IV JORNADA
DEL INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE ROSARIO

(Retreat-IBR-2022)

Viernes 25 de Noviembre de 2022

Rosario

Organiza:
Fundación del Instituto de Biología Molecular y Celular de Rosario (FIBR)

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR)

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Organización:

Natalia Gottig, Claudia Banchio, Pablo Tomatis, Diego Serra, Darío Krapf, Marta Vijande, Marina Avecilla, Franco Savoretti, Jimena Zoni, Andrés Binolfi & Eleonora García Véscovi

Fundación del Instituto de Biología Molecular y Celular de Rosario (FIBR),
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR)

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No fue fácil, no es fácil…

Cuando me pidieron que escriba un texto para la edición 2022 del ya clásico *Retreat-IBR*, acepté muy contenta la invitación. Nunca imaginé lo difícil que me resultaría resumir en unos pocos párrafos las vivencias y experiencias vividas en el IBR durante tantos años de mi vida.

El título que elegí no es trivial. No fue fácil crear y hacer funcionar al IBR. El objetivo siempre fue hacer ciencia de calidad. Para eso, en sus comienzos, unos pocos integrantes hacíamos todo, ¡todo! Desde planificar los experimentos, hacer las compras, buscar las cartas, hasta limpiar los pisos luego de las inundaciones. Aun en ese contexto aparentemente adverso, ¡fuimos los primeros en Argentina en secuenciar un fragmento de DNA!

Convencidos y comprometidos en mejorar nuestra situación edilicia, durante más de 15 años, varios de nosotros, cada uno desempeñando diferentes roles, planificamos, gestionamos y trabajamos intensamente para conseguir un nuevo edificio; éste, en el CCT. Ninguna etapa fue fácil, pero la felicidad del éxito alcanzado fue tan enorme que compensó los esfuerzos realizados y las horas dedicadas a esa misión.

Una característica del IBR desde sus inicios fue la existencia de una forma diferente de gestión institucional. Si bien el IBR siempre tuvo un Director y un Vice-Director (requisitos de CONICET), fue pionero en incorporar un consejo directivo (el famoso CD-IBR) como organismo de gestión. Ese CD estaba conformado, además del director y el vice, por dos investigadores de cada una de las tres divisiones que conformaban el instituto en aquel momento: Microbiología, Biología Molecular y Biología del Desarrollo. Este modelo de gestión fue tomado y replicado en otras UE del CONICET.

Si bien estoy convencida que las contribuciones a las instituciones y a la sociedad en su conjunto son realizadas por individuos, independientemente de su género, hay una situación que no puedo soslayar. Dado que en la división Biología del Desarrollo (donde yo estaba) éramos sólo dos investigadores, fui la primera mujer en integrar el CD del IBR. Años más tarde, se constituyó la Fundación del IBR, organizadora de este evento. Luego de varias exitosas presidencias, fui la primera mujer designada por el Consejo Asesor para presidir la FIBR los años 2018 y 2019, puesto que dejé para asumir la Vice-Dirección del IBR, en plena pandemia. En la actualidad, tanto el

instituto como la fundación, cuentan con la presencia de figuras femeninas en la mayoría de sus órganos de gestión y toma de decisiones.

En estos momentos tampoco es fácil. Hacer ciencia de calidad en nuestro querido país es complejo y suele ser desalentador. Circunstancias y situaciones difíciles nos impactan cotidianamente, minuto a minuto. Sin embargo, es claro y evidente que la Comunidad IBR tiene la fortaleza necesaria para convertir grandes desafíos en grandes oportunidades. Hemos alcanzado grandes éxitos, juntos, cada uno en su lugar, haciendo lo que hay que hacer. No es fácil… pero, ¡estoy persuadida que con compañerismo, compromiso, motivación, creatividad e inspiración podremos seguir haciendo Ciencia de calidad en Argentina! En buena parte depende de nosotros.

Nora B. Calcaterra

PROGRAMA

8:30-9:00 -Espacio para colgar los pósters. Explanada CCT

9:00-10:00 - Bienvenida e introducción a la jornada - Salón IRICE

10:00-12:00 - Sesión de posters (con café-desayuno)

12:00-14:00 -Tiempo libre

14:00-17:00 - Charlas Temáticas (con café-merienda)- Salón IRICE

a) ***14:00-14:30*** *Bioquímica Estructural*

"La Biología Estructural/ Bioimagenología integrativa en el IBR" Dra. Natalia Lisa

b) ***14:30-15:00*** *Microbiología*

-14:30-14:45 "Mecanismos moleculares implicados en la oxidación de manganeso en *Pseudomonas* utilizadas en procesos de biofiltración de aguas de consumo." Dra. Natalia Gottig

-14:45-15:00 "Biología y Bioquímica de *Trypanosoma cruzi* durante el proceso de infección". Dra. Victoria Alonso

c) ***15:00-15:30*** *Ciencia aplicada/traslacional*

"De cómo desde la Academia se creó una EBT: La precuela de DETx MOL S.A." Dra. Adriana Giri

d) ***15:30-16:00*** *Biomedicina*

"Secuenciación de alto rendimiento: nuestra experiencia en el descubrimiento de nuevos virus" Dra. Elisa Bolatti

16:00-16:15 - Descanso

e) ***16:15-16:45*** *Biología vegetal*

"La historia de Flavodoxina: de cianobacteria a plantas superiores" Dr. Rocío Arce

f) ***16:45 -17:00*** *Unidad Técnica- CPAs*

"Unidad de Microscopía IBR y capacitación en nuevas técnicas" CPA. Rodrigo Vena.

17:00 a 18:00 - Homenaje a miembros del IBR que se han jubilado y han cesado su actividad en el Instituto

Estela Valle, Silvana Boggio, Mónica Arévalo.

18:00 - Festejo (música, baile y algo más…) a cargo de los Becarios del IBR. Explanada CCT

Resúmenes

- *El número de poster está indicado en el margen superior izquierdo de cada resumen*

- *El lugar para colocar los posters de cada grupo de investigación estará indicado en la sesión*

Role of oleic acid in alpha-synuclein aggregation in a *Caenorhabditis elegans* model of Parkinson disease.

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Parkinson disease (PD) is the second-most common neurodegenerative disorder, and is currently incurable. The progressive loss of dopaminergic neurons in the substantia nigra is accompanied by the aggregation of the protein alpha-synuclein (aSyn) into filamentous inclusions called Lewy bodies.
Numerous reports indicate that aSyn interacts with lipids and while it is generally accepted that the interaction is relevant for the protein's normal functioning, it has been shown that perturbations in lipid metabolism induce and/or accelerate disease progression. In particular, unsaturated fatty acids (UFAs) such as oleic acid (OA) increased aSyn inclusion formation and toxicity in yeast and mammalian cell models and the genetic or pharmacological inhibition of stearoyl-CoA-desaturase enzymes (SCD) reversed those features.
Here we use a *Caenorhabditis elegans* PD model to characterize the *in vivo* aggregation of aSyn under genetic backgrounds and environmental conditions that promote changes in UFA homeostasis. *C. elegans* strain overexpressing a YFP-aSyn construct formed aggregates that were visualized by fluorescence microscopy and these were absent in control YFP strains. To assess the effect of OA in aSyn aggregation, we used RNAi to decrease the levels of one of the enzymes responsible for its synthesis, the Fat-7 stearoyl-CoA-desaturase. NMR analysis of *C. elegans* lipid extracts showed modified lipid profiles consistent with Fat-7 inhibition. Interestingly, these animals showed a nearly complete absence of aggregates, supporting the role of UFAs in aSyn aggregation. Our work highlights the advantages of *C. elegans* to study *in vivo* the molecular basis of aSyn aggregation and the onset of PD.

Acknowledgements: we thank Enrique Morales and Rodrigo Vena for technical assistance. We thank M. Eugenia Goya for gently giving us C. elegans strains.

ZN(II) AFFINITY IS CRUCIAL IN THE EVOLUTION OF THE NDM CARBAPENAMASE

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The New Delhi metallo-β-lactamase (NDM) is one of the most prevalent carbapenemases worldwide present in Gram-negative organisms. NDM is a membrane-bound periplasmic Zn(II)-dependent enzyme that efficiently hydrolyzes all β-lactam antibiotics (βLA) (1). During an infection, the host immune response withholds metal ions from microbial pathogens by releasing metal-chelating proteins such as calprotectin. This impacts directly on the periplasmic Zn(II) levels. Under metal limitation conditions, NDM loses activity against β-lactams by dissociation of the Zn(II) cofactor, and is degraded in the periplasm by proteases (2).

There are 43 reported natural NDM variants that differ by a few mutations outside the active site. Our group reported that most of the first 16 variants show a better tolerance to Zn(II) starvation. Among the most frequent substitutions, M154L increases the affinity of the protein for Zn(II), and A233V stabilizes the apoenzyme (the non-metalated form) against the action of proteases (3).

In this work, we aim to assess the possible evolutionary paths of NDM to understand the forces driving its evolution. We evaluated the resistance phenotypes with minimum inhibitory concentrations (MICs) of the new NDM variants (NDM-17 to -42), and constructed laboratory variants to explore some possible evolutionary pathways. None of the variants increased resistance to the six different βLAs tested. However, most of the variants were more capable of conferring resistance than NDM-1 under Zn(II) limitation conditions. This suggests that NDM is evolving to endure the Zn(II) starvation imposed by the immune system during the infection, instead of incorporating mutations that enhance the catalytic efficiency.

Among the laboratory variants tested, the ones with substitution E152K in combination with the more common substitutions (M154L and A233V) show a remarkably high stability upon Zn(II) starvation. Given this, we focused our analysis on the molecular characterization of laboratory variants NDM-EA (E152K A233V), NDM-EM (E152K M154L) and NDM-EMA (E152K M154L A233V). We proved that the E152K substitution enhances the resistance at low Zn(II) levels of these variants by increasing the *in vivo* stability of the apo form. We performed Zn(II) affinity measurements in spheroplasts that reveal that E152K impairs the Zn(II) affinity in combination with the M154L and A233V substitutions. The lower frequency of the substitution E152K compared to A233V could be attributed to its deleterious impact on the metal affinity.

Overall, our results suggest that NDM accumulates substitutions in order to maintain the resistance in Zn(II) limitation conditions. This is achieved either by *in vivo* stabilization of the apoenzyme or by an increase in the Zn(II) affinity with respect to NDM-1.

Acknowledgements: ANPCyT, NIH, CONICET
References:
1.Bahr, G. *et al*. (2021) Chem Rev 14;121(13):7957-8094.
2.González, L., Bahr, G., Nakashige, T. *et al.* (2016) *Nature Chem Biol* 12, 516–522
3.Bahr, G. *et al*. (2018) Antimicrob Agents Chemother 62:e01849-17.

ACCUMULATION OF A MISFOLDED PROTEIN IN CHLOROPLASTS TRIGGERS AN ORGANELLE-SPECIFIC RESPONSE

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A quality control system of heat shock proteins, chaperones, and proteases ensures that proteins entering the chloroplast are properly folded. Yet, the system may be overloaded in stressful situations and can get overwhelmed by unfolded proteins. In this case, a genetic program called the unfolded protein response (UPR) is unleashed and consists of increased levels of the components of the quality control network. In a laboratory setting, the UPR can be triggered by chemicals, but it is suspected that the molecular signature of the chemically induced UPR is different from other more physiological stresses. In this work, we used aggregation-prone versions of ferredoxin NADP+ reductase (Taq3-FNR and ΔC-FNR) that show a marked reduction in solubility. Genetic constructs containing the Taq3-FNR, ΔC-FNR, or wild-type FNR coding sequences were used to transform *Nicotiana benthamiana* and *Arabidopsis thaliana*. We confirmed that Taq3-FNR protein accumulates in chloroplasts at a lower concentration than WT-FNR, suggesting increased turnover. The levels of the chloroplastic ClpB3 disaggregase were used as a marker for the UPR. Leaves overexpressing Taq3-FNR and ΔC-FNR showed a significant accumulation of ClpB3. We then analyzed alterations in the leaf proteome by proteomics using a Q-Exactive HF mass spectrometer. Data were analyzed by MaxQuant/Perseus and Proteome Discoverer. We then performed network and GO enrichment analysis using String and Cytoscape. We found that, in the presence of Taq3 and ΔC-FNR, the plant cell responds by activating the expression of molecular chaperones (such as ClpB3), proteases (FtzH), and proteins involved in retrograde signaling to the nucleus (GUN5). Our results suggest that Taq3-FNR and ΔC-FNR generate a specific response in chloroplasts, due to their presence in the stroma. Also, since most proteins with elevated levels during the UPR are nuclear-encoded, the response should involve chloroplasts-to-nucleus communication, a common feature in other UPRs.

