Heat shock proteins in *Schistosoma mansoni*

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1. INTRODUCTION

Schistosoma mansoni causes human schistosomiasis with over 200 million people infected across 78 countries and approximately 700 million people at risk of infection[1–3]. *S. mansoni* has a complex lifecycle that involves passage through a freshwater molluscan intermediate host and a mammalian definitive host[4,5]. When voided in the faeces, and upon contact freshwater, the schistosome eggs hatch releasing miracidia that locate and penetrate a suitable snail host. In the snail, miracidia undergo asexual reproduction resulting daughter sporocysts that have the capacity to generate thousands of cercariae for release[6]. The cercariae then swim with their bifurcated tail to locate the mammalian definitive host[4,7]. Upon penetration, the cercariae lose their tail and the 'head' transforms into a schistosomulum (somule) which then migrates via the lungs to the hepatic portal system where they develop into adult worms. This passage through, and transfer between, two different hosts exposes the schistosome to significant changes in local environment to which the parasite must adapt to survive, grow and develop.

Heat shock proteins (HSPs) are evolutionarily conserved proteins that are expressed constitutively and can also be induced by stress in all living cells[8]. HSPs are involved in a variety of cellular processes and play a vital role in the regulation of cellular homeostasis in both stressed and unstressed conditions[9,10]. Considering that the lifecycle of the parasite provides a unique physiological stress involving increases in temperature and salt concentration as the schistosome moves from a freshwater to warm -blooded environment, it is plausible that HSPs play a vital role in ensuring the parasites' survival during these transitions.

2. AIM

The aim of my research is to identify the presence and ascertain the functional role of selected HSPs in *Schistosoma mansoni*

3 – MATERIALS AND METHODS

Bioinformatics: Human HSPs were used as query for a pBLAST search on WormBase Parasite[11] to identify the number of HSP family (HSP 10, 40,60,70 and 90) members in *S. mansoni*, other *Schistosoma* species as well as free living flatworms. Normalised HSP 90 expression data was obtained from schisto.xyz[12]

Shedding and Transformation: *S. mansoni* cercariae were obtained from infected *Biomphalaria glabrata* snails exposed under intense light. Cercariae were then mechanically transformed into somules.

Immunolocalisation: Cercariae, 3 h, 24 h *in vitro* transformed somules, adult male and female worms were fixed in acetone, permeabilised in Triton X100, blocked in goat serum and incubated with anti-HSP antibodies for three days. Samples were washed, incubated in Alexa fluor-488 secondary antibody (1:500) and rhodamine phalloidin (1:500) for two days, washed and mounted in Vectashield. Parasites were visualised on a Zeiss LSM 800 confocal microscope.

4. RESULTS

Comparative analysis of *S. mansoni* HSPs

A total of 47 *S. mansoni* HSPs were identified across the five HSP families. Compared to schistosomes and humans, a higher number of HSPs were observed in the free living flatworms; *Macrostomum lignano* and *Schmidtea mediterranea* (Figure 1).

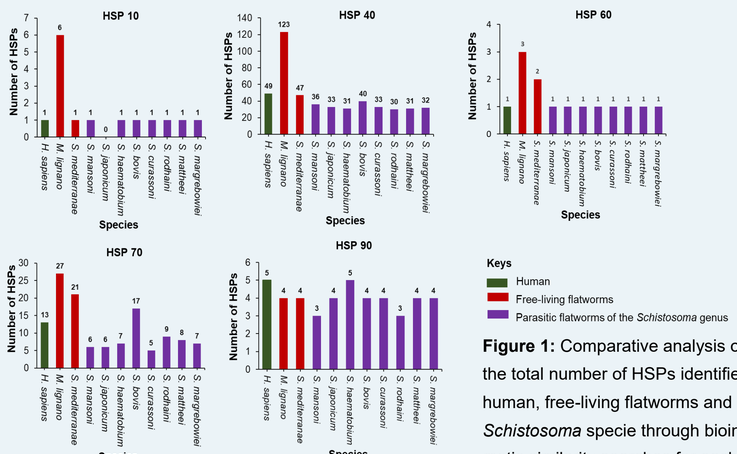

Figure 1: Comparative analysis of the total number of HSPs identified in human, free-living flatworms and *Schistosoma* specie through bioinformatic similarity searches for each HSP family

Differential *S. mansoni* HSP gene expression

HSP gene expression data revealed that *S. mansoni* HSP genes were differentially expressed across the various developmental life stages of the parasite, with most genes being highly expressed in the miracidia and sporocyst life stages (Figure 2).

