



# Exploring the function of protein kinases in schistosomes: perspectives from the laboratory and from comparative genomics

Anthony J. Walker\*, Margarida Ressurreição and Rolf Rothemel

Laboratory of Molecular Parasitology, School of Life Sciences, Kingston University, Kingston upon Thames, UK

## Edited by:

Paul J. Brindley, The George Washington University, USA

## Reviewed by:

Amparo Latorre, University of Valencia, Spain

Paul J. Brindley, The George Washington University, USA

## \*Correspondence:

Anthony J. Walker, Laboratory of Molecular Parasitology, School of Life Science, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK  
e-mail: t.walker@kingston.ac.uk

Eukaryotic protein kinases are well conserved through evolution. The genome of *Schistosoma mansoni*, which causes intestinal schistosomiasis, encodes over 250 putative protein kinases with all of the main eukaryotic groups represented. However, unraveling functional roles for these kinases is a considerable endeavor, particularly as protein kinases regulate multiple and sometimes overlapping cell and tissue functions in organisms. In this article, elucidating protein kinase signal transduction and function in schistosomes is considered from the perspective of the state-of-the-art methodologies used and comparative organismal biology, with a focus on current advances and future directions. Using the free-living nematode *Caenorhabditis elegans* as a comparator we predict roles for various schistosome protein kinases in processes vital for host invasion and successful parasitism such as sensory behavior, growth and development. It is anticipated that the characterization of schistosome protein kinases in the context of parasite function will catalyze cutting edge research into host-parasite interactions and will reveal new targets for developing drug interventions against human schistosomiasis.

**Keywords:** *Schistosoma*, kinome, *Caenorhabditis elegans*, kinase function, cell signaling, schistosomiasis

## PROTEIN KINASES AND SCHISTOSOMES—AN OVERVIEW

Protein kinases are pivotal regulators of cellular function. When activated, these signaling enzymes phosphorylate transcription factors and other intracellular proteins leading to alteration in gene expression and/or other cellular behavior/activities. Elucidating functional roles for protein kinases in humans and other organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* has sometimes been challenging, particularly because protein kinase pathways can drive multiple functions and influence one another through cross talk (e.g., Krishna and Narang, 2008), the nature of which is often dependent upon “input” signal(s). This presents a conundrum when trying to characterize the functions of protein kinases in organisms that have only relatively recently entered the protein kinase research arena. Schistosomes, which are human blood parasites, arguably fall into this category. The importance of schistosomes, particularly *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobium*, is highlighted by the fact that they are responsible for causing the neglected tropical disease human schistosomiasis in over 200 million people worldwide (Steinmann et al., 2006).

The life cycle of these schistosomes is complex (Walker, 2011). Paired adult male and female worms living in the blood vessels of the human definitive host produce eggs that are excreted and hatch in fresh water releasing a miracidium that infects an aquatic intermediate host snail. In the snail the miracidium develops into a mother and then daughter sporocysts that produce cercariae; when released into water these cercariae search out and penetrate

the skin of the human host. Each cercaria then transforms into a schistosomule which subsequently enters the circulation and develops into juvenile and then adult worms that pair to stimulate full maturation and egg laying. Considerable morphological and physiological differences exist between these separate schistosome life-cycle stages (Walker, 2011) and thus protein kinase function may be different in each individual life stage, adding a further layer of complexity to role characterization. Also, each life stage will intercept different “input” signals from its environment (Walker, 2011). Such signals will include: light and host-derived surface molecules in the free-living swimming stages (cercariae and miracidia); signaling molecules (e.g., growth factors) in the blood of the endoparasitic stages of both the snail (post-miracidia and mother/daughter sporocyst) and human (schistosomule and adult worm) host; and differences in osmolarity and/or temperature during infection of/release from the host. Furthermore, an immortalized schistosome cell line does not exist meaning that cellular experiments need to be conducted with whole schistosomes, fractions, or lysates thereof. Nevertheless, while challenging, defining roles for kinases in this fascinating parasite will be rewarding, enabling questions concerning cellular regulation, development and homeostasis, particularly during key life-stage transitions to be answered. Such research is also important to develop strategies focusing on drug-mediated kinase modulation in the parasite for therapeutic intervention.

*Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* genome and transcriptome projects [e.g., (Hu et al., 2003; Verjovski-Almeida et al., 2003; Berriman et al., 2009;

The *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009; Protasio et al., 2012; Young et al., 2012] have provided crucial sequence and expression data to support research into schistosome signaling, and 252 putative protein kinase genes have been found in *S. mansoni* (Andrade et al., 2011). However, the number of protein kinases encoded by these genes remains unknown because of the possibility of alternative splicing. This phenomenon, which increases proteome complexity, likely provides over 900 protein kinases from 445 protein kinase genes in humans, with 209 genes encoding a single kinase (Anamika et al., 2009). Alternative splicing results in potentially diverse functions because protein kinase splice variants can possess different domain architectures (Anamika et al., 2009).

Schistosomes possess putative kinases from all eight main eukaryotic protein kinase groups (Andrade et al., 2011). They also possess upstream receptors and endogenous signaling molecules (Osman et al., 2006; Khayath et al., 2007; Berriman et al., 2009; Oliveira et al., 2009; The *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009; Zamanian et al., 2011; Young et al., 2012). Importantly, in the context of host-parasite interactions, schistosomes have been shown to respond to human insulin (You et al., 2009), transforming growth factor (TGF)- $\beta$ 1 (Osman et al., 2006), and tumor necrosis factor (TNF)- $\alpha$  (Oliveira et al., 2009), demonstrating that they can bind host signaling molecules and transduce input signals through intact pathways. Here, perspectives on unraveling kinase function in schistosomes are provided that stem from techniques in cell biology developed for other organisms and from comparative genomics using *C. elegans* as a model.

