A Divergent Member of the Transforming Growth Factor β Receptor Family from Schistosoma mansoni Is Expressed on the Parasite Surface Membrane*

(Received for publication, November 21, 1997, and in revised form, February 9, 1998)

Stephen J. Davies‡, Charles B. Shoemaker§, and Edward J. Pearce¶

From the ‡Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853 and the §Department of Immunology and Infectious Disease, Harvard School of Public Health, Boston, Massachusetts 02115

To optimize reproductive success under the limitations determined by conditions within an individual host, parasitic helminths have evolved mechanisms that allow them to detect and respond to host factors such as species, age, sex, reproductive condition, and immune status. Using the model helminth Schistosoma mansoni, we have explored the possibility that parasitic helminths express signal-transducing receptor molecules on their surfaces. Here, we present the identification of a schistosome member of the transforming growth factor β receptor family of cell-surface receptors, the first member of this family to be identified in a platyhelminth. The putative protein kinase domain of the schistosome receptor displays up to 58% amino acid identity to kinase domains of other type I receptor serine-threonine kinases, and contains a potential “GS domain,” suggesting it is a divergent member of the type I receptor subfamily. This receptor is expressed on the surface of the parasite’s syncytial tegument and expression of receptor messenger RNA and protein is up-regulated following infection of the mammalian host. The receptor protein can be isolated in a phosphorylated form from adult parasites, which together with its surface location, suggests that it functions in transducing signals across the parasite surface membrane.

An estimated 200 million people throughout tropical and temperate regions of the world are infected with parasitic trematodes of the genus Schistosoma (1). These parasites possess complex life cycles, using humans and other mammals as definitive hosts, while various species of aquatic snails serve as intermediate hosts for larval stages. After infection of the definitive host by infective stage cercariae of Schistosoma mansoni, the immature parasites undergo a complex program of development to become sexually mature adult males and females at approximately 6 weeks post-infection. During development, the parasites migrate from their point of entry in the skin to the circulatory system and then pass via the heart and lungs to their final destination in the mesenteric veins of the hepatic portal circulation. The severe hepatic and intestinal pathology associated with schistosomiasis is caused by the vigorous host immune response to large quantities of schistosome eggs produced by pairs of adult parasites residing in the mesenteric veins.

Previous studies have suggested that successful completion of schistosome development and migration is dependent on the receipt of appropriate host-derived signals by the parasite, and on transmission of signals between male and female parasites (2–5). Since the schistosome surface is composed of a continuous cytoplasmic tegument bound externally by a lipid bilayer, we hypothesized that schistosomes may possess surface-exposed, membrane-bound receptor proteins that allow signals in the surrounding milieu to be detected. To test this hypothesis, we developed a procedure to specifically label surface proteins expressed on the outer membrane of the parasite tegument using a polar, water-soluble form of biotin (6). Parasite surface proteins could then be isolated from solubilized tegument membranes using a streptavidin affinity matrix and analyzed for potential receptor molecules. As an initial screen for receptor-like proteins, we analyzed purified parasite surface molecules for the presence of protein kinase activity, since many cell-surface receptors are known to possess intrinsic kinase activity, e.g. members of the receptor tyrosine kinase family. Using this approach, we observed that a protein kinase activity specific for serine and threonine residues copurified with schistosome surface proteins (6). The susceptibility of the kinase to biotinylation indicated that it must be exposed at the parasite surface, while the presence of a kinase domain suggested that the protein may span the parasite surface membrane, since protein kinase domains are usually intracellular. Subsequent studies using Triton X-114 to phase-separate purified surface molecules into detergent and aqueous phases have found that the surface-associated kinase partitions into the detergent phase, adding weight to the conclusion that it is an integral membrane protein. Based on these observations, we hypothesized that the surface-associated kinase may be a schistosome member of the receptor serine-threonine kinase (RSTK) family of proteins, since RSTKs are the only transmembrane proteins known to possess intrinsic serine-threonine kinase activity (7).

The RSTKs constitute a large family of cell-surface receptors, with examples now identified in widely divergent taxa of metazoa, including Caenorhabditis elegans, Drosophila melanogaster, and various vertebrates (7). Members of the RSTK...
family can be further classified as either type I or type II receptors based on structural and functional characteristics. Mammalian members of this family include the type I and type II receptors for growth factors such as transforming growth factor β, the activins and inhibins, bone morphogenetic proteins, and other factors that play prominent roles in growth and development. To test our hypothesis that a RSTK may be expressed on the schistosome surface membrane, we attempted to clone and characterize schistosome members of this protein family. Here we report the identification of a schistosome RSTK (S. mansoni Receptor Kinase-1, or SmRK-1) which is expressed on the parasite surface and which may be responsible for, at least in part, for the serine-threonine kinase activity associated with the parasite surface membrane.

