International Journal for Parasitology 39 (2009) 675–681

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Revised karyotyping and gene mapping of the *Biomphalaria glabrata* embryonic (Bge) cell line

Edwin Odoemelam [a], Nithya Raghavan [b], Andrè Miller [b], Joanna M. Bridger [a], Matty Knight [b,*]

[a] *Laboratory of Nuclear and Genomic Health, Centre for Cell and Chromosome Biology, Biosciences, School of Health Sciences and Social Care, Brunel University, West London UB8 3PH, UK*
[b] *Biomedical Research Institute, 12111 Parklawn Dr., Rockville, MD 20852, USA*

ARTICLE INFO

Article history:
Received 23 September 2008
Received in revised form 3 November 2008
Accepted 19 November 2008

Keywords:
Biomphalaria glabrata
Schistosomiasis
Chromosomes
Karyotyping
Bge cell line
Fluorescence in situ hybridization (FISH)
Bacterial artificial chromosomes (BACs)
Mapping

ABSTRACT

The fresh water snail *Biomphalaria glabrata* (2*n* = 36) belongs to the taxonomic class Gastropoda (family Planorbidae) and is integral to the spread of the human parasitic disease schistosomiasis. The importance of this mollusc is such that it has been selected as a model molluscan organism for whole genome sequencing. In order to understand the structure and organisation of the *B. glabrata*'s genome it is important that gene mapping studies are established. Thus, we have studied the genomes of two *B. glabrata* embryonic (Bge) cell line isolates 1 and 2 grown in separate laboratories, but both derived from Eder L. Hansen's original culture from the 1970s. This cell line continues to be an important tool and model system for schistosomiasis and *B. glabrata*. Using these cell line isolates, we have investigated the genome content and established a revised karyotype based on chromosome size and centromere position for these cells. Unlike the original karyotype (2*n* = 36) established for the cell line, our investigations now show the existence of extensive aneuploidy in both cell line isolates to the extent that the total complement of chromosomes in both greatly exceeds the original cell line's diploid number of 36 chromosomes. The isolates, designated Bge 1 and 2, had modal chromosome complements of 64 and 67, respectively (calculated from 50 metaphases). We found that the aneuploidy was most pronounced, for both isolates, amongst chromosomes of medium metacentric morphology. We also report, to our knowledge for the first time using Bge cells, the mapping of single-copy genes peroxiredoxin (*BgPrx4*) and P-element induced wimpy testis (*piwi*) onto Bge chromosomes. These *B. glabrata* genes were mapped onto pairs of homologous chromosomes using fluorescence in situ hybridization (FISH). Thus, we have now established a FISH mapping technique that can eventually be utilized for physical mapping of the snail genome.

1. Introduction

The fresh water snail *Biomphalaria glabrata* (diploid, 2*n* = 36) is a major intermediate host for the platyhelminth parasite *Schistosoma mansoni* that causes schistosomiasis. Humans are the obligate definitive host of the parasite. This disease is endemic in 74 tropical countries, (in regions of Africa, the Caribbean, the Middle East and South America) and causes vast morbidity and debilitation in terms of public health and socio-economic importance (LoVerde et al., 2004; Friedman et al., 2005).

The work of Hansen (1976) in establishing the *B. glabrata* embryonic (Bge) cell line aided the efforts that led to most of what we currently know today about the molecular genetic interactions between trematode and the intermediate snail host in vitro. Before the cell line was established, much of the work focused on maintaining molluscan organs in vitro (Benex, 1961, 1965). Development of cell lines from other molluscs, such as the oyster *Crassostrea gigas* and the hard clam, *Meretrix lusoria*, have been attempted and primary cultures were successfully maintained for only up to 5 months (Chen and Wen, 1999) thus making Bge cells the only established cell line from molluscs. Despite this overall advantage these cells are an underutilized resource. Bge cells' competence as a model for the in vitro development of *S. mansoni* was demonstrated when in the presence of these cells, miracidia were able to transform and, most significantly, complete the intramolluscan cycle from miracidium to cercaria (Ivanchenko et al., 1999; Coustau and Yoshino, 2000). By co-culturing these cells with the helminth parasites, it has been possible to examine the in vitro response to parasitic antigens and excretory–secretory (ES) products (Coustau and Yoshino, 2000). Indeed, some have shown that ES products from *S. mansoni* can stimulate the p38 signalling pathway of Bge cells, a response that is associated with stress factors, such as UV light, osmotic changes and heat shock (Sano et al., 2005; Humphries and Yoshino, 2006).