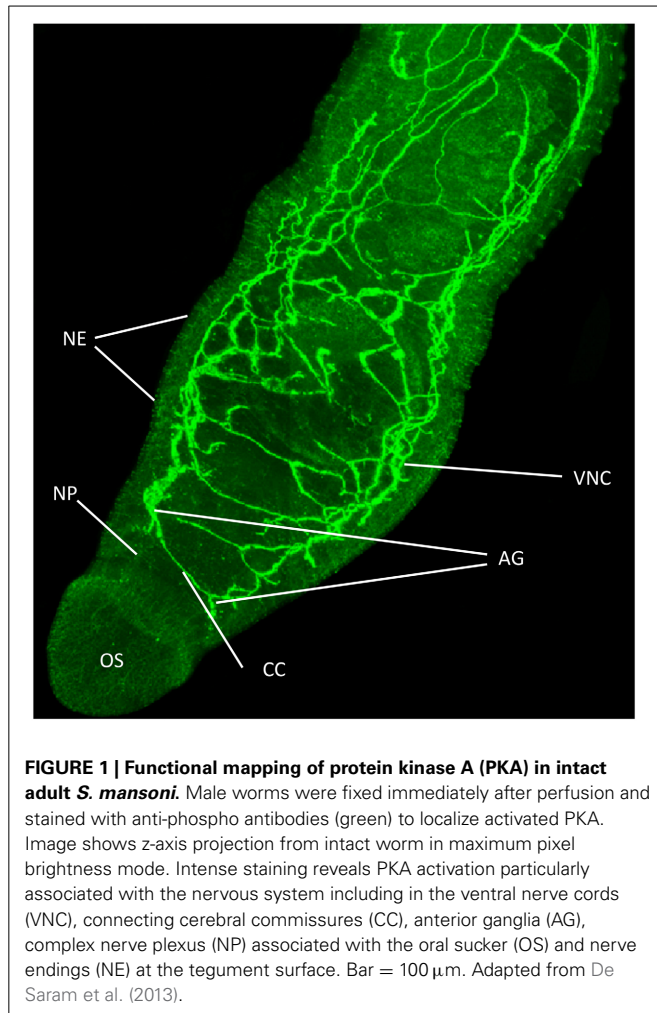
## SCHISTOSOME FUNCTIONAL KINOMICS—PERSPECTIVES FROM THE LABORATORY

### STUDYING ACTIVATED PROTEIN KINASES IN SCHISTOSOMES

In our laboratory we have employed “smart” phospho-specific antibodies (Bonetta, 2005) to detect functionally activated protein kinases in life stages of *S. mansoni*. These antibodies, first produced by Cell Signaling Technology (CST; www.cellsignal.com) detect key phosphorylation sites (Tyr, Thr, or Ser) within the kinase that are critical for function. Although such antibodies are generated against phosphorylated human peptide sequences (typically 11–13 amino acids around the phosphorylation site), a number of such sequences are well conserved in kinases of invertebrates allowing certain antibodies to be used after careful validation. For example, anti-phospho antibodies against the mitogen-activated protein kinases (MAPKs) have been used widely in *D. melanogaster* and *C. elegans* (e.g., Gabay et al., 1997; You et al., 2006; Zhuang et al., 2006), and in snails (Plows et al., 2004, 2005) including *B. glabrata*, host to *S. mansoni* (Zahoor et al., 2008). In our approach (Ressurreição et al., 2011a) we first align the predicted *Schistosoma* kinase protein sequence (from www.GeneDB.org) with the orthologous sequence for the respective human kinase and search for conserved site(s) containing the important phosphorylation motif(s). We next select appropriate antibodies (e.g., from CST) and screen them using western blotting with extracts from untreated live parasites, and live parasites

treated with an activator for the kinase in question (e.g., anisomycin for p38 MAPK). Increased immunoreactivity of the kinase from the exposed parasite samples provides the first indication of antibody suitability and of functional conservation of the phosphorylated site in schistosomes. Antibodies are then further validated before they are used routinely. This involves experiments such as inhibition assays to block upstream activators of the kinase or block direct kinase activation (Ludtmann et al., 2009), and if possible immunoprecipitation of the phosphorylated kinase followed by kinase assay. In principle, by raising antibodies against phospho-peptides identical to schistosome protein kinase sequences it is possible to produce schistosome-specific anti-phospho antibodies; however, this requires significant investment without knowing whether the phospho site is functionally active. A major benefit of using anti-phospho antibodies is that it is possible to study protein kinase activation with small quantities of protein (e.g., from ~750 schistosomules or one adult worm pair) using western blotting. While for some schistosome protein kinases activity has been determined using activity assays with conserved kinase substrates (Wiest et al., 1992; Swierczewski and Davies, 2009, 2010), the quantity of protein needed is usually considerably greater. A further benefit of phospho-specific antibodies is that they often work in immunohistochemistry allowing the exclusively activated kinase (rather than the just the protein) to be visualized within the intact parasite using fluorescence-based confocal laser scanning microscopy (Figure 1). For this we have coined the term “functional mapping” (De Saram et al., 2013) given that only the activated kinase is detected. This can be valuable to elucidate the apparent distribution of the activated kinase within the parasite enabling hypotheses concerning function to be formulated and subsequently tested (see below). To date we have employed anti-phospho antibodies in *S. mansoni* to explore the kinetics of protein kinase C (PKC) and p38 MAPK activation in miracidia during development to mother sporocysts (Ludtmann et al., 2009; Ressurreição et al., 2011b), to help demonstrate a role for p38 MAPK in miracidia motility (Ressurreição et al., 2011a), and to study protein kinase A (PKA) function in adult worms (De Saram et al., 2013). Our current research using anti-phospho antibodies is focusing further on these pathways and others [e.g., AKT and extracellular signal-regulated kinase (ERK)] in cercariae, schistosomules, and adult worms.

