Published in Tropical Medicine & International Health 1, issue 5, 640-645, 1996
which should be used for any reference to this work

Ascaris suum: molecular cloning of an intermediate filament*

Marco Bisoffi[1] and Bruno Betschart[2]

[1] *Department of Medical Parasitology, Swiss Tropical Institute, Basel, Switzerland*
[2] *Institute of Zoology, University of Neuchâtel, Neuchâtel, Switzerland*

Summary

It has been proposed that intermediate filament proteins are involved in force transduction from the muscle cells through the hypodermis to the cuticle of nematodes. An additional role of intermediate filaments as excretory/secretory components of parasitic nematodes is under discussion. We report on the molecular characterization of the cDNA clone AsIF of the intestinal nematode parasite *Ascaris suum*, encoding a member of the intermediate filament protein family by sequence comparison with intermediate filaments of other nematodes. We also show the precise location of the product encoded by AsIF within the organism by immunoelectron microscopy.

keywords *Ascaris suum*, intermediate filament proteins, molecular cloning, cDNA

correspondence Bruno Betschart, Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel, Switzerland

Introduction

Nematodes are successful parasites of vertebrates including man. This is represented by the fact that approximately 25% of the world population are infected with the parasitic nematode *Ascaris lumbricoides* (Crompton 1989). After a tissue-dwelling period during the larval stages, the adult *Ascaris* worms are located in the intestines, where they constantly move against the nutrition flow of the host. This movement consists of the typical undulatory propulsions of nematode striated muscles that exert their force on the extracellular cuticle, which, as the nematode's hydroskeleton, counteracts the muscle propulsions (Wharton 1986). Interposed between muscle cells and the acellular cuticle are a basement membrane and a thin cellular tissue, the hypodermis. Due to the relatively large size of *Ascaris*, its hypodermis can be isolated by a combination of enzymatic treatment and mechanical removal (Fetterer & Wasiuta 1987). In the free-living nematode *Caenorhabditis elegans*, the hypodermis has been shown to contain bundles of different filaments as part of the cytoskeletal scaffold of the cell (Francis & Waterston 1985). Proteins of the intermediate filament (IF) superfamily (Fuchs & Weber 1994) are part of this scaffold and have been discussed in the context of transmission of tension from the muscle cell to the cuticle (Francis & Waterston 1991). IFs of *Ascaris* have been reported to occur in the hypodermis, body musculature, pharynx, intestine, ovary, uterus, and testis, but not in the cuticle (Bartnik *et al.* 1986).

Materials and methods

In the context of the identification of nematode parasite antigens of importance in the host–parasite

*Nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDJB Nucleotide Sequence Databases under access number X92100.

interplay, we have screened (Huynh *et al.* 1985) a cDNA library in λZAP II (Stratagene, USA) of *A. suum*, derived from mRNA of the hypodermis, using antibodies raised in mice against non-collagenous structural surface components of the cuticle (Betschart *et al.* 1990). We cloned the cDNA in pBluescript (Stratagene, USA), and after subcloning, in the sequencing vector M13mp18/19. No sequence corresponding to the eukaryotic TATA promoter (Corden *et al.* 1980) or any in-frame initiator methionine was present in the 5′ part of the clone, but several termination codons were present in the non-coding 3′ region. Sequence analysis of the 1609-bp insert revealed an open reading frame encoding 526 amino acids. The nucleotide sequence and the deduced amino acid sequence of the clone were used to screen the GenEMBL and Swissprot databanks, respectively. The highest similarities were found with other nematode proteins that are members of the IF superfamily (Fuchs & Weber 1994).

Amino acid sequence similarities of the clone with some of these IF proteins were as follows: 70 and 72%, respectively, with IF proteins a_2 and a_3 of *C. elegans* (Dodemont *et al.* 1994), 76, 77 and 78%, respectively, with the filarial parasites *Onchocerca volvulus* (Zhang & Miller 1994), *Brugia malayi* (Chandrashekar *et al.* 1995), and *Acanthocheilonema viteae* (Seeber *et al.* 1994). The obvious affiliation of our clone to this protein group prompted us to term the clone 'AsIF', for *Ascaris suum* intermediate filament.

