

REVIEW

Structure–function analysis of apical membrane-associated molecules of the tegument of schistosome parasites of humans: prospects for identification of novel targets for parasite control

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Neglected tropical diseases are a group of some 17 diseases that afflict poor and predominantly rural people in developing nations. One significant disease that contributes to substantial morbidity in endemic areas is schistosomiasis, caused by infection with one of five species of blood fluke belonging to the trematode genus *Schistosoma*. Although there is one drug available for treatment of affected individuals in clinics, or for mass administration in endemic regions, there is a need for new therapies. A prominent target organ of schistosomes, either for drug or vaccine development, is the peculiar epithelial syncytium that forms the body wall (tegument) of this parasite. This dynamic layer is maintained and organized by concerted activity of a range of proteins, among which are the abundant tegumentary annexins. In this review, we will outline advances in structure–function analyses of these annexins, as a means to understanding tegument cell biology in host–parasite interaction and their potential exploitation as targets for anti-schistosomiasis therapies.

LINKED ARTICLES

This article is part of a themed section on Annexins VII Programme. To view the other articles in this section visit <http://dx.doi.org/10.1111/bph.2015.172.issue-7>

Abbreviations

Anx, annexin; NTDs, neglected tropical diseases; RA, radiation attenuated; *Sm*, *Schistosoma mansoni*; TEMs, tetraspanin-enriched microdomains; TSP, tetraspanin

Table of Links

TARGETS		
Other protein targets ^a	Transporters ^b	Enzymes ^c
Heat shock proteins	Calcium pump	Calpain

This Table lists key protein targets in this document, which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (^{a,b,c}Alexander *et al.*, 2013a,b,c).

Neglected tropical diseases (NTDs)

NTDs include some 17 lesser known chronic infections that affect poor and disenfranchised people, primarily, but not exclusively, in developing nations (Hotez *et al.*, 2007; Hotez and Fenwick, 2009). Chronic infections caused by NTDs lead to many adverse outcomes in affected populations and contribute substantially to human morbidity. In addition to microbial and protozoan diseases, NTDs include a number of helminth infections, such as diseases caused by flatworm parasites, notably schistosomiasis, echinococcosis and liver fluke diseases, as well as roundworm parasites, such as the major soil transmitted helminth infections (ascariasis, trichuriasis and hookworm diseases). Although no individual NTD rivals the major infectious threats of HIV, malaria or tuberculosis in terms of global impact of disease, collectively, the NTDs contribute substantially to morbidity throughout the world (Engels and Savioli, 2006).

A number of factors present major challenges for the development of new treatments for NTDs. Firstly, NTDs are chronic diseases, which may reside in affected people as life-long infections. Secondly, NTDs are not always associated with human mortality and the burden of these diseases can be subtle, hidden among such other measures of disease burden as hindered development, poor cognitive function and chronic ailments. Thirdly, as stated, NTDs often affect the poorest of the poor, people often unable to pay for medical treatments, especially for chronic illnesses. Hence, these diseases receive less attention than other, immediately life-threatening infectious diseases. Lastly, poor development of infrastructure systems in impoverished countries also irreversibly impacts on efficient drug distribution for the treatment of these NTDs (Chimbari *et al.*, 2004).

Among the NTDs of major interest is the suite of diseases known as human schistosomiasis. These diseases are caused by infection with any of a number of species of the genus *Schistosoma*, a taxon of platyhelminth trematodes, commonly known as blood flukes (and historically, known as the agents of bilharzia) (Ross *et al.*, 2002). Five species are the main contributors to human schistosomiasis, *Schistosoma mansoni* (*Sm*), *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. haematobium*. Transmission of the parasites to humans takes place in freshwater, typically in regions of poor sanitation where human excreta contaminate water bodies. The egg hatches in freshwater to liberate a larva, which searches for and infects a species of snail. Schistosomes, like other trema-

todes, display high host specificity for their snail host. The distribution of a schistosome species is largely dependent on the geographic distribution of its snail host.

Schistosomes infect over 200 million people in approximately 74 nations, the majority of which are in Sub-Saharan Africa and the Middle East (Steinmann *et al.*, 2006). Distinct foci of schistosomiasis also occur in Asia (China, the Philippines, along the Mekong River and Indonesia), as well as South America (notably Brazil and some Caribbean Islands). Disability-adjusted life years lost to human schistosomiasis in 2010 were measured at 48/100 000, an increase of 20% on estimates made in 1990 (Murray *et al.*, 2013).

