



Schistosomes—proteomics studies for potential novel vaccines and drug targets

Ricardo DeMarco¹ and Sergio Verjovski-Almeida²

¹ Departamento de Física e Informática, Instituto de Física de São Carlos, Universidade de São Paulo, 13560-970 São Carlos, SP, Brazil

² Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, 05508-900 São Paulo, SP, Brazil

Schistosomiasis is a major health problem and, despite decades of research, only one effective drug, Praziquantel is currently available. Recent expansion of sequence databases on *Schistosoma mansoni* and *S. japonicum* has permitted a wealth of novel proteomic studies on several aspects of the organization and development of the parasite in the human host. This unprecedented accumulation of molecular data is allowing a more rational approach to propose drug targets and vaccine candidates, such as proteins located at the parasite surface. Successful preliminary trials of two vaccine candidates that have been detected at the parasite surface by proteomics give grounds for believing that such an approach may provide a fresh start for the field.

Introduction

Schistosomes are blood-dwelling trematode parasites and the causative agents of schistosomiasis, a tropical disease affecting 200 million individuals, being endemic in 74 countries in tropical regions of Africa, Asia and South America [1]. It is not totally understood how the parasite succeeds in escaping the host immune system, giving rise to chronic infections possibly persisting for decades. Considering that scenario, development of new treatments for schistosomiasis would benefit millions of people living in developing countries.

Despite the testing of drugs from different classes over the past decades [2], Praziquantel remains the strategic cornerstone for the treatment of schistosomiasis worldwide, which heavily relies on massive chemotherapy [3]. Praziquantel is effective against all *Schistosoma* species infecting humans, has been extensively tested, does not display severe side effects and is currently inexpensive and available [4,5].

Reports of treatment failure in Senegal and Egypt [6,7], where isolates with reduced susceptibility to Praziquantel were obtained, have raised concerns about development of drug resistance. Further *in vitro* experiments confirmed the development of parasite resistance to Praziquantel compared with susceptible isolates [8–11]. It is still controversial whether the low cure rates observed

can be correlated with the emergence of Praziquantel-resistant parasites, because the interpretation of data is complicated by other factors, such as reinfection of patients in the field, varying sensitivity of diagnostics techniques utilized and the presence of non-susceptible immature worms in the patients [12,13]. Despite these uncertainties, development of novel, effective drugs against schistosomiasis is presently needed to provide a range of treatments using different drug classes [3].

In addition, there is currently no effective vaccine against schistosomiasis; considering the peculiarities of schistosome infection, even the development of vaccines that partially diminish the worm burden or female fecundity could have a significant impact on the control of the disease. Several candidates have been proposed in the 1980s and 1990s. Their discovery was based mainly on the recognition of these proteins by protective antibodies/serum or high immunogenicity [14–19]. Despite promising preliminary results, tests with six selected antigens (glutathione-S-transferase (SmGST), triose phosphate isomerase (TPI), paramyosin, 23 kDa integral membrane tetraspanin protein (Sm23), myosin heavy chain (IrV5) and 14 kDa fatty acid binding protein (Sm14)) by independently contracted laboratories in the *Schistosoma mansoni*/mouse experimental system showed disappointing results [20]. In addition, all these antigens, with the exception of Sm23, are not expected to display an extracellular location and are instead cytosolic or cytoskeletal components, which raises doubts about

Corresponding author: Verjovski-Almeida, S. (verjo@iq.usp.br)

the mechanisms of how they trigger the immune effector system [21]. Currently, Phase I and II clinical trials in humans are underway using *S. hematobium* glutathione-S-transferase; on the basis of previous results it is hoped that vaccination with this antigen should help to limit pathology and improve the efficiency of Praziquantel [22].

It is clear that development of new drugs and vaccines for schistosomiasis would provide important alternatives for Praziquantel treatment. New drugs would permit combined treatments to increase the efficacy of Praziquantel or alternate treatments that would avoid development of resistance. Development of an effective vaccine would be quite desirable since, unlike chemotherapies, it might provide longer term protection. Therefore, drugs and vaccines appear to be complementary to each other and strategies to stop the dissemination of the disease may involve both components.

