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MOLECULAR BIOLOGY OF SCHISTOSOME - A STEP TOWARDS RECOMBINANT VACCINES

Israel Schechter

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

The *objectives* of our research are to study the regulation of gene expression at different stages of the life cycle of schistosome, to understand the molecular basis of host-parasite relationship, and use this information to develop protective vaccines against Bilharzia.

cDNA libraries of cercaria and worm **(S.mansoni)**, constructed in λ gt11 were screened with antibody probes revealing stage specific gene expression (precipitating many proteins from worm mRNA translation products and a few proteins from cercaria, or vice-versa). Several stage specific clones were isolated. Two clones encoding the homologues of a heat-shock protein (HSP70) and a calcium-binding protein (CaBP) are decribed below.

The HSP70 cDNA (1.05 Kb long) was cloned from a worm library and its nucleotide sequence determined. The predicted amino acid sequence shows 76% homology with the carboxyl-terminal portion of human HSP70. Quantitative Southern blots revealed about three gene copies per haploid genome. From nuclear DNA libraries construted in EMBL4 we isolated genomic clones, some of which have two HSP70 related genes, in complete agreement with the gene dosage estimate. Northen blots showed selective expression of HSP70 in worm mRNA but not in cercaria mRNA. Newport and colleagues recently reported that with anti-HSP70 antibodies they detected the HSP70 protein in both worm and cercaria. This apparent inconsistency was resolved by looking into the hepatopancreas of infected snails. We found that sporocysts synthesize HSP70 mRNA, but the level of expression probably depends on their maturation stage. It seems that high expression occurs in embryos and it decreases to nearly zero levels in mature cercaria. It is conceivable that the HSP70 protein in the free swimming cercaria was programmed by mRNA in the sporocyst, and the protein has a longer life time than the mRNA. These experiments raise several important topics. 1) The fact that snail maintenance and shedding of cercaria (triggered by light) occur at the same room temperature indicate that at least one of the HSP70 genes is developmentally regulated. 2) Schistosome exhibit a complex pattern of HSP70 gene expression: High in sporocyst, practically zero in cercaria and again high in worm. 3) One HSP70 gene may be developmentally regulated during sporocyst-cercaria transformation (actually it may regulate this process via HnRNA splicing), and another gene may be triggered by heat during cercaria-worm transformation.

The putative CaBP cDNA (0.36 Kb long) was cloned from a cercaria library, and we determined its nucleotide and derived amino acid sequences. Northern blots

showed selective expression in cercaria but not in sporocyst or worm. To understand the nature of the encoded protein we used computer programs that brought up significant (but not striking) homology (~ 30%) to members of the calcium binding protein family. However, further analyses revealed clear resemblance to the domain structure and organization of CaBP molecules: 1) The schistosome CaBP contains two calcium binding loops of correct size (12 residues) and composition (six residues carry side chain oxygen to bind the calcium ion), 2) The distance between the loops (24 residues apart) is identical to the spacer length conserved in CaBP molecules. In addition, the schistosome CaBP programmed by mRNA shows Ca^{++} — dependent electrophoritic mobility (increased with Ca^{++} —ions and decreased with EGTA), like other CaBP molecules.

The CaBP gene was cloned and sequenced. The gene and cDNA sequences were compared and primer extension experiments were performed. The results established that the cDNA contains the complete coding sequence (69 codons), a portion of the 5' untranslated region (34 nucleotides upstream to the initiator-Met codon), the entire 3' untranslated region (56 nucleotides), and the poly-(A) tail. The structure of the gene is similar to that of eukaryotes. The promotor is composed of CAAT and TATA boxes as well as a cap site; one short intron (91 nucleotides long) interrupts the coding sequence. To our knowledge this is the first cDNA with defined 5' terminus and complete gene structure determined for helminth parasites. The CaBP is interesting because: 1) Most of the metabolic (activation of regulatory enzymes like Kinases) and physiological (contraction, secretion, etc) events triggered by calcium ions are mediated via CaBP molecules, 2) The preferential expression of this CaBP in cercaria raises questions as to what function(s) specific to cercaria it regulates, and whether all or only a few cells express the CaBP molecule.

A general issue revealed by the CaBP is the rapid change in gene expression during schistosome metamorphosis. We found that the CaBP mRNA is missing in infected hepatopancreas just prior to shedding, but is readily detected in cercaria at one hour after shedding. Other genes which are turned on (like the CaBP) or shut off within the short time interval (~1hr) of transition from snail to free swimming cercaria were identified. This system is likely to provide information on the mechanism of stage - specific gene activation/inactivation during the life cycle of the parasite.

Clone 21LT isolated from a worm cDNA library annealed with the mRNA of sporocyst, cercaria and worm. The unique feature of this clone is that it also hybridized with *normal* snail mRNA. Thus, the 21LT cDNA is a potential candidate for studying molecular mimicry at the nucleotide and protein levels. So far biological mimicry between snail and schistosome is known for shared sugar epitopes defined by serology. This system can provide relevant information on the molecular basis of host-parasite relationship.

Surface antigens of the schistosome have been studied extensively to develop protective vaccines against Bilharzia. Recently it was shown that internal and excreted proteins of the parasite can elicite immunity levels comparable to those achieved by surface antigens. We isolated several clones encoding internal antigens differentially expressed in cercaria or worm. We plan to evaluate the immune-protection conferred by these proteins when administered alone or in a mixture. Immunity directed against both the invasive stage and the adult worm may be advantageous, and a mixture of antigens may provoke higher protection than each antigen alone.