We anticipate that the above tools will also help enable certain protein kinase-mediated signaling pathways to be delineated. Putative pathway maps have been constructed incorporating some schistosome protein kinases including MAPK pathways and others (Wang et al., 2006; Dissous et al., 2007; Berriman et al., 2009; Beckmann et al., 2010b). These are largely predicted using *in silico* data for schistosome pathway components, comparative mechanisms in vertebrates and, in some cases, interaction data from schistosomes obtained using valuable yeast two/three-hybrid screening and immunoprecipitation experiments [e.g., for Src and syk tyrosine kinases and polo-like kinases (Plks) (Quack et al., 2009; Beckmann et al., 2010a,b; Long et al., 2012)]. However, experimental data delineating routes of pathway activation in schistosomes remain negligible and need to be expanded to develop functional pathway maps.



### DECIPHERING PROTEIN KINASE FUNCTION IN INTACT SCHISTOSOMES

Current strategies for interfering with protein kinases to elucidate function directly within schistosomes focus largely on pharmacological inhibition and conventional RNA interference (RNAi) by double stranded RNA. Probably the most controversial aspect of using kinase inhibitors is their potential to affect “non-target” kinases, particularly at high concentrations (Anastassiadis et al., 2011). However, when functional experiments are performed on intact parasites inhibitor IC<sub>50</sub>'s have little meaning as their values are normally derived from cell-free or single-cell assays. To help mitigate possible non-target effects, careful selection of kinase inhibitors is needed, particularly as they often have different specificities. Sometimes sites of protein-inhibitor interaction have been mapped as illustrated by SB203580 and its target p38 MAPK (Gum et al., 1998; Wang et al., 1998). Such knowledge is valuable as it enables one to ascertain whether the critical residues are conserved in the schistosome kinase, as was found for *S. japonicum* p38 MAPK during our recent work (Ressurreição et al., 2011a). Moreover, performing inhibition experiments with subsequent kinase activity screening (see above) further validates inhibitor use. Experiments with inhibitors/activators have supported roles for kinases in *S. mansoni* including in: (1)

miracidia/sporocysts, in which p38 MAPK (Ressurreição et al., 2011b) and PKC (Ludtmann et al., 2009) inhibition restrict and accelerate miracidia-to-mother sporocyst development, respectively, and activation of p38 MAPK attenuates miracidial ciliary motion (Ressurreição et al., 2011a); (2) cercariae and adult worms, in which PKA inhibition kills the parasite (Swierczewski and Davies, 2009, 2010); and (3) adult worms, in which PKA activation stimulates neuromuscular movement (De Saram et al., 2013), insulin and venus kinase receptor inhibition restricts feeding, egg-laying, and results in death (Vanderstraete et al., 2013), and SmTK4 and Plk inhibition suppresses gametogenesis (Beckmann et al., 2010a; Long et al., 2010). Moreover, inhibition and transcriptomic analysis have recently been used to identify a co-operative role for Src kinase and TGF $\beta$  in eggshell formation (Buro et al., 2013). By coupling outcomes of pharmacological experiments with *in situ* functional mapping (De Saram et al., 2013)/*in-situ* hybridization (Long et al., 2012), further confidence in terms of identified role can be achieved. Thus, inhibitor-based assays have an important place in functional schistosome kinomic research when used appropriately, and are particularly useful in short-term experiments as RNAi-mediated knockdown of a protein can take several days.