**Experimental Procedures**

Parasites—*Biomphalaria glabrata* snails infected with *S. mansoni* (NMRI strain) sporocysts were obtained from Biomedical Research Institute (Bethesda, MD). Cercariae were obtained by exposing infected snails to illumination in a 30 °C water bath for 1 h. Schistosomula were obtained by intraperitoneal transformation of cercariae as described previously (8). Adult parasites were recovered from female C57BL/6J mice (Tac-onic Farma) infected with cercariae 6 weeks (or more) previously using the ring method (9).

Isolation of SmRK-1 cDNA and Sequence Analysis—Total RNA was isolated from adult *S. mansoni* parasites using RNAzol B (Tel-Test, Inc.) and first-strand cDNA synthesis performed using M-MLV reverse transcriptase (Life Technologies, Inc.) and an oligo(dT)12–18 primer. A pair of degenerate primers (forward primer: 5'-AACACGATATTGCTCAYMNGA-3'; reverse primer: 5'-TCTTGAGGACCATAACGTTTTGTGCNAC-3'; corresponding to conserved sequences from subdomains VIB and VIII of other RSTK kinase domains, respectively) was used to amplify schistosome RSTK sequences from parasite cDNA by PCR. PCR products were cloned into the pCRII vector (Invitrogen), sequenced directly to isolate a larger clone fragment from an adult male *S. mansoni* cDNA library constructed in Agt11 (10). Phage forward and reverse primers were used in combination with internal primers to amplify the cDNA insert as two overlapping fragments from the recombinant phage. These fragments were cloned into pCRII and sequenced. Additional 5′ sequence was isolated using 5′-rapid amplification of cDNA ends. DNA sequencing was performed using dye terminator chemistry and a 373A sequencer (Applied Biosystems Inc.). Sequence analysis was performed using LASERGENE (DNASTAR Inc.). For phylogenetic analyses, the SmRK-1 kinase domain sequence was aligned with those of other RSTK kinase domains using the Clustal function of the Megalign program (DNASTAR Inc.). The Protist program from the PHYLIP package (11) was used to compute distance measures for the sequence data using the Dayhoff PAM matrix, and an unrooted tree was generated using the Neighbor-Joining algorithm (Neighbor program PHYLIP).

**Southern Hybridization Analysis of Genomic DNA—**Genomic DNA was isolated from cercariae using standard procedures. Following digestion with restriction enzymes and electrophoresis, DNA was transferred to nylon membrane (Schleicher & Schuell) and hybridized to a 1.4-kilobase fragment of the SmRK-1 cDNA labeled with [32P]dATP (Amersham) by the random primer method. Bound probe was detected by autoradiography.

Expression of Recombinant SmRK-1 and Production of Antibodies—A DNA fragment encoding most of the intracellular portion of the SmRK-1 protein (amino acids Met289–His1796) was amplified using primers that incorporated a prokaryotic ribosome-binding site (12) and a 6-histidine tag at the N and C termini, respectively. The PCR product was cloned into pCRII and modifications were confirmed by sequencing before subcloning into the prokaryotic expression vector pKK223-3 (Amersham Pharmacia Biotech). The His-tagged cytoplasmic domain of SmRK-1 was then overexpressed in *Escherichia coli* Top10F(·) cells (Invitrogen) and purified under denaturing conditions (8 M urea) on a Ni-NTA-agarose column (Qiagen). Recombinant protein was dialyzed extensively against phosphate-buffered saline and used to raise polyclonal anti-SmRK-1 antisera in mice and rabbits.

Surface Labeling of Schistosomiasis with Biotin—Freshly recovered adult *S. mansoni* were surface-labeled with a polar, water-soluble form of biotin that does not cross lipid bilayers, as described previously (6). Briefly, parasites were incubated in a 0.5 mg/ml solution of sulfo-oxidinomobidiotin (Sulfo-NHS-Biotin, Pierce), dissolved in Dulbecco’s phosphate-buffered saline supplemented with 0.1 mM CaCl2 and 1 mM MgCl2 (DPBS+), for 30 min at 4 °C. After removal of the biotin solution, the parasites were washed twice with Dulbecco’s modified Eagle’s medium to quench remaining biotin, and three times with DPBS+ prior to examination.

Metabolic Radiolabeling of Schistosome Proteins—Parasites were metabolically radiolabeled with [35S]methionine and [35S]cysteine as described previously (6). Briefly, freshly recovered *S. mansoni* were incubated for 6 h at 37 °C, 5% CO2 in Dulbecco’s modified Eagle’s medium (minus methionine and cysteine), 10 mM HEPES, 2 mM l-glutamine, 50 μM pyruvate, 150 μM Na2HCO3, 5% fetal bovine serum, 100 μg/ml streptomycin (worm culture medium), containing 500 μCi/ml [35S]methionine and [35S]cysteine (Trans-5-S-label, ICN). Labeling of parasite proteins with [32P]orthophosphate was achieved by incubating freshly recovered adult *S. mansoni* in phosphate-free worm culture medium containing 667 μCi/ml [32P]orthophosphate (NEN Life Science Products Inc.) for 3 h at 37 °C, 5% CO2. Unincorporated radioactivity was removed by extensive washing of parasites before protein extracts were prepared.

