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GENOMIC REGULATION IN IMATURE FEMALES OF *SCHISTOSOMA MANSONI*

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Sexual maturation in females of *Schistosoma mansoni* is still poorly understood. Although several factors may be involved, experimental evidence exists indicating that the male parasites might control the development of the female vitellaria through the secretion of steroid hormones, possibly ecdysones, as shown by Nirde (1983, *FEBS Lett.*, *151*: 223-227).

Because the eggs laid by the females are the main agents in the pathology of schistosomiasis, it is important to understand what elements participate in oogenesis and their mode of action. Such knowledge may permit interruption of the process through the use of analogues or antagonists.

The occurrence of a gene encoding female specific proteins (F-10), expressed only in mature females has been recently reported by Simpson (1987, *Mol. Biochem. Parasitol.*, *22*: 169-176). Indirect evidence suggests that the F-10 gene encodes proteins present in egg shells, but it is quite probable that the F-10 gene consists of a complex transcription unit involved in the formation of the vitellaria itself.

Although the complete sequence of the F-10 gene has been carried out by Rodriguez (manuscript in preparation), little is known about the regulation of its expression. In the present report we describe preliminary attempts to characterize lipid and protein mediators of the F-10 gene expression.

Lipid extracts of adult *Schistosoma mansoni* were obtained through extraction with methanol, partition with hexane and chromatography on silicic acid columns. These lipid fractions were incubated with live imature females obtained by unisexual infections in mice, for periods ranging from 1-2 days. After incubation, total RNA was extracted in hot phenol followed by ethanol precipitation. The RNA was spotted on nitrocellulose sheets which were then hybridized with a ^{32}P F-10 probe prepared by nick translation. In order to estimate total RNA synthesis, the parasites were incubated in the presence of ^{3}H-Uridine and with the different lipid extracts. RNA was extracted as above and counted for radioactivity in a liquid scintillation spectrometer.

Interaction of schistosome soluble proteins with the F-10 gene was analyzed by the gel shift technique of Fried (1981, *Nucl. Acids Res.*, *9*: 6505-6525) using the ^{32}P F-10 obtained by nick translation.

The results in Fig. 1 show that when hexane extracts were incubated with imature females, the transcripts of the F-10 gene could be detected through hybridization as indicated by the arrow. The active principle contained in the hexane extract probably is not a steroid since steroids would have partitioned into the more polar methanol phase. On the other hand, the more polar compounds contained in the aqueous phase was able to stimulate total RNA synthesis as shown in the Table. This fraction which must have contained the ecdysteroids did not, however, promote the transcription of the F-10 gene since this RNA population did not hybridize with the F-10 probe. So far we have no information as to the identity of these different compounds.

Steroid hormones usually exert their action by binding to cytoplasmic protein receptors. The complex hormone: receptor then binds to specific sequences of the genome initiating transcription.

