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***Schistosoma mansoni*: TGF- β Signaling Pathways**

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Abstract

Schistosome parasites have co-evolved an intricate relationship with their human and snail hosts as well as a novel interplay between the adult male and female parasites. We review the role of the TGF- β signaling pathway in parasite development, host-parasite interactions and male-female interactions. The data to date supports multiple roles for the TGF- β signaling pathway throughout schistosome development, in particular in the tegument which is at the interface with the host and between the male and female schistosome, development of vitelline cells in female worms whose genes and development are regulated by a stimulus from the male schistosome and embryogenesis of the egg. The human ligand TGF- β 1 has been demonstrated to regulate the expression of a schistosome target gene that encodes a gynecophoric canal protein in the schistosome worm itself. Studies on signaling in schistosomes opens a new era for investigation of host-parasite and male-female interactions.

Index Descriptors

Host-parasite interactions; Male-female interactions; *Schistosoma mansoni*; Smad; TGF- β

Schistosome parasites are multicellular eukaryotic organisms with a complex life cycle that involves mammalian and snail hosts. The vertebrate endoparasitic life cycle begins when a schistosome parasite (cercariae) penetrates the skin of a host, it transforms into a schistosomule and begins its life as an endoparasite. To continue its development it must take advantage of signals from the host. Schistosome parasites stay in the skin and after two days enter the circulation and move to the lungs where they remain for further development. About day 8-15, the parasites leave the lungs and move to the portal circulation of the liver where the male and female worms mate. The male worm, through a set of unknown signals, stimulates female gene expression that results in reproductive development and subsequent egg production. The worm pairs move against the flow of blood into their final niche in the mesenteric veins. This stage of the parasite life cycle takes place in a milieu of host signaling molecules. Co-evolution has allowed the schistosome parasite to sense and respond to host mediators. As with all multicellular eukaryotic organisms, development proceeds through the elaboration and response to extracellular signals. In the case of an endoparasite such as the schistosomes, one must consider both external and internal signaling mechanisms in parasite-host and male-female signaling pathways.

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Signals from host environment

For the schistosome parasite to migrate from the skin to the portal circulation and undergo development at the same time, it must receive signals from the host environment and from within that are transduced to regulate cell proliferation and development. Even after maturation, the adult worms receive signals from the host that regulate development. For example, IL-7 was shown to be an important cytokine in regulating schistosome development. It was identified as being important in the initial phases of *S. mansoni* infection by demonstrating that a single injection of recombinant human IL-7, at the site of cercarial penetration one day prior to infection resulted in: the impairment of parasite migration to the lungs, an increased number of surviving adult worms, and more severe liver pathology (Wolowczuk, et al., 1997). IL-7 has a dramatic effect on adult worm development. In its absence, adult male and female worms, as judged by organ systems and egg production, are fully developed yet stunted in size (Wolowczuk, et al., 1999, Wolowczuk, et al., 1999). Interestingly, (Hernandez, et al., 2004) provided evidence that male worm but not female worm development is affected by host immune signals and that this in turn affects the ability of the male to transduce signals to the female that regulate her reproductive development.

The identification of various growth factor receptors in *S. mansoni*, represents further evidence that schistosomes are responsive to stimuli emanating from the host environment. Epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, is another example of growth factor receptors that are expressed on the surface of parasites (Ramachandran, et al., 1996) and has been shown to respond to human EGF (Vicogne, et al., 2004). The role of host factors in schistosome development has been reviewed (Davies and McKerrow, 2003, Dissous, et al., 2006, Escobedo, et al., 2005, Knobloch, et al., 2007).

Together, these observations suggest the presence of signaling pathways by which the parasites can detect host signals and respond to them in a way that presumably increases their survival.

Biological interplay between male and female parasite

Female schistosome physical and reproductive development is dependent on the presence of mature male worms (Armstrong, 1965, Erasmus, 1973, Michaels, 1969, Moore, et al., 1954, Shaw, 1977, Vogel, 1941). Females from single sex infections are underdeveloped in that they are stunted and exhibit an immature reproductive system. The ootype, uterus and oviducts are developed in the immature female (Erasmus, 1973, Neves, et al., 2005, Shaw, 1977). However, the ovary remains small and the ova are devoid of their characteristic cortical granules, the Mehlis' gland is not fully formed and the vitellaria, whose cells produce the eggshell precursors and nutrients for the egg, is not developed. The male stimuli for female growth and for reproductive development appear to be independent (Armstrong, 1965). The male stimuli for growth and reproductive development are independent of sperm transfer and fertilization, and not species-specific (LoVerde, 2002, LoVerde and Chen, 1991, LoVerde, et al., 2004, Popiel, 1986). The nature of the male stimulus responsible for female sexual maturation has yet to be determined. However, an intimate association between the male and female worm that is achieved by the female residing within a ventral groove, the gynaecophoric canal, of the male is necessary. The male stimulus requires contact and is spatially distributed throughout the gynaecophoric canal. However, its effect (vitelline gland development) is local (Popiel and Basch, 1984).

The stimulus from the male worm is not only necessary for female worms to complete physical and reproductive development but also for the female to maintain her mature state (Clough, 1981, Popiel, 1986). The fact that a continuous male stimulus is necessary to maintain female reproductive activity makes this an important target for controlling morbidity by limiting (preventing) egg production.

If one considers the architecture of the female parasite, it seems that the transduction of the signal likely involves a number of intercellular as well as intracellular substrates to transduce the signal from the surface of the female to the target vitelline cell [see (Knobloch, et al., 2007)].

The female worm is composed of a tegument that is in direct contact with the host environment, and the male parasite. The tegument overlays circular and longitudinal muscle bundles beneath which are found the organ systems including the vitellaria in mature parasites, or vitelline primordia in immature parasites (Erasmus, 1987). The signal transferred directly from the male could be by a male ligand that binds to a receptor on the surface (tegument) of the female or by a number of other pathways (LoVerde, et al., 2004). In any case, the ligand binds the receptor and transduces a signal from the tegument to the target vitelline cell receptor which may be on the vitelline cell membrane and through intercellular substrates to the vitelline cell nucleus where vitelline cell gene expression is activated (LoVerde, 2002, LoVerde, et al., 2004).

Growth, migration and development of the parasitic worms in their host are believed to be controlled, in part, by signals received from the host. *Schistosoma mansoni* worms in their niche in the definitive host are bathed in host molecules (hormones, antibodies, cytokines, growth factors, etc.). Data to date indicate that schistosomes are in a dynamic process of receiving and responding to host molecules. Receptors present on the parasite surface process the repertoire of signals in such a way that promotes development, and guides the worms through their journey from site of infection to their final destination (Davies and McKerrow, 2003, Salzet, et al., 2000). Furthermore, the absolute prerequisite for the female worm to reside within the gynaecophoric canal of the male worm, in order to develop and maintain its reproductive activity, highlights the significance of a set of self signals on the growth and development of the parasite and differentiation of its tissues (LoVerde, et al., 2004). The diverse effects produced by members of the TGF- β superfamily on a wide array of cell types stimulated the investigation of this signaling pathway as a plausible means of signal transmission involved in schistosome growth and maturation.