"Rol de las proteínas que contienen un dominio CCT en la productividad vegetal"

D. Renzi, M. L. Sossi, M. I. Zanor

Para aumentar la productividad de los cultivos es importante identificar correctamente los mecanismos moleculares que limitan dicha productividad. El grupo de genes que codifican proteínas con un motivo conservado denominado CCT (CONSTANS, CONSTANS like (CO-like) y Timing of CAB expression1) participan en la regulación de la floración y el ritmo circadiano. Las proteínas CCT se pueden dividir en tres grupos, el grupo CMF que tiene un solo dominio CCT, y el grupo CO-like y PRR que además del dominio CCT tienen una B-Box o un dominio regulador de pseudorespuesta, respectivamente. El progreso reciente en la caracterización de algunas proteínas CCT mostró que regulan no solo el crecimiento y desarrollo de las plantas, sino también la tolerancia al estrés. Los resultados obtenidos hasta la fecha sugieren que los genes que codifican proteínas con un solo dominio CCT funcionan integrando señales ambientales dinámicas con transiciones de desarrollo, provocando cambios morfológicos y respuestas de estrés que maximizan el éxito reproductivo y la tolerancia a entornos desfavorables. Nuestro grupo ha caracterizado un gen de tipo CMF llamado FITNESS, que controla la productividad en Arabidopsis, la tolerancia al estrés ambiental y los patógenos. Existe información limitada sobre la función de los genes CMF en la familia Solanaceae y especialmente en tomate. En tomate existen 7 genes que pertenecen al grupo CMF y ninguno de ellos ha sido estudiado hasta el momento. El objetivo de este trabajo es identificar y caracterizar el gen ortólogo de FITNESS en tomate (SlFITNESS), y analizar la productividad y la respuesta al estrés en plantas de tomate con niveles disminuidos del transcripto.

P5

Understanding molecular mechanisms of action of PthA4^{AT}, a small TAL effector of *Xanthomonas citri* subsp. *citri* for the identification of citrus host resistance genes

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Transcription-activator-like effectors (TALE) are secreted by Xanthomonas, acting as eukaryotic transcription factors that can target both susceptibility or resistant genes in the plant cells. They recognize specific DNA sequences in the host promoters through a domain consisting of a variable number of ~34 amino acid repeats following a well characterized code. The main virulence factor of *Xanthomonas citri* subsp. *citri* (*X. citri*), the bacteria causing citrus canker, is PthA4, a 17.5-repeats TAL effector activating susceptibility genes in *Citrus*. Recently, a natural variant of *X. citri* triggering a hypersensitive response (HR) in *C. limon* and *C. sinensis* was isolated, and the effector triggering this HR was shown to be a 7.5- repeat PthA4-derivative TALE (PthA4^{AT}), likely activating a resistance gene on these citrus hosts. To further refine PthA4^{AT} targets in the plant cell as an initial step towards the identification of the *C. sinensis* resistance gene responsible for this HR, a library of artificial PthA4^{AT} TALEs were built. Introduction of PthA4^{AT} and specific artificial variants into the reference strain *X. citri* 306 abolished its ability to cause canker symptoms in *C. sinensis*. Notwithstanding, other artificial variants of PthA4^{AT} promotes canker disease. Using a combination of synthetic biology, phenotypic analyses and molecular markers of HR and canker disease, a couple of artificial PthA4^{AT} TALEs were selected. to proceed to the next step. This knowledge will help to rationally exploit the plant immune system with a biotechnological approach to manage citrus canker.

REGULATION OF LIPID BIOSYNTHESIS PRECURSORS AND CELL DIVISION IN MICOBACTERIA: PHYSIOLOGICAL ROLE OF MAF PROTEIN

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Considering infectious diseases, tuberculosis (TB) is one of the most significant causes of dead in the world. The bacterium *Mycobacterium tuberculosis* (*Mtb*) is the etiological agent of TB. Actually, this disease has been reestablished since the breach of extensive antibiotics treatments, the continuous appearance of multi-resistant (MDR) and extremally resistant strains. Also, the HIV and SARS-CoV2 pandemics, and the deterioration of public health systems in undeveloped countries have been contributing to TB propagation. Consequently, is necessary the identification of new drugs targets and de development of novel compounds efficient against MDR strains, with the aim of complement conventional therapies.

Mycobacteria possess a cellular wall unusually rich in lipids, and some of them are essential for bacteria viability and the pathogenicity of this microorganisms. The main components of this cellular envelop are mycolic acids, and acids and alcohols methyl-branched. Therefore, the biosynthetic pathways that generate these compounds offer an attractive target for the development of new antimycobacterial agents.

These complex lipids, which conform the cell wall are synthetized by enzymes previously characterized; however, very few information is disponible about the enzymes that provide precursors for *the novo* fatty acid biosynthesis and the production of meromycolic acids. In *Mtb*, the enzymatic complexes acyl-CoA carboxylases (AcCCase) generate malonyl-, methyl-malonyl-CoA, and carboxylated long-chain acyl-CoA as substrates for the biosynthetic vias. In our laboratory, three different *Mtb* AcCCase complexes were characterized at biochemical, genetic and structural level. However, less is known about the modulation of this activity. Recently, we identified the Rv3282 gene, which codified for a protein denominated Maf, found adjacent to a cluster of genes involved in the production of a AcCCase subunits. Maf protein has sequence similarity to inhibitors of septum formation, but the function of *Mtb* Maf is unknown. In this work we overexpress Maf protein in *E. coli*, and then was purified using Ni-affinity columns. The activity of one *Mtb* AcCC complex was reconstructed *in vitro*, and the presence of Maf protein resulted in a dramatic increase of the activity. Nevertheless, the AcCCase activation due to Maf was not maintained during the aging of the sample, suggesting an instability of the protein or the necessity of a co-factor. Actually, a deletion mutant strains on *maf* gene is under construction, in order to physiologically characterize the role of this protein. Also, by using pull-down assays, we started to perform interaction studies, between Maf and the different subunits of the AcCCase complex. The information of these studies provides new insights into the regulatory mechanism for the biosynthesis of lipids in Mtb and open the opportunity to identified molecules that could work as antimycobacterial compounds.

P7

CONTROL OF BIOFILM FORMATION BY A *SALMONELLA*-SPECIFIC TRANSCRIPTION FACTOR UNDER ENVIRONMENTAL CONDITIONS

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Salmonellosis is a food-borne disease caused by *Salmonella*, that include illness ranging from gastroenteritis to enteric/typhoid fever, with millions of cases occurring worldwide every year. One of the key aspects of *Salmonella* life cycle that contributes to its high prevalence is its ability to persist in the environment and to form biofilms in abiotic surfaces and in the host tissues. This multicellular behavior allows the pathogen to survive hostile environmental conditions, and confers resistance to both host defenses and antimicrobial agents. This lifestyle's change, from motile to sessile cells attached to diverse solid surfaces, implies a drastic metabolic modification, and depends on the master transcriptional regulator CsgD. This regulator activates the production of the two major components of extracellular matrix in *Salmonella*, the curli fiber and the exopolysaccharide cellulose, but also other extracellular components important for biofilm development, such as colanic acid and the large cell-surface protein BapA. Because of its homology to a well-known *csgD* transcriptional activator, we analyzed the role of BioR, a *Salmonella*-specific transcription factor, in biofilm formation. Using the Congo Red dye to evaluate biofilm production in solid media, and the Cristal Violet dye to quantify the extracellular matrix production in liquid media, we determined that this factor is required for *Salmonella* adhesion and biofilms formation. To gain insight into the optimal conditions of BioR expression, we use *lacZ* and *gfp* transcriptional fusions to its natural promoter, as well as specific antibodies. We found that this regulator is maximally expressed when *Salmonella* grows at low temperatures and in minimal media. Also, we searched for genes that were differentially expressed in the presence or absence of BioR, analyzing changes in the bacterial transcriptome through an RNA-Seq approach. We uncover that BioR controls the expression of genes encoding several matrix components, as well as enzymes that take part of diverse metabolic processes. In conclusion, we identified a *Salmonella*-specific regulator that modulates biofilm formation at low temperatures and under nutrient limitations, favoring the of pathogen's persistence under extreme conditions in non-host environments.

INTRA-MOLECULAR INVERSIONS OF A *bla*$_{OXA-58}$ AND Tn*aphA6*-CONTAINING ADAPTIVE MODULE CONFERRING CARBAPENEM- AND AMINOGLYCOSIDE-RESISTANCE MEDIATED BY PAIRS OF pXERC/D SITES IN *Acinetobacter baumannii* PLASMIDS

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Acinetobacter baumannii is an important opportunistic bacterial pathogen responsible of a variety of infections in healthcare institutions. It can rapidly evolve multi-drug resistance (MDR), and in this context, resistance to last-resort carbapenems (carb^R) represents a major concern worldwide. The most frequent cause of carb^R in *A. baumannii* are acquired *C*arbapenem-*H*ydrolyzing class *D* β-*L*actamases (CHDL, OXA-type), with the respective *bla*$_{OXA}$ genes carried by plasmids.
We characterized a number of epidemiologically related MDR *A. baumannii* strains of the clonal complex CC15 predominant in our geographical region. The carb^R strains of this collection (Ab242 and Ab825) housed different iteron plasmids, and in both strains, we found a bi-replicon carrying a *bla*$_{OXA-58}$- and Tn*aphA6*-containing adaptive module conferring carbapenem and amikacin resistance. Notably, this resistance module is bordered by several 28-bp sequences potentially recognized by the XerC and XerD tyrosine recombinases of their hosts (designated pXerC/D-like sites), suggesting functions of this site-specific recombination (SSR) system in their horizontal mobilization. Sequence analysis of the genetic context of this resistance module in Ab242 and Ab825 plasmids indicated an inverted orientation between them. In addition, transformation of sensitive *Acinetobacter* strains allowed us to identify that some pXerC/D-like sites located in different plasmids from Ab242 could constitute recombinationally active pairs, mediating the fusion as well as the resolution of plasmid co-integrates in this strain[1]. Since we identified 17 pXerC/D-like sites in Ab242 and Ab825 plasmids, we analyzed whether some of these sites were active in SSR and how they mediate mobilization of the modules they encompass.
We used a combination of methodologies that included transformation of susceptible *Acinetobacter* strains with total plasmids obtained from Ab242 and Ab825, and the characterization of the plasmids recovered from the carb^R transformants using specific primers to detect any pXerC/D hybrid sites by PCR. We identified that sites 2 and 7 were involved in the reversible intra-molecular inversion of the resistance module and sites 7 and 9 mediated the reversible formation of plasmid co-integrates in both strains. Thus, these results provide first empirical evidences that some of the pXerC/D-like sites present in both Ab242 and Ab825 plasmids could actually conform recombinationally-active pairs. The dynamic state of plasmid architectures resulting from both intra- and inter-molecular recombination mediated by different pXerC/D active pairs supports our previous proposals[1,2] of their contribution to the evolution of *Acinetobacter* plasmids, allowing an efficient spread of carbapenem resistance.

1. Cameranesi *et al.* 2018 Front Microbiol 9:66. doi:10.3389/fmicb.2018.00066.
2. Cameranesi *et al*. 2020 Microb Genom 6(9):mgen000360. doi:10.1099/mgen.0.000360.

Tobacco necrosis virus A overcomes local cell death response in *Nicotiana tabacum*

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Tobacco necrosis virus strain A (TNVA) belongs to the genus *Alphanecrovirus* in the *Tombusviridae* family. Symptoms induced by TNVA include necrotic lesions on inoculated leaves in a wide host range. The necrotic cell-death response to certain viruses is poorly understood. This study characterizes the mechanisms underlying the local cell death triggered by TNVA in *Nicotiana tabacum*, showing that it shares several components with the hypersensitive response (HR) mediated by resistance proteins. TNVA perception triggers the accumulation of local viral-derived small interference RNA and the regulation of biological processes related to pathogen associated molecular patterns (PAMPs)-triggered immunity (PTI), including hydrogen peroxide accumulation, cell wall reinforcement, the activation of unfolded protein response and the induction of SA and SA-dependent pathways. However, these antiviral defences do not prevent either local virus multiplication or systemic movement, leading to disease development. Moreover, SA-deficient *NahG* tobacco plants challenged with TNVA showed an increase in virus accumulation on non-inoculated leaves, which was correlated with the development of systemic necrosis, highlighting the role of SA signalling in TNVA-induced defence. On the other hand, SA treatment enhances local defence response to TNVA infection and suppresses systemic necrosis in *N. benthamiana.* Taken together, our data suggest that TNVA induces an impaired plant defence response by modulating host factors to persist at low levels in distal tissues.