New opportunities generated by the recent expansion of schistosome molecular databases

Improvement of the Sanger DNA-sequencing technique during the 1990s allowed its automation and the development of several strategies for high-throughput sampling of DNA and cDNA, resulting in an exponential increase in the number of sequences deposited in public databases. The use of such strategies permitted the intensive sequencing of schistosomes' genome and transcriptome over the past few years and has greatly influenced the approaches employed to understand parasite biology. Two large-scale, independent, sequencing projects on *S. mansoni* and *japonicum* transcriptomes [23,24] allowed, for the first time, a broad sampling of components from the parasites' molecular systems. Analysis of the gene collection represented by the ESTs is based on the inferred functions, which are deduced from alignments of the predicted protein products with proteins of known function from other organisms. Such analyses have allowed the proposal of an initial list with several vaccine and drug target candidates [23]. More recently, a draft of the *S. mansoni* genome has been made publicly available and is currently being annotated [25]. The combination of transcriptome and genome data allows a comprehensive description of the schistosome genes and provides an invaluable repository for further studies.

In fact, the availability of schistosome large-scale sequencing data in conjunction with technical advances is permitting a series of post-genomic experiments aimed at better understanding the parasite biology and searching for genes that are crucial for parasite development, differentiation and parasitism. Microarray platforms with oligonucleotide probes designed from the transcriptome sequencing data allow monitoring of the expression of a very large fraction of the parasite genes [26]. Silencing of those newly described schistosome genes is now possible with the demonstration of its feasibility using RNAi technology [27,28] that provides a way of inferring function, even for schistosome-specific genes that do not have any known ortholog genes.

Proteomics also benefited from the expansion of schistosome databases concomitantly with the improvement of technology. Since no organisms phylogenetically close to schistosomes have been extensively sequenced, only a small portion of schistosome proteins are expected to have the high degree of identity with non-schistosome protein sequences in the public databases. Therefore, before large-scale sequencing of the parasites' transcriptomes a considerable number of spectra generated by mass spectrometry of schistosome peptides could not be reliably associated with an orthologous protein from public databases. In that scenario, generation of large databases of schistosome coding sequences [23,24] has greatly improved the level of identification in proteomic studies of these organisms.

In fact, several proteomics studies have used EST assemblies, or gene prediction data based on the genome, for peptide searches [29–32] or have used full-length clones of schistosome genes that resulted from further transcriptome sequencing [33]. Using these resources, several strategies have been employed to search for potential novel vaccines and drug targets, as we will describe in detail in the next sections and is summarized in Table 1.

Selecting vaccine candidates using data from high throughput studies

Expansion of the molecular databases referred to in the previous sections should, in principle, permit the use of a novel approach for identification of vaccine targets, termed reverse vaccinology. It consists of *in silico* analysis of genomes from pathogens to select

TABLE 1

Summary of different aspects approached by proteomic studies with potential implication for the development of schistosomiasis therapies.

Characteristics studied	Life stages sampled ^a	Organism	References
Proteins expressed in different life cycle stages	c, s, a, e	<i>Schistosoma mansoni</i>	[42]
Proteins expressed in different life cycle stages	c, s, a, e, l	<i>Schistosoma japonicum</i>	[33]
Proteins differentially expressed between genders	am, af	<i>Schistosoma japonicum</i>	[43]
Proteins differentially expressed between adult worm tegument and carcass	a	<i>Schistosoma mansoni</i>	[30]
Proteins present at different fractions of the adult worm tegument	a	<i>Schistosoma mansoni</i>	[29]
Proteins exposed at adult worm surface	a	<i>Schistosoma mansoni</i>	[47]
Protein content of adult and schistosomule tegument	s, e	<i>Schistosoma japonicum</i>	[33]
Protein content of adult tegument	a	<i>Schistosoma bovis</i>	[50]
Proteins exposed at adult worm surface	a	<i>Schistosoma bovis</i>	[51]
Host proteins present in parasite tissue	c, s, a, e, l	<i>Schistosoma japonicum</i>	[52]
Host proteins present in parasite tissue	a, e	<i>Schistosoma mansoni</i>	[47,53]
Cercarial secretions	c	<i>Schistosoma mansoni</i>	[31,55,57]
Cercarial secretions	c	<i>Schistosoma japonicum</i>	[56]
Egg secretions	e	<i>Schistosoma mansoni</i>	[32]
Larval secretions	l	<i>Schistosoma mansoni</i>	[54]

^a c, cercaria; s, schistosomulum; a, adult worm; am, male adult worm; af, female adult worm; e, egg, l, miracidium (larva).

