Differential expression of AP-1 transcription factor genes *c-fos* and *c-jun* in the helminth parasites *Taenia crassiceps* and *Taenia solium*

J. MORALES-MONTOR[1]*, G. ESCOBEDO[1], M. RODRIGUEZ-DORANTES[2], N. TÉLLEZ-ASCENCIO[2], M. A. CERBÓN[2] *and* C. LARRALDE[1]

[1] *Departamento de Inmunología, Instituto de Investigaciones Biomédicas, and* [2] *Departamento de Biología, Facultad de Química, U.N.A.M. AP 70228, México D.F. 04510, México*

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SUMMARY

Homologues of *c-fos* and *c-jun* from total DNA of *Taenia crassiceps* and *Taenia solium* were cloned and sequenced. The amino acid alignment analysis revealed that *c-fos* DNAs from *T. crassiceps* and *T. solium* were highly homologous (96%), and both have high homology compared to several mammalian *c-fos* proteins (93% to mouse, 96% to rat and 86% to human). The *c-jun* protein alignment showed higher homology (*T. crassiceps* and *T. solium* have 98%), when compared with mouse, rat and human, being 92%, 98% and 93% respectively. RT–PCR amplification of the parasite's total RNA, showed that *T. crassiceps* expressed both AP-1 complex genes, while *T. solium* only expressed *c-fos*. Southern blot hybridization analysis confirmed the true origin of each amplified gene. AP-1 transcription gene expression is regulated by oestradiol in the same fashion as their mammalian counterparts only in *T. crassiceps*. To study if AP-1 genes are involved in a physiological function of the cyst, reproduction was studied *in vitro*. Oestradiol treatment stimulated reproduction in *T. crassiceps* but not in *T. solium* cysticerci. This is the first report of the detection and functionality of AP-1 transcription factor genes in any species of helminth parasite.

Key words: AP-1 transcription factor, *Taenia crassiceps*, *Taenia solium*, *c-fos* gene, *c-jun* gene.

INTRODUCTION

The metacestode stage of the tapeworm *Taenia solium* causes neurocysticercosis, the Third World major brain disorder that is estimated to affect 50 million people world-wide, and is an emergent disease in the United States (White, 1997). *T. solium* also infects pigs, the intermediate host, leading to major economic loss and transmission to humans (Sciutto *et al.* 2000). Slow data retrieval and high costs in *T. solium* research have led to the use of an experimental disease in mice with another cestode, *Taenia crassiceps*, which exhibits extensive similarities to *T. solium* (Larralde *et al.* 1990; Toledo *et al.* 2001).

Experimental intraperitoneal (IP) cysticercosis of mice is caused by the IP injection of *T. crassiceps* (Culbreth, Esch & Kuhn, 1972) and is characterized by the parasite's rapid rate of asexual reproduction to reach grams of parasite mass per infected host in a few months (Larralde *et al.* 1995). Parasite proliferation is faster in female than in male mice, and is favoured by oestradiol and hindered by androgens (Huerta *et al.* 1992; Bojalil *et al.* 1993; Terrazas *et al.* 1994). The importance of sexual factors for murine cysticercosis is further stressed by a remarkable feminization process that ensues in chronically infected male mice: serum oestradiol levels are increased 200 times their normal values, while those of testosterone are 90% reduced relative to control values (Larralde *et al.* 1995). The infected peritoneum shows a strikingly mild and transient inflammatory process (Padilla *et al.* 2001) and the systemic immune response shows a progressive downregulation of the TH1 cytokine profile and upregulation of TH2 when massive levels of parasite loads are reached (Terrazas *et al.* 1998; Toenjes *et al.* 1999). Sex hormones are proposed to be involved in the regulation of murine *T. crassiceps* cysticercosis by way of thymus-dependent immune mechanisms that obstruct or favour parasite growth (Bojalil *et al.* 1993; Terrazas *et al.* 1994). IL-6 is proposed to favour the expression of P-450 aromatase in infected males, thus shunting testosterone to oestradiol (Morales-Montor *et al.* 2001). Hints that sex hormones may be involved in *T. solium* disease of humans and pigs are progressively being reported (Sotelo & del Bruto, 2000; Morales *et al.* 2002).

In addition to the effects of sex-steroids on the host immune system (Morales-Montor *et al.* 2002), they may also directly affect the parasite if their usual target genes involved in cell proliferation and

* Corresponding author: Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, AP 70228, México DF 04510, México. Tel: +5255 5622 3854. Fax: +5255 5622 3369. E-mail: jmontor66@biomedicas.unam.mx

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Table 1. Sequences of the primers used for PCR amplification of total *Taenia crassiceps*, *T. solium*, mouse and pig RNA reverse transcribed

(Primers were designed based on the most conserved regions of several species sequenced genes or based using the sequence of cloned *T. crassiceps* genes (*c-fos* and *c-jun*). GenBank accession numbers: *c-fos* (gi55933, gi50399, gi15131232), *c-jun* (gi57819, gi6754401, gi186624), TNF-α (gi395369, gi7305584, gi25952110, gi164694, gi29569762), β-actin (gi38648901, gi6671508, gi5016088, gi27371027, gi156758, gi29603620, gi156758, gi28573581) and VDR (gi184894). Each PCR product was purified and sequenced as described in the Materials and Methods section.)

Gene	Sense primer	Antisense primer
c-fos	5′-CCCCTGTCAACACACAGGAC	5′-CCGATGCTCTGCGCTCTGC
c-jun	5′-CAGATCCCGGTGCAGCACCCG	5′-GGTGGCACCCACTGTTAACGTGG
TNF-α	5′-GGCAGGTCTACTTTGGAGTCATTGC	5′-ACATTCGAGGCTCCAGTGAATTCGG
VDR	5′-TGAATTCCATGAAACACCTGTGGTTCTT	5′-GACTCTAGAGGGCTCACACTCACCTCCCCT
β-actin	5′-CTACAATGAGCTGCGTGTGG	5′-GGTCTCAAACATGATCTGGG

differentiation (such as *c-fos* and *c-jun*) are present in the parasite. These parasite genes could regulate the expression of many other genes in a variety of tissues and cell types (Hyder, Shipley & Stancel, 1995; Hyder *et al*. 1992) and their over-expression in the cysticercotic mice could well influence the outcome of the infection as well as explain its strong dependence on sex-steroids. The specific aim of this study was to investigate whether *T. crassiceps* and *T. solium* cysticerci express sequences of the AP-1 complex proto-oncogenes *c-fos* and *c-jun* (both strongly regulated by oestradiol) and, if so, because oestradiol has been demonstrated to play a major role during cysticercosis, to study if the AP-1 gene complex is involved in regulating reproduction of both *T. crassiceps* and *T. solium* cysticerci *in vitro*.

MATERIALS AND METHODS

Parasites

Total RNA was extracted from cysticerci of the fast growing ORF strain of *Taenia crassiceps* isolated as described by Freeman (1962). The cysticerci used in these experiments were cultivated for 5 days after being collected from the peritoneal cavity of donor female mice. The collected cysticerci were alive and quite clean from debris but were, however, washed several times with NTS to minimize contamination with host cells. *T. solium* live cysticerci were dissected and freed from all surrounding tissues of an infected pig, immediately after being humanely killed in the Facultad de Medicina Veterinaria y Zootecnia, UNAM. The cysticerci were washed several times with saline to minimize contamination with host cells.