TGF β signaling pathway

Intracellular signal transduction pathways convey information from the cell surface to the nucleus and this enables the cell to respond to stimuli from its environment by changes in gene expression. These same pathways are thought to function at the parasite surface and transduce signals not only to the cells associated with the host-parasite interface but to the nuclei of cells throughout the parasite body to regulate gene expression important in differentiation, homeostasis, parasite migration, immune evasion and reproductive development. This review will focus on the transforming growth factor β (TGF- β) pathways and their role(s) in parasite development, host-parasite interactions and male-female interactions.

The TGF- β superfamily comprises a large number of structurally related polypeptide growth factors produced by diverse cell types, capable of regulating a vast array of cellular processes including cell proliferation, lineage determination, differentiation, motility, adhesion, and apoptosis (Massague, 1998). The TGF- β superfamily includes TGF- β s, activins, bone morphogenetic proteins (BMPs), nodal, myostatin, anti-Mullerian hormone (AMH) and growth/differentiation factors (GDFs). TGF- β -related factors are synthesized as single polypeptide chains, which homodimerize and are proteolytically cleaved by furin-type enzymes to produce C-terminal mature polypeptide dimers. The propeptide dimer (called latency associated protein; LAP) remains non-covalently associated to the active dimer maintaining it in an inactive complex (TGF- β latent complex), which contains a third protein called Latent TGF- β binding protein (LTBP) (Dubois, et al., 1995). The term TGF- β ligand activation refers to the release of the active peptide from the latent complex (Annes, et al.,

2003). The general model of TGF- β signaling from cell surface to nucleus has been established (Derynck and Zhang, 2003, Shi and Massague, 2003) (Fig. 1). Upon activation, TGF- β family members bind to and signal through a family of transmembrane receptor serine/threonine kinases. The receptor family is divided into two subtypes, type I (e.g. T β RI) and type II (e.g. T β RII). Type I receptors are also called activin receptor-like kinases or ALKs. In the prototypical signal transduction pathway of TGF- β , the binding of a ligand to its type II receptor in concert with a type I receptor leads to the formation of a ligand-receptor complex. Most receptor complexes bind several ligands, and several type I receptors form combinatorial interactions with type II receptors, thus creating signal diversity. Type I receptors are distinguished by the presence of a 30-amino acid regulatory segment, which contains a stretch of alternating glycine and serine residues (GS region), located immediately upstream of the kinase domain. Following ligand binding, the type II receptor kinase phosphorylates serine and threonine residues in the GS region and thereby activates the type I receptor cytoplasmic domain. Phosphorylation switches this region from serving as a docking site for an inhibitor signaling molecule, FKBP12 (Huse, et al., 1999), to a site for Smad* proteins (Derynck, et al., 1996), a family of intracellular transducers (Huse, et al., 2001). Activated T β RI then transduces the signal to a member of the Smad family that can carry the signal to the nucleus and regulate transcription of selected genes in response to the ligand (Kloos, et al., 2002). Smads are a class of proteins that function as intracellular signaling effectors for the TGF- β family. Smads fall into 3 categories: (1) Receptor-regulated Smads (R-Smads) that serve as substrates of TGF β family type I receptor kinases, where Smad1, 5 and 8 serve as substrates for BMP and AMH receptors, while Smad2 and 3 are substrates for TGF- β , activin and nodal receptors; (2) Common-Smads (Co-Smads) that act as partners for the activated R-Smads (e.g. Smad4 in vertebrates and MEDEA in *Drosophila*). Co-Smad, although not required for nuclear translocation of R-Smad, is required for the formation of transcriptional complexes; and (3) Inhibitory Smads (I-Smads) that block the signal to the nucleus by binding to the type I receptors and/or Smad4, thus preventing phosphorylation of the R-Smad and/or its association with Smad4 (Heldin, et al., 1997) e.g. Smad6 and 7 in vertebrates and Dad in *Drosophila*. Smad proteins consist of two globular domains, an N-terminal Mad homology region (MH1) (with the exception of I-Smads) and a C-terminal MH2 domain, connected by a highly divergent, flexible linker region. The MH1 domain contains a DNA-binding β -hairpin structure, which is conserved in all R-Smads and the Co-Smad (Shi, et al., 1998). The MH1 domain recognizes the sequence CAGA and certain GC rich sequences. However the affinity of these sequences is too low for effective binding *in vivo*. By forming transcriptional complexes with other DNA-binding factors such as FAST/FoxH1, c-Jun, and CBP/p300, Smads are able to regulate expression of a target gene (Feng and Derynck, 2005) for review). In addition, The MH1 domain is also required for nuclear import through its N-terminally located nuclear localization signal. The linker region contains multiple phosphorylation sites (e.g. ser/thr-pro residues that are phosphorylated by MAPK kinases and calcium-calmodulin dependent protein kinase II), which provide crosstalk points with other signaling networks as parts of an overall cellular regulation mechanism of signaling networks. One example of such crosstalk is demonstrated in cellular control of TGF β signaling through Ras-mediated pathway, where Erk MAPK phosphorylates the linker region of R-Smads, resulting in blocking their nuclear translocation (Kretzschmar, et al., 1997, Kretzschmar, et al., 1999) and consequently inhibiting the TGF β antiproliferative responses. However, this Ras-mediated down-regulation of TGF β signaling through Erk phosphorylation seems to be restricted to epithelial cells, since in other cell types, Ras has been reported to promote TGF β signaling (Ellenrieder, et al., 2001, Hayashida, et al., 2003, Suzuki, et al., 2007). The highly conserved MH2 domain is involved in the formation of Smad heterooligomeric complexes in addition to participation in

*Smads received their name by combining the name of the intracellular transducers of TGF β signaling from *Drosophila melanogaster* termed MAD (Mothers against decapentaplegic) and from *Caenorhabditis elegans* termed Sma (identified through genetic screens of mutants of small body size phenotype) to describe the proteins in vertebrates.

