

Localization of Alfa and Beta Proteasome Subunits in *Entamoeba histolytica* Trophozoites

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Proteasomes are ubiquitous cell components that participate in many biochemical regulatory pathways by timely removing critical proteins that include damaged, abnormal, or foreign proteins. Previous studies have shown that *Entamoeba histolytica* has α subunit proteasome with homology to human α proteasome gen, moreover, a 20S proteasome activity has been reported. A recent work has also shown that amoebic proteasome is only localized in the cytoplasm. Presently, we used immunocytochemical, confocal and electron microscopy techniques to determine the precise cell localization of α and β proteasome subunits in virulent *E. histolytica* trophozoites. For immunocytochemistry, we used *Methanosarcina thermophila* antibodies against α and β proteasome subunits. For confocal microscopy, trophozoites were fixed in 4% paraformaldehyde and permeabilized with acetone at -20°C , then incubated with the respective antibodies at 37°C . Second antibody used for α subunit was rhodamine-labeled and for β subunit FITC was used. Samples for immunoelectron microscopy were fixed 4% paraformaldehyde and 2.5 M glutaraldehyde in cacodylate buffer and embedded in LR-White resin. Ultrathin sections were placed on nickel grids and incubated with the respective antibodies, for immunodetection, the second antibody was labeled with 10 nm gold particles. Sections were analyzed with a Jeol 100SX TEM. Results with confocal microscopy showed that either α or β proteasome subunits were present at the membranes and inside amoeba vacuoles. Based on these date, we conclude that amoebic proteasomes, as in most of the eukaryotic cells, have both, cytoplasmic and nuclear localization. This feature suggests that proteasomes in *E. histolytica* may have functions on the growth, cell cycle and encystation, among others.

References:

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- DeMartino, G. N. and C. A. Slaughter. 1999. The Proteasome, a Novel Protease Regulated by Multiple Mechanisms. *J. Biol. Chem.* 274:22123-22126.

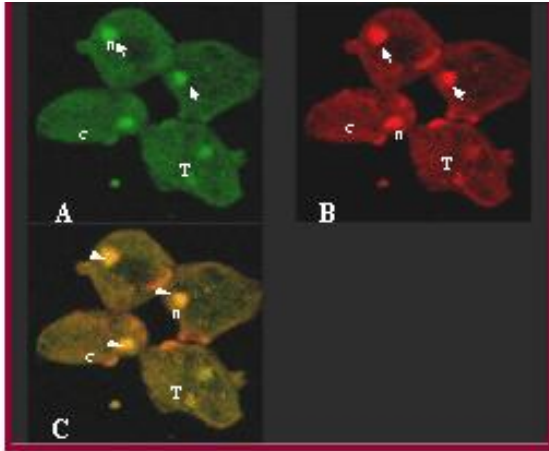


Fig.1. Confocal microscopy of *Entamoeba histolytica* trophozoites showing the distribution of proteasome's alpha and beta subunits. Monoclonal anti-alpha and polyclonal anti-beta antibodies against *Methanosarcina thermophila* proteasome were used. Secondary antibodies were labeled with rhodamine for alpha and FITC for beta subunits. Labels were detected in both, cytoplasm (c) and nucleus (n) of *E. histolytica* trophozoites (T), with co-localization. A) FITC labeled beta subunit (arrows), B) Rhodamine labeled alpha subunit (arrows) and C) Alpha and beta subunits (arrow-heads).

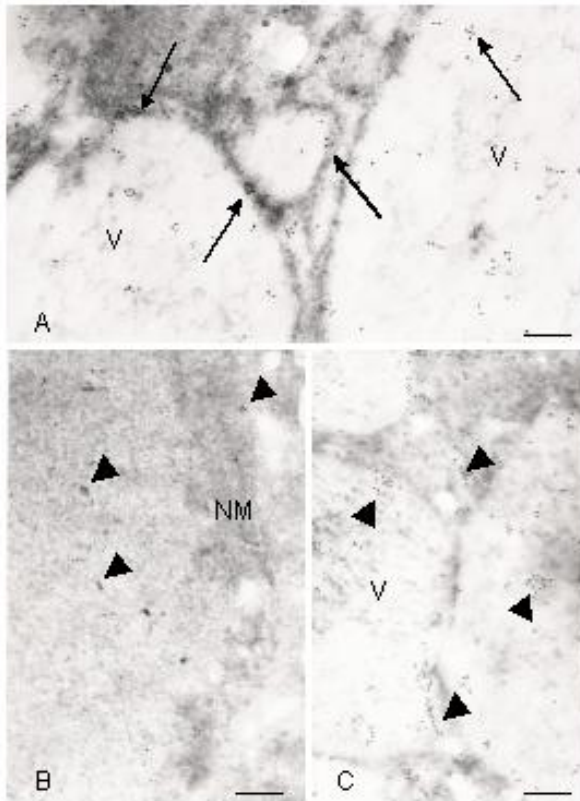


Fig.2. Immunoelectronmicroscopy of *E. histolytica* trophozoites using monoclonal (A) and polyclonal (B and C) gold labeled antibodies against alpha and beta subunits of human proteasomes. A) Cytoplasm and vacuoles (V) show uniformly distributed gold labeled alpha subunits (arrows). B and C) Trophozoites show cytoplasm, vacuoles (V) and nucleus labeled with the beta subunit (arrow-heads). Nuclear membrane (NM). Bars = 0.5 μ m