In addition, we searched the literature for reports on intermediate filaments that might not be present in the common databanks and found two intermediate filament proteins of *A. lumbricoides*, IF protein A (IFA) and IF protein B (IFB), which had been sequenced on the amino acid level (Weber *et al.* 1989). Amino acid sequence comparison of AsIF with IFA and IFB revealed an almost total identity of 98% with IFB and a similarity of 76% to IFA. The amino acid sequence comparison of AsIF with IF proteins IFB of *A. lumbricoides*, Bm of *B. malayi*, and Cea2 of *C. elegans* is shown in Figure 1. The alignment shows that clone AsIF is characterized by the occurrence of the typical structure common to all IF proteins: a non-helical head domain (amino terminus), helical rod domains interrupted by short non-helical linker domains, and the non-helical tail domain (carboxy terminus). Pruss and co-workers (1981) have reported on a monoclonal antibody, IFA, that recognizes an antigenic determinant at the end of the coil 2 domain that occurs in all intermediate filament proteins described so far, including human IFs, such as vimentin, desmin, and lamin proteins (Steinert & Roop 1988). This epitope also occurs in AsIF and is boxed in Figure 1.

In order to obtain a specific detection probe for AsIF we amplified a 1000-bp fragment of the clone, corresponding to amino acids 196–526 in Figure 1, by the polymerase chain reaction using nucleotide primers featuring EcoRI and BamHI restriction endonuclease sites for the direct subcloning into the pGEX plasmid (Pharmacia, Sweden). The latter allows expression of the cDNA in *Escherichia coli* as fusion protein with glutathione S-transferase (GST) of the parasitic helminth *Schistosoma japonicum*, and allows a glutathione-agarose-based purification of the fusion protein (Smith & Johnson 1988). We used the purified fusion protein to immunize BALB/c mice and to produce specific polyclonal anti-AsIF antibodies for the detection of corresponding antigenic determinants in extracts of different nematodes in immunoblot experiments (Towbin *et al.* 1979). The specificity of the antibody for AsIF was verified on Western blots using GST alone as an antigen. No reactivity could be detected (data not shown). Protein extracts of fresh adult *A. suum* worms were prepared enriched for proteins of the body wall muscle cells (Francis & Waterston 1985), whereas for *C. elegans* and *B. malayi*, total extracts were prepared for immunoblot analysis. The results obtained are shown in Figure 2A.

Results

In all protein preparations of the *A. suum* body wall, extracted with buffers containing sodium chloride (NaCl), deoxycholate (DOC), potassium thiocyanate (KSCN), and urea, as well as in the total extracts of the filarial parasite *B. malayi* and the non-parasitic nematode *C. elegans*, a distinct reaction at 69–71 kDa was observed, whereas the pre-immune serum did not react to proteins of the NaCl extract of *A. suum* (Figure 2A). The molecular size of the observed reaction corresponds well to IF proteins