protein interactions with type I receptors (R-Smad phosphorylation/activation) (Huse, et al., 2001), with a cytoplasmic protein that assists Smad2 anchoring to the activated type I receptor (Smad Anchor for Receptor Activation; SARA) (Wu, et al., 2000), with nucleoporins for nucleocytoplasmic translocation (Xu, et al., 2002), and with other nuclear partners (DNA binding factors, transcription co-activators or co-repressors) for the assembly of transcriptional complexes (Massague and Wotton, 2000). Receptor-mediated phosphorylation occurs at the carboxy-terminal motif SXS, only present in R-Smads. L45 loop and L3 loop are two structural motifs present in the kinase domain of type I receptor (Feng and Derynck, 1997) and the MH2 domain of the corresponding R-Smad (Lo, et al., 1998), respectively, determine the specificity of the interaction between these two members of the signaling cascade. The sequence of the L3 loop is highly conserved and invariant among R-Smads of similar signaling specificity (e.g. Smad2 and Smad3 that are activated by T β R-I and activin type I receptor, or Smad1, Smad5 and Smad8 activated by BMP or BMP-like type I receptors) (Lo, et al., 1998). Upon phosphorylation by type I receptor, R-Smad undergoes conformational changes, in which a mutual inhibitory status exerted by the MH1 and MH2 domains on each other is relieved, resulting in transformation of the phosphorylated R-Smad from an inactive to an active state. The activated R-Smad then forms a complex with the co-Smad, Smad-4 and the newly formed Smad complex is translocated into the nucleus where it binds to a target gene response element and in concert with other factors regulates gene expression (activation or repression). Cooperation between Smads and other transcription regulators permits crosstalk between the TGF- β signaling pathways and other known pathways (Labbe, et al., 1998, Zhang, et al., 1998).

Members of the schistosome TGF β signaling pathway

In vertebrates the ligand and membrane receptor interactions determine which receptor substrates (Smad proteins) will be activated. The Smad complexes (R-Smad and Co-Smad) translocate to the nucleus where they interact with a set of partner and regulator proteins that are cell type-specific and in the end will determine which genes are to be regulated and thus the outcome of the response to the signal. Identifying the components of the TGF- β signaling family, the partners and target genes is critical to understanding the role of the TGF- β signaling pathway in the biology of the schistosome. To date, two members of the schistosome TGF β family of ligands (Freitas, et al., 2007); LoVerde et al., unpublished data); two TGF- β receptors including SmT β RI, (Davies, et al., 1998) and SmT β RII, (Forrester, et al., 2004, Osman, et al., 2006, Verjovski-Almeida, et al., 2003) ; four members of the schistosome Smad family including SmSmad-1 (Beall, et al., 2000), SmSmad-2 (Beall, et al., 2000, Osman, et al., 2001), SmSmad-4 (Osman, et al., 2004), and SmSmad1B (formerly identified as SmSmad-8, (Verjovski-Almeida, et al., 2003) (Carlo, et al., 2007) and six scaffolding/regulatory proteins including SmSARA (unpublished results, (Verjovski-Almeida, et al., 2003), SmGCN5 (Carlo, et al., 2007), SmCBP (Carlo, et al., 2007), SmFKBP12 (Knobloch, et al., 2004), SmElF2 α (McGonigle, et al., 2002) and Sm14-3-3 ϵ (McGonigle, et al., 2001) have been identified.

TGF β superfamily ligands

Two TGF- β ligands from *S. mansoni* have been identified (Freitas, et al., 2007) and unpublished results]; An Inhibin/activin-like molecule (SmInACT) and a BMP-like molecule (SmBMP) (Fig. 2).

SmInAct is said to be 161 amino acids long and contains many of the hallmarks for a TGF- β ligand. It has a "RXRR" furin proteolytic cleavage site that separates the functional C-domain from the propeptide (Fig. 2A). Surprisingly, Freitas et al. (Freitas, et al., 2007) reported 31 residues (approx 3.5 kDa) as the N-terminal propeptide (a dimer of the propeptide constitutes the Latency-associated peptide; LAP). In case of other TGF β ligands, the LAP, which is needed

for secretion and processing of the bioactive component, is around 50 kDa. Perhaps there is something novel about the schistosome ligand. The ORF that encodes the mature peptide (monomer) contains 9 canonical cysteine residues and invariant proline and glycine residues that are necessary for the proper dimerization and tertiary structure of the TGF- β orthologue (Fig 2A). The native mature peptide occurs as a 28 kDa homodimer in egg and adult worm extracts as determined by western blot analysis. The mature peptide is 27% identical to both DAF-7 from *C. elegans* and dActivin from *Drosophila melanogaster* and 29% identical to human TGF β -1. Phylogenetic analysis shows that SmInAct clusters with TGF- β orthologues from *C. elegans* (DAF-7), *Brugia malayi* (Bm-TGH-2) and *Strongyloides stercoralis* (Ss-TGH-1). SmInAct is developmentally regulated as it is found in eggs and both mature and immature (single-sex) adult male and female worms. However, it is most abundant in mature female worms and eggs.

The *S. mansoni* BMP-like molecule (SmBMP) shows 44 % similarity to human BMP6. The closest orthologue, the chicken homologue of Xenopus Vg1 gene, shows 55% identity. It encodes a mature polypeptide (monomer) of 102 amino acids (11.5 kDa) giving a dimer of 23 kDa which is typical for members of the TGF- β family. It exhibits a furin cleavage site to cleave the propeptide from the mature form of the molecule (Figure 2B). The *S. mansoni* BMP-like ligand has 7 canonical cysteines, including the one that normally forms the interchain disulfide. However, it lacks two cysteines that are characteristic to some members of the superfamily, such as TGF- β s and activins (Shi and Massague, 2003).

Receptor Serine/threonine kinases: SmT β RII and SmT β RI

The type II receptor is essential for ligand binding, and upon ligand binding, it activates the type I receptor via phosphorylation. It is the type I receptor that specifically phosphorylates the regulatory Smads (Forrester, et al., 2004, Osman, et al., 2006). SmT β RII (=SmRK2) was first identified as an EST (Verjovski-Almeida, et al., 2003) and subsequently isolated and characterized (Forrester, et al., 2004, Osman, et al., 2006). The complete cDNA encodes a protein of 815 amino acids, which contains the entire predicted coding sequence of TGF- β type II receptor. Sequence comparisons demonstrated that the schistosome receptor shows highest similarity to mammalian Activin type II receptors (rat, mouse and human, respectively). These results were based on comparison of the kinase domains of the isolated clone and other members of type II receptor subfamily. However, the N-terminal extracellular domain (ECD) and the trans-membrane (TM) domains showed greatest similarity to Activin type II receptors from the spotted green puffer, *Tetraodon nigroviridis*, and the zebra fish, *Danio rerio*, and to BMP type II receptor of the giant pacific oyster, *Crassostrea gigas*. Further analysis predicted a single transmembrane domain spanning amino acid residues 139 to 164. The protein sequence apparently lacks membrane anchoring motifs such as glycosylphosphatidylinositol- (GPI) anchoring signals or signal peptides. The SmT β RII gene is 26 kbp and consists of 9 exons. Two transcripts are produced from the same gene by alternative splicing of the last two exons (exons 8 and 9) (Forrester, et al., 2004, Osman, et al., 2006). The shorter is 550 amino acid long [SmRK2; 62.2 kDa deduced size (Forrester, et al., 2004)] and the larger is 815 amino acid [SmT β RII; 92.6 kDa deduced size (Osman, et al., 2006)]. Consistent with this observation, two major protein bands (92 and 62 kDa) were observed in western blots using specific anti-type II receptor antiserum (Osman, et al., 2006).