Preparation of Parasite Extracts—Following surface labeling or metabolic radiolabeling, parasite surface membranes and the underlying tegumental cytoplasm were solubilized by gently incubating whole parasites in lysis buffer (20 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.5% Nonidet P-40, 10 μg/ml aprotinin, 0.5 μg/ml leupeptin, 5 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride) for 30 min on ice, after which the supernatant was removed as the “ tegument extract.” The phosphate inhibitors sodium fluoride (10 mM) and Na2VO3 (1 mM) were also included in the lysis buffer when preparing extracts from [32P]orthophosphate-labeled worms. The remains of the parasites were then homogenized in a further volume of lysis buffer, incubated on ice for 30 min, and microcentrifuged at 4 °C for 15 min, the resulting supernatant being called the “carcass extract.” The protein concentration of parasite extracts was determined using the Protein-Gold Assay Reagent (Integrated Separation Systems).

**Immunological Techniques—**To perform immunoprecipitations from parasite extracts, samples were first precleared by incubating with 25 μl of Protein G-Sepharose CL-4B beads (Amerham Pharmacia Biotech) and 5 μl of preimmune serum for 1 h at 4 °C. Precleared supernatants were incubated for 2–12 h with 5 μl of antisera at 4 °C. 25 μl of Protein G-Sepharose beads were then added and incubation continued for a further 1 h to collect immune complexes. Beads were pelleted in a microfuge and washed 5 times with lysis buffer. Bound immune complexes were then eluted in SDS-PAGE sample buffer containing 2.5% 2-mercaptoethanol at 100 °C for 5 min and analyzed by 10% SDS-PAGE gels. Immunoblots were performed using 1 μg/ml of the primary antibody and 1:250 dilution of secondary antibody. The proteins were visualized on autoradiography. For immunoblotting, mouse and rabbit anti-SmRK-1 antisera were used at a dilution of 1:1000 and detected with peroxidase-conjugated sheep anti-mouse immunoglobulin (Ig) (Amersham Pharmacia Biotech) or peroxidase-conjugated donkey anti-rabbit Ig G (Jackson Immunoresearch Laboratories, Inc.) diluted to 1:5000. For detection of biotinated proteins on blots, streptavidin-peroxidase conjugates bound to blots were detected using 4-chloro-1-naphthol substrate (Kirkegaard & Perry), 20 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.1% Tween 20 for 25 min at room temperature. Antibody- and streptavidin-peroxidase conjugates bound to blots were detected using ECL Detection Reagents (Amersham Pharmacia Biotech).

**Confocal Microscopy—**Whole-mount adult schistosomes were prepared for confocal microscopy as described by Brownlee et al. (13). Briefly, parasites were fixed in 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, for 4 h at 4 °C, and then washed in phosphate-buffered saline, 0.1% bovine albumin, 0.2% Triton X-100, 1 mM NaN3 (PBT) for 24 h at 4 °C. Parasites were then incubated with rabbit anti-SmRK-1 antisera diluted 1:500 in PBT for 72 h at 4 °C, washed in PBT for 24 h at 4 °C, and incubated with rhodamine-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc.) diluted to 1:5000. For detection of biotinated proteins on blots, streptavidin-peroxidase conjugates were incubated in 1 μg/ml of labeled streptavidin (Kirkegaard & Perry), 20 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.1% Tween 20 for 25 min at room temperature. Antibody- and streptavidin-peroxidase conjugates bound to blots were detected using ECL Detection Reagents (Amersham Pharmacia Biotech).

Reverse Transcriptase-PCR Assay for SmRK-1 Transcripts—Total RNA was isolated and cDNA synthesis performed as described above. To detect SmRK-1 transcripts, the forward primer 5′-TCTGAGAATCTA-GAAAACATTCAT-3′ and reverse primer 5′-GATCCTAGTTGAA-TA-3′ (corresponding to nucleotides 1314 to 1336 and 1414 to 1435 of the SmRK-1 cDNA sequence, respectively) were used to amplify a 1.2-kilobase fragment of the adult *S. mansoni* cDNA containing the putative SmRK-1 transcript.
121-bp segment of the SmRK-1 cDNA sequence. As a control, a 230-bp segment of the message for a S. mansoni calcium-binding protein (CaBP) which is preferentially expressed in larval stages (14) was amplified using the forward primer 5'-TTCAGTATATTGCATTAGAATGG-3' and reverse primer 5'-TTATGACATAAAAATCATCAG-3' (corresponding to nucleotides −20 to +4 and +278 to +301 of the CaBP genomic sequence).

