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# A STAGE-SPECIFIC OPEN READING FRAME FROM THREE-DAY OLD ADULT WORMS OF *TRICHINELLA SPIRALIS* ENCODES ZINC-FINGER MOTIFS

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**Summary:**

The aim of the study was to isolate genes coding for stage-specific antigens of *T. spiralis*. Such antigens may then be associated with local and systemic immune responses against adult *T. spiralis*. Recombinant clones were obtained with an adult stage specific probe from a cDNA library of three-day old adult *T. spiralis*. Several cDNA clones encoding the same peptide were identified and their stage specificity was confirmed by northern blot analysis. Three independent clones were fully sequenced, and the resulting sequence found to code for a 487 amino acid peptide with a deduced molecular weight of ≈ 55 kDa. Sequence analysis showed that the 55 kDa peptide contained putative DNA binding motifs, suggesting that this protein may be involved in transcriptional regulation during the early development of the parasite.

**KEY WORDS :** *Trichinella spiralis*, three-day old adult, cDNA library, DNA sequencing, cloning.

***Résumé :*** MOTIFS EN DOIGT DE ZINC D'UNE PROTÉINE SPÉCIFIQUE DU STADE ADULTE (3 JOURS) DE *TRICHINELLA SPIRALIS*

*Le but de cette étude a été de participer à l'isolement de gènes codant pour des antigènes spécifiques de stade de* Trichinella spiralis. *De tels antigènes peuvent interagir de façon spécifique avec le système immunitaire et induire une réponse locale contre les adultes de* T. spiralis. *Des clones recombinants ont été obtenus à partir d'ADNc du stade adulte purifié au jour 3 après infestation expérimentale. Plusieurs clones d'ADNc codant pour le même peptide ont été sélectionnés. La spécificité de stade a été montrée par une hybridation spécifique des ARNm du stade Adulte (J3). Trois clones indépendants ont été séquencés, et la séquence nucléotidique résultante a permis de mettre en évidence l'existence d'un cadre de lecture unique codant pour un peptide de 487 acides aminés ayant une masse moléculaire déduite de 55 kDa. L'analyse de la séquence en aa montre l'existence d'un double motif original en doigt de zinc suggérant que cette protéine pourrait être impliquée dans une régulation transcriptionnelle précoce des embryons de* Trichinella.

***MOTS CLÉS :*** Trichinella spiralis, *adultes J3, banque d'ADNc, clonage, séquençage.*

## INTRODUCTION

*Trichinella spiralis* (Stichosomida, trichinellidae) is a parasitic nematode distributed worldwide which infects a wide variety of mammals. Infection in humans occurs when raw or undercooked meat from infected animals is ingested (Dupouy-Camet *et al.*, 1998). Over the last 20 years, the number of outbreaks of trichinellosis in China has increased in several provinces (Liu & Boireau, 2002), and it is estimated that around 20 million individuals are infected with *T. spiralis*. The *Trichinella* cycle occurs within the same host with minor variations amongst species. After ingestion of meat contaminated with muscle larvae (ML), the parasites are released inside the duodenum and invade the intestinal tract where four molts occur within three to four days giving rise to the adult worm (Ad). Newborn larvae (NBL) are produced after four to 10 days post-infection according to the host species (Pozio *et al.*, 1992). They migrate *via* the lymphatic vessels to muscle tissue where they remain and transform into ML.

Human trichinellosis continues to be a serious zoonotic disease, based on the number of human cases worldwide and the cost of pig control. Two alternatives have been developed to reduce the cost of control of trichinellosis in China, Mexico and Eastern European countries. One is the use of anti-helminth drugs such as albendazole in pig breeding units. However, the need to use several injections and the health risk generated by residual drug in meat dramatically reduce the benefit of this method. The second is to develop vaccines for pigs based on the

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use of antigens from invasive stages of the parasite (*i.e.* Ad and NBL). If such stages can be blocked, muscle invasion will be prevented, as occurs in naturally immunized mammals (Lawrence *et al.*, 1998; Negrao-Correa, 2001; Stewart *et al.*, 1999). However, these parasite stages are difficult to obtain in large amounts from infected hosts and it is not feasible to reproduce the *Trichinella* life cycle *ex vivo*. An alternative is to express genes encoding Ad or NBL antigens in expression systems such as bacteria, yeast or insect cells. Previous studies have shown that some recombinant proteins of *Trichinella* ML can be recognized by naturally or experimentally infected mammals and are able to induce some protection against infection (Robinson *et al.*, 1995; Sun *et al.*, 1994; Zarlenga & Gamble, 1990).