ORFs encoding potentially antigenic exposed proteins, followed by large-scale screening of these antigens in immunization protocols using recombinant expression [34]. This high throughput approach has been successfully used to propose promising vaccine targets for prokaryote pathogens such as *Neisseria meningitidis* [35] and *Streptococcus agalactiae* [36]. Reverse vaccinology has also been used for identifying potential vaccine candidates in the protozoa *Trypanosoma cruzi* and *Leishmania* [37–39]. Use of this approach in more complex eukaryotic pathogens such as schistosomes, however, suffers from several drawbacks: (1) deduction of ORFs from genomes of most eukaryotes is not straightforward owing to mRNA splicing. Prediction programs are useful but tend to accumulate errors, especially at the extremities of ORFs that usually encode signals for secretion. A definitive description of the ORF complement of the organism is dependent on complete sequencing of the parasite's transcriptome, a complex, labor-intensive endeavor, which is not yet complete in *S. mansoni*; (2) several of the secreted proteins from multi-cellular organisms will be produced by internal cells of the parasite and therefore not exposed to the external environment; (3) schistosomes display a complex life cycle with very different stages and not all of them occur inside the mammalian host. Therefore, it is expected that several genes would have a stage specific expression, meaning that several external proteins will never be in contact with the host immune system.

Considering these shortcomings, a direct *in silico* screening of the schistosome genome for antigens, using the same criteria as in prokaryotes and protozoa, might be a time-consuming and relatively inefficient way to identify new vaccine targets. The use of proteomic information should help one to delineate a subset of proteins exposed to the host, and/or present in intra-mammalian stages that may provide a more appropriate starting point for vaccine trials, as discussed in the next section.

There is evidence that CD4⁺ T cells of individuals living in endemic areas who are naturally resistant to schistosomiasis do respond to *S. mansoni* antigens such as Sm14 protein [40]. This raises the possibility that a very recent, high throughput CD4⁺ T cell expression screening method [41] could be employed to identify promising novel *S. mansoni* vaccine candidates. This screening method has been successfully used to identify *Mycobacterium tuberculosis* (Mtb) antigens as candidates for a subunit vaccine against tuberculosis [41]. In *S. mansoni*, the screening could use Sm-specific CD4⁺ T cell lines from naturally resistant donors, which would be stimulated with different parasite extracts. These stimulated lines would be used for screening a *S. mansoni* cDNA library expressed in *Escherichia coli* and processed and presented by autologous dendritic cells from the donors. With an approach analogous to that of Coler *et al.* [41], the identity of novel T cell antigens would be recovered by sequencing the expression library cDNA clones that encode *S. mansoni* proteins causing CD4⁺ T cell proliferation and IFN- γ production.

Using proteomics to determine stage specific proteins

One of the most straightforward uses of proteomics is to compare the abundance of different proteins along the life cycle of the parasite. Comparing the abundance of proteins between intra-mammalian and free-living or intra-molluscan stages would point towards those with specific functions in the development and

adaptation of the parasite that could be interesting targets for treatments. Soluble extracts from cercariae, lung worms, adult and eggs from *S. mansoni* have been compared using bi-dimensional (2D) electrophoresis and 24 of the 32 most abundant proteins detected in those life stages had been previously characterized as a result of their high immunogenicity, including four previous vaccine candidates [42]. Subsequently, Liu *et al.* [33] performed a sampling of five life stages from *S. japonicum* (cercariae, hepatic schistosomula, adults, eggs and miracidia) and produced extensive lists with hundreds of proteins with apparent stage specificity. The information contained on these lists may provide an additional parameter for selection of promising targets for development of therapeutics.

In addition, comparisons between protein extracts from adult male and female were performed to detect gender-specific proteins in *S. japonicum*. Proteins involved in development, sexual maturation, signaling and hormone receptors have been described as differentially displayed in male or female [33,43]. Development of therapeutic agents targeting the proteins involved in sexual development or reproduction may have a positive impact in the control of the disease, considering that blockage of egg laying may reduce schistosome-induced host liver pathology, as well as impair transmission of the parasite.

Probing the schistosome surface by proteomics

Exploring the surface of the intra-mammalian stages of schistosomes for vaccine and drug targets is a very attractive approach. One of the reasons for this is that these proteins are easily accessible, meaning that eventual drugs that are developed do not have to permeate the parasite membrane to reach their targets and that the antigen is exposed, facilitating antibody binding. In addition, proteins located at the parasite/host interface are likely to be associated with mechanisms of escape from the host immune system or other adaptation to parasitism; and neutralization of their functions could make the parasite more vulnerable to host defenses. Therefore, proteomics studies of the schistosome tegument, which is a syncytial cytoplasmic layer covering the parasite, have been performed in the hope of describing potential drug targets and vaccine candidates.

