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Short communication

Differentially expressed sequences from a cestode parasite reveals conserved developmental genes in platyhelminthes☆

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Cestodes are the etiological agents of major parasitic diseases both in humans and in domesticated animals [1,2]. Despite the considerable attention received by some disease-causing cestode species, such as *Echinococcus* spp., and *Taenia* spp., little is known about the molecular biology of the developmental transition from larval forms into adult parasites. In the light of the recently proposed metazoan phylogenies, platyhelminthes have a much more derived condition than previously thought, being placed within the lophotrocozoan branch [3,4]. In this context, inclusion of data from the currently neglected cestode strobilation phenomena would offer a more complete picture on the extent of evolutionary conservation of developmental mechanisms in bilaterian metazoans.

As a first step toward this aim, we are using *Mesocestoides corti* as a model organism to study the development of the cestode strobilar stage. The research potential of *M. corti* has already been recognized [5–7]. We have improved culture conditions that induce larvae (tetrathyridia) to differentiate into strobilated worms [8] and are currently conducting a morphological and histological analysis of *M. corti* in vitro strobilation (unpublished observations).

Here, we have adapted the cDNA representational difference analysis technique [9], which enables the isolation of genes with an altered expression between tissues or cell samples, to isolate differentially expressed sequences between tetrathyridia and strobilated forms obtained from in vitro cultures. First strand cDNA was synthesized using the SMART^{TM} PCR cDNA Synthesis kit (Clontech Inc.) with 200U of superscript II RNAse H^{-} (Invitrogen Life Technologies) and the PCR primer (5′-AAGCAGTGGTAACAACGCAGAGT-3′), which allowed us to start the libraries with only 1 μg of total RNA. The amplified cDNAs were digested with *Sau*3AI and then subjected to cDNA RDA as previously described [10]. In the Forward library, cDNAs from segmented worms were used as tester and cDNAs from tetrathyridia as drivers. In the Reverse library, tetrathyridia cDNAs were used as tester and cDNAs from segmented worms as drivers. *M. corti* RDA-subtracted cDNA libraries were constructed after two rounds of subtraction, using a driver:tester ratio of 100:1 and 800:1 in the first and second rounds, respectively.

As a first approach to verify the efficiency of cDNA subtraction in both libraries, we used the second differential products (DP2) from both the Forward and the Reverse cDNA fragment pools as probes against the SMART cDNA synthesis products of tetrathyridia and segmented worms (Fig. S1—Supplemental Material) The DP2 Reverse probe

Abbreviations: RDA, representational difference analysis; RT-PCR, reverse transcription polymerase chain reaction

☆ *Note*: Nucleotide sequence data reported in this paper are available in the GenBank^{TM}, EMBL and DDBJ databases under the acession numbers CX863392–CX865174.

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hybridized only to SMART cDNA synthesis products from tetrathyridia, suggesting an enrichement for tetrathyridia-specific sequences, while DP2 Forward probe hybridized with both SMART cDNA products, suggesting a more limited subtraction efficiency. For an additional evaluation, we prepared cDNA macroarrays with clones from both libraries and hybridized with probes prepared from the non-subtracted cDNA synthesis products from larval and segmented stages (Fig. 1A). Indeed, spots of PCR-products from Reverse library-derived clones hybridized more intensely with the non-subtracted tetrathyridia cDNA probe, as expected. However, the opposite was not true when the hybridization signals from the Forward library clones were compared. Unexpectedly, it was possible to distinguish a set of high intensity Forward library spots displaying a stronger hybridization signal with the tetrathyridia-derived cDNA probe (see Fig. 1A-a and A-c). Interestingly, a considerable amount of the total cDNA synthesis reactions corresponds to a major unidentified band, which is even more abundant in the cDNA from segmented worms used as a probe (Fig. 1A-b and A-d). Therefore, considering that the probes were normalized by their specific activities (cpm/μg), the majority of the cDNA species in the cDNA probe from segmented worms are under-represented in relation to the cDNA probe from tetrathyridia. Therefore, we reasoned that the high intensity spots could represent non-differentially expressed sequences contaminating the DP2 Forward library. To address this question, Forward library selected clones were individually tested in virtual northern blot experiments using amplicons (after R-adapter ligation) of *Sau*3A-digested Smart cDNA synthesis products as probes (Fig. 1B), which are devoid of the above-mentioned prominent band, since it is not cleaved by *Sau*3AI (see Fig. 1B). Two clones from the set of sequences presenting high intensity spots as non-differentially expressed candidates and another four clones as differentially expressed candidates were selected and the virtual Northern blot results obtained with these probes confirmed the macroarray hybridization pattern.

To compare the transcripts present in both developmental stages, we sequenced randomly selected clones from both libraries (Tables S1–S3—Supplemental Material). We have generated 1363 reads from the Forward library and 601 reads

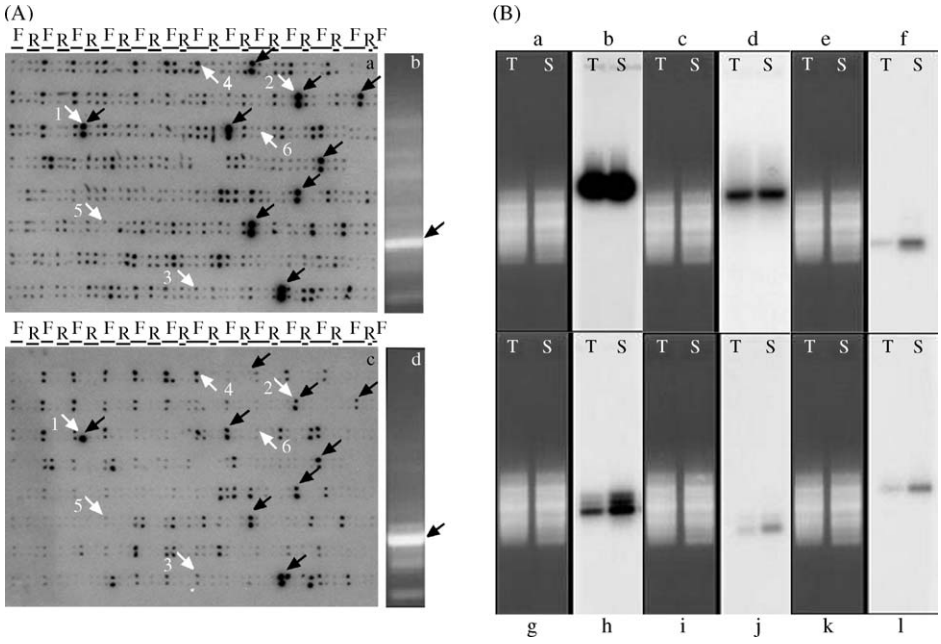

Fig. 1. (A) Macroarrays of PCR-amplified fragments from DP2 Forward and DP2 Reverse clones. A total of 248 randomly selected clones from DP2 Forward (F) and 136 clones from DP2 Reverse (R) libraries were amplified by PCR, and the amplicons were spotted, as duplicates, onto two nitrocellulose membrane replicas (a and c), resulting in 768 spots per membrane. The transferred PCR products were hybridized with radioactively labeled non-subtracted SMART cDNA synthesis products from tetrathyridia (a) or from segmented worms (c). The SMART cDNA synthesis products from tetrathyridia (b) and segmented worms (d) are depicted after agarose gel electrophoresis fractionation. The white arrows (a and c) point to spots from the DP2 Forward library corresponding to clones which were selected for virtual Northern blot analysis, presumed to be non-differential contaminants (1 and 2) or differentially expressed sequences (3–6). Black arrows (a and c) depict DP2 Forward PCR products duplicates that presented a stronger signal upon hybridization with tetrathyridia cDNA probe than with segmented worms cDNA probe (see text for details). Black arrows (b and d) depict a highly expressed major band more abundant in the segmented worm cDNA synthesis products than in the tetrathyridia cDNA products. (1) poly(A) binding protein (PABP); (2) apoptotic PDCD4-related sequence; (3) deoxynucleoside kinase; (4) 26S proteasome component; (5) annexin; (6) SET/TAF-1/PP2A inhibitor. (B) Virtual Northern blot analysis of selected clones from the DP2Forward library. After R-adapter ligation, the amplicons of *Sau*3A-digested Smart cDNA synthesis products from tetrathyridia (T) and segmented worms (S) were fractionated by agarose gel electrophoresis in six replicas (a, c, e, g, i and k), transferred to nitrocellulose membranes, and hybridized with radiolabeled probes prepared from sequences whose expression was evaluated (after macroarray analysis) to be non-differential (b and d) or augmented (f, h, j, and l) in segmented worms. (b) poly(A) binding protein (PABP); (d) apoptotic PDCD4-related sequence; (f) deoxynucleoside kinase; (h) 26S proteasome component; (j) annexin; (l) SET/TAF-1/PP2A inhibitor.