Conventional RNAi has become an invaluable tool for studying protein function in various life stages of *Schistosoma* species (Kalinna and Brindley, 2007; Stefanić et al., 2010; Rinaldi et al., 2011). This approach has been used to silence a large number of schistosome proteins including leucine aminopeptidase, involved in hatching of miracidia (Rinaldi et al., 2009), tetraspanins 1 and 2 that regulate tegument integrity (Tran et al., 2010), cathepsin B, important to schistosome growth (Correnti et al., 2005), and aquaporin, involved in excretion of metabolic waste across the tegument (Faghiri et al., 2010). Challenges with RNAi in schistosomes, however, remain and are considered elsewhere (Stefanić et al., 2010; Dalzell et al., 2012). These include the transient nature of RNAi, variable knockdown between individual parasites, and “knock-down” rather than “knock-out” of gene function, all of which can complicate phenotype analysis. To-date, there are relatively few reports of schistosome protein kinases being targeted by RNAi. These include knockdown of PKA, SmTK4, fibroblast growth factor receptor, TGF $\beta$  receptor II and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II, found important for viability, gametogenesis, maintenance of neoblast-like cells, male-female reproductive development, and regulation of praziquantel induced calcium influx in adult worms, respectively (Osman et al., 2006; Swierczewski and Davies, 2009; Beckmann et al., 2010a; Collins et al., 2013; You et al., 2013). Importantly, the interconnected nature of kinase signaling is such that phenotypes caused by RNAi-mediated kinase depletion presumably reflect the aggregate biological consequence of dysregulation of several pathways (Sopko and Andrews, 2008). Furthermore, phenotypes may also be masked by increased compensatory expression and subsequent activation of other isoforms and pathways in the face of suppression of any one isoform. Thus, although conventional RNAi is valuable for schistosome kinomics research, interpreting phenotype outcomes can be more challenging than for single gene/single function proteins. Nevertheless, in principle, as proposed for mammals (Moffat and Sabatini, 2006), it should



be possible to perform high-throughput RNAi-based screening to delineate schistosome signaling pathways, using downstream phosphorylation events as “readouts” for depletion of “upstream” components such as kinases. Although an immortalized schistosome cell line might appear essential for such experiments, we consider progress could also be achieved using primary cell cultures derived from mechanically or enzymatically dissociated schistosome tissues, and possibly even totipotent stem cells recently identified in this parasite (Collins et al., 2013; Wang et al., 2013).

## SCHISTOSOME FUNCTIONAL KINOMICS—PERSPECTIVES AND PREDICTIONS FROM COMPARATIVE GENOMICS

Given that protein kinases have been conserved through evolution, valuable insights into their possible functions in schistosomes can also be gleaned by considering roles for orthologous kinases in organisms in which functional genomics is more advanced. Here, selected *S. mansoni* protein kinases displaying orthology to protein kinases of the well-characterized *C. elegans* have been chosen (Table 1) to illustrate how comparative genomics can help build hypotheses for testing protein kinase function in schistosomes.

*Caenorhabditis elegans* dauer larvae exhibit distinct morphological and behavioral characteristics in response to environmental duress. AKT-1 and AKT-2, components of the insulin-like pathway, phosphorylate and inhibit the FoxO transcription factor DAF-16 (Paradis and Ruvkun, 1998; Hertweck et al., 2004) regulating dauer formation and lifespan (Hu, 2007), such that DAF-16/FoxO activation in the intestine affects lifespan whereas neuronal activity affects dauer formation (Libina et al., 2003). The orthologous *S. mansoni* Akt gene (Smp\_073930) is particularly expressed in the schistosomules and adult worms (Table 1); it is therefore possible to predict that Akt may regulate lifespan in *S. mansoni*. Interestingly, Smp\_172240 is orthologous for a c-Jun N-terminal kinase (JNK-1) in *C. elegans*, which acts together with the insulin-like pathway and converges with DAF-16 to further promote lifespan regulation. In fact, *C. elegans* Jnk-1 mutants have reduced lifespan and increased tolerance to heat stress. JNK-1 modulates translocation of DAF-16 to the nucleus where it promotes expression of specific genes to combat environmental stress (Oh et al., 2005). Compared to cercariae, JNK-1 is more highly expressed in 3 h schistosomules (Table 1), which coincides with the considerable environmental change experienced by schistosomes upon entering the warm-blooded definitive host. The presence of *Schistosoma* insulin-like and JNK pathway components, vital to lifespan regulation in *C. elegans*, warrants investigations to decipher whether AKT and JNK regulate growth and development in schistosomes and whether these proteins co-operate in a similar manner. Thus, during our on-going investigations into AKT signaling in schistosomes we shall use the *C. elegans* knowledge base to build and test hypotheses concerning AKT function in different *S. mansoni* life stages.

