Ubiquitin-specific proteases are differentially expressed throughout the *Schistosoma mansoni* life cycle

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**Abstract**

**Background:** The ubiquitination process can be reversed by deubiquitinating enzymes (DUBs). These proteases are involved in ubiquitin processing, in the recovery of modified ubiquitin trapped in inactive forms, and in the recycling of ubiquitin monomers from polyubiquitinated chains. The diversity of DUB functions is illustrated by their number and variety of their catalytic domains with specific 3D architectures. DUBs can be divided into five subclasses: ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs or UBPs), ovarian tumour proteases (OTUs), Machado-Joseph disease proteases (MJDs) and JAB1/MPN/Mov34 metalloenzymes (JAMMs).

**Methods:** Considering the role that the ubiquitin-proteasome system has been shown to play during the development of *Schistosoma mansoni*, our main goal was to identify and characterize SmUSPs. Here, we showed the identification of putative ubiquitin-specific proteases using bioinformatic approaches. We also evaluated the gene expression profile of representative USP family members using qRT-PCR.

**Results:** We reported 17 USP family members in *S. mansoni* that present a conservation of UCH domains. Furthermore, the putative SmUSP transcripts analysed were detected in all investigated stages, showing distinct expression during *S. mansoni* development. The SmUSPs exhibiting high expression profiles were SmUSP7, SmUSP8, SmUSP9x and SmUSP24.

**Conclusion:** *S. mansoni* USPs showed changes in expression levels for different life cycle stages indicating their involvement in cellular processes required for *S. mansoni* development. These data will serve as a basis for future functional studies of USPs in this parasite.

**Keywords:** Ubiquitination, Deubiquitination, Deubiquitinating enzymes (DUBs), Differential expression, Schistosome development

**Background**

The covalent modification of proteins by the addition or removal of ubiquitin, a highly conserved protein comprising of 76 amino acids, changes the molecular function of the target protein and can therefore influence its interactions with other proteins. In turn, these interactions can regulate many biological processes, including DNA repair, cell-cycle control, endocytosis, transcription and protein degradation by the proteasome [1–4].

Deubiquitinating enzymes (DUBs) catalyse the removal of ubiquitin from ubiquitin-conjugated proteins and from its precursor proteins [5, 6]. DUBs can be divided into five subclasses, of which four are cysteine proteases and one comprises a group of metalloproteases. They are named as ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs or UBPs), ovarian tumour proteases (OTUs), Machado-Joseph...
disease proteases (MJDs) and JAB1/MPN/Mov34 metalloenzymes (JAMMs) [7]. Of these, USPs represent the largest subclass with approximately 56 members in humans. The USP catalytic domain is highly divergent in size (295–850 residues). High sequence homology is mainly observed in two regions that surround the catalytic Cys and His residues: the so-called Cys Box domains, containing 19 amino acids, and the His Box domains, containing 60–90 amino acids [8–10].

As is the case for most cellular enzymes, the activity of DUBs can be controlled through multiple mechanisms. Several DUBs require assembly into large multimolecular complexes for full activation; this is exemplified by proteasomal DUBs. Other DUBs, including USP1, USP7, USP12 and USP46, are allosterically regulated by co-activator complexes or proteins, such as the WD40-repeat proteins, RAD50 and cullin-3 [7, 11]. Cross-talk between phosphorylation and ubiquitination is a significant aspect of intracellular signalling networks [12, 13]. Differential phosphorylation and dephosphorylation of some USPs can result in enhanced or reduced activity of these enzymes. Other post-translational modifications are emerging as modifiers of their activity, including ubiquitination and SUMOylation [7]. Furthermore, DUBs are prone to reversible inactivation caused by reactive oxygen species (ROS) [14].

Schistosomes are parasitic worms that require several coordinated morphological and biochemical changes that guarantee adaptation to various environments such as water and the internal milieu of their vertebrate and invertebrate hosts [15, 16]. Our group was the first to observe that the ubiquitin–proteasome system plays a crucial role in regulating cercariae to schistosomula transition in Schistosoma mansoni [17, 18]. Subsequent studies also revealed both differential expression of 20S proteasome subunits and specific patterns of ubiquitinated proteins during S. mansoni egg maturation, highlighting the importance of controlled protein turnover during embryo development [19].