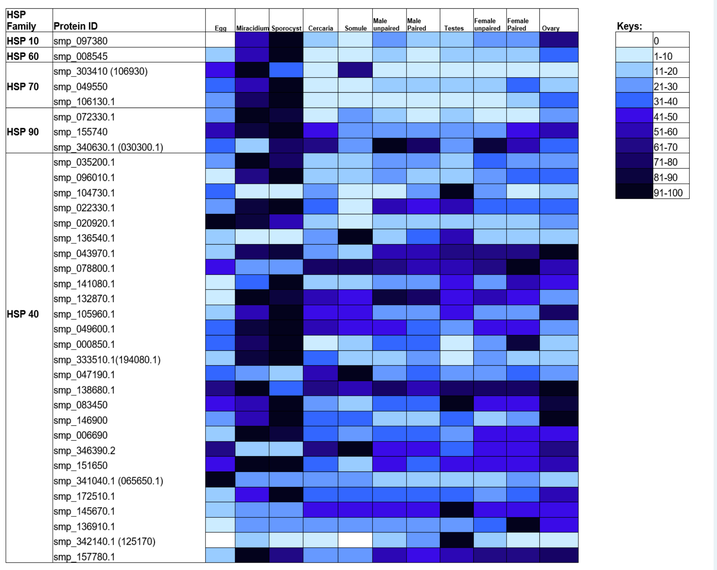

Figure 2: Heat map showing the comparative analysis of the different *S. mansoni* HSP expression levels during the development of the parasite from gonad to adult worms using quantitative data obtained from schisto.xyz. The normalised expression value were calculated as a percentage relative to the maximum expression level for each gene that were assigned a value of 100%. White represents no expression and the darkest blue represents the maximum (100%) expression.

Immunolocalisation of HSPs in *S. mansoni*

Confocal microscopy of *S. mansoni* cercariae, 3 h and 24 h somules, incubated with anti-HSP antibodies and detected with Alexa Fluor-488 secondary antibody (green) (figure 3), revealed that HSPs localised mainly in the tegument, sub-tegument, cephalic ganglia, acetabulum, gland duct and in some cases the cercarial spines (HSP 60), head/tail junction (HSP 70 and 90) and nervous system in the cercarial tail. In adult male and female worms, HSP 10, 60 and 90 were localised in the female ovary, male testes and tubercles whereas HSP 40 and 70 were prominent in the tegument and tubercles.