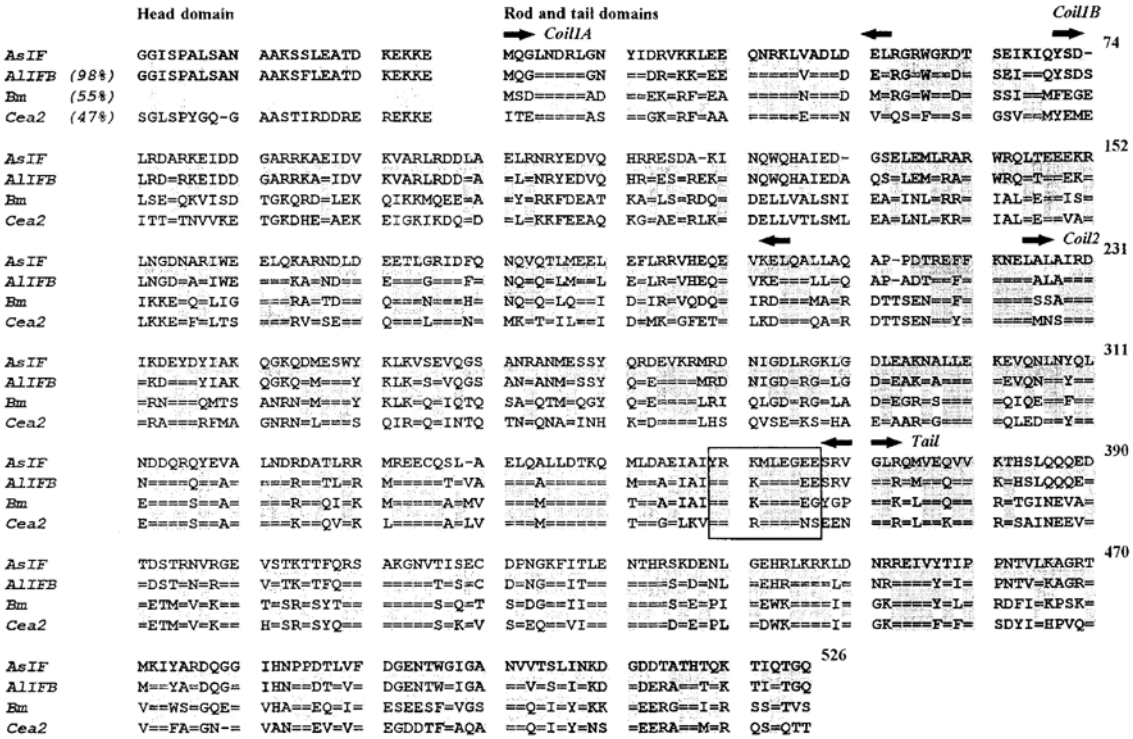

Figure 1 Sequence comparison of IF proteins of *A. suum* (AsIF), *A. lumbricoides* (AlIFB; Weber *et al.* 1989), *B. malayi* (Bm; Chandrashekar *et al.* 1995), and *C. elegans* (Cea2; Dodemont *et al.* 1994). The percentages indicate the identities to the AsIF sequence. The head domain is partially represented, the coiled regions of the rod domain and the tail domain are indicated by arrows. The numbers refer to amino acid residues of the *A. suum* sequence (AsIF). Shaded areas indicate amino acid identity with the AsIF sequence, =indicates identity in all four proteins. The boxed sequence marks the epitope common to all IF proteins (Pruss *et al.* 1981). Gaps (dashes) have been introduced to allow maximal alignment.