RESULTS

Isolation and Sequence Analysis of SmRK-1 cDNA—PCR with degenerate primers based on conserved amino acid sequences from kinase domains of other RSTKs resulted in the amplification of a single 176-bp sequence from adult schistosome cDNA. Cloning and sequence analysis revealed that the fragment contained part of an open reading frame and that the predicted translation product showed considerable amino acid identity to comparable regions of other RSTKs, suggesting that the sequence was indeed derived from a novel schistosome RSTK. After confirming by PCR that the sequence was present in adult male S. mansoni cDNA (data not shown), the fragment was used as a probe to screen an adult male S. mansoni cDNA library. A screen of 1.2 × 10^9 plaques resulted in the identification of a single positive recombinant phage containing a 3.9-kilobase insert. The cDNA was sequenced and found to contain a single open reading frame of 1782 bases beginning with an ATG codon 19 nucleotides downstream of the 5' end, and a long 3' untranslated region terminating in a poly(A) tail of 27 residues. Since the initiator ATG codon was not in the context of a Kozak consensus sequence (15) and the 18 nucleotides of sequence upstream remained open, an additional 250 bp of 5' sequence was isolated by 5'-rapid amplification of cDNA ends (data not shown). However, no other potential initiator ATG codons were detected in the additional 5' sequence and an in-frame stop codon occurred 45 nucleotides 5' of the first ATG, leading us to conclude that the entire open reading frame was contained within the initial cDNA isolate. This open reading frame encodes a 594-mer protein which we call SmRK-1.

The predicted amino acid sequence of SmRK-1 is shown in Fig. 1, panel A. Hydrophobicity analysis using the Kyte-Doolittle method (16) revealed two hydrophobic segments: one at the N terminus and another between residues 137–158. The N-terminal region could constitute a short signal peptide (17) with potential signal peptidase cleavage sites after residues 13 or 16 (18), while the region between residues 137 and 158 may represent a transmembrane domain (Fig. 1, panel A). The predicted molecular mass of the entire SmRK-1 polypeptide is 66.3 kilodaltons (kDa). Removal of the signal peptide would reflect by the lack of a cytoplasmic tail downstream of the N terminus, and the predicted molecular mass of the signal peptide would be 594 amino acids.

The partial GS domain are highlighted in reverse type; the three cysteine residues closest to the N-terminal end transmembrane domain correspond to the cysteine box. The residues of the partial GS domain are shaded. The predicted ATP-binding site at residues 309–331 and the putative serine-threonine kinase active site at residues 421–440 are underlined. B, unrooted tree showing the phylogenetic relationship between the kinase domain of SmRK-1 and those of other RSTKs. The type I receptors cluster on the left side of the tree, the type II receptors cluster on the right side. SmRK-1 is highlighted in bold type. Full names and GenBank accession numbers for the other sequences included in the analysis are: GgRPK1, Gallus gallus receptor protein kinase 1, D13432; MnBRK1, Mus musculus BRK1, U04672; DmSAX, Drosophila melanogaster saxophone, L3350; DmAALK1, Homo sapiens activin receptor-like kinase 1, Z22534; DmAALK2, H. sapiens activin receptor-like kinase 2, Z22534; DmAATRI, D. melanogaster antitrypsin 5, DmAATRI, M. musculus TGFB type I receptor, D25540; CeDCAF1, C. elegans DCAF1, M32877; OcAMHR, Oryctolagus cuniculus anti-Mullerian hormone receptor, U15075; DmAATRII, D. melanogaster activin receptor II, L22176; HsAcTIII, H. sapiens activin receptor type II, X62381; MnActRIIB, M. musculus activin receptor type II, M84120; HsTBR1, H. sapiens TGFB receptor type II, M85079.

protein sequence, a high degree of similarity (up to 58% identity) to RSTKs of the type I subfamily was found. Significant levels of amino acid identity were restricted to the kinase domain, with little similarity to other proteins detected elsewhere in the sequence. Type II RSTKs were less similar, showing approximately 40% identity over the kinase domain. The similarity between SmRK-1 and other type I RSTKs is also reflected by the lack of a cytoplasmic tail downstream of the
The kinase domain of SmRK-1, a typical feature of type I receptors (7). Phylogenetic analysis of the SmRK-1 kinase domain sequence further demonstrated its overall similarity to type I receptors but also confirmed its divergent nature, indicating that it is not orthologous to any other currently known RSTKs (Fig. 1, panel B).

Type I RSTKs are further distinguished from type II receptors by the presence of a "GS domain," a 30-amino acid glycine- and serine-rich region immediately upstream of the kinase domain that functions in regulation of receptor kinase activity (19). This domain contains the core sequence hy$_2$-3GSGSG (where hy represents either of the hydroxyamino acids serine or threonine), plus 1–2 additional downstream threonines, which are conserved in all type I receptors. While elements of this domain are evident in SmRK-1, the core sequence contains G, which defies classification at present (20).

Confirmation of Schistosome Origin of SmRK-1—To confirm the parasite origin of the SmRK-1 sequence and examine its genomic organization, a 1.4-kilobase fragment of the SmRK-1 cDNA was used as a probe in Southern analysis of S. mansoni genomic DNA isolated from free-living cercarial stages (Fig. 2). Single bands were obtained when probe was hybridized under stringent conditions to cercarial genomic DNA digested with EcoRI and HindIII, confirming that the SmRK-1 cDNA is of schistosome origin and was not inadvertently isolated from contaminating mouse host tissue. These data also suggested that the SmRK-1 gene is represented by a single copy in the S. mansoni genome. Two bands were obtained when probe was hybridized to genomic DNA digested with ClaI, and this is consistent with our observation that a ClaI site is located in the 3′-untranslated region of the SmRK-1 cDNA.