Considering the aforementioned results, the main objective of this work was to identify the putative and non-annotated genes encoding ubiquitin-specific enzymes using bioinformatic approaches. For this we took advantage of the available S. mansoni sequences as 81 % of the parasite’s genome has been assigned to specific chromosomes [20]. Additionally, we evaluated the gene expression profile of 17 identified members of the USP family. These may be involved in important cellular processes during the life cycle of this parasite.

Methods

Ethics Statement

All experiments involving animals were authorized by the Ethics Committee for Animal Care of the Federal University of Ouro Preto (CEUA/UFOP protocol no. 2011/55). The experiments were performed in accordance with national and international regulations accepted for laboratory animal use and care. Mice (Balb/c strain, age 6 weeks, weight ~16–18 g) were kept under environmentally controlled conditions (temperature ~25 °C; humidity ~70 %) with free access to water and rodent diet.

Parasites

The 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 larvae were recovered by sedimentation on ice. Adult parasites were obtained by liver perfusion of mice after 50 days of infection. Livers of infected mice were macerated in phosphate buffer (Na2HPO4 63 mM, KH2PO4 330 mM, pH 8.2) and trypsinised, and the homogenate was then incubated for 2.5 h at 37 °C in a water bath. Eggs were recovered in saline solution after sequential sieving of the liver homogenate through 360- and 180-μm meshes. Mechanically transformed schistosomula (MTS) were prepared as described by Harrop and Wilson (1993) [21], using a protocol that mimics skin-transformed S. mansoni schistosomula [22]. Briefly, cercariae were recovered and washed in RPMI 1640 medium (Invitrogen, Sao Paulo, Brazil) before vortexing at maximum speed for 90 s. The cercariae were immediately cultured in 169 medium for 3.5 h at 37 °C with 5 % CO2. Then, the recovered schistosomula were washed in RPMI 1640 until no tails were detected. For subsequent incubations, the parasites were maintained in M169 medium supplemented with 10 % FBS, penicillin/streptomycin at 100 μg/mL and 5 % Schneider’s medium [23] at 37 °C in a 5 % CO2 incubator for 3.5, 24, 48 and 72 hours.

Computational analysis of USPs

The putative SmUSPs were identified and selected by mining S. mansoni sequences in GenDB database (version 5.0, available at http://www.genedb.org/genedb/smanson/) using the basic local alignment search tool (BLAST) algorithm BLASTp and Homo sapiens reference USP proteins as queries [24]. Best Blastp hits showing cutoff values < e−12 were selected. Reference proteins from Mus musculus, Rattus norvegicus, Drosophila melanogaster and Caenorhabditis elegans were searched in the NCBI (National Center for Biotechnology Information) using BLASTp tool and non-redundant database to obtain a full set of putative orthologue USP proteins to compare with the S. mansoni putative proteins. Analyses of protein families, domains and active sites were performed using the Pfam (version 27.0, available at http://pfam.sanger.ac.uk) and Conserved Domains Database (CDD) (http://
Expression analysis of identified USPs

Total RNA from cercariae, schistosomula, adult worms and eggs was obtained using a combination of TRIzol (GIBCO, Sao Paulo, Brazil) and chloroform extraction; it was then column-purified using the SV Total RNA Isolation System (Promega, Belo Horizonte, Brazil). The preparation was treated three times with RNase-free DNase I (1 unit each treatment), as described by the manufacturer (RQ1 DNase; Promega). The RNA was quantified using a spectrophotometer, and an aliquot containing 1 μg of total RNA was reverse-transcribed using an oligo(dT) primer from the ThermoScript RT-PCR System (Invitrogen) as described by the manufacturer. The efficiency of DNase I treatment was evaluated by PCR amplification of a cDNA reaction mix lacking the ThermoScript enzyme. *S. mansoni*-specific primers were designed using the GeneRunner® program. Despite the fact that USPs have well-conserved sequences, primers were designed to less conserved regions. The sequence accession numbers and their primer pairs are shown in Additional file 1: Table S1. Reverse-transcribed cDNA samples were used as templates for PCR amplification using SYBR Green Master Mix UDG-ROX® (Invitrogen) and a 7300 Real-Time PCR System (Applied Biosystems, Rio de Janeiro, Brazil). *S. mansoni* EIF4E was used as an endogenous control (GeneDB ID: Smnp_001500) (forward primer: 5’-TGTTCCACCACGGTCTCG-3’, reverse primer: 5’-TCGCCCTCCATGCTTAGG-3’) [32]. The efficiency of each pair of primers was evaluated according to the protocol developed by the Applied Biosystems application (the cDNA dilutions used were 1:4, 1:16, 1:64, 1:256 and 1:1024). The absence of non-specific products was confirmed by the presence of a single peak in the dissociation curves. For all investigated transcripts, three biological and technical replicates were performed, and their gene expression levels normalized using the EIF4E transcript as a reference according to the 2^ΔΔCt method [33] using the Applied Biosystems 7300 software.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism software package, version 5.0 (Irvine, CA, USA). The normality of the data was established using one-way analysis of variance (ANOVA). Tukey post-tests were used to investigate significant differences in the expression of transcripts throughout the investigated stages. In all cases, the differences were considered significant when the p values were < 0.05.

