Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: (12): Prospects for immunological intervention in human schistosomiasis

Artikel: Gender-specifically expressed genes in "Schistosoma mansoni"
Autor: Kunz, W. / Symmons, P.
DOI: https://doi.org/10.5169/seals-313847

Acta Tropica *44*, Suppl. 12, 90–93 (1987)

Institut für Genetik, Universität Düsseldorf, FRG

Gender-specifically expressed genes in *Schistosoma mansoni*

W. KUNZ, P. SYMMONS

Summary

Using a genomic gene bank in phage lambda and two cDNA banks in the expression vector lambda-gt11 we have cloned and characterized genes that are expressed preferentially or exclusively in females. One of these genes transcribes two predominant RNA molecules of 0.8 and 3.9 kb which comprise more than 5% of the mRNA population of adult female worms. Transcription of these two RNAs occurs in close proximity on the genome, probably in an overlapping fashion. Experiments are presently in progress to sequence the genes and to produce antibodies against their polypeptide products which will be used to determine in which tissue and at what time in development these genes are expressed. The gene products are probably used for egg shell formation. The final long-term perspective of this project is to interfere with the schistosome parasite's cycle and to reduce its pathogenicity by interrupting egg production.

Although it now seems fairly well established that mammalian hosts are able to mount an immune response against *Schistosoma,* the exact mechanism responsible remains uncertain. This uncertainty is reflected in the wide range of different approaches being pursued by the many laboratories seeking ways to combat *Schistosoma* immunologically.

Our laboratory which has only recently started to investigate *Schistosoma,* has no intention of competing with laboratories that are searching directly for potential immunogens. Instead, we are intrigued by the very marked differences between the two sexes of this worm. The absolute dependence of the female maturation on the presence of males immediately suggests a possible target for interfering in the parasitic cycle. Finding a way to decrease the extent of female maturation and hence egg production would provide a means of reducing the pathogenicity of *Schistosoma.* Of course, if it were possible to prevent egg production entirely, the cycle would be interrupted.

Correspondence: Prof. Dr. W. Kunz, Institut für Genetik, Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, FRG

Very little is known at the molecular level about the differences between males and females. Thus the initial task we have set ourselves is to identify and clone those genes whose expression differs markedly between males and females.

Two techniques are being used to identify genes that are differentially expressed in the two sexes. In one, antibodies which define gender-specific antigens are being used to screen two expression vector libraries (one constructed with cDNA from males, the other with cDNA from females). In the other approach a genomic gene bank is screened differentially with cDNA made from polyA mRNA from females and from males. Clones which hybridize with cDNA from only one sex are chosen for further investigation.

Subsequent to cloning and characterization of gender-specifically expressed genes at the nucleic acid level, we plan to ask in which organs of males or females these genes are expressed by using in situ hybridization techniques. These techniques will also allow us to ask at what stage in development the genes are expressed and whether expression is dependent on contact with the oppsite sex. Genes whose pattern of expression is deemed interesting can be investigated further at the gene product level using immunohistological techniques.

Investigation of our gene libraries with both screening techniques (antisera and mRNA) has already yielded results:

1. Western blot analyses using antisera from chronically infected mice of two different strains (CF1 and NMRI) have revealed a female-specific antigen of 56 kDa. This antigen appears to be recognized only by antibodies of the IgG_1 subclass. To characterize the DNA sequences coding for this antigen, we screened our expression vector banks with the IgG_1 serum fraction and isolated 13 positive clones. These clones are presently further examined to check whether any of them contain sequences coding for the 56 kDA female-specific antigen.
2. With the other approach (screening a genomic bank with cDNA made from polyA RNA) we have isolated a clone which codes for part of two major transcripts of 0.8 and 3.9 kb. These transcripts are present only in females; they are completely absent in the RNA of male worms. These two transcripts are very abundant in the mRNA population of adult female worms, since in our female-cDNA bank more than 5% of the clones were positive. The genomic clone was isolated in a screen of 20,000 genomic bank clones which equals about half of the genome. This clone gave a very strong hybridization signal with female cDNA, and since only very few clones gave a positive signal of this magnitude we conclude that only one or a few genes per haploid set can code for these transcripts.

We have further characterized our genomic clone by restriction enzyme mapping and hybridization of the restriction fragments with cDNA made from polyA RNA. Surprisingly, the coding sequences are restricted to a very short fragment of about 350 base pairs within the 11 kb genomic clone, and this short

fragment contains sequences complementary not just to one, but to both predominant female-specific transcripts. Since both transcripts are longer than 350 base pairs, it follows that both genes must have at least one other exon and that these exons must lie elsewhere in the genome. Using in vitro transcribed single stranded RNA from this 350 base pair fragment, we have determined that both RNA molecules are coded on the same DNA strand.

In a recent publication [1] Simpson and Knight describe a predominant female-specific transcript of approximately 1 kb length which they estimate to comprise about 10% of the total RNA in females. A cDNA clone containing part of this transcript has kindly been given to us by Simpson, and we have found that it contains sequences homologous to the coding region of our genomic clone. The small discrepancy in the size estimates between Simpson's and our group (1 kb as opposed to 0.8 kb) may be explained by technical reasons, or it might reflect the use of different strains of *S. mansoni*. However, Simpson's cDNA clone does not hybridize to the 3.9 kb transcript we observe. This suggests that the two transcripts share no sequence homology.

DNA sequencing of the coding portion of our genomic clone is presently in progress.

The fact that the exons of two different genes are transcribed in very close proximity raises the question how the transcription units are arranged relative to each other. Is one of the two RNA molecules transcribed on a sequence that is the intron of the other gene?

If transcription of the precursors of the two genes would occur in an overlapping fashion, it would appear likely that the two genes are expressed under coordinate control.

In addition to this predominant female-specific clone we characterized three other genomic clones which are also expressed in adult females, although far less frequently. In contrast to the predominantly expressed sequences the expression of the latter sequences is not exclusively female-specific: a very faint signal on Northern blots of male RNA indicates that RNA molecules homologous to these clones are present also in adult male worms, although females contain much more.

The coding regions of these three clones share no sequence homology with our predominant female-specific clone and are again very short. However, on the Northern blots they also hybridize with an RNA size class of 0.8 kb. This means that in adult female schistosomes different RNA molecules are expressed which belong to the same size class.

As already mentioned there are several clones in our expression vector library, which contain cDNA copies of either the 0.8 kb RNA or the 3.9 kb RNA of our predominant female-specific clone. Experiments are presently in progress to immunize rabbits (mice) with the polypeptide products made by the recombinant expression vectors. The antisera should contain antibodies which recognize the protein counterparts of the predominant female-specific *Schistosoma*

mRNAs which can then be localized in tissue sections. Since the vitellarium is the most abundant tissue in adult females and since this organ is restricted to female worms, we strongly suspect that the protein products of the predominant female-specific messages are produced in the vitellarium and that they are involved in egg manufacture, probably in egg shell formation, and expect the projected immunohistochemical studies to provide confirmation thereof. Furthermore, Western blot and immune precipitaiton experiments will be able to solve the problem of why no highly abundant female-specific polypeptide counterpart to the predominant messages can be found by SDS-PAGE analysis of total *Schistosoma* adult worm proteins.

1 Simpson A. J. G., Knight M.: Cloning of a major developmentally regulated gene expressed in mature females of *Schistosoma mansoni*. Molec. biochem. Parasit. *18*, 25–35 (1986).