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Short technical report

Whole mount *in situ* hybridization methodology for *Schistosoma mansoni*

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ABSTRACT

The genome sequence for *Schistosoma mansoni* has been determined, allowing the complete protein complement to be predicted. However, few functional genomics techniques have been developed for use in *S. mansoni*, limiting the usefulness of the sequence data. Here we describe a whole mount *in situ* hybridization (WISH) method that can be used to identify the tissue-specific expression of transcripts in *S. mansoni*. Using this protocol we determine the tissue-specific expression of *tetraspanin 2*, a female-enriched tetraspanin, *phenol oxidase*, the secretory *Cu/Zn superoxide dismutase*, and an Argonaute family member. The localization of these transcripts by WISH correlates with prior studies performed using immunohistochemistry and/or *in situ* hybridization on tissue sections. WISH can be adapted to screen multiple transcripts, thus identifying novel targets for drugs or vaccines.

Schistosomiasis, or bilharzia, affects more than 200 million people, making it a major cause of morbidity and mortality worldwide. Although the genome sequence for *Schistosoma mansoni* has been determined [1], our understanding of schistosomes at the molecular level is still quite rudimentary and the function of most predicted genes/proteins is unknown. Bioinformatics can predict the function of some proteins based solely on their sequence, but many schistosome genes have no homologs outside of the genus [1], posing problems for understanding the function of many schistosome-specific proteins. Since many of the techniques used in other model systems to characterize gene function have not yet been adapted for use in *S. mansoni*, new methodologies need to be developed. Because schistosomes have multiple tissues and organs systems involved in processes such as digestion, neural function, and reproduction, understanding the tissue and/or cellular expression pattern of a gene may provide useful information about the function of its encoded protein. *In situ* hybridization (ISH) experiments on histological sections are used routinely for adult schistosomes, but they are labor intensive, requiring the analysis of many sections to determine the localization of a transcript [2]. Immunohistochemistry is another useful methodology, but requires a great deal of time and resources (e.g., recombinant protein production, purification and antibody production), thus rendering it less than optimal as a screening tool to analyze multiple genes. Whole mount *in situ* hybridization (WISH) experiments are routinely performed to characterize gene expression patterns in numerous animal models, from hydra to higher vertebrates [3,4]. Because of the complex tissue architecture of schistosomes, WISH would be a valuable method to determine the expression patterns of schistosome-specific gene products. Although a WISH protocol has been described for schistosomes [5], we have developed an alternative method that provides high-resolution transcript localization while maintaining the structural integrity of worms during the fixation and incubation steps. Here we present this streamlined WISH protocol that will be useful as a moderate-throughput screening tool to localize transcripts. To demonstrate the functionality of this WISH protocol we have examined the tissue expression of several transcripts: *phenol oxidase* (*po*), *tetraspanin 2* (*tsp2*), a female-specific tetraspanin (*fs-tsp*), an Argonaute (Ago) family member, *ago2*, and a secretory form of *Cu/Zn superoxide dismutase* (*sp-sod*).

A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and NIH Swiss mice according to previously described methods [6]. Mice were infected through tail exposure to cercariae and adult parasites were harvested through portal vein perfusion at 49 days post infection. Maintenance and experiments using vertebrate animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Rush University Medical Center (IACUC number 08-058; DHHS animal welfare assurance number A3120-01).

Transcripts were amplified from a mixed adult male and female cDNA library using primers shown in Table 1. PCR products were cloned into the pCRII vector using the TOPO TA Cloning Kit (Invitrogen) or into the pJC53.2 vector [7] through standard cloning