Results and Discussion

Here, we report 17 USP family members in *S. mansoni* and a particular emphasis was given to their structures and conserved domains. Phylogenetic analysis was conducted to understand how closely related these proteases are when compared to their orthologs. In addition, the *S. mansoni* orthologue proteins were compared throughout the parasite's life cycle and their expression profile evaluated by qRT-PCR.

Conservation of UCH domains in *S. mansoni* USPs

Putative members of the USP family in *S. mansoni* were retrieved by mining the parasite databases. Our analyses revealed that *Sm*USPs are conserved at the amino acid level compared to their orthologues from diverse organisms, such as *D. melanogaster*, *C. elegans*, *H. sapiens*, *M. musculus* and *R. norvegicus*. Furthermore, by comparing the *S. mansoni* predicted genes and their related ESTs (Expressed Sequence Tag) from *S. japonicum* in NCBI, up to 54% sequence similarity could be found (Additional file 1: Table S2). A total of 18 USPs were identified in *S. mansoni*. The number of USPs annotated for *H. sapiens* (~50) is much higher possibly due to the differences in complexity between these organisms (Table I). In addition, the *S. mansoni* orthologue proteins were compared to eukaryotic protein-coding genes, generated from the GENES database in KEGG, confirming their identification. Structures of the UCH domains differed comparing fifteen subclades, and their length varied from 300 to 700 amino acids among orthologs. We report that this parasite possesses 12 out of 18 USP genes with complete UCH domains. This difference in UCH domains may indicate diversity in substrate specificity as described for other organisms [34], the presence of non-functional USP or incomplete sequences deposited on parasite databases. The *S. mansoni* USP proteins exhibiting a complete UCH domain displayed the well-conserved Cys and His boxes, which include the catalytic triad formed by Cys, His and Asp residues (Fig. 1). It is reported that USPs
constitute the largest DUB sub-family with approximately 56 members in humans and 16 in yeast. Such diversity highlights their involvement in diverse cellular processes [35].

In addition, conserved domains present in each USP were investigated (Fig. 2). USPs contain a diverse range of ancillary domains whose roles are poorly characterized for the majority of catalogued and annotated USPs [35]. This characteristic has been conserved for S. mansoni USPs. SmUSP2, SmUSP9x, SmUSP10, SmUSP14, SmUSP16, SmUSP22, SmUSP30, SmUSP36-42 and SmUSP46 contain only the UCH domain (PF00443). SmUSP5, SmUSP39 and SmUSP49-44 exhibit the zf-UBP domain (PF02148), which is a relatively small motif responsible for docking interactions with their target molecules [36]. Furthermore, SmUSP5 and SmUSP24 possess the UBA (ubiquitin associated) domain (PF00627) found in diverse proteins involved in the ubiquitin/proteasome pathway, DNA excision-repair, and cell signalling via protein kinases [37]. SmUSP7 contains three unique domains: MATH (Meprin and TRAF-Homology) (PF00917), USP7_ICP0 (PF12436) and USP7_C2 (PF14533). The MATH domain, which participates in protein-protein interactions, is also found in intracellular TRAFs (tumour necrosis receptor-associated factors) and extracellular meprins [38]. USP7_ICP0, found only in SmUSP7, is known to interact with the herpesvirus 1 trans-acting transcriptional protein ICP0/VMW110 [39]. The third domain, USP7_C2, is found at the C-terminus of USP7, and its function is unclear. Only SmUSP8 contains the IPPT domain (PF01715) related to ATP binding and the USP8_dimer (PF08969) found at its N-terminus and likely involved in homodimer formation [40]. SmUSP15 and SmUSP20 also contain the DUSP (domain present in ubiquitin-specific protease) (PF06337), located at the N- and C-terminal sides of their