The first evidence for the presence of surface-associated serine-threonine kinase activity was from a study that employed surface biotinylation to isolate proteins (Davies and Pearce, 1995). One of these proteins that demonstrated serine-threonine kinase activity was shown to be a TGF- β type I receptor called SmRK-1 (= SmT β RI) (Davies, et al., 1998). SmRK-1 is a 780 amino acid protein (86.7 kDa). The protein kinase domain of the receptor shows 58% identity to kinase domains of other type I receptor serine-threonine kinases and contains a

characteristic GS domain. SmRK-1 is phosphorylated and contains an amino-terminal N-linked glycosylation site that is glycosylated (Davies and Pearce, 1999). Phylogenetic analysis of SmRK-1 kinase domain demonstrated its relationship with type I receptors but suggested that it is a divergent member of the TGF- β type I receptor subfamily (Davies, et al., 1998). SmT β RI is expressed in lung and adult stages but not 3 and 24 hr stages (Beall, et al., 2000).

Comparison of *S. mansoni* TGF β type I and II receptors with other type I and II receptors of the superfamily

Structural studies carried out over the course of the past several years (Allendorph, et al., 2006, Greenwald, et al., 1999, Hart, et al., 2002, Kirsch, et al., 2000, Kirsch, et al., 2000, Thompson, et al., 2003) have shown that TGF- β s, BMPs, and activins each bind and assemble their receptors in a distinct manner. TGF- β s, on the one hand, have been shown to bind their type II receptor on the underside of the fingers (Groppe, et al., 2006). BMPs and activins, on the other hand, bind their type II receptor on the knuckle (Kirsch, et al., 2000). The BMP type I receptor, BMPRIa, has been shown to bind to the wrist (Kirsch, et al., 2000b), and while no structural information is yet available, it has been proposed based on mutagenesis and other data that the TGF- β type I receptor (T β RI) binds TGF- β (Zuniga, et al., 2005) and the activin type Ib receptor (ActRIb) binds activin (Harrison, et al., 2004) in a manner that differs somewhat from that of BMPRIa. These differences might account for the strong interdependence of type I-type II receptor binding that has been demonstrated for the TGF- β system (Zuniga, et al., 2005) and the weak independence that has been demonstrated for the BMP system (Greenwald, et al., 2003).

The alignments of the *S. mansoni* type I and type II receptor extracellular domains with others of the TGF- β superfamily that have been structurally characterized are shown in Figure 3. These reveal full (type II receptor, top) or nearly full (type I receptor, bottom) conservation of the four disulfide bonds that define the three finger toxin fold of the receptors (blue asterisks) (Greenwald, et al., 1999). There are, however, important differences. The *S. mansoni* type II receptor, for example, has one additional disulfide in the loop 1 region (red asterisks), not two as in the human (h) TGF β type II receptor (red asterisks). The *S. mansoni* type II receptor-ED also has an additional disulfide in the C-terminal region (green asterisks), which is also present in the activin type II receptor (green asterisks), but not the TGF- β type II receptor. The *S. mansoni* type I receptor has similar differences. It lacks one of the four disulfides characteristic of the three finger toxin fold (blue asterisks), and the additional disulfide in the N-terminal region (red asterisks) is between adjacent cysteines, rather than separated by a several residues as in the TGF- β type I receptor and the BMP type Ia receptor.

The type I and type II receptor sequence alignments further show that the *S. mansoni* type I and II receptors share little conservation at sites identified as contacting the growth factor based on structural studies of the growth factor-receptor complexes.

These important differences, together with differences in the manner of receptor binding for the TGF- β , BMP, and activin subfamilies, raise the possibility that the *S. mansoni* receptors may well interact with host TGF- β 1 in a manner that is entirely distinct relative to the host TGF- β receptors. If proven true then this opens the door for the development of novel antagonists that would interfere with interaction of host ligands with the *S. mansoni* receptors, but would not with cognate host receptors.

In this regard, recent data indicates that SmT β RII was able to interact productively with the SmT β RI in the presence of the human ligands TGF- β 1 and BMP-2 but not activin-A nor BMP-4 (Osman, et al., 2006).

BMP- Pathway R-Smads, SmSmad1 and SmSmad1B

The SmSmad1 protein is composed of 455 amino acids with a molecular mass of 50.8 kDa (Beall, et al., 2000). SmSmad1 contains conserved MH1 and MH2 domains that showed high homology to *Drosophila* MAD, 90 and 84% identity, respectively (Beall, et al., 2000).

The SmSmad1B protein is composed of 380 amino acids with a molecular mass of 43 kDa (Carlo, et al., 2007). SmSmad1B contains conserved MH1 and MH2 domains separated by a short, 42 amino acid linker region, making it the smallest R-Smads in terms of size. Importantly, the L3 loop in the MH2 domain of SmSmad1B resembles the L3 loop of R-Smads specific for transducing BMP-like signals (i.e. amino acid residues H340 and D343). The *SmSmad1B* gene (>10.7kb) is composed of 5 exons separated by 4 introns.

Phylogenetic data suggest that *SmSmad1* and *SmSmad1B* were paralogue genes; they originated from the duplication of a common ancestor gene after the split of the Platyhelminths, arthropods and vertebrates (Carlo, et al., 2007).

The expression level of SmSmad1 and SmSmad1B mRNA was evaluated by performing quantitative RT-PCR on cDNA prepared from total RNA isolated from various schistosome developmental stages (Carlo, et al., 2007). The results demonstrate that *SmSmad1* and *SmSmad1B* are expressed in eggs, primary sporocysts, 30d infected snails, 3d, 7d, 15d, 21d, 28d and adult stages where both exhibit the highest transcript levels in cercariae and to a lower extent in different developmental stages in the intermediate host, *Biomphalaria glabrata* snails. On the other hand, expression levels demonstrate significant drop in the stages representing different time points in mammalian host, as early as 3 days post infection with a gradual decrease thereafter up to 21-day old parasites, which represent the trough of the expression curves of both R-Smads. The levels then display a slight increase to reach maximum levels of expression in the mammalian host in paired adult worms. Expression of SmSmad1 was not detected in 3hr and 24hr stages (Beall, et al., 2000) and not determined for SmSmad1B (Figure 4).

