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Schistosoma japonicum: A method for transformation by electroporation

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Abstract

Despite of our knowledge of genetic make up of schistosomes, a number of genes have not been characterized largely due to lack of effective transformation protocols. Here we present electroporation as a strategy for effective introduction of plasmids DNA into schistosomula and adults. Using plasmids of pEGFP-C1 as an expression vector, we first verified that the CMV promoter could direct EGFP to express in primary culture cells from *Schistosoma japonicum.* Subsequently, the plasmids were introduced into schistosomula and adults by electroporation and EGFP expression was demonstrated using molecular and microscopical methods. Our findings indicate that electroporation is an effective method for transformation of *S. japonicum*.

Index Descriptors and Abbreviations: Schistosome; *Schistosoma japonicum*; Transformation; Electroporation; Transgenic parasite; EGFP, enhanced green fluorescent protein; RT-PCR, reverse transcription-polymerase chain reaction; PBS, phosphate-buffered saline; MF-PBS, magnesium-free phosphate-buffered saline; EDTA, ethylene diamine tetraacetic acid; FBS, fetal bovine serum; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; DAB, diaminobenzidine tetrahydrochloride

1. Introduction

Schistosomiasis is an important and highly prevalent health problem in many countries of the world affecting more than 200 million people (Chitsulo et al., 2000). Despite our molecular and biological knowledge of this trematode, a number of genes playing important roles during the development and in the host–parasite interaction have not been identified largely due to lack of effective transformation protocols. After transcriptome analysis of *Schistosoma mansoni* and *Schistosoma japonicum* had been achieved (Hu et al., 2003; Verjovski-Almeida et al., 2003), the question now arises of how to take advantage of this wealth of new information and to characterize the function of a large number of new cDNA sequences from *S. mansoni* and *S. japonicum*. Development of effective methods that permit efficient gene transfer to schistosomes are crucial to this.

The techniques employed thus far for introducing DNA constructs and RNA into *S. mansoni* have included both particle bombardment and electroporation (Correnti and Pearce, 2004; Davis et al., 1999; Heyers et al., 2003; Rossi et al., 2003; Wippersteg et al., 2002a,b). In these studies, GFP and luciferase genes could transiently express in the sporocyst and adult stage of *S. mansoni* as well as in schistosomula. This findings laid the foundation for our approach to introducing plasmid DNA into *S. japonicum* by electroporation, which has been shown to be effective for introducing nucleic acids into prokaryotic or eukaryotic cells and protozoan parasites, as well as into whole organisms (Canatella et al., 2004; Ngo et al., 1998; Robinson and Beverley, 2003; Yamazaki et al., 2000). We have used plasmid pEGFP-C1 as an expression vector and demonstrated

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that the CMV promoter offered by the plasmid itself could direct expression of the reporter gene in primary culture cells of *S. japonicum*. Furthermore, we optimized the parameters of electroporation for introducing plasmid DNA into schistosome.

2. Materials and methods

2.1. Preparation of schistosomula for cell culture

Preparation of schistosomula and cell cultures was done as described previously(Dong et al., 2002). Briefly, a Chinese strain of *S. japonicum* was maintained in the snail *Oncomelania hupensis* and in mice. Schistosomula used for cell culture were obtained by perfusion at day 21 post-infection and washed five times with PBS containing antibiotics (1000 IU/ml penicillin G and 1000 μg/ml streptomycin).

The schistosomula were washed three times in 50 ml of a mixture containing equal volume of 0.25% trypsin and 0.02% EDTA (trypsin and EDTA were dissolved in calcium- and MF-PBS) and then transferred to a small glass bottle. After settling for 15 min at room temperature, the supernatant was removed. The worms were minced in 0.2 ml of the trypsin/EDTA mixture by using scissors and then transferred to a 10 ml conical centrifuge tube. The volume made up to 10 ml with the mixture and the fragments were digested for at least 4 h at 4 °C. During cold digestion, the solution containing the fragments was regularly shaken. The solution was then homogenized with a Pasteur pipette and the cell suspension was settled for 30 min. The suspension containing the cells and fraction suitable for culture was transferred to another tube. After centrifugation at 1200 rpm for 15 min, the cell pellet was resuspended in RPMI 1640(Gibco) and placed in a flask. Cells of parasites from one mouse were inoculated in a moist environment onto the surface of separate culture flasks, established for more than 8 h, and then were maintained in culture medium(RPMI 1640+10% FBS containing100 IU/ml penicillin G and 100 μg/ml streptomycin) at 37 °C in 5% CO_2.

