The epidermal growth factor receptor (EGF-R) plays an important role in development and cell differentiation, and homologues of EGF-R have been identified in a broad range of vertebrate and invertebrate organisms. This work concerns the functional characterization of SER, the EGF-R-like molecule previously identified in the helminth parasite Schistosoma mansoni (Shoemaker, C. B., Ramachandran, H., Landa, A., dos Reis, M. G., and Stein, L. D. (1992) Mol. Biochem. Parasitol. 53, 17–32). Transactivation assays performed in epithelial Madin-Darby canine kidney cells co-transfected with SER and a Ras-responsive reporter vector indicated that SER was able to trigger a Ras/ERK pathway in response to human epidermal growth factor (EGF). These results were confirmed in Xenopus oocytes showing that human EGF induced meiosis reinitiation characterized by germinal vesicle breakdown in SER-expressing oocytes. Germinal vesicle breakdown induced by EGF was dependent on receptor kinase activity and shown to be associated with phosphorylation of SER and of downstream ERK proteins. 125I-EGF binding experiments performed on SER-expressing oocytes revealed high affinity (2.9 × 10^–9 M) of the schistosome receptor for human EGF. Phosphorylation of the native SER protein present in S. mansoni membranes was also shown to occur upon binding of human EGF. These data demonstrate the ability of the SER schistosome receptor to be activated by vertebrate EGF ligands as well as to activate the classical ERK pathway downstream, indicating the conservation of EGF-R function in S. mansoni. Moreover, human EGF was shown to increase protein and DNA synthesis as well as protein phosphorylation in parasites, supporting the hypothesis that host EGF could regulate schistosome development. The possible role of SER as a receptor for host EGF peptides and its implication in host-parasite signaling and parasite development are discussed.

The epidermal growth factor receptor (EGF-R) is a major key mediator of cell communication during animal development and homeostasis. EGF-R was the first receptor tyrosine kinase to be cloned (1), and its structure and activation pathways have been studied extensively. EGF-R represents the archetype of receptor tyrosine kinase with an extracellular ligand-binding part with two cysteine-rich repeats and an intracellular domain containing tyrosine kinase activity (2). In mammals, four isoforms of EGF-R have been characterized (EGF-R/ErbB-1, HER2/ErbB-2, HER3/ErbB-3, and HER4/ErbB-4), and a number of different ligands, including epidermal growth factor (EGF)-like molecules, can selectively bind each isoform (3). Ligand binding activates the receptor by inducing the formation of homo-heterodimers. Dimerization triggers trans-phosphorylation and subsequent autophosphorylation of receptor molecules on tyrosine residues that provide docking sites for diverse effector and adaptor proteins. These partners (Grb2/Sos, pp53-P13K, PLCγ, and JAK) are active in different signal transduction cascades, such as the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase, antiapoptotic kinase Akt, and several transcriptional regulatory pathways (reviewed in Ref. 4). Different homodimer-heterodimer combinations formed by EGF-R family members drive a complex signaling network within the MAPK pathway. The ERK pathway is the most recurrent and is mainly responsible for the mitogenic action of EGF receptors. Dysregulation of EGF-R signaling is therefore strongly onogenic, and the direct implication of EGF-R isoforms in various cancers has been widely demonstrated. For this reason, EGF-R currently represents one of the major drug targets in human cancer therapy (5).

In invertebrates, EGF-R isoforms appeared to be expressed in more limited numbers. A single isoform has been characterized in Caenorhabditis elegans (LET-23) (6) as well as in Drosophila melanogaster (DER) (7, 8). A single cognate ligand (LIN-3) would be present in the worm (9), and four distinct cognate ligands (Vein, Gurken, Spitz, and Argos) would be present in the fly (10). These observations indicated that the EGF-R signaling module has grown in complexity from invertebrates to mammals. However, except for C. elegans and D. melanogaster models, few data are available at present about the role of the EGF-R family in invertebrate development.