Total RNA isolation

Total RNA was isolated from *T. crassiceps* and *T. solium* cysticerci, together with mouse testes and pig muscle (as controls for specific *c-jun* or *c-fos* amplification), using the single-step method based on guanidine isothiocyanate/phenol/chloroform extraction with Trizol reagent (Life Technologies, CA). In brief, parasites, mouse testes and pig muscle were disrupted in Trizol reagent (1 ml/0·1 g of tissue), and 0·2 ml of chloroform was added per ml of Trizol. The aqueous phase was recovered after a 10 min centrifugation at 14 000 ***g***. RNA was precipitated with isopropyl alcohol, washed with 75% ethanol and redissolved in RNAse-free water. RNA concentration was determined by absorbance at 260 nm and its purity was verified after electrophoresis on 1·0% denaturing agarose gel in the presence of 2·2 M formaldehyde.

RT–PCR amplification

Total RNA from parasites, mouse testes and pig muscle was reverse transcribed followed by specific PCR amplification of *c-fos* and *c-jun* gene sequences. Nucleotide sequences of the primers used for *c-fos*, *c-jun* and β-actin amplification are shown in Table 1. To demonstrate that cysticerci were not contamined by host cell DNA or RNA, we used primers of pig TNF-α and the mouse VDR gene, which correspond to the mouse variable region of the IgG (Table 1). Briefly, 2 μg of total RNA (either host or parasite) were incubated at 37 °C for 1 h with 400 units of M-MLV reverse transcriptase (Applied Biosystems, Boston, MA) in 20 μl of reaction volume containing 50 mM of each dNTP and 0·05 μg oligo (dt) primer (Gibco, NY). Ten μl of the cDNA reaction was subjected to PCR in order to amplify specific sequences of the specified genes. The 50 μl PCR reaction included 10 μl of previously synthesized cDNA, 25 μl of 10× PCR-buffer (Biotecnologias Universitarias, Mexico) 1 mM $MgCl_2$, 0·2 mM of each dNTP, 0·05 μM of each primer, and 2·5 units of *Taq* DNA polymerase (Biotecnologias Universitarias, Mexico). After an initial denaturation step at 94 °C for 4 min, temperature cycling was initiated as follows: 94 °C for 55 sec, 50 °C for 55 sec and 72 °C for 45 sec during 30 cycles for *c-fos*, while for *c-jun* was: 94 °C for 55 sec, 57 °C for 55 sec and 72 °C for 45 sec during 30 cycles. β-actin was amplified by using same

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T.crassiceps     1:----------------------AUGAGCAGCTACACGAAGCCTGTGTTTCT-------AT
T.solium         1:----------------------AUGAGCAGCTACACGAAGCCTGTGTTTCT-------AT
T.crassiceps    61:TTAGCGTTGTTCTCTTTCCCTCCCCCCTCTTCCGAGTGAGAAAAAAAAGG-GGGGCCTAA
T.solium        61:TTAGCGTTGTTCTCTTTCCCTCCCCCCTCTTCCGAGTGAGAAAAAAAAGG-GGGGCCTAA
T.crassiceps   121:ATTCCCACCAACATAAACTAA--TGACATACAATGATGAAATTCTGTTTTCACCTCTGCC
T.solium       121:ATTCCCACCAACATAAACTAA--TGACATACAATGATGAAATTCTGTTTTCACCTCTGCC
T.crassiceps   181:TGTGACAGGGAATGCAAAAATAGCA--AGTGGCCTATTTCCACGAATCCCCGCCTCCCTG
T.solium       181:TGTGGGACTTAGCTCAAAAATAGCA--AGTGGCCTATTTCCACGAATCCCCGCCTCCCTG
                      ** *** ***
T.crassiceps   241:CCCTCCCCCCTCCCGCTATGTACGATCACTGAACTGCAATGGAGGCTAACGCAGAGGCAG
T.solium       241:CCCTCCCCCCTCCCGCTATGTACGATCACTGAACTGCAATGGAGGCTAACGCAGAGGCAG
T.crassiceps   301:GAGCCAGCCGGGATCAGCCCCCCGTTTTGCGC-AGATCTGTCCGTCTCTAGTGCCAACTT
T.solium       301:GAGCCAGCCGGGATCAGCCCCCCGTTTTGCGC-AGATCTGTCCGTCTCTAGTGCCAACTT
T.crassiceps   361:T-ATCCCCACGGTGACAGCCATCTCCACCAGCCCAGACCTGCAG-TGGCTGGTGCAGCCC
T.solium       361:T-ATCCCCACGGTGACAGCCATCTCCACCAGCCCAGACCTGCAG-TGGCTGGTGCAGCCC
T.crassiceps   421:-----ACTCTGGTCTCCTCCGTGGCCCCATCGCAAGACCAGA----GCGCCCCATCCTTA
T.solium       421:-----ACTCTGGTCTCCTCCGTGGCCCCATCGCAAGACCAGA----GCGCCCCATCCTTA
T.crassiceps   481:C--GGACTCCCACC-----AGCGACAGTCAGTTATATCCTGGCCGCCAG--AGCGGG--A
T.solium       481:C--GGACTCCCACC-----AACAGGAGTCAGTTATATCCTGGCCGCCAG--AGCGGG--A
                                    * *******
T.crassiceps   541:GTGGTGAAGACCATGTC-----AGGCGGCAGGGC--AGGTTTCCGGTATCGT-AGCTAGG
T.solium       541:GTGGTGAAGACCATGTC-----AGGCGGCAGGGC--AGGTTTCCGGTATCGT-AGCTAGG
T.crassiceps   601:TCGATAACGA-----------AGATCCATCCGCTGTCCTAATCCTCTCTAGTCCGTCTTG
T.solium       601:TCGATAACGA-----------AGATCCATCCGCTGTCCTAATCCTCTCTAGTCCGTCTTG
T.crassiceps   661:GAATGCCCA-----CCGTTTGGCTTCGCGACCCTTGGGACCCTCAGAATGGAGACGAGGG
T.solium       661:GAATGCCCA-----CCGTTTGGCTTCGCGACCCTTGGGACCCTCAGAATGGAGACGAGGG
T.crassiceps   721:GGACACATGAGTTCTGCGAGG-----------------------------------------
T.solium       721:GGACACATGAGTTCTGCGAGG-----------------------------------------
T.crassiceps   781:-------------ATCTGCGGTTTCCTATCCCAGAGGTGACCGGCCCAGT---CAGTCTA
T.solium       781:-------------ATCTGCGGTTTCCTATCCCAGAGGTGACCGGCCCAGT---CAGTCTA
T.crassiceps   841:ACCCGGCTTGTCCTCTGCGGAAGGACAGGAGGCCGAGGGCAAGTGGCTACGTTCGGGGGT
T.solium       841:ACCCGGCTTGTCCTCTGCGGAAGGACAGGAGGCCGAGGGCAAGTGGCTACGTTCGGGGGT
T.crassiceps   901:GTGTTTGTTCTACACTGAAGCACCAAAGTTCCATCTTCCAAGACTCAAAGCTGTTCTCAG
T.solium       901:GTGTTTGTTCTACACTGAAGCACCAAAGTTCCATCTTCCAAGACTCAAAGCTGTTCTCAG
T.crassiceps   961:GTCCCAGACGCCACTGACCTCTTACAGCTGGGAACCTTTCTTCCCGTCCCCTCTGCGCCC
T.solium       961:GTCCCAGACGCCACTGACCTCTTACAGCTGGGAACCTTTCTTCCCGTCCCCTCTGCGCCC
T.crassiceps  1021:CCACCCCCCTTCCCAAGTCCGA-TCTGGAAAATCACCCGCTGCAGGCGGGTTCCTTGTAA
T.solium      1021:CCACCCCCCTTCCCAAGTCCGA-TCTGGAAAATCACCCGCTGCAGGCGGGTTCCTTGTAA
T.crassiceps  1081:GCGCAGT-TTCCAGGCTGCACGTATTCAGACCCCCATCTCCCCAGCACCGACTTGCTTTC
T.solium      1081:GCGCAGT-TTCCAGGCTGCACGTATTCAGACCCCCATCTCCCCAGCACCGACTTGCTTTC
T.crassiceps  1141:TCCTCCCCCCCCCCCCCCCGAGCTCACCTCACTTTGTAATTCTGAGCTCCCCCCCTACCTC
T.solium      1141:TCCTCCCCCCCCCCCCCCCGAGCTCACCTCACTTTGCCCCCCTGCGCTGCACCCTCAG---
                                                  *****   *   * *   ** *
T.crassiceps  1201:AGTCCGCCCTCTGGTCTCAGCTC---AAAACTAAACATACGACCCCTTCAGGCATACTTG
T.solium      1201:AGTTGGCTAGCTGTTCCCCACGCGGAAGAACTAAACATACGACCCCTTCAGGCATACTTG
                     **  ***   *  * ** *     *
T.crassiceps  1261:TAGGGTGGTTTTGCACAATGTTTATC--CGTCAGTGTCAACGGGGACTGTCGCCTTGATA
T.solium      1261:TAGGGTGGTTTTGCACAATGTTTATC--CGTCAGTGTCAACGGGGACTGTAATTGGCCTA
                                                                 ********
T.crassiceps  1321:GCTCTAAGTGGCTAAGGGTCGGG---GAGTAGGTGGGGGGCTGTGCTGCCGTCCTTTAAA
T.solium      1321:GCTCTAAGTGGCTAAGGGTCGGG---GAGTAGGTGGGGGGCTGTGCTGCCGTCCTTTAAA
T.crassiceps  1381:ACACGAATT-TATGAATGAACCCAGTACTG---TAGTTATTGTACACTTATTTACAATCC
T.solium      1381:ACACGAATT-TATGAATGAACCCAGTACTG---TAGTTATTGTACACTTATTTACAATCC
T.crassiceps  1441:T-TCACTTTTCTCTGCACGC--TGCTTCCAGCCCCGAC---TCCCAGGCAAGGAGCTGGA
T.solium      1441:T-TCACTTTTCTCTGCACGC--TGCTTCCAGCCCCGAC---TCCCAGGCAAGGAGCTGGA
T.crassiceps  1501:GAGAGGGGCTGAGAAGCTGACCCCCCCTTTTTCTAAAGTTTCTTG---ATTTGGGAATGT
T.solium      1501:GAGAGGGGCTGAGAAGCTGACCCCCCCTTTTTCTAAAGTTTCTTG---ATTTGGGAATGT
T.crassiceps  1561:GGGCAAGCTTTCCTTTAGGAACAG-AGGCTTCGAGCCTAAATCAGGTTTTAAGGC-TGCG
T.solium      1561:GGGCAAGCTTTCCTTTAGGAACAG-AGGCTTCGAGCCTAAATCAGGTTTTAAGGC-TGCG
T.crassiceps  1621:TACTTG--CTTC--TCCTAA----TACCAGAGACTCAAAAAAAAAAAAAAAAGTTC-----
T.solium      1621:TACTTG--CTTC--TCCTAA----TACCAGAGACTCAAAAAAAAAAAAAAAAGTTC-----
T.crassiceps  1681:---------------------------------CAGATTGC-TGGACAATGACCCGGGTC
T.solium      1681:---------------------------------CAGATTGC-TGGACAATGACCCGGGTC
T.crassiceps  1741:TCATCCCTTGACCCTGGGAACCGG--GTCCACATTGAATCAGGTGCGAATGTTCGCTCGC
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Fig. 1. (*cont.*)