There is a distinct dichotomy in schistosomiasis in relation to the host responsiveness to various life stages. On the one hand, the invasive larvae and adult parasites are largely able to avoid immunosurveillance of the hosts. To that effect, these parasites employ a series of strategies including rapid development, stealth-like host interfaces and immunosuppression (Wilson, 2009). On the other hand, the active secretion of immunogenic molecules by eggs provokes an intense immune response (Burke *et al.*, 2009). This phenomenon is characterized by a strong granulocytic response around the egg in affected tissues that may lead to fibrosis, particularly in the liver. The intense response enables the escape of the eggs from the host. The cellular infiltrate forces a schistosome egg across the vascular endothelium and into tissues of luminal organs, such as the intestinal lining, the bladder wall or genital organs, driving the egg ultimately into the lumen, from which the egg is voided into the environment. The bulk of chronic disease in schistosomiasis is related to host responses against parasite eggs deposited in the blood vessels surrounding the gut (*Sm* and *S. japonicum*) or bladder and genital organs (*S. haematobium*) (Ross *et al.*, 2002). However, it has proven more effective to direct control towards killing adult worms or the invasive larvae that establish infection so that the deposition of eggs is stopped.

Schistosomes belong to the Clade Lophotrochozoa of the Kingdom Animalia. The multicellular animals are monophyletic (Walker *et al.*, 2011) and there are substantial similarities among the many cellular, biochemical and molecular adaptations in different animal clades. The search for effective treatments against schistosomiasis thus needs to exploit key molecular and conformational differences between target molecules of these parasites and their hosts. This review explores work focused on the search for novel molecular targets of therapeutics and prophylactics, and examines new

insights from studies of a primary site of host interaction, the schistosome tegument. Some annexins of schistosomes are abundant molecules in the proteome of the schistosomes. These proteins are found in close association with the apical membrane of the tegument of the parasites. In view of their abundance, distribution and distinctive structure, these proteins are of interest, both as targets and as vehicles to understand the dynamic nature of the apical membrane complex of these parasites, a complex that is crucial for survival of the parasites in their hosts.

Treatments for schistosomiasis – drugs

There currently exist few drugs for treatment of schistosomiasis: praziquantel, metrifonate, oxamniquine and artemether (Cioli *et al.*, 1995; Ross *et al.*, 2002; Bartley *et al.*, 2008). All of these drugs have proven useful for therapeutic treatment of individuals in the clinic or of communities in mass drug administration. Of the four drugs, oxamniquine is only effective against schistosomiasis mansoni; resistance to this drug by the parasite is known and the mechanism of resistance elucidated (Valentim *et al.*, 2013). Metrifonate is only effective against urinary schistosomiasis, caused by *S. haematobium*, and its use is hampered by a complex administration schedule with multiple doses required over a 2 week period. Frequently, this therapy is met with a low rate of compliance among patients. Furthermore, the drug is labile in warm climates and is thus less useful in field settings. Combination therapy using praziquantel and metrifonate has been effective for urinary schistosomiasis (Danso-Appiah *et al.*, 2009).

Artemether is a β -methyl ether derivative of artemisinin, a compound derived from the sweet wormwood *Artemisia annua*. Artemisinin and its derivatives are highly effective against haematophagous parasites, notably malaria, but they have also proven effective against schistosome infection (Liu *et al.*, 2012). One recent report suggests that artemisinin is acted upon by elemental iron in the iron-rich environment of haematophagous parasites and the complex, in turn, inhibits calcium transport (Shandilya *et al.*, 2013). Concerns about resistance to artemisinin and its derivatives by the more insidious human disease of falciparum malaria has precluded the use of artemether against schistosomiasis where the two diseases are co-endemic (Bergquist *et al.*, 2005; Utzinger *et al.*, 2007).

The current drug of choice for treatment of schistosomiasis is praziquantel. This drug has been used in mass treatment campaigns in many countries and remains a primary tool in the war against the disease (Knopp *et al.*, 2013). The mode of action of praziquantel remains unknown, although recent developments strongly suggest a role for the drug in calcium homeostasis in the parasites and notably in calcium transport complexes (Greenberg, 2005; You *et al.*, 2013). The drug remains highly effective for a wide range of flatworm diseases of humans and domestic animals. Praziquantel has been deployed for mass drug administration in endemic regions and has been successful in pushing the disease from high to low endemicity (Geary, 2012). This major achievement has been facilitated in part by reductions in costs associated with manufacture of the drug, and the development of public-private partnerships that have led to the distribution of the

drug to many impoverished communities where schistosomiasis is endemic.

Despite its high efficacy, praziquantel has limitations (Geary, 2012). The drug is only effective against adult or pre-adult forms (Greenberg, 2005). Furthermore, praziquantel confers no protection against subsequent infection and people may become reinfected within days of treatment (Ross *et al.*, 2002). Treatment failures for *S. mansoni* and *S. haematobium* infections have been observed, and the presence of resistant strains has been demonstrated experimentally (Greenberg, 2013). Although widespread resistance to praziquantel has not been observed clinically, the application of the drug in mass treatment campaigns may result in new resistant forms emerging and new replacement drugs and formulations are needed (Geary, 2012).

Prevention of schistosomiasis – vaccines

Many experts within the schistosomiasis community argue that continued application of a single drug, praziquantel, for single treatments and as a mass control strategy is problematic and not likely to lead to effective control of the disease. The alternative, a subunit vaccine, has thus been promoted as an important alternative strategy for the control and elimination of schistosomiasis (Bergquist *et al.*, 2008; McManus and Loukas, 2008; Loukas *et al.*, 2011; Kupferschmidt, 2013).