TCBDF5, UN FACTOR BROMODOMIO ESENCIAL DE TRYPANOSOMA CRUZI

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Entre las ocho proteínas bromodominios presentes en el genoma de T. cruzi, TcBDF5 es la única que tiene dos bromodominios, además de una copia del dominio MRG, el cual está definido como un módulo de interacción proteína-proteína. Esta proteína se considera esencial para el desarrollo de Leishmania mexicana; y tanto en este organismo como en Trypanosoma brucei, BDF5 se ha encontrado localizado en amplias áreas del genoma relacionadas con la iniciación de la transcripción asociada con muchas otras proteínas coligadas con la transcripción, así como la replicación y reparación del ADN. Usando la técnica CRISPR/Cas9, intentamos eliminar el gen tcbdf5. Después de tres eventos de transformación y selección independientes, solo pudimos obtener parásitos hemicigotos, con una copia funcional del gen y la otra eliminada. Estos resultados sugieren fuertemente que TcBDF5 es esencial para T. cruzi. Los epimastigotes obtenidos (Dm28c::tcbdf5+/-) mostraron un aumento en el tiempo de duplicación, en comparación con la cepa de tipo silvestre. Para entender mejor su función, diferentes versiones de la proteína fueron sobre expresadas en una forma inducible por el plásmido pTcINDEX. La expresión de la proteína de tipo salvaje inhibió drásticamente el crecimiento de los parásitos, al igual que la expresión de una versión mutante en un residuo esencial para la función del dominio MRG (R623A). En contraste, los mutantes en la asparagina considerada esencial para la función de bromodominio (N91A, N300A) afectaron ligeramente el crecimiento. Estos resultados sugieren que tener el dominio MRG podría ser más esencial para la función del TcBDF5 que el propio bromodominio. Curiosamente, las proteínas con la mutación N91A mostraron niveles más bajos de expresión, lo que sugiere que podría estar desestabilizando la estructura del dominio y facilitando su degradación.

HYPONASTIC LEAVES 1 IS REQUIRED FOR PROPER ESTABLISHMENT OF AUXIN GRADIENT IN APICAL HOOKS OF ARABIDOPSIS

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Seedlings germinating under the soil surface have evolved to an exquisite developmental program termed skotomorphogenesis. In darkness, seedlings rapidly elongate the hypocotyl toward the surface in a desperate search of light, while protecting the apical meristem against mechanical damage by forming a hook between the hypocotyl and the two closed cotyledons. A proper skotomorphogenic growth must be achieved until seedlings reach the light to ensure survival as they depend on limited seed reserves. In our previous work we uncovered that microRNA biogenesis is necessary for proper skotomorphogenesis in Arabidopsis. By studying mutants in the core components of miRNA microprocessor, DICER LIKE 1 (DCL1), HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE), we surprisingly found a microprocessor-independent function of HYL1 as a repressor of hook development. Our findings have led to hypothesized a possible connection between HYL1 action in skotomorphogenesis and the well-established and crucial function of the phytohormones auxins in hook development. In this work we dissect different aspects of auxin biology in HYL1 mutants (auxin sensitivity, transcriptional responses, biosynthesis and transport) and found that HYL1 is needed to establish the auxin gradient in apical hooks. Our research led us to propose that HYL1 might integrate light/dark and auxin signals to control skotomorphogenic growth in Arabidopsis.

P12

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Cholesterol is an essential metabolite present in virtually all eukaryotic organisms. It mediates highly relevant biological processes such as the regulation of membrane fluidity and the synthesis of steroid hormones and bile acids. Therefore, proper cholesterol trafficking to different subcellular locations is crucial for cell viability and proper organism functioning. Non-vesicular cholesterol transport is mediated by a multi-domain membrane protein called STARD3 that binds cholesterol through its cytosolic domain (START). Recent studies have reported that STARD3 co-localizes and interacts with methionine sulfoxide reductase A, an enzyme that reduces methionine sulfoxide side-chains, suggesting that methionine oxidation could modulate the sterol binding properties of the START domain of STARD3 and hence cholesterol transport. Our goal is to understand the regulation of non-vesicular cholesterol transport employing the nematode *Caenorhabditis elegans* as a model organism. By applying a set of biophysical and structural biology methodologies, we have characterized the cholesterol-binding properties of the START domain of *C. elegans* STARD3 and solved its crystal structure in the absence and presence of cholesterol, providing a high-resolution picture of a cholesterol-START complex for the first time. We propose that a conserved Met residue is involved in lipid binding. Further studies aimed to characterize the interaction between STARD3 and MSRA *in vitro* and *in vivo* will shed light on the possible role of this Met residue as a redox regulatory switch for cholesterol trafficking.

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The IS6770 insertion sequence modifies the promoter region structure of the gene *kup* encoding for the potassium symporter in *Enterococcus faecalis* JH2-2.

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Enterococcus faecalis is a ubiquitous colonizer of the gastrointestinal tract of animals, however, this microorganism evolved in the last decade from an avirulent commensal to nosocomial multiresistant bacteria. The intrinsic robustness of *E. faecalis*, which allows it to tolerate different environmental stress conditions, is directly related to the pathogenic potential of this bacterium. In our laboratory demonstrated that Kup and KimA proteins, two members of the K^+ transporter Kup family, catalyze the uptake of K^+ inside the cells and are involved in the response to osmotic stress in *E. faecalis*. These genes are located in a chromosomal region of 16 kbp, including the *opuABCD* operon, which encodes for putative osmolyte transporters, and two putative cation transporters. Insertion Sequences (IS), which codify for transposases, are mediators of genetic diversity in prokaryotes. They could induce rearrangements including deletions, duplications, inversion and activation of genes. The IS6770 is composed of a unique Orf that codifies for a putative transposase (member of the IS30 element) and 30 nucleotides of the imperfect inverted repeat. Even though, IS6770 was identified in *E. faecalis* strain associated to clinical isolated, in the vancomycin resistant strain V583 is absent. In the laboratory strain JH2-2 we detected 12 copies of the IS6770, two of them located downstream of both the *kup* and the *kimA* gene forming a genetic structure that constitutes a putative composite transposon. The presence of the IS6770, located 79 bp upstream of the *kup* gene initiation, modified the structure of the promotor region. To analyse whether the presence of IS6770 in the JH2-2 strain modified the transcriptional expression of the *kup* gene, transcriptional fusions of *kup* upstream regions of different sizes with a fluorescent reporter gene were performed, and then compared with transcriptional fusions of the promoter region of the V583 strain (IS6770 deficient strain). It was observed that while the promoter region amplified from the V583 strain allowed the expression of the fluorescent protein, the upstream region amplified from JH2-2, which includes part of the IS6770, showed no activity. However, the region containing part of the *kup* promoting region and a full copy of IS6770 from JH2-2 did show activity. Thus, these experiments suggest that the promoter region required for *kup* gene expression was either displaced by the IS6770 insertion or replaced by an internal promoter region located in the IS. Regarding the study of the influence of pH stress on the transcriptional activity of the *kup* promoter, increased expression of the fluorescent protein mCherry for PkupL, and of GFP for PkupV19 was detected in culture medium at pH 5. However, when

assaying the response of *kup* gene expression to acid stress, no increase in fluorescence levels was seen as the external pH decreased.

Genetic polymorphisms in G-Quadruplex affect transcription of human disease-related genes

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Keywords: G-Quadruplex, promoter, SNPs, diseases.

G-quadruplexes (G4) are nucleic acid secondary structures that can be transiently folded within proximal promoter regions (PPRs) during transcription when single-stranded G-rich regions are exposed. The work goal is to identify single nucleotide polymorphisms (SNPs) associated with human diseases, located in PPRs (here -1000 pb from transcription start site) and may affect the folding of putative G4 (PG4), hereafter called SNP-PG4. First, we performed a bioinformatic analysis by downloading flanking sequences (+/- 50 bp) of each SNP located within the PPR and associated with human diseases from COSMIC, ClinVar, dbSNP and HGMD databases. Nearly 3% of the 427479 sequences with at least two variants contained PG4s. To identify those SNPs most affecting G4 folding we selected sequences containing one PG4 in either the reference or variant versions; i.e., SNPs that disrupt or promote G4-folding. Then we used a G4 folding predictor (Quadron - 1) to choose those PG4s with higher scores. SNP-PG4 within PPRs of 5 genes were further analyzed. Spectroscopic analyses by Circular Dichroism (CD) demonstrated that selected PG4 sequences fold *in vitro* as G4 and that SNPs cause quantitative spectral changes. Moreover, ^{1}H NMR spectroscopy confirmed the formation of G4s identified for all cases and SNP´s induction to quantitative and qualitative changes. qPCR stop assays and CD melting indicated that SNPs induce G4 stability changes. Finally, PG4s cloning into pGL3-promoter vector revealed that firefly luciferase reporter activity was altered by SNPs when transfected into HEK-293 cells. Results gathered in this work suggest that SNPs in the PPRs of these genes may alter G4 folding thus modifying transcriptional activity. In view of this, SNP-PG4s should be considered as a novel molecular etiology mechanism for the predisposition or establishment of human diseases, as well as potential targets for chemotherapeutic treatments.

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Unveiling the mechanism of activation of the VraSRT system of *Staphylococcus aureus* by vancomycin and peptidoglycan fragments

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Abstract:

Staphylococcus aureus is the leading cause of nosocomial and community-acquired infections. The VraSRT system acts as a sentinel that can rapidly sense cell wall peptidoglycan damage and coordinate a response that leads to resistance to β-lactam and glycopeptide antibiotics. VraS is a membrane histidin-kinase and VraR a cytoplasmatic response regulator. However, the role of VraT, another membrane protein, is yet unknown but essential for manifestation of resistance. We still do not understand how VraS is activated in response to cell wall-active antibiotics. To evaluate whether the system is activated by direct interaction of glycopeptides or of cell-wall derived fragments with either VraS or VraT, a vancomycin-derived photoprobe (VPP) and peptidoglycan fragments were used.

Using a *S. aureus* reporter strain, which has a shuttle vector that allows expression of GFP under the control of the VraSRT operator region, we corroborated that β-lactam and glycopeptide antibiotics activate the VraSRT system. Peptidoglycan from *S. aureus* ATCC29213 was obtained and subjected to cleavage with Mutanolysin, Lysostaphin or both enzymes to generate different peptidoglycan fragments. Working with the reporter strain, we demonstrated that theses fragments do not activate the system.

A vancomycin-derived photoprobe (VPP), with a benzophenone photoaffinity label and a biotin linker, was used to evaluate if there was direct interaction of the antibiotic with VraS and/or VraT. VPP activated the VraSRT system and we evidenced formation of a VraS-VPP adduct by Western blot. MS/MS analysis of the purified VraS and VraS-VPP complex did not allow identification of the site of crosslinking.

In conclusion, the VraSRT system was not activated by peptidoglycan fragments. VraS interacted directly with vancomycin, but the exact site of interaction could not be determined. VraT participation in activation of the system is not as a receptor of the antibiotic.

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ANÁLISIS DEL EFECTO DEL ESTRÉS OXIDATIVO DURANTE EL PLEGAMIENTO DEL TUBO CARDÍACO

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El pez cebra (*Danio rerio*) es un pequeño vertebrado utilizado como modelo para el estudio del desarrollo del corazón y enfermedades cardiovasculares (1). El plegamiento del tubo cardíaco es un proceso morfogenético conservado y anormalidades en dicho proceso están asociadas a defectos cardíacos congénitos en humanos (2). Un aumento en los niveles de las especies reactivas del oxígeno promueve la activación constitutiva de la quinasa II dependiente de Ca2+ y calmodulina (CaMKII), evento asociado con varias enfermedades cardíacas (3). Dicha activación involucra la oxidación de la enzima, siendo esta modificación postraduccional reversible vía la enzima Metionina Sulfóxido Reductasa A (MSRA) (4). En el presente trabajo nos proponemos analizar cómo la exposición a condiciones de estrés oxidativo afecta el proceso de plegamiento del tubo cardíaco, y si dicho estrés modula la expresión e interacción regulatoria de Camk2 y Msra en el tejido cardíaco. Para analizar el efecto del estrés oxidativo sobre la morfogénesis del corazón utilizamos una línea reportera de pez cebra [Tg(myl7:eGFP)^{twu34}](5), que expresa la proteína GFP en el tejido cardíaco, y observamos que el tratamiento oxidativo interfiere con el plegamiento del tubo cardíaco de manera dosis-dependiente. Realizamos un análisis *in sílico* de los genes camk2 y msra del pez cebra y encontramos siete genes para camk2 (a, b1, b2, d1, d2, g1, g2), y un gen para msra. Para analizar los niveles de expresión de dichos genes diseñamos oligos específicos y realizamos ensayos cuantitativos de la reacción en cadena de la polimerasa (qPCR) durante el plegamiento del tubo cardíaco, observando un aumento de 30 veces para el transcripto camk2d2 al final de dicho proceso. En conjunto, estos hallazgos contribuyen a comprender el papel de la regulación oxidativa durante el desarrollo cardíaco normal y anormal.