SER, the Schistosoma mansoni EGF-R homologue, is one of the three receptor tyrosine kinases that have been characterized in this trematode parasite (11, 12). SER is present predominantly in schistosome muscles, suggesting that it could play a key role in host-parasite interaction. This work concerns the functional characterization of SER, the EGF-R-like molecule previously identified in the helminth parasite Schistosoma mansoni (Shoemaker, C. B., Ramachandran, H., Landa, A., dos Reis, M. G., and Stein, L. D. (1992) Mol. Biochem. Parasitol. 53, 17–32). Transactivation assays performed in epithelial Madin-Darby canine kidney cells co-transfected with SER and a Ras-responsive reporter vector indicated that SER was able to trigger a Ras/ERK pathway in response to human epidermal growth factor (EGF). These results were confirmed in Xenopus oocytes showing that human EGF induced meiosis reinitiation characterized by germinal vesicle breakdown in SER-expressing oocytes. Germinal vesicle breakdown induced by EGF was dependent on receptor kinase activity and shown to be associated with phosphorylation of SER and of downstream ERK proteins. 125I-EGF binding experiments performed on SER-expressing oocytes revealed high affinity (2.9 × 10^–9 M) of the schistosome receptor for human EGF. Phosphorylation of the native SER protein present in S. mansoni membranes was also shown to occur upon binding of human EGF. These data demonstrate the ability of the SER schistosome receptor to be activated by vertebrate EGF ligands as well as to activate the classical ERK pathway downstream, indicating the conservation of EGF-R function in S. mansoni. Moreover, human EGF was shown to increase protein and DNA synthesis as well as protein phosphorylation in parasites, supporting the hypothesis that host EGF could regulate schistosome development. The possible role of SER as a receptor for host EGF peptides and its implication in host-parasite signaling and parasite development are discussed.

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participate in muscle development and functions (13). Similar to other EGF-R family members, the intracellular tyrosine kinase domain is the most highly conserved region, whereas the extracellular domain is poorly conserved. Previous work has shown that recombinant SER expressed in insect cells possessed kinase activity and was capable of autophosphorylation, but no evidence was given regarding its function in the parasite. In the present study, we have studied the expression of SER to analyze the signaling pathways triggered upon its activation and its potential role as a receptor for host ligands. The expression of the SER recombinant protein has been examined in two different vertebrate cellular systems (epithelial MDCK cells and Xenopus oocytes) to analyze the affinity of the parasite receptor for human EGF and its ability to phosphorylate and activate an EGF-dependent signaling pathway. SER analysis was extended to the different schistosome life stages, and the effect of human EGF on parasite metabolism was studied in vitro on sporocysts, the only proliferative stage that develops in the mollusc intermediate host.

The possible role of SER as a receptor for host EGF peptides and its implication in host-parasite signaling and parasite developmental stages is discussed.

EXPERIMENTAL PROCEDURES

Parasites—A Puerto Rican strain of S. mansoni was maintained by passage through albino Biomphalaria glabrata snails and Mesocercus auratus hamsters. Mircacidia were transformed in vitro into sporocysts by incubation in minimum salt medium at 28 °C for 18 h. Adult schistosomes were collected by portal perfusion from infected hamsters.

Cloning of SER—Total RNA was isolated from S. mansoni adult worms by the method of Chirgwin et al. (16) and purified by centrifugation through a cesium chloride gradient. The first-strand cDNA was synthesized using the Thermoscript RT-PCR System (Invitrogen) and the oligo(dT)15 primer, SERfwd (5′-AAAAACTTAAAAAGTTTGT-TTACTACCTACTCTG-3′) and SERrev (5′-GGGGGCCGATATTCGCA-GCGAGGTACTAGTATTTTGG-3′) complementary to the N- and C-terminal sequence of the SER cDNA (GenBank™ accession no. M66396) were used as primers in RT-PCR to amplify the schistosome cDNA. PCR was carried out with Taq Platinum HiFidelity polymerase (Invitrogen). Final products were purified, cloned into TOPO 4.0 TA cloning vector (Invitrogen), and then inserted in-frame into the pcDNA3.1-V5/His expression vector (Invitrogen) and pcDNA3.1-V5/His expression vector (Invitrogen), and then inserted in-frame into the pcDNA3.1-V5/His expression vector (Invitrogen). SER was expressed in insect cells.