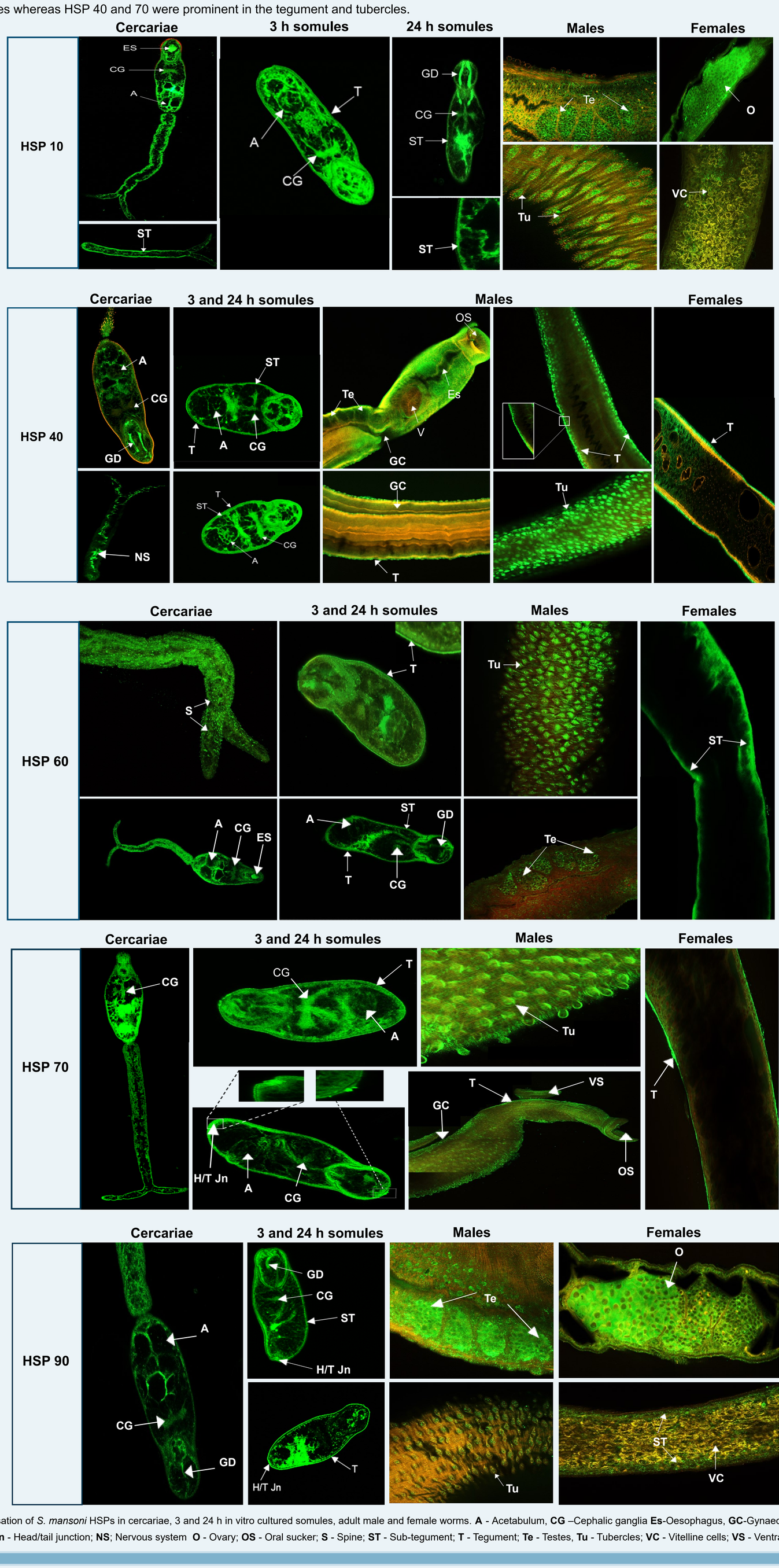

Figure 3: *In situ* localisation of *S. mansoni* HSPs in cercariae, 3 and 24 h in vitro cultured somules, adult male and female worms. **A** - Acetabulum, **CG** –Cephalic ganglia **Es**-Oesophagus, **GC**-Gynaecophoric canal, **GD** - Gland duct; **H/T Jn** - Head/tail junction; **NS**; Nervous system **O** - Ovary; **OS** - Oral sucker; **S** - Spine; **ST** - Sub-tegument; **T** - Tegument; **Te** - Testes, **Tu** - Tubercles; **VC** - Vitelline cells; **VS** - Ventral sucker

5. Conclusion

Most investigations on schistosome HSPs have been restricted to the study of individual HSPs. This is the first study to investigate five HSP families and the findings here support the presence of HSPs in *S. mansoni* and provide insight into their *in situ* distribution. These findings provide a framework for our further work on schistosome HSPs that aims to study their importance to schistosome growth and survival, work which is currently ongoing.

6. References :

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