Optimism for a vaccine rests on observations from the 1970s on host responses to radiation-attenuated (RA) cercariae in experimental infections (Bickle *et al.*, 1979a,b). A cercaria is the larval stage that penetrates human skin to initiate infection. This stage transforms rapidly in human skin to become a host-adapted larva, the schistosomulum. This larva then follows a set pattern of migration and development over the following days and weeks, passing along vasculature through the lung and liver. In the liver, a male parasite will mate with a female and carry her to mesenteric or pelvic circulation, the final destination being parasite species specific (Wilson, 2009). It was shown that infection of humans with live, RA parasites led to strong protection against subsequent challenge infections with normal cercariae (Correa-Oliveira *et al.*, 2000; Ribeiro de Jesus *et al.*, 2000). Vaccination of animal models with RA cercariae has thus led to an adult worm burden reduction in experimental schistosomiasis of 60–70% (Bickle *et al.*, 1979a,b; Caulada-Benedetti *et al.*, 1991; Coulson *et al.*, 1998; McManus, 1999; Dillon *et al.*, 2008). The molecular mechanism of protection with RA is unclear; however, the immune response appears to result from transcriptional suppression in the attenuated parasites during the early stage of development (Dillon *et al.*, 2008). Transcriptional suppression in RA was observed for a variety of genes including those encoding tegument proteins, members of signalling pathways associated with GPCRs, neurotransmitters and cytoskeletal components. The major lessons learned from these studies are that parasite killing is largely dependent on host-parasite interaction during the host establishment phase of the parasites, that is, within the first week after infection. During this time, the cercaria undergoes an extensive remodelling of its surface body wall, the

tegument and becomes transcriptionally active for a series of molecules associated with surface dynamics and nutrient absorption (Gobert *et al.*, 2009b), compared with the cercaria. Indeed, some of the promising vaccine candidates come from this tissue, and it seems that vaccine targeting of this layer is crucial for parasite killing.

Despite the high level of protection available with radiation-attenuated vaccines, the unstable lifespan, delivery problems and safety problems of these modified cercariae makes them unsuitable for further development as a vaccine (Bergquist *et al.*, 2008). Therefore, efforts have been directed to discover and identify suitable protective antigens from schistosomes, leading to the development of recombinant vaccines, DNA vaccines, peptide-epitope-based vaccines, multivalent vaccines and chimeric vaccines (McManus and Loukas, 2008).

Of the vaccines trialled, a number have been promoted for human trials, including the Bilvax vaccine based on a 28 kDa *S. haematobium* glutathione-S-transferase, which has entered phase 3 clinical trials, and a *S. mansoni* tetraspanin (Sm-TSP-2) (Tran *et al.*, 2006), which has entered phase 1 trials (Loukas *et al.*, 2011; Kupferschmidt, 2013). Other vaccines presented at a recent vaccine discovery workshop sponsored by the Bill and Melinda Gates Foundation in the United States (Kupferschmidt, 2013) identified additional vaccines still in experimental development, including Sm14, a fatty acid binding protein, a calpain (Smp80) from *S. mansoni*, and Sj23, a TSP, a triose-phosphate isomerase, an insulin receptor, and paramyosin from *S. japonicum* (Zhu *et al.*, 2004; 2006; Siddiqui *et al.*, 2005; Tendler and Simpson, 2008; You *et al.*, 2012). An advantage of vaccination strategies against the zoonotic *S. japonicum* is that the parasite is found in a variety of domesticated animals, including water buffalo and goats in China. Researchers involved in controlling this species in China and the Philippines have developed vaccines for use in animals as transmission-blocking vaccines, based on modelling of transmission dynamics in endemic regions (McManus *et al.*, 2009). Antigen discovery studies are still progressing using a variety of immunomics and proteomic approaches. It is now widely appreciated that targeted approaches are required for antigen discovery, and there is continuing interest in considering fundamental cell biological and developmental understanding with molecular advances.

The tegument of schistosomes

The tegument, or body wall, of schistosomes is a dynamic host-adapted interface between the parasite and its vascular environment. The tegument is a highly polarized syncytium and possesses functional analogy with transporting epithelia, including the gut lining or the syncytiotrophoblasts of the human placenta. The tegument plays significant roles in nutrient uptake, immune evasion and modulation, excretion, osmoregulation, sensory reception, and signal transduction (Jones *et al.*, 2004; Kusel *et al.*, 2007; Castro-Borges *et al.*, 2011). Given the importance of the schistosome tegument in nutrition and immune evasion, proteins of this surface layer are recognized as prime candidates to target for vaccine and therapeutic drug development (Loukas *et al.*, 2007).