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LA INHIBICIÓN DE FACTORES DE TRANSCRIPCIÓN TCP POR MICROARN319 ES NECESARIA PARA EL CRECIMIENTO NORMAL DE RAÍCES DE ARABIDOPSIS

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El microARN miR319, conservado en la evolución de las angiospermas, regula genes codificantes para factores de transcripción de la familia TCP. El módulo miR319-TCP controla la proliferación y diferenciación celular en hojas y otros órganos del vástago. El modelo funcional actual sugiere que el microARN modula cuantitativamente a los *TCP*s durante el crecimiento y desarrollo de la hoja, determinando su tamaño final. Este trabajo estudia el rol del módulo en el desarrollo de la raíz. Al impedir la interacción con miR319, encontramos que el aumento de los TCP resulta en meristemas radiculares y raíces más cortos. Mediante análisis a nivel celular y molecular, mostramos que un alto nivel de actividad TCP altera el número de células y la expresión de ciclinas, pero no el largo final de las células, indicando que, en la raíz, suprimir la represión de los *TCP*s por miR319 afecta significativamente la proliferación celular. Por el contrario, las mutantes múltiples *tcp* no mostraron ningún defecto en el crecimiento de la raíz pero sí alteraciones fuertes en la morfogénesis de las hojas. Por lo tanto, en contraste con el mecanismo de acción cuantitativo que este módulo ejerce en hojas, nuestros resultados proponen que miR319 elimina los transcriptos *TCP* de las células de raíz. Presentamos, entonces, nuevos conocimientos sobre las funciones del sistema regulatorio miR319-TCP en el desarrollo de Arabidopsis, distinguiendo el *modus operandi* en la raíz de aquél observado en el vástago.

OXIDACIÓN Y FOSFORILACIÓN DE ALFA-SINUCLEÍNA, LA INTERACCIÓN ENTRE DOS MODIFICACIONES POST-TRADUCCIONALES ASOCIADAS A LA ENFERMEDAD DE PARKINSON

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Introducción: Uno de los eventos patológicos característicos de la enfermedad de Parkinson (EP) es la acumulación de la proteína alfa-sinucleína (AS) agregada en inclusiones intraneuronales denominadas cuerpos de Lewy (LB). Varias modificaciones post-traduccionales (MPTs) de AS, incluyendo la oxidación de metioninas y la fosforilación de serinas, han sido correlacionadas con su agregación. AS posee cuatro metioninas, dos localizadas en el extremo N-terminal y dos en el C-terminal. La oxidación de estos residuos da lugar a una mezcla de dos isómeros de sulfóxidos de metionina (MetOx), denominados R y S. Estos MetOx son reducidos por una familia de enzimas enantioselectivas denominadas Metionina Sulfóxido Reductasas (MSR). Estudios realizados por nuestro grupo han demostrado que mientras los MetOx de la región N-terminal son reducidos eficientemente, los de la región C-terminal permanecen oxidados, desconociéndose los motivos por los que esto ocurre. En cuanto a la fosforilación del residuo de serina 129 (S129), estudios previos han demostrado que, cuando AS se encuentra agregada, un 90% de las moléculas poseen el residuo de S129 fosforilado, mientras que, cuando la proteína se encuentra soluble, solo el 4% posee dicha modificación.

Objetivos: El objetivo del este proyecto es caracterizar los mecanismos de óxido-reducción de AS catalizada por las MSR *in vitro* y la interacción entre los estados de oxidación y fosforilación de AS. Utilizamos RMN de alta resolución para analizar el estado de oxidación y fosforilación de AS simultáneamente y delinear las cinéticas de dichos procesos.

Resultados: La cinética de reducción de AS reveló diferencias en la especificidad de sustrato entre distintas MSRs y confirmó que los MetOx C-terminales no eran reconocidos por las enzimas. Experimentos complementarios realizados con beta-sinucleína, un homólogo de AS, sugieren que estos MetOx no se reducen debido al impedimento estérico introducido por prolinas adyacentes. La cinética de desfosforilación de AS reducida y oxidada mostró que la velocidad de desfosforilación es más lenta en el segundo caso. Esto sugiere que, la desfosforilación de S129 está comprometida por la presencia de sulfóxidos vecinos, M116 y/o M127. En base a esto, sugerimos que la acumulación de los MetOx de la región C-terminal interfiere con la desfosforilación favoreciendo el depósito de la especie fosforilada de AS en los LB.

Tc*BDF6: una proteína imprescindible para la infectividad de *T.cruzi

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La acetilación de proteínas en residuos de lisinas, es una modificación postraduccional dinámica, que regula diversas funciones de las proteínas. El único dominio conocido capaz de reconocer lisinas acetiladas es el bromodominio (BD), que funciona como un andamio para el ensamblaje de complejos macromoleculares. En *Trypanosoma cruzi*, existen 8 secuencias codificantes con BD en *T. cruzi*: *Tc*BDF1-8. Debido a que sus secuencias son muy divergentes y no pueden ser relacionados filogenéticamente con los BDs de mamíferos, podrían encontrarse inhibidores selectivos con potencial actividad tripanocida. Como objetivo de este trabajo nos propusimos generar mutantes de *Tc*BDF6 y evaluar su morfología, replicación e infectividad. Para ello, el gen codificante para *Tc*BDF6 fue interrumpido mediante la técnica de CRISPR-Cas9. Luego de varias rondas de selección, se seleccionaron mediante clonado, líneas mutantes heterocigotas (Dm28cBDF6$^{-/+}$) y homocigotas (Dm28cBDF6$^{-/-}$), lo que indica que se trata de una proteína no esencial para epimastigotes. Sin embargo, las líneas mutantes mostraron diferencias en cuanto al crecimiento y a la morfología de los epimastigotes, en comparación con los parásitos "wild type". Ambas líneas produjeron tripomastigotes metacíclicos (TM) *in vitro.* Cuando se infectaron células Vero con TM Dm28cBDF6$^{-/+}$, se produjo un atraso en el desarrollo de amastigotes y en la liberación de tripomastigotes, la cual comenzó el día 20 post-infección (en la cepa Dm28c ocurrió a los 7 días). Los tripomastigotes liberados mostraron diferencias morfológicas, pero infectaron células Vero de forma normal, liberando nuevamente tripomastigotes al día 7 post-infección. Por el contrario, los TM Dm28cBDF6$^{-/-}$ fueron completamente incapaces de infectar células Vero. Estos resultados sugieren que *Tc*BDF6 participa, en la infectividad y replicación intracelular del parásito, sugiriendo que podría llegar a ser un interesante blanco terapéutico para el tratamiento de la enfermedad de Chagas.

DISEÑO Y PRODUCCIÓN DE PARTÍCULAS SIMILARES A VIRUS (VLP) DEL VIRUS ZIKA.

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Las partículas similares a virus (VLP) son viriones vacíos. Es decir, son partículas definidas por las cubiertas de un virus, pero sin contener el genoma viral. Por lo tanto, están constituidos por una cápside, una envoltura viral o ambas estructuras combinadas. Una definición más amplia abarca cualquier tipo de nanopartícula derivada de las cubiertas de un virus. Las VLP se ensamblan a partir de las proteínas estructurales virales dentro de células hospedadoras apropiadas. Debido a la falta de genomas virales, estas partículas no se replican, pero retienen el potencial de infectividad de los virus parentales, por lo que pueden usarse como sistemas de nanopartículas para entrega de fármacos o vacunas, como también para estudiar procesos inmunológicos o virales. En nuestro laboratorio, nos hemos abocado al desarrollo de VLP del virus Zika (ZIKV), un Flavivirus, que poseen envoltura y cápside. La envoltura está conformada por fosfolípidos de la célula hospedadora y dos proteínas codificadas por el genoma viral, cuyas formas maduras se denominan M y E. Es importante destacar que las señales responsables de la interacción del virus con la maquinaria secretora celular se localizan en prM (preproteína de M) y E, por lo que la expresión de sólo estas proteínas es suficiente para que puedan formarse y secretarse partículas virales con propiedades estructurales y antigénicas similares a los viriones. Específicamente, diseñamos y desarrollamos una construcción compuesta de pcDNA 3 (un vector de expresión en células eucariotas) con las secuencias que codifican para prM y E de ZIKV. Luego transfectamos células eucariotas HEK-293 con dicho constructo para expresar tales proteínas virales. Pudimos detectar mediante técnicas inmunoquímicas y de imunofluorescencia, usando un anticuerpo anti proteína E comercial, la expresión de dicha proteína. Estos resultados nos sugieren la factibilidad de la producción y liberación de las VLP de ZIKV, lo que nos alienta a su aplicación en nuestros estudios sobre el ciclo de replicación de este virus.

Long-range quenching of C-terminus flexibility stabilizes carbapenemase NDM *in vivo*

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The New Delhi metallo-β-lactamase (NDM) is a periplasmic Zn(II)-dependent carbapenemase. To date, it distributes worldwide and no clinical inhibitors are available, making NDM a bigger problem [1]. During an infection, the innate immune response from the host withholds essential metal ions affecting bacterial growth. As a result, the periplasmic Zn(II) levels decrease and NDM accumulates as apo-form (without Zn(II) in the active site) [1,2]. Apo-NDM is inactive and becomes unstable *in vivo*, being prone to degradation. However, there are NDM clinical variants that show a better performance under Zn(II) limitation conditions [3,4]. The improved resistance is associated with the fact that some substitutions improve the Zn(II) affinity, and prevent accumulation of unstable apo form. Instead, other substitutions, such as E152K, reduce apo-NDM degradation rates *in vivo* [3] Recent results show that the susceptibility of apo-NDM to degradation is due to the flexibility of C-terminal stretch upon Zn(II) dissociation. However, position 152 is located 25 Å away from the C-terminus, hence the structural bases by which the substitution E152K stabilizes apo-NDM *in vivo* are unknown.

The crystal structure of the di-Zn(II)-NDM-9 (natural variant with substitution E152K) shows a salt bridge between residues K152 and D223. By thermal shift assay and molecular dynamics simulations we show that this salt bridge is maintained in the apo-form. The study of backbone dynamics by NMR revealed that apo-NDM-9 lacks the dynamics present in apo-NDM-1 that makes it unstable *in vivo*. We conclude that the substitution E152K induces a long-range effect in the dynamics of the C-terminus. We verified that apo-NDM-9 is not affected *in vitro* neither *in cell* by the periplasmic protease Prc, confirming the observation from the NMR experiments.

These results reinforce that the *in vivo* stability of apo-NDM is a feature optimized during the natural evolution of NDM variants under Zn(II) starvation conditions.

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Análisis del efecto de la supresión y restauración de la endocitosis sobre la incorporación de hemoglobina en epimastigotes de *Trypanosoma cruzi*

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Trypanosoma cruzi, el agente causal de la enfermedad de Chagas, es incapaz de sintetizar hemo. *Tc*HRG (*Trypanosoma cruzi Heme Responsive Gene*) es una proteína involucrada en la incorporación y la homeostasis del hemo libre en el parásito[1,2]. La hemoglobina (Hb) constituye una fuente de hemo importante para el parásito y hay evidencia de que *T. cruzi* incorpora proteínas a través de endocitosis[3].

Con el objetivo de profundizar el conocimiento sobre las posibles vías de incorporación de hemo derivado de Hb en *T. cruzi*, analizamos el efecto de la supresión y la restauración de la endocitosis en la incorporación de Hb en epimastigotes de *T. cruzi* (cepa Y). Para esto, utilizamos tres líneas de parásitos: la línea WT, una línea incapaz de realizar endocitosis, la línea Δ*Tc*Act2, y una línea control en la cual el gen de Act2 fue reinsertado al genoma (*Tc*Act2.Ty). Cada línea se cultivó en ausencia de fuente de hemo y en el mismo medio suplementado con hemina (hemo libre) o con Hb. En presencia de Hb, los parásitos Δ*Tc*Act2 lograron crecer de forma óptima y alcanzar niveles intracelulares de hemo similares a los parásitos cultivados con hemina y a las líneas control y WT, sugiriendo la existencia de un mecanismo alternativo a la endocitosis que permite suplir los requerimientos de hemo del parásito. Además, el patrón de expresión de *Tc*HRG no se vio modificado en los parásitos Δ*Tc*Act2 respecto a la línea WT o control.

