A Zinc Finger Gene from Onchocerca volvulus Encodes a Protein with a Functional Signal Peptide and an Unusual Ser-His Finger Motif*

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The filarial parasite Onchocerca volvulus is the causative agent of river blindness. In order to identify genes potentially involved in parasite development we cloned a zinc finger-encoding gene from this species. The ovzf-1 gene represents one member of a family of related zinc finger genes. The predicted ovzf-1 translation product of 447 amino acids includes a hydrophobic signal peptide, which is followed by 13 contiguous finger motifs. The domains of fingers II–XIII display several conserved amino acids and a typical Kruppel-like Cys2-His2 motif. The first finger domain has the two conserved Cys residues replaced by Ser residues; however, it includes all additional amino acids typical of zinc finger domains. The N-terminal domain functions as a signal peptide, as it directs secretion of a reporter protein and a truncated Ovzf protein. Expression of an Ovzf protein via the secretory pathway was also confirmed by demonstrating attachment of N-linked carbohydrates to the recombinant protein. Although the recombinant Ovzf protein also includes a signal peptide, immunofluorescence analyses localize it inside a specific compartment of the infected insect cell. Expression of ovzf mRNA is developmentally regulated; no specific transcript is detected in adult female worms but in the infective L3. Identification of a secreted protein that might function in modulating gene expression of host cells provides an interesting tool for the study of parasite-host interaction on a biochemical and molecular level.

The filarial nematode Onchocerca volvulus (Leuckart 1893), the causative agent of river blindness, infects an estimated 20 million humans who inhabit equatorial Africa, Central America, and Yemen (1). The infective larvae (L3) are transmitted to the human host by a female blackfly of the simulium species (2). In their final host the larvae undergo two molting steps and develop into an adult worm. The adult female worms of O. volvulus, which reside within nodules for periods of more than 10 years produce microfilariae that are for the most part responsible for pathogenesis of the disease (Refs. 3 and 4 and references therein).

It is of considerable interest to identify genes of the filarial nematode O. volvulus that are expressed at critical stages of the life cycle and during development (4). Parasite transcription factors with likely functions in parasite development and differentiation are ideal candidates for stage-specifically regulated genes. Upon identification of such putatively regulatory molecules it is of interest to study their pattern of expression during parasite development. The infective larva L3 is of particular interest, as this form is expected to express or secrete factors that allow the larvae to adjust to the physiological conditions provided by the mammalian host. Little is known about early development of O. volvulus, and to our knowledge no factors have yet been identified that play a regulatory role in parasite development. The characterization of these regulatory molecules is important for the understanding of physiological events involved in parasite development. Such regulatory proteins expressed in the infective L3 larvae are also of particular immunological interest, since upon infection and entry into the human host they are directly presented to the immune system.

We are interested in identifying and characterizing genes of O. volvulus that play a regulatory role in parasite development. Given the important function of zinc finger proteins in development and differentiation and the conservation of function among zinc finger proteins in diverse species (5–9) we have started the isolation of zinc-finger encoding genes (ovzf-1) of the Cys2-His2 type in O. volvulus (10). Here we describe the characterization of the complete ovzf-1 gene.

Expression of O. volvulus zinc finger mRNA is developmentally regulated. Expression was detected in the infective L3 larvae but not in the adult female worm. The corresponding Ovzf-1 protein has two unusual features; it includes a novel finger-like domain, which has the two conserved Cys residues replaced by Ser residues, and it possesses an N-terminal hydrophobic signal peptide that is capable of directing the expression of a reporter protein via the secretory pathway.

MATERIALS AND METHODS
Cloning and Screening

A genomic fix II library prepared from adult female O. volvulus was screened using a 507-bp fragment (representing nucleotides 756–1262) of the previously described O. volvulus zinc finger gene fragment ovzf-1 (10). Three distinct phage were isolated, their corresponding DNA was digested with EcoRI, and the various fragments were subcloned into pBluescribe vectors (Stratagene) for further analyses. Two cDNA clones (ovzf-2A and ovzf-2B) were amplified by PCR1 from L3 cDNA using the

1 The abbreviations used are: PCR, polymerase chain reaction; bp, basepair(s); PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis.
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ovzf-1-specific primers Cov7/ Cov25 and Cov7/ Cov9, respectively (see below).