TGF β /Activin Pathway R-Smads, SmSmad2

SmSmad2 cDNA encodes a 649 amino-acid protein representing the largest Smad2 protein identified to date with a molecular mass of 71.3 kDa (Beall, et al., 2000, Osman, et al., 2001). NCBI blast search showed that SmSmad2 exhibits a high degree of homology to Smad2 gene products of *Xenopus laevis* (Graff, et al., 1996) and humans (Eppert, et al., 1996, Nakao, et al., 1997, Riggins, et al., 1996, Zhang, et al., 1996). Homology is restricted to the MH2 domain (69% identity, 77% similarity) and to a lesser extent, the MH1 domain (61% identity and 70% similarity). The SmSmad2 MH2 domain also contains a conserved L3 loop. The L3 loop in SmSmad2 has the specific sub-type residues R613 and T616, which dictates the interaction of Smad2 and Smad3 with the T β R-I and the activin type I receptors. Another characteristic feature maintained in the MH2 domain of SmSmad2 is the conservation of all the 5 sub-type-specific residues required for the interaction of Smad2 and Smad3 with SARA (Smad-Anchoring for Receptor Activation), I527, F532, Y552, W554 & N567 (Wu, et al., 2000). In western blots of adult worm extracts two proteins of about 70 and 72 kDa are recognized by the anti-SmSmad2 specific antibodies. The 72 kDa band is the phosphorylated form of SmSmad2 (Osman, et al., 2001).

In the mammalian host stages, SmSmad2 mRNA was found to be detected as early as 4 days post-infection. Compared to the mRNA level of an internal control, SmSmad2 mRNA exhibits relatively constant levels throughout development. It appears that the BMP-related Smads, SmSmad1 and SmSmad1B, exhibit relatively lower levels of expression (45% lower) compared to the TGF- β -related SmSmad2 in the late stages of infection (28-day, 35-day and

adult worms) whereas the SmSmad2 exhibits relatively lower levels of transcripts in the intramolluscan stages, cercariae and egg compared to SmSmad1 and SmSmad1B (Osman, et al., 2001, Osman, et al., 2004) (Carlo, et al., 2007) (Fig 4).

Co-Smad, SmSmad 4

Smad4, a common mediator Smad (co-Smad), performs a central role in transmitting signals from TGF- β , BMP and activins. SmSmad4 encodes a 738 amino-acid protein with a predicted molecular mass of 79.6 kDa. Western blot analyses of adult worm extracts identify native SmSmad4 as ~78 kDa in size. NCBI BLASTP search showed that SmSmad4 exhibits the highest homology with *Drosophila* Medea for the MH1 domain, while its MH2 domain exhibited the highest homology with mouse and human Smad4. As mentioned before, Erk phosphorylation of the linker region of R-Smads inhibits agonist-induced nuclear accumulation of Smad1 (Kretzschmar, et al., 1997), Smad2 and 3 (Kretzschmar, et al., 1999), which represents a counterbalance mechanism to regulate TGF- β signaling in epithelial cells. Interestingly, while none of the schistosome R-Smads; SmSmad1, SmSmad1B, or SmSmad2 contain Erk phosphorylation motifs, SmSmad4 linker region was found to contain 3 Erk1/2 phosphorylation motifs (PXS/TP). Osman et al. (Osman, et al., 2004) showed that SmSmad4 was phosphorylated *in vitro* by an active mutant of mammalian Erk2, and this phosphorylation significantly inhibited the interaction of SmSmad4 with the receptor-activated SmSmad2. Thus, this observation suggests that in schistosome parasites, the regulation of TGF- β signaling by Erk phosphorylation is exerted at SmSmad4 rather than R-Smads as is the case in the mammalian model. Such regulation may be cell type-specific such that down regulation may result in certain cell types while stimulation of TGF β signaling cascades could be the elicited effect of SmSmad4 phosphorylation by Erk in other cell types.

SmSmad4 mRNA levels exhibited little variation throughout development of the parasite from the cercariae to the mammalian stages including sexually immature parasites (single-sex female and male worms) stages to the egg stage. In contrast, in the infected snail stage, which represents secondary sporocysts, SmSmad4 showed a relatively low level.

Accessory Proteins in Schistosome TGF- β Signaling

Accessory factors play an important role in regulating the signal in the TGF- β pathway. In schistosomes a number of co-factors such as the immunophilin SmFKBP12 (Knobloch, et al., 2004), Sm14-3-3 ϵ (McGonigle, et al., 2001, McGonigle, et al., 2002), Sme2IF α (McGonigle, et al., 2002) SmSIP (McGonigle, et al., 2001), SmGCN5 (de Moraes Maciel, et al., 2004) and Smp300/CBP (Bertin, et al., 2006) have been identified and characterized to various degrees.

SmFKBP12

In eukaryotes, FK506-binding proteins with a molecular weight of 12 kDa (FKBP12s) influence a variety of signal transduction pathways that regulate cell division, differentiation and ion homeostasis. Among these, TGF- β signaling is modulated by FKBP12 via binding to TGF- β -family type I receptors (Wang, et al., 1996). FKBP12 binds to the GS domain, resulting in blocking the phosphorylation and activation of type I receptors by the corresponding type II receptors (Chen, et al., 1997). Knobloch et al. (Knobloch, et al., 2004) demonstrated by yeast two-hybrid analyses a direct binding of SmFKBP12 and SmT β RI, which was specifically inhibited by the drug FK506. SmFKBP12 and SmT β RI co-localized to the vitellaria of female parasites. These data suggest that SmFKBP12 may regulate SmT β RI activity in female reproductive tissues. Whether it functions to inhibit TGF- β signaling as occurs in mammals is yet to be determined.

SmSARA

SARA is a protein that has been shown to recruit Smad2 to the cell membrane for interaction with the T β RI subunit of the receptor complex (Tsukazaki, et al., 1998). A DNA fragment predicted to encode SmSARA was identified in the Brazilian cDNA sequencing project (Verjovski-Almeida, et al., 2003) and this EST was used to obtain a full length cDNA (LoVerde et al., unpublished data). The schistosome SARA, SmSARA is 1,187 amino acids in length. It is 37 to 39% homologous to SARA from several higher eukaryotes. It does not possess a well-defined Smad2 binding domain or the normal N-terminal FYVE domain, indicating that it may not act like the characterized SARA proteins from higher organisms. Preliminary experiments indicate that SmSARA may be able to bind directly to SmSmad1, however. This suggests that SmSARA is not the ortholog of the described SARA proteins but rather a related protein that functions to stimulate receptor binding to Smad1 rather than Smad2.

