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Characterization and analysis of the cuticlin gene family of the human parasite *Onchocerca volvulus*

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Abstract

Onchocerciasis, or African River blindness caused by the filarial parasite *Onchocerca volvulus* is the second leading cause of infectious blindness world-wide, with over 18 million people infected. The parasite has a complex life cycle with developmental stages in both the black fly vector (L1, L2 and L3) and human host (infective L3, L4, adult, microfilaria). The parasite is protected by an external exoskeleton called the cuticle, which is a complex structure composed primarily of two classes of proteins: collagens and cuticlins. Of these two types of proteins, only the cuticlins are present in the parasite and not in the human host. Because the cuticle defines the host-parasite interface, characterization of these molecules is important in understanding the first line of parasite defense. More specifically, the composition of the *O. volvulus* cuticle during the dynamic process of the L3 to L4 molt may elucidate a mechanism for targeting the disruption of the infection cycle. Expressed sequence tag (EST) analysis of an *O. volvulus* molting L3 cDNA library reveals three distinct cuticlin gene family members present in the River Blindness Genome Project dataset. Although the three genes are most abundantly expressed in the molting L3 stage, PCR analysis indicates that they are differentially expressed in other *O. volvulus* life cycle stage cDNA libraries (L2, L3, adult female, adult male and microfilaria). Genomic copies of these three cuticlin gene family members have been obtained and the intron-exon structure determined for each gene. The genes contain between 5 and 9 exons ranging in size from 72 to 647 base pairs. The intron sizes of the three genes range from 84–1660 base pairs, with some of the largest *O. volvulus* introns represented. The cDNA copies of each gene, which range in size from 42–48 kDA, have been cloned in pieces (12–15 kDA) into a plasmid expression vector, and the highly insoluble protein fragments have been expressed. The proteins have been used for antibody production and patient serum ELISAs using both infected and putatively immune sera. Additionally, the proteins were identified in *O. volvulus* worms using immuno-light microscopy and immuno-gold electron microscopy localization. ^

Subject Area

Molecular biology|Pathology

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