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T.solium      1741:TCATCCCTTGACCCTGGGAACCGG--GTCCACATTGAATCAGGTGCGAATGTTCGCTCGC
T.crassiceps  1801:CTTCTCTGCCTTTCCCGGCAGCCGGCGCCTCCCCTCCGCC--CCGGTTCCCCCCCTGCGC
T.solium      1801:CTTCTCTGCCTTTCCCGGCAGCCGGCGCCTCCCCTCCGCC--CCGGTTCTAATTCTGAGC
                                                          *****   *
T.crassiceps  1861:TGCACCCTCAG---AGTTGGCT---AGCTGTTCCCCACGCGGAAGGT-----CAATCCCT
T.solium      1861:TCCCCCCCTACCTCAGTCCGCC---CTCTGGTCTCAGCTC---AAGT-----CAATCCCT
                    * *   ** *       **  *   **   *  * ** *     *
T.crassiceps  1921:CCC----TCCTTTACACAGGATGTCCATATTAGGACATCTGCGTCAGCAGGTTTCCAC--
T.solium      1921:CCC----TCCTTTACACAGGATGTCCATATTAGGACATCTGCGTCAGCAGGTTTCCAC--
T.crassiceps  1981:GGCCGGTCCCTGTTGTTCTGGGGGGGGGACC--ATCTCCGAAATCCTACCCGGCCGCGG-
T.solium      1981:GGCCGGTCCCTGTTGTTCTGGGGGGGGGACC--ATCTCCGAAATCCTACCCGGCCGCGG-
T.crassiceps  2041:TCTAGGAGACCC---CCTAAGATCCCAAATGTGAACACTCATAGGTGAAAGA---TGTAT
T.solium      2041:TCTAGGAGACCC---CCTAAGATCCCAAATGTGAACACTCATAGGTGAAAGA---TGTAT
T.crassiceps  2101:GCCAAGACGGGGGT---------TGAAAGC-------CTGGGGCGTAGAGTTGACGAC-A
T.solium      2101:GCCAAGACGGGGGT---------TGAAAGC-------CTGGGGCGTAGAGTTGACGAC-A
T.crassiceps  2161:GAGCGCCCGCAGAGGGCCTTGGGGCGCGCTTCCCCCCCCTTCCAGTTC--CGCCCAGTGA
T.solium      2161:GAGCGCCCGCAGAGGGCCTTGGGGCGCGCTTCCCCCCCCTTCCAGTTC--CGCCCAGTGA
T.crassiceps  2221:C--GTAGGAAGTCCATCCATTCACAGCGCTTCTATAAAGGCGCCAGCTGAGGCGCC----
T.solium      2221:C--GTAGGAAGTCCATCCATTCACAGCGCTTCTATAAAGGCGCCAGCTGAGGCGCC----
T.crassiceps  2281:TACTACTAAAAAAAAAA----------------------------------------
T.solium      2281:TACTACTAAAAAAAAAA----------------------------------------
                  * *   ** ****  *
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B

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Rattus rattus: 1   MMYQGFAGEYEXXXXXXXXXXXXXXXLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANF 60
Mus musculus: 1   MMYQGFAGEYEXXXXXXXXXXXXXXXLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANF 60
Homo sapiens: 1   ************************************************************ 60
Taenia crassiceps: 1   MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANF 60
Taenia solium: 1   MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNAQDFCTDLAVSSANF 60

Rattus rattus: 61  VPTVTAISTSPDLQWLVQPTLISSVAPSQNRG-HPYGVXXXXXXXXYSRPAVLKAPGGRG 120
Mus musculus: 61  VPTVTAISTSPDLQWLVQPTLISSVAPSQNRG-HPYGVXXXXXXXXYSRPAVLKAPGGRG 120
Homo sapiens: 61  ************************************************************ 120
Taenia crassiceps: 61  IPTVTAISTSPDLQWLVQPTLVSSVAPSQTRAPHPYGLPTPSTGAYARAGVVKTMSGGRA 120
Taenia solium: 61  IPTVTAISTSPDLQWLVQPALVSSVAPSQTRAPHPFGVPAPSAGAYSRAGVVKTMTGGRA 120

Rattus rattus: 121 QSIGRRGKVEQLSPXXXXXXXXXXXXNKMAAAKCRNRRRELTDTLQAETDQLEEEKSALQ 180
Mus musculus: 121 QSIGRRGKVEQLSPXXXXXXXXXXXXNKMAAAKCRNRRRELTDTLQAETDQLEEEKSALQ 180
Homo sapiens: 121 ************************************************ETDQLEEEKSALQ 180
Taenia crassiceps: 121 QSIGRRGKVEQLSPEEEEKRRIRRERNKMAAAKCRNRRRELTDTLQAETDQLEDEKSALQ 180
Taenia solium: 121 QSIGRRGKVEQLSPEEEEKRRIRRERNKMAAAKCRNRRRELTDTLQAETDQLEDEKSALQ 180

Rattus rattus: 181 AEIANXXXXXXXXXFILAAHRPACKMPEELRFSEELAAATALDLGAPSPAA----AEETF 240
Mus musculus: 181 AEIANXXXXXXXXXFILAAHRPACKMPEELRFSEELAAATALDLGAPSPAA----AEETF 240
Homo sapiens: 181 AEIANXXXXXXXXXFILAAHRPACKMPEELRFSEELAAATALDLGAPSPAAA----EETF 240
Taenia crassiceps: 181 TEIANLLKEKEKLEFILAAHRPACKIPNDLGFPEEM-SVTSLDLTGGLPEATTPESEEAF 240
Taenia solium: 181 TEIANLLKEKEKLEFILAAHRPACKIPDDLGFPEEMSVASLDLTGGLPEVATPESEEAFT 240

Rattus rattus: 241 ALPXMTEAPPAVPPKEP--SGSGLELKAEPFDELLFSTGPR----EASRSVPDMDLPGAS 300
Mus musculus: 241 ALPXMTEAPPAVPPKEP--SGSGLELKAEPFDELLFSTGPR----EASRSVPDMDLPGAS 300
Homo sapiens: 241 ALPXMTEAPPAVPPKEP--SGSGLELKAEPFDELLFSTGPR----EASRSVPDMDLPGAS 300
Taenia crassiceps: 241 TLPLLNDPEPK-PSLEPVKNISNMELKAEPFDDFLFPASSRPSGSETARSVPDVDLSG-- 300
Taenia solium: 241 LPLLNDPEPKPSVEPVKSISSMELKTEPFDDFLFPASSRPSGSETARSVPDMDLSGSFYA 300

Rattus rattus: 301 SFYASDWEPLGAGSSG------ELEPLXXXXXXXXXXXXXXXXXXFVFTYPEADAFPSCAA 360
Mus musculus: 301 SFYASDWEPLGAGSSG------ELEPLXXXXXXXXXXXXXXXXXXFVFTYPEADAFPSCAA 360
Homo sapiens: 301 SFYASDWEPLGAGSSG------ELEPLXXXXXXXXXXXXXXXXXXFVFTYPEADAFPSCAA 300
Taenia crassiceps: 301 SFYAADWEPLHSSSLGMGPMVTELEPLCTPVVTCTPSCTTYTSSFVFTYPEADSFPSCAA 360
Taenia solium: 301 ADWEPLHSGSLGMGPMATELEPLCTPVVTCTPSCTAYTSSFVFTYPEADSFPSCAAAHRK 360

Rattus rattus: 361 AHRKGXXXXXXXXXXXXXPTLLAL 384 96%
Mus musculus: 361 AHRKGXXXXXXXXXXXXXPTLLAL 384 93%
Homo sapiens: 361 AHRKGXXXXXXXXXXXXXPTLLAL 384 82%
Taenia crassiceps: 361 AHRKGSSSNEPSSDSLSSPTLLAL 384
Taenia solium: 361 GSSSNEPSSDSLSSPTLLAL 380
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Fig. 1. For legend see opposite page.

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conditions as those for *c-jun*. An extra primer extension temperature at 72 °C for 10 min was done for every gene. Then 25 μl of the total PCR reaction products of each sample were electrophoresed on 2% agarose gel. PCR products were visualized by staining with ethidium bromide. A single band was detected in all cases, and the size of each specific amplification product as expected was 274 nt (*c-fos*), 325 nt (*c-jun*) and 327 nt (β-actin).

Probe development

Fifty μl samples of the PCR reaction were electrophoresed on 2% agarose gels. The PCR products obtained were visualized by staining with ethidium bromide, and were detected by using a 100 nt ladder as molecular weight marker (Gibco, BRL, NY). In all cases, the bands correponding to *T. crassiceps c-fos* and *c-jun*, and *T. solium c-fos*, together with VDR and TNF-α from mouse and pig respectively were excised directly from the gel, purified by electroelution, and cloned into the bluescript SK(+/−) (Stratagene, La Jolla, CA) vector. These specific cDNAs were used as probes to perform the *T. crassiceps* and *T. solium* phage library screening and the Southern blot hybridization analysis.

Southern blot analysis

T. crassiceps and *T. solium* cysticerci were homogenized gently and digested by proteinase K (100 units/ml) for 18–20 h at 37 °C, with continuous shaking. After the protein fraction was extracted with phenol/chloroform, the DNA was precipitated with cold ethanol and assessed for high molecular weight integrity by electrophoresis. DNA aliquots (20 μg) were digested with *Eco*RI, separated in 1% agarose gels, denatured with 0·25 M NaOH, and transferred to Hybond nylon membranes. cDNA parasite probes, labelled with [α-^{32}P]CTP, 0·6 kb *c-fos* and *c-jun* were synthesized from linearized cDNA. The membranes were hybridized with the probe overnight at 65 °C and then washed with 0·3 × SSC/0·1% SDS for about 2 h at 65 °C, followed by exposure to X-ray film. The signals were quantitated by densitometric scanning (Molecular Dynamics, Sunnyvale, CA). PCR products of the VDR and TNF-α gels were blotted in the same way as parasite genomic DNA to reveal a very low rate of amplification, and to be sure that no contamination at all from host cells occurred.

DNA sequencing

The DNA sequence was determined by using a Thermo Sequenase cycle sequencing kit (Biorad) and an automatic sequencer (Model LIC-4200, Aloka Co., Japan). The sequence data were analysed using DNASIS Software (Hitachi Software Engineering, Tokyo, Japan). Homology searching on the nucleotide and protein database was carried out with the Blast program at the National Center for Biotechnology Information (Bethesda, Maryland). Pairwase sequence alignment and protein identities were performed using CLUSTLAW 1.6 software.