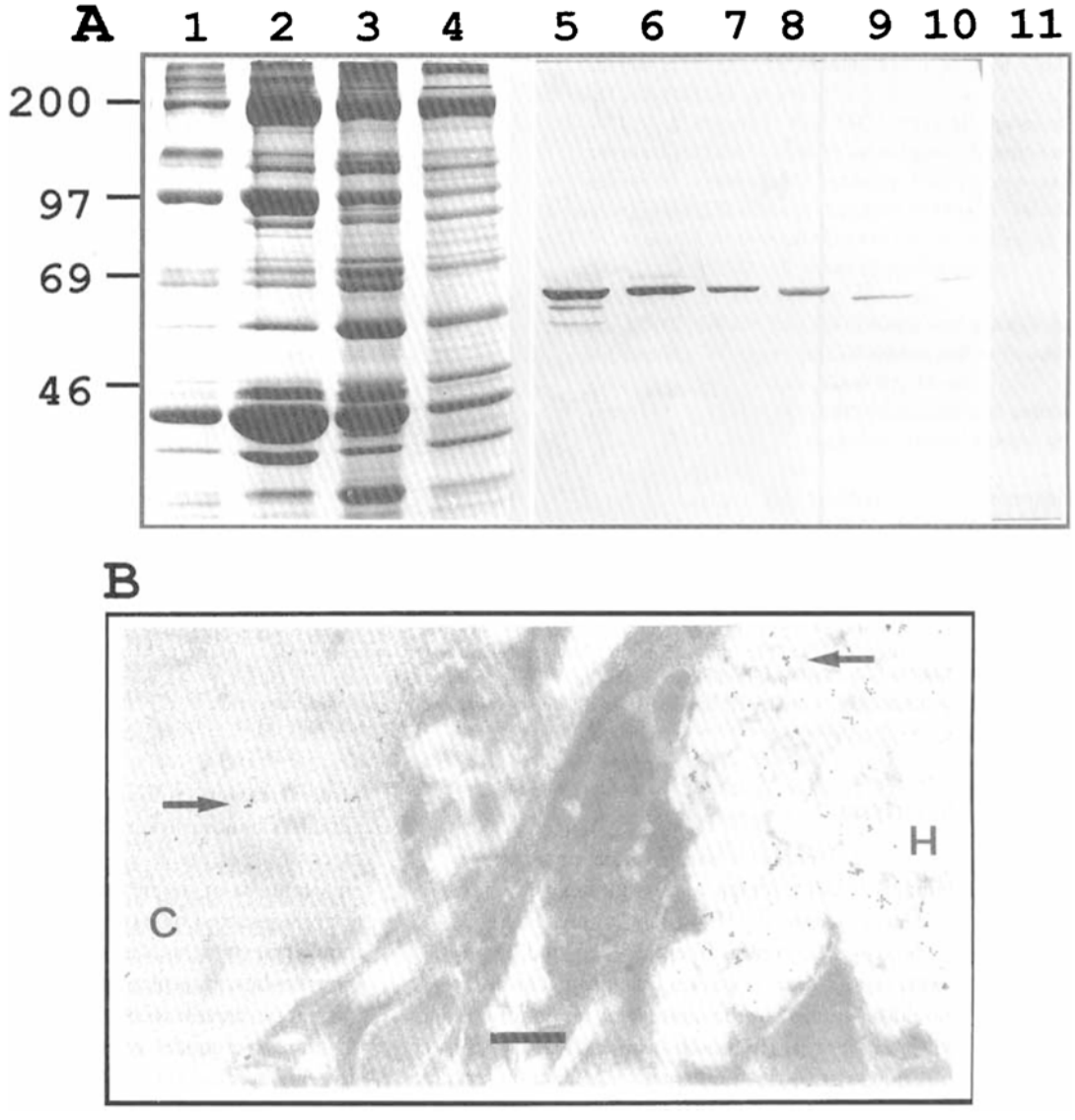

Figure 2 A and B

detected earlier in immunoblot experiments of filarial parasites (Seeber *et al.* 1994), *C. elegans* (Francis & Waterston 1991), and *A. lumbricoides* (Bartnik *et al.* 1986). A precise location of antigenic determinants corresponding to the polypeptide encoded by the AsIF cDNA was obtained by immunoelectron microscopical experiments on ultrathin sections of *A. suum* adult worms (Figure 2). The anti-AsIF antibodies specifically labelled epitopes within the hypodermis (Figure 2B and C) and to a lesser extent the muscle tissue (Figure 2C), and the cuticle (Figure 2B). The detection of AsIF epitopes in these zones is in agreement with published data (Bartnik *et al.* 1986). The detection of IF epitopes in the basal zone of the cuticle could indicate a possible involvement of IF in force transmission from the muscle to the cuticle in agreement with the hypothesis of Francis and Waterston (1991). Functional studies to further substantiate this possibility are needed.