* Corresponding author. Tel.: +1 301 881 3300x26; fax: +1 301 770 4756.
E-mail address: mknight@afbr-bri.com (M. Knight).

0020-7519/$36.00 © 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2008.11.011

The importance of *B. glabrata* as an intermediate host of a major human pathogen is such that a proposal submitted by the snail genome project to the National Human Genome Research Institute (NHGRI) was accepted and its genome is currently being sequenced by the Genome Sequencing Center (GSC, Washington University in St. Louis, USA) (reviewed by Raghavan and Knight, 2006). The AT content of *B. glabrata* is estimated to be ~64% based on the analysis of bacterial artificial chromosome (BAC) end sequence data (Adema et al, 2006) and trace reads of *B. glabrata* genome sequences currently deposited in GenBank. The genome size of *B. glabrata* is approximately 931 Mb and is based on Feulgen image analysis densitometry of haemocyte samples (Gregory, 2003). This is approximately three times smaller than that of the 3000 Mb human genome (Venter et al., 2001) and three times larger than that of the 270 Mb *S. mansoni* genome (El-Sayed et al., 2004). Yet, compared with other molluscs, the genome is relatively small, e.g. *Aplysia calfornica* at 1800 Mb, and *Lymnaea stagnalis* at 1195 Mb (Raghavan and Knight, 2006). Currently in the GenBank database there are 824 nucleotide sequences, 643 protein sequences, 619 genome survey sequences (GSS), and 52,624 expressed sequence tags (ESTs), from *B. glabrata,* in addition to the 155,319 trace reads that have been deposited by the GSC. To date, numerous gene libraries have been constructed, e.g. cosmid (Knight et al., 1999), cDNA (Raghavan et al., 2003; Lockyer et al., 2007), two BAC libraries (*B. glabrata* BB02; Arizona Genomics Institute; Adema et al., 2006 and *B. glabrata* BS90; Raghavan et al., 2007) and a fully sequenced *B. glabrata* mitochondrial genome of 13,670 nucleotides (DeJong et al., 2004).

The complete genome sequence of *B. glabrata* will be of great importance to further understand how host–parasite relationships are elicited and may be controlled. An additional feature of the snail genome project is to develop techniques to analyse *B. glabrata* on a biochemical, genomic and chromosomal level. The latter will be crucial in constructing a physical, cytogenetic map (via the use of fluorescence in situ hybridisation (FISH) for physical mapping) of this organism (Langer et al., 1981).

Research into *B. glabrata* chromosomes has somewhat stagnated in recent years. Patterson and Burch (1978) performed the pioneering work in this field. They identified the basic chromosome number of planorbidae snail family (which includes *B. glabrata*) as $2n = 36$. Another important schistosome intermediate host, genus *Bulinus* exhibit diploid, tetraploid, hexaploid and even octoploid levels of polyploidy (Goldman et al., 1984). Raghunathan (1976) described the karyotype of *B. glabrata* by organising chromosomes into groups of metacentric, submetacentric, acrocentric and telocentric (in accordance with centromere position as stipulated by Levan et al. (1964), as well as confirming a diploid number of 36 chromosomes (Levan et al., 1964). Subsequently, Goldman et al. (1984) produced another karyotype of *B. glabrata*. Both karyotypes were derived from the snail and not the Bge cell line. However, Bayne et al. (1978) performed a detailed analysis of the Bge cell line developed by Hansen (1976) with respect to its antigenic determinants, karyotype, behavioural and enzyme characteristics prior to depositing the cells at the American Type Culture Collection (ATCC®, Manassas, USA). The Bge cells that were deposited by Dr. C. Bayne are currently available from ATCC® (Catalog No. CRL-1494™) where they are described as only being loosely adherent, in contrast to their original morphology, described as monolayer forming fibroblast-like cells (Hansen, 1976; Bayne et al., 1978). During the past several, years we have independently purchased different vials of Bge cells from ATCC® and to date have failed to propagate these particular cells in our laboratories (Biomedical Research Institute, Rockville, USA and Brunel University, West London, UK). Personal communication with technical support at ATCC® indicated that the cells are currently not being actively propagated due to lack of demand and also low availability of their stocks. Because of the failed attempts to propagate commercially purchased Bge cells from ATCC®, we obtained Bge cells from two different sources, the original ATCC® depositor Dr. C. Bayne (Oregon State University, Corvallis, USA), and Dr. E.S. Loker (University of New Mexico, Albuquerque, USA). Our interest was to characterize the isolates from these two different sources prior to using them for any molecular analysis, since slight differences were observed in their physical characteristics, for example in their ability to adhere, form monolayers, and in their generation time.

