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Review

Contribution of proteomics of *Leishmania* spp. to the understanding of differentiation, drug resistance mechanisms, vaccine and drug development

Daniel Paape[a,*], *Toni Aebischer*[b]

[a]*Centre for Immunology and Infection, Department of Biology/Hull York Medical School, University of York, YO10 5DD, UK*
[b]*Robert Koch-Institut, 13353, Berlin, Germany*

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ABSTRACT

Leishmania spp., protozoan parasites with a digenetic life cycle, cause a spectrum of diseases in humans. Recently several *Leishmania* spp. have been sequenced which significantly boosted the number and quality of proteomic studies conducted. Here a historic review will summarize work of the pre-genomic era and then focus on studies after genome information became available. Firstly works comparing the different life cycle stages, in order to identify stage specific proteins, will be discussed. Identifying post-translational modifications by proteomics especially phosphorylation events will be discussed. Further the contribution of proteomics to the understanding of the molecular mechanism of drug resistance and the investigation of immunogenic proteins for the identification of vaccine candidates will be summarized. Approaches of how potentially secreted proteins were identified are discussed. So far 30–35% of the total predicted proteome of *Leishmania* spp. have been identified. This comprises mainly the abundant proteins, therefore the last section will look into technological approaches on how this coverage may be increased and what the gel-free and gel-based proteomics have to offer will be compared.

Contents

* *Corresponding author.*
E-mail address: Daniel.paape@york.ac.uk (D. Paape).

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1. Introduction

Genomics and transcriptomics were until recently unmatched in their power to enable highly parallel insight into the biology of cells and organisms. However, proteomics and more recently metabolomics have come of age and contribute and expand this knowledge.

The idea that mRNA levels are indicators of the respective protein expression/abundance, is often untrue and mRNA abundance can be misleading [1,2] and do not inform about post-translational protein modifications that can dramatically change protein function. This is especially true for the trypanosomatids as these organisms regulate their protein expression and abundance post-transcriptionally [3]. The three major human disease causing trypanosomatids — conveniently referred to as TriTryps — are unicellular organisms of the genera *Leishmania* spp. and *Trypanosoma brucei* and *Trypanosoma cruzi* which cause morbidity and mortality in humans and life stock in the middle East, south and south-east Asia, middle and South-America as well as countries surrounding the Mediterranean sea and sub-Saharan Africa (data from WHO). *Leishmania* the causative agent of the leishmaniases has as the other trypanosomatids a digenetic life cycle and oscillates between the extracellular promastigote form transmitted by sand flies and the intracellular amastigote form occurring in vertebrate hosts. Currently there are 350 million people at risk of leishmaniases with a prevalence of 12 million people and an estimated 1.5–2 million new infections each year. There are 60,000–70,000 deaths each year mostly due to the most severe form of the leishmaniases the visceral leishmaniasis caused by *Leishmania donovani* in India but also by *Leishmania infantum* in northern Africa and southern Europe [4,5]. For a detailed review on *Leishmania* transmission and distribution see Alexander et al. and Reithinger et al. respectively [4,5]. Less severe disease outcomes are the mucocutaneous and the cutaneous leishmaniasis caused by *Leishmania braziliensis* and *Leishmania major* as well as *Leishmania mexicana*, respectively.

Here, we like to review the information available on the proteome of *Leishmania* spp., a field that has greatly advanced since the genomic information became available in 2005 [6] and complement a recent review on proteomics of TriTryps [7] by the latest data.

The focus will be a historical review of how proteomics in *Leishmania* spp. advanced over time and its contribution to the understanding of molecular changes in the different life cycle stages, drug resistance and to the identification of vaccine candidates and novel drug targets.

1.1. *Leishmania spp. proteome profiling in pre-genomic era*

Leishmania spp. have a core genome of 8178 genes shared between the three currently sequenced and annotated genomes of *L. major*, *L. infantum* and *L. braziliensis*, and only few genes are species specific, 5, 27 and 49, respectively [8]. Although it is known that host factors play also a part in disease outcome, it is reasonable to assume that for the course of an infection differences in gene content or expression level in parasite species and isolates determine their respective virulence. Since the parasites regulate gene expression mainly at post-transcriptional stages, proteomics is thought to yield critical insight into the mechanisms of stage differentiation, species differences, virulence and drug resistance.

The first proteome maps of *Leishmania* were published in the early 1980s [9–11] long before the term proteomics was coined [12]. Handman et al. as well as Saravia and colleagues were investigating species differences and virulence factors [9–11]. However, at the time no protein was identified as mass spectrometric technologies were not yet developed for large biomolecules, the genome/s of *Leishmania* were not yet sequenced and identifications were relying on for example Edman degradation. Identification of large biomolecules became only possible from the late 1980s onward when two groups independent of each other developed matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) [13,14] analyses. The spectrum of analysis methods was then widened by the development of electrospray ionization (ESI) [15]. Several studies have led to stepwise improvement of methodology to investigate the parasites' proteomes. Suitable lysis methods of *Leishmania* to resolve as many protein-species as possible were identified [16]. A number of detailed 2DE proteome maps were then produced. For example, Brobey and colleagues reported normalized maps with 1650 and 1530 protein spots for *Leishmania amazonensis* and *L. major*, respectively and showed 4 of these as only present in *L. amazonensis* [17]. Similarly, Gongora and colleagues produced highly resolved proteome 2DE maps of *Leishmania* (*Viannia*) *guyanensis* and *Leishmania* (*Viannia*) *panamensis* promastigotes [18] and identified 'landmark protein spots'. These landmark protein spots were analyzed by mass spectrometry and identified as 2 isoforms of heat shock protein 70 (hsp70), chaperonin (hsp60), ribosomal protein S12, kinetoplastid membrane protein 11 and a 13 kDa small myristoylated protein-3, putative (annotated as hypothetical at the time) [18]. This enabled a comparison with clones of *L. guyanensis* that differed in metastatic capacities highlighting four protein species that may be involved in the different biologic behavior [18].

However, it was the sequencing of the genomes of *L. major*[6], *L. infantum* and *L. braziliensis*[8], with data available since the beginning of this millennium that enabled the relatively straight forward identification of protein-species excised from 2DE gels by mass spectrometry or from peptides separated by liquid chromatography (LC) as discussed below.