In this study we identified and characterised a gene coding for an adult antigen using an Ad specific probe to screen a cDNA library from *T. spiralis*. The gene codes for a peptide with a deduced MW of ≈ 55 KDa that contains DNA binding motifs.

## MATERIALS AND METHODS

### Parasite purification

The Chinese *T. spiralis* strain ISS 534 was isolated from a naturally infected pig. The worms were maintained in OF1 mice and Wistar rats. ML were recovered from experimentally infected mice 30 days post infection (pi) by standard pepsin digestion. After recovery, the worms were washed three times with saline solution (0.9 % NaCl) and then used to orally inoculate 300 OF1 mice with 1,000 ML per mouse.

### Isolation of parasite RNA and northern blot analysis

*T. spiralis* (Ts) Ad worms were obtained from OF1 mice three days after infection with ML (Le Guerhier *et al.,* 1997). Examination of 100 worms under 100 × magnification confirmed that the worms were under and that no NBL were present in the females. Purified *Trichinella* ML were incubated for 20 hours at 37° C in cell culture medium ((RPMI, GIBCO) with 10 % calf serum). To obtain NBL, $1 \times 10^5$ Ad were recovered from mice on day 5 pi and maintained in cell culture medium with antibiotics (200 UI/ml penicillin and 200 µg/ml streptomycin) for 24 h. The released NBL were separated from the adults by differential sedimentation and filtration. After several PBS washes, the Ad, NBL or ML were collected by centrifugation. Ad, NBL or ML total RNA was isolated by the acid phenol method and mRNA was purified through oligo-dT-cellulose chromatography. RNA quality was monitored by formaldehyde gel electrophoresis.

Hybridisation between Ad and ML Ts total RNA was performed after denaturation in MOPS 10 ×, 40 % formaldehyde and deionized formamide at 65° C for 10 min, before separation through 1.0 % agarose gel electrophoresis. Total RNA from M12 mice cells (ATCC) was used as a control. RNA was transferred to hybond NX (Amersham) membranes, which were hybridised overnight at 42° C with alpha$^{32}$P labelled probe (T1-2D7). After high stringency washes (65° C), the filters were exposed to X-ray films for four days at – 80° C.

### cDNA libraries

Lambda ZAP cDNA libraries of three-day old Ad, NBL and ML were prepared as previously described (Vayssier *et al.,* 1997). Briefly, the first strand cDNA was synthesised by using MMLV reverse transcriptase. After second strand synthesis, cDNA was purified by a CHROMASPIN-400 column and ligated to a lambda ZAP DNA vector. Vector-attached cDNAs were packaged with Gigapack II Gold packaging extracts (Stratagene), and amplified in *Escherichia coli* XL1-Blue MRF'. Transformants were grown in LB medium containing $MgSO_4$ and ampicillin (100 mg/mL). The resulting cDNA library was amplified and cloned cDNA fragments were PCR-amplified with $T_3$ and $T_7$ primers ($T_3$ primer 5'-AATTAACCCTCACTAAAGGG-3'; $T_7$ primer 5'GTAATACGACTCACTATAGGGC-3').

### Preparation of adult subtracted cDNA probe

Adult subtracted cDNA clones were obtained with PCR-select cDNA Subtraction Kit (Clontech) using Ad cDNA as the "Tester" and ML cDNA as the "Driver" as suggested by the manufacturer. The Ad subtracted clones were sequenced and identified by hybridization with cDNAs from *T. spiralis* Ad, NBL and ML cDNA libraries.