2.2. Transfection of the schistosomula cell with pEGFP-C1

Cells of schistosomula were seeded in 100 mm culture flask to reach 60–80% confluence and grown for 24 h. The pEGFP-C1 (5 μg, BD Biosciences Clontech) and Lipofectamine 2000 (5 μl, 1 mg/ml, Invitrogen) were mixed, diluted with 100 μl of RPMI1640, and incubated at room temperature for 20 min. The pEGFP-C1 mixture was added to the cells without changing the culture medium (RPMI1640) and the cells were continuously cultured for 48 h before fluorescent microscopy.

2.3. Mechanical transformation of cercariae into schistosomula

The mechanical disruption technique described by Colley and Wikel (1974) produced nearly complete separation of the cercariae heads and tails after 14 passages of the cercariae suspended in 5 ml of wash medium (RPMI1640 containing 100 IU/ml penicillin G and 100 μg/ml streptomycin) through a 21 g needle fitted to a 10 ml syringe. Following disruption, the suspension was incubated in polypropylene tubes in a 30 °C water bath for 40 min. After incubation the suspension was centrifuged at 1500 rpm for 3 min and the supernatant discarded. The pellet was resuspended in fresh medium and the tubes were left to stand for 10 min to sediment the organisms and to leave most of the tails in suspension; the tail-rich supernatant was discarded and then the remaining material was incubated in culture medium at 37 °C in 5% CO_2 before electroporation.

2.4. Preparation of adult schistosomes

A Chinese strain of *S. japonicum* was maintained in mice in the laboratory. Male adult worms were obtained by perfusion at 42 days post-infection with monosexual infection and washed three times with RPMI1640 containing 100 IU/ml penicillin G and 100 μg/ml streptomycin. The adults were then incubated in culture medium at 37 °C in 5% CO_2 before electroporation.

2.5. Electroporation of the cultured schistosomula and adults

It has been reported that older schistosomula might actually be better targets for transformation than younger parasites (Correnti and Pearce, 2004). Therefore, the schistosomula cultured for 18 h were harvested for electroporation and 100 schistosomula and 10 male adults were placed in chilled electroporation cuvettes (0.4 cm gap) in 500 μl PBS containing 80 μg plasmids. Parasites were pulsed with a capacity discharge electroporation generator (Bio-Rad Gene Pulser). Immediately after electroporation, the schistosomula and adults were transferred into pre-warmed culture medium and cultured at 37 °C, 5% CO_2 for 48 h before microscopy.

2.6. Isolation and characterization of nucleic acids and protein

DNA was obtained using a commercial kit (Dopusen). Total RNA was isolated by TRIzol Reagent (Invitrogen). Protein was prepared by ultrasonification (3 × 100 W) and boiling for 10 min in Lammli-buffer (Sambrook et al., 1989) with 1%PMSF (Sigma) and 10% (v/v) protease-inhibitor mix (complete mini, Roche). After centrifugation for 5 min at 4 °C, the supernatant was loaded onto a 12% SDS-PAGE. Western blot analysis was done by electroblotting on nitrocellulose membranes (Protran, Schleicher & Schuell). After blocking with 3%(w/v) BSA (Serva) in PBST (1 × PBS, 0.2% Tween 20) for 1 h, the blot was incubated for 2 h with a monoclonal mouse-anti-GFP antibody (BD Biosciences Clontech, JL-8) diluted 1:1000 in PBST (3% BSA) at room temperature. As secondary