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Table I Putative ubiquitin-specific proteases in S. mansoni

| USP   | S. mansoni putative orthologs | KEGG Orthology Entry (KO) | KEGG Orthology name | Length (aa) | Domain and Pfam number | Orthologue length (aa) in Homo sapiens | Pfam e-value |
|-------|-------------------------------|---------------------------|---------------------|-------------|------------------------|---------------------------------------|--------------|
| USP2  | Smp_212390                    | K11833                    | USP2_21             | 563         | UCH (PF00443) (171)    | 605                                   | 3.1e-66      |
| USP5  | Smp_069960                    | K11836                    | USP5_13, UBP14      | 916         | zf-UBP                 | 855                                   | 2.6e-16      |
| USP7  | Smp_089180                    | K11838                    | USP7, UBP15         | 1412        | MATH                   | 1102                                  | 3.1e-12      |
| USP8  | Smp_152000                    | K11839                    | USP8, UBP5          | 1027        | IPPT                   | 1118                                  | 1.7e-16      |
| USP9x | Smp_153690                    | K11840                    | USP9_24             | 811         | UCH                    | 918                                   | 9.7e-22      |
| USP10 | Smp_005280                    | K11841                    | USP10, UBP3         | 597         | UCH (91)               | 802                                   | 5.9e-31      |
| USP14 | Smp_084740                    | K11843                    | USP14, UBP6         | 178         | UCH (52)               | 494                                   | 3.9e-42      |
| USP15 | Smp_128770                    | K11835                    | USP4_11_15, UBP12   | 945         | DUSP                   | 951                                   | 3.8e-14      |
| USP16 | Smp_074200.1                  | K11844                    | USP16_45            | 622         | UCH (44)               | 823                                   | 4.9e-30      |
| USP20 | Smp_021300                    | K11848                    | USP20_33            | 881         | UCH (34)               | 914                                   | 6.1e-66      |
| USP22 | Smp_074400                    | K11366                    | USP22_27_51, UBP8   | 489         | UCH (181)              | 525                                   | 3.5e-52      |
| USP24 | Smp_198740                    | K11840                    | USP9_24             | 2235        | UCH (229)              | 2620                                  | 4e-17        |
| USP30 | Smp_122960.1                  | K11851                    | USP30               | 595         | UCH (58)               | 517                                   | 1.4e-21      |
| USP36-42 | Smp_046430                  | K11855                    | USP36_42            | 799         | UCH (178)              | 1123                                  | 2e-49        |
| USP39 | Smp_017890                    | K12847                    | USP39, SAD1         | 577         | zf-UBP                 | 565                                   | 1.5e-12      |
| USP46 | Smp_000710                    | K11842                    | USP12_46            | 414         | UCH (47)               | 366                                   | 8e-67        |
| USP48 | Smp_196290                    | K11858                    | USP48               | 1281        | UCH (95)               | 1035                                  | 2.3e-12      |
| USP49-44 | Smp_123630                  | K11834                    | USP44_49            | 823         | zf-UBP                 | 640                                   | 2.7e-18      |

All enzymes are classified as ubiquitin thioesterase
UCH domains, respectively. The function of DUSP is unknown; however, it may play a role in protein/protein interaction or substrate recognition [35]. SmUSP48 is unique among the investigated molecules as it contains the ubiquitin domain (PF00240) at its C-terminus. Understanding the interaction of these protein domains provides insights into the diverse biological activities exhibited by SmUSP enzymes. Furthermore, the presence of the DUSP domain is not indicative of catalytic activity. A recent report showed that Usp39 lacks catalytic activity in vitro and is unable to cleave ubiquitin from ubiquitylated Aurora B in vivo [41].

A phylogenetic tree generated with the neighbour-joining method was used to separate the putative SmUSPs from their respective orthologues in *H. sapiens*, *M. musculus*, *R. norvegicus*, *C. elegans* and *D. melanogaster* (Fig. 3). Most of the *S. mansoni* USP proteins clustered with their orthologues into 15 subclades: USPs 2, 5, 7, 8, 10, 14, 15, 22, 30, 36–42, 39, 46, 48 and 49–44. The putative USP proteins 9x, 16 and 24 did not cluster in the same clade as their orthologues due to the incompleteness of their UCH domains. One exception was SmUSP20s, which exhibits the complete domain but did not cluster with their orthologs.