Por otra parte, mediante microscopía confocal de super resolución, se detectó *Tc*HRG endógena como una señal intensa en las cercanías de la base del flagelo y se observaron señales más tenues dispersas sobre todo el cuerpo del parásito, las cuales colocalizaron parcialmente la mitocondria *T. cruzi*.

En base a resultados previos propusimos dos modelos de incorporación de Hb: la Hb podría ingresar por endocitosis o bien ser degradada extracelularmente, tras lo cual el hemo liberado ingresaría facilitado por *Tc*HRG. Tomados en conjunto, estos resultados favorecen el modelo degradación extracelular y posterior ingreso de hemo libre facilitada por *Tc*HRG como principal vía de ingreso de hemo del parásito. Finalmente, la localización dual de *Tc*HRG endógena abre la posibilidad de que esta tenga un rol no sólo en el transporte de hemo libre desde el medio extracelular sino también en el transporte intracelular de hemo.

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EXPRESSION AND CHARACTERIZATION OF RECOMBINANT CAZyme IN *LACTOCOCCUS LACTIS* NZ9000 TO ENHANCE SILAGE QUALITY.

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The main components of plant cell walls that constitute forages include cellulose, lignin, and hemicellulose (which is primarily formed by xylan). These are the building blocks for livestock feed. Silage fermentation is crucial for agroindustry and society because ruminants can generate meat and milk from plant biomass that is unsuitable for human consumption. However, how efficiently plant cell walls could be digested has a significant impact on how successful this process is. In hardwoods and grasses, xylan, which is composed of -1,4-linked xylopyranosyl residues, is the second most prevalent polysaccharide. It is hydrolyzed by Xylanases (EC 3.2.1.8) present in many fungi, yeasts as well as bacteria. By enhancing fermentation and digestibility, increasing metabolizable energy, and causing a shift in structural carbohydrates, the incorporation of enzymes in the silage promotes the degradation and also is beneficial once the silage reaches the rumen. One of the most widely common lactic acid bacteria used in the manufacturing of fermented foods is *Lactococcus lactis*, which is generally regarded as safe (GRAS). Thus, its incorporation into biotechnological procedures and the manufacture of commercial enzymes could simplify the downstream processing while reducing contamination hazards. In this context, the aim of this work was the over-expression of the XynA xylanase in *L. lactis* NZ9000 strain and its biochemical characterization to assess its potential for ensiling improvement. The *xynA* gene from *Bacillus subtilis* was codon-optimized, synthesized, and cloned in the pNZ8048 plasmid under the control of the Pnis promoter. Protein over-expression was detected, in medium supernatant. XynA was purified to homogeneity by Ni-affinity chromatography and its biochemical properties were characterized. Xylanase activity was examined by the DNS assay, by measuring the amount of reducing sugars liberated from solubilized beechwood xylan. We found that XynA activity is maximum at 50°C however the enzyme is stable up to 40°C thus defining the optimal temperature to 40°C. Concerning pH dependance, maximum activity was found between pH 5 and 6 with a stability range between pH 4,5 - 8. These characteristics are consistent with what has been reported so far about numerous xylanases (XynA) from several organisms. The measured parameters for the purified XynA protein are consistent with the pH and temperature found in silage practices.

Estudio de las cuproproteínas TcSCO1, TcSCO2, TcSCO3 y su aporte al funcionamiento de la citocromo C Oxidasa de *Trypanosoma cruzi* Dm28c

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En *Trypanosoma cruzi*, el agente etiológico de la enfermedad de Chagas se ha descrito la presencia de una cadena respiratoria ramificada con una citocromo c oxidasa tipo aa3 (COX) como la principal oxidasa terminal [1-3]. Recientemente la importancia de COX quedo evidenciada cuando la supervivencia, proliferación e infectividad del parásito se vio comprometida cuando la actividad de COX fue afectada [4].

COX es un complejo multiproteico en cuyo ensamblaje participan diversas proteínas [5]. Dentro de las cuales se encuentran las metalochaperonas SCO (Synthesis of Cytochrome c Oxidase), pertenecientes a la superfamilia de Thioredoxinas y cuya función ha mostrado ser esencial no solo por su participación en el suministro de cobre para el ensamblaje del núcleo catalítico de la citocromo C oxidasa (COX), sino también en la homeostasis del cobre y la homeostasis redox celular en diversos organismos [6-9].

En nuestro laboratorio hemos identificado mediante análisis bioinformático, a 3 genes que codificarían para dichas proteínas: TcSCO1/TcSCO2 (BCY84_12119; BCY84_03573, BCY84_19910) y TcSCO3 (BCY84_01670) cuya función es incierta.

En el presente trabajo, nos planteamos los siguientes estudios:

1. Validación de la función de estas proteínas en *T. cruzi* usando ensayos de complementación en células de *S. cerevisiae* deficientes para estas proteínas. Para ello obtendremos mutantes ∆SCO1 y ∆SCO2 mediante deleción de los genes empleando el casette KanMX4.

2. Por otra parte, analizaremos la expresión de TcSco1, TcSco2 y TcSCO3 en los distintos estadios del ciclo de vida de *T. cruzi* y además en epimastigotes sometidos a distintas condiciones de disponibilidad de Cu^{1+} analizándolos mediante RT-sqPCR.

3. Adicionalmente evaluaremos el efecto de la sobreexpresión de TcSCO1, TcSCO2 y TcSCO3 en diferentes estadios del ciclo de vida del parásito mediante transfección con el vector pTcINDEX.

4. Analizaremos el crecimiento de epimastigotes mutantes en distintas condiciones de Cu y evaluaremos la actividad COX midiendo el consumo de O_2.

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CHARACTERIZATION OF CARDIAC LOOPING IN ZEBRAFISH

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Cardiac looping is a highly conserved process among vertebrates that correctly positions the heart chambers relative to one another (1). Abnormalities in this process generate congenital heart defects in humans (2). Zebrafish (*Danio rerio*) is a vertebrate model organism with many applications for the study of heart morphogenesis and offers advantages over traditional mammalian models (3). The aim of the present work is to characterize the looping of the cardiac tube in zebrafish embryos. We described this process by imaging embryonic hearts during cardiac looping stages (24 - 57 hours post fertilization) using a fluorescent microscope in combination with a zebrafish reporter line [Tg(myl7:eGFP)^{twu34}] (4), which expresses a green fluorescent protein (GFP) in the myocardium. We measured morphological variables, such as the size of the cardiac chambers, their relative positions, and their degree of overlap for the characterization of the heart looping. We also record the relative speed of blood flow, the onset of red-colored erythrocytes and heart beat rates. All these cardiovascular parameters play a relevant role in the normal development of the heart (5). Additionally, we analyzed phenotypic characteristics of the embryo to accurately establish their developmental stage. This research puts forward a detailed characterization of cardiac looping morphogenesis and the hemodynamic changes associated, which will serve as the basis for detecting and evaluating how abnormalities influence cardiac morphology and physiology, as well as discerning stages of critical changes during this process.

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ISOLATED STRAIN FROM RESIDUAL SLUDGE WITH THE CAPACITY TO REMEDIATE THEM AND WITH BIOCONTROLLING CHARACTERISTICS

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Olein production through the acidulation of neutralization sludge generated by the refining of crude oil, produces residues with a very high organic load (COD around 170 g/L), acid pH (1.5-2.5) and high sulfate content. They also present water-soluble components such as sterols, tocopherols, degraded oxidized compounds, pigments, salts, colored compounds and immiscible material such as phosphatides, traces of fatty acids and triglycerides that do not degrade during acidulation. In addition to trouble caused by the high values of COD, SSE and high levels of sulfate which prevent their treatment in a biodigester, these residues have phytotoxic effects even after being neutralized. In order to treat these sludge, microorganisms capable of reducing COD, SSE and phytotoxicity were isolated and characterized. One of these isolates, named I-43, obtained from vegetable oils acid residues, previously characterized by its exoenzyme production capacity, showed the ability to decrease these parameters and also showed activity as a biocontroller.
The evaluation of the toxicity of the treated residue was followed by the use of lettuce seedlings *(Lactuca sativa L)* and onion bulbs *(Allium cepa)*. In both models, it was shown that the residue treatment with the bacterial isolate I-43 reduced its toxicity, allowing its final disposal in soils destined for that purpose. In parallel, isolate I-43 was analyzed as a biocontroller. Mushrooms used include *Rhizoctonia solani, Fusarium verticillioides, Fusarium sp., Fusarium graminearum* and *Macrophomina phaseolina*. Isolate I-43 showed significant biocontrol capacity for *Macrophomina phaseolina.* Finally, in order to identify the isolate I-43, 16sRNA and GyrA genes were amplified using specific oligonucleotides. Since, these sequences were not enough to identify the isolate, the I-43 genome was sequenced. The taxonomic classification was obtained by MiGA, a data management and processing system for microbial genomes and metagenomes. According to this tool, the dataset most likely belongs to the order *Bacillales* (p-value: 0.002) and probably belongs to the family *Bacillaceae* (p-value: 0.019).

EL SISTEMA MIR396-GRF-GIF EN PLANTAS: UNA TECNOLOGÍA PARA AFRONTAR SITUACIONES ADVERSAS.

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El crecimiento de los órganos de las plantas esta estrictamente regulado por su programa de desarrollo y la interacción con el ambiente. Los GRFs (del inglés, Growth-Regulang Factor) son una familia de factores de transcripción compuesta por nueve miembros en Arabidopsis thaliana, siete de los cuales se encuentran regulados negativamente por un miRNA, el miR396. Se tiene evidencia que los GRFs tienen una importante participación en el desarrollo de las hojas y órganos florales, específicamente en etapas tempranas donde tiene lugar una intensa proliferación celular. Plantas sobreexpresantes al miR396 tienen hojas más pequeñas, similar a aquellas líneas grf KO. Se han generado versiones de GRFs insensibles a la represión post-transcripcional del miR396 y se ha demostrado que estos alelos llamados rGRF promueven el crecimiento y tamaño de las hojas, fenotipo similar a aquellas plantas con miR396 KO. La capacidad que tienen los GRF por sí mismos a aumentar el tamaño de las hojas, sugiere que puede esta herramienta puede ser utilizada con aplicaciones biotecnológicas. En este trabajo caracterizamos el crecimiento de líneas modificadas en el sistema miR396-GRF frente a ambientes limitantes, como lo son un ambiente salino o una sequia.

Screening de actividades anti-*Trypanosoma cruzi* de diversas familias de compuestos.

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La enfermedad de Chagas es transmitida por el kinetoplástido *Trypanosoma cruzi*, parásito que desarrolla un ciclo de vida complejo alternando entre un huésped invertebrado (triatominos hematófagos) y uno vertebrado (en este caso el Hombre). Con el objetivo de identificar nuevas entidades químicas para desarrollar medicamentos para tratar e, idealmente, curar la enfermedad (controlando o eliminando *T. cruzi* del paciente infectado) ensayamos diversas familias de compuestos para determinar su efecto anti-*Trypanosoma cruzi.* Estos compuestos (monoaminas alifáticas N- sustituidas, diaminas alifáticas N,N' disustituidas, derivados de 1,2,3 triazoles, colecciones ya testeadas en otros Tritryps) fueron diseñados y sintetizados por el grupo dirigido por el Dr. Labadie en el IQUIR .
Hasta el momento los experimentos realizados consisten en: 1- Determinar su IC_{50} sobre epimastigotes en cultivo axénico; 2- determinar su toxicidad en células de mamífero (para calcular un índice de selectividad); 3- desarrollar experimentos para analizar su efecto sobre las moléculas blanco para las que fueron diseñados o realizar estudios de metabolómica dirigida para tratar de identificar las vías metabólicas afectadas. En nuestro laboratorio desarrollamos los puntos 1 y 2.

1- Para determinar IC_{50} de los compuestos estudiados, los epimastigotes son cultivados en medio LIT (Liver Infusion-Tryptose) suplementado con Suero Fetal Bovino (SFB) 10 % y hemina 5 µM, a una densidad inicial de 2 x 10^6 parásitos/mL, durante 72 h, en presencia de distintas concentraciones de los compuesto a analizar. Al finalizar el período de incubación se determina el crecimiento de los parásitos tratados (contador hematológico Counter 19, Wiener Lab y cámara de Neubauer) y se grafican las curvas de regresión no lineal (log(inhibitor) vs. response - Variable slope – programa GraphPad Prism 5) para determinar IC_{50} (IC_{25}, IC_{90}).