A first approach to determine abundant proteins of the tegument was developed by Van Balkom *et al.* [30], using one-dimensional (1D) SDS-PAGE and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) of two samples: (1) a crude tegument preparation released by freeze-thaw and (2) the resulting stripped *S. mansoni* adult worms. Comparison of these two samples revealed that 43 proteins were detected only in the tegument, and on the basis of these observations it was proposed that they were enriched in this tissue [30]. Among those proteins, we can highlight sperm-coating protein-like (SCP-like) proteins [23,44] and ATP-diphosphohydrolase [45], which have been proposed to play a role in host parasite interaction, and SGTP-4, which is implicated in acquisition of glucose from host serum [46].

The strategy of directly using a crude extract, released by freeze-thaw, for proteomics and comparing its content with the protein content from stripped worms has some potential drawbacks. It should be noted that stripped *S. mansoni* adult worms may contain in their subtegumental cell bodies a number of proteins intended for exportation to the tegument; therefore, the list of proteins

shared by tegument and stripped worms may include proteins that in fact perform functions specific to the tegument. For the majority of tegumental proteins, however, the expected concentration in the total mass of stripped worms would be too low for the current detection limits of mass spectrometers, and the majority of them should remain undetected in this fraction. Another problem of using crude, non-fractionated tegument extracts is the possible contamination with subtegumental cell bodies. In fact, detection in the tegument of proteins related to the protein synthesis pathway (which is expected to occur at the subtegumental cell bodies, not at the tegument), such as elongation factor 1- α and eukaryotic translation initiation factor 2- α subunit [30] suggests that the tegumental sample may have been in fact contaminated with underlying cell bodies.

A more detailed proteomic study of the *S. mansoni* tegument was performed by Braschi *et al.* [29] who divided the tegument into four fractions on the basis of sequential extractions with buffers of increasing solubilizing power. These fractions were separated using 2D electrophoresis, except for the last, insoluble, fraction that was separated by 1D SDS-PAGE; samples were submitted to LC-MS/MS, followed by identification by MS/MS. The latter two fractions obtained by this procedure contained the integral membrane and membrane associated proteins of the tegument [29]. In those fractions, four different enzymes and seven transporters have been identified and represent the most interesting targets owing to their peripheral location. Three out of four enzymes detected are involved in hydrolysis of organic phosphate compounds. In addition, three different tetraspanins have been detected in those fractions and were hypothesized to provide spatial organization for other tegument plasma proteins.

In an attempt to determine which components of the tegument apical membrane are exposed to the host environment, Braschi and Wilson [47] performed an incubation of intact *S. mansoni* adult worms with two non cell-permeable biotinylation reagents of variable carbon chain length and later recovered the biotinylated proteins using a streptavidin column. A total of 24 schistosome proteins were identified and divided into two groups, one with 9 proteins that were labeled by both reagents with long and short carbon chain, which must represent the more exposed proteins in the tegument, and another group with 15 less exposed proteins that were labeled only by the short carbon chain reagent.

Examining the lists generated by these three studies, it is possible to detect at least four proteins in common to all of them, namely an ATP-diphosphohydrolase, a phosphodiesterase, a tetraspanin (CD63-like tetraspanin) and a dysferlin. It is possible to infer that these four tegument membrane proteins must have high abundance to be detected in those three studies. In addition, several other proteins were detected by at least two of these approaches, demonstrating their consistency.

Interestingly, two of the tegumental proteins detected in proteomic studies, Sm29 and CD63-like protein tetraspanin (Sm-TSP-2), have been recently used in independent experiments as vaccine candidates, causing high reductions of worm burden (>50%) and of liver egg burden (>60%) in animal models [48,49]. Although none of these works has used the proteomic data as a starting point for antigen selection, these results show the potential of tegument proteins as candidates for vaccine development, highlighting the

importance of those additional tegument proteins newly detected by proteomics, which were discussed in the previous paragraphs.

A shotgun proteomic approach has also been used to explore the tegument of adults and hepatic schistosomula of *S. japonicum* [33] and 373 tegumental proteins have been identified (134 from adult females, 58 from adult males, 156 from mixed-sex adults, and 159 from hepatic schistosomula). These included 9 proteins previously characterized as tegument proteins and components of a Ca^{2+} ion signaling pathway that have been pointed out by the authors as potential drug targets [33].