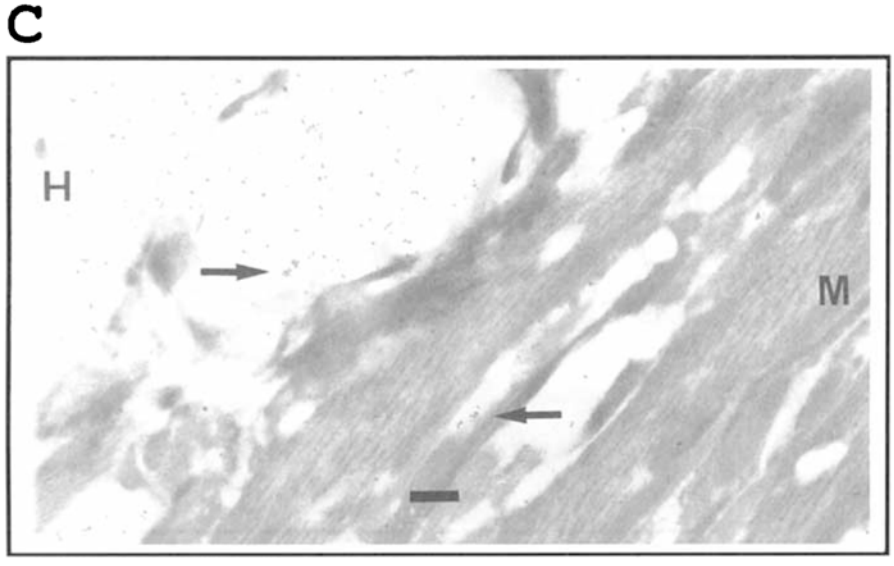

Figure 2 A, Immunoblot analysis of *A. suum* extracts, analysed in SDS-PAGE (lanes 1–4), and electroblotted onto nitrocellulose (lanes 5–11). Lanes 1, 5, and 11, NaCl extract; lanes 2 and 6, DOC extract; lanes 3 and 7, KSCN extract; lanes 4 and 8, urea extract; lane 9, *B. malayi* total extract; lane 10, *C. elegans* total extract. Lanes 5–10, mouse anti-AsIF antibodies; lane 11, pre-immune serum. Numerals on the left indicate molecular size in kDa. Immunoelectron microscopy with the anti-AsIF antibodies on B, section of *A. suum* hypodermis/inner cuticle (× 32 400), and C, hypodermis/muscle (× 20 600). C, cuticle; H, hypodermis; M, muscle. Arrows indicate gold particles.

Discussion

Due to the remarkable conservation of protein domains (based on high homologies in amino acid sequences) within the IF protein family (Fuchs & Weber 1994), common IF epitopes shared between parasites and their hosts are likely to occur and should be studied in the context of immunological symptoms potentially based on autoimmunity during infections with parasitic nematodes (Unnasch *et al.* 1988; Chandrashekar *et al.* 1995). Zhang and Miller (1994) and Seeber *et al.* (1994) recently reported that cloned and expressed IF proteins of *O. volvulus* are recognized by sera of patients infected with this filarial agent indicating that IFs are antigenic and exposed during infection. In this context it is interesting to note that our results, based on immunoelectron microscopy using the anti-AsIF antibodies, indicate antigenic IF determinants to occur in the inner part of the cuticle (Figure 2B) as well as in more external layers of the extracellular matrix (not shown). This is consistent with recent results in the filarial parasite *B. malayi*, where IF proteins have been found to be secreted through the cuticle (Chandrashekar *et al.* 1995). However, the mechanism of the release of IF proteins to the environment of the nematode, i.e. whether they are actively secreted or passively exposed during the moulting events, as well as their exact role in the parasite–host interplay in the context of auto-immunity, remain to be determined. We propose *A. suum* as a model system for the study of IF proteins because this large nematode allows the dissection and isolation of single tissues, such as the cuticle, and the IF containing muscle and hypodermis (Fetterer & Wasiuta 1987; Bartnik *et al.* 1986).

Acknowledgements

The authors wish to thank Dr T. Geary and Dr R. Klein (The Upjohn Company, Kalamazoo, USA) for providing the *A. suum* λZAP II hypodermal library. We are grateful to Regina Zahn, Jolanda Bonato, and Georg Scheidegger for technical support. This work was supported by a grant from the Swiss National Research Foundation (No. 31-33961.92).