Treatment of T. crassiceps *and* T. solium *with oestradiol: hormone dose-response and temporal curves*

Culture grade E_2 was obtained from Sigma. For *in vitro* tests, water-soluble E_2, was dissolved in AIM-V free-serum culture medium, and sterilized by passage through a 0·2 mm millipore filter. The experiments employed parasite-loaded wells: 6 wells were used as untreated controls, 6 wells were supplemented with the solvent in which the hormone was diluted and 6 wells were treated with different concentrations of E_2 (5, 10, 20 and 40 μg/ml). Hormone was prepared in a final volume of 100 μl and added to 1 ml of medium in each well. The range of concentrations of oestradiol tested in this study was based on our previous studies of serum levels found in feminized mice (Larralde *et al*. 1995) and were chosen to approximate those levels *in vitro*. In hormone dose-response-time curves, only the number of buds per cysticercus as a function of days in culture was assessed as the response variable (1–10 days in culture). From the dose-response-time curves of oestradiol, an optimal dose was selected to be used in further experimentation: the dose of each hormone at the shortest time in which the differences with the respective control values was maximal. Reproduction was measured as the number of buds that each cyst produced in response to treatment and these were counted directly under an inverted light microscope.

RESULTS

The alignment of the *c-fos* gene sequences and predicted amino acid translation of *T. crassiceps* and *T. solium* with their mammalian counterparts is shown in Fig. 1. *T. crassiceps* and *T. solium* genes

Fig. 1. *Taenia crassiceps* and *T. solium c-fos* gene sequence and predicted amino acid sequence alignment. (A) Nucleotide sequence of *T. crassiceps* and *T. solium c-fos* gene. The positions of the start codon and the oligonucleotides used for PCR amplification are underlined. The nucleotide numbers are by reference to the start of the 5′ UTR when the *T. crassiceps* and *T. solium* sequence is aligned with other known mammalian *c-fos* sequences. Asterisks (*) indicate a change in nucleotide sequence. (B) Alignment of the *c-fos* protein sequences in rat (*Rattus rattus*), mouse (*Mus musculus*), human (*Homo sapiens*), *T. crassiceps*, and *T. solium*. Amino acids are represented by single-letter codes. Dotted lines represent undentified amino acids, and the X represents highly variable regions, meaning that X can be any amino acid.

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T.crassiceps      1:AUGTTGGTAACCCTAAGATCCTAAAACAGAGCATGACCTTGAACCTGGCCGACCCGTAGC
T.solium          1:AUGTTGGTAACCCTAAGATCCTAAAACAGAGCATGACCTTGAACCTGGCCGACCCGTAGC
T.crassiceps     61:C-AGAACTCAGCACCGCCGG-AGAGCCGCTGTTGCTGGG-----ACTGGTCTGCGGGCTC
T.solium         61:C-AGAACTCAGCACCGCCGG-AGAGCCGCTGTTGCTGGG-----ACTGGTCTGCGGGCTC
T.crassiceps    121:CAAGGAACCGCTGCTCCCCGAGAGCGCTCCGTGAGTGACCGCGACTTTTCAAAGCTCGGC
T.solium        121:CAAGGAACCGCTGCTCCCCGAGAGCGCTCCGTGAGTGACCGCGACTTTTCAAAGCTCGGC
T.crassiceps    181:ATCGCGCGGGAGCCTACCAACGTGAGTGCTAGCGGAGTCTT----AACCCTGCGCTCCCT
T.solium        181:ATCGCGCGGGAGCCTACCAACGTGAGTGCTAGCGGAGTCTT----AACCCTGCGCTCCCT
T.crassiceps    241:GGAGCGAACTGGGGAGGAGGGCTCAGGGGGAAGCACTGCCGTCTGGAGCGCACGCTCCTA
T.solium        241:GGAGCGAACTGGGGAGGAGGGCTCAGGGGGAAGCACTGCCGTCTGGAGCGCACGCTCCTA
T.crassiceps    301:AACAAACTTT----GTTACAGAAGCGGGGACGCTTCGCCGAGGGCTTCGTGCGCGCCCTG
T.solium        301:AACAAACTTT----GTTACAGAAGCGGGGACGCTTCGCCGAGGGCTTCGTGCGCGCCCTG
T.crassiceps    361:GCTGAACTGCACCGAAACTTCTGCGCACAGCCCAGGCTAACCCCGCGTGAAG-GCCAGCA
T.solium        361:GCTGAACTGCACCGAAACTTCTGCGCACAGCCCAGGCTAACCCCGCGTGAAG-GCCAGCA
T.crassiceps    421:ACTTTCCTGACCACAGACGGACGAAACGACCTTCTACGACGATGCCCTCAACGCCTCGTT
T.solium        421:ACTTTCCTGACCACAGACGGACGAAACGACCTTCTACGACGATGCCCTCAACGCCTCGTT
T.crassiceps    481:CCTCCAGTCCGAGAGCGGTGCCTACGGCTACATCGGAGGCCGGGCGGCAGAGCCGCAGAC
T.solium        481:CCTCCAGTCCGAGAGCGGTGCCTACGGCTACATCGGAGGCCGGGCGGCAGAGCCGCAGAC
T.crassiceps    541:GGTGCCGGAGATG-CCGGGAGAGACGCCGCCCTGTCCCCCATCGACATGGAGTCTCAGGA
T.solium        541:GGTGCCGGAGATG-CCGGGAGAGACGCCGCCCTGTCCCCCATCGACATGGAGTCTCAGGA
T.crassiceps    601:GCGGATCAAGGCGGAGAGGAAGGCGCATGAGAAACCGCATCGCTGCCTCCAAGTGCCGGA
T.solium        601:GCGGATCAAGGCGGAGAGGAAGGCGCATGAGAAACCGCATCGCTGCCTCCAAGTGCCGGA
T.crassiceps    661:AAAGGAAGCTGGAGCGGATCGCCCGGCTAGAGGAAAAAGTGAAAACCTTGAAAGCGCAAA
T.solium        661:AAAGGAAGCTGGAGCGGATCGCCCGGCTAGAGGAAAAAGTGAAAACCTTGAAAGCGCAAA
T.crassiceps    721:CTCCGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGG-CACAGCTTAAACAGA
T.solium        721:CTCCGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGG-CACAGCTTAAACAGA
T.crassiceps    781:AAGTCATGACCCCGCTTCCCGGCGGAGCAGGAGGTGACGACTGGGCGCGGGTATCCCCCC
T.solium        781:AAGTCATGACCCCGCTTCCCGGCGGAGCAGGAGGTGACGACTGGGCGCGGGTATCCCCCC
T.crassiceps    841:GCTTCCCGGC----------GCGCTGTTGCGGCCGCGGTGGCCTCAGTAGCAGGCGCTGG
T.solium        841:GCTTCCCGGC----------GCGCTGTTGCGGCCGCGGTGGCCTCAGTAGCAGGCGCTGG
T.crassiceps    901:CGGCGGTGGTGGCTACAGCGCCAGCCTGCACAGTGAGCCTCCGGTCTACGCCAACCTCAG
T.solium        901:CGGCGGTGGTGGCTACAGCGCCAGCCTGCACAGTGAGCCTCCGGTCTACGCCAATAT-AC
                                                                    **   *
T.crassiceps    961:CAACTTCAACCCGGGTGCGCTGA-GCAGCGGCGGTGGGCTGGAGCATTTACTGCTGTGTC
T.solium        961:TCATACCAGTTCGCACAGGCGGCTGAAGTTGGGCGGGGCTGGAGCATTTACTGCTGTGTC
                     ** ***  *** ***** * *  *  ** * **
T.crassiceps   1021:ACCTCCGCGGCACAGCCGGTCAGCGGGCCGGGCTGCAGCCGCCTCCGGTGCAGCACCCGC
T.solium       1021:ACCTCCGCGGCACAGCCGGTCAGCGGGCCGGGCTGCAGCCGCCTCCGGTGCAGCACCCGC
T.crassiceps   1261:GGCTGGCCTTTCCCTCGCAGCCGCAGCAGCAGCAGCAAGCCGTGTTGAGATGATGCTTCG
T.solium       1261:GCCTGGCCTTTCCCTCGCAGCCGCAGCAGCAGCAGCAAGCCGTGTTGAGATGATGCTTCG
T.crassiceps   1321:ACGTGCCGGAGATGCCGGGAGAGACGCCGCCCCTGTCCCCTATCGACATGGAGTCAGCAG
T.solium       1321:ACGTGCCGGAGATGCCGGGAGAGACGCCGCCCCTGTCCCCTATCGACATGGAGTCAGCAG
T.crassiceps   1381:TTGCAAA-CGTTTTGAGAACAGACTGTCAGGGCTGACGCATTGCCGCCTCCAAGTGCCGG
T.solium       1381:TTGCAAA-CGTTTTGAGAACAGACTGTCAGGGCTGACGCATTGCCGCCTCCAAGTGCCGG
T.crassiceps   1441:AAAAGGAAGCTGGAGCGGATCGCTCGGCTAGAGGAAAAAGTGAAAACCTTGAAAGCGCAA
T.solium       1441:AAAAGGAAGCTGGAGCGGATCGCTCGGCTAGAGGAAAAAGTGAAAACCTTGAAAGCGCAA
T.crassiceps   1501:AACTCCGAGCTGGCATCCACGGCCAACATGCTCAGGGAACAGGTGGCACAGCTTAAGCAG
T.solium       1501:AACTCCGAGCTGGCATCCACGGCCAACATGCTCAGGGAACAGGTGGCACAGCTTAAGCAG
T.crassiceps   1561:AAAGTCATGAACCACGTTAACAGTGGGTGCCAACTCATGCTAACGCGGGGCAATGGTCAG
T.solium       1561:AAAGTCATGAACCACGTTAACAGTGGGTGCCAACTCATGCTAACGCGGGGCAATGGTCAG
T.crassiceps   1621:GAGCGGATCAAGG-CAGAGAGGAAGCGCATGAGGAAC-----AAGAAAAAAAAATAAC---
T.solium       1621:GAGCGGATCAAGG-CAGAGAGGAAGCGCATGAGGAAC-----AAGAAAAAAAAATAAC---
T.crassiceps   1681:-AGAGACAAACTTGAGAACTTGACTGGTTGCGACAAAAAAAAAAAAAAA----------
T.solium       1681:-AGAGACAAACTTGAGAACTTGACTGGTTGCGACAAAAAAAAAAAAAAA----------
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Fig. 2. (*cont.*)

demostrated homology to several known *c-fos* genes sequenced. The *c-fos T. crassiceps* and *T. solium* genes have a high sequence homology between them (96%), while when compared with mouse, rat and human, the scores are lower, but still they are as high as (93%, 96% and 82%) respectively. Comparison of the predicted amino acid sequence of *c-fos* and *c-jun* with the mammalian proteins indicates that *c-fos* and *c-jun* share 92% amino acid identity. Molecular cloning of both genes in both parasites, together with their respective mRNAs, and determination of the full length protein sequence, has confirmed that both belong to the group of proteins of the *c-fos* and *c-jun* family.

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B

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Rattus rattus: 1   MTAKMETTFYDDALNASFLQSESGAYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL 60
Mus musculus: 1   MTAKMETTFYDDALNASFLQSESGAYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL 60
Homo sapiens: 1   MTAKMETTFYDDALNASFLQSESGAYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL 60
Taenia crassiceps: 1   MTAKMETTFYDDALNASFLQSESGAYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL 60
Taenia solium: 1   MTAKMETTFYDDALNASFLPSESGPYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL 60

Rattus rattus: 61  LTSPDVGLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE 120
Mus musculus: 61  LTSPDVGLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE 120
Homo sapiens: 61  LTSPDVGLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE 120
Taenia crassiceps: 61  LTSPDVGLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE 120
Taenia solium: 61  LTSPDVGLLKLASPELERLVIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE 120

Rattus rattus: 121 LHSQNTLPSVTSAAQPXXXXXXXXXXXXXXXXXXXXXXXXXXXLHSEPPVYANLSNFNXXX 180
Mus musculus: 121 LHSQNTLPSVTSAAQPXXXXXXXXXXXXXXXXXXXXXXXXXXXLHSEPPVYANLSNFNXXX 180
Homo sapiens: 121 LHSQNTLPSVTSAAQPXXXXXXXXXXXXXXXXXXXXXXXXXXXLHSEPPVYANLSNFNXXX 180
Taenia crassiceps: 121 LHSQNTLPSVTSAAQPVSGAGMVAPAVASVAGAGGGGGYSASLHSEPPVYANLSNFNPGA 180
Taenia solium: 121 LHSQNTLPSVTSAAQPVNGAGMVAPAVASVAGGSGSGGFSASLHSEPPVYANLSNFNPGA 180

Rattus rattus: 181 XXXXXXXXXXXXXXXXFXXXXXXXXXXXXXXXXXXXXXXXXXRLQALKEEPQTVPEMPG 240
Mus musculus: 181 XXXXXXXXXXXXXXXXFXXXXXXXXXXXXXXXXXXXXXXXXXRLQALKEEPQTVPEMPG 240
Homo sapiens: 181 XXXXXXXXXXXXXXXXAXXXXXXXXXXXXXXXXXXXXXXXXXRLQALKEEPQTVPEMPG 240
Taenia crassiceps: 181 LSCGGGAPSYGAAGLAFPSQPQQQQQPPQPPHHLPQQIPVQHPRLQALKEEPQTVPEMPG 240
Taenia solium: 181 LSSGGGAPSYGAAGLAFPAQPQQQQQPPHHLPQQMPVQHPRLQALKEEPQTVPEMPGETP 240

Rattus rattus: 241 ETPPLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELAST 300
Mus musculus: 241 ETPPLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELAST 300
Homo sapiens: 241 ETPPLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELAST 300
Taenia crassiceps: 241 ETPPLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELAST 300
Taenia solium: 241 PLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANM 300