Sequence Analyses

Pluripurified plasmons were sequenced in double-stranded form by the dyeoxy chain termination method using [α-35S]dATP and Sequenase 2.0 (Amersham Buchler) (11). In addition to plasmid-specific primers (T3, T7, SP6) and primers AC372 (5′-TTTACCTGTTGATGACGTT-3′) and HisR (5′-GGATCCATATGATGATGATGATGATGATGCTTTACAC-3′), designed for the expression vector pB5V8HS (12), new oligonucleotides were synthesized to allow sequencing of all inserts in both orientations. The DNASIS program (Hitachi) was applied for DNA sequence analysis.

Isolation of Onchocerca volvulus from Onchocercomata

FRESHLY excised nodules from onchoceriosis patients in Benin, West Africa, were incubated in 0.5% collagenase A (Boehringer Mannheim, FRG) in RPMI medium at 30°C for 8–36 h. Intact motile worms were prepared as described (13), and the isolated worms were immediately frozen in liquid N2.

RNA Isolation

Twenty-six O. volvulus adult female worms were thawed in lysis buffer A (60% guanidiniothiocyanate, 0.5% Na-laurylsarcosine, 50 mM lithium citrate, 0.5% β-mercaptoethanol) and immediately homogenized using a glass homogenizer. Cell debris were removed by centrifugation at 2,000 × g for 20 min. (Beckman) j-21, rotor JA-20. The supernatant was layered on top of a CsCl gradient (3.0–5.7 M CsCl in 0.1 M NaCl, EDTA) and centrifuged at 28,000 rpm for 22 h (Beckman LB 8–70M, rotor SW55). The pellet was resuspended in 100 μl of double-distilled H2O, and the yield was determined photometrically at 260 nm. The purity of the RNA was verified by ethidium bromide staining following agarose gel electrophoresis (14). All solutions were prepared with DEPC-treated double-distilled H2O.

Reverse Transcription and PCR Amplification

cDNA was synthesized from O. volvulus adult female RNA, using digo(dT)25 (25 μg/ml, Pharmacia Biotech Inc.), and reverse transcriptase (RT superscript, Life Technologies, Inc.) according to the recommendations of the manufacturer. The specific primers Cov7 (5′-ATGCTCTGGCTTTTTATTACTCTGTA-3′) and Cov8 (5′-CCGCA-CACATCGCACTTGAACG-3′, positions 903–980) and Cov7 and Cov9 (5′-TTTACCATATGATGATGATGATGATGCTTTACAC-3′, positions 990–967), respectively, were employed in PCR reactions using cDNA prepared from adult worms and third stage larvae (provided by Dr. K. D. Erttmann, Bernhard Nocht Institute) as template (Fig. 1). Positions of oligonucleotides refer to the ovzf-2 sequence (Fig. 4B). Two rounds of PCR amplifications were performed, each consisting of 25 cycles (95°C for 1 min, 72°C for 1 min for the first round and 55°C annealing temperature during the second round) yielding dyes ovzf-2A and ovzf-2B, respectively. Primer Cov8 (5′-CGGATGATATGCGGATGATACT-3′, positions 98–75) and the phage-specific T3 primer were used to amplify a 48-bp S′-untranslated fragment of ovzf-2 from the same cDNA source. The OvGST1 control fragment was amplified with digonucleotides G3t-938 and G9t-967, provided by Dr. E. Liebau, Bernhard Nocht Institute. The PCR products were separated in a 1% agarase gel and were further subcloned into the pCR TOPO-1 vector (Invitrogen).

Construction of Baculovirus Expression Plasmids

Step 1—The entire 942-bp ovzf-2B cDNA fragment was reamplified by PCR, using primers Cov7exp (5′-ATTGCGAGTATGCGGATGATACT-3′) and Cov3exp (5′-TTTACCTGTTGATGACGTT-3′), nucleotide positions 49–74) and Cov9exp (ttttccggcccTTTACCATATGATGATGATGATGATGCTTTACAC-3′, positions 990–961). Unspecific nucleotides are shown in lowercase letters, and BglII and SmaI restriction sites are underlined (nucleotide positions refer to the ovzf-2B sequence; see Fig. 4G). The PCR product was digested with BglII and SmaI and ligated into the BglII/SmaI-cleaved baculovirus expression vector pB5V8HS (12), yielding the expression construct ovzf-2exp.