References

Bartnik E, Osborn M & Weber K (1986) Intermediate filaments in muscle and epithelial cells of nematodes. *Journal of Cell Biology* **102**, 2033–2041.

Betschart B, Marti S & Glaser M (1990) Antibodies against the cuticlin of *Ascaris suum* cross-react with epicuticular structures of filarial parasites. *Acta Tropica* **47**, 331–338.

Chandrashekar R, Curtis KC, Li BW & Weil GJ (1995) Molecular characterization of a *Brugia malayi* intermediate filament which is an excretory–secretory product of adult worms. *Molecular Biochemistry and Parasitology* **73**, 231–239.

Corden J, Wasylyk B, Buchwalder A, Sassone-Corsi P, Kedinger C & Chambon P (1980) Promoter sequences of eukaryotic protein-coding genes. *Science* **209**, 1406–1414.

Crompton DWT (1989) Biology of *Ascaris lumbricoides*. In *Ascariasis and its prevention and control* (eds DWT Crompton, MC Nesheim & ZS Pawlowski), Taylor & Francis, London, pp 9–44.

Dodemont H, Riemer D, Ledger N & Weber K (1994) Eight genes and alternative RNA processing pathways generate an unexpectedly large diversity of cytoplasmic intermediate filament proteins in the nematode *Caenorhabditis elegans*. *EMBO Journal* **13**, 2625–2638.

Fetterer RH & Wasiuta M (1987) *Ascaris suum*: Partial isolation and characterization of hypodermis from the adult female. *Experimental Parasitology* **63**, 312–318.

Francis GR & Waterston RH (1985) Muscle organization in *Caenorhabditis elegans*: Localization of proteins implicated in thin filament attachment and I-band organization. *Journal of Cell Biology* **101**, 1532–1549.

Francis GR & Waterston RH (1991) Muscle cell attachment in *Caenorhabditis elegans*. *Journal of Cell Biology* **114**, 465–479.

Fuchs E & Weber K (1994) Intermediate filaments: Structure, dynamics, function and disease. *Annual Review of Biochemistry* **63**, 345–382.

Huynh TV, Young RA & Davies RW (1985) Constructing and screening cDNA libraries in lambda gt10 and lambda gt11. In *DNA Cloning Vol. 1* (ed DM Glover), IRL, Oxford, pp 49–78.

Pruss RM, Mirsky R & Raff MC (1981) All classes of intermediate filaments share an antigenic determinant defined by a monoclonal antibody. *Cell* **27**, 419–428.

Seeber F, Höfle W, Kern A & Lucius R (1994) *Onchocerca volvulus* and *Acanthocheilonema viteae*: cloning of cDNAs for muscle-cell intermediate filaments. *Parasitological Research* **80**, 699–702.

Smith DB & Johnson KS (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* **67**, 31–40.

Steinert PM & Roop DR (1988) Molecular and cellular biology of intermediate filaments. *Annual Review of Biochemistry* **57**, 593–625.

Towbin H, Staehelin T & Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences of the USA* **76**, 4350–4354.

Unnasch TR, Gallin MY, Soboslay PT, Erttmann KD & Greene BM (1988) Isolation and characterization of expression cDNA clones encoding antigens of *Onchocerca volvulus* infective larvae. *Journal of Clinical Investigation* **82**, 262–269.

Weber K, Plessmann U & Ulrich W (1989) Cytoplasmic intermediate filament proteins of invertebrates are closer to nuclear lamins than are vertebrate intermediate filament proteins; sequence characterization of two muscle proteins of a nematode. *EMBO Journal* **8**, 3221–3227.

Wharton DA (1986) Movement and Co-ordination. In *A Functional Biology of Nematodes* (ed P Calow), Croom Helm, London, pp 35–59.

Zhang D & Miller DJ (1994) Molecular and serological analysis of cDNAs encoding an intermediate-filament protein from *Onchocerca volvulus*. *Molecular and Biochemical Parasitology* **67**, 175–178.