Research line No. 2

Biochemistry and molecular biology of parasites

Programs:

2.1 Characterization and purification of hydrolytic enzymes from *Entamoeba histolytica*.

2.2 Research studies on the genetic organization of *Entamoeba histolytica*.

2.3 Research studies on repetitive DNA from *T. cruzi* and *Plasmodium* sp.

Program 2.1 Characterization and purification of hydrolytic enzymes from *Entamoeba histolytica*.

We are presently working with proteolytic and phospholipasic enzymes from *Entamoeba histolytica* because of their possible participation in the invasivity and cytopathic effect of this protozoan. We are characterizing factors which affect the expression of these enzymes on cultivated amoebas.

The massive culture of entamoebas will provide us with the necessary biomass for the purification by affinity chromatography of the above mentioned enzymes, which will also be useful for future biochemical and immunological studies. Our research group also plans to study other aspects of cell biology and genetics of *Entamoeba* in collaboration with other scientists from México and the United States of América.

Specific projects

Characterization of a fibrinolytic activity from *Entamoeba histolytica*.
I. Cervantes, A. Alagón and R. López-Revilla
1983/P/A/DEP

Mass production of *Entamoeba* in PEHPS-1 media.
J. Vargas, S. Saíd-Fernández and A. Alagón
1983/P/S/DEP

Purification and characterization of a phospholipase from *Entamoeba histolytica*.
J. Vargas, A. Alagón and S. Saíd-Fernández
1985/P/S/DEP

Characterization of a hialuronidase from *Entamoeba histolytica*.
M.A. Torti and A. Alagón
1985/P/S/DBP

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**Program 2.2 Studies on the genetic organization of *Entamoeba histolytica***.

Scientific interest on the protozoan *Entamoeba histolytica* is due to two factors: first, *Entamoeba* is the agent responsible for amoebic dysentery and second, it has interesting biological properties. It shows great polymorphism both at the morphological and at the biochemical level; one can find variations in the levels of specific enzymes in different cultures of the same
Scientific interest on the protozoan Entamoeba histolytica is due to two factors: first, Entamoeba is the agent responsible for amoebic dysentery and second, it has interesting biological properties. It shows great polymorphism both at the morphological and at the biochemical level; one can find variations in the levels of specific enzymes in different cultures of the same
strain. We are interested in studying this organism's genome with the objective of describing some of its properties at the level of genetic expression.

The new techniques on separation of high molecular weight DNA by pulse field gradient electrophoresis should allow the mapping of some Entamoeba genes at the level of chromosomes and should also allow the investigation of possible rearrangements of the genome which have already been observed in other protozoa. For this purpose we plan to clone some genes of interest, such as those which code for phospholipase, fibrinolysine or some abundant membrane proteins. We are also interested in cloning repetitive DNA elements of unknown function that could provide useful markers for regions with potential instability in the chromosome. In order to obtain these clones we are constructing genomic and cDNA libraries of Entamoeba in collaboration with the Instituto Politécnico Nacional (National Polytechnical Institute from México). In the long run we are interested in the possibility of genetic manipulation of amoebas through exogen DNA transformation. It is our belief that these techniques will allow us to better comprehend the phenomena of phenotype variability in amoebas.

Specific projects

Cloning of repetitive DNA elements and ribosomal genes from Entamoeba histolytica.
J. Cruz, A. Alagón and P. M. Lizardi
1986/I/S/DBP

Isolation and characterization of chromosomes from Entamoeba histolytica.
J. Cruz, M. Reyes, M.L. Villarreal, A. Alagón and P.M. Lizardi
1986/I/S/DBP

Cloning of important structural genes in Entamoeba histolytica.
P.M. Lizardi, A. Alagón and I. Meza
1986/I/S/DBP
Program 2.3 Studies on the repetitive DNA of *T. cruzi* and *Plasmodium*.

It is known that in the genome of various parasite species repetitive DNA sequences are found which represent a large percentage of total nuclear DNA. Usually repetitive DNA sequences are species-specific, therefore making possible the taxonomic identification of the organism. Recently the detection of ten to thirty cells from *T. cruzi*, by means of hybridization methods, has been demonstrated (González et al., 1984). In collaboration with Dr. Nadia Nogueira and Dr. Antonio González in New York University Medical School we continue some studies on *T. cruzi*’s repetitive genes.

In a project initiated by Dr. Lizardi at the Rockefeller University, four *P. falciparum* repetitive DNA elements were sequenced. These sequences showed specific hybridization for the species, in other words, the elements did not form hybrids with DNA from other *Plasmodium* species, such as *P. vivax* and *P. malariae*. The usefulness of these repetitive DNA clones in malaria diagnostic assays has been demonstrated in hybridization tests with monkey’s blood infected with the parasite. This research project continues at CIIGB and besides, we have initiated a parallel project with the objective of isolating and characterizing repetitive DNA clones from *P. vivax*, which is the most prevalent *Plasmodium* species of malarial infection in México. We also expect to obtain, from this parasite, clones of species-specific repetitive sequences, with similar potential application in diagnostic assays.

**Specific projects**

Structure and sequence of some repetitive genes in *T. cruzi*.
P.M. Lizardi, A. González and N. Nogueira
1988/P/S/DBP

Sequence and chromosomal localization of *P. falciparum* repetitive DNA elements.
M.T. Tusie, A. González and P.M. Lizardi
1986/P/S/DBP
Cloning of DNA repetitive elements from *P. vivax* and *P. malarie*.

I. Tusie, A. Alagón and P.M. Lizardi

1986/P/S/DBP