Smp\_065290, orthologous with CAM Kinase-1 in *C. elegans* and responsible for thermosensory behavior (Table 1), has raised expression in cercariae compared to 3 h schistosomules, consistent with cercariae being stimulated by a rising

temperature gradients (Cohen et al., 1980). Although *C. elegans* CAMK-1 mutants display thermosensory defects, the mechanisms by which CAMK-1 operates remain to be characterized fully (Satterlee et al., 2004). Disruption of this gene in schistosomes would be interesting to reveal whether it mediates host searching/cercarial penetration, possibly together with JNK-1 (orthologous to Smp\_172240) that might play a role in thermosensation given its importance to thermotolerance in *C. elegans* (Table 1).

*Schistosoma mansoni* novel PKC $\epsilon$  (Smp\_131700; Table 1) is orthologous to *C. elegans* novel PKC-1 that plays a role in salt attraction (Adachi et al., 2010). Expression analysis (Table 1) suggests this gene is important in cercariae and early schistosomules. Increasing salinity promotes transformation of cercariae to schistosomules (Samueleson and Stein, 1989), thus, Smp\_131700 provides an interesting target for phenotype disruption studies focusing on successful host infection by schistosomes. In *C. elegans*, PKC-1 is involved with ASE-right (ASER) neurons, which sense the Cl ion of NaCl (Adachi et al., 2010). ASER also express the guanylate cyclase receptor, gcy-22, essential in Cl ion sensing, and *C. elegans* gcy-22 mutants show impaired ability of ASER in response to varying salt concentrations (Ortiz et al., 2006). Interestingly, PKC-1 has other roles in *C. elegans* including nose touch stimulation and locomotion with evidence suggesting it acts through the conserved ERK pathway (Hyde et al., 2011). It is thus tempting to predict a further role for *S. mansoni* PKC $\epsilon$  in mechanosensation during contact of cercariae to host skin and in schistosome locomotion. Future immunolocalization studies may reveal PKC $\epsilon$  in the nervous system of *S. mansoni* as in *C. elegans*.

Other examples illustrated in Table 1 include MAPK15, MEK1, casein kinase 1 (CK1), and vaccinia-related kinase (VRK), disruption of which in *C. elegans* is lethal to larvae, embryos, or both, and a G-protein coupled receptor kinase (GRK) that plays a role in chemotaxis, the hyperosmotic response and olfaction. The death-associated protein kinase (DAPK), expressed at high levels in all stages except 24 h schistosomules, plays an important role in epidermal and cuticle integrity and maintenance in *C. elegans* (Table 1). Given the importance of the schistosome tegument to host immune evasion and parasite survival (Van Hellemond et al., 2006), and the potential for similar effects in schistosomes, studies focusing on this kinase are warranted.

## SCHISTOSOME FUNCTIONAL KINOMICS—FUTURE PERSPECTIVES

By integrating various approaches such as those detailed above and benefiting from emerging tools like schistosome transgenesis (Mann et al., 2011), it should be possible to unravel the complexity of protein kinase signaling in schistosomes. Certain protein kinases in schistosomes may ultimately be found to have roles comparable to those in other organisms including *C. elegans*, which can currently be used to generate functional hypotheses to test [see also further lethality predictions (Andrade et al., 2011)]. However, it is worth noting that common functionality may differ particularly as different organisms possess different complements of protein kinases and downstream target proteins, and because tissue expression may differ. To illustrate differences, *in silico* reconstruction of the *S. mansoni* and *S. japonicum*

Table 1 | Selected examples of orthologous protein kinases in *S. mansoni* and *C. elegans* with functional annotations shown for *C. elegans*.