2- Para determinar la citotoxicidad sobre células Vero, se cultivan las células durante 24 a 72 h en presencia de los compuestos (para determinar un IC_{50} o para determinar la concentración máxima a la que el compuesto no presenta citotoxicidad). Las células (Vero: African Green monkey kidney cells) se siembran a una densidad de 15.6 x 10^3 cél/cm^2 en medio DMEM alta glucosa suplementado con SFB 2%. A las 24 h, se les agrega los compuestos a testear y se incuba por 1, 2 o 3 días. Al finalizar el período de incubación se determina viabilidad celular por medida de detoxificación de MTT (bromuro de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolio) a concentración de 0,5 mg/mL durante 1 h a 37 °C, y midiendo absorbancia del color violeta del formazán producido (y disuelto de DMSO) a 540 nm.

3- Como ejemplo, se mostrará las determinaciones de metabolómica dirigida para algunos compuestos estudiados: para este tipo de determinación, los parásitos (0,5 x 10^6 epimastigotes/mL) se incubaron por 96 h en DMEM alta glucosa suplementado con SFB 2% y hemina 5 µM en presencia del compuesto estudiado a una concentración igual al IC_{50} correspondiente. El medio así condicionado es separado de los parásitos por centrifugación y filtración a través de membrana de poros de 0,22 µm. El medio fue analizado mediante ^1H NMR agregando DMSO deuterado (DMSO-*d6*) como estándar interno, y agua deuterada (D_2O) para bloquear la muestra. Los metabolitos analizados (acetato de sodio, piruvato de sodio, glicina, succinato, lactato de sodio, etanol y alanina) fueron identificados inequívocamente mediante "spiking" de la muestra con

metabolitos puros para determinar sus corrimientos químicos en estas condiciones experimentales de forma precisa.

En el póster se mostrarán los protocolos experimentales usados y ejemplos de los resultados obtenidos con algunos de los compuestos estudiados.

Prevention of craniofacial abnormalities in a zebrafish model of Treacher-Collins Syndrome by Metformin

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Treacher Collins Syndrome (TCS) is a hereditary mandibulofacial dysostosis with variable expressivity caused primarily by mutations in the *TCOF1* gene. Previously, we have described increased reactive oxygen species (ROS) levels together with an association between the expression of CNBP, a central protein of cranial development sensitive to ROS, and the severity of the TCS in a zebrafish model of the disease. CNBP phosphorylation by AMP-activated protein kinase (AMPK) prevents its proteasome-mediated degradation. Metformin, an anti-diabetic biguanide, is an activator of AMPK and a modulator of ROS. Our working hypothesis is that Metformin prevents TCS severe manifestations due to its effects on cell metabolism. Here our aim was to evaluate the effects of Metformin on embryonic cranial development in the zebrafish TCS model and explore its mechanism of action.

TCS model was generated by specific Morpholino injection of zebrafish embryos. Negative controls were injected with the standard universal Morpholino. Incubations with varying concentrations of Metformin diluted in embryo medium were carried out from 6 to 24 hours post-fertilization. Embryo craniofacial cartilage morphology was analyzed by Alcian Blue staining of larvae. 1 mM Metformin induced statistically significant morphological improvements of the craniofacial cartilages of TCS-like larvae. Analysis of embryo ROS levels (using DCFH-DA) and neuro-epithelial cell death (by Acridine Orange staining) showed normalization in TCS-like embryos treated with 1 mM Metformin. Expression of redox-response genes (*sod2*, *cat*, *nfe2l2α*) analyzed by qPCR agreed with these results. Also, CNBP protein levels responded to Metformin treatment. These results suggest that Metformin was able to prevent TCS manifestations in the zebrafish model.

Identificación de proteínas involucradas en el transporte de cobre en *Trypanosoma cruzi*.

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Trypanosoma cruzi posee un ciclo de vida complejo que incluye procesos de adaptación a distintos hospedadores. Este parásito debe suplir su cuota de cofactores metálicos para garantizar su supervivencia y, además, presentar un control estricto sobre su importación, distribución intracelular y almacenamiento para evitar su toxicidad. Actualmente estudiamos el transporte y distribución de cobre (Cu) en *T. cruzi.* Como primera aproximación, realizamos curvas de crecimiento de epimastigotes en condiciones de estrés de Cu por exceso y por defecto, agregando quelantes de Cu. Se observó una disminución significativa pero no deletérea del crecimiento de los parásitos a elevadas concentraciones de $CuSO_4$ (mayores a 250 μM) y de quelante de cobre (mayores a 250 μM de BCS). Este amplio rango de tolerancia a los cambios en las [Cu], permite postular que el parásito presenta un mecanismo capaz de tolerar los excesos de Cu y garantizar su disponibilidad ante la disminución excesiva del mismo. Utilizando métodos bioinformáticos (TriTrypDB y herramientas asociadas) identificamos marcos abiertos de lectura que codificarían para proteínas con homología a transportadores y chaperones de Cu. Entre ellas las proteínas putativas a Pic2, Sco1, Sco2, Cox11, Cox17 y Cox19 de mitocondria y 3 copias de una ATPasa de Cu de tipo P del sistema de endomembrana involucradas en la exportación de Cu. También se identificaron 6 copias de homólogas a ferro-cuprorreductasas de membrana que podrían estar involucradas en la reducción de Cu^{2+} a Cu^{+} previo a su importación y de un posible transportador a nivel de membrana plasmática. Con esta información diseñamos estrategias para validar la función de estas proteínas homólogas por complementación en *S. cerevisiae* y para estudiar por qPCR la expresión de estos genes ante la respuesta a cobre. Hasta la fecha, hemos podido validar la función del gen que codifica para la CuATPase por complementación de función en levaduras; y que la mayoría de estos genes ven alterada su expresión ante cambios en la disponibilidad de Cu en el medio de cultivo. Por otro lado, no se han identificado en el genoma de *T. cruzi* genes que codifiquen para algunas proteínas que intervienen en la distribución de Cu que han sido descriptas y que están conservadas en otros organismos, como, por ejemplo, alguna posible chaperona citosólica para la distribución de Cu intracelular. En función de los resultados preliminares obtenidos postulamos que *T. cruzi* tendría una vía de incorporación de Cu no conservada.

FAR-RED LIGHT PRODUCES CHANGES IN THE PROTEIN EXPRESSION PATTERN OF XANTHOMONAS CITRI SUBSP. CITRI

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Living organisms perceive and respond to a wide variety of stimuli present in their environments, including the quality and quantity of **light** [1]. This perception of light is possible due to the presence of **photoreceptor** proteins, which cover the visible to near infrared range of the spectrum of electromagnetic radiation, between 380 and 750 nm [2]. In plant-pathogen interactions light modulates not only the plant metabolism and their defense responses, but also the virulence of the pathogen; and its effect on the physiology of chemoheterotrophic bacteria is a novel paradigm that has attracted worldwide interest for its potential implications.
***Xanthomonas citri* subsp. *citri* (Xcc)** is the bacterium responsible for type A citrus canker, a disease that causes significant damage to citrus crops [3]. The Xcc genome presents four genes encoding putative photoreceptors: three blue light sensing proteins (one with LOV domain and two with BLUF domain) and a single **bacteriophytochrome** (BphP), responsible for the perception of red/far red light. The gene encoding the latter (XAC4293) is found within the same operon and overlapping 4 nucleotides with the gene encoding a heme oxygenase (BphO, XAC4294), the enzyme responsible for the synthesis of the BphP chromophore, biliverdin (BV).
The effect of blue light on the physiology of Xcc was described in previous works carried out by our group [4], [5]. Here, we propose to study the effect of far-red light on the Xcc **proteome** by a label-free quantification (LFQ) approach. To assess this, wild-type strain, ΔbphP and ΔbphOP mutant strains were grown in liquid XVM2 medium at 28 °C up to late log phase in the dark or far-red light. For choosing the most suitable total protein extraction method, three different protocols (named as: phenol, lysis, and centrifugation methods) were tested with the wild-type strain in a continuous dark condition. Peptide separations and MS/MS analysis were performed by a nanoHPLC Ultimate3000 and a Q-Exactive HF mass spectrometer, respectively, at the Mass Spectrometry Unit of the Institute of Molecular and Cellular Biology of Rosario (UEM-IBR), Argentina.
Our results showed that the phenol method yielded the highest amount of total protein and a better performance on SDS-PAGE gels, so it was chosen as the extraction method for the LFQ analysis. Our preliminary data analysis suggests that far-red light and BphP modifies the Xcc proteome and some of the differentially express proteins might be relevant for virulence and/or colonization of the plant-pathogen into the host tissue.

Keywords: Light - Photoreceptors - Xanthomonas - Bacteriophytochrome - Proteome

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"EVENTOS MOLECULARES ACTUANTES EN LA HIPERPOLARIZACIÓN DE LA MEMBRANA PLASMÁTICA DE ESPERMATOZOIDES MURINOS"

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Los espermatozoides de mamíferos no pueden fecundar el óvulo sin sufrir ciertos cambios fisiológicos desencadenados durante su recorrido por el tracto reproductivo femenino, conocidos como capacitación. A nivel molecular, la capacitación involucra la reorganización de la membrana plasmática, modificaciones postraduccionales de proteínas y cambios en la permeabilidad de la membrana iónica que generan hiperpolarización de la membrana plasmática (*E*m). Esta hiperpolarización asociada a la capacitación se desencadena por apertura de canales de potasio SLO_3, y es necesaria y suficiente para que los espermatozoides adquieran capacidad de respuesta acrosómica. La regulación de este mecanismo no es clara, pero evidencias previas utilizando farmacología inhibitoria apuntaban al rol de PKA en este proceso. Nuestros resultados contraponen estas evidencias, permitiendo que el proceso de hiperpolarización ocurra en ausencia de la actividad de PKA. Mediante mediciones de potencial de membrana por fluorimetría poblacional utilizando $DISC_3(5)$, mostramos que el inhibidor peptídico permeable específico para PKA, sPKI no afecta la hiperpolarización de *E*m durante la capacitación. Esta hiperpolarización no ocurre en ratones KO para $SLO_{3,}$ ni bajo condiciones de inhibición farmacológica de este canal, confirmando que en ausencia de actividad de SLO_3 la inhibición de PKA no produce hiperpolarización. Sin embargo, en contraposición, la inhibición de la síntesis de AMPc por medio de TDI-10229, inhibidor específico de la adenilato ciclasa (ADCY10), impide la hiperpolarización de *E*m asociada a capacitación, mientras que el agregado a medios no capacitantes de análogos permeables de AMPc desencadenan hiperpolarización. Esto indica la necesidad de AMPc en este evento, pero independiente de PKA. En este sentido, mostramos que la inhibición mediante dimetil-amiloride del intercambiador de Na^+/H^+ (sNHE) presente en espermatozoides impide la hiperpolarización, aún en presencia de análogos permeables de AMPc, obteniéndose igual resultado en ratones KO de sNHE. Sin embargo, la estimulación con NH_4Cl, de modo de alcalinizar el medio intracelular, restaura la hiperpolarización de *E*m. Teniendo en cuenta que la activación de canales SLO_3 es sensible a alcalinización intracelular, proponemos que el canal sNHE, específico de espermatozoide, el cual tiene un sitio putativo de unión a AMPc, podría ser target de la actividad de ADCY10, alcalinizando el entorno intracelular durante la capacitación, activando $SLO_{3,}$ de manera independiente de PKA.