### Stage specificity analysis

Total cellular DNA was isolated from $1 \times 10^6$ M12 mice cells applying the Genomic DNA Preparation Kit (Qiagen) according to the manufacturer's specifications. 40 µg of purified mice DNA or plasmid of *T. spiralis* Ad, NBL and ML cDNA libraries were digested with *Eco*RI or with *Eco*RI and *Xho*I respectively, and resolved on a 1 % agarose gel. DNA was then transferred to 0.45 µm nitrocellulose filter with a vacuum blotter (Appligene). The filter was hybridised for 12 h at 55°C with the $^{32}$P-labelled subtracted probe T1-2D7 in hybridisation buffer (6X SSC, 5X Denhart's solution, 0.1 % SDS), washed twice at high stringency and exposed to X-ray films for 24 h at – 80° C.

| Stages | RNA (µg) | mRNA (µg) | mRNA/RNA (100 %) | cDNA (µg) | Initial library titer ($10^6$/mL) | Final titer after amplification ($10^{10}$/mL) |
|---|---|---|---|---|---|---|
| NBL | 256 | 3.8 | 1.4 % | 0.45 | 1.20 | 1.6 |
| Ad | 1,100 | 14.0 | 1.2 % | 0.80 | 2.20 | 0.2 |
| ML | 1,383 | 10.8 | 0.7 % | 1.25 | 2.69 | 0.7 |

Table I. – Characteristics of the various cDNA libraries obtained from the three main antigenic stages of *Trichinella spiralis*, ISS534.

### SCREENING OF THE LAMBDA ZAP AD CDNA LIBRARY

The Ad cDNA library (2 × $10^4$ pfu/plate) was propagated in XL1-BLUE cells and plated according to standard methods (Sambrook *et al.*, 1989). Selected plasmids were immobilised on nylon membranes (Amersham) before hybridization. Approximately 2 × $10^5$ plaques were screened with the Ad stage-specific radiolabelled DNA probe T1-2D7. The membranes were exposed to X-ray films overnight at – 80° C. After two rounds of hybridisation screening several positive clones were obtained that were suspended in SM buffer (50 mM Tris-HCl, pH 7.5 100 mM NaCl, 10 mM MgSO4) and stored at 4° C.

### CHARACTERIZATION OF POSITIVE CLONES

Plasmid DNA from positive clones was prepared using the *in vivo* excision procedure. A mixture of phage, fresh XL1-BLUE cells and helper phage was added to the LB broth and incubated at 37° C for 3 h. Later, cultures were heated at 65° C-70° C for 20 min and then centrifuged. The supernatant containing the plasmids was incubated with SOLR cells and then plated out on LB plates containing ampicillin and incubated at 37° C overnight. Positive plasmid DNA was digested with *Eco*RI and *Xho* I endonucleases and analysed on 1 % agarose gel electrophoresis.

### DNA SEQUENCING

Plasmid DNA was sequenced by using dideoxy chain-termination method with an automatic sequencer (ABI 377).

### DATA ANALYSIS

DNA sequences were analysed by DNAstar software (DNASTAR.Inc.WI.USA). The predicted features of deduced amino acid (aa) sequence were determined using the same software. Homology of cDNA was analyzed with GenBank database using BLAST (McGinnis *et al.*, 2004).

## RESULTS

### PREPARATION OF *T. SPIRALIS* AD, ML AND NBL CDNA LIBRARIES

Total RNA and mRNA were purified from three parasite preparations (Ad, ML and NBL). Interestingly, the ratio of mRNA/RNA increased from the ML stage to the NBL stages which appeared the most likely to synthesise a greater level of mRNA (Table I). The three-day old Ad cDNA library contained 2.2 × $10^6$ independent clones. After amplification and freezing at – 80° C, the library was randomly tested to analyse the average size of inserts. For this purpose several clones were selected and amplified with T3/T7 primers. Most of amplified DNA fragments (69 %) had a size ranging 1.5 ~ 2 × $10^3$ bp (Fig. 1). Two other cDNA libraries were prepared (NBL and ML) with a similar number of independent clones before amplification (1.2 and 2.69 × $10^6$, respectively).

