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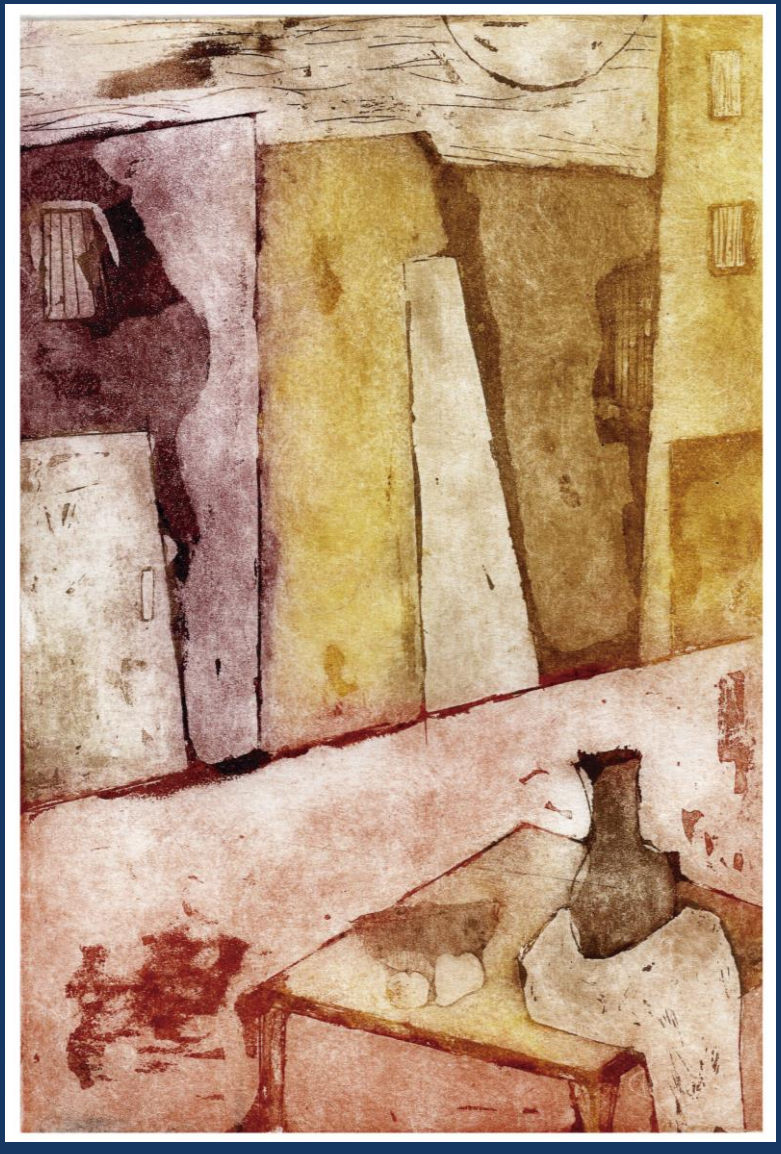

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La Tapa (Ver pág. 4)
Atardecer en la tarde
Antonella Ricagni

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Tel. 5287-3827 Int. 73919 y 4523-6619
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Abstract/Resumen: Autophagy is a conserved process along evolution and is essential for the maintenance of cellular homeostasis. However, trypanosomatids seem to possess a less complex route, or different proteins not yet identified. This process can be up-regulated during stress, starvation, or infection. In mammals, two kinases differentially regulate the process of autophagy: mTor and a phosphatidylinositol 3-kinase, Vps34, which interact with a regulatory subunit, Vps15. Metacyclogenesis is a fundamental process in the life cycle of the protozoan Trypanosoma cruzi, the etiological agent of Chagas disease. This process involves the transformation of non-infective epimastigotes into infective metacyclic trypomastigotes. Several changes occur during metacyclogenesis, and autophagy was found to be strongly upregulated during this process. In this work, we demonstrate that parasites overexpressing TcVps34 or TcVps15 proteins enhance both, autophagy and metacyclogenesis. TcVps34 or TcVps15 overexpressing epimastigotes were able to differentiate to metacyclic forms in a higher proportion than wild-type cells. Parasites overexpressing these proteins showed a more intense labeling with the autophagosome marker Atg8.1 and higher levels of monodansycadaverine (MDC) staining, a specific in vivo marker for autophagic vacuoles, in the intermediate forms of differentiated parasites, in comparison to control parasites. To extend this study we are also performing assays with DQ-BSA, to evaluate degradative compartments, since the induction of autophagy is characterized by an increase in the number of lysosomes/autolysosomes required for the lysis of trapped components. In addition, we will study the effect of autophagy regulatory drugs such as rapamycin and wortmanin in transgenic parasites during metacyclogenesis. Taken together, these data demonstrate the key role of phosphatidylinositol 3-phostate pathway in autophagy, T. cruzi differentiation and cell cycle progression.

