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LXII ANNUAL MEETING OF ARGENTINE SOCIETY OF CLINICAL INVESTIGATION (SAIC)

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LA TAPA

María Esther Gené, **Imagen ígnea**, 1996.

Acrílico sobre tela, 110 x 95 cm. Cortesía de la Comisión Nacional de Energía Atómica, Predio TANDAR, Centro Atómico Constituyentes. Presidente de la Comisión Organizadora de la Exposición Permanente: Dr. A.J.G.Maroto.

María Esther Gené nació en Buenos Aires. Cursó Historia del Arte y Estética con Blanca Pastor y Nelly Perazo. Se inició en el taller de Centa Bertier y continuó su formación con Miguel Dávila. Participó del grupo de investigación plástica que dirigió Emilio Renart. Integró el Grupo Gen y formó el Grupo Fusión. Realizó numerosas exposiciones colectivas e individuales (Museos Municipal de Bellas Artes de Luján, Fernán Félix de Amador, de Arte Moderno de la Ciudad de Buenos Aires, Fundaciones San Telmo y Banco Mayo, Fundación Andreani, Patio Bullrich, Galería Kristel K., Salón ICCED de Pintura, entre otros). Sus obras se encuentran en colecciones privadas de Argentina, México, Alemania, España, Uruguay y EE.UU.

[1] Comisión Nacional de Energía Atómica. Artistas Plásticos con la CIENCIA, Centro Atómico Constituyentes, Predio TANDAR, Buenos Aires, 1999; En: *http://www2.cnea.gov.ar/xxi/artistas/artistasplasticos.htm*

in death, depending on severity of stress. These results could help to explain the mechanisms underlying in SUDEP.

(259) **STOICHIOMETRY AND KINETICS OF ACTIVATION AND POTENTIATION OF NICOTINIC ALPHA7BETA2 HETEROMERIC RECEPTORS**

Beatriz Elizabeth Nielsen (1), Teresa Minguez (2), Isabel Bermudez (2), Cecilia Bouzat (1)
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The α7 nicotinic receptor (nAChR) is a promising drug target for neurological and inflammatory disorders. It has been considered the homomeric member of the family. The recent discovery of α7β2 receptor in brain led to the urgent need of its functional characterization. Our main goal is to determine the stoichiometry of the heteromeric α7β2 receptor and its activation and potentiation profile. We generated receptors with fixed stoichiometry by two different approaches. One involved the generation of concatemeric α7β2 pentamers of different stoichiometries, and the other involved co-expression of unlinked α7 and β2 subunits, with the α7 subunit carrying a reporter mutation. Receptors were expressed in mammalian cells and function was evaluated by single-channel recordings. We found that α7 can assemble with one, two or three β2 subunits to form functional receptors. As the number of β2 subunits in the pentamer increases, the durations of openings and activation episodes, called bursts, increase progressively whereas channel conductance remains constant. We proposed that the prolonged bursts observed for α7β2 can be used as the signature of the presence of heteromeric receptors in native tissues. The prolonged activation episodes and reduced desensitization of α7β2 may have an important impact on calcium-dependent intracellular signaling and neuronal excitability. By using mutant subunits, we demonstrated that activation of α7β2 occurs through the α7/α7 binding-site interface. Among α7 positive allosteric modulators (PAMs), which emerge as novel therapeutic tools, type I PAMs were more selective for α7 than for α7β2 whereas PNU-120596, a type II PAM, similarly potentiated all α7-containing receptors. Statistically significance differences were established at p-values<0.05.This first single-channel study of α7β2 provides basis for deciphering the role and functional location of this novel receptor and opens doors for the development of selective therapeutic drugs.

Keywords: alpha7beta2 nicotinic receptors, concatemers, patch-clamp.

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(320) **IDENTIFICATION AND CHARACTERIZATION OF A NEW CLASS OF C22 STEROL DESATURASE FROM *TETRAHYMENA THERMOPHILA***

Nicolas P. Garcia Siburu (1), María L. Sánchez Granel (2), Clara B. Nudel (2), Alejandro D. Nusblat (2), Antonio D. Uttaro (1)
(1) IBR; FCByF, UNR. (2) NANOBIOTEC; FFyB, UBA.