Distinct expression of SmUSPs is observed during *S. mansoni* development

SmUSP transcript levels were analysed by qRT-PCR at different developmental stages of *S. mansoni*: cercariae, MTS-3.5 h, MTS-24 h, MTS-48 h, MTS-72 h, paired adult worms and eggs. Three biological and technical replicates were performed. The putative SmUSP transcripts analysed were detected in all investigated stages (Fig. 4). A 2-fold change in the expression of USP genes, relative to EIF4E transcript levels, was considered of biological relevance [42, 43]. According to their expression profile, SmUSPs were divided into three groups, to account for their high (0.5–5), medium (0.2–1.5) and low expression (0.02–0.5) levels. The SmUSPs exhibiting high expression profiles were SmUSP7, SmUSP8, SmUSP9 and SmUSP24 (Fig. 4a). Those showing medium expression were SmUSP2, SmUSP15, SmUSP20, SmUSP30, SmUSP36–42, SmUSP39, SmUSP46 and SmUSP49–44 (Fig. 4b). The SmUSPs displaying low expression profiles were SmUSP10, SmUSP14, SmUSP16, SmUSP22 and SmUSP48 (Fig. 4c).

The expression profile of *Smusps* has been previously described [44]. Of the 17 analysed transcripts, none of the USPs was up-regulated in the cercariae and MTS-
Fig. 2 Schematic diagram of conserved protein domains in *S. mansoni* USPs. A comparative analysis of the domains in SmUSP2, SmUSP5, SmUSP7, SmUSP8, SmUSP9x, SmUSP10, SmUSP14, SmUSP15, SmUSP16, SmUSP20, SmUSP22, SmUSP24, SmUSP30, SmUSP36-42, SmUSP39, SmUSP46, SmUSP48 and SmUSP49-44.

LEGEND:

- **UCH** (PF00443)
- **zf-UBP** (PF02148)
- **DUSP** (PF06337)
- **UBA** (PF0627)
- **USP7_C2, USP8_dimer** (PF14531, PF08969)
- **ubiquitin** (PF00240)
- **MATH** (PF00917)
72 h stages (USP2, USP5, USP7, USP8, USP9x, USP10, USP14, USP15, USP16, USP20, USP22, USP24, USP30, USP36-42, USP39, USP46, USP48, USP49-44). In contrast, Smusp2, Smusp39, Smusp46 and Smusp49-44 were up-regulated in early schistosomula (p < 0.05). Extending the culture period to 24 h, Smusp8 transcripts were observed at increased levels (p < 0.05). Concerning MTS-48 h, Smusp24 was up-regulated in this stage (p < 0.05). Smusp7, Smusp9x and Smusp15 transcripts presented at high levels in adult worms (p < 0.05). Smusp20 was up-regulated in three stages: MTS-3.5 h, MTS-48 h and adult worms (p < 0.05). Smusp30 was also up-regulated in three stages: MTS-24 h, MTS-48 h and adult worms (p < 0.05). Smusp36-42 was up-regulated in early schistosomula (MTS-3.5 h MTS-24 h and MTS-48 h) (p < 0.05). Furthermore, Smusp7, Smusp8, Smusp9x and Smusp24

Fig. 3 Phylogenetic tree of S. mansoni USP Conservation of SmUSP2, SmUSP5, SmUSP7, SmUSP8, SmUSP9x, SmUSP10, SmUSP14, SmUSP15, SmUSP16, SmUSP20, SmUSP22, SmUSP24, SmUSP30, SmUSP36-42, SmUSP39, SmUSP46, SmUSP48 and SmUSP49-44. Multiple alignments were performed using Mega 5.0 with bootstrap analysis. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed [57]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was drawn to scale, with branch lengths representing the evolutionary distances used to infer the phylogenetic tree.
were more highly expressed USPs in *S. mansoni*. Overall, upon examining the expression levels of all USP genes, it was found that the *Sm*USPs were more abundant in schistosomula and adult worms when compared with cercariae and eggs. This finding corroborates with previous data from our group, which demonstrated low levels of free, non-conjugated ubiquitin in the cercariae stage followed by its increase during schistosomula development up to adult worms [44]. In parallel, western blot analysis revealed the accumulation of ubiquitinated proteins in cercariae and schistosomula cultured up to 5 days relative to other intra-mammalian stages, suggesting that *Sm*USPs are less active at this stage [44]. This also coincides with the reported lower proteasomal proteolysis observed during early schistosomula compared with that found for the adult worm stage [17]. Whether the accumulation of ubiquitinated proteins are due to low levels of USPs transcripts, decreased
proteosomal activity or their combined effects remains to be elucidated. These data reinforce the hypothesis that a major function of USP members in *S. mansoni* is the regulation of protein stability during the cercariae to adult worm development. The high levels of ubiquitinated conjugates found in early schistosomula likely indicates ubiquitination linked to alternative protein fates, such as sub-cellular localization and lysosome mediated degradation [45–47]. The importance of the ubiquitin cycle to protein stability is now recognized in all eukaryotic cells as a key mechanism for maintenance of cell viability [48].