To further confirm the parasite origin of SmRK-1, anti-SmRK-1 antibodies were generated against the cytoplasmic portion of the molecule and used to immunoprecipitate SmRK-1 from extracts of adult schistosomes metabolically radiolabeled with $[^{35}S]$methionine and $[^{35}S]$cysteine. Tegument (Teg) and carcass (Carc) extracts were prepared and SmRK-1 immunoprecipitated with mouse anti-SmRK-1 antiserum (a-SmRK). Control immunoprecipitations were performed using preimmune mouse serum (Pre). A protein with a calculated molecular mass of 65 kDa, consistent with the predicted molecular mass of SmRK-1, was specifically immunoprecipitated by the anti-SmRK-1 antiserum (arrow) from both tegument and carcass extracts. The position of a 68-kDa molecular mass standard is indicated. IP Ab, immunoprecipitating antibody.

SmRK-1 is expressed on the parasite surface and could therefore contribute to the surface-associated kinase activity, we examined whether native, parasite-expressed SmRK-1 was susceptible to selective surface labeling of parasite proteins. We have previously shown that the water-soluble, membrane-impermeable probe sulfosuccinimidobiotin can be used to selectively and specifically biotinylate the surface proteins of adult schistosomes (6). When freshly isolated adult schistosomes were subjected to surface labeling, SmRK-1 could be immunoprecipitated in a biotinylated form from extracts of these parasites, indicating that the receptor is expressed on the schistosome surface (Fig. 4).

To further examine the distribution of SmRK-1 expression at the schistosome surface, whole adult parasites were fixed and probed with anti-SmRK-1 antibodies. Indirect immunofluorescence and confocal scanning laser microscopy were used to observe anti-SmRK-1 antibody binding to whole-mount parasites. A punctate pattern of staining was observed on the anterior dorsal surfaces of male schistosomes (Fig. 5, panels A and C). These foci of immunofluorescence correspond to the tubercles on the dorsal surfaces of male parasites. A similar pattern of fluorescence was observed when frozen sections of adult parasites were probed with anti-SmRK-1 antibodies (data not shown). SmRK-1 expression could not be detected in other regions of male parasites or in female parasites, suggesting that SmRK-1 is preferentially expressed on the surface membranes of males. Host Ig bound to the surfaces of adult schistosomes was visualized simultaneously as a marker for the parasite surface (Fig. 5, panels B and C), since host antibodies become bound to the surface of adult parasites during infection (21, 22). Host Ig was observed in the same focal plane as SmRK-1 staining, providing further evidence that SmRK-1 is expressed at the parasite surface.

Phosphorylation of SmRK-1—To examine the possibility that SmRK-1 may participate in a signaling mechanism within the schistosome tegument, we assessed whether native SmRK-1 is phosphorylated in intact parasites. Freshly recovered adult schistosomes were incubated in medium containing $[^{32}P]$orthophosphate to label parasite phosphoproteins and SmRK-1 was immunoprecipitated from tegument extracts of these parasites (Fig. 6). SmRK-1 was immunoprecipitated in a phosphorylated form, suggesting that it participates in a protein kinase cascade and may therefore be active in a signaling mechanism. Several other phosphoproteins were also detected in anti-SmRK-1 immunoprecipitates that were absent in the tegument.
idobiotin and tegument extracts were prepared. SmRK-1 was immuno-
fluorescence over the dorsal surfaces of male parasites was observed.
were fixed, probed with rabbit anti-SmRK-1 antiserum followed by
soni
A
the surfaces of male schistosomes.
ected to blotting and probing with peroxidase-labeled streptavidin.
expression is confined to the tubercles, while host antibod-
ies are bound primarily to the intervening surface membrane, produc-
invasive larval stage. Reverse transcriptase (RT)-PCR assay (Fig. 7).
section (24): genetic heterogeneity in the host population. This variation in
environment, genetic heterogeneity in the parasite population, and phe-
tions may partly account for the persistence of parasitic hel-
The slight degradation of paramyosin observed in the schisto-
sample is due to the presence of high levels of proteases in this
malignant host and in adults (ad), but not in extracts of eggs, schistosomula (som), or 15 days postinfection (15d). The blot was stripped and reprobed with anti-paramyosin antibo-
odies to demonstrate the equivalence of loading in each lane (middle
panel). The position of a 68-kDa molecular mass standard is indicated. IP Ab, immunoprecipitating antibody.

preimmune serum precipitates. The possibility that these spe-
cies represent specifically co-precipitating molecules that inter-
act with SmRK-1 is currently being addressed.
Stage-specific Expression of SmRK-1—Immunoblot analysis of
extracts from various life cycle stages was performed using
anti-SmRK-1 antibodies to determine whether stage-specific
regulation of SmRK-1 expression occurred during the parasite
life cycle (Fig. 7). SmRK-1 expression was detected from 21
days postinfection of the mammalian host onwards, but not in
eggs or larval stages. A monoclonal antibody specific for
paramyosin, a protein found in the tegument cytoplasm, was
used as a control for protein loading in each lane. These data
correlate with those obtained using a reverse transcriptase-
PCR assay for SmRK-1 transcripts (Fig. 7). SmRK-1 message
was readily detected in adult RNA but not in RNA from 3-h-old
schistosomula. In contrast, message for a schistosome CaBP
which is expressed in cercariae and early schistosomula (14),
was readily detected in RNA from 3-h schistosomula, while
only a weak signal could be amplified from adult RNA.