### SPECIFICITY OF A SELECTED AD PROBE

Sequence analysis showed that after depletion of Ad cDNA with ML cDNA, 14 of 27 Ad subtracted cDNA clones contained cDNA from the same gene. So the

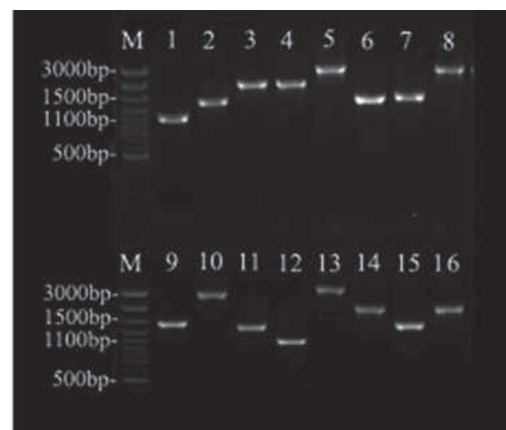


Fig. 1. – Quality control of the cDNA library after freezing and storage at – 80° C. PCR analysis of cDNA inserts from the adult worm cDNA library was performed randomly.
M: molecular weight marker 100 bp ladder.
Lanes 1-16: cloned inserts from the adult cDNA library.

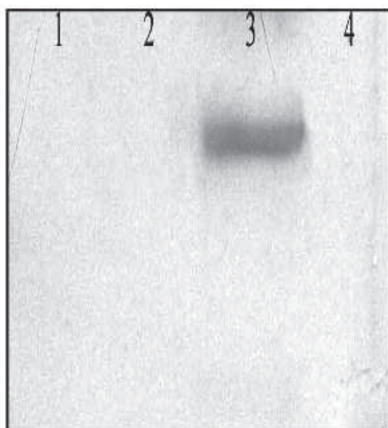


Fig. 2. – Analysis of the stage specificity of the subtracted probe T1-2D7.
Lane 1: muscle larvae cDNA; Lane 2: newborn larvae cDNA; Lane 3: adult cDNA; Lane 4: mice genomic DNA.

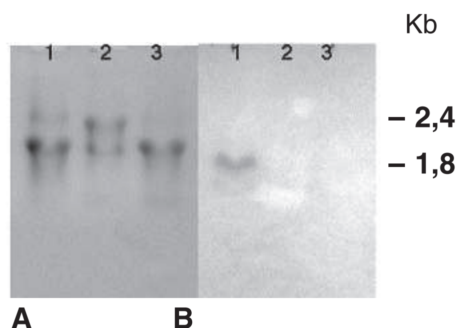


Fig. 3. – Northern Blot analysis with the T1-2D7 probe.
Lane 1: total RNA of *T. spiralis* Ad (40 mg); lane 2: total RNA from mouse M12 cells (40 mg); lane 3: total RNA of *T. spiralis* ML (40 mg). A: migration of total RNA; B: hybridization to T1-2D7 probe.

high redundant clone T1-2D7 was chosen and the Ad specificity was demonstrated by hybridisation with cDNA from Ad, NBL and ML stages. No hybridisation was observed with mouse genomic DNA used as the control, nor with NBL or ML cDNA but a strong signal was revealed with Ad preparation (Fig. 2). The T1-2D7 probe was also used to hybridise Ad or ML total RNA with total RNA from M12 mouse cells as control (Fig. 3). Hybridisation was only observed when total Ad RNA was used, thus confirming the stage specificity of the T1-2D7.

### NUCLEOTIDE AND AA SEQUENCE ANALYSIS OF THE AD SPECIFIC ORF

The adult cDNA library was screened using the T1-2D7 probe and 16 positive clones were recovered. Most of the cDNA inserts were around 1.7 Kb. Three independent clones were fully sequenced on both strands. The resulting nucleotidic sequence (AdTs1) was 1,621 bp long, including a 5'-untranslated region (UTR) of 90 bp, an open reading frame (ORF) encoding a 487 aa protein, a short (20 bp) 3' UTR with a polyadenylation signal (AATAAA) at position 1558 and a 3' poly (A) tail. A putative start codon was identified at nucleotides 91-93 and a TGA was located at nucleotides 1552-1554 (Fig. 4). The T1-2D7 nucleotide sequence is located between nucleotide 538 and 1621 (Fig. 4).

Analysis showed that the DNA from the main ORF encoded a peptide (AdTs1) with a calculated molecular mass of ≈ 55 kDa and an isoelectric point of 4.9. Two putative zinc finger domains were located between positions 207 and 273 of the amino acid sequence. The deduced amino acid sequence had no homology with those of Ts antigens reported previously in the GenBank, but showed 42 % identity with nuclear hormone receptors of rodent between amino acids 209 and 270 (Fig. 5).