ORF319, A SPI-2 ENCODED SALMONELLA-SPECIFIC ANTIVIRULENCE FACTOR THAT CONTROLS BIOFILM FORMATION

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La celulosa es uno de los componentes principales de la matriz extracelular de *Salmonella*. Se la considera un factor de antivirulencia porque interfiere con la proliferación de este patógeno dentro de macrófagos y con su virulencia en ratones. Su síntesis es estimulada por CsgD, el regulador maestro en la formación de biopelículas en enterobacterias. Previamente identificamos un factor de transcripción específico de *Salmonella* codificado en SPI-2, MlrB, que reprime la transcripción de *csgD* dentro de las células hospedadoras y al gen que se encuentra corriente abajo, *orf319*. Aunque la función de Orf319 es desconocida y la deleción del gen que codifica para esta proteína no ejerce efectos en la formación de biopelículas ni en la sobrevida dentro de macrófagos, su sobreexpresión aumenta notablemente la producción de matriz extracelular, induce la transcripción de *csgD* y reduce la proliferación de *Salmonella* dentro de macrófagos. Proponemos que Orf319 funciona como un factor de antivirulencia en el control de la producción de celulosa, ya que la deleción del gen que codifica para la celulosa sintasa, *bcsA*, restaura los niveles de sobrevida de *Salmonella* dentro de macrófagos dejando sin efecto la sobreexpresión de *orf319.* Por otra parte, el efecto de Orf319 en la formación de biopelículas y en los niveles de expresión de *csgD* no requiere la presencia de MlrA, MlrB o ningún otro factor involucrado en la regulación de la transcripción de *csgD.* Estas observaciones sugieren un rol directo de Orf319 en el control transcripcional del regulador maestro en la formación de biopelículas. Análisis *in silico* mostraron la presencia de dos residuos de cisteína conservados en homólogos de Orf319, involucrados en la formación de clusters Fe-S. La sustitución de estos residuos por alaninas suprimió los efectos de activación de *csgD* y formación de biopelículas, indicando su relevancia para la función biológica de Orf319. Nuestros descubrimientos proporcionan una relación novedosa entre los factores de transcripción de SPI-2 en el control de la virulencia de *Salmonella* y la producción de matriz extracelular.

Un nuevo, rápido y eficiente método de metaciclogenesis *in vitro* de *Trypanosoma cruzi*.

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Durante su ciclo de vida, *T. cruzi* alterna entre hospedadores vertebrados (hombre) e invertebrados (triatominos). La metaciclogenesis (transformación de la forma epimastigotes a trypomastigotes metaciclicos) que ocurre en el triatomino, es un proceso esencial que capacita al parasito para la infección exitosa del hospedador vertebrado, y es gatillado por: estrés nutricional, contenido de hemo, procesos de adhesión, osmolaridad, pH, entre otros. Hasta el momento, el medio artificial TAU es el más utilizado para mimetizar el proceso de metaciclogenesis *in vitro* bajo condiciones químicamente definidas, sin embargo, resulta en bajas concentraciones de trypomastigotes metaciclicos cuando los parásitos han sido cultivados artificialmente durante largos periodos de tiempo. En el presente trabajo nos propusimos optimizar la metaciclogenesis *in vitro* utilizando diferentes condiciones de cultivo. Brevemente, epimastigotes de *T. cruzi* (cepa Dm28c) fueron mantenidos 7 días en fase exponencial de crecimiento a 28°C, medio LIT pH:7, 10% SFB. Posteriormente, se cultivaron 12 días a 28°C con y sin agitación en: M16, LIT o TAU a pH: 4, 5 o 6 (Ci: 5.10^6 parásitos/ml), período en el que se cuantificó el porcentaje de metaciclogenesis. Los tripomastigotes metacíclicos obtenidos se utilizaron para infecciones de células Vero (MOI: 10:1, 24 hs). Al D4 y D5 p.i. se cuantificó la tasa de infección y liberación de trypomastigotes sanguíneos. Resultado: observamos mayores tasas de metaciclogenesis en epimastigotes cultivados en medio LIT y M16, pH:6 (LIT: 58±13; M16: 38±4; TAU:5±3), en un proceso dependiente de la adherencia del parásito al sustrato. En las mismas condiciones, la infectividad *in vitro* fue mayor, en relación al medio TAU (LIT:33±12, M16:26±2, TAU:2±1). Concluimos que los medios LIT y M16 (pH:6) son más eficientes y prácticos para ser utilizados en procesos de metaciclogenesis *in vitro*, que el tradicionalmente utilizado medio TAU.

LA REGULACIÓN POST-TRADUCCIONAL MUTUA ENTRE FACTORES DE TRANSCRIPCIÓN Y SUS CO-REGULADORES ASEGURA UN DESARROLLO ROBUSTO DE LAS HOJAS EN PLANTAS

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Uno de los aspectos más fascinantes del desarrollo vegetal es su robustez frente a las múltiples variaciones y perturbaciones internas y ambientales. Esta continuidad se basa en la actividad de los meristemas, grupos celulares especializados en el mantenimiento de las células madre y el suministro de células en proliferación para el desarrollo de tejidos y órganos. El equilibrio entre el mantenimiento de las células madre, la proliferación y la posterior diferenciación es crucial para lograr trayectorias de desarrollo reproducibles, asegurando el crecimiento y la supervivencia, a veces durante miles de años.
Entre los actores que contribuyen a este equilibrio, las redes complejas de factores de transcripción desempeñan un papel fundamental en el control de la homeostasis de los órganos. En este trabajo, investigamos un módulo de factores de transcripción y co-reguladores con funciones conservadas en el desarrollo de las angiospermas, cuya expresión espacio-temporal está determinada por una intrincada regulación transcripcional y postranscripcional. A través del análisis funcional, de expresión y de localización subcelular, tanto para variantes expresadas en líneas transgenicas estables y transitoriamente (en Arabidopsis y *Nicotiana benthamiana*, respectivamente), revelamos que este módulo está regulado por refuerzo mutuo a nivel postraduccional: la presencia del co-regulador aumenta el nivel de proteína del factor de transcripción, mientras la asociación entre las dos potencia fuertemente la localización nuclear del co-regulador. Además, demostramos que aumentar la actividad del módulo, o bien interferir con su regulación postraduccional, repercute negativamente en el desarrollo foliar.
Sugerimos que la mutua activación de este módulo reduce el ruido y refuerza los estados regulatorios, ayudando a asegurar un desarrollo foliar robusto frente a condiciones ambientales cambiantes.

STUDY OF THE CLINICAL EVOLUTION OF β-LACTAMASE PDC IN A HYPERMUTABLE LINAGE OF *PSEUDOMONAS AERUGINOSA*

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Abstract

Traditional studies on the evolution of antibiotic resistance development use approaches that can range from laboratory-based experimental studies, to epidemiological surveillance, and to sequencing of clinical isolates. However, evolutionary trajectories also depend on the environment in which selection takes place, compelling the need to more deeply investigate the impact of environmental complexities and their dynamics over time. We have previously explored the in-patient adaptive long-term evolution of a *Pseudomonas aeruginosa* hypermutator lineage in the airways of cystic fibrosis patient treated with different antibiotics during more than 25 years of chronic infection. Chronological tracking of mutations from different subpopulations demonstrated parallel evolution events in the PDC β-lactamase (Pseudomonas-derived cephalosporinase). Multiple mutations within blaPDC shaped diverse coexisting alleles, depending on the antibiotic selective pressures. Importantly, the combination of the cumulative mutations in blaPDC resulted in a continuous enhancement of its catalytic efficiency and high level of

cephalosporin resistance leading to a “gain of function” of collateral resistance towards ceftolozane (TOL), a fifth generation cephalosporin that was not prescribed to this patient Despite the diversity of coexisting alleles along the years, there were three most prevalent variants (PDC-461, 462 and 463), which share three conserved mutations (A89V, Q120K and V211A) combined in triple and quadruple mutant alleles, but no simple nor double mutants could be found. These enzymes showed greater resistance and catalytic efficiencies against both cephalosporines, with respect to parental enzyme PDC-3. In fact, the mutant PDC-461 containing the core of three conserved mutations showed the highest resistance and activity. Docking and molecular dynamics calculations showed an expansion in the cavity of the active site of PDC-461, which is due to Q120K mutation that opens the active site pocket.

Here, to elucidate the role of the conserved mutations and to reconstruct the evolutionary pathways, we engineered single and double mutants that combines the three core mutations and study their evolution of the resistance against CAZ. Importantly, the Q120K single mutant conferred the same MIC that the evolved enzyme PDC-461. However, it was much less stable than the rest of the mutants in its native periplasm environment, indicating that the PDC variants evolve towards accumulation of compensatory mutations to restore the protein stability.

Our current investigations aim at exploring the tradeoff between PDC activity and stability in the evolutionary pathways in order to gain a deeper understanding of the evolution of *P. aeruginosa* resistance driven by decades of antibiotic treatment in the natural CF environmental setting.

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Zika virus proteins regulate the expression of the secretory protein Synaptotagmin-9.

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Despite the fact that the flavivirus life cycle has been extensively study, there are still many opened questions to anwser. One of them consists in the mechanism for the release of the new virions, as well as the secretion pathways for certain viral proteins, mainly the Non Structural protein 1 (NS1). Previous reports have suggested that these processes may involve vesicular transport, in which vesicles containing new virions or secretable isoforms of the viral protein NS1 are transported from the Endoplasmic Reticulum and Golgi apparatus to the cell membrane. However, the cellular proteins involved in these processes are still missed. In this context, we decided to study the role of a cellular protein barely studied until now, called Synaptotagmin-9 (SYT9). Previous studies determined that SYT9 plays a role in the intracellular trafficking of protein-laden vesicles in different tissues. Therefore, we first evaluated the effect of the secretable NS1 viral protein over the expression of SYT9. Our results showed that the presence of this viral protein significantly increases the expression of SYT9 using *in vitro* transfection models. In addition, we demonstrated a marked subcellular redistribution of SYT9 and an intense colocalization pattern of both proteins by immunofluorescence assays. Interestingly, the changes of SYT9 expression in the presence of NS1 were also observed in ZIKV infected cells. Subsequently, we wanted to asses a potential contribution of SYT9 in virus assembly and release. For this, we initiated the analysis of the impact of one of the structural ZIKV proteins, the envelope (Env) protein, on SYT9 expression. We could show that Env protein induces an increase in SYT9 abundance together with changes in its subcellular localazation with an intense co-localization with Env. Again, these observations were also found in ZIKV infected cells. Furthermore, we evaluated the expression of SYT9 in the presence of a viral protein not related to secretion processes such as the Non Structural protein 4A (NS4A). The presence of NS4A did not induce clear changes in either the levels or localization of SYT9, suggesting that our previous findings were not an artefact due to the expression of any viral protein. Altogether our results, suggest that SYT9 plays a role during ZIKV infection, probably being involved in the transport of vesicles loaded with viral proteins and/or new virions produced in infected cells. Although much more studies are necessary, this work shows for the first time the association of a member of the synaptotagmin family protein with the life cycle of flaviviruses, laying the bases for further research to identify potential new therapeutic targets to control viral dissemination in the infected host.

P38 **EFFECT OF PHOSPHATIDYLCHOLINE ON NSCS DIFFERENTIATION AND PROLIFERATION UNDER STRESS CONDITIONS**

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Neuroinflammation is a common feature of many neurodegenerative diseases, and causes an imbalance in neural stem cells (NSCs) growth and differentiation, preventing important processes such as neurogenesis. Thus, it is essential to generate a favourable condition for NSCs and conduct them to differentiate towards functional neurons. Here, we show that inflammation has no effect on NSCs proliferation but induces an aberrant neuronal differentiation that gives rise to dystrophic, non-functional neurons. This is perhaps the initial step of brain failure associated to many neurological disorders. Interestingly, we demonstrated that phosphatidylcholine (PtdCho)-enriched media enhances neuronal differentiation even under inflammatory stress by modifying the commitment of post-mitotic cells. The pro-neurogenic effect of PtdCho increases the population of healthy normal neurons. In addition, we provide evidences that this phospholipid ameliorates the damage of neurons and, in consequence, modulates neuronal plasticity. These results contribute to our understanding of NSCs behavior under inflammatory conditions.

SCL28 PROMOTES ENDOREPLICATION AND CELL EXPANSION IN ARABIDOPSIS BY ACTIVATING A GROUP OF SIAMESE-RELATED CYCLIN-DEPENDENT KINASE INHIBITORS.

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Plant organ growth initiates with proliferating cells generated by the mitotic cell cycle (MCC). Then, cells enter an expansion and differentiation program. During this later stage, plant cells often switch to an alternative cell cycle named endoreplication (ER), in which cells replicate their DNA without progressing through mitosis nor cytokinesis, generating somatic polyploidy. The transition from MCC to ER involves regulatory mechanisms that specifically inhibit mitotic cyclin/cyclin-dependent kinase (CDKs) complexes causing cells to oscillate between S and G1 without engaging into mitosis.
Previously, we´ve shown that the *Arabidopsis thaliana SCL28* transcription factor promotes mitotic G2/M progression. To extend this characterization we performed a transcriptome analysis of *scl28-3* a mutant in this. Among the differentially expressed genes we found genes related to cell elongation and differentiation, including cell wall and cytoskeleton assembling related genes. Also, we found downregulated 6 members of *SIAMESE/SIAMESE-RELATED* (*SIM/SMR*) family in the mutant. These proteins have been reported to trigger the transition from the MCC to ER by its association with specific CDKs. In this work we characterize the role of SCL28 in both roots and leaves development. We found that this transcription factor is involved in switch from the proliferation to the cell expansion phases, promoting ER and regulating cell wall and cytoskeleton assembly genes.