Step 2—A functional assay was developed to analyze whether the hydrophobic N-terminal region of the Ovzf-2 protein can direct protein expression via the secretory pathway. The human factor H-like plasma protein 1 (FHL-1) (15, 16) is expressed in the baculovirus system as a secreted protein (17). Thus, an expression construct was created in which the region representing the FHL-1 signal peptide was replaced with the sequence encoding the N-terminal region of Ovzf-2. A 54-bp fragment coding for the Ovzf-2 hydrophobic region (Ov-SP, nucleotides 49–102) was amplified with primers Cov7exp and Cov3exp (5′-TTTACCTGTTGATGATGATGATGATGACGTT-3′, positions 102–75; the PsiI restriction site is underlined). This PCR fragment was used to replace the nucleotides encoding the signal peptide of FHL-1 (17), yielding expression plasmid Ov-SP/H.

Step 3—In order to recombinantly express a truncated fragment of the Ovzf-1 protein encoding the N-terminal finger domains 1–111, the ovzf-1 expression fragment was generated. The second exon of ovzf-1 was PCR-amplified with primers Cov51exp (5′-TTTACCTGTTGATGATGATGATGATGACGTT-3′, positions 114–141) and Cov9exp and subsequently cleaved with PsiI (underlined in Cov51exp) and EcoRI (using the internal EcoRI site at positions 492–497 of the ovzf-1 sequence) (Fig. 2). The 381-bp ovzf-1 expression fragment was ligated into the PsiI/EcoRI-cleaved pB5V8HS vector, downstream of the 54-bp sequence encoding the Ovzf-2 signal peptide.

Cell Culture

Spodoptera frugiperda intestinal cells (Sf9) were grown in monolayer cultures in Grace’s medium (BioWhittaker) supplemented with 10% fetal calf serum (Life Technologies), penicillin (100 units/ml), streptomycin (100 μg/ml), and fungizone (250 ng/ml), at 27°C and 95% humidity. Sf9 cells infected with recombinant baculovirus were grown in serum-free X-Press medium (BioWhittaker), supplemented with antibiotics.

Transfection and Expression of Recombinant Ovzf-2exp, Ovzf-1t, and Ov-SP/H

Plasmid DNA used for transfection of insect cells was purified using Nucleobond AX cartridges (Macherey & Nagel). Sf9 cells were cotransfected with 0.25 μg of viral DNA (BaculoGOLD, Pharmingen) and 2 μg of plasmid DNA employing a calcium phosphate precipitation method modified for insect cells (18). After 4 h the cells were washed, and 3 ml of medium were added. Infected cells were incubated for at least 7 days. For expression of recombinant protein 1 × 106 Sf9 cells growing in 140-mm cell culture dishes (Nunc) were infected with recombinant virus at a multiplicity of infection of 5. In a positive control, Sf9 cells were infected with recombinant virus that represents the signal peptide and the secreted FHL-1 protein (H-SP/H) (17). Four days after infection, recombinant proteins were analyzed in the cell culture supernatant, and Sf9 cells were harvested by centrifugation at 1,000 × g. The cells were resuspended in lysis buffer C (0.1 M NaH2PO4, 1 M urea, pH 8.0), supplemented with protease inhibitors (1 μg/ml each of antipain dihydrochloride, apratoin, leupeptin (all Boehringer Mannheim), chymostatin, trypsin inhibitor, pepstatin, elastinal (all Sigma), and Na2-EDTA) and homogenized in a glass homogenizer at 4°C. For concentrations of recombinant Ovzf-2exp and Ovzf-1t the proteins in the cell culture medium were precipitated with 80% cold aceton, washed in 70% ethanol, and resuspended in 10% volume of lysis buffer C.

Purification of Recombinant Proteins

Secretron recombinant proteins were purified from the culture medium by nickel chelate chromatography as described previously (12).