0438 - BIOCHEMICAL CHARACTERIZATION OF DIHYDROXYACETONE KINASE FROM TRYPANOSOMA CRUZI (TCDAK)

Juan Matias VIECENZ (1) | Patricia A. GARAVAGLIA(1) | Laura Mónica TASSO(1) | Cristina G. MAIDANA(1) | Gabriela GARCIA(1) | Joaquin Jb CANNATA(2)

INSTITUTO NACIONAL DE PARASITOLOGÌA "DR MARIO FATALA-CHABEN" ANLIS MALBRAN; CONICET (1); INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB-UNSAM-CONICET) (2)

Abstract/Resumen: Dihydroxyacetone (DHA) is used as carbon source by many cell types; however, at high concentrations, it is toxic and some microorganisms are susceptible to this compound. DHA detoxification is dependent on a functional dihydroxyacetone kinase (DAK) which converts DHA to DHA-phosphate (DHAP). Trypanosoma cruzi, the etiological agent of Chagas disease, possess two genes encoding for putative ATP-dependent DAKs (TcDAKs). Previous studies have shown that T. cruzi epimastigotes are able to grow in the presence of DHA and DAK activity was found in their lysates. Nevertheless, TcDAK has not been characterized so far. With this aim, recombinant TcDAK, expressed in E. coli by pJexpress vector and purified by IMAC, was used to determine kinetic properties of this enzyme. DAK activity was measured using DHA and ATP as substrates in the presence of 2 mM $MgCl_2$ in a coupled assay by enzymatic reduction of DHAP with NADH in the presence of glycerol-3-P dehydrogenase. TcDAK exhibited Michaelis-Menten kinetics for DHA (Km= 6.3 µM and Vmax= 12.79 µmoles/min/mg of protein). On the other hand, it showed sigmoideal kinetics for Mg-ATP (S0.5 =125.03 µM, Vmax= 1.18 µmoles/min/mg of protein and Hill coefficient= 1.99). TcDAK activity in the presence of other metals different than Mg^{++} was also evaluated. All of them gave lower activities than that observed with Mg-ATP (Ca^{++}> Cd^{++}> Mn^{++}> Co^{++}> Zn^{++}). Additionally, TcDAK expression was evaluated in epimastigote and trypomastigote lysates by western blot using an anti-recombinant TcDAK serum. Epimastigotes and trypomastigotes showed the presence of two proteins recognized by the specific antiserum of molecular masses of 60 and 49 kDa, respectively, suggesting that trypomastigotes might have a TcDAK cleaved isoform. These results endorse the hypothesis that T. cruzi possess an active TcDAK which enables the parasite to use DHA as carbon source.

0509 - A COLLABORATIVE TRANSLATIONAL RESEARCH FRAMEWORK TO STUDY THE ASSOCIATION BETWEEN THE ALTERATION OF THE VAGINAL MICROBIOTA AND PRETERM BIRTH: PRELIMINARY RESULTS.

María Del Carmen ESANDI (1) | Olga Lorena GERMAN(1) | Ignacio BERGE(1) | Ariana BRUZZONE(2) | Martin ORESTI(1) | María Luz BENVENUTTI(3) | Paula LÓPEZ ULLÁN(4) | Jimena GALAVOTTI(3) | Laura GONZALEZ(3) | Natalia GONZALEZ(5) | Sergio MENDOZA(5) | Adriana GARDELLINI(6) | Gabriela SERRALUNGA(7) | Luis GOMEZ(8) | María Eugenia ESANDI(9) | Cecilia BOUZAT(1) | Marta BERTIN(10)

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Abstract/Resumen: Preterm birth (PTB) constitutes a major health problem in our country. There is a variety of potential etiological mechanisms for spontaneous PTB, including infectious and inflammatory pathways, stress-related influences and genetic predispositions. Uncovering the multifactorial processes and the interplay of risk factors that lead to spontaneous birth is necessary to identify effective strategies for preventing PTB. Our study analyzes the possible association between the vaginal microbiota and preterm birth. We conducted a prospective cohort study on 163 pregnant women treated in Hospital Dr. Penna Bahia Blanca (October, 2017- August, 2019), to analyze the alteration of vaginal microbiota by Nugent score (dysbacteriosis/ vaginosis), the assessment of Candida spp. and other pathogenic microorganisms at weeks 24/28 of pregnancy. Follow-up until delivery, with identification and recording of PTB or premature membrane rupture (PMR) events. The average age was 26 ± 6.1. Sixty seven % of women had a vaginal microbiota alteration: 35 % dysbacteriosis-vaginosis, 27 % candidiasis, and 41 % a sexual transmitted pathogen like Chlamydia trachomatis, Mycoplasma genitalium or Trichomonas vaginalis. Mixed infections were detected in 33 % of women and 28 % were positive for HPV. Sixty nine % concluded the follow-up until delivery and 14 % presented at least one of the outcomes of interest: 7 % PTB; 6 % PMR and 1 % PTB and PMR. The frequency of PTB was higher in the presence of some types of infections, however, a higher number of cases is still required for a statistical significance analysis. These results are of clinical and public health relevance due to high rates of vaginal microbiota alteration found in the analyzed population of pregnant women. In addition, the implementation of a translational research represented a huge challenge that impacted positively on public health by improving the systematizing care processes in and between hospital units as well as on primary health care attention.

0534 - PROTEINS BEARING CYCLIC NUCLEOTIDE BINDING DOMAINS IN TRYPANOSOMA CRUZI.

José Leonardo ESCALONA | Guillermo DI MARIO | Gabriel FERRI | Martin EDREIRA