Fig. 4. SmRK-1 is expressed at the parasite surface. Freshly
recovered adult S. mansoni were surface-labeled with sulfo-succinimidobiotin and tegument extracts were prepared. SmRK-1 was immuno-
precipitated with mouse anti-SmRK-1 antiserum (a-SmRK) and sub-
jected to blotting and probing with peroxidase-labeled streptavidin. The position of a 68-kDa molecular mass standard is indicated. IP Ab, immunoprecipitating antibody.

Fig. 5. Expression of SmRK-1 is localized to the tubercles on the
surfaces of male schistosomes. A, whole-mount adult S. mansoni were fixed, probed with rabbit anti-SmRK-1 antiserum followed by
rhodamine-conjugated donkey anti-rabbit IgG antibodies, and exam-
ined using confocal scanning laser microscopy. A punctate pattern of
fluorescence over the dorsal surfaces of male parasites was observed. The areas of fluorescence correspond to the tubercles found on the
dorsal surfaces of male S. mansoni. B, the same specimen as in A was
simultaneously probed with fluorescein-conjugated goat anti-mouse
IgG + IgM antibodies to detect surface-bound host antibodies as a
marker for the schistosome surface membrane. A typical reticulate pattern of
fluorescence was observed. C, the fluorescence images in A and B were assigned false colors and superimposed. Red represents the
SmRK-1 staining and green represents the surface-bound host antibod-
ies. SmRK-1 expression is confined to the tubercles, while host antibo-
dies are bound primarily to the intervening surface membrane, produc-
ing the reticulate pattern of staining.

Fig. 6. SmRK-1 is phosphorylated in the tegument of adult S.
mansoni. Freshly recovered adult parasites were labeled with
[^32P]orthophosphate and tegument extracts were prepared. SmRK-1 was then immunoprecipitated with rabbit anti-SmRK-1 antiserum (a-
SmRK) and subjected to SDS-PAGE and autoradiography. A phospho-
protein with the predicted molecular mass of SmRK-1 was
detected in anti-SmRK-1 immunoprecipitates (arrow), but not in control
immunoprecipitations performed with preimmune rabbit serum (Pre). Several other phosphorylated proteins were also detected in anti-
SmRK-1 immunoprecipitates but not in control immunoprecipitates.
These additional bands may represent co-precipitating proteins that
interact with SmRK-1. The positions of molecular mass standards are indicated in kDa. IP Ab, immunoprecipitating antibody.

DISCUSSION
In comparison to pathogens such as viruses, bacteria, and
protozoa, a distinguishing feature of helminth infections is the
aggregated distribution of infections observed in affected popu-
lations, where a minority of individuals are found to harbor
the majority of parasites (23). This aspect of helminth infec-
tions may partly account for the persistence of parasitic hel-
minths in host populations, since heavily infected, susceptible
individuals may act as reservoirs of infection for the rest of
the population. This variation in infection intensity within a single
population is believed to be the combined result of three inter-
acting factors (24): genetic heterogeneity in the host population,
genetic heterogeneity in the parasite population, and phenet-
otypic plasticity on the part of the parasite in response to
prevailing intrahost conditions which vary from host to host.
Phenotypic plasticity, or parasite responsiveness to host factors

Fig. 7. Stage-specific expression of SmRK-1. Extracts were prepared from various stages of the S. mansoni life cycle and subjected to
immunoblotting with rabbit anti-SmRK-1 antiserum (a-SmRK, upper
panel). 50 µg of protein were loaded in each lane. SmRK-1 was detected after 21 days post-infection (21d) of the mouse host and in adults (ad),
but not in extracts of eggs, schistosomula (som), or 15 days postinfection (15d). The blot was stripped and reprobed with anti-paramyosin antibo-
dies to demonstrate the equivalence of loading in each lane (middle
panel).

In preparation...
such as species, age, sex, reproductive condition, and immune status is advantageous to the parasite in allowing it to maximize reproductive success under the limitations determined by conditions within an individual host. Helminth life history traits influenced by host factors include migration routes taken through host tissues and organs, entry into hypobiotic or dormant states, length of prepatent period, longevity, final body size, and fecundity. Clearly, phenotypic plasticity requires that parasites be able to receive and respond to signals from their hosts.