T. thermophila does not require sterols, but synthesizes as a surrogate the triterpenoid "tetrahymanol". If sterols are available, they are avidly incorporated by the ciliate and bioconverted to 7,22-*bis*-dehydrocholestrol, whereas tetrahymanol synthesis is completely inhibited. This bioconversion requires four desaturating activities; three of them were previously characterized by our groups. Here we describe the identification of the last fourth enzyme, the C22 desaturase, instrumental in the development of microbial systems for the synthesis of tailored steroids. All known C22 sterol desaturases belong to the P450 oxygenases, essential enzymes for the synthesis of ergosterol in fungi or phytosterols in plants. *T. thermophila* has 44 putative P450 oxygenases, all of them sharing low similarity to canonical C22 desaturases. The ciliate enzyme was induced by sterols, required NAD(P)H and was inhibited by azide and cyanide, but not by azoles, typical properties of oxygenases belonging to the superfamily fatty-acid desaturases/hydroxylases (FAD/H). Analysis of a differential transcriptome carried out on *T. thermophila*, grown in presence or absence of sterols, allowed us to detect several FAD/H significantly induced by sterols. The two more induced ones, named Des1 and Des2 (sharing 68% similarity) were selected for further characterization. Expression of Des1 or Des2 in a *Saccharomyces cerevisiae* C22 desaturase mutant, both rescued the synthesis of the yeast ergosterol. Additional genetic approaches performed on the ciliate have unambiguously confirmed the C-22 activity of these two isoenzymes. It is the first description of a C22 sterol desaturase which is not a P450 oxygenase. These results also expand the repertoire of FAD/H specificities.

Keywords: sterols, bioconversion, desaturases, ciliates

(1099) **ISOLATION AND CHARACTERIZATION OF STRONG CONSTITUTIVE PROMOTERS ACTIVE IN *Lactococcus lactis* AND *Escherichia coli*.**

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Lactic acid bacteria are an increasingly interesting model for the expression of recombinant proteins of industrial interest because of their GRAS (Generally Recognized As Safe) condition. If the protein to be overproduced is non-toxic to the bacterium, constitutive promoters can be used for expression. The objective of the this work was to isolate strong promoters of constitutive expression from *L. lactis*, also active in *E. coli*. The advantage of these promoters is that they would allow the construction and preliminary analysis of DNA constructs in *E. coli*, a very easy to work bacteria, for later use in lactic acid bacteria. To this end, a library of *L. lactis* genomic DNA fragments was generated by digestion with different restriction enzymes and subsequently, the 400-2000 bp fragments were cloned into a reporter vector for promoter regions (pTLGR). *E. coli* competent cells were transformed with the ligation mixture and the presence of a DNA fragment with strong constitutive promoter activity was revealed by the appearance of red colonies, because of the expression of the red fluorescent protein of the reporter vector. A plasmid purification was carried out from these red colonies and used to transform *L. lactis*competent cells. *L. lactis* colonies showing a rosaceous color were selected, plasmids purified and sequenced. We identified 4 different DNA regions capable of acting as strong constitutive promoter regions in both,*L. lactis* and *E. coli*. The putative promoter regions were identified in the *L. lactis* genome. Moreover, 3 of the 4 regions identified were reported in the literature as strong promoter regions in transcriptomic studies of *L. lactis*. Preliminary analysis showed that the four identified promoter regions would be considerably more active than the most active constitutive promoter identified in *L. lactis* to date.

Keywords: strong constitutive promoter, *Lactococcus lactis*, *Escherichia coli*

(1140) ***BRUCELLA ABORTUS* EXPLOITS HOST CELL ALPHA-ENOLASE VIA THE VIRB EFFECTOR BPE123 TO PROMOTE INTRACELLULAR REPLICATION**

Susana María Morrone Seijo, Francisco Fernando Guaimas, María Inés Marchesini, Diego José Comerci
IIB-Intech. UNSAM CONICET.

Brucella abortus, the causative agent of bovine brucellosis, invades and replicates within cells inside a membrane-bound compartment known as the *Brucella* containing vacuole (BCV). *Brucella* Type IV Secretion System (VirB) is a major virulence factor, which translocate effector proteins from the BCV into host cell cytoplasm, thus modulating several signaling pathways to favor bacterial intracellular survival and replication. BPE123 is a *B.abortus* VirB sustrate previusly identified by our group. In an attempt to identify host cell proteins interacting with BPE123, a pull-down assay was performed and human alpha-enolasa (ENO1) was identified as a potential interaction partner of BPE123. This interaction was confirmed in vivo by immunoprecipitation assay and by confocal microscopy analysis of ENO1 redistribution in the presence of BPE123. Aditionally, quantitative analysis of confocal micrographs of macrophages infected with *B. abortus* showed that ENO1 associates to BCVs in a