Inhibition of N-Linked Glycosylation of the Ovzf-2exp and Ovzf-1t Proteins by Treatment with Tunicamycin

Sf9 cells were plated and infected as described above. Twenty-four hours postinfection, the cell culture medium was replaced with fresh X-Press medium, which included 2 μg/ml tunicamycin (Sigma). The cells and the cell culture medium were harvested 3 days after tunicamycin treatment. Both the cell pellet and the concentrated cell culture medium were resuspended in 0.1 culture volume of lysis buffer C.

SDS-PAGE and Western Blot Analyses

Proteins were separated on a 12% denaturing polyacrylamide gel (19) and were visualized either by staining with Coomassie G (20) or by silver staining (21). In addition, proteins were electroblotted onto nitrocellulose membranes by a semi-dry technique (22). Membranes were blocked with 5% (w/v) BSA for 30 min and incubated overnight with rabbit antiserum. Recombinant Ovzf proteins were detected by Western blotting using rabbit antiserum at a dilution of 1:100. This antiserum had been raised against a synthetic peptide that represents an N-terminal fragment of the Ovzf-2exp protein (NH2-YARIDQLEQKNDTKEVDVI-COOH, amino acid positions 29–47) (Eurogentec, Belgium). For detection of the fusion protein Ov-SP/H and the control protein H-SP/H, antiserum raised against the human factor H-like protein 1 was used at a dilution of 1:2000 (17). After washing in PBS, membranes were incubated with peroxidase-labeled swine anti-
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Isolation and Genomic Organization of the ovzf-1 Gene—In order to isolate the complete gene encoding the O. volvulus zinc finger protein 1 (ovzf-1) (10) a genomic library prepared from adult female O. volvulus worms was screened with an ovzf-1 probe. Three distinct clones were isolated. The genomic fragments representing the ovzf-1 gene were subcloned, and their nucleotide sequence was determined. The structure of the ovzf-1 gene is shown in Fig. 1. The organization of ovzf-1 was confirmed by comparison of the genomic sequence with that of the cDNA clone isolated from L3 larvae (10), indicating that the identified genomic region includes the transcription start site and the promoter element of the ovzf-1 gene (Fig. 4). The presence of a TATA-like box (AATAATAT) at positions −28 to −22 (25) and of a CCAAT box at positions −88 to −84 (26) is in agreement with the predicted transcription start site. Thus, the nucleotide sequence of the isolated ovzf-1 gene includes 240 bp of the promoter region.

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Predicted Amino Acid Sequence of the ovzf-1 Gene Product—Upon splicing of the intron the ovzf-1 gene encodes an open reading frame of 447 amino acids. Exon 1, which includes the translation initiation codon at position 60, encodes 18 mostly hydrophobic amino acids, and exon 2 encodes 429 amino acids followed by the stop codon at positions 1401–1403. The resulting gene product has a calculated molecular mass of 53.3 kDa. Hydrophobicity analysis of the 18 amino acids encoded by exon 1 revealed a pattern indicative of a signal peptide (31, 32), suggesting that the Ovzf-1 protein is expressed via the secretory pathway.

The amino acid sequence encoded by exon 2 of ovzf-1 represents 12 Krüppel-like contiguous zinc finger motifs of the type Cys2-His2 (9). These conserved Cys- and His- residues have been shown to bind zinc ions, thus forming a finger-like structure. Similar zinc finger domains have been shown to interact with nucleic acids and were found in several nucleic acid bind-
ing proteins (5, 6, 33). The first finger-like domain of the Ovzf-1 protein (amino acid positions 53–74) has the two Cys residues replaced with Ser residues. With this exception, this domain displays several characteristics reported for Cys2-His2 zinc finger structures (Fig. 2): the spacing of 12 amino acids between the second “Cys” (Ser) and the first His residue and the conservation of a Phe and a Leu residue at positions 4 and 10 of the finger domain (5, 33) as well as a Glu and a Lys residue (positions 3 and 7) of the linker region separating two adjacent finger domains (H-C-link) (34). Thus, this first domain is likely to form a finger structure as well.