Detection of Phosphorylated SER Protein in Schistosome Membrane—Extracts were prepared from [35S]methionine-labeled oocytes following the procedure described previously (20). Oocytes were washed with 600 ng ml−1 [35S]methionine, and then incubated in ND96 medium supplemented with streptomycin/penicillin (50 μg ml−1 each) for 8 h, lysed in buffer A (50 mM HEPES, pH 7.4, 500 mM NaCl, 0.5 mM MgCl2, 1 mg/ml bovine serum albumin, 10 μg ml−1 leupeptin, 10 μg ml−1 aprotinin, 10 μg ml−1 soybean trypsin inhibitor, 10 μg ml−1 benzamidine, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate), and centrifuged at 4 °C for 15 min at 10,000 g. Membrane pellets were resuspended and incubated for 15 min at 4 °C in buffer A containing 1% Triton X-100 and then centrifuged under the same conditions. Supernatants were incubated with anti-V5 antibodies (1:100; Invitrogen) overnight at 4 °C. Protein A-Sepharose beads (5 mg; Amer sham Biosciences) were added for 1 h at 4 °C. Immune complexes were collected by centrifugation, rinsed three times, resuspended in Laemmli sample buffer, and subjected to a 7.5% SDS-PAGE. Gels were dried and autoradiographed. The detection of tyrosine-phosphorylated SER protein was performed in oocytes expressing SER or SERTK−, and HER cRNA were injected in each oocyte 4 h before stimulation by recombinant human EGF (R&D Systems). GVBD was detected by the appearance of a white spot at the center of the animal pole after 15 h of human EGF treatment. Student’s t test was used to assess the significance of the observed differences.

Immunoprecipitation and Western Blot Analyses of SER Expressed in Xenopus Oocytes—Expression of SER in oocytes was confirmed by immunoprecipitation of membrane extracts from [35S]methionine-labeled oocytes according to the procedure described previously (20). Oocytes were washed with 600 ng ml−1 [35S]methionine, and then incubated in ND96 medium containing 1% Triton X-100 and then centrifuged under the same conditions. Supernatants were incubated with anti-V5 antibodies (1:100; Invitrogen) overnight at 4 °C. Protein A-Sepharose beads (5 mg; Amer sham Biosciences) were added for 1 h at 4 °C. Immunocomplexes were collected by centrifugation, rinsed three times, resuspended in Laemmli sample buffer, and subjected to a 7.5% SDS-PAGE. Gels were dried and autoradiographed. The detection of tyrosine-phosphorylated SER proteins was performed in oocytes expressing SER or SERTK− for 4 h and stimulated or not for 5 min by EGF (49.8 ng, final concentration). Briefly, 30 oocytes were lysed in 300 μl of buffer A, and membrane extracts were immunoprecipitated by anti-V5 antibodies under the same conditions as described above. Immunocomplexes were analyzed by Western blotting using anti-V5 (1:10,000; BD Biosciences) antibodies and the ECL advance detection kit (Amersham Biosciences).

The detection of EGF proteins in oocyte homogenates was performed by Western blotting using anti-ERK2 mouse antibodies (Santa Cruz Biotechnology) as described previously (21).