## DISCUSSION

Stage specific antigens of *T. spiralis* play different roles in protective immune responses (Boireau *et al.*, 1997; Ortega-Pierres *et al.*, 1996). Crude soluble antigen preparations derived from Ad, NBL and ML can induce specific humoral and cellular responses that give protection against larval challenge in mice or pigs (el-Shazly *et al.*, 2002; Xu *et al.*, 1997). Among these, Ad and NBL antigens have been shown to be immunogenic in pigs (Darwish *et al.*, 1996; Tu *et al.*, 2001). In the present study, an Ad stage-specific ORF was selected by screening an Ad Ts cDNA library with a substractive adult specific probe. BLAST analysis revealed that AdTs1 had no homology with *Trichinella* antigens published in the GenBank.

The guanine-cytosine (GC) content of this new *T. spiralis* gene (42 %) is very similar to those previously described (Sugane & Matsuura, 1990; Su *et al.*, 1991; Vayssier *et al.*, 1999), but is higher than the average GC percentage of the overall genome of parasitic nematodes (35 %) (Hammon & Bianco, 1992). Interestingly, some similarities were found between the AdTs1 protein and nuclear hormone receptors of mammals (rodents and human) or other species (ecdysteroid receptor from *Tenebrio molitor* or nuclear hormone receptor of *Drosophila melanogaster*). Among the conserved motifs, two possible zinc-binding motifs separated by 15 aa were predicted in the AdTs1 protein. The alignment of DNA binding domains of the mouse glucocorticoid receptor (Danielsen *et al.*, 1989) with a similar domain of AdTs1 protein indicates an identity of 42 % (Fig. 5). If AdTs1 functions as a DNA binding protein, it may activate or inactivate downstream genes responsible for a particularly host response phenotype. Several zinc finger motifs have been identified in *Caenorhabditis elegans* and these are thought to be involved in blas-

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aacgcttttgcgtcgacacgtctcttcattttcatttgcttcgctctttttctgctgcttctgttcgaaaacgtggaaatagcagcatag   90
 M   D   K   N   R   L   T   E   T   D   F   P   I   D   Y   S   L   P   G   T   S   G   L     23
ATG GAT AAG AAC CGT TTG ACT GAA ACT GAT TTT CCA ATC GAC TAC AGT CTA CCC GGT ACC AGT GGT TTG   159

 S   N   K   P   S   N   N   N   A   L   T   G   A   N   V   E   R   R   A   H   S   F   S     46
TCG AAC AAA CCG TCG AAC AAT AAT GCT TTG ACT GGC GCA AAC GTT GAA AGA AGA GCG CAT TCA TTC TCT   228

 V   S   G   L   R   A   P   D   E   S   S   F   G   R   R   S   F   T   I   S   E   Y   T     69
GTA TCC GGT TTG CGG GCA CCA GAT GAA TCC TCC TTC GGC AGG AGA AGT TTT ACA ATC AGC GAA TAT ACG   297

 A   D   Q   V   M   E   A   L   R   R   Y   G   N   L   L   G   Q   S   F   L   Q   Y   G     92
GCC GAT CAG GTT ATG GAA GCT CTC AGA AGA TAT GGG AAT CTT TTG GGA CAA TCT TTC CTG CAG TAT GGA   366

 S   H   P   M   A   D   E   E   L   L   Y   H   Q   V   S   E   L   T   S   P   D   R   N    115
AGC CAT CCG ATG GCT GAT GAA GAA CTG CTT TAT CAT CAA GTT AGT GAG CTT ACC TCT CCT GAT CGC AAT   435

 P   L   A   F   A   Y   A   S   T   R   I   F   T   N   H   L   Q   S   A   Y   S   S   M    138
CCG CTG GCA TTT GCG TAT GCT AGC ACC CGA ATT TTT ACA AAC CAC CTG CAG TCA GCG TAC AGC AGT ATG   504

 E   G   G   T   S   A   S   S   V   N   D   V   R   N   S   E   A   D   S   V   T   P   D    161
GAA GGT GGA ACA TCC GCT TCG TCT GTG AAT GAT GTA CGG AAT TCA GAA GCG GAT TCT GTT ACT CCT GAT   573

