DNA Polymorphism of Schistosomes and their Snail Hosts

Andrew JG Simpson, Emmanuel Dias Neto, Teofânia HDA Vidigal, Heloisa B Pena*, Omar S Carvalho, Sérgio DJ Pena*

Centro de Pesquisas "René Rachou"- FIOCRUZ, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil *Departamento de Bioquimica, ICB-UFMG, Belo Horizonte, MG, Brasil

Analysis of the genomes of schistosomes and one of their intermediate hosts, Biomphalaria glabrata, *using Random Amplified Polymorphic DNA (RAPD) demonstrated that intraspecific genetic polymorphism in the parasite is limited but in the snail is highly pronounced. This suggests an important role for the snail in the determination of the epidemiology of the disease. In addition to their intraspecific stability, schistosome derived RAPDs exhibit a high level of interspecific polymorphism and are thus ideal for the construction of phylogenetic trees. For the detection of intraspecific polymorphisms extensive variation in the mitochondrial DNA is being exploited for the development of a PCR based test for* Schistosoma mansoni. *Gene level polymorphims are being analyzed by Low Stringency Single Specific Primer PCR.*

Key words: schistosomes - *Biomphalaria glabrata* - DNA - polymorphisms - PCR

The biological interaction of the schistosome with its hosts is fundamentally influenced by their respective genetic constitutions. Although we have a profound and rapidly expanding knowledge of the human genome, that of the schistosome and its intermediate snail hosts is extremely limited. In order to gain insight into the extent of genetic variability of the schistosome and its intermediate hosts and the relevance of such variation both to the biology of the disease and efforts towards its control we have undertaken PCR based studies of the genetic polymorphisms of these organisms.

The technique that we exploited most is a recent development of the PCR (polymerase chain reaction) named Arbitrarily Primed-PCR (AP-PCR) (Williams et al. 1990, Welsh & McClelland 1990). This methodology results in the amplification of anonymous regions of the organism's genome and provides an indication of sequence change whilst avoiding problems of bias inherent in the study of a small number of genes by direct sequencing. Simply, the proportion of amplified fragments [known as Randomly Amplified Polymorphic DNAs (RAPDs)] that exhibit polymorphism (usually presence or absence) reflect the genetic polymorphism of the population being studied. A preliminary analysis of species from the *Schistosoma haematobium* and *S. mansoni* groups (Dias Neto et al. 1993a) using a number of primers showed that quite distinct patterns of RAPDs are always obtained with DNA isolated from adult worms from different species. Comparison of DNA from different strains of the same species, on the other hand, produced RAPD profiles with very little variation. Recent analysis of individual organisms from any given strain has found that approximately 5% of the bands are polymorphic (Dias Neto et al. unpublished observations). These data had a number of implications. Firstly, they indicated the applicability of the technique for the phylogenetic genetic analysis even for the closely related schistosome species within each group. Thus, a more formal and extensive analysis using RAPDs produced from nine species and involving more than 500 individual DNA fragments was undertaken (Kaukas et al. 1994). A phylogenetic tree calculated on the basis of band sharing ordered the best characterized species as one would predict from biological characteristics. Secondly, the quite distinct RAPD profiles produced by different species and their reproducibility showed that they were highly suitable for the identification of species of organism in survey situations particularly as the technique works well even with individual cercariae or miracidia. Thirdly, it was immediately clear that schistosomes do not exhibit extensive intraspecific genetic variation. Within species the similarity of RAPD profiles of strains isolated from quite distinct geographic areas was striking and contrasts with data that we have collected from several other groups of organisms. When studying *Trypanosoma cruzi*, for example, we found that all DNA fragments amplified from six primers were polymorphic within the species

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(Steindel et al. 1993). Lastly, the fact that polymorphisms can be detected in individual free living stages of the schistosome life cycle now makes the detailed studies of population genetics of schistosomes a possibility.

The results obtained when AP-PCR was applied to the study of the genome of *Biomphalaria glabrata* were in complete contrast to those obtained with schistosomes in that analysis of DNA prepared from snails obtained from different geographical regions of Brazil showed extremely high levels of polymorphism (Vidigal et al. 1994). Nevertheless, when specimens from the same region were compared a low level of polymorphism was observed. The results showed that *B. glabrata* exhibits a remarkable level of genetic variability that we explain on the basis of genetic drift due to the mollusks ability to reconstitute populations from one or very few specimens. The contrast between the genetic variability of the schistosome and its intermediate host indicates that the genetics of the snail may play at least as important a role in the determination of the epidemiology of schistosomiasis as those of the parasite it self.

An important DNA polymorphism exhibited by the schistosome is that which distinguishes the male from the female parasite. We developed a new PCR technique called Low Stringency-PCR (LS-PCR) in order to exploit this polymorphism for schistosome sex determination of cercariae shed from single sex infections of *B. glabrata*. a fundamental step in undertaking genetic crosses with schistosomes in the laboratory. It had already been shown that specific PCR amplification of a highly repeated region of the female specific W chromosome identifies females (Gasser et al. 1991). However, a mechanism for providing an internal control was needed to show that the absence of the female specific amplification indicated that the cercariae being analyzed were males. We showed that this could be achieved by using the female specific primers under low stringency conditions so that with male DNA, a series of non-specific bands are reproducibly amplified (Dias Neto et al. 1993b). These bands contrast with the specific pattern that is maintained when female DNA is amplified, even under the altered conditions. Thus distinct male and female patterns are produced using the same pair of primers allowing confident sex determination.

Current approaches to the more detailed analysis of the of DNA polymorphism in schistosomes and its relevance to disease include the analysis of the mitochondrial DNA (mtDNA) of the schistosome. Mitochondrial DNA is usually maternally inherited and is typically highly variable as it is not subject to the same proof reading mechanisms as genomic DNA. In the study of schistosomes the use of mtDNA may be extremely valuable because of the lack of rearrangement. Thus, the study of the mtDNA of eggs produced from a given patient will directly reflect the female population of adult worms of that individual. This is not the case with genomic markers (other than those on the W chromosome) because of the usually sexual reproduction of these parasites. It has already been shown that the schistosome mtDNA is highly variable in terms of size (Despres et al. 1991). Detailed sequence analysis of the variable region is now being undertaken to accurately define the basis of this size polymorphism. This had permitted the construction of PCR primers which we have found to amplify highly variable mtDNA fingerprints due to a complex arrangement of repeats within the polymorphic region (data not published). This test offers great promise for the analysis of natural populations.

Another potentially very powerful approach that we are also beginning to apply to the study of the schistosome is another low stringency PCR technique that we have developed named LSSP-PCR (Low Stringency Single specific Primer- PCR) that has the ability to detect point mutations in gene sized fragments (Pena et al. 1994). The approach we are taking is to apply this to the study of individual genes of interest such as those that encode proposed vaccine candidates to establish whether or not the gene exhibits polymorphism in natural populations. This not only has importance in terms of providing further data concerning the genetic variability of schistosome populations but also implications for the possible application of a schistosome vaccine.

In conclusion, the potential now exists for significant head way to be made in the field of schistosome genetics using DNA based techniques particularly those that involve low stringency PCR in its various forms. The preliminary data that we have achieved appear solid and are entirely consistent with previous studies of schistosomes and their intermediate hosts using more traditional approaches. It is hoped that our ongoing investigations of inter and intra-specific genetic variation in schistosomes will not only contribute to our deeper biological understanding of the organism but also aid in the rational design and development of means of control.

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