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Cloning and characterization of thioredoxin peroxidase (TPx) in {\it Onchocerca volvulus\/}

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Abstract

About 400 million individuals worldwide are currently infected with filarial parasites. To find a cure or prevention for the infections, studies of the mechanisms of pathogenesis and the survival stratagies of the parasites are necessary. At this early stage of investigation, the lack of an animal model and an in vitro culture system make it impossible to define the important genes for the parasites by any functional analysis (forward genetics). For the purpose of unraveling the survival strategies of O. volvulus, the best approach is to gain a broad knowledge of the genes that are expressed in the parasite and then select those that may be important for survival (reverse genetics).^ cDNA libraries of infective third stage larvae of the two major filarial parasites, Onchocerca volvulus and Brugia malyai, were constructed. EST (Expressed Sequence Tag) analysis was perfomed on randomly selected clones. 200 ESTs were analyzed from each cDNA library. Seven identical ESTs out of the first 200 ESTs from the cDNA library of Onchocerca volvulus infective stage larvae were found to be very similar to a newly discovered antioxidant enzyme: Thioredoxin Peroxidase (TPx). Two genes that match with TPx were also identified from the cDNA library of Brugia malayi infective third stage larvae through more EST analysis of the ongoing Brugia malayi genome project.^ The detoxification ability of parasites has always been interesting to the parasitologist because it may be a parasite survival strategy in the face of host generated oxidative stress. In this thesis, I report my success in characterizing the TPx gene and protein of Onchocerca volvulus and I discuss its possible role in parasite survival.^ The complete sequence of the TPx cDNA of O. volvulus reveals an ORF (Open Reading Frame) that encodes a polypeptide of 199 amino acid residues with a calculated molecular weight of 21,890 daltons. The TPx mRNA represents roughly 2% of total transcripts in the infective third stage larvae of Onchocerca

volvulus. The TPx gene was expressed in the pRSET prokaryotic gene expression system and the expressed product was shown to have antioxidant activity. The antiserum raised against the expressed TPx gene product recognized a native protein from both the infective third stage larvae and adult extract that has a molecular weight of 22 kD, in agreement with the calculated molecular weight. The localization of the O. volvulus TPx protein at different stages of parasite development was detected immunochemically using the O. volvulus TPx specific antiserum. It was found to be surface located in infective third stage larvae, microfilariae and probably young adult worms. Therefore, TPx protein in O. volvulus may protect the parasites from being damaged by host generated oxidative stress. The results of immunohistochemistry combined with the results of in situ hybridization for mRNA detection, support the hypothesis that microfilariae are protected by a layer of TPx protein on their surface which is provided by the adult female worms. Evidence suggests that the expression of the TPx is induced initially inside of the intermediate host (blackfly). ^

Subject Area

Biology, Molecular|Biology, Cell|Biology, Microbiology|Health Sciences, Pathology

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