The schematic structure of the Ovzf-1 zinc finger protein is shown in Fig. 4A. The N-terminal hydrophobic signal peptide is followed by a stretch of 34 amino acids, 13 finger-like domains each consisting of 28–30 amino acids, and a C-terminal stretch of 32 amino acids. The amino acid sequences of the individual domains predicted for the Ovzf-1 protein are shown in Fig. 4C.

The ovzf-1 product represents an unusual protein; the hydrophobic pattern of the N-terminal region, which is encoded on a separate exon, is indicative of a secreted protein, whereas the presence of several contiguous zinc finger domains suggests a nucleic acid binding protein, which most likely is contained in the cell nucleus.

Developmental Expression of O. volvulus Zinc Finger Genes—The expression of O. volvulus zinc finger genes was analyzed by Northern blotting and by RT-PCR. ovzf mRNA was not detected in RNA prepared from adult female worms. By Northern blot analysis no specific signal was detected even upon extended exposure (data not shown). The application of RT-PCR increased the sensitivity of detection; however, again no ovzf transcript was detected in cDNA prepared from adult worms, even after two rounds of PCR amplification (Fig. 3, lanes 1 and 2). However, expression of the OvGST1 mRNA (35) was detected in RNA prepared from adult female O. volvulus (Fig. 3, lanes 3 and 4). Since zinc finger proteins play an important role in cell differentiation and development, we analyzed expression of zinc finger genes in the infectious third larval stage of O. volvulus (L3). Using primers specific for ovzf-1, a unique 855-nucleotide fragment was amplified from reverse transcribed L3 mRNA, indicating stage-specific expression of ovzf mRNA (Fig. 3, lane 5). The identity of this L3-derived fragment (ovzf-2A) was verified by demonstrating the existence of the internal EcoRI site at positions 49–903 (Fig. 3 and Fig. 4, lane 6) and by sequence analysis. With a different pair of ovzf-1-derived primers, an additional 942-bp fragment was amplified from the same L3 cDNA. The nucleotide sequence of this ovzf-2B fragment is shown in Fig. 4B. The sequence is distinct but highly related to that of ovzf-1. In the overlapping region of ovzf-1 and ovzf-2, the identity is 89% on the nucleotide level and 87.3% on the amino acid level, whereas the sequences of ovzf-2A and ovzf-2B are identical in their overlapping 855 nucleotides (positions 49–903).

Comparison of the ovzf-1 and ovzf-2 Gene Products—Similar to the predicted Ovzf-1 protein, the product of ovzf-2 also possesses a hydrophobic signal peptide and an unusual first finger domain. A homology comparison of the two related proteins Ovzf-1 and Ovzf-2 is shown in Fig. 4C. Within the predicted...
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Fig. 3. Developmental expression of ovzf mRNA in the infective L3 larvae. Expression of ovzf was analyzed in adult worm and the infective larva L3 by reverse transcription-PCR using ovzf-1-specific primers Cov7 and Cov25. Using RNA isolated from adult female parasites as template, no specific band was identified, even after two rounds of PCR amplification (lane 2). However, a specific fragment was detected after the first round of PCR amplification using primers specific for the O. volvulus glutathione-S-transferase gene (ovgst1) (lanes 3 and 4). In L3 cDNA, a specific 855-bp fragment was amplified with ovzf-1 primers Cov7 and Cov25 after the second round of amplification (lane 5). The demonstration of the internal EcoRI site at positions 492–497 confirms the identity of this fragment (lane 6).

The Recombinant Ovzf-2exp Protein Is Glycosylated—The amino acid sequence deduced from the ovzf-2 cDNA shows five consensus sites for N-glycosylation. Since N-linked glycosylation is a hallmark for expression via the secretory pathway, we wanted to demonstrate that the recombinant Ovzf-2exp protein, which was located inside the cells, has attached carbohydrates. Sf9 cells were infected with ovzf-2 baculovirus for 96 h. In a parallel experiment, the medium of infected cells was removed after 24 h and replaced with fresh medium containing tunicamycin, a potent inhibitor of N-linked glycosylation. The mobilities of the recombinant Ovzf-2exp proteins, expressed in the absence or presence of tunicamycin, were compared by SDS-PAGE and Western blotting (Fig. 7). Inhibition of N-linked glycosylation resulted in an increase in mobility of approximately 10 kDa (43 kDa in the presence of tunicamycin, compared with 53 kDa in the absence of tunicamycin), indicating that the Ovzf-2exp protein expressed in the absence of tunicamycin is glycosylated (Fig. 7, compare lanes 3 and 4). The attachment of carbohydrate moieties to recombinant Ovzf-2exp is in agreement with the hypothesis that Ovzf-2exp is expressed via the secretory pathway.