In this study, we have analysed the chromosomes of Bge cell lines from these two different sources, named here as Bge 1 (E.S. Loker Laboratory) and Bge 2 (C. Bayne Laboratory) and constructed a revised karyotype that reveals extensive aneuploidy in the cell line isolates. Additionally we demonstrate, to our knowledge for the first time, chromosomal mapping of non-repetitive (single-copy) *B. glabrata* genes onto homologous chromosomes isolated from Bge cells.

2. Materials and methods

2.1. Bge cell culture

Bge cells used in this study were obtained from the laboratories of Dr. E.S. Loker (Bge 1) and Dr. C. Bayne (Bge 2). Both cultures were derived from Hansen's original Bge cell line (Hansen, 1976), and were grown in the absence of carbon dioxide, at 26 °C in medium which comprised of 22% Schneider's *Drosophila* medium (Invitrogen, Paisley, UK), 0.13% galactose (Invitrogen, Paisley, UK), 0.45% lactalbumin hydrolysate (Invitrogen, Paisley, UK) and 14.1 μM phenol red. The medium was sterilised using a 0.22 μm pore filter (Fisher Scientific UK Ltd., Loughborough, UK) and the antibiotic gentamicin (Invitrogen, Paisley, UK) was added post-filtration at a concentration of 20 μg/ml. The Bge medium was made complete by adding 10% FBS (v/v, Hyclone, Cramlington, UK) which had previously been inactivated at 56 °C for 30 min. The Bge cells were passaged when their confluence had reached approximately 80%, and then reseeded at a 1:12 dilution. Since trypsinization of either cell line isolates over 10–15 passages resulted in the loss of viability of the cells and ultimately led to cell death, we resorted to releasing the cells using either a cell scraper or by firm tapping. While propagating the Bge cells from the two different sources in our laboratory we observed differences in their characteristics such as doubling time, cell adherence and morphology.

2.2. Bge cellular fixation and slide preparation

Bge cells were arrested in metaphase using the mitotic inhibitor colcemid. Colcemid (10 μg/ml) dissolved in Hank's balanced salt solution (Invitrogen, Paisley, UK) was added to T75 flasks (Fisher Scientific UK Ltd., Loughborough, UK) of Bge cells (at the stage of 70–80% confluence). The cells were incubated with the colcemid for 1.5 h at 26 °C and the cells were subsequently dislodged from the flasks via the application of either a cell scraper (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) or by firmly tapping the flask. The cells were then centrifuged at 400*g* at 15 °C. The cell pellet was resuspended by vigorously tapping the tube, followed by the addition of hypotonic potassium chloride solution (0.005 M) and subsequently fixed with methanol and acetic acid (3:1 v/v). Twenty microliters of this cellular suspension was then applied onto a glass slide to achieve metaphase chromosome spreads. Glass slides of fixed Bge cellular suspensions were stained with the DNA intercalator DAPI. Ten microliters of 2 μg/ml DAPI in Vectorshield anti-fade mountant (Vector Laboratories, Peterborough, UK) was applied to the slide and sealed with a 22×50 mm coverslip.