Ultrastructure of schistosome tegument

The tegument is formed as a single syncytium that covers the entire body and is continuous with other epithelia (Figures 1–2), notably the foregut lining (Silk *et al.*, 1969). This surface cytoplasmic layer is a highly ordered structure with distinct transporting regions, secretory components and absorptive adaptations. A peculiarity of the layer is the presence of a dual membrane complex that forms the apical extremity of the tegument cytoplasm (Hockley, 1973; Hockley and McLaren, 1973; Castro-Borges *et al.*, 2011).

The developmental activity of cercarial transformation referred to above appears first and foremost to involve alteration of the apical membrane of these parasites soon after invasion (Hockley and McLaren, 1973; Skelly and Shoemaker, 1996; 2001; Keating *et al.*, 2006). The single-unit membrane of the cercaria, with its highly immunogenic glycocalyx, becomes replaced by a host-adapted dual membrane system, consisting of the membrane proper, overlain by an additional unit membrane, the membranocalyx. Although the membranocalyx is depauperate of parasite-derived proteins, the underlying membrane is decorated with abundant membrane proteins (Braschi and Wilson, 2006). Membrane repair and maintenance is an ongoing process, as evidenced by abundant cytoplasmic inclusions and molecule associated with the apical membranes.

The advantage to schistosomes in possessing a syncytial tegument is poorly understood, but appears to be an important strategy that ensures survival of parasites in the vascular environment. Invaginations of the surface membrane complex, as well as in the basal membrane of the cytoplasm (Hockley, 1973; Gobert *et al.*, 2003; Skelly and Wilson, 2006), are structural evidence of high turnover of these membranes (Brouwers *et al.*, 1999), a process that is related to nutrient uptake and a way of avoiding the host immune response by internalizing antibodies and removing possible antigenic molecules from the surface (Skelly and Wilson, 2006). Membrane internalization and translocation events are driven by a complex interplay of multiple membrane proteins including the TSP-enriched microdomains (TEMs) (Tran *et al.*, 2010; Jia *et al.*, 2014). The TEMs are protein complexes formed about a membrane-resident TSPs, which act as scaffold proteins for the multiple fusion and scission activities of plasma membrane (Hemler, 2008). For *S. mansoni*, TEM residents include a variety of proteins strongly linked to the apical plasma membrane, including schistosome annexins B30, Sm29, a dysferlin, calpain, fructose-biphosphate aldolase, heat shock protein 70 and actin (Jia *et al.*, 2014).

The tegument is supported by cell bodies that lie embedded in the parasite parenchyma (Hockley, 1973; Gobert *et al.*, 2003). The apical cytoplasm of the tegument and the cell bodies are linked by cytoplasmic bridges, which traverse the muscle bundles lying beneath the parasite tegument (Figure 1B) (Hockley, 1973; Gobert *et al.*, 2003). Tegumentary cell bodies contain the synthetic machinery of the syncytium, including endoplasmic reticulum and Golgi apparatus, and produce abundant vesicular products that are trafficked to the tegument along cytoplasmic bridges (Figure 3).

The molecular interactions driving membrane formation during transformation and in repair and renewal are far from understood, but the abundance of adaptor and chaperone