Despite the presence of a functional signal peptide the recombinant Ovzf-2exp protein is detected predominantly in the cell pellet. In order to find out whether the zinc finger domain may serve as a retrieval or retention signal, we expressed a truncated fragment of Ovzf-1. This Ovzf-1 fragment includes the first three finger domains with two potential N-linked glycosylation sites. The recombinant Ovzf-1 fragment was secreted into the culture medium and compared by SDS-PAGE and Western blot (Fig. 7, lane 1), and as observed for the recombinant Ovzf-2exp protein the mobility of the secreted protein was affected by tunicamycin treatment. Inhibition of N-linked glycosylation resulted in a change in mobility from 35 to 29 kDa (lane 2). Similarly, binding of Galanthus nivalis agglutinin to the recombinant Ovzf-1 protein expressed in the absence (lane 3) but not in the presence of tunicamycin (lane 4) demonstrates the attachment of specific mannose residues to N-glycan chains (37).

Detection of the Recombinant Ovzf-2exp Protein by Immunofluorescence—The recombinant Ovzf-2exp protein is detected predominantly in the cell pellet. In order to localize the Ovzf-2exp protein inside infected insect cells the a-46 antisera was used for immunohistochemistry. Methanol-fixed cells were incubated with a-46 antisera and were subsequently exposed to fluorescein isothiocyanate-linked a-rabbit antibody. Fluorescence staining revealed that the recombinant Ovzf-2exp protein is present in a distinct cellular compartment (Fig. 9) located close to the nucleus. No recombinant Ovzf-2exp protein
Fig. 4. Domain structure of the O. volvulus zinc finger protein-1 (Ovzf-1). The signal peptide is indicated by an orange box, and the N- and C-terminal domains are indicated by blue boxes. The structure of the finger domains is indicated. B, nucleotide sequence of the L3 cDNA amplificate ovzf-2. The nucleotide sequence of the L3 amplificate ovzf-2B includes 48 bp of 5'-untranslated sequence and an open reading frame of 942 bp. This open reading frame encodes the signal peptide, 34 amino acids, and a stretch of 9½ contiguous finger domains. Again, the first
Finger-like domain has the conserved Cys residues replaced with Ser residues. The overall sequence conservation
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**DISCUSSION**

We have cloned a zinc finger gene from the parasitic nematode O. volvulus that encodes a 53.3-kDa protein. The Ovzf-1 gene represents an approximately 3.2-kilobase pair genomic DNA and is organized into two exons that are separated by a single intron of about 1 kilobase pair. The predicted protein of the size markers are indicated in kDa on the left.

The recombinant Ovzf-2exp protein of 53 kDa and the Ovzf-1 protein of 35 kDa are expressed via the secretory pathway. Attachment of N-linked carbohydrate moieties occurs dur-

Fig. 6. **Kinetics of recombinant Ovzf-2exp expression in insect cells.** Recombinant baculovirus with the ovzf-2exp cDNA under control of the polyhedrin promoter was used to infect insect cells. Cells were harvested at the indicated times, and the cell lysate was separated by 0.1% SDS, 12% polyacrylamide gel electrophoresis. Recombinant Ovzf-2exp protein was visualized by Western blotting using rabbit antiserum (α-46). The mobility of the size markers is indicated in kDa on the left.

Fig. 7. **Tunicamycin inhibits N-glycosylation of the recombinant Ovzf-2exp protein.** Sf9 cells were infected with recombinant ovzf-2exp baculovirus. 24 h postinfection the cell culture medium was replaced with fresh medium in either the absence or presence of tunicamycin (2 μg/ml). After 4 days of incubation, the cell pellet was resuspended in lysis buffer C. The recombinant Ovzf-2exp proteins were separated by SDS-PAGE and visualized by Western blotting using the α-46 antiserum.