The cellular roles of DUBs are as wide as that of the ubiquitin-proteasome system itself given the involvement of the Ub system in intracellular signalling [7]. Here, we selected some examples to illustrate their broader functional categorization. The proteasome has both ubiquitin ligases and DUBs that associate with it and several DUB-ligase pairs interact directly, including BRCC36-BRCA1, BAP1-BRCA1, USP4-Ro52, USP7-MDM2, USP8-GRAIL, USP20-pVHL, USP33-pVHL and USP44-APC [49]. Although the E3 repertoire in *S. mansoni* is not known, previous analyses from our group suggest the conservation of the MDM2, GARIO and APC in the parasite genome (data not shown), indicating a role for USP44-44 in cell cycle progression. USP7 removes ubiquitin not only from p53 itself but also from the p53 E3 ubiquitin-ligase MDM2. These combined effects determine functional p53 levels, creating an important role for USP7 in p53-dependent stress responses. Our group showed that the p53 orthologues, p63 and p73 [50], are up-regulated in MTS-3.5 h and MTS-5 days [51]. We also observed a differential expression profile for *Smusp7*, *Smusp8* and *Smusp9x* were highly expressed throughout the parasite cycle. USP8 and USP9 appear to be involved in the control of mammalian cell proliferation, inducing apoptosis [52], indicating a role of these enzymes in parasite remodelling. *Smusp14* transcript level is higher in cercariae when compared with adult worms, suggesting that *SmUSP14* can inhibit proteasome function noncatalytically, as previously observed for its yeast orthologue Ubp6 [53]. These data could explain, at least in part, the low rate of proteasome-dependent proteolysis detected in cercariae when compared with adult worms [17]. *Smusp20* expression is up-regulated in intra-mammalian stages, particularly in MTS-3.5 h. Orthologues of USP20 seem to be involved in hypoxia signalling, and the possibility of this protease to regulate the schistosomula development will be investigated [54]. Additionally, *Smusp24* and *Smusp30* were three times more abundant in MTS-48 h when compared with adult worms. Considering that USP24 was recently identified as a novel regulator of DDB2 (damage-specific DNA-binding protein 2) stability [55] and the implication of USP30 in the maintenance of mitochondrial morphology [56], *SmusP24* and *SmusP30* could be necessary during parasite remodelling. Furthermore, the transcription levels of *Smusps36–42* are similar when comparing MTS-3.5 h, 24 and 48 h, reinforcing the hypothesis that specific *SmUSPs* can be important for early schistosomula development.

Conclusions

In conclusion, we describe the USP enzyme repertoire in *S. mansoni* and their regulated expression in the parasite life cycle. This differential profile of transcripts can reflect the stage-specific subset of their target ubiquitinated substrates during the parasite’s life cycle. Our results raise a number of interesting questions concerning the regulation of *SmUSP* activities and their role during schistosome development. USPs are temporally and spatially controlled and most often act as part of multi-protein complexes [56, 55]. Moreover, a single USP can act upon various substrates, and its activity can be regulated by several DUBs. Further experiments shall clarify the functions of putative *SmUSPs* in *S. mansoni*.

Additional file

Additional file 1: **Table S1.** Sequence accession numbers and primer pairs. **Table S2.** Putative ubiquitin-specific proteases in *S. mansoni* and *S. japonicum*.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

RVP performed transcriptome analyses, RNA isolation and qRT-PCR experiments. MSG performed transcriptome and bioinformatics analyses. RPO and DMS helped with qRT-PCR experiments. LKJP participated in *S. mansoni* life cycle maintenance and schistosomula culture. EHB, LKP, WCB and RGS conceived the study and participated in design and coordination. RVP, MSG and RGS wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements

The authors thank the following transcriptome initiatives: the São Paulo Transcriptome Consortium, the Minas Gerais Genome Network and the Wellcome Trust Genome Initiative (UK). This work was supported by the following Brazilian research agencies: FAPESP (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, CBB - APQ-02101-11), NaBio UFOP (Núcleo de Bioinformática da Universidade Federal de Ouro Preto) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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