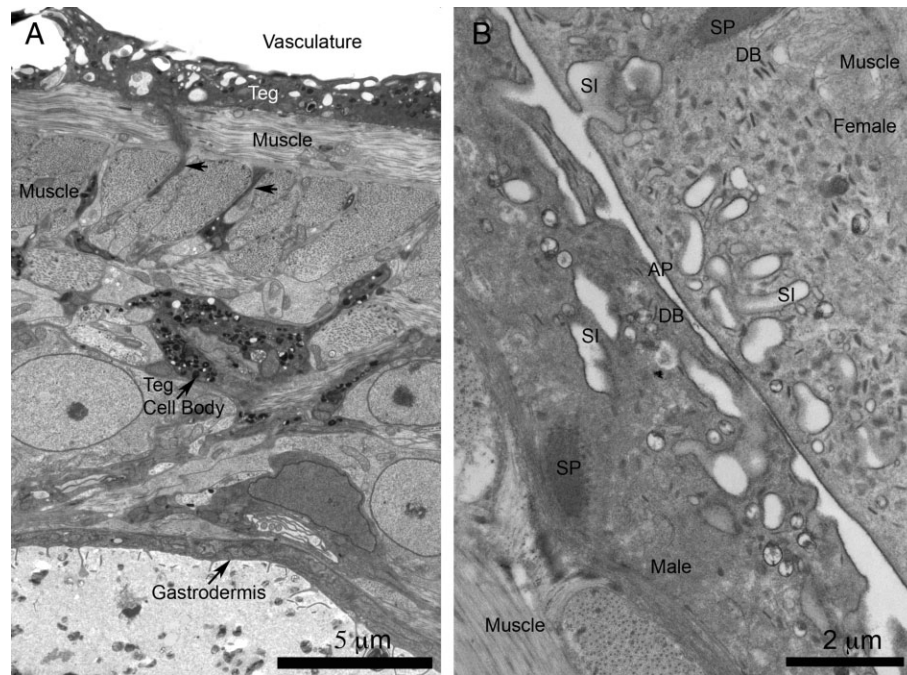


Figure 1

Tegument of *Schistosoma mansoni* by transmission electron microscopy. (A) Low magnification image from a cross-section of an adult. The image shows the apical cytoplasm of the tegument (Teg), which is the interface with the host vasculature. The syncytial cytoplasm rests on bands of musculature and is supported and maintained by tegumentary cell bodies. That depicted is rich in vesicles that are transported to the apical cytoplasm along cytoplasmic bridges (arrows). The parasite digestive system, lined by a syncytial epidermis called a gastrodermis, lies deep within the body. (B) High magnification view of the teguments of paired male and female adults. The apical membrane complex (AP) consists of a plasma membrane overlain by a subsidiary membrane-like structure, the membranocalyx, evident only after special fixation/staining of TEM tissues using uranyl acetate. The apical cytoplasm infolds frequently as surface invaginations, sometimes with secondary caveola-like outpocketings appearing. Other bodies decorate the tegument, including discoid bodies (DB). Tegumentary spines (SP), used for adhesion, are observed. Original figures.

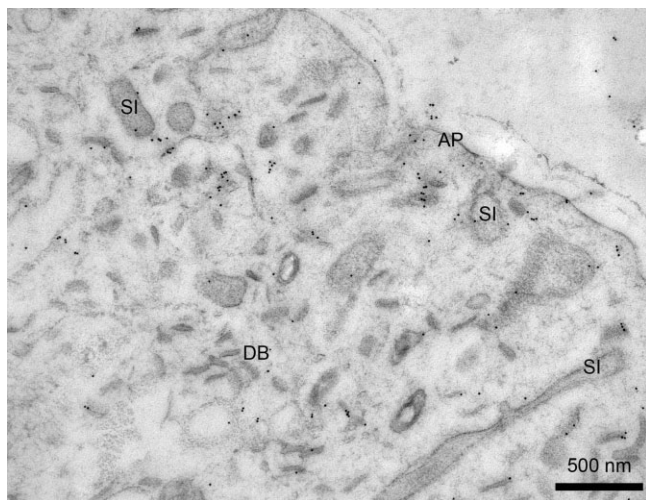


Figure 2

Immuno-electron microscopy of *Sm-Anx-B22*, transmission electron microscopy, using indirect immunocytochemistry incorporating 10 nm protein-A gold particles. *Sm-Anx-B22* was localized to surface invaginations (SI) and other membrane compartments associated with the apical plasma membrane complex (AP). After Leow *et al.* (2014).

proteins associated with the apical membrane complex (Table 1) and abundance of membrane vesicles (Figures 1–2) suggest a continuous cycle of renewal and repair throughout adult life of the parasite.

Tegument proteins as vaccine targets

In mice immunized with tegument extract of newly transformed *S. mansoni* schistosomula, an induced Th1-type protection has been observed, which damages the adult worm tegument layer and reduces egg number and parasite burden in challenge infections (Smithers *et al.*, 1990). Therefore, it is currently believed that tegument proteins of schistosomes are a priority in antigen discovery. Proteins potentially exposed at its surface during intra-mammalian stages are possibly the most susceptible targets for vaccine development (Loukas *et al.*, 2007). A challenge in studies of schistosome biology is the elucidation of when and how during the infection targets are exposed to the host immune recognition. According to models of the *S. mansoni* tegument, the primary vaccine target *Sm-TSP-2* (tetraspanin-2) occurs in the plasma membrane, that is, it lies hidden from the host under the membranocalyx (Wilson, 2012). Recent immunolocalization data suggest the molecule is even more hidden from the host, in adult parasites at least, occurring predominantly in association with surface invaginations of the hosts and in subsidiary membranes (Schulte *et al.*, 2013).

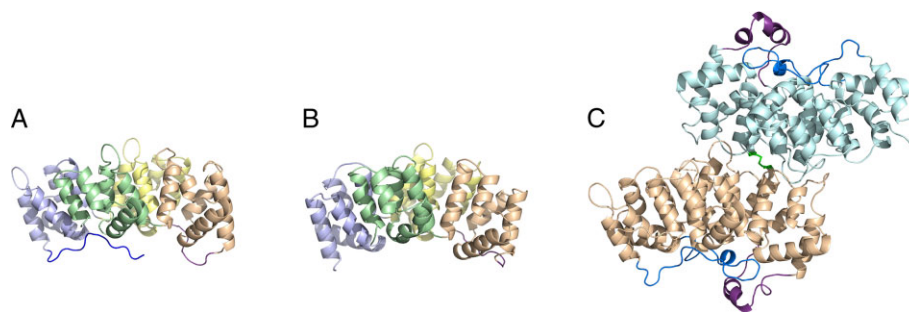


Figure 3

Cartoon representations of (A) human annexin A5, (B) α 1-giardin from *Giardia intestinalis* and (C) the dimerized *Schistosoma mansoni* annexin Sm-Anx-B22. For annexin A5 and α 1-giardin, the annexin repeats are shown in different colours. For all annexins shown the N-terminus is coloured blue and the II/III linker in magenta. Note the distinctly longer linker in Sm-Anx-B22, which packs against the N-terminal domain. The disulphide bond between Cys173 (molecule 1) and Cys173 (molecule 2) is rendered in green. Protein structures were rendered with PyMOL (DeLano, 2002).

