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REGULATION OF GENE EXPRESSION IN THE **SCHISTOSOMA MANSONI** FEMALE **MR-17**

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Abstract-The maturation of females of **S. mansoni** is a process which depends on the presence of male parasites. Preliminary results indicated that the males may secret hormones, possibly steroid or steroid-like compounds, which regulate the expression of genes directly connected to events pertaining to oogenesis. Because the eggs are important agents in the pathology of schistosomiasis, it is of interest to elucidate the biochemical events associated to this developmental stage. The present work describes the efforts to characterize proteins which regulate the expression of a gene encoding a major protein precursor of egg shells and which is only transcribed by adult females. It is hoped that the receptor for the male hormone will be detected among these regulatory proteins.

Female **S.mansoni** can only evolve fully when paired to the male parasites. It has been shown that unisexual infections with female cercariae, produce stunted parasites, which do not display a functional vitellaria and hence cannot lay eggs. Among the hypothesis trying to explain how the male schistosomes can influence the sexual maturation of females, the three most accepted are: a) physical contact b) nutritional dependence and c) hormonal regulation. Although these hypothesis are not necessarilly mutually exclusive, evidence is accumulating suggesting that hormones may mediate this process. Whether the hormones are secreted by the male parasites alone is not yet known. The reports of Nirde (1983,FEBS Lett.,151:223-227) and Haseeb (1986, J.Chem.Ecol.,12,1699-1712) showed, using two different experimental approaches, that ecdysones, the insect molting hormone, occur in all but one stage of **S. mansoni**, namely the miracidia and that these ecdysteroids concentrated mainly in the vitellaria of females. Furthermore the original findings of Shaw (1977,Exp.Parasitol.,42,14-20) have shown that lipid extracts of adult male schistosomes could induce maturation of immature female parasites. It should not be surprising, therefore, that steroids regulate the changes associated to sexual maturation, since analogous situations abound in nature.

Recently Simpson (1985, Mol.Biochem.Parasitol., 18,25-35) has described a gene (F-10) which is only transcribed by adult female schistosomes. We have been able to show (1987, Mem. Inst. Oswaldo Cruz,82,209-212)

that semi-purified lipid extracts of adult worms could induce F-1O RNA synthesis in immature worms, suggesting that the F-1O gene contains steroid regulatory elements. Indeed, the results of Rodrigues (1989,Mol.Biochem.Parasitol.,32,7-14) sequencing the F-1O DNA, revealed that in the 3' untranslated region of the transcript, a hexanucleotide motif, TGTCCT, occurs. According to the results of Beato (1987, J.Steroid Biochem.,27,9-14), this motif has been found in several genes which are responsive to steroids, including ecdysones. Taking advantage of this information, an oligonucleotide with the following sequence was synthesized:

5' TCTCCACTGTCCTATTTTTC 3'

This sequence was derived from the region of the F-1O gene which contained the hexanucleotide motif. The antisense strand was also synthesized in order to obtain a double stranded oligonucleotide. This oligonucleotide was then end-labelled using the T4-polynucleotide kinase method and the ^{32}p-labelled molecule was used in experiments measuring the retardation of electrophoretic migration of the oligonucleotide probe after incubation with protein extracts obtained from various preparations of schistosomes.

Polyacrylamide gel electrophoresis revealed that the protein extracts were complex mixtures containing aprroximately 30 proteins. When incubated with the oligonucleotide probe,extracts from adult male schistosomes, adult females and immature females produced each different patterns of gel retardation, indicating that the probe was binding different ligands in the individual preparations. Upon addition of competing DNA (to avoid non-specific ionic interactions), consisting of synthetic double stranded poly d(A) d(U) or poly d(I) d(C), however, only the proteins from the adult male schistosomes remained bound to the probe. Addition of the unlabelled oligonucleotide blocked this interaction. These results indicated that the male proteins bound with high affinity to a sequence known to respond to the steroid: receptor complex. The ligand(s) in this case could in fact be repressors, since the male F-1O gene must be inactive at all times. Alternatively, only the males would posses the steroid receptor protein and after forming a complex with the hormone, it would be transferred to the females, possibly using the same route as that for insemination. Work in progress is being concentrated on the characterization of the oligonucleotide binding proteins.