ducted to the surface via the esophagus and excretory pore (8). Anchoring of TES-120 onto epicuticle-bound TES-32 would explain how TES-120 forms an evenly distributed layer covering the entire surface of the worm. Mammalian membrane-associated mucins such as MUC-1 are tethered to the cell surface by integral membrane proteins, derived either from the same gene transcript (31), or like GlyCAM-1 (32), an independent anchor protein as postulated for *Toxocara*.

Parasitic nematodes have evolved a multiplicity of evasive strategies to survive in a immunologically competent host (33, 34). One mode of immune evasion is the ability of *T. canis* larvae to shed the entire surface coat in response to binding by antibodies (35) or eosinophils (36), thus permitting parasites to physically escape immune attack (37). The identification of a mucin on the surface may, in addition, explain a generally nonadhesive property of the parasite. Membrane-associated mucins are closely concerned with the adhesion status of cells, both through electrostatic charge and the steric effects of long extended chains protruding from the surface. Thus, MUC-1-transfected cells lose homotypic adhesion (38) and overexpression of MUC-1 correlates with metastatic potential (39). This is also reflected in the inhibition of T-cell adhesion and cytotoxicity by CD43 and MUC-1 expression (40, 41) and even in the direct blocking of the ability of eosinophils to adhere and kill schistosome parasites in *in vitro* tests (42). It has been suggested that membrane-associated mucins such as MUC-1 may protect the lumenal surface of endothelial cells from damage from immune activity such as the macrophage oxidative burst. Thus, it is possible that in mimicking the surface of endothelial cells the surface coat disguises *T. canis* infective larvae, effectively exploiting a loophole in host immunity.

TES-120 is also released from *Toxocara* larvae in some quantity. The possibility is, therefore, raised that the soluble mucin interacts with host cells, perhaps through the oligosaccharide structures that are related to mammalian carbohydrates (43), in a manner that blocks extravasation, activation, and inflammation in response to infection. These possibilities are now being actively investigated as one route to understanding the ability of this parasite to survive for many years in the tissues of a mammalian host.

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