Analysis of the schistosome proteome has vastly increased the speed of identification of tegument proteins (Braschi and Wilson, 2006; Mulvenna *et al.*, 2010; Castro-Borges *et al.*, 2011; Jia *et al.*, 2014) (Table 1). A series of experiments have allowed different proteins to be assigned to the distinct membrane fractions of the apical membrane complex (Wilson, 2012), although these assignments are likely to be crude and require further analysis by refined localization tools. A range of molecules has been identified including glucose transporters, proteases and other enzymes, receptors, chaperones and structural proteins (Mulvenna *et al.*, 2010; Castro-Borges *et al.*, 2011; Wilson, 2012). Further confirmation of the co-location of many of these molecules has come from interaction studies of Sm-TSP-2 (Jia *et al.*, 2014), which show strong interactions, as stated, with a range of surface-linked molecules including annexin B30, alkaline phosphatase, actin, an aldolase, calpain, HSP70, dysferlin and Sm29, a schistosome-specific molecule. These interacting partners widen the pool of available molecules for vaccination studies.

Among the dominant surface-related proteins is *S. mansoni* annexin B30 (hereafter Sm-Anx-B30) (Castro-Borges *et al.*, 2011; Cantacessi *et al.*, 2013; Jia *et al.*, 2014). This molecule is strongly associated with the tegument (Tararam *et al.*, 2010) and the Sm-TSP-2 TEMs, although how it binds to other proteins is undetermined. Sm-Anx-B30 lies in direct association with the apical plasma membrane (C. Leow, unpubl. obs.). Three other *S. mansoni* annexins, namely, Sm-Anx B7a, B22 and B5a, have been shown in various studies of different schistosomes to be located to the tegument. The abundance, as well as the peculiar features of annexins of some parasite groups, makes them potential targets for therapies.

Schistosome annexins

Annexins are a family of proteins that are able to bind to acidic phospholipid membranes. Their membrane-binding mode includes formation of a ternary complex involving the protein, the calcium ions and the membrane. The survey of group B annexins from different invertebrate taxa revealed that the proteins occur in the vast majority of species studied so far (Cantacessi *et al.*, 2013). The abundant annexin proteins are conspicuously evident in many parasite groups,

including a series of arthropod vectors of disease, as well as basal metazoans, but are apparently absent from others, notably the Mollusca. Using structure-based amino acid sequence alignments and phylogenetic analyses, the recent analysis provided a robust classification for this protein group, enabling information on structure–functional relationships of these proteins, as well as to assign names to sequences with ambiguous annotations in public databases (Cantacessi *et al.*, 2013). It was immediately apparent in phylogenetic analyses that gene duplication in divergent clades was the major evolutionary event in annexins' genesis, particularly in schistosomes. The highest representation of annexin was found in *S. mansoni* with 13 annexins, many distributed on two chromosomes, suggesting linkage (Cantacessi *et al.*, 2013).

Evidence gained from tissue-specific transcriptional and proteomic profiling of adult parasites suggests that the different schistosome annexins are expressed differentially throughout the body of the parasites (Gobert *et al.*, 2009a). As stated, Sm-Anx-B7a, B22 and B30 are distinctly associated with the syncytial tegument (Braschi and Wilson, 2006; Mulvenna *et al.*, 2010). Our tissue-specific transcriptomic survey of female *S. japonicum* indicated that different annexins were expressed preferentially by different cell types, with the gut lining expressing annexin B7 and B22, while the vitelline gland expressed annexin B5 (Gobert *et al.*, 2009a). Although *S. japonicum* is a distinctive parasite, as it diverged early from other species of *Schistosoma*, similar patterns of annexin expression might reasonably be expected to be conserved within the genus. The abundance of annexin B7 and B22 in gut and tegument allows the postulate that these molecules may be epithelial annexin in these parasites, and thus associated with syncytial epithelia. Importantly, both the gastrodermis and the tegument are predicted to have high membrane turnover and reshaping (Nawaratna *et al.*, 2011).