Spectroscopic and functional characterization of synthetic biosensors based on the CueR

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The Cu/Au/Ag CueR cytoplasmic sensor, a member of the MerR family of transcriptional regulators, is the main contributor to Cu homeostasis in Gram-negative bacteria. This biological sensor forms a dimer with two symmetrical metal binding sites formed by residues from both monomers. Cu(I), Ag(I) or Au(I) are coordinated in a linear array by two conserved cysteine residues (C112 and C120) that define the metal-binding loop (MBL). Within the metal coordination environment there is also a key serine residue (S77) from the other monomer that restricts the access of +2 ions to the metal binding site. S77 replacement for cysteine, the residue found in a similar position in all MerR members responding to +2 ions, allows the mutant CueR77 sensor from *Salmonella* to expand the spectrum of inducer metals to include Hg(II), Zn(II), Pb(II), Cd(II) or Co(II) ions. To understand the molecular bases directing metal recognition in this non-selective sensor, we introduced the same substitution (S77C) in the structurally characterized *Escherichia coli* (EC) CueR ortholog and evaluated its ability to interact with different divalent metals, both *in vivo* and *in vitro* comparing with the parental sensor. EC-CueR77 binds up to two equivalents of Hg(II), Cd(II) or Co(II) per dimer and all these metal ions are almost equally effective in switching the conformation of the regulator to its active form to induce transcription of its target genes. By contrast, the wild-type sensor only binds Hg(II), although with less affinity, and was unable to acquire the active conformation in these conditions. In addition, we generated a CueR77 derivative carrying the MBL of the *Bacillus megaterium* MerR mercury sensor (CueR77-LRB). This non-selective variant resulted fully insensitive to Cu(I) and poorly respond to Au(I) or Zn(II), but retained almost intact its ability to detect Pb(II) or Cd(II). Using competition assays with a specific Cu(I) chelator, we observed a significant decrease in its affinity for Cu compared with the parental sensor. Our results suggest that both the S77 residue and the native MBL region of CueR were coordinately selected during evolution of this biological sensor to avoid the interaction with +2 ions such as Zn(II) and guarantee a proper control of Cu homeostasis in bacteria.

Evaluation of a biotechnological tool for the control of *Pectobacterium carotovorum*, causative agent of potato soft rot

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Iron incorporation is problematic for terrestrial organisms, and many infectious agents have developed mechanisms to hack host ferroproteins. The most abundant iron-containing plant protein is ferredoxin (Fd), which delivers redox equivalents to numerous essential oxido-reductive pathways, including photosynthesis. One of the phytopathogens that have taken advantage of this abundance to their benefit is *Pectobacterium carotovorum* (*Pcc*), a necrotrophic bacterium that causes soft rot in numerous crops of agro-economic importance, such as potatoes and onions. Several observations showed that this mechanism of iron acquisition via Fd could be key to the success of *Pcc* infection, suggesting that plants with decreased Fd levels could be resistant to soft rot. Unfortunately, Fd is an essential gene, and its deficiency has catastrophic phenotypic consequences for the plant. In this context, many cyanobacteria and algae contain an iron-free protein called flavodoxin (Fld), which can functionally replace Fd, and whose expression is induced in situations of iron starvation. Although the Fld-encoding gene is absent from the plant genome, the introduction of a cyanobacterial Fld directed to chloroplasts increased tolerance to environmental stresses including iron limitation. Therefore, we tested the virulence of *Pcc* in transgenic plants with decreased levels of Fd and expressing Fld. We worked on leaves of two plant species in which Fd levels were decreased by different methods: Arabidopsis knockout mutants in the gene encoding the major leaf ferredoxin (Fd2); and tobacco plants using transient virus-induced gene silencing (VIGS). We evaluated the disease progression by monitoring the onset of symptoms and their spread, estimation of bacterial populations and electrolyte leakage in leaf tissue. Our results show that Fd deficiency would limit the availability of iron during *Pcc* infection and affect the development of the disease.

Medición de los niveles de α-tubulina acetilada mediante citometría de flujo en *Trypanosoma cruzi*

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Los tripanosomátidos tienen un citoesqueleto que puede ser considerado simple pero altamente ordenado, además está organizado y constituido por microtúbulos estables. La isoforma más abundante en todas las estructuras microtubulares de estos protozoos es la α-tubulina acetilada en la lisina 40 (K40). *Tc*ATAT es responsable de acetilar α-tubulina en *T. cruz*i y hemos visto que el correcto balance de los niveles de acetilación de los microtúbulos es crucial para la progresión del ciclo celular y la diferenciación en este parásito.
La Tricostatina A (TSA) es un compuesto derivado del ácido hidroxámico, capaz de unirse irreversiblemente al sitio activo de las lisina desacetilasas (KDACs), impidiendo su unión a histonas y provocando su hiperacetilación. En el caso de *T. cruzi*, se ha reportado también un incremento en el grado de acetilación de la tubulina al tratar con TSA aunque no se conoce con exactitud cuál es la KDAC responsable de desacetilar tubulina.
Al ser la isoforma acetilada la más abundante, es difícil poder cuantificar cómo cambia el porcentaje de esta isoforma al alterar los niveles en *T. cruzi* ya sea mediante el uso de compuestos químicos o manipulación genética. En este trabajo se llevó optimizo su cuantificación mediante un ensayo de citometría de flujo de epimastigotes de la cepa Dm28c (Control), incubados con TSA 50 µM durante 48 hs y sobreexpresantes de *Tc*ATAT inducidos con tetraciclina 0,5 µg/mL durante 48 hs. Se utilizaron anticuerpos primarios anti a-tubulina y anti a-tubulina acetilada de ratón y anti-ratón conjugado a FITC. Los valores de fluorescencia media se relativizan al control.
Mediante los datos obtenidos se pudo observar un incremento en el grado de acetilación de los microtúbulos luego del tratamiento con TSA. También se pudo observar un incremento, en los sobreexpresantes de *Tc*ATAT. Esta técnica resultó muy útil para estandarizar la cuantificación de la isoforma de tubulina acetilada y puede luego utilizarse para la medición de los niveles de otras isoformas o isotipos de tubulina.

STUDY OF THE ENZYME-HOST COMPATIBILITY AND THE INCORPORATION OF OXA BETA-LACTAMASES INTO OUTER MEMBRANE VESICLES

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OXA enzymes belong to class D of β-lactamases, representing around 15% of the total universe of β-lactamases. Some OXAs are capable of hydrolyzing carbapenems, "last resort" antibiotics, which makes the bacteria carrying them a threat of major concern. OXA-23, OXA-24 and OXA-48 are the most clinically relevant enzymes from this family. OXA-48, initially isolated from *Klebsiella pneumoniae*, is also found in other species from Enterobacteriaceae, while OXA-23 and OXA-48 are frequently found in *Acinetobacter baumannii*. Furthermore, OXA-23 and OXA-24 are putative lipoproteins; since they have a lipobox sequence which anchors them to the outer membrane, whereas OXA-48 is a soluble periplasmic protein, without a lipobox sequence. In order to understand the features determining the host specificity of these enzymes, we study the effect of their expression on different bacteria (*A. baumannii, Escherichia coli,* and *Pseudomonas aeruginosa*). We analyzed the resistance phenotype by measuring MICs against different carbapenems, and the fitness cost by growth curves. We observed that, although some of these enzymes conferred great resistance, the fact that they negatively affected the bacterial growth excludes them from some bacterial strains.
Some β-lactamases can be incorporated into outer membrane vesicles (OMVs), which makes them less susceptible to degradation and allows them to protect coexisting bacterial populations, playing a key role in polymicrobial infections. We studied the incorporation of OXAs into OMVs, focusing on the role of their cellular localization. We studied OXA-23 as a model of a lipoprotein and OXA-48 as a model of a soluble periplasmic protein. We purified OMVs of *E. coli* and *A. baumannii* expressing OXA-23 and OXA-48 and their levels were determined by immunoblotting. Both enzymes were incorporated in OMVs but the proportion varied in each of the studied bacterial host, indicating that there are protein- and host-dependent features that play a role in OXAs levels into vesicles. We observed that membrane anchoring is not the only factor that favors the selective packaging of OXAs into OMVs, and this incorporation involves specific enzyme-host interactions.

IDENTIFICATION OF GENES INVOLVED IN MN(II) OXIDATION PROCESS IN *PSEUDOMONAS RESINOVORANS* STRAIN MOB-513

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The presence of soluble manganese Mn(II) affects the quality of groundwater, a source of drinking water for many populations, and is an important environmental concern. Biological sand filter technology, based on bacterial oxidation of metals to form insoluble oxides that can be filtered out of the water, is widely used for groundwater potabilization. Bioaugmentation of biological sand filters with Mn(II)-oxidizing bacteria (MOB) is used to increase Mn removal efficiencies from groundwater. The environmental isolate *Pseudomonas resinovorans* strain MOB-513 improves Mn groundwater removal. Interestingly, previous studies showed that this bacterium can oxidize Mn(II) only in the biofilm lifestyle and that c-di-GMP, a second messenger crucially involved in *Pseudomonas* biofilm formation, increases biofilm-formation and Mn(II)-oxidizing capabilities in MOB-513.

In order to identify genes involved in Mn(II) oxidation, transposon mutagenesis was performed in MOB-513. A total of 30.000 transformants were obtained and 428 were white indicating the lack of Mn(II) oxidation in these clones, on the other hand 284 clones showed a higher capacity of Mn(II) oxidation than MOB-513 wild type.

For these mutant clones two sets of arbitrary-primed PCR were used to amplify the DNA flanking the transposon insertion performing colony PCR assays. The PCR products were purified and sequenced. This strategy allows the identification of several genes that encode transcription factors, two component system response regulators and proteins involved in biofilm formation. Future studies with these mutants may allow to determine the specific roles of the mutated genes in Mn(II) oxidation and also if they have a link with c-di-GMP regulatory network.

Keywords: Manganese oxidation - Bioaugmentation - *Pseudomonas* - Transposon mutagenesis - Biofilm

Bases genéticas de caracteres de calidad de fruto de tomate y su aplicación en programas de mejoramiento

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Abstract

El tomate (*Solanum lycopersicum* L) es uno de los cultivos hortícolas más importantes a nivel mundial, para el cual se ha reportado heterosis y efecto recíproco (RE). La heterosis ocurre cuando el híbrido supera a sus líneas parentales. Los híbridos recíprocos son obtenidos al invertir la dirección del cruzamiento de los genotipos parentales, y la existencia de diferencias fenotípicas entre ellos determina la presencia de RE. Este trabajo tuvo como objetivo evaluar el desempeño en condición híbrida de cinco cultivares de tomate con distinto origen genético y explorar los mecanismos genéticos de la heterosis y el RE en caracteres de calidad de fruto de tomate. Cinco cultivares (Querubín FCA [Q], Gema FCA [G], RIL17, Purple Pear, y Green Zebra) fueron crecidos en dos condiciones de cultivo para estimar la influencia ambiental en los fenotipos. Doce caracteres agronómicos y 28 metabolitos de frutos fueron evaluados. Dado que el genotipo fue más importante que el ambiente, los 40 caracteres fueron medidos en los 20 híbridos obtenidos al cruzar los cinco cultivares siguiendo un diseño de cruzamientos dialélico completo en invernadero. Los híbridos incrementaron la variabilidad fenotípica observada entre los genotipos parentales. Los metabolitos, y en particular los aminoácidos, mostraron mayor presencia de heterosis y RE. Se detectó un gran número de correlaciones fenotípicas entre caracteres. Debido a la existencia de heterosis, RE, efecto materno y paterno, cuatro cruzamientos fueron seleccionados para analizar sus perfiles transcriptómicos por cDNA-AFLP. A partir de los resultados obtenidos, los genotipos Q, G, QxG y GxQ fueron seleccionados para estudiar sus perfiles de expresión génica y detectar genes expresados diferencialmente en frutos al estado rojo maduro, usando la técnica RNA-Seq. Los híbridos recíprocos mostraron las mayores diferencias significativas a nivel transcriptómico. Usando un enfoque multi-ómico, se encontraron varios genes relacionados a metabolitos que influyen en el sabor y el contenido nutricional de los frutos de tomate. Los fenotipos parentales fueron explicados principalmente por transