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ORIGINAL PAPER

Characterization of export receptor exportins (XPOs) in the parasite *Schistosoma mansoni*

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Abstract Several proteins and different species of RNA that are produced in the nucleus are exported through the nuclear pore complexes, which require a family of conserved nuclear export receptors called exportins (XPOs). It has been reported that the XPOs (XPO1, XPO5, and XPOT) are directly involved in the transport processes of noncoding RNAs from the nucleus to the cytoplasm and/or from cytoplasm to the nucleus. All three genes are present in fungi, plants, and deuterostome metazoans. However, protostome metazoan species lack one of the three genes across evolution. In this report, we have demonstrated that all three XPO proteins are present in the parasite protostome *Schistosoma mansoni*. As this parasite has a complex life cycle presenting several stages in different hosts and environments, implying a differential gene regulation, we proposed a genomic analysis of XPOs to validate their annotation. The results showed the conservation of exportin family members and gene duplication events in *S. mansoni*. We performed quantitative RT-PCR, which revealed an upregulation of *Sm*XPO1 in 24 h schistosomula (sixfold when compared with cercariae), and similar transcription levels were observed for *Sm*XPO5 and *Sm*XPOT in all the analyzed stages. These three XPO proteins have been identified for the first time in the protostome clade, which suggests a higher complexity in RNA transport in the parasite *S. mansoni.* Taken together, these results suggest that RNA transport by exportins might control cellular processes during cercariae, schistosomula, and adult worm development.

Introduction

Schistosomes have a complex life cycle, alternating passages into two independent hosts and exhibiting significant morphological and physiological differences. For the completion of the parasite life cycle, the regulation of gene expression, especially at the transcriptional and post-transcriptional levels, is extremely important (Han et al. 2009).

During transcription in *Schistosoma mansoni*, mRNA capping, splicing, and polyadenylation events have been reported (Protasio et al. 2012). However, the mechanisms regulating the nuclear export of RNAs remain poorly understood. Different RNA species produced in the nucleus are exported through the nuclear pore complexes by export receptors (also known as karyopherins or importin-β family members). This nucleocytoplasmic transport occurs by various mechanisms: small RNAs (tRNAs and microRNAs) directly bind to export receptors, whereas large RNAs (ribosomal RNAs and mRNAs) have a more complex mechanism (Köhler and Hurt 2007). Furthermore, nuclear export is an important step in quality control, as faulty or unprocessed mRNAs are not

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only useless but potentially harmful if translated in the cytoplasm (Lacker and Bahler 2008). Only functional mRNAs are exported into the cytoplasm, and this quality control step is closely coupled to RNA processing and the ribonucleoprotein composition. The family of nuclear receptors has several members that share a common characteristic: their regulation by the small Ran GTPase. Exportins bind nuclear cargo only, and together with RanGTP, this complex is translocated to the cytoplasm, where it dissociates upon hydrolysis of RanGTP by RanGAP and releases the cargo (Köhler and Hurt 2007). To date, eight exportins have been characterized: exportin 1 (XPO1), exportin 2 (XPO2), exportin 3 (XPO3), exportin 4 (XPO4), exportin 5 (XPO5), exportin 6 (XPO6), exportin 7 (XPO7), and exportin T (XPOT) (Lipowsky et al. 2000; Stuven et al. 2003; Mingot et al. 2004).

XPO1 is the major receptor for the export of proteins from the nucleus and is also required for the transport of many RNAs (rRNAs, snRNAs, and several specific mRNAs) (Hutten and Kehlenbach 2007). mRNA export, in general, is mediated by the export receptor Tip-associated protein, which is not a protein of the importin-β superfamily (Fried and Kutay 2003). However, it has been shown that export of a certain subset of mRNAs is mediated by XPO1 (Schütz et al. 2006). XPO5 is involved in a different nucleocytoplasmic transport pathway that includes nuclear export of microRNA (miRNA) precursors (pre-miRNAs), which is a rate-limiting step in miRNA biogenesis (Yi et al. 2005). Recent reports have shown that inactivation of XPO5 results in nuclear accumulation of pre-miRNAs and perturbation of gene expression (Leisegang et al. 2012). Recently, it has been suggested that XPO1 also participates in the transport of miRNAs in the opposite direction from the cytoplasm to the nucleus in *Drosophila melanogaster* and *Caenorhabditis elegans* (Castanotto et al. 2009; Bussing et al. 2010). It has been shown that XPO5 and XPO1 proteins participate in the transportation process of miRNAs, but until now, no evidence that indicates that endo-siRNAs are transported from the nucleus to the cytoplasm has been found (Bussing et al. 2010; Castanotto et al. 2009; Kim et al. 2009; Nilsen 2008). XPOT is responsible for the nuclear export of tRNAs in eukaryotes (Rodriguez et al. 2004) and recognizes features that are structurally conserved among tRNAs (Cook et al. 2009). In yeast, an XPOT homolog, Los1p, has been shown to display a similar function (Hellmuth et al. 1998).

XPO1, XPO5, and XPOT have been reported in fungi, plants, and deuterostome metazoans. However, the protostome metazoans such as nematodes and arthropods have lacked one of the three proteins across their evolution, suggesting that possibly all protostomes have specialized in shifting the RNA specificity from the missing XPO protein to the two existing ones in order to sustain the normal exportation and importation process into the cells (Murphy et al. 2008). However, in this study, we demonstrate the presence of all three of these XPO genes, XPOT, XPO5, and XPO1, in the protostome parasite *S. mansoni* genome (*Sm*XPO1, *Sm*XPO5, and *Sm*XPOT) using bioinformatics approaches. In addition, we performed quantitative RT-PCR to compare the relative levels of mRNA transcription in cercariae mechanically transformed schistosomula cultivated from 3.5 to 72 h, adult worms, eggs, and miracidium. Thus, the presence of these three transcripts and the expression profile of the XPOs in all the stages investigated suggest that these proteins might have a role in the control of gene expression during the life cycle of *S. mansoni*.

Fig. 1 Evolutionary distribution of *Sm*XPO1, *Sm*XPO5, and *Sm*XPOT ▶ proteins: Consensus phylogenetic tree based in amino acid residues of proteins XPO1, XPO5, and XPOT. Bootstrap tests were performed to assess the reliability of branches in the consensus tree with 2,000 replicates, and bootstrap proportions of ≥50 % were considered to be reliable. The tree showed three distinct clades (separated by *brackets*), one for each group of orthologous proteins. Proteins *highlighted* in *gray box* represent the proteins identified in this work. The tree construction and "bootstrap" analysis were performed in MEGA 4.0

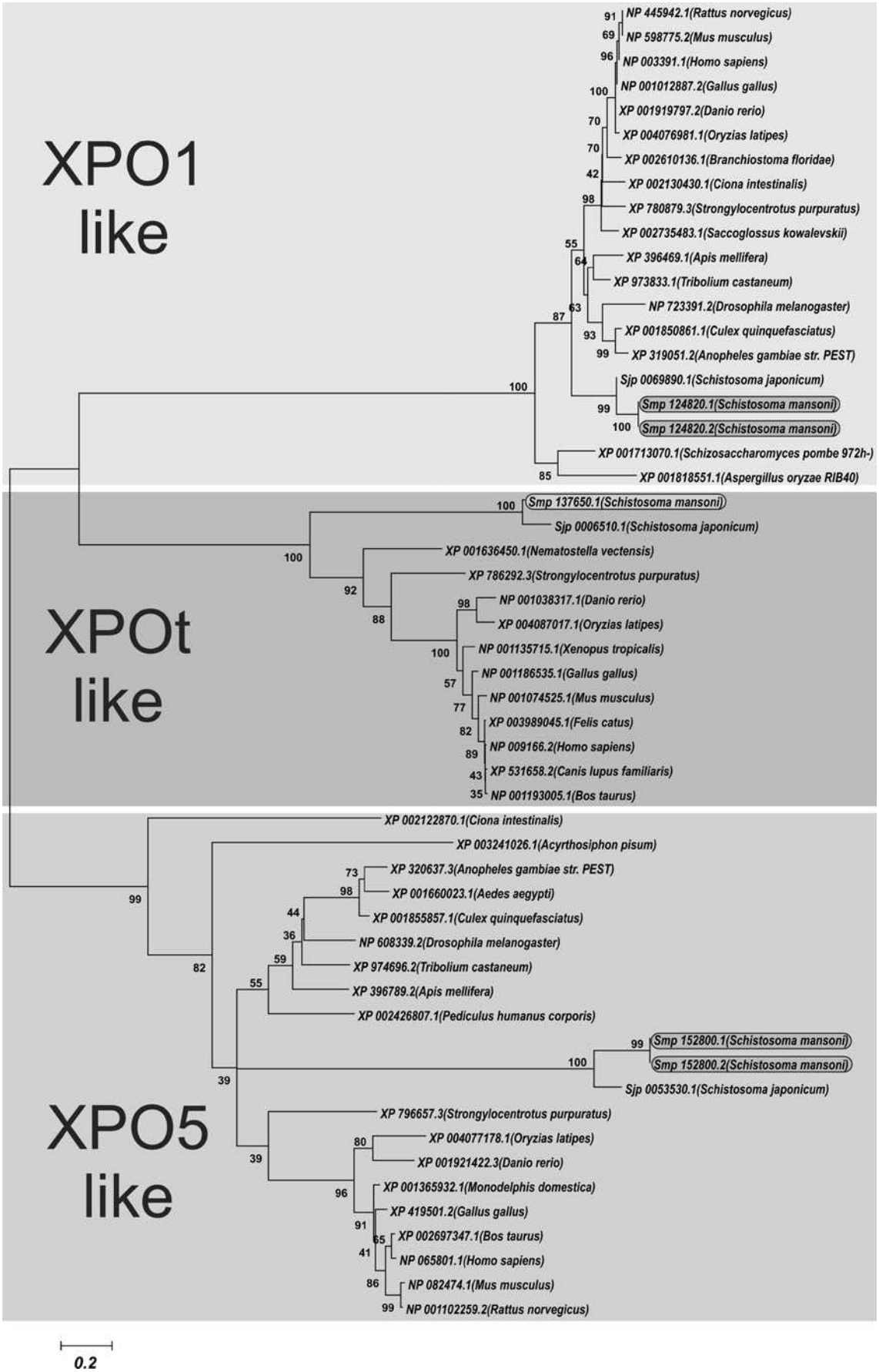
Materials and methods

Ethics statement

All experiments were authorized by the Ethical Committee for Animal Care of University of OuroPreto and were in accordance with the national and international regulation accepted for laboratory animal use and care.

Parasites

S. mansoni parasite (LE strain) was maintained by routine passage through *Biomphalaria glabrata* snails and BALB/c mice. The infected snails were induced to shed cercariae under light exposure for 2 h, and the cercariae were recovered by sedimentation on ice. Adult worm parasites were obtained by liver perfusion of mice after 50 days of infection. Mice livers were triturated with trypsin and incubated for 2.5 h, and the eggs were recovered by saline wash. To obtain miracidium samples, eggs were placed in water under light exposure and hatched miracidia were recovered in the saline solution. The mechanically transformed schistosomula (MTS) were prepared as described by Harrop and Wilson (1993). Briefly, cercariae were recovered and washed in RPMI 1640 medium (Invitrogen, Sao Paulo, Brazil) before vortexing at maximum speed for 90 s and immediately cultured for 3.5 h at 37 °C in a 5 % CO_2 incubator. Then, the recovered schistosomula were washed with RPMI 1640 until no tails were detected. For subsequent incubations, the parasites were maintained in M169 medium supplemented with 10 % fetal bovine serum,


XPO1 like
XPOt like
XPO5 like
NP 445942.1(Rattus norvegicus)
NP 598775.2(Mus musculus)
NP 003391.1(Homo sapiens)
NP 001012887.2(Gallus gallus)
XP 001919797.2(Danio rerio)
XP 004076981.1(Oryzias latipes)
XP 002610136.1(Branchiostoma floridae)
XP 002130430.1(Ciona intestinalis)
XP 780879.3(Strongylocentrotus purpuratus)
XP 002735483.1(Saccoglossus kowalevskii)
XP 396469.1(Apis mellifera)
XP 973833.1(Tribolium castaneum)
NP 723391.2(Drosophila melanogaster)
XP 001850861.1(Culex quinquefasciatus)
XP 319051.2(Anopheles gambiae str. PEST)
Sjp 0069890.1(Schistosoma japonicum)
Smp 124820.1(Schistosoma mansoni)
Smp 124820.2(Schistosoma mansoni)
XP 001713070.1(Schizosaccharomyces pombe 972h-)
XP 001818551.1(Aspergillus oryzae RIB40)
Smp 137650.1(Schistosoma mansoni)
Sjp 0006510.1(Schistosoma japonicum)
XP 001636450.1(Nematostella vectensis)
XP 786292.3(Strongylocentrotus purpuratus)
NP 001038317.1(Danio rerio)
XP 004087017.1(Oryzias latipes)
NP 001135715.1(Xenopus tropicalis)
NP 001186535.1(Gallus gallus)
NP 001074525.1(Mus musculus)
XP 003989045.1(Felis catus)
NP 009166.2(Homo sapiens)
XP 531658.2(Canis lupus familiaris)
NP 001193005.1(Bos taurus)
XP 002122870.1(Ciona intestinalis)
XP 003241026.1(Acyrthosiphon pisum)
XP 320637.3(Anopheles gambiae str. PEST)
XP 001660023.1(Aedes aegypti)
XP 001855857.1(Culex quinquefasciatus)
NP 608339.2(Drosophila melanogaster)
XP 974696.2(Tribolium castaneum)
XP 396789.2(Apis mellifera)
XP 002426807.1(Pediculus humanus corporis)
Smp 152800.1(Schistosoma mansoni)
Smp 152800.2(Schistosoma mansoni)
Sjp 0053530.1(Schistosoma japonicum)
XP 796657.3(Strongylocentrotus purpuratus)
XP 004077178.1(Oryzias latipes)
XP 001921422.3(Danio rerio)
XP 001365932.1(Monodelphis domestica)
XP 419501.2(Gallus gallus)
XP 002697347.1(Bos taurus)
NP 065801.1(Homo sapiens)
NP 082474.1(Mus musculus)
NP 001102259.2(Rattus norvegicus)
0.2

penicillin (100 μg/mL), streptomycin (100 μg/mL), and 5 % of Schneider's medium (Basch and DiConza 1977) at 37 °C in a 5 % CO_2 incubator for 3.5, 24, 48, and 72 h.

Identification and computational analysis of exportins

Sequences of exportin genes were searched through the *S. mansoni* genome database version 5.0 from GeneDB (http://www.genedb.org/genedb/smansoni/) and the *S. mansoni* transcriptome project (http://verjo18.iq.usp.br/schisto/) using orthologs from *D. melanogaster*, *C. elegans*, and *Homo sapiens* as queries. The BLASTp algorithm, underpinned by the Pfam (v26.0) database, was used for searches of conserved protein domains or motifs from *S. mansoni* sequences.

Multiple alignments of SmXPO1.1, *Sm*XPO1.2, *Sm*XPO5.1, *Sm*XPO5.2, and *Sm*XPOT were performed by ClustalX 2.0 (Larkin et al. 2007). Phylogenetic analyses were conducted in MEGA 5 (Kumar et al. 2004). Phylogenetic tree of these sequences were inferred using the neighbor-joining method under the JTT model (Saitou and Nei 1987). The bootstrap consensus tree inferred from 2,000 replicates was used to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 30 % bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2,000 replicates) is shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated from the dataset.

Expression analysis of exportins

The total RNA samples from cercariae, schistosomula, adult worms, eggs, and miracidium were obtained using a combination of Trizol reagent (Gibco, Sao Paulo, Brazil) and chloroform for extraction and then purified on column using the "Nucleo Spin® RNA II Kit"(Macherey-Nagel, Belo Horizonte, Brazil). The preparation was treated with RNase-free DNase I. RNA was quantified using a spectrophotometer, and the cDNA were obtained using 1 μg of total RNA by reverse transcription using the "High Capacity cDNA Reverse Transcription Kit"(Applied Biosystems, Sao Paulo, Brazil). Primers for the predicted sequences were designed using the program Vector NTI (Invitrogen) and GeneRunner®. The sequence accession numbers and their pair of primers are in the supplementary table (Table S1).

Reverse-transcribed cDNA samples were used as templates for PCR amplification using SYBR Green Master Mix UDG-ROX® (Invitrogen) and 7300 Real Time PCR System (Applied Biosystems). Specific primers for the *S. mansoni* eIF4E gene were used as an endogenous control (GeneDB ID: Smp_001500) (forward 5′TGTTCCAACCACGGTCTCG3′, reverse 5′TCGCCTTCCAATGCTTAGG3′) (Liu et al. 2012). The efficiency for each pair of primers was evaluated according to the protocol developed by the Applied Biosystems application (cDNA dilutions were 1:10, 1:100, and 1:1,000). For the investigated transcripts, three biological replicates were performed and their gene expressions were normalized against the eIF2B transcript according to the $2^{-\Delta Ct}$ method using the Applied Biosystems 7300 software (Livak and Schmittgen 2001).

Statistical analysis

The statistical analyses were performed using GraphPad Prism version 5.0 software package (Irvine, CA, USA). Normality of the data was established using one-way analysis of variance. Tukey post hoc tests were used to investigate significant differential expression of transcripts throughout the investigated stages. The differences were considered significant when p values were <0.05.

Results

Conservation of exportins in *S. mansoni*

Silico analysis identified three members of exportin family and the presence of two spliced forms for XPO1 and two for XPO5 (*Sm*XPO1.1, *Sm*XPO1.2, *Sm*XPO5.1, *Sm*XPO5.2, and *Sm*XPOT). Phylogenetic analysis showed conservation among orthologs, suggesting a conservation of mechanisms for RNA transport (Fig. 1). Our data revealed that *S. mansoni* exportins were very similar to those found in the protostomes *C. elegans* and *D. melanogaster.* A conservation of the exportin proteins was observed across deuterostome and protostome organisms such as *C. elegans*, *D. melanogaster*, and *H. sapiens*, indicating a high degree of similarity (Fig. 2).

Expression profiles of exportins in different stages

The gene expression analysis of *Sm*XPO1, *Sm*XPO5, and *Sm*XPOT was performed using quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR). For the analysis of each gene, three biological replicates were used. The results were expressed by normalizing the RNA levels to the levels of the constitutively expressed eIF4E gene (Fig. 3). For the spliced forms *SmXPO1.2* and *SmXPO5.2*, we did not obtain specific primers to analyze their expression profiles and postulated that there could be possible errors in the gene data annotation. The results showed that all XPOs were expressed more in the schistosomula stage, and we

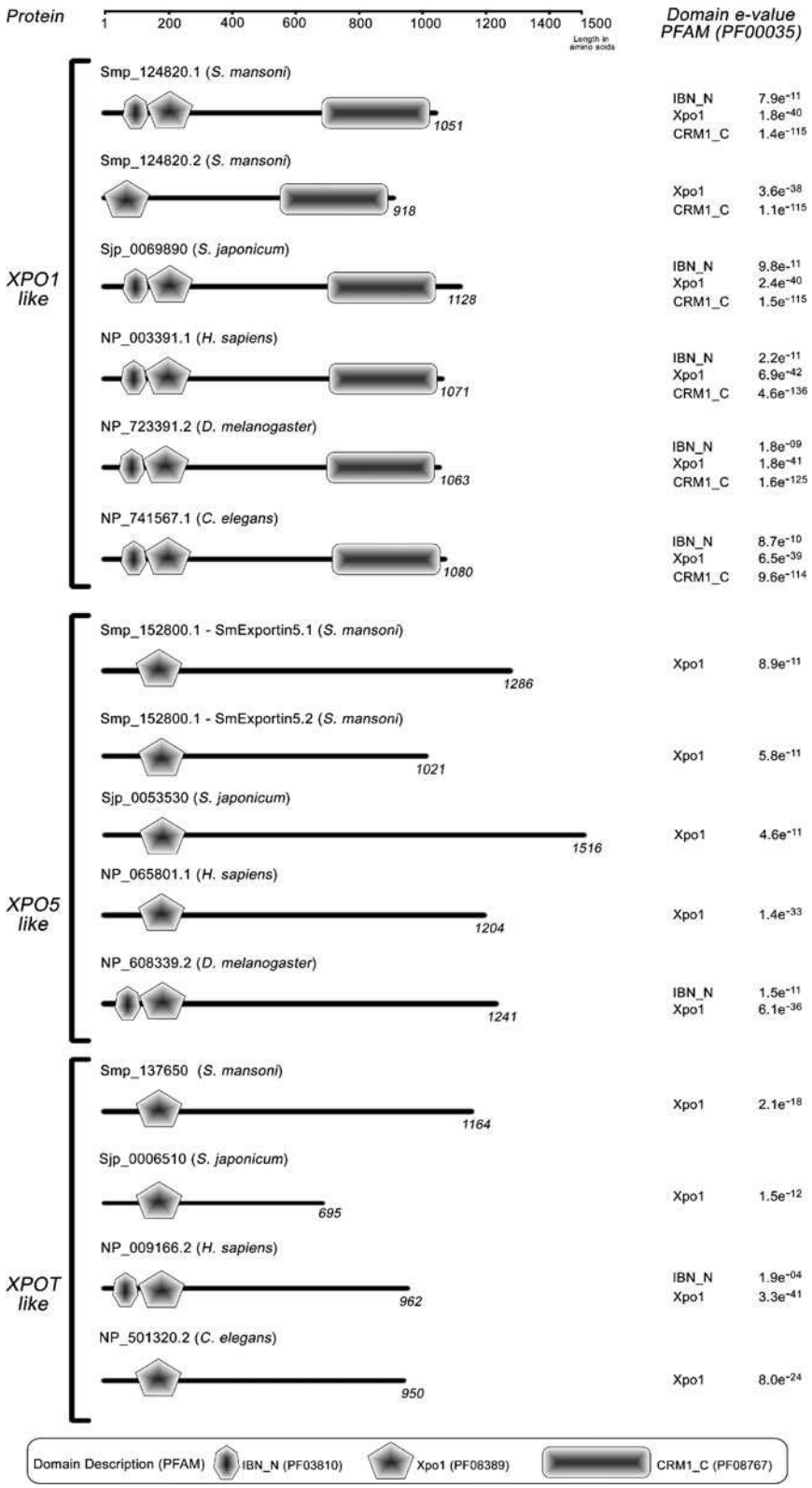


Fig. 2 Evolutionary conservation of *Sm*XPO1, *Sm*XPO5, and *Sm*XPOT domains: The orthologous proteins *Sm*XPO1, *Sm*XPO5, and *Sm*XPOT found in *D. melanogaster, C. elegans*, *H. sapiens*, and those identified in *S. mansoni* were compared according to their disposition and presence of conserved domains in the primary structure. The domain descriptions were represented in the structure by polygons as indicated in the figure, as well by PFAM numbers. The length of each protein is indicated at the end of each sequence, and the comparative values of each domain is shown in the *right hand side* of the sequences

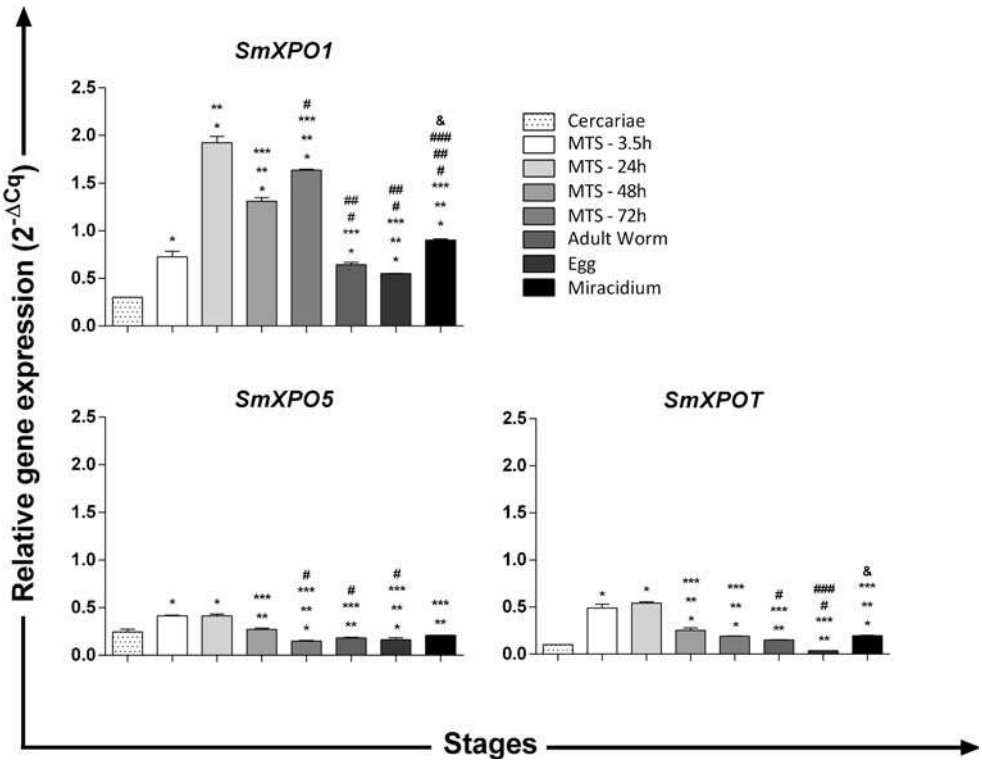


Fig. 3 Expression profiles of *Sm*XPO1 through *S. mansoni* life cycle: The *SmXPO1*, *SmXPO5*, and *SmXPOT* mRNA expression levels were measured, based on three replicates, in the following stages: cercariae, MTS-3.5, 24, 48, and 72 h, adult worms, eggs, and miracidium using quantitative RT-PCR. Expression levels were calibrated according to the comparative $2^{-\Delta Ct}$ method using the constitutively expressed eIF4E gene as an endogenous control (one-way variance analysis followed by Tukey pairwise comparison $p<0.05$). *Different from cercariae, **different from MTS-3.5 h, ***different from MTS-24 h, #different from MTS-48 h, ##different from MTS-72 h, ###different from adult worms, &different from eggs, and &&different from miracidium

observed an upregulation of *SmXPO1* in schistosomula compared with cercariae after 24, 48, and 72 h.

Discussion

XPO1, XPO5, and XPOT homologs have been reported in animals, fungi, and plants. An exception has been shown in protostome organisms such as nematodes and arthropods, which lose one of the three exportins during the course of evolution (XPO5 or XPO1). A recent report suggests that due to evolutionary loss in protostome organisms, exportin proteins undergo specialization to maintain the normal process of export and import in the cells (Murphy et al. 2008). Interestingly, we identified three proteins in *S. mansoni* which correspond to the following exportins: XPO1 (*Sm*Exportin1.1 and *Sm*Exportin1.2), XPO5 (*Sm*Exportin5.1 and *Sm*Exportin5.2), and XPOT (*Sm*ExportinT). The identification of these three XPOs have been described for the first time in protostome clade, suggesting a higher complexity in RNA transport in the parasite *S. mansoni.*

Phylogenetic analysis was employed using protein orthologs previously identified in protostomes and deuterostomes. The analysis revealed three different groups of exportins (XPO1, XPO5, and XPOT), and those putative proteins matched with the respective protein orthologs in ecdysozoans and lophotrochozoans such as *D. melanogaster, C. elegans*, and *Schistosoma japonicum*. We observed that there is more evolutionary proximity among proteins in the XPO1 and XPOT groups when compared with their respective paralogs. These results corroborate with the report from Murphy et al. (2008), which showed a higher proximity between the XPO1 and XPOT orthologs when compared with their XPO5 paralogs in animals. The similarity of the exportins identified in *S. mansoni* to its orthologs was also analyzed by domain conservation. The conserved domains of XPO1, XPO5, and XPOT revealed high degrees of similarity among the identified parasite proteins and its orthologs in *C. elegans*, *D. melanogaster*, and *H. sapiens,* suggesting a conserved mechanism for exporting different RNA species through nuclear pore complexes.

The transport of molecules from nucleus to cytoplasm and the intracellular conditions are crucial for gene expression in eukaryotic cells. The transport through nuclear pore complexes requires a family of conserved nuclear transport receptors, which recognize a short signal peptide on a cargo protein or motifs in RNA cargoes (Köhler and Hurt 2007). In this work, the transcript levels measured by qRT-PCR showed that all exportins are expressed in the different stages of the *S. mansoni* life cycle, suggesting an important role of exportins in transporting proteins and different RNAs. A comparative study of transcriptome profiles of mechanically and skin-transformed schistosomula showed that these two preparations differ only in the expression of 38 genes (out of ~11,000) and provided evidence that MTS is a good substitute for the natural skin-transformed ones (Berriman et al. 2013). As the exportin genes

are not in the list of 38 genes that differ in expression, our MTS preparations generated reliable results of expression. However, we did not obtain specific primers to evaluate the expression profiles for the spliced forms of *Sm* XPO1 and *Sm* XPO5 (*Sm* XPO1.2 and *Sm* XPO5.2). *Sm* XPO1.2 differs from *Sm* XPO1.1 because the first domain is absent in the beginning of the IBN_N domain, and *Sm* XPO5.2 differs from *Sm* XPO5.1 by 265 amino acids. We postulate that if there are errors in the gene annotation of these two isoforms or if molecular mechanisms, such as trans-splicing, occurred to generate the second isoform, then it would be difficult to distinguish one isoform from the other using specific primers.

Our results showed that XPO1 is the most expressed export receptor in all the parasite life cycle stages compared with XPO5 and XPOT, implying that XPO1 is the most versatile of all nuclear export receptor in *S. mansoni*. Whereas XPO5 and XPOT are involved in the export of microRNAs and tRNAs, respectively, XPO1 has an important role in the transport of several proteins with leucine-rich nuclear export signals, snRNAs involved in the mechanism of splicing, rRNA subunits and specific mRNAs (Nakielny and Dreyfuss 1999). Also known as Chromosomal Maintenance 1, XPO1 was originally identified in yeast as a protein required to maintain higher order chromosome structure and was also found to play a role in centrosome duplication and spindle assembly, especially in response to DNA damage (Nguyen et al. 2012). The transformation of the cercariae to the next developmental stage, the schistosomula, is accompanied by a series of specific biochemical and morphologic adaptations that rapidly prepare the parasite for survival in a new environment, and this transformation might seem the ideal point to observe gene regulation events (Blanton and Licate 1992). Studies have shown an apparent increase of protein synthesis following transformation that might involve an alteration in protein synthesis. The level of protein synthesis during the first 3 h after transformation is low (Nagai et al. 1977; Yuckenberg et al. 1987), and it is followed by an increase in schistosomula during the first 24 h after transformation (Blanton and Licate 1992). Our results corroborate with these findings, as we observed a sixfold upregulation of *Sm* XPO1 in 24 h schistosomula compared with cercariae. These results suggest a higher requirement of this receptor for transport of proteins and RNAs due to the increase of protein levels in 24 h schistosomula and reflect an alteration in gene expression in the cercariae–schistosomula transformation.

In *S. mansoni*, it was suggested that the regulation of gene expression by post-transcriptional modification occurred by blocking translation during the cercariae to schistosomula transformation (Blanton and Licate 1992). Furthermore, the fact that miRNA pathway components (*Sm* Dicer1 and *Sm* Ago2/3/4) are present and are differentially expressed during schistosomula development (Gomes et al. 2009) led us to focus on the exportins. Encoded in the genome of most eukaryotes, miRNAs have been proposed to specifically regulate up to 90 % of human genes (Perron and Provost 2008). Furthermore, the machinery of RNA silencing mediated by miRNA is dependent on several proteins (Song and Joshua-Tor 2006). In the initiation stage, the primary miRNA is transcribed by a type II RNA polymerase and is cleaved into pre-miRNA by RNase III Drosha (Lee et al. 2003). Next, the pre-miRNA is transported actively, with the expenditure of GTP, from the nucleus to the cytoplasm, using the Ran-GTP/Exportin-5 complex. Two proteins have been reported to be responsible for such transport: XPO5 and XPO1. The pre-miRNA, after transportation to the cytoplasm, is processed by the Dicer enzyme, a protein coupled with double-stranded RNA linker, into miRNA which leads to RNA silencing (Saito et al. 2005). In this report, we showed that XPO5 is expressed in all life cycle stages analyzed and considering that miRNAs account for ~1 % of the genome (Köhler and Hurt 2007), pre-miRNAs are likely to be the main cargos for XPO5. Furthermore, RNAi against XPO5 in HeLa cells resulted in a reduction in the concentration of let-7a miRNA precursor, supporting the evidence that XPO5 is important for miRNA biogenesis (Lund et al. 2004). Moreover, RNAi of XPO5 did not result in nuclear accumulation of pre-miRNA, suggesting that pre-miRNAs are stabilized by XPO5 (Yi et al. 2003). Studies that show similar amounts of mRNA in cercariae and schistosomula, where there is no increase in mRNA levels and the increase in protein level correlates to mRNA availability (Blanton and Licate 1992), indicate that there is a post-transcriptional gene regulation in *S. mansoni.* As miRNAs can bind to the 3′-UTR region in mRNAs, either inhibiting translation or leading to mRNA degradation, we propose that these small noncoding RNAs are one way of blocking the translation of mRNAs in cercariae and this translational block is gradually lifted following the transformation to schistosomula.

Approximately, 40 different tRNAs exist in eukaryotic cells and the tRNA export pathway is targeted by XPOT. An XPOT homolog in *Saccharomyces cerevisiae*, Los1p, binds directly to tRNA (Arts et al. 1998). In higher eukaryotes, splice-end trimming modifications and amino-acylation appear to ensure that nonfunctional tRNAs do not obtain into the cytoplasm. Amino-acylation produces charged tRNAs that are exported more efficiently than uncharged ones (Arts et al. 1998; Lund and Dahlberg 1998), which can affect the affinity of the tRNA–exportin interaction. The nuclear export signals in the tRNAs are not linear motifs, but they are coded in secondary and tertiary structural elements and properly processed 5′ and 3′termini, which suggests that XPOT can monitor the correct structural integrity of tRNAs before export (Lipowsky et al. 1999; Arts et al. 1998). Our data shows significant expression profiles of *Sm* XPOT indicating that this important tRNA export pathway is present in *S. mansoni*. Further, although XPO5 is thought to be an auxiliary export

receptor for tRNAs, it has been shown that the main role of XPO5 is to export microRNAs (Calado et al. 2002; Bohnsack et al. 2002).

In this work, against the suggestion that nematodes, arthropods, and possibly all protostome species lack one of the XPO components adapting the RNA specificity transport (Murphy et al. 2008), we identified, analyzed, and characterized all three XPO putative proteins in *S. mansoni* parasite, a protostome species. The characterization and validation of exportins in *S. mansoni* led us to conclude that these conserved nuclear export receptors are important for driving the nuclear protein and RNA export in the parasite. The major export receptor, XPO1, seems to be essential for coordinating the transport of most RNAs, transcription factors, translation factors, and other proteins, indicating that it contributes to the gene regulation in *S. mansoni*. Furthermore, the different gene expression and protein levels during cercariae–schistosomula transformation may be related to RNA and protein transport along with the mechanisms of post-transcriptional gene regulation that can involve translation inhibition by miRNAs. More studies are needed to accumulate data that can support all the mechanisms and factors that are involved in the alteration of gene expression from cercariae to schistosomula transformation. These findings might be important to clarify the biological function of all three XPO genes in *S. mansoni* genome and also in their respective RNA pathways and may open a new avenue for the understanding of the schistosomiasis disease.

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