EGF Binding Assays—Oocytes were injected with 60 nl of SER cRNA (equivalent to 60 ng) or with 60 nl of water 48 h before EGF binding assays. Sets of 10 oocytes were placed in 24-well plates and preincubated in ND96 medium and 1% bovine serum albumin for 1 h at 0 °C. A 10−4 M human EGF solution containing [125I]labeled human EGF (250 μCi ml−1; specific activity, 1268 Ci mmol−1; Amersham Biosciences) was prepared, and serial dilutions of the radioactive mixture were made in ND96 medium/bovine serum albumin. Oocyte groups were incubated for 4 h (until equilibrium) at 0 °C in a total volume of 200 μl in the presence of 10−6, 6.6 × 10−7, 3.3 × 10−7, 5 × 10−7, and 5 × 10−8 M EGF final concentrations. At the end of the incubation, aliquots of media were removed and used to determine the amount of free EGF. Oocytes were washed with 300 μl of ND96 medium and then incubated in ND96 medium/bovine serum albumin, and the radioactivity bound to oocytes was determined. Nonspecific binding to water-injected oocytes represented <10% of total binding and was deducted from the values of bound radioactivity. Scatchard analysis was performed using EnzFitter software (Biosoft). The experiment was repeated twice using independent plasmid preparations to assess reproducibility. cRNA in Vitro Synthesis—Capped mRNA (cRNA) was synthesized using the T7 Message Machine Kit (Ambion). SER and SERTK−, pcDNA3.1 plasmids were linearized by the enzyme PmeI, and pOBER plasmid (19) containing the human EGF-R (HER) was digested by NotI. cRNA was transcribed from linearized plasmids. Oocytes were precipitated by 2.5 M LiCl, washed in 70% ethanol, resuspended in 20 μl of diethyl pyrocarbonate-treated water, and then quantified by spectrophotometry. Finally, 1 μg of cRNA was analyzed on a denaturing agarose gel. Gel staining with 10 μg ml−1 ethidium bromide allowed confirmation of the size of cRNA and verification of the absence of any transcripts.

Expression of SER in Xenopus Oocytes—After anesthesia with MS 222 (1 g l−1; Sandoz), Xenopus laevis ovaean fragments were surgically removed and placed in ND96 medium (96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, and 5 mM Hepes adjusted to pH 7.4 with NaOH) supplemented with streptomycin/penicillin (50 μg ml−1 each; Eurobio, Saint Germain-en-Laye, France) and then centrifuged at 4 °C for 5 min at 15,000 g. Membrane pellets equivalent to 100 parasites were resuspended in 1 ml of PY buffer with or without 1 μg EGF and incubated at 37 °C for 10 min.
Myelin basic protein was an active kinase, able to phosphorylate MBP (bovine with anti-V5 antibodies confirmed that the recombinant protein was an active kinase, able to phosphorylate MBP (bovine myelin basic protein) substrate and to autophosphorylate, as already shown by Ramachandran et al. (13) (data not shown). After centrifugation, membrane pellets were homogenized in 100 μl of PT supplemented with 1% Triton X-100 and incubated for 30 min at 0 °C. Membrane lysates were complemented with 4 volumes of kinase buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 12.5 mM MgCl2, 1 mM dithiothreitol, 1 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl fluoride) containing 50 μM ATP and incubated for 30 min at 30 °C before immunoprecipitation by purified rabbit anti-SER antibodies directed against the ligand binding domain (residues 357–517) of the receptor (1 μg ml⁻¹, kindly provided by P. Skelly; Ref. 13) and protein A-Sepharose beads. Immune complexes were analyzed in 7.5% SDS-PAGE gel and revealed by Western blotting using anti-PY20 (1:10,000; BD Biosciences).

Quantitative RT-PCR—Total RNA was extracted from miracidia, sporocysts, cercariae, and adult male and female worms using the technique of Chirgwin et al. (16) and then reverse-transcribed using the Thermoscript™ RT-PCR System (Invitrogen). cDNAs were used as templates for PCR amplification using the SYBR® Green PCR Master Mix and the ABI PRISM 7000 sequence detection system (Applied Biosystems). Primers specific for S. mansoni tubulin (GenBank™ accession no. 80214, positions 851–873 and 925–904) and SER (GenBank™ accession no. M86396, positions 1002–1022 and 1102–1081) were designed by the Primer Express Program (Applied Biosystems) and used for amplification in triplicate assays. For graphical representation of quantitative PCR data, raw cycle threshold values (Ct values) obtained for male worms, female worms, miracidia, and cercariae were de ducted from the Ct value obtained for sporocyte transcript levels using the ΔΔCt method (22), with tubulin gene levels serving as the internal standard.

