PN-AAZ-777
56029 CS-139



U.S. - Israel CDR Program

Room 720 SA-18

Agency for International Development

Washington, D.C. 20523

U.S.A.

Progress Report

Grant No. DPE-5544-G-SS-6034-00

Microtubule Associated Proteins (MAPs) - A possible Chemotherapeutic Target Against Trypanosomes (CS-139)

During the first months of the grant, we have concentrated on developing methods for purification and solubilization of Leishmania microtubules. Using promastigotes of L. major WR309, we found that both the abundant subpellicular microtubules and the flagellar microtubules were cold stable and therefore resistant to the classical treatments established for purification of brain and mammalian cell microtubules and their MAPs. Since cytoskeletal elements are known for their detergent stability, we tested various detergents on Leishmania homogenates for solubilization of cell membranes and organelles. We found that a Hoechst detergent called 3-14 offered the best preparation of microtubules since in contrast to Triton X-100, CHAPS, Nonidet P-40 and Hoechst 1566 this detergent seemed to effectively remove the plasma membrane which is strongly associated with the subpellicular microtubules.

Phenothiazines which selectively cause the disintegration of the subpellicular microtubules in Trypanosomes (leaving the flagellar axoneme

Rec'd in SC: NOV 13 199

intact) had no effect on L. major promastigotes as assessed by SDS-PAGE or electron microscopy (EM).

The insoluble preparation of microtubules obtained by the 3-14 detergent consisted of tubulin and several unidentified proteins that could qualify as MAPs. The microtubules in contrast to those of brain were cold stable, insensitive to colchicine and to calcium ions but they could be depolymerized to a certain extent by high ionic strength. A Leishmania subpellicular microtubule preparation was sonicated in the presence of 0.75 M NaCl. The soluble fraction was enriched in tubulin and contained a few other proteins in the molecular weight ranges of 70, 60, 35 and 20 kDa. In order to check whether these proteins were involved in microtubule polymerization and or in the promotion of associations between microtubules, it was necessary to isolate the various proteins. This was achieved by fractionation of the salt solubilized dialyzed proteins on a cation-exchange column (mono s (Pharmacia) or phospho cellulose). Tubulin was not retained on the column and proteins in the molecular range of 35 and 48 kDa were released by increasing the ionic strength of the eluting buffer.

The amount of tubulin that was obtained from each preparation was very scarce. For polymerization studies it was therefore necessary to use phosphocellulose purified calf brain tubulin, an approach that was successful in the study of MAPs from Crithidia fasciculata.

Favorable conditions for microtubule polymerization were applied for the polymerization studies. These consisted of incubating calf brain tubulin

at 37°C with GTP, Taxol (a drug known to enhance and stabilize microtubule polymerization) and MAPs, in this case cation exchange purified Leishmania proteins.

Calf brain tubulin without added parasite proteins formed long strands of single microtubules. Addition of Leishmania proteins created bundles of microtubules in which the strands were aligned to one another reminiscent of the subpellicular network seen in the parasite. This deservation suggests a role for the purified proteins, that were strongly associated with the insoluble microtubules, in the specific organization of the subpellicular microtubules in these organisms.

During a period of 3 months, we had with us Mr. H. Waitheka from Prof. Njogu's laboratory in KETRI. Together with Mr. Waitheka we carried out parallel experiments on Trypanosomes (T. brucei KETRI 2693). The blood form of this parasite has a prominent array of subpellicular microtubules that are less stable than those of Leishmania. Starting with live organisms detergent insoluble microtubules were prepared. Salt solubilization and cation exchange chromatography led to the separation of tubulin and associated proteins with molecular weights of 66-70, 30-45 and 20 kDa. The Trypanosome MAPs were also effective in bringing about the bundling of single calf brain microtubules.

We have observed several differences in the behaviour of Trypanosome and Leishmania microtubules and studies of these will be pursued in the future.

Purified MAPs of Trypanosomes were also injected into rabbits in order to obtain polyclonal antibodies for further studies. We had no luck as yet with the isolation of monoclonal antibodies towards Leishmania MAPs.

The work performed up-to-date will be summarized shortly and we will send you preprints when available.

R. Goldman

Rachel Goldman

Principal Investigator