Phenotypic plasticity by a parasitic helminth in response to a host factor was recently demonstrated by Amiri et al. (2), who showed that the host cytokine tumor necrosis factor α was a regulator of fecundity for S. mansoni. Furthermore, transmission of signals between male and female schistosomes is a well recognized but poorly defined phenomenon (25). Like other parasitic platyhelminths, the schistosome surface tegument is composed of a layer of cytoplasm, bound externally by a lipid bilayer, which therefore forms a potentially intimate interface between the parasite and its environment. These observations led us to hypothesize that parasitic flatworms may express membrane-bound receptor proteins on their surface membranes that engage external factors in the surrounding milieu and transduce signals into the tegumental cytoplasm, thus providing a mechanism by which signals in the immediate environment might be detected. Using the S. mansoni model system, we have now identified a candidate for just such a receptor in that (i) the predicted amino acid sequence of this candidate protein displays a considerable level of identity to members of a known family of signal-transducing receptors, and (ii) the protein is expressed at the parasite surface, where we would expect interactions between external ligands and parasite receptors to occur.

With the identification of SmRK-1, platyhelminths now become the most primitive organisms known to possess RSTKs, an important step toward elucidating the evolutionary origins of this receptor family. In other organisms, signaling by TGFβ-like molecules requires the presence of both a type I and type II RSTK (26, 27). Ligand is first engaged by the type II receptor, which is a constitutively active kinase. The ligand-type II receptor complex then recruits a type I receptor, which is subsequently phosphorylated by the type II receptor (28). Phosphorylation of the type I receptor within the GS domain by the type II receptor results in activation of the type I receptor kinase activity, which then propagates the signal intracellularly by phosphorylating cytoplasmic Smad proteins (29). Since the GS domain of the type I receptor plays a critical role in regulation of type I receptor kinase activity by the type II receptor (19), it will be of interest to determine if type I-like receptors such as Daf-1 and SmRK-1, which possess atypical GS domains (Fig. 1, panel A), are regulated by type II receptors in the same way as other type I receptors. To facilitate the examination of this issue, we are attempting to identify schistosome type II receptors that may act as partners for SmRK-1. Our observation that SmRK-1 becomes phosphorylated in adult parasites (Fig. 6) suggests that it may interact with other protein kinases in the schistosome tegument. Alternatively, SmRK-1 phosphorylation may occur as the result of an autoprophosphorylation mechanism.

We have found that when intact adult schistosomes are specifically surface-labeled with a polar, water-soluble form of biotin, the SmRK-1 protein becomes biotinylated (Fig. 4), indicating that it is expressed on the parasite surface. Experiments using immunofluorescence microscopy further confirmed the localization of the SmRK-1 protein to the parasite surface, where SmRK-1 expression was found to be restricted to the tubercles on the dorsal surfaces of male worms (Fig. 5). We have not been able to detect SmRK-1 expression elsewhere in male tissues or in female parasites using these techniques. However, we have detected SmRK-1 protein in carcass extracts of adult parasites (Fig. 3), suggesting that SmRK-1 may be expressed in other schistosome tissues. Alternatively, this result may represent SmRK-1 protein contained within small amounts of residual tegumental material that was not removed from the parasite carcasses during the preparation of the tegument extract.

Our results indicate that the SmRK-1 protein is expressed at detectable levels only after the second week of infection of the mammalian host (Fig. 7). The timing of SmRK-1 expression during the schistosome life cycle suggests that the protein is either: (i) involved in host-parasite interactions at the final destination of the parasite within the mammalian host, since SmRK-1 protein is only expressed after arrival of the parasite in the hepatic portal circulation, or (ii) SmRK-1 is involved in male-female interactions, since differentiation of the separate sexes coincides with initiation of SmRK-1 expression. The expression of SmRK-1 on the tubercles of males suggests that the SmRK-1 ligand may be of host origin, since during the normal course of infection, the tubercles of the adult male are tightly apposed against the blood vessel endothelial cell membrane (30). A ligand of endothelial cell origin secreted at the luminal surface would therefore be in close proximity to the SmRK-1 receptor. Unfortunately, the lack of homology between SmRK-1 and other RSTKs does not allow us to make deductions about the nature of the ligand, although it is most likely a member of the TGFβ family of growth factors. The hypothesis that a platyhelminth receptor may bind a vertebrate transforming growth factor β-like molecule is plausible, since it has already been shown that the Daf-4 RSTK from C. elegans is capable of binding human bone morphogenetic protein-2 and bone morphogenetic protein-4 when expressed in mammalian cells (31). While an interaction between Daf-4 and these growth factors would not occur under natural circumstances, this result emphasizes the highly conserved nature of the RSTK signaling system, which makes it an ideal candidate system for transducing signals between parasites and their hosts. It is also of interest to note that the Daf-1 and -4 RSTKs from C. elegans are involved in controlling a developmental switch in the nematode life cycle which occurs in response to conditions in the animal's environment. When C. elegans larvae are subjected to starvation or overcrowded conditions, development proceeds to an arrested third stage dauer larva. Mutations in the daf-1 and daf-4 genes result in constitutive dauer larva formation regardless of environmental conditions. Control of developmental responses to environmental signals by RSTK signaling mechanisms might therefore be a widespread phenomenon among the various invertebrate phyla. The identification of the SmRK-1 ligand will be an important step toward elucidating the function of this receptor and may provide new insights into the molecular interactions that occur between parasitic helminths and their hosts.