Sporocyst Labeling—200 transformed sporocysts were incubated for 24 h in 96-well plates in minimum salt medium at a concentration of 1000 sporocysts ml⁻¹ in the presence of radioactive precursors (2 μCi of [1-35S]methionine or 1 μCi of [3H]thymidine (Amersham Biosciences)) and different doses (10⁻⁶ to 10⁻⁵ M, final concentration) of human EGF (R&D Systems). After incubation, parasites were collected, and 35S radioactivity was measured in the trichloroacetic acid-precipitated material using a scintillation liquid (Beckman) and a Packard scintillation counter. [3H]Thymidine incorporation was determined after filtration onto GF/C filters (Packard) using a cell harvester (Packard) and a Packard microplate scintillation counter. 32P Labeling was performed under similar conditions with the addition of 2 μCi of [γ-32P]ATP (3000 Ci mmol⁻¹). Labeling was stopped after 5 min of incubation by the addition of 0.2 volume of 10% SDS and boiling for 2 min. Proteins were precipitated with 5% trichloroacetic acid, and radioactivity was determined.

Results

Human EGF Activates SER in MDCK Epithelial Cells—The full-length SER sequence (GenBank™ accession no. M86396) was amplified by RT-PCR from adult schistosome RNA, cloned in-frame into the pcDNA3.1.5/V5/His expression vector and expressed in MDCK epithelial cells. In vitro kinase assays on immune complexes formed after precipitation of cell extracts with anti-V5 antibodies confirmed that the recombinant protein was an active kinase, able to phosphorylate MBP (bovine myelin basic protein) substrate and to autophosphorylate, as already shown by Ramachandran et al. (13) (data not shown). Transactivation assays were further performed in SER-expressing MDCK cells co-transfected with the Py-Luc reporter vector. This vector contains Ras-responsive elements with three tandem copies of a polyoma virus enhancer-derived sequence with ETS/AP1 binding sites linked to the thymidine kinase promoter and the Luciferase reporter gene (18). This co-transfection system has been demonstrated to be a sensitive assay to analyze functional activity of receptor tyrosine kinase (17). Results in Fig. 1 indicated that in SER-transfected cells, human EGF is able to increase the Ras-dependent transcriptional response in a dose-dependent manner, with maximum luciferase activity detected with EGF concentrations of ≥10⁻⁹ M. In control cells transfected with the empty vector, a basal level of luciferase activity was detected, and its moderate increase in the absence of EGF was attributed to activation of the endogenous canine EGF-R. These results already suggested that SER expressed in mammalian cells was able to trigger a Ras pathway in response to EGF, but the level of protein expression in MDCK cells was too low to pursue the analysis of receptor activation and phosphorylation in this expression system.

EGF Induces GVBD in SER-expressing Oocytes—Xenopus oocytes are frequently used to express proteins, and they have already been shown to express with efficacy S. mansoni proteins (23). Therefore, we considered these cells as an alternative model for the expression of the SER receptor. Results presented in Fig. 2 demonstrated that translation of SER RNA, in vitro-transcribed from SER-containing plasmids, was efficient in oocytes. After 48 h of incubation, the presence of the [35S]methionine-labeled SER protein was detected in oocyte membranes. The labeled band of 215 kDa exhibited an apparent molecular mass larger than that expected for the recombinant protein (192 kDa), which could be attributable to glycosylation, a processing required for translocation of the receptor to the cell surface and subsequent acquisition of function (24).