<i>S. mansoni</i> Gene	Kinase type	Group	Family	Subfamily	Target ortholog(s) in <i>C. elegans</i>	Function of orthologous kinase in <i>C. elegans</i> . Notes curated from Uniprot ( <a href="http://www.Uniprot.org">www.Uniprot.org</a> ), Wormbase ( <a href="http://www.wormbase.org">www.wormbase.org</a> ), and other referenced sources, including results of phenotype disruption experiments with <i>C. elegans</i>	References	<i>S. mansoni</i> QTE			
								Cerc	3h S	24h S	Adult
Smp_073930	Serine/Threonine Kinase	AGC	AKT		Q9XTG7 (akt2) and Q17941 (akt1)	Expressed in neurons, muscle cells of pharynx, rectal gland cells, and spermatheca. Simultaneous knockdown of akt-1 and akt-2 result in dauer formation/weak extension to life span. Role in immunity	Paradis and Ruvkun, 1998; Hertweck et al., 2004; Evans et al., 2008				
Smp_172240	Serine/Threonine Kinase	CMGC	MAPK	JNK	Q8WVG9 (jnk-1)	Expressed in neuronal cell bodies and processes. Analysis of mutant phenotype infers role in determination of adult lifespan and response to heat and oxidative stress. JNK kinase mutants exhibit locomotory defects	Kawasaki et al., 1999; Oh et al., 2005				
Smp_065290	Serine/Threonine Kinase	CAMK	CAMK1		Q9TXJ0 (CAM kinase 1)	Expressed in head and tail neurons and vulval muscles. Disruption results in changes in thermosensory behavior and temperature-dependent defects in AFD-specific gene expression	Eto et al., 1999; Kimura et al., 2002; Satterlee et al., 2004				
Smp_210820	Serine/Threonine Kinase	AGC	GRK	Beta ARK	Q09639 (grk2)	Broadly expressed in adult worm nervous system. Analysis of mutant phenotype infers role in chemotaxis, hyperosmotic response and olfaction	Fukuto et al., 2004				
Smp_131700	Serine/Threonine Kinase	AGC	PKC	Novel	P34885 (protein kinase C-like 1B)	Expressed in neurons that sense and respond to environmental signals. Disruption results in: attenuated response to nose touch stimulation, defects in salt attraction, disrupted chemotaxis, reduced dauer formation, reduced protection from hemiasterlin toxicity, reduced neuropeptide secretion	Okochi et al., 2005; Sieburth et al., 2007; Adachi et al., 2010; Zubovych et al., 2010; Hyde et al., 2011				
Smp_181490	Serine/Threonine Kinase	CAMK	DAPK	DAPK	O44997 (dapk-1)	Expressed in epidermis, muscles and neurons. Disruption reveals that beginning at L3, animals display progressive defects in morphology of the epidermis and cuticle, particularly the nose, tail, vulva, and the dorsal midline in the region of the posterior pharyngeal bulb. Thickened cuticle (5–10 times) at the expense of underlying epidermis. Up-regulates innate immune responses to damage. Decreases starvation-induced autophagy	Kang et al., 2007; Tong et al., 2009				

(Continued)