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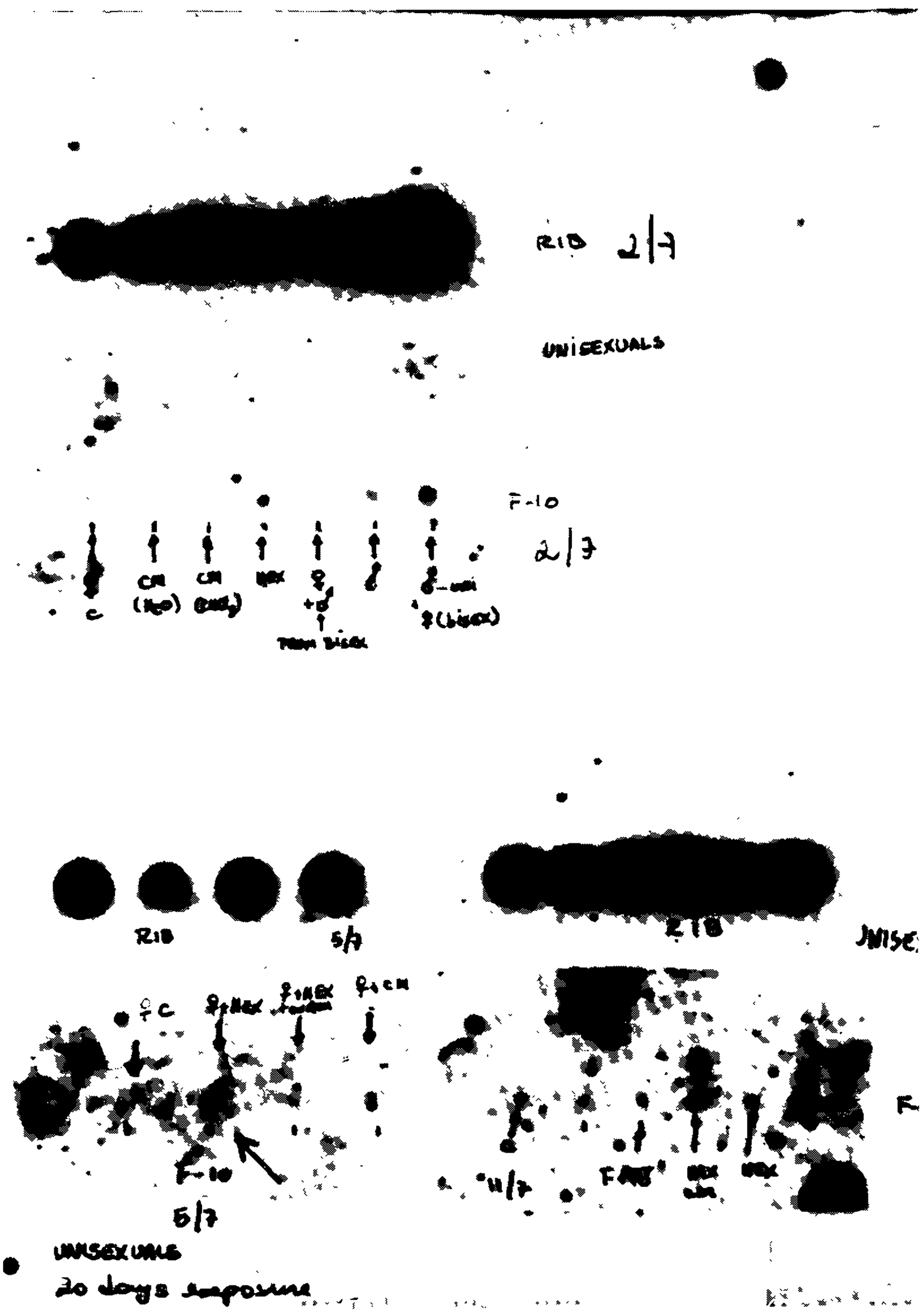

Fig. 1: 20 imature females obtained through unisexual infections were incubated in RPM I containing the different lipid extracts for several days with constant changes in the incubation medium. Total RNA was then extracted and spotted on nitrocellulose. This was hybridized against a probe consisting of ^{32}P-F-10 or against ribosomal RNA genes as a positive control (Rib). C = females incubated without lipid extracts. Hex = hexane extracts. αAm = alfa amanitin. CM = chloroform: methanol lipid extracts.

TABLE

Effect of lipid extracts on total RNA synthesis by immature females of *Schistosoma mansoni*

Treatment	CPM in total RNA
None	26.500
Extract containing polar steroids	58.200
α amanitin (2.1 μM)	10.900

20 parasites were incubated with ^{3}H-Uridine for several days and then total RNA was extracted and counted for radioactivity.

Soluble protein extracts of schistosomes contain proteins which bind specifically to the F-10 gene as shown by the results in Fig. 2. In these experiments ^{32}P-F-10 was used for incubation with the total protein extract. The mixture was then fractionated on a 4% polyacrylamide gel under non-denaturing conditions. The complex formed between proteins and the DNA altered the electrophoretic migration of the F-10 DNA as indicated in Fig. 2. That the complex consisted of protein: DNA, was shown by control experiments in which a proteinase K treated schistosome extract failed to produce the gel shift of the F-10 DNA. In Fig. 2, it can also be seen that binding of the proteins to F-10 protects it against degradation by DNase I. Thus it should be possible with this technique, to localize specific regions in the F-10 directly associated with regulation of its expression. Also, knowing that complexes formed between steroids and receptors bind to the DNA regulatory sites it should be possible to affinity purify the receptors in females for the male hormones.

Fig. 2: interaction of schistosome proteins with the F-10 gene. A total soluble protein extract of adult schistosomes was incubated with the ^{32}P F-10 DNA for 1h at 0 C and then fractionated on a 4% acrylamide gel under non-denaturing conditions. 1-5 = protein extract containing respectively 0.2, 0.4, 2,4 and 10 μg protein. 6 and 7 contained no extract but 7 contained 2 μg poly A poly U double stranded DNA as a control for non specific interactions (1 to 5 contained this synthetic DNA as well). 8 and 9 were treated with DNase I with the difference that 8 was preincubated with the protein extract prior to DNase treatment.