Studies were also performed to explore the protein content of the tegument of the ruminant parasite *S. bovis* [50]. One of these studies used 2D immunoblots with infected sheep serum to identify the most antigenic proteins in the tegument preparation. The spots from a parallel 2D electrophoresis corresponding to those regions identified in the 2D immunoblot were excised and 5 different proteins were determined by mass spectrometry. All of those proteins are soluble and include orthologs of *S. mansoni* vaccine candidates glutathione-S-transferase and GAPDH [50]. Considering that the approach that has been utilized shows a very limited recovery of integral membrane proteins, it is understandable that only soluble proteins have been sampled. A more recent approach used trypsinization of whole adult worms coupled with LC-MS/MS to detect tegumental proteins exposed in *S. bovis* [51]; although this methodology is more appropriated to detect the exposed membrane proteins in the tegument, the authors reported mostly cytosolic and cytoskeletal proteins, which suggests leakage of intra-parasite contents possibly due to damage to the tegument. In the face of these results, a cautious interpretation of data from these studies is advisable and further experiments, for example testing the accessibility of the proteins to non cell-permeable chemical modifiers, are warranted to indicate whether any of the proteins detected are, in fact, associated with the tegument.

An additional proteomic study found several host proteins to be associated with the *S. japonicum* tegument and other stages of the parasite life cycle [52]. Although it cannot be ruled out that detection of some of these proteins can be derived from simple contamination of samples with host tissue, detection of host proteins in independent experiments with *S. mansoni* tegument [47] and egg hatching fluid [53] reinforce the idea that schistosomes might have some mechanism to actively capture host proteins. It has been proposed that presence of these host proteins in schistosome tissues could reflect an escape mechanism from host defense attacks [52]. Identification of the effector proteins for this mechanism would open the way for development of new treatments aimed at exposing the parasite to the host defense system.

Overall, proteomics approaches have so far increased the amount of information regarding surface-exposed proteins in schistosomes, especially in the adult worms. Nevertheless, it is clear that a challenge still lies ahead in terms of better cell-fractionation and tegument membrane-proteins purification protocols. Mass spectrometry in the proteomics field is only as good as the quality of the input protein fractions that are used. In particular, establishment of novel effective, high-yield fractionation and purification methods to obtain a homogeneous membrane fraction from early-stage schistosomula could bring a considerable improvement in terms of identification of surface-exposed

proteins of the parasite along its development from somula to adult worms inside the human host.

Schistosome secretions

The existence of morphologically distinct intra-mammalian life stages of the parasite reflects intricate events of interaction with the human host. It is expected that some of these processes are mediated by parasite's secreted proteins and that the analysis of these products will help to understand and manipulate the mechanisms of parasitism. In addition, these products are also in direct contact with the host, being likely candidates for vaccines. *S. mansoni* larval secretions have also been the subject of a proteomic study [54], but are of less interest in the scope of this review, since the larvae exist only within the mollusc intermediate host, and a possible treatment of molluscs in the open field to eliminate parasites at this stage would be difficult to implement.

Cercarial secretions are implicated in the invasion process of human skin by schistosomes and have been initially studied by two independent groups. The first study [55] has utilized 1D SDS-PAGE LC-MS/MS to analyze secretions from stimulated *S. mansoni* cercariae, using either human skin lipid stimulation or tail shearing transformation by passages through a syringe; also non-induced cercariae were used. The authors identified 72, 85 and 15 proteins that were secreted by cercariae submitted to these three conditions, respectively [55]. In the lipid-stimulated sample 12 glycolytic enzymes, six previous vaccine candidates and three cercarial elastases were detected [55]. A second study [31] has utilized *S. mansoni* cercariae mechanically transformed by vortexing, followed by incubation in culture medium. Released products were separated by 2D electrophoresis and further analyzed by MS/MS, being 48 spots associated with protein products. Among these, the authors identified one novel metalloproteinase, a dipeptidyl peptidase, a new serine protease inhibitor and four novel immunomodulators (including the SCP-like protein) [31]. These studies obtained a diverse list of detected proteins in cercarial secretions, and because they utilized different approaches to mimic the process of stimulation of the parasite when invading the human skin, there is still controversy of which would better represent the actual secretions from cercariae in its process of invasion of the human skin (<http://www.mcponline.org/cgi/eletters/5/5/835>).