Table 1 | Continued

<i>S. mansoni</i> Gene	Kinase type	Group	Family	Subfamily	Target ortholog(s) in <i>C. elegans</i>	Function of orthologous kinase in <i>C. elegans</i> . Notes curated from Uniprot ( <a href="http://www.Uniprot.org">www.Uniprot.org</a> ), Wormbase ( <a href="http://www.wormbase.org">www.wormbase.org</a> ), and other referenced sources, including results of phenotype disruption experiments with <i>C. elegans</i>	References	<i>S. mansoni</i> QTE			
								Cerc	3h S	24h S	Adult
Smp_180400	Serine/Threonine Kinase	CK1	CK1	CK1D	Q8WQ99 (csnk-1)	Disruption results in 'dumpy' and 'small' phenotypes, egg variability, embryo and larval lethality, locomotory irregularity, paralysis and reduced brood size	Fraser et al., 2000; Simmer et al., 2003; Lehner et al., 2006				
Smp_141380	Serine/Threonine Kinase	CK1	VRK		Q19848 (vrk-1)	Expressed in germ cells, ventral nerve cord and vulval cells. Embryonically lethal. Analysis of mutant phenotype revealed VRK-1 essential for formation of the vulva, uterus and uterine seam cells and in development and maintenance of the somatic gonad and thus the germ line. Adults are sterile. Depletion leads to mitotic defects, including impaired nuclear envelope formation and baf-1 delocalization	Gorjánác et al., 2007; Klerkx et al., 2009				
Smp_134260	Serine/Threonine Kinase	CMGC	MAPK	ERK7/ MAPK15		Disruption results in larval arrest and is lethal to embryos	Rual et al., 2004				
Smp_026510	Serine/Threonine Kinase	STE	STE7	MEK1	Q10664 (mek-2)	Gene disruption reveals multiple phenotypes including: germ cell abnormalities, larval arrest, lethality, sterility, and developmental abnormality	e.g., (Sönnichsen et al., 2005; Ceron et al., 2007; Green et al., 2011)				

Relative quantitative transcript expression (QTE) data for cercariae (Cerc), 3 and 24 h schistosomules (3 and 24 h S), and adult worms were extracted from GeneDB ([www.GeneDB.org](http://www.GeneDB.org)) (Protasio et al., 2012); representing 0–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, 0.8–1.0 relative expression, respectively. Smp protein identifiers were queried in PhylomeDB ([www.phylomedb.org](http://www.phylomedb.org)) to obtain orthologous identifiers for *C. elegans* proteins, and protein sequence compared using BLAST (<http://blast.ncbi.nlm.nih.gov>). Curated data on Uniprot ([www.Uniprot.org](http://www.Uniprot.org)) and Wormbase ([www.wormbase.org](http://www.wormbase.org)) were used for phenotype annotation.

MAPK pathways (Wang et al., 2006; Berriman et al., 2009) shows that while many of the *Schistosoma* cytosolic signaling mechanisms are intact when compared to those of humans, the number of orthologous downstream transcription factors appear fewer, with members such as CREB, p53, c-Myc, and c-Jun strikingly absent. Thus, while hypothetical frameworks for kinase signaling in schistosomes may be built, they do need to be tested.

Schistosome protein kinase research is arguably in its infancy and many questions remain concerning the functional biology of these important enzymes in this parasite. To help address these issues, and to stimulate research on schistosome protein kinases, it is proposed here that key areas for on-going fundamental schistosome kinomic research should include:

- (i) Identification of upstream activators and downstream targets of schistosome protein kinases to enable functional pathway characterization. To include consideration of “input” signals such as host growth factors;
- (ii) Functional elucidation of protein kinases beyond the commonly used “gross” phenotypes (e.g., dead/alive, moving/not moving) often evaluated;
- (iii) Consideration of all life stages to fully appreciate the complexity of protein kinase signaling in this parasite. This is important because outcomes from one life-stage may offer novel insights into cellular regulation in another life-stage.

By expanding research in the above areas, it should be ultimately possible to integrate fundamental research outcomes and develop a systems level understanding of protein kinase function in schistosomes. In doing so, opportunities will emerge along the way to consider individual kinases as targets for drug-mediated chemotherapy for schistosomiasis. This may include making use of drugs that are available/being considered for therapy of other diseases such as cancer [e.g., (Ali et al., 2009)], modification of such drugs, or development of new multi-species drugs that specifically target *Schistosoma* protein kinases.

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