Rattus rattus: 301 ANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQTF 98%                          334
Mus musculus: 301 ANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQTF 92%                          334
Homo sapiens: 301 ANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQTF 93%                          334
Taenia crassiceps: 301 ANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQTF                         334
Taenia solium: 301 LREQVAQLKQKVMNHVNSGCQLMLTQQLQTF                                331
```

Fig. 2. *Taenia crassiceps* and *T. solium c-jun* gene sequence and predicted amino acid sequence alignment. (A) Nucleotide sequence of *T. crassiceps* and *T. solium c-jun* gene. The positions of the start codon and the oligonucleotides used for PCR amplification are underlined. The nucleotide number are by reference to the start of the 5′ UTR when the *T. crassiceps* and *T. solium* sequence is aligned with other known mammalian *c-jun* sequences. Asterisks (*) indicate a change in nucleotide sequence. (B) Alignment of the *c-fos* protein sequences in rat (*Rattus rattus*), mouse (*Mus musculus*), human (*Homo sapiens*), and cestodes *T. crassiceps*, and *T. solium*. Amino acids are represented by single-letter codes. Dotted lines represent unidentified amino acids, and the X represents highly variable regions, meaning that X can be any amino acid.

On the other hand, *T. crassiceps* and *T. solium c-jun* genes (Fig. 2) have a higher sequence homology (98%). Interestingly, the homology is also higher when compared with both helminth gene sequences to their mammalian counterparts (mouse 93%, rat 98%, and human 93%) (Fig. 2). Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the Accession numbers: AY436613, AY436614, AY436615 and AY436616.

In view of the fact that different mRNAs can have varying half-lives and may undergo selective degradation, the quantity and integrity of total RNA extracted from the different sources used in this study was compared. The same amount of RNA (1 μg) that was non-degraded was used for RT–PCR amplification in each tissue studied. The RT–PCR amplification of the *c-fos* sequences for *T. crassiceps* and *T. solium* is shown in Fig. 3A. It is clear that a specific fragment (274 nt) that corresponds in molecular weight to that of *c-fos* was obtained in both control tissues, as well as in *T. crassiceps* and *T. solium*. Note that in both parasites the intensity of the band for *c-fos* amplification is 10 times stronger than the control tissue, using the same total RNA amount. β-actin was constantly expressed in all tissues amplified. In Fig. 3A the amplification by RT–PCR of the *c-jun* gene is also shown in *T. crassiceps* but not in *T. solium*. A specific fragment (325 nt) that corresponds in molecular weight to that of *c-jun* was obtained from mouse testes and pig muscle total RNA. β-actin was constantly expressed in all tissues amplified. By

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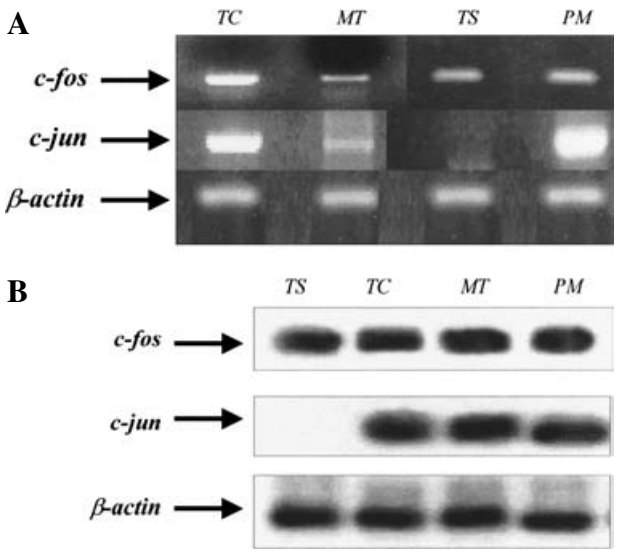

Fig. 3. Analysis of the expression of *c-fos* and *c-jun* gene in *Taenia crassiceps* and *T. solium* cysticerci. (A) A representative RT–PCR of total RNA from *T. crassiceps* (TC), *T. solium* (TS), mouse (MT) testes and pig muscle (PM) showing the detection of *c-fos* and *c-jun*. β-actin was used as the constitutively expressed control gene in all lanes. (B) Representative Northern blot of total RNA from *T. crassiceps* (TC), mouse testes (MT), *T. solium* (TS) and pig muscle (PM), showing the detection of *c-fos* and *c-jun*. β-actin was used as a control of RNA integrity, as well as a contitutively expressed gene to show that *T. solium* does not express the *c-jun* gene.

using degenerated oligonucleotides of the *c-jun* gene as primers, no expression of *c-jun* gene was detected in *T. solium* either (not shown).

As a demonstration that *c-fos* and *c-jun* play a physiological role in both species of cysticerci, and that the lack of expression in *T. solium* found by RT–PCR was not artifactual or due to a trivial reason such as non-specificity of the oligonucleotides used, Northern blot analyses were performed. In Fig. 3B it is shown that both *T. crassiceps* and *T. solium* expressed the *c-fos* gene at similar levels. However, when *c-jun* gene expression was assessed, it was found that only *T. crassiceps* expressed this gene (Fig. 3B). It is important to mention that β-actin was expressed in all RNA samples analysed, supporting the notion that *T. solium* cysticerci do not express the *c-jun* gene.

The fact that *c-fos* and *c-jun* genes are regulated in the same way as their mammalian counterparts in both species of cysticerci, was demostrated by way of RT–PCR amplification of cultured *T. crassiceps* and *T. solium* cysticerci in the presence of oestradiol, a steroid hormone that is known to induce *c-fos* and *c-jun* expression in mammalian cells. In Fig. 4A the pattern of expression for *c-fos*, is shown and it is clear that E_2 treatment strongly augmented *c-fos* expression in *T. crassiceps*, but not in *T. solium*. In Fig. 4A, a representative Northern blot is shown for *c-jun*, in which it is clear that E_2 stimulates *c-jun* expression in *T. crassiceps*, but has no effect whatsoever in *T. solium*.

In Fig. 4B a Southern blot analysis is depicted of *c-jun* and *c-fos* in *T. solium* and *T. crassiceps* cysticerci. Southern blot and hybridization probing of *Eco*RI-digested *T. crassiceps* and *T. solium* genomic DNA identified DNA fragments in each sample that hybridized with the *c-fos* and *c-jun* cDNA probes. *T. crassiceps* and *T. solium* genomic DNA produced restriction fragments of 2·37 kb when hybridized with the *c-fos* probe, while when hybridized with *c-jun* both produced a 4·5 fragment (not shown).

When treated with E_2, *T. crassiceps* cysticerci exhibited amplified copy number of the *c-fos* gene, while *T. solium* did not. The level of *c-fos* amplification in *T. crassiceps* ranged from 2·4 to 3·6. The same pattern was seen when the *c-jun* gene was similarly examined in the same samples in both parasites: an increased (1·9 to 2·3) copy number in *T. crassiceps* *c-jun* gene as the oestradiol dose increased, while there was no change in the level of *c-jun* gene in *T. solium*. The β-actin gene remained constant.

In Table 2, the effect of oestradiol on *in vitro* reproduction of *T. crassiceps* and *T. solium* is shown. The number of buds was augmented in *T. crassiceps* cysticerci by 120% when they were stimulated with 40 μg of E_2 compared with untreated cysticerci. On the other hand, when *T. solium* cysticerci were treated with E_2, no effect on reproduction was recorded, as compared with untreated cysts. E_2 did not affect viability in any species of cysticerci tested, and reproduction was correlated with AP-1 complex gene expression in *T. crassiceps*.

