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Minireview

Schistosome biology and proteomics: Progress and challenges

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Abstract

The recent availability of schistosomal genome-sequence information allows protein identification in schistosome-derived samples by mass spectrometry (proteomics). Over the last few years, several proteome studies have been performed that addressed important questions in schistosome biology. This review summarizes the applied experimental approaches that have been used so far, it provides an overview of the most important conclusions that can be drawn from the performed studies and finally discusses future challenges in this research area.

Index Descriptors and Abbreviations: ESI, electrospray ionisation; MALDI, matrix assisted laser desorption ionization; MS, mass spectrometry; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gelelectrophoresis; TOF, time-of-flight; Tegument; Host–parasite interaction; Proteome

1. Introduction

Schistosomes are parasitic helminths that cause schistosomiasis (also known as bilharziasis), a tropical disease, which is one of the most important parasitic diseases in tropical and subtropical areas, afflicting over 200 million people (Gryseels et al., 2006). Next to the clinical importance of this disease, schistosomes are scientifically interesting, as these worms are able to maintain themselves for decades in the blood vessels of their mammalian hosts. Despite their relatively large size (1-cm long with a diameter of 0.5-mm) and their abundant exposure to immune cells present in the blood of the host, this parasite apparently prevents an adequate immune response of its host (Pearce and MacDonald, 2002; Maizels, 2005; Gryseels et al., 2006). However, the underlying molecular mechanisms involved in this immune modulation of the host are not yet completely understood. For these reasons, schistosomes are studied by many research groups not only to combat schistosomiasis, but also to unravel the complex interaction with the host, in order to increase the knowledge on the mammalian immune system.

Current research on schistosome biology is still hampered by multiple experimental difficulties (Wilson et al., 2007). First, schistosomes are parasites with a complex life-cycle, involving two distinct hosts and many distinct life-cycle stages that differ drastically from each other, which implies that research cannot be focused on a single life-cycle stage. Second, although schistosomes can be cultured for very long periods (Basch, 1981), they cannot be multiplied *in vitro* yet, as cultured organisms do not produce offspring. All attempts performed in the past decades to propagate schistosomes *in vitro* have so far failed, and

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therefore, parasites have to be isolated from sacrificed infected laboratory animals. This drastically limits the number of parasites that can be used for experimental studies. Third, schistosomes are multi-cellular eukaryotes containing distinct tissues, such as reproductive organs, secretory glands, etc., each containing a specific set of diverse cell types, for all of which a cell line is lacking that can be cultivated *in vitro*.

Multiple methods have been developed for various schistosomal life-cycle stages to manipulate gene expression, such as recombinant expression, RNA interference and gene ablation (Wippersteg et al., 2002; Correnti et al., 2005; Brindley, 2005; Grevelding, 2006; Kines et al., 2006). However, the above mentioned experimental limitations seriously hamper the application of these genetic manipulation techniques in schistosomes and it has not been possible to produce stable transfected mutants producing transgenic offspring yet. Therefore, schistosomal mutants can only be studied for the limited time span that this life-cycle stage can be maintained *in vitro*. All together, functional characterization of schistosomal proteins is difficult because (i) the limited amount of biomaterial hampers classical biochemical methods, and (ii) the impossibility to multiply schistosomes *in vitro* complicates the application of methods to manipulate gene expression.

1.1. The schistosome genome and transcriptome

The *Schistosoma mansoni* genome has now been sequenced to approximately six-fold coverage by the schistosomal genome project, but due to the large genome size (ca. 300 Mb) and the many repetitive elements derived from retro-transposons, assembly into chromosomes and the identification of a full set of open reading frames encoding bonafide genes will be a major challenge (Wilson et al., 2007) (see also the review on the schistosome genome in this special issue). Therefore, a comprehensive list of genes encoded in the genome is still lacking, but analysis of the transcriptome of schistosomes suggests that the *S. mansoni* genome contains approximately 14,000 genes (Verjovski-Almeida et al., 2003; Liu et al., 2006). Interestingly, about half of these genes lack sequence similarity to any other protein in any species other then schistosomes. Furthermore, it is often not possible to annotate the schistosomal proteins that share homology with proteins of other organisms, because the function of about half these proteins of other organisms is also not known (Verjovski-Almeida et al., 2003; Wilson et al., 2007). Therefore, the *S. mansoni* genome contains many genes with a yet unknown function as well as many genes that thus far seem to be specific for schistosomes.

Large-scale transcriptome studies on multiple stages of *S. mansoni* and *S. japonicum* demonstrated that many of the predicted genes are indeed transcribed in one or more life-cycle stages of the parasite (Verjovski-Almeida et al., 2003; Liu et al., 2006). Furthermore, approximately 20% of the transcribed genes seem to be stage-specifically expressed (Verjovski-Almeida et al., 2003), which fits with the many stage-specific differences in structure and metabolic capacity. In addition, microarray studies were successfully used to identify genes that are expressed in a gender-specific manner or specifically in certain life-cycle stages (Hoffmann et al., 2002; Fitzpatrick et al., 2005; Vermeire et al., 2006; Moertel et al., 2006; Gobert et al., 2006). Microarray studies are a powerful tool to detect genes specifically expressed in a certain life-cycle stage, and those genes are of interest because these genes are likely to be involved in stage-specific functions in the parasite. However, the correlation between transcript abundance and protein abundance is not always straight forward, and therefore, interpretation of the results of microarray studies should occur with caution. In addition, this technique does not provide information on the actual protein abundance or its subcellular localization, and therefore, this technique provides limited information for the functional characterization of the transcribed genes. In this respect, the recent development of analytical techniques to determine protein compositions in complex biological samples (proteomics) in combination with the availability of sequences of the schistosomal genome, now allows studies on identification and localization of schistosomal proteins in (isolated fractions of) distinct life-cycle stages of the parasite (see below).

2. Analytical procedures used in schistosome proteomics

Proteomics, or the characterization of all proteins expressed in a particular tissue or cell at a given moment (the proteome), encompasses a wide variety of techniques and approaches. When considering the application of proteomics in schistosome research, there are several aspects to be discussed.

2.1. Sample preparation

Chronologically, the first step in proteomics experiments is the preparation of the (protein) sample (see Fig. 1). Given the high dynamic range of protein abundance in an organism (>10 orders of magnitude) (Qian et al., 2006), this step is crucial for the success of any experiment. First of all, the source (developmental stage, gender, excreted proteins, etc.) has to be established. Obviously, the choice of the protein source is defined within the research question. One can choose to investigate a specific specimen in detail, but also comparisons between for example male and female (Cheng et al., 2005), different developmental stages (Curwen et al., 2004) or different structural elements in adult worms (van Balkom et al., 2005; Braschi and Wilson, 2005; Braschi et al., 2006b) are interesting topics in schistosome proteomics research.

Then, depending on the research question and the organism, organ, cell type or organelle of interest, one can choose to pre-fractionate in order to enrich a sample