Structure–function observations of schistosome annexins

Observations made by us and others in the recent past point towards a potential use of parasite annexins as therapeutic

Table 1

Apical membrane complex-associated proteins of schistosomes

Membranocalyx associated	Proteins	Putative role
Inter-membrane space	Tetraspanin-2	Mediators of tetraspanin-enriched microdomain in surface membrane complex; membrane remodelling
	8 kDa low-molecular weight protein?	Secreted protein
	Sm13	Uncertain, membrane protein
	Sm29	Uncertain role, but potential ligand of tetraspanin
	Sm200	Uncertain role
	Bound host proteins-CD48, 90, immunoglobulins, complement factors	Host molecules
	8 kDa low-molecular weight protein?	Secreted protein
	Annexin B30	Membrane maintenance
	Alkaline phosphatase	Hydrolase
	Carbonic anhydrase	Metalloenzyme
Plasma membrane	8 kDa low-molecular weight protein?	Secreted protein
	CD59	Complement inhibition
	PDE	PDE
	Apyrase	Apyrase
	Tetraspanins	Membrane recycling, tetraspanin-enriched microdomains
	Glucose transporter (SGTP-4)	Glucose uptake
	Aquaporins	Water and small solute transport
	Na/K ATPase	Na/K ATPase
	Anion channel	
	Plasmolipins	Tetraspanin myelin proteins, membrane rafts
Cytosolic	Dysferlin	Calcium-dependent membrane fusion events; component of tetraspanin-enriched microdomains in tegument
	Calpain?	Protease
	Tegument allergen-like proteins (Sm 22.6, Sm21.7)	Dynein-like motor function; calcium binding
	GAPDH	GAPDH
	Heat shock protein (HSP70)	Chaperone
	Actin	Cytoskeleton
	Dynein light chains	Dynein-like motor function

Evidence or proposed location in the tegument is derived from the review of proteomic analyses of the tegument of *Schistosoma mansoni* by Wilson (2012). In many cases, the link between the protein and unit membrane is inferred and further experimental evidence is required. The model is based on a static two-dimensional structure only and ignores the dynamic nature of the schistosome tegument. The membrane complex is a dual membrane system, consisting of a unit membrane overlaid by an additional membrane structure, the so-called membranocalyx. Only one annexin is listed here, although it is known that multiple annexins are present in the tegument of schistosomes.

targets. These findings include (i) immunoreactivity of some parasite annexins (Hongli *et al.*, 2002; Palm *et al.*, 2003; Weiland *et al.*, 2003; Gao *et al.*, 2007; Weeratunga *et al.*, 2012; Leow *et al.*, 2014); (ii) localization of certain parasite annexins to areas of potential exposure and/or structural integrity (Braschi and Wilson, 2006; Jia *et al.*, 2014); and (iii) the existence of a unique structural feature, including the extended helical linker between repeats II and III (Figure 3), in parasite annexins that differentiates them from host annexins (Hofmann *et al.*, 2010; Weeratunga *et al.*, 2012; Leow *et al.*, 2014).

The extended linker region is a primary source of variation between some group B and group A annexins. Many

group B annexins, including those from the cestode *Taenia solium*; annexin B36 (nex-4) from the model nematode *Caenorhabditis elegans*; and some Group E annexins, including α -12- and α -19 giardin, possess an unusually long linker segment between repeats II and III on the concave side of the protein (Hofmann *et al.*, 2010) (Figure 3). Whereas the typical length of this linker in annexin ranges from 10 to 15 amino acids, the linker peptide of these groups B and E range from 25 to 38 amino acids (Hofmann *et al.*, 2010). Secondary structure predictions consistently indicate that this elongated linker region adopts an α -helical structure, and the recent crystal structure of Sm-Anx-B22 provided the anticipated experimental proof (Leow *et al.*, 2014). We hypothesize that

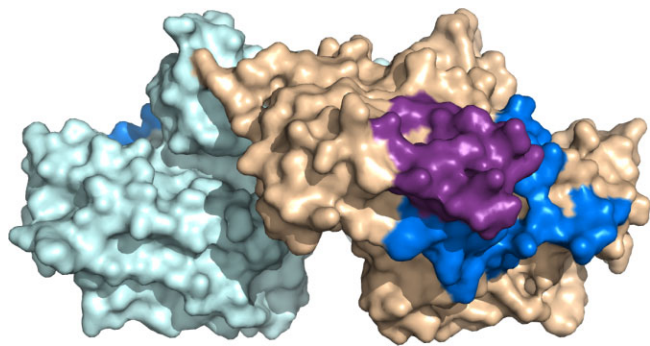


Figure 4

Surface-rendered model of *Schistosoma mansoni* annexin *Sm-Anx-B22*. A distinctive groove, lying at the junction of the two partners of the dimer (coloured light blue and tan) may give the protein an adaptor function. The N-terminus (dark blue) and II/III linker region (magenta) of one partner are shown. Figure prepared with PyMOL (DeLano, 2002).

this additional α -helical element on the concave side of the molecule may provide a target for immunological therapeutics (Hofmann *et al.*, 2010). It is tempting to speculate that other parasite annexins with an extended II/III linker peptide may adopt a very similar conformation. A comparison of the extent of the N-terminal domains for annexins with the unique linker shows that such a fold may be possible for most of them.