A more recent study has performed a proteomic analysis of *S. japonicum* cercarial secretions [56]. The authors identified 361 unique proteins in the secretions; fifteen of them were identified as proteases. Interestingly, the analyses did not identify the presence of peptide sequences orthologous to *S. mansoni* cercarial elastase, the most abundant serine protease enzyme secreted by *S. mansoni* cercariae. By contrast, *S. japonicum* cathepsin B2 (SjCB2), an ortholog of the *S. mansoni* cysteine-class cathepsin CB2 was found; this was the only protease found with potential to degrade host skin or connective tissue. The authors proposed that cysteine proteases represent an archetypal tool for tissue invasion among primitive metazoa and that the use of serine proteases arose later in schistosome evolution [56].

More recently, a study of *S. mansoni* cercariae invasion using human skin samples has been performed and permitted confirmation, by mass spectrometry, of some of the proteins sampled in the previous proteomics studies, such as the serine protease

inhibitor, SCP-like protein (venom allergen like), GST and glycolytic enzymes in cercarial secretions [57]. The detection of cytosolic enzymes in secretions of the parasite suggests that the acetabular glands of cercariae are holocrine, meaning that cytosolic components of the degrading cell will be released by the gland. This may be an explanatory mechanism to the observed prominent recognition of some cytosolic antigens by sera of infected patients [57].

Schistosome eggs are responsible for acute pathogenic responses that occur in the liver. It is hypothesized that antigens secreted by eggs induce a granulomatous inflammation and that such response would be important for excretion of eggs [58]. Secretions from eggs have been studied by a proteomic approach [32] and a total of 188 proteins have been identified. Several proteins involved in cell signaling, redox balance and energy production have been identified [32]. Several of the detected proteins, such as histones and cytoskeletal proteins, are not expected to be found in secretions, raising doubts as to whether the secreted fraction has been contaminated with leakage products from dead eggs. The authors utilize the Secretome P program to validate the identification of individual proteins, but even with this additional filter the schistosome histones are still predicted to be secreted. These findings suggest that perhaps stricter, controlled protein isolation protocols and analytical parameters are still necessary to determine the actual set of secreted egg proteins. Further studies of proteins at the host-parasite egg interface may bring new information that would help understanding immune modulation and the pathology of schistosomiasis.

Schistosome novel proteins

Large-scale sequencing of the *S. mansoni* and *japonicum* transcriptomes [23,24] has revealed that over 50% of the transcripts had no matches in the public sequence databases. A recent re-annotation revealed that, as of now, approximately 55% of *S. mansoni* genes still have no homology outside of the genus [25]. Of the remaining 45% with a homolog, almost half have no functional assignment after GO analysis. Therefore, informative homology might be as low as 26% and consequently, three out of four schistosome gene products have unknown functions [25]. The latter might be a good starting point for selecting an effective and safe drug or a vaccine target; one could aim at identifying a protein that has a crucial, so far unknown function in the parasite that has no homolog in the human host.

Cellular functions are the result of the coordinated action of several proteins in macromolecular assemblies and the analysis of protein-complex composition is an important step in postulating possible functions for proteins. Mass spectrometry based proteomic tools coupled to tandem affinity purification (TAP) protocols have proven to be successful in the identification of multicomponent complexes formed under native conditions [59]. The TAP procedure is an affinity purification technique originally developed in yeast [60] that produces tagged fusion-protein targets and enables the purification of the target protein in complex to its partners under close-to-physiological conditions. Protein complex composition is then determined by mass spectrometric protein identification. It has been successfully applied in the analysis of protein-protein interaction networks in prokaryotic and in eukaryotic cells [61,62] and a recent dual-affinity tags modification of

TAP has improved the procedure specificity and has resulted in a tenfold increase in protein–complex yield [63]. This allowed purification of protein complexes that were hitherto not amenable to TAP, and permitted the use of less starting material, enabling systematic interaction proteomics projects.

Protein complexes in the cell usually bring together protein partners in a functional pathway, such as enzymes, their modulators and regulators. We believe that the field of schistosome proteomics could aim at finding crucially important, novel drug and vaccine targets among the proteins of as yet unknown function that would be determined by TAP or dual-tag TAP to be part of certain protein complexes. A challenge for this kind of experiments resides in the development of novel protocols that would enable a stable transformation of worms with expression

vectors encoding *S. mansoni* tagged or dual-tagged fusion-proteins.

Conclusions

Several proteomic studies in schistosomes have been performed in the past five years in an attempt to identify proteins involved in crucial processes for the parasite biology. Integration of proteomic data with those generated by microarrays is permitting a change of paradigm for the proposal of new targets for schistosomiasis treatment. In fact, databases integrating the molecular information for schistosomes are now becoming available [64–66] and should enable the entire scientific community to elect candidates for further investigation of their potential as drug targets or vaccine candidates.

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