To further demostrate that *c-fos* and *c-jun* genes were of parasite origin and not obtained artifactually by host cell contamination, we eliminate all possible host cell contamination after parasites being extracted directly from host tissues by culturing them for 1 week in a free-serum medium, which maintains parasites, but not mammalian cells. Parasite cultures were then checked for possible DNA and RNA host cell traces, amplifing a highly specific gene product, which has evolved only in mammals, such as the fragment VDRHC (Variable Region of the Heavy Chain of the immunoglobulins), which showed no amplification in DNA or RNA from the parasite, but gave positive in the host tissues (not shown). The same result was obtained when another highly evolved gene (as TNF-α) was used as a template: the mouse and pig tissues amplified it, whereas *T. solium* and *T. crassiceps* did not (not shown). β-actin was expressed to a similar level as in mouse and pig tissues, demostrating DNA and RNA integrity.

DISCUSSION

This study reports the expression of *c-fos* in *T. crassiceps* and *T. solium* cysticerci and of *c-jun* only in *T. crassiceps*. The presence of both *c-fos* and *c-jun*

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Table 2. *In vitro* reproduction and AP-1 relative expression of *Taenia crassiceps* and *T. solium* cysticerci in the absence (C) or presence of a dose of 40 μg of oestradiol (E_2) during five days in culture

Species of cysticerci	Number of buds (av. per cultured well)		Relative expression *c-fos*/β-actin		Relative expression *c-jun*/β-actin	
	C	E_2	C	E_2	C	E_2
T. crassiceps	0	25*	1·2	120·8*	1·6	90·3*
T. solium	0	0	1·5	1·8	0	0

* $P<0{\cdot}01$.

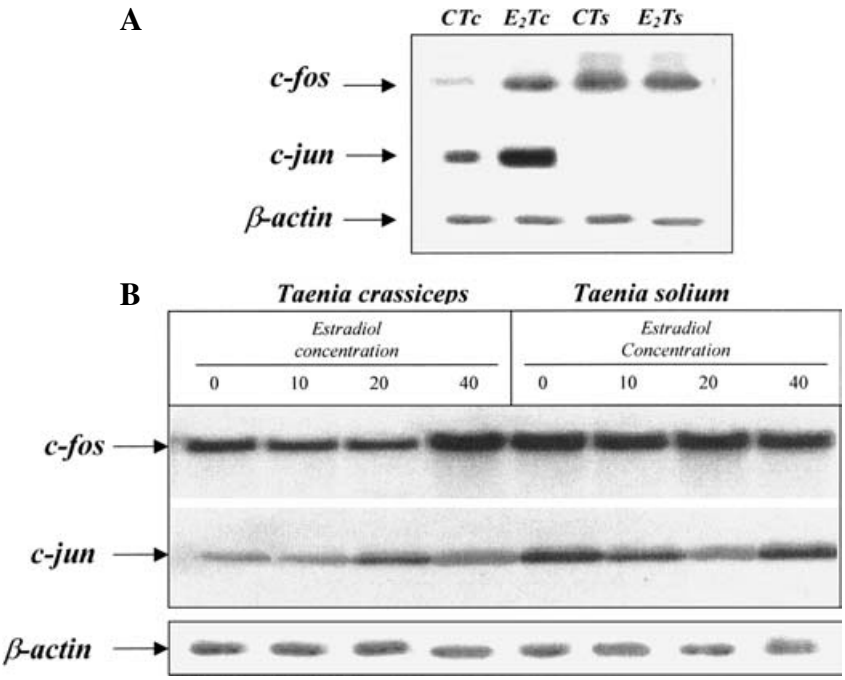

Fig. 4. Northern and Southern blot analyses of *c-fos* and *c-jun* gene expression in cultured and estradiol-treated cysticerci. (A) Representative Northern blot of *c-fos* and *c-jun* expression in response to 40 μg of oestradiol (E_2) from Tc (*Taenia crassiceps*) and *T. solium* compared against unstimulated *T. crassiceps* (CTc) and unstimulated *T. solium* (CTs) cysticerci. (B) Southern blot analysis of genomic DNA for *c-fos* and *c-jun* in *T. crasiceps* and *T. solium* cysticerci. β-actin was used as a control of DNA integrity, and to show that regulation of AP-1 complex genes by estradiol is specific, as well as a contitutively expressed gene to show that *T. solium* possesses the *c-jun* gene, but does not express it. In both blots, β-actin was used as a control, constitutively expressed gene and remained unaltered.

could help to explain the high asexual proliferation rate of *T. crassiceps* in female and feminized male mice, as well as the strong effects of sex-steroids leading to increased parasite loads (Bojalil *et al.* 1993; Larralde *et al.* 1995; Morales-Montor *et al.* 2001, 2002). The sequenced parasite *c-fos* and *c-jun* gene fragments code for the proteins' DNA binding domains, that are the most highly conserved among species, as shown also by our finding that they are identical in *T. solium* and *T. crassiceps*. Most certainly, these parasite genes must be important in the physiology, development and asexual reproduction of the parasite, as they are for many other organisms. Possibly these parasite genes are also important for the host, should the proteins coded reach the host cells and influence their activities in a variety of processes such as inflammation, immunity and cell proliferation (Barnes & Adcock, 1998).

To show the integrity, size and expression level of these genes in parasite RNA, we performed Northern blot analysis. Our results reinforced the notion that *c-fos* is expressed in both species of tapeworm, but *c-jun* is only expressed in *T. crassiceps*, and not in *T. solium*, which is very intriguing. Lack of *c-jun* expression in *T. solium* could result either from host or parasite regulation factors, since Southern blot analysis showed a transcript for *c-jun* in *T. solium*, which demostrates that it does have the gene, but it is not being expressed.

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It is well known that *c-fos*/*c-jun* heterodimers are the most functional at the transcription level (Chinenov & Kerppola, 2001). Thus, *T. solium* cysticerci expressing only *c-fos* would limit the effective transcription of the genes involved in asexual reproduction that characterizes *T. crassiceps* cysticerci. Indeed, the lack of *c-jun* expression in *T. solium* cysticerci is congruent with the fact that this parasite is incapable of asexual reproduction. Although *c-jun* can form a functional homodimer, so far it has not been demonstrated that *c-fos* functions in this way. In this regard, it is very intriguing to understand how *c-fos* alone functions in *T. solium*. It would be important to ascertain which of both possibilities is operating, since *T. solium* cysticerci frequently degenerate and calcify spontaneously and harmessly in its human host's central nervous system for unknown reasons. If these reasons involve regulation of the expression of parasite transcription factors, the possibility of therapy to shut down these genes is opened.

The connection between sex steroids and parasitism is progressively being recognized as decisive in the outcome of several parasite infections (Alexander & Stimson, 1988; Klein, 2000). For instance, in mammals, sex steroids participate in a large number of physiological processes such as sexual behaviour (Maggi & Pérez, 1985), reproduction, and development (Knobil *et al*. 1988). Oestradiol regulates these processes by inducing DNA replication and cellular proliferation through changes in the expression of several proto-oncogenes such as *c-fos* (Kushner *et al*. 2000) and a variety of genes from the *jun* family (Ransone & Verma, 1990; Nephew *et al*. 1993).

Likewise, the influence of *c-fos* and *c-jun* genes on reproductive behaviour affect the evolution of the host and parasite species (McLennan & Brooks, 1991). The inclusion of gene complexes, such as AP-1 in the parasite, with such potent and wide effects on various cellular processes involved in host–parasite relationships may help in understanding a number of major questions in parasitology, like host–parasite specificity, sexual dimorphism, the possible relation with oncogenesis, antigenic parasite variation, and the type of effective immune responses, to name a few.

Comparison of the amino acid sequence encoded by the conserved regions of the taeniid genes further indicates that both *c-fos* and *c-jun* proteins are highly conserved in relation to other parasite genes. Members of the *c-fos* and *c-jun* gene family have two exons encoding two DNA binding domains that are alternatively spliced, producing transcripts that encode proteins having either one or two binding protein domains, which is further indicative that these are transcription factors proteins. Elucidation of features common to the genes encoding these proteins will lead to a better understanding of the role that they play in the parasites' biology.

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