 S   S   A   R   W   I   E   L   R   S   V   K   Q   A   T   A   Q   G   E   T   S   S   T    184
TCA TCT GCA CGG TGG ATA GAA CTG CGT TCT GTA AAA CAA GCA ACA GCA CAA GGG GAG ACA AGT TCC ACT   642

 N   T   S   S   N   T   V   N   D   N   S   A   D   F   P   L   L   T   R   F   Q   D   V    207
AAT ACC AGT AGC AAT ACA GTC AAC GAT AAC AGC GCT GAT TTT CCT CTG TTG ACT CGG TTT CAA GAT GTC   711

 R   C   M   V   C   G   D   C   A   H   G   F   H   Y   G   V   V   T   C   E   G   C   K    230
CGT TGT ATG GTT TGT GGT GAT TGC GCT CAC GGT TTT CAT TAC GGC GTA GTT ACT TGT GAA GGT TGC AAA   780

 G   F   F   R   R   T   V   Q   R   N   L   R   Y   S   C   H   K   S   S   R   C   E   I    253
GGG TTT TTT CGA AGA ACG GTG CAA CGC AAC CTT CGA TAT TCT TGT CAC AAA TCT TCC CGG TGT GAA ATT   849

 N   R   Q   T   R   T   R   C   Q   A   C   R   Y   Q   Q   C   L   M   L   A   C   H   Y    276
AAC CGC CAG ACC AGA ACC CGG TGT CAG GCA TGC CGA TAC CAA CAA TGC TTG ATG TTG GCA TGT CAC TAT   918

 D   V   R   I   D   Q   R   R   K   G   T   P   Q   F   N   V   E   D   D   S   S   A   I    299
GAT GTG CGA ATT GAC CAA CGT CGA AAG GGC ACT CCC CAA TTT AAC GTA GAG GAT GAC AGT TCG GCA ATT   987

 S   K   M   Q   N   L   L   N   S   I   G   D   A   F   D   T   L   I   S   S   A   N   D    322
TCA AAA ATG CAG AAC CTG CTC AAC TCT ATT GGC GAT GCT TTC GAC ACG CTG ATC AGT TCC GCC AAC GAC  1056

 N   D   N   D   T   A   T   Q   S   Q   C   F   N   A   F   T   S   A   L   P   E   F   S    345
AAC GAC AAT GAC ACT GCC ACG CAA TCC CAG TGT TTC AAT GCA TTC ACC AGT GCT CTA CCG GAG TTT TCA  1125

 K   L   D   P   V   D   Q   E   I   L   Q   H   D   S   M   E   E   I   Q   T   L   V   K    368
AAG CTA GAT CCG GTT GAT CAA GAA ATT TTA CAG CAC GAC TCT ATG GAA GAG ATT CAA ACT TTA GTG AAA  1194

 A   Y   L   L   I   D   K   D   T   S   T   I   G   M   T   D   L   D   S   V   L   L   K    391
GCC TAT TTG TTG ATC GAT AAA GAC ACT TCG ACG ATA GGA ATG ACT GAT CTG GAC AGT GTT TTA CTG AAG  1263

 V   K   A   E   M   E   E   L   Q   V   Q   P   M   E   V   A   L   L   C   C   I   C   F    414
GTG AAA GCA GAA ATG GAG GAG CTG CAA GTG CAA CCA ATG GAA GTG GCT TTG TTG TGC TGT ATT TGT TTT  1332

 Y   S   P   I   R   E   N   L   K   A   V   Q   Q   V   E   A   V   Q   D   E   C   L   Q    437
TAT TCT CCA ATA CGT GAA AAT TTG AAA GCA GTT CAG CAA GTT GAA GCT GTG CAA GAC GAA TGC TTG CAG  1401

 A   L   Q   A   Q   V   H   L   R   C   A   E   N   P   Q   I   L   Q   E   F   Y   F   Y    460
GCG TTG CAA GCA CAA GTC CAC TTA AGA TGT GCA GAA AAT CCA CAG ATT TTG CAA GAA TTT TAT TTT TAC  1470