Sm14-3-3 ϵ

The 14-3-3 family of proteins are multifunctional molecules that participate in a wide range of cellular functions including the interaction with a number of signal transduction molecules. McGonigle et al. (McGonigle, et al., 2001) identified Sm14-3-3 ϵ as an interacting partner with SmT β RI in a yeast two-hybrid screen. They went on to demonstrate that Sm14-3-3 ϵ and SmT β RI interact with each other *in vitro* in a phosphorylation dependent manner. Subsequently, they demonstrated that Sm14-3-3 ϵ would interact with human T β RI *in vitro* and *in vivo* and would enhance TGF- β -induced transcription. This identified the role of 14-3-3 proteins as activators of TGF- β signaling and suggested that this regulation mechanism occurs in schistosomes as well. Thus 14-3-3 ϵ serves as a modulator of the TGF- β receptor signaling.

Sme2Ifa

S. mansoni eukaryotic translation initiation factor 2 α subunit (e2IF α) was identified as a molecule that interacts with SmT β RI and human (h) T β RI and II (McGonigle, et al., 2002). The strongest interaction is with kinase inactive receptors especially hT β RII. It turns out that both hT β RI and hT β RII phosphorylate SmeIF2 α *in vitro* at novel phosphorylation sites. SmeIF2 α , unlike Sm14-3-3 ϵ , overexpression inhibits the hTGF- β -driven response. Co-expression of Sm14-3-3 ϵ and SmeIF2 α abrogated the inhibitory effect of SmeIF2 α and restored TGF- β signaling to basal levels (McGonigle, et al., 2002). Thus eIF2 α serves as a modulator to fine tune TGF- β receptor signaling.

SmSIP

SmRK1-interacting protein (SIP) is a novel schistosome SH3 domain-containing protein, which is thought to function as an adaptor molecule that links SmT β RI to other signaling protein (McGonigle, et al., 2001). SIP expression is developmentally regulated and coincides with SmT β RI (= SmRK1). It seems that the SH3 domain is not necessary for the SIP-SmT β RI Interaction.

SmGCN5 and SmCBP/p300

In the nucleus, activated Smad complexes regulate transcription of the target genes in cooperation with transcriptional co-activators and co-repressors. General Control Nonderepressible-5 protein (GCN5) and CREB binding protein (CBP) have intrinsic histone acetyltransferase (HAT) activity, which facilitates transcription by decreasing chromosome condensation through histone acetylation and by increasing accessibility of transcription factors with the basal transcription machinery (Bannister and Kouzarides, 1996). Carlo et al. (Carlo, et al., 2007) demonstrated that the BMP-related R-Smads, SmSmad1 and SmSmad1B interact at relatively higher levels with SmGCN5 compared to that achieved with the TGF- β -related SmSmad2. SmSMAD4 alone did not interact with SmGCN5. However, addition of

SmSmad4 to the SmR-Smads resulted in decreased levels of interaction. For SmSmad2, including SmT β RI-QD (a constitutively active form) but not SmT β RI-wt in the binding reaction, not only significantly increased the interaction of SmSmad2 with SmGCN5, but also revealed the interaction of SmSmad4 with SmGCN5 and demonstrated its participation in the formation and probably the stabilization of the transcriptional protein complex. The above results are consistent with the report that human GCN5 interacts with both TGF- β - and BMP-related R-Smads (Kahata, et al., 2004). SmCBP interacted with SmSmad1 and SmSmad2 but not with SmSmad1B, or SmSmad4. When SmSmad4 was included in the reactions, a reduction in interaction level with GST-SmCBP1 was observed with both SmSmad1 and SmSmad2. However, like the case with SmGCN5, the addition of SmT β RI-QD, boosted the interaction of SmSmad2 and SmCBP and demonstrated the presence of SmSmad4 in the protein complex (Carlo, et al., 2007). Thus, it seems that complex interaction patterns with transcription co-activators are influenced by different factors depending on the context of the developmental event and/or the response to a signal of host or parasite origin.

Functional Role of *S. mansoni* TGF β components

In situ hybridization assay revealed that SmInAct localizes to the subtegument of male worms and the vitelline cells and ovary of the mature female parasite. In addition, qPCR and western blot analyses demonstrated that the transcript is also present in the egg stage (Freitas, et al., 2007). That SmInAct plays a role in female reproduction and egg embryogenesis was demonstrated by analyses of female worms from bisexual infections recovered from infertile infections from IL-7R^{-/-} mice in which the SmInAct protein was undetectable compared to mature females from normal infections in which the ligand was very abundant (Freitas, et al., 2007). Further, the use of RNAi to knock down SmInAct demonstrated that the RNAi treated worms failed to develop compared to controls and in spite of the fact that both the treated and control treated worms produced the same number of eggs. When the eggs themselves were treated with RNAi to knockdown SmInAct expression, only 6.4% of the egg developed compared to 17.2% for the controls (Freitas, et al., 2007). Thus, it is clear that SmInAct plays an important role in the embryogenesis of the egg both inside the mature female as the vitelline cells, which surround the zygote at eggshell formation and express the ligand, provide the required nutrients for the embryo during the 5-day period it takes for development of the miracidium outside of the female worm.

SmT β RII and SmT β RI have been shown to be expressed at the parasite surface and in the case of SmT β RI, expression was up-regulated after infection of the mammalian host (Davies, et al., 1998, Forrester, et al., 2004). In the presence of human ligands, TGF- β 1 and BMP-2 but not activin-A nor BMP-4, SmT β RII is able to interact productively with SmT β RI (Osman, et al., 2006). Thus, there is evidence that SmT β RII will selectively bind host ligand and in the presence of appropriate host ligand interact with SmT β RI. In a set of experiments, Beall and Pearce (Beall and Pearce, 2001) demonstrated that SmT β RI (=SmRK-1)-human (h)T β RI chimera (where the extracellular domain was from SmT β RI and the intracellular domain was from hT β RI) that the hT β RII could bind TGF- β 1 and TGF- β 3 but not BMP-7 and productively interact with SmT β RI extracellular domain to transduce a signal. hTGF β 1 but not BMP-7 was shown to allow the interaction with full length SmT β RI (Beall and Pearce, 2001).