REFERENCES

1. World Health Organization (1990) The Control of Schistosomiasis, WHO Technical Report 830, WHO, Geneva.
2. Amiri, P., Locksley, R. M., Parslow, T. G., Sadick, M., Rector, E., Ritter, D., and McKerrow, J. H. (1992) Nature 356, 604–607.
3. Basch, P. F., and Rhine, W. D. (1983) J. Parasitol. 69, 567–569.
4. Basch, P. F. (1988) Comp. Biochem. Physiol. B 90, 389–392.
5. Gupta, B. C., and Basch, P. F. (1987) J. Parasitol. 73, 481–486.
6. Davies, S. J., and Pearse, E. J. (1995) Mol. Biochem. Parasitol. 70, 33–44.
7. Massague, J., Attisano, L., and Wrana, J. L. (1994) Trends Cell Biol. 4, 172–178.
8. Lazzaro, J. K., Stein, M. J., David, J. R., and Sher, A. (1982) Exp. Parasitol. 53, 39–44.
9. Smithers, S. R., and Terry, R. J. (1965) Parasitology 55, 695–700.
10. Shoemaker, C. B., Ramachandran, H., Landa, A., dos Reis, M. G., and Stein, L. D. (1992) Mol. Biochem. Parasitol. 53, 17–32.
11. Felsenstein, J. (1989) Cladistics 5, 164–166
12. Gold, L. (1988) Annu. Rev. Biochem. 57, 199–233
13. Brownlee, D. J., Fairweather, I., Johnston, C. F., Thorndyke, M. C., and Skuce, P. J. (1995) Parasitology 110, 143–153
14. Ram, D., Grossman, Z., Markovics, A., Avivi, A., Ziv, E., Lantner, F., and Schechter, I. (1989) Mol. Biochem. Parasitol. 34, 167–175
15. Kozak, M. (1986) Cell 44, 283–282
16. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
17. von Heijne, G. (1985) J. Mol. Biol. 184, 99–105
18. von Heijne, G. (1986) Nucleic Acids Res. 14, 4683–4690
19. Wieser, R., Wrana, J. L., and Massague, J. (1995) EMBO J. 14, 2199–2208
20. Georgi, L. L., Albert, P. S., and Riddle, D. L. (1990) Cell 61, 635–645
21. Sogandares-Bernal, F. (1976) J. Parasitol. 62, 222–226
22. Gearner, G. W., and Kemp, W. M. (1994) J. Parasitol. 80, 275–283
23. Maizels, R. M., Bundy, D. A., Selkirk, M. E., Smith, D. F., and Anderson, R. M. (1993) Nature 365, 797–805
24. Poulin, R. (1996) Adv. Parasitol. 37, 107–134
25. LoVerde, P. T., and Chen, L. (1991) Parasitol. Today 7, 303–307
26. Wrana, J. L., Attisano, L., Carcamo, J., Zentella, A., Dody, J., Laiho, M., Wang, X. F., and Massague, J. (1992) Cell 71, 1003–1014
27. Letsou, A., Arora, K., Wrana, J. L., Simin, K., Twombly, V., Jamal, J., Staehling-Hampton, K., Hoffmann, F. M., Gelbart, W. M., Massague, J., and O'Connor, M. B. (1995) Cell 80, 899–908
28. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) Nature 370, 341–347
29. Massague, J. (1996) Cell 85, 947–950
30. Hockley, D. J. (1973) Adv. Parasitol. 11, 233–305
31. Estevez, M., Attisano, L., Wrana, J. L., Albert, P. S., Massague, J., and Riddle, D. L. (1993) Nature 365, 644–649

S. mansoni Receptor Serine-threonine Kinase

24. Poulin, R. (1996) Adv. Parasitol. 37, 107–134
25. LoVerde, P. T., and Chen, L. (1991) Parasitol. Today 7, 303–307
26. Wrana, J. L., Attisano, L., Carcamo, J., Zentella, A., Dody, J., Laiho, M., Wang, X. F., and Massague, J. (1992) Cell 71, 1003–1014
27. Letsou, A., Arora, K., Wrana, J. L., Simin, K., Twombly, V., Jamal, J., Staehling-Hampton, K., Hoffmann, F. M., Gelbart, W. M., Massague, J., and O'Connor, M. B. (1995) Cell 80, 899–908
28. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) Nature 370, 341–347
29. Massague, J. (1996) Cell 85, 947–950
30. Hockley, D. J. (1973) Adv. Parasitol. 11, 233–305
31. Estevez, M., Attisano, L., Wrana, J. L., Albert, P. S., Massague, J., and Riddle, D. L. (1993) Nature 365, 644–649