 E   Q   C   P   N   Y   N   T   F   I   Y   S   F   S   F   N   F   Y   T   D   L   C   F    483
GAA CAA TGT CCA AAT TAT AAT ACT TTT ATA TAT AGT TTT TCC TTC AAT TTT TAC ACT GAT TTA TGC TTT  1539

 V   F   V   L   *                                                                           487
GTT TTT GTT TTA TGA  atgaataaagtaatttaaacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa  1621
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Fig. 4. – Consensus nucleotidic sequence of three independent cDNA clones and the deduced amino acid sequence.
The underlined sequence denotes the start codon. The asterisk indicates the stop codon. The box indicates the polyadenylation signal. The T1-2D7 nucleotide sequence is located between nucleotides 538 and 1621. The GenBank accession number of the sequence is AF260232.

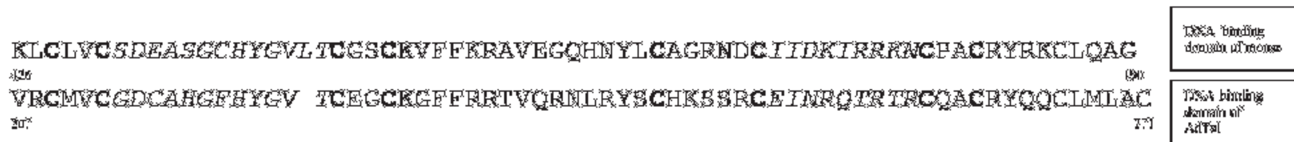


Fig. 5. – Double zinc fingers motif. Alignment of the DNA binding domain of the mouse glucocorticoid receptor (GR) (Danielsen *et al.*, 1989) with the homologous domain of the AdTs1 protein.
In bold characters, the conserved cysteine residues involved in the fixation of zinc finger (Four cysteine residues are necessary to fix one zinc atom). In italic the resulting loops after the fixation of the zinc atom.

tomere asymmetry (Levitan *et al.*, 1994; Tabara *et al.*, 1999) or development of neurons (Hughes *et al.*, 2002). Also a *C. elegans* gene, *nhr-2*, was characterised as a member of the nuclear hormone receptor family (Sluder *et al.*, 1997) strongly expressed during embryogenesis. The NHR-2 protein was located earlier in embryonic nuclei until the 16-20 cell stage. The high abundance of AdTs1 nucleotidic sequence in the subtracted cDNA library of three-day old Ad and the 53.3 % identity between the typical double zinc finger domains of AdTs1 (aa 207-273) and NHR-2 protein (aa 112-174) suggest the stage specific *AdTS1* gene could be homologous to *nhr-2* of *C. elegans*. If this is so, AdTs1 should be considered as a marker of early embryogenesis in *Trichinella*.

The ratio of mRNA/RNA increased enormously between the invasive ML and the NBL with an intermediate with Ad. This result was confirmed by other independent experiments with *T. britovi*, the ratio of mRNA/RNA is 0.13 % in ML, 0.5 % in Ad and 2.1 % in Ad/NBL. The increase of mRNA during NBL development should be correlated with the expression of new proteins and new epitopes, as the NBL is the only extra-cellular stage during the whole cycle of *Trichinella*.

Hydrophobicity and mobility analysis of various segments of the deduced aa sequence of the AdTs1 protein revealed the presence of several possible antigenic determinants according to the method of Hopp & Woods (1981). Such putative antigenic fragments were located between aa 52 and 64 (APDESSFGRRSFT), 108 and 116 (ELTSPDRNP), 137 and 163 (SMEGGTSASSVNDVRNSEADSVTPDSS), 226 and 260 (CEGCKGFFRRTVQRNLNYSCHKSSRCEINRQTRTR). These fragments may be useful either in the induction of immune responses or as immunodiagnostic reagents (eg as shown for the Hepatitis B surface antigen (Prince *et al.*, 1982)). The latter was chemically synthesised on the basis of software analysis and prediction analysis showed that it had antigenic activity in radio-immunoassays. To our knowledge this is the first report of an ORF of the Ts Ad stage. Since several putative antigenic determinants were found, the synthesis of these sequences should be useful to test the antigenicity of AdTs1.

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