It has been demonstrated that a constitutively active form of SmT β RI phosphorylated SmSmad 2 and allowed its interaction with SmSmad4. This receptor-induced SmSmad2/SmSmad4 heterooligomeric complex formation resulted in the nuclear translocation of the Smad complex and in activation of target genes (by measuring reporter gene expression) (Beall, et al., 2000, Beall and Pearce, 2001, Osman, et al., 2001, Osman, et al., 2004, Osman, et al., 2006). In fact, it has been demonstrated in *in vitro* studies, in *in vivo* studies and in the schistosome worm itself that hTGF β 1 binds to SmT β RII that forms a complex with SmT β RI, and through

phosphorylation, activates it. This induces the interaction of SmT β RI with SmSmad2, which results in the phosphorylation of SmSmad2 and its dissociation from the receptor complex, and hence the formation of a heterooligomeric complex with SmSmad4 and activation of a reporter construct or upregulation of transcription in a target gene (Beall, et al., 2000, Beall and Pearce, 2001, Osman, et al., 2001, Osman, et al., 2004, Osman, et al., 2006). Thus, there is evidence that a host ligand is capable of transducing a signal through the schistosome TGF- β signaling pathway. Whether this occurs during infection is yet to be demonstrated.

Smad1 and Smad1B bind to T β RI but are not phosphorylated and therefore are not able to dimerize productively with SmSmad4 (Beall, et al., 2000, Carlo, et al., 2007, Osman, et al., 2004). This strongly argues for a second T β RI that will interact with SmSmads 1 and 1B of the BMP pathway (Beall and Pearce, 2001, Carlo, et al., 2007). However, SmSmad1 and SmSmad1B do interact with SmSmad4 (Carlo, et al., 2007, Osman, et al., 2004).

Identification of SmGCP as a *S. mansoni* TGF- β target gene

An 86 kDa glycoprotein termed gynecophoral canal protein (SmGCP) has been shown to localize to the surface of the gynecophoric canal of the male (the site of interaction between the mating pair) and to the entire surface of the en copula female but not to non-mated males nor to immature females (Aronstein and Strand, 1985, Bostic and Strand, 1996). SmGCP is involved in promoting intimate contact of the female with the tegument of the gynecophoric canal of the male. A blast search with the protein sequence revealed striking homology to beta-ig-h3 (Transforming Growth Factor β induced gene-Human clone 3). Beta-ig expression is induced in response to TGF- β 1 and is thought to mediate cell adhesion (Ferguson, et al., 2003). The expression of TGF- β and Beta-ig parallel each other in tissue, location and time (Ferguson, et al., 2003). Based on sequence similarities, Osman et al (Osman, et al., 2006) assumed that SmGCP may undergo a TGF- β dependent induction similar to what occurs with the human homologue beta-igh3. They examined this hypothesis and found that SmGCP was indeed regulated by TGF- β As the distribution of T β RII, the TGF- β ligand binding receptor, is localized to the tegument of the gynecophoric canal (Forrester, et al., 2004), spatial expression was reasonable.

The first step in evaluating SmGCP gene as a target for TGF- β regulation was to determine its expression pattern throughout development. A semi-quantitative RT-PCR was performed and SmGCP was found to exhibit an expression peak at 28 days-post infection (Osman, et al., 2006), which coincides with worm mating.

The next question in this regard was to determine the responsiveness of SmGCP to induction by members of the TGF- β family. Total RNA was extracted from adult worms that were incubated in culture media supplemented with either 1 nmole of TGF- β 1 or 5 nmole of BMP2 then subjected to semi-quantitative RT-PCR to identify differences in gene expression levels due to different treatment regimens. Levels of transcripts for SmGCP and previously identified members of TGF- β signaling pathways were examined using this approach. Compared to untreated worm controls, an approximately 2-fold induction of SmGCP expression level was observed in adult worms treated with human TGF- β 1, while BMP2 treatment induced a slight decrease, if anything, in SmGCP levels.

The above data indicates that SmGCP expression is induced by human TGF- β 1. To determine whether this is a primary effect dependent on direct stimulation of TGF- β signaling pathway or a secondary event incited through stimulation of a different signaling network as part of a more generalized cellular response and not attributed to TGF- β treatment in particular, Osman et al. (Osman, et al., 2006) investigated the effect of blocking TGF- β signaling on the expression of SmGCP, by employing RNAi to knock down type II receptor as the initial event of TGF- β signaling.

Under these conditions, TGF- β type II receptor-specific 20-30 bp dsRNA (siRNA) was delivered into 26-day and 33-day old worm pairs and transformed parasites were left untreated or treated with human TGF- β 1. RT-PCR data demonstrated that SmT β RII-specific siRNA treatment did diminish transcription levels of SmT β RII about 3-4 fold (25-30% of the levels of untreated samples) in tested organisms. Not only did SmT β RII-siRNA treatment result in a concomitant reduction in SmGCP levels (there was 2-3 fold reduction in levels of SmGCP compared to levels of untreated samples, but SmGCP also failed to respond to human TGF- β 1 induction. No significant differences were detected in levels of SmT β RI, SmSmad1, SmSmad2 or SmSmad4 compared to untreated controls. Thus a host ligand (hTGF β 1) could bind to a T β RII receptor that resulted in a signal that specifically regulated the expression of a target gene that may function in worm pairing.

Role of TGF β pathway in parasite development, host-parasite interactions and male-female interactions

Schistosomes have a complex life cycle in which the parasite moves from a freshwater environment as a non-feeding free-living larvae, to an endoparasitic environment within the vasculature of a vertebrate host (homeotherm) to a free-living non-feeding freshwater environment to the endoparasitic environment of a snail (poikilotherm) host. As the parasite moves through these different units of environment, it responds to signals through transduction pathways that convey information from the parasite surface to various cells to enable the parasite to respond to stimuli from its host environment by changes in gene expression. In addition to the environmental stimuli, signals are also being transduced between the cells within the parasite. Together these external and internal signals regulate development, host-finding behavior, site-finding behavior, reproductive activity and immune evasion among other biological activities. In addition, schistosomes have evolved an interesting biological interplay among the male and female parasites such that male schistosomes via an unknown stimulus regulate female-specific gene expression and thus female reproductive development and egg production. Signaling pathways are prime candidates for transduction of the male stimulus to the vitelline cells of the female parasite.

The data presented in this review supports multiple (pleiotropic) roles for the TGF- β signaling pathway throughout the schistosome life cycle, especially in female reproductive development and egg embryogenesis involving the vitelline cells. Western blot and RT-PCR analyses have demonstrated that the components of the TGF- β signaling pathway studied to date are expressed variously throughout the schistosome life cycle with some showing more developmental regulation than others. For example, both SmT β RII and SmT β RI are expressed throughout schistosome development (daughter sporocysts (infected snails), cercariae, 5d, 15d-21d schistosomula, 28d-45d worms, adult male and female worms) with T β RII showing 3-5 fold higher transcription levels (Beall, et al., 2000, Osman, et al., 2006). However, T β RI transcripts were not detected in 3h and 24h schistosomula (Beall, et al., 2000). SmSmad1 and SmSmad1B are expressed throughout development with the highest levels in cercariae whereas SmSmad2 is expressed more or less constitutively throughout development except for 3h schistosomula which show minimal expression (Osman, et al., 2001) although Beall et al. (Beall, et al., 2000) did not detect SmSmad2 transcripts in 3 and 24 hr schistosomula. SmSmad4, the common Smad, is expressed more or less the same throughout development except for in the intramolluscan stages of *S. mansoni* where it is 2-3 fold less abundant (Osman, et al., 2004). However, SmSmad4 is 3-5 fold more abundant in each developmental stage relative to SmSmad1 and SmSmad2 (Osman, et al., 2004) indicating that it is involved in a number of diverse developmental processes throughout the parasite life cycle.