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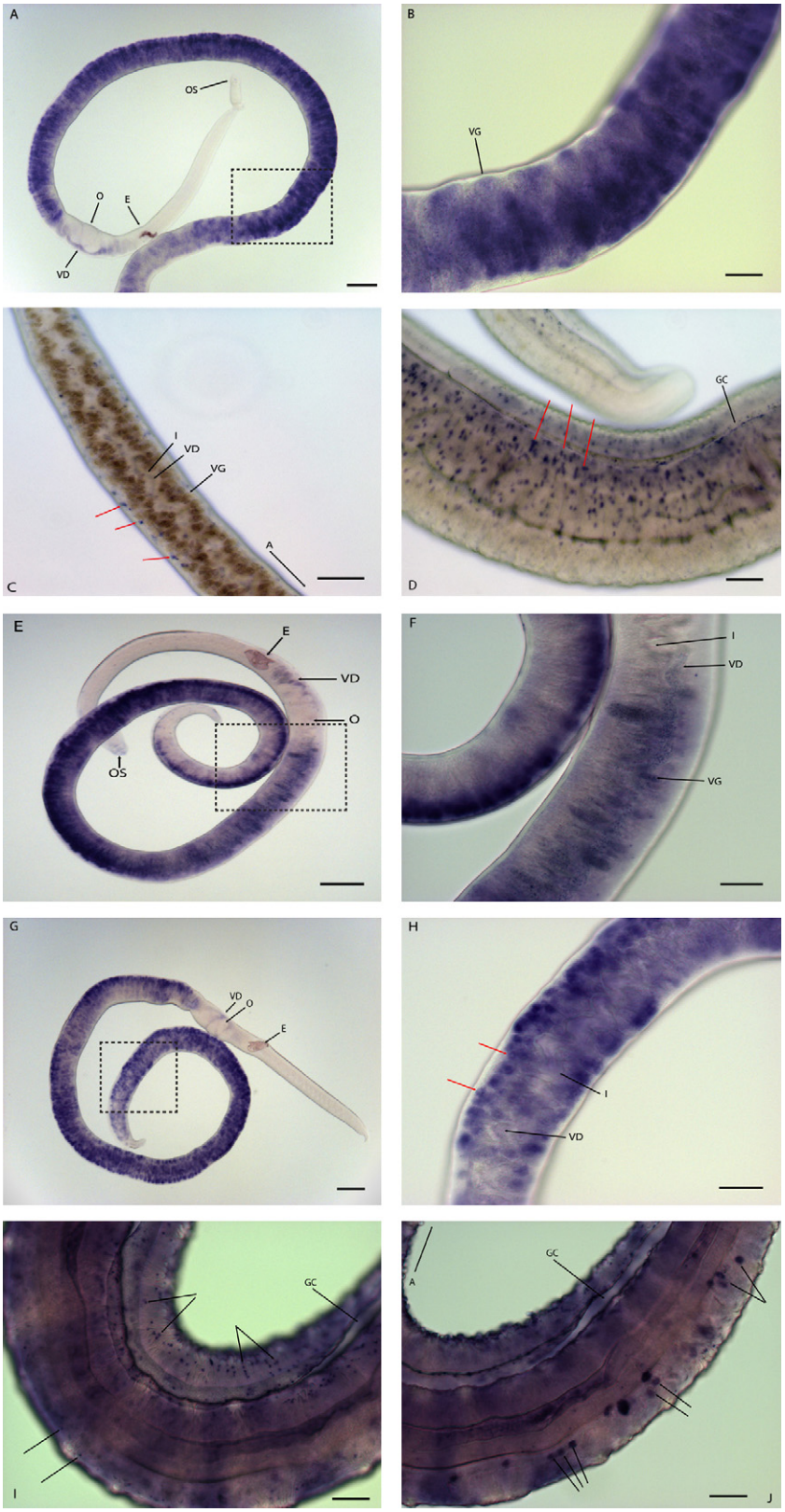

Fig. 1. Localization of *po*, *tsp-2*, *fs-tsp*, and *sp-sod* transcripts by whole mount *in situ* hybridization. (A and B) *po* localized by WISH (3 h development). (A and B) Female worms showing staining in the vitellaria but not in the other reproductive organs; (B) is a higher magnification view of the boxed image in (A). (C and D) *tsp2* localized by WISH (4 h development). *tsp2* localizes to sub-tegumental cells in females (C) and males (D). Red arrows indicate punctate blue staining representing the localization of *tsp2* transcripts. (E and F) Localization of *fs-tsp* (3 h development). *fs-tsp* localizes to the vitelline duct and vitellaria but does not localize to subtegumental cells; (F) is a higher magnification image of the boxed area in (E). (G–I) localization of *sp-sod* (3 h and 22 h development time for panels (G and H) and (I and J), respectively). A female enriched *sp-sod* localizes to the immature vitellaria of females (indicated by red arrows) and the vitelline duct downstream of the vitellaria (G and H) but is not detected in the oviduct, ovary, or uterus in the female worm; (H) is a higher magnification view of the image shown in (G). (I) Localization of *sp-sod* in the intestine and sub-tegumental cells of a male worm. (J) Localization of *sp-sod* in a subset of cells in the parenchyma that appear to be associated with the intestinal epithelium. (A and B) and (E–I) were visualized using a Zeiss Axio Star plus microscope, (C and D) were visualized using a Zeiss Axio Observer Z1 microscope. Abbreviations: A, anterior end of worm; E, egg; GC, gynecophoral canal; I, intestine; OS, oral sucker; O, ovary; T, testes; VG, vitelline gland; VD, vitelline duct. Scale bars: (B–D, F, and H–J) 50 μm; (A, E, and G) 150 μm.