Besides their use in examining protein production, oocytes have been shown to represent a suitable model for investigating receptor tyrosine kinase activation and transduction cascades. They are physiologically arrested at the G2-M transition of the first meiotic prophase, and this blockade can be abolished upon activation of receptor tyrosine kinase-dependent pathways. Ras activation triggers the MAPK cascade and causes phosphorylation of the ERK2/MAPK, which is necessary and sufficient to induce meiosis reinitiation characterized by oocyte GVBD (25). The results presented in Fig 3A indicate that human EGF-induced GVBD in a high percentage of oocytes expressing the human EGF-R (HER). GVBD resulted essentially from HER activation because no GVBD was detected in noninjected control oocytes or in HER-expressing oocytes in the absence of EGF. When similar experiments were performed
with SER-expressing oocytes, we observed that human EGF was also able to induce GVBD in such oocytes, at levels comparable with that found for HER-expressing oocytes. The percentage of GVBD induced by 16.6 nM EGF in HER oocytes (61.6 ± 35.4%) was not significantly different from that found in SER oocytes (40.8 ± 27.5%) at the same ligand concentration. In these experiments, we could demonstrate that SER kinase activity was directly implicated in GVBD induction by analyzing oocytes injected with a SER sequence mutated in its tyrosine kinase domain (SERTK⁻). Results in Fig. 3A show that a single mutation in the Mg²⁺-binding site of the receptor kinase domain completely abolished GVBD in SERTK⁻-expressing oocytes. Results of immunoprecipitation confirmed that the mutated SER protein was expressed at a similar level as the native receptor (Fig. 2).

**SER Activates ERK Phosphorylation in Oocytes**—To determine the nature of the signaling pathway triggered by SER, we first demonstrated that the induction of GVBD in oocytes requires receptor autophosphorylation. Results in Fig. 3B confirm the recognition of SER by anti-phosphotyrosine antibodies only when oocytes expressed the active receptor and were activated by EGF. Further analyses have concerned the effect of EGF on the phosphorylation of ERK proteins. Results in Fig. 3C indicate a shift of phosphorylated ERK2 proteins in SER- and HER-expressing oocytes after the addition of EGF. ERK phosphorylation appeared to be dependent on SER kinase activity because the dead mutation in SERTK⁻ was sufficient to totally inhibit the shift of ERK proteins in EGF-treated oocytes.

**Determination of the Affinity of SER for Human EGF**—Results from experiments of transactivation in MDCK cells and maturation of oocytes already suggested that SER could bind EGF from vertebrates with high affinity. To confirm the ability of the parasite receptor to bind and be activated by human EGF, Schistosome membrane preparations were incubated with EGF and subjected to kinase assay conditions before analysis by SDS-PAGE. Results in Fig. 5A show that a limited number of proteins in schistosome mem-
brane extracts react with anti-phosphotyrosine antibodies but indicate the presence in EGF-treated membranes of a major tyrosine-phosphorylated protein with an apparent molecular mass of 190 kDa, corresponding to the size of the activated EGF-R. Immunoprecipitation of EGF-treated membrane extracts by anti-SER antibodies conclusively identified the 190-kDa protein as SER (Fig. 5B) and confirmed phosphorylation of the native SER protein upon EGF binding. The apparent molecular mass of SER detected in these assays is slightly different from that found by previous authors using the same preparation of antibodies (170 kDa, Ref. 13), and this is probably due to different conditions of protein analysis. The disparity in size of native and recombinant SER (Fig. 2) probably results from differential glycosylation in adult schistosomes and Xenopus oocytes.

Potential Role of EGF in Host-Parasite Interactions—As a preliminary step toward a functional study of SER in schistosomes, we have analyzed the relative expression of SER transcripts in the different S. mansoni parasite stages living in both the definitive and intermediate hosts using the technique of real-time RT-PCR. Results presented in Fig. 6 confirm the high representation of SER in adult schistosomes, particularly in male worms, in which muscular structures are the most abundant. Data also indicate that SER is expressed throughout the parasite life cycle but that lower amounts of transcripts are present in larval stages as compared with adult forms. EGF-R has been demonstrated to be involved in diverse proliferative processes. The observation that SER was also expressed in sporocysts, the only larval stage responsible for asexual reproduction and proliferation of schistosomes in the mollusc host, prompted us to analyze a potential effect of EGF on sporocysts. Experiments shown in Fig. 7 indicate that in vitro incubation of sporocysts with human recombinant EGF stimulated both [35S]methionine and [3H]thymidine incorporation in a dose-dependent manner, with a maximal effect at a 10^{-8} M concentration. Additionally, after a short period (5 min) of incubation with the optimal EGF dose, we observed a 2-fold increase in the level of 32P incorporation as compared with control sporocysts, demonstrating that human EGF could activate a signaling pathway dependent on phosphorylation in the parasite. Further experiments confirmed that a similar dose of human EGF (10^{-8} M) was also able to stimulate (2-fold) protein synthesis in in vitro-cultured adult schistosomes (data not shown).