Immunolocalization and *in situ* hybridization studies demonstrate that in adult male and female worms SmT β RII, SmT β RI, SmSmad1B, SmSmad2, and SmSmad4 are localized to the

tegument and SmT β RII and SmT β RI are surface exposed suggesting that they can sample the external environment of the host (Carlo, et al., 2007, Davies and Pearce, 1995, Davies, et al., 1998, Forrester, et al., 2004, Knobloch, et al., 2004, Osman, et al., 2001, Osman, et al., 2004, Osman, et al., 2006). This is supported by the fact that hTGF β 1 can transduce a signal from the SmT β RII receptor to a target gene in the adult worm (Osman, et al., 2006). The fact that the T β Rs are surface exposed also opens the possibility of communication between the male and female parasite.

SmInACT, SmT β RII, SmT β RI, SmSmad1B, SmSmad2, and SmSmad4 are expressed in and localize to the vitelline cells and female reproductive organs suggesting involvement in female reproductive development and egg production (Forrester, et al., 2004, Freitas, et al., 2007, Knobloch, et al., 2004, Osman, et al., 2001, Osman, et al., 2004, Osman, et al., 2006). The vitelline cells produce the eggshell precursor proteins and the nutrients needed for the zygote to embryonate into a miracidium (LoVerde, et al., 2004). That T β RII and T β RI are located in the vitelline cells and that SmInACT is expressed in the vitelline cells and acts on egg embryogenesis argues that the TGF- β pathway transduces signals between cells within the reproductive system to regulate parasite development (Freitas, et al., 2007, Knobloch, et al., 2004, Osman, et al., 2006). Recent studies employing a T β RI kinase inhibitor (TRIKI) demonstrated that female mitotic activity was reduced up to 40% and egg production 30% in TRIKI-treated worms (Knobloch, et al., 2007). In a previous study the same authors demonstrated that Herbimycin A, an inhibitor of protein tyrosine kinases (PTKs), also blocks mitotic activity and egg production of paired females (Knobloch, et al., 2006). The authors in arguments presented in a paper in this volume suggest that this may indicate a cross talk between PTKs and TGF β signaling pathways that regulates vitelline cell development and thus egg production (see (Knobloch, et al., 2007) for discussion).

This is an exciting time as current research effort in a number of laboratories is aimed at understanding the role of signal transduction in the interaction of the male and female parasites and the interaction of the parasite with its host environment. Evidence is accumulating that the TGF- β pathway plays a role in female reproductive development, egg embryogenesis and in the host-parasite interaction. We can expect that this line of research will identify useful targets for control of parasite development and /or parasite caused morbidity and will begin to unravel the intricate relationship the schistosome has evolved with its human and snail hosts.

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Abbreviations

ALKs	activin receptor-like kinases
ActRIb	activin type Ib receptor
GS region	alternating glycine and serine residues
AMH	anti-Mullerian hormone
BMPs	bone morphogenetic proteins
Co-Smads	Common-Smads
cDNA	complementary deoxyribonucleic acid
CBP	CREB binding protein
EGFR	Epidermal growth factor receptor
e2IFα	eukaryotic translation initiation factor 2 α subunit
ERK	extracellular signal-regulated kinase
FKBP	FK506-binding protein
GCN5	General Control Nonderepressible-5 protein
GPI	glycosylphosphatidylinositol
GCP	

	gynecophoral canal protein
GDFs	growth/differentiation factors
HAT	histone acetyltransferase
h	human
I-Smads	Inhibitory Smads
IL-7	Interleukin-7
LAP	latency associated protein
LTBP	Latent TGF- β binding protein
MH	Mad homology region
MAPK	mitogen-activated protein kinase
Sm	Schistosoma mansoni
SARA	Smad Anchor for Receptor Activation
SmInACT	SmInhibin/activin-like molecule
ECD	N-terminal extracellular domain
TM	trans-membrane domain
RK	Receptor Kinase
R-Smads	Receptor-regulated Smads
RT-PCR	Reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
RNAi	RNA interference

siRNA	short interfering RNA
SIP	SmRK1-interacting protein
TGF-β	Transforming Growth Factor beta
TβRI	TGF-b type I receptor
TβRII	TGF-b type II receptor
beta-ig-h3	Transforming Growth Factor β -induced gene-Human clone 3

Figure. 1.

Hypothetical TGF- β signaling pathways in *Schistosoma mansoni*. In this illustration, a surface-exposed (tegumental) TGF- β type II receptor (RII; e.g. SmT β RII) binds a self and/or a host TGF- β /Activin ligand (e.g. hTGF- β 1, or SmInAct) [left side], or a self and/or a host BMP ligand (e.g. hBMP2 or SmBMP) [right side]. The ligand/RII complex triggers the interaction with a type I receptor (RIA; left e.g. SmT β RI, or RIB; right e.g. an unidentified, BMPRI-like receptor I), phosphorylates and activates it. The activated type I receptor relays the signal down to a R-Smad (RIA to SmSmad2 and RIB to SmSmad1 or 1B), where the R-Smad is activated via phosphorylation at the SXS C-terminal motif, a process that is blocked by an unidentified Schistosome Smad7 orthologue. The activated R-Smad interacts with the co-Smad (SmSmad4) in the subtegument. The Smad complex translocates to the nucleus of the sub-tegumental cell, binds a nuclear partner (e.g. SmGCN5, or SmCBP/p300) and the assembled transcription complex binds to the SBE in the promoter of the target gene and regulates target gene transcription. See text and (LoVerde, 2002, LoVerde, et al., 2004) for further details.

Figure 2. Diagram showing SmInAct and SmBMP-like molecules. A. SmInAct, B. SmBMP-like ligand. Green arrow identifies furin cleavage sites. (*) identifies conserved cysteine residues that involved in intra- and inter-chain disulfide bonds.

Figure 4.

Semi-quantitative RT-PCR analysis of schistosome Regulatory receptor Smad genes. Bar-graph comparing the relative band-intensity of SmSmad1 (blue), SmSmad2 (purple) and SmSmad1B (yellow) to Sm α -Tubulin throughout various stages of schistosome development. Values were calculated from three independent experiments.