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The sequence conservation of zinc finger proteins in evolution emphasizes their important role in development and regulation of differentiation. Similar to zinc finger genes identified in other organisms (7, 38–40) the ovzf-1 gene is a member of a family of related zinc finger genes. The ovzf-2 sequence was cloned on the cDNA level, and three different genomic clones (ovzf-1, ovzf-4, and ovzf-5) have been isolated from the same genomic library (data not shown). The complex pattern of bands identified in O. volvulus DNA by Southern blot analysis is in agreement with the existence of several ovzf genes (10). In general, zinc finger genes show a remarkable conservation in sequence and a very low degree of polymorphism. Although the genomic library was generated from DNA obtained from several adult female O. volvulus the overall sequence conservation observed for zinc finger genes makes it highly unlikely that the identified isolates represent polymorphic forms of a single gene.

The recombinant Ovzf-2exp protein of 53 kDa and the Ovzf-1 protein of 35 kDa are expressed via the secretory pathway. Attachment of N-linked carbohydrate moieties occurs dur-
ing protein processing in the endoplasmic reticulum and in the Golgi complex. Thus, the identification of N-linked carbohydrates by inhibition of glycosylation as well as by demonstrating the binding of G. nivalis agglutinin is a strong indication for expression of both Ovzf-2exp and Ovzf-1t proteins via the secretory pathway (Fig. 8). A crucial requirement, however, is the presence of a signal peptide located at the N terminus of the protein. The signal peptide of Ovzf-2 is capable of directing secretion of a reporter protein (human FHL-1) as well as the truncated Ovzf-1t protein to the cell culture medium. Proper processing and cleavage of the heterologous Ovzf-2 signal peptide confirms the role of this N-terminal region as a signal peptide. A comparison of the two FHL-1 proteins, which were secreted by either the internal FHL-1 or the heterologous Ovzf-2 signal peptide are identical in size and display the same N terminus (Xaa-Gln-Asp-Ile), which was confirmed by amino acid analysis.

Expression of a nearly full-length ovzf-2exp cDNA in the baculovirus system shows that the majority of the recombinant protein is detected inside the cells and only a small fraction is detected in the culture medium. As demonstrated by immunofluorescence analysis, the recombinant protein is contained in a distinct cellular compartment and is possibly released by a specific stimulus. So far we have been unable to define conditions that result in Ovzf-2exp secretion. The Ovzf-2exp protein does not contain a hydrophobic stretch of amino acids indicative of a membrane-spanning domain. In addition, sequence comparison does not reveal any common retention or retrieval sequence motif required for protein localization within the endoplasmic reticulum or the Golgi complex (41, 42). However, the existence of such a retrieval sequence within the domains of fingers IV–XIII is concluded, because the truncated recombinant Ovzf-1t protein is efficiently secreted and is predominantly detected in the culture medium (Fig. 8).

The isolation of genes with structural motifs common to transcription factors was initiated to identify parasite genes that are involved in development and differentiation. The expression of the ovzf-2 transcripts in the infective L3 larvae but not in the adult female parasite demonstrates the significance of our approach to identify developmentally regulated genes in O. volvulus.

Similar to the intracellular state described for O. volvulus L2 larvae, an analogous form is reported for the parasitic nematode Trichinella spiralis, which invades the skeletal muscle of the host and can survive for decades in this intracellular state. Proteins secreted from the parasite into the cytoplasm have been described and are believed to participate in muscle cell dedifferentiation and nurse cell formation (43). A secreted 43-kDa protein with an N-terminal signal peptide and a putative DNA-binding helix-loop-helix motif has been described in T. spiralis (44). The question of whether this parasite-derived protein is involved in the regulation of muscle-cell dedifferentiation is controversial, as only a part of the antisera that were raised against different regions of the protein detect the native protein in the nuclei of infected myocytes (45). It is intriguing to speculate whether proteins secreted by intracellular parasites can regulate gene expression of affected muscle cells. The Ovzf proteins are good candidates to characterize such an interaction between parasite and host on the cellular as well as on the molecular level.
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