DISCUSSION

Much knowledge has been accumulating in recent years on the importance of EGF signaling in embryonic development and physiology. Progress on the developmental role of this growth factor has come, in large part, from the two inverte-
brate models, Drosophila (7, 8, 10) and C. elegans (6, 9). The identification of an EGF-like receptor molecule in S. mansoni (SER) (11, 13) led us to investigate its role in mitogenic and differentiation processes in the parasite and its possible involvement in host-parasite signaling. In this work, we analyzed the functional activity and ability of this receptor to bind host EGF and addressed the question of EGF signaling in S. mansoni development as well as in host-parasite interactions. The SER protein shares sequence and structural homologies with other members of the EGF-R family. The tyrosine kinase domain of SER is well conserved and has previously been shown to be catalytically active (13). The recombinant protein, expressed in insect cells, was capable of tyrosine autophosphorylation; however, no information was given regarding its potential to bind and be activated by EGF ligands (13). For this reason, we have undertaken functional characterization of the SER receptor by analyzing its EGF-dependent activity and determining its affinity for human EGF. Because no technique is currently available to transfect schistosomes with efficacy, we have chosen to express the SER protein in two vertebrate cellular systems, MDCK epithelial cells and the oocytes of X. laevis. Transactivation in MDCK epithelial cells is a sensitive assay to analyze the functional activity of receptor tyrosine kinase by measuring their ability to induce a transcriptional response through specific Ras-dependent promoter elements (17). It was recently used to study signaling and biological activities of the MET receptor (27). This transactivation system requires a limited number of surface receptors, and the cotransfection with a luciferase vector containing ETS/AP1 binding sites allows a very sensitive detection of the activation of the Ras/MAPK pathways in the cell. The second system we have used offers other advantages, which are the high efficacy of protein expression and the facility to observe MAPK activation optically by the induction of GVBD in oocytes. Opresko and Wiley (19) have shown that the human receptor HER entirely retained its specific biological activities in the context of oocytes and was able to induce oocyte maturation. The fact that no endogeneous EGF-R was present in Xenopus oocytes was an additional reason to use this expression system for studying SER activity.

Results obtained in MDCK cells indicated that luciferase activity increased in SER-transfected cells as compared with control cells when stimulated by human EGF. The signal intensity was ligand dose-dependent, and a plateau was reached for nanomolar concentrations of EGF in both control and SER-expressing cells. This sensitive system allows the determination of the level of receptor activation and might be appropriate for further analyses of EGF ligand or specific inhibitor effects on SER activation. Such data, demonstrating that SER was able to trigger a Ras/MAPK pathway dependent on EGF, were then confirmed in the second expression system. We showed that EGF specifically stimulated maturation of oocytes expressing SER as a membrane protein, as demonstrated by analysis of labeled oocyte membrane extracts and immunopre-
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In this model, the use of a dead kinase receptor allowed us to demonstrate the importance of SER kinase activity in the observed process. Without affecting the level of receptor expression in membranes, a single mutation in the Mg\(^{2+}\)-binding site (DFG motif) of the SER thymidine kinase domain completely abolished the appearance of GVBD, confirming that receptor kinase activity was essential for oocyte maturation. Taken together, these results indicated that the recombinant parasite receptor tyrosine kinase was functionally active in the context of both epithelial cells and oocytes and strongly suggested that it could interact properly with the protein partners of the vertebrate Ras/ERK pathway. Western blot analysis of oocyte lysates clearly demonstrated that SER activation induces its phosphorylation and effectively triggers a Ras pathway and ERK protein phosphorylation, a process dependent on the existence of a functional kinase domain in the SER protein. At this stage, further analysis of the C-terminal region of SER is necessary to better characterize the activated signaling cascade. However, the absence of significant homology of the SER intracellular region to EGF-R homologues, with the exception of the kinase domain (11), makes such an analysis difficult. Indeed, although SER contains a large number of tyrosine residues in its C-terminal portion that represent potential phosphorylation sites, a precise sequence analysis of this region (residues 1333–1717) confirmed the absence of strictly conserved binding sites for the well-known EGF-R cascade partners (28). Further work is now concerned with tyrosine scanning and site-directed mutagenesis to identify functional sites for SER signaling and the characterization of potential partners able to activate ERK cascade in schistosomes.