The crystal structure of *Sm-Anx-B22* confirms the presence of the predicted α -helical segment in the II/III linker and also reveals a covalently linked head-to-head dimer (Leow *et al.*, 2014). *Sm-Anx-B22* and its homologues from *S. japonicum* (Cantacessi *et al.*, 2013) and *S. bovis* (de la Torre-Escudero *et al.*, 2012) are the only B annexins known to date that possess an exposed cysteine residue in the IIIDE loop (Cys173), a position where most other annexins possess a serine residue. In *Sm-Anx-B22*, the involvement of Cys¹⁷³ in an inter-molecular disulphide bond as well as several intimate electrostatic side chain interactions add to the stabilization of the unique head-to-head dimer topology where the dimer interface is exclusively located in module II/III. Structurally, this is significantly different to other annexin head-to-head dimers (Hofmann *et al.*, 2010), where the dimer interface comprises the entire convex surface of both molecules.

In addition, from the calcium-bound crystal structure of *Sm-Anx-B22*, canonical as well as novel calcium binding sites can be identified, which seems to be a recurring motif in parasite annexins. Intriguingly, the dimer arrangement observed in the annexin B22 crystal structure revealed the presence of two non-anticipated prominent features: a potential non-canonical membrane-binding site and a potential binding groove opposite of the former (Figure 4).

Annexins in schistosomes

A variety of roles have been proposed for annexins. In vertebrates, annexins are known to display a broad range of

biological activities including response to inflammation, membrane traffic and adhesion, anticoagulation, signal transduction, developmental processes and membrane repair (Bouter *et al.*, 2011; Draeger *et al.*, 2011). In parasites, annexins are suggested to be involved in maintenance of membrane structure (Peattie *et al.*, 1989; Tararam *et al.*, 2010), anti-inflammatory activity (Zhang *et al.*, 2007) and fibrinolytic activity (de la Torre-Escudero *et al.*, 2012). Annexins may thus have distinct roles in enabling survival of parasites when they are within the hosts. Some annexins are speculated to be involved in redox reactions and the regulation of reactive oxygen molecules in plants (Hofmann *et al.*, 2003) (Konopka-Postupolska *et al.*, 2011) and in mammals (Tanaka *et al.*, 2004; Madureira *et al.*, 2011; Madureira and Waisman, 2013).

Localization of *Sm-Anx-B22* by fluorescence and electron microscopy in different species of schistosomes (Tararam *et al.*, 2010; de la Torre-Escudero *et al.*, 2012; Leow *et al.*, 2014) demonstrates that the molecule is strongly associated with the tegument and the plasma membrane structures of the apical regions of the tegument of adult parasites (Figure 2). The molecule is expressed in human-parasitic phases of the parasite life cycle, suggesting a major role in surface membrane dynamics during life in the human host. Although *Sm-Anx-B22* shares many structural similarities with other annexins, its dimeric nature as well as the unique extended linker region suggests that this molecule is co-adapted to function in the peculiar syncytial environment of the tegument of these parasites (Leow *et al.*, 2014). Among the peculiarities, there is a prominent groove that occurs within the dimeric species (Figure 4). This groove is postulated to enable the *Sm-Anx-B22* dimer to assume an adaptor function, linking the apical membrane complex with proteins.

Sm-Anx-B22 possesses another unique feature, namely, the external arrangement of the II/III linker that is reflexed over the N-terminal region of the molecule. There is now substantial evidence that annexins, and indeed other molecules of the schistosome tegument, adopt unique conformations that might be exploited for therapeutics or prophylaxis as they distinguish the parasite proteins from homologous proteins in the host.

The question remains as to how these unique regions might be targeted if we are to develop anti-schistosomiasis therapies directed against these molecules. Undoubtedly, as with all areas of investigation concerning annexins from a wide variety of organisms, more structure–function analyses of the proteins in cells is required. For schistosomes the peculiarities of the annexins, including the extended II/III linker regions, non-canonical calcium binding sites and other molecular anomalies are of interest, not only for enhancing fundamental understanding of membrane dynamics, but also for designing anti-parasite targets.

Being highly adapted to life within hosts, helminth parasites present considerable difficulties in functional genomics analyses. They are not easily cultivated outside of the host and require molecular signalling from their host to develop fully. Furthermore, transgenesis studies for schistosomes remain in their infancy, although these parasites are amenable to RNA-interfering technologies. Thus, studies of annexins of schistosomes present some challenges. Two important

outcomes of the recent comparative analysis of invertebrate annexins (Cantacessi *et al.*, 2013) is the occurrence of common structural motifs in some group B (invertebrate) and group E (*Giardia*) annexins and the growing diversity of annexins among the single celled protists. The encouraging result suggests that there is substantial information to be gained from comparative studies among parasites that are less tractable in laboratory models and readily culturable invertebrate model species and parasites. These comparative structure–function investigations as models for understanding annexins' function at the host–parasite interface, as heterologous expression systems of parasite annexins and as targets of inhibitor and drugs assays, will prove invaluable as we move towards developing targeted therapies for parasites of socio-economic importance.

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Conflict of interest

None.

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