Results of SER activation by human EGF already suggested a relatively high affinity of the parasite receptor for the vertebrate hormone. Indeed, human EGF concentrations sufficient to obtain optimal activation of the Ras/ERK pathway in MDCK cells or oocytes expressing SER were very similar to those required for optimal activation of the endogenous canine receptor in epithelial cells or the human receptor expressed in oocytes. Binding experiments in oocytes confirmed that the calculated equilibrium affinity of SER expressed in oocytes was of the same order of magnitude (10–9 M) as that of the human fibroblast receptor (26). To our knowledge, these results constitute the first direct demonstration that a schistosome growth factor receptor is able to bind the corresponding vertebrate ligand. The demonstration that the SER protein immunoprecipitated from EGF-treated membrane extracts was effectively phosphorylated on tyrosine residues also confirmed the ability of the native schistosome protein to bind and be activated by EGF. Previous studies have already shown the ability of SmRK1, a S. mansoni divergent member of the transforming growth factor β receptor family, to activate a conserved pathway in response to human transforming growth factor β. However, in these transfection studies, no evidence was given for a direct interaction of transforming growth factor β with SmRK1; a functional recruitment of the type I SmRK1 receptor by the mammalian type II receptor is more likely (29, 30), with the mammalian receptor binding the ligand. Together, these data confirm the conservation of signaling pathways in helminths and support the hypothesis that host factors could regulate schistosome development by interaction with specific parasite receptors.

Further experiments were performed to analyze in vitro the effect of human EGF on S. mansoni parasites, considering in priority its influence on the metabolism of sporocysts. In vitro-transformed from miracidia, sporocysts are devoid of contamination by host components and constitute the most suitable stage for analysis of metabolic activities, such as protein and DNA synthesis. The procedures described have been already used with success to show that mollusc host molecules could stimulate proliferative activity in these infective schistosome larvae (31). Results presented in this work demonstrate that human EGF could increase protein and DNA synthesis as well as protein phosphorylation in primary sporocysts. A maximal effect is observed after incubation with 10–8 M EGF; higher EGF doses are less efficient, probably because of toxicity. Similar assays performed on adult worms, which are more complex organisms, also indicated that human EGF could increase in vitro methionine incorporation 2-fold. Therefore, it seems that in both larval and adult stages, binding sites for exogenous EGF are accessible on the parasite. The observation that SER was predominantly present in schistosome muscles (13) would not be a priori in agreement with this hypothesis. However, considering the high affinity of SER for the human hormone, we could not exclude the possibility that SER molecules, present in a rather limited number on the surface and therefore not previously detected by immunofluorescence (13), could be sufficiently active to induce host EGF signaling in the context of host-parasite relationships. Our preliminary results showing that EGF was able to stimulate protein synthesis might be in agreement with a positive effect of host EGF on schistosome biology. Also, our results showing the stimulating effect of EGF on sporocysts indicated the possibility that the SER molecule was accessible to host components at this parasite stage and therefore suggested its potential recognition by mollusc EGF ligands and its importance in sporocyst differentiation.

In conclusion, our data demonstrate the conservation of EGF signaling in S. mansoni, i.e. the ability of the schistosome receptor to be activated by vertebrate EGF ligands and to activate a vertebrate classical MAPK pathway downstream, raising the question of the role of co-evolution in host-parasite adaptation processes.

As in the case of a variety of organisms, SER is supposed to play an important role in development and could thus be considered an interesting target for anti-schistosome chemotherapy. Three-dimensional structural analysis of the catalytic domain of SER and comparison with other EGF-R family members would be helpful for the discovery of SER-specific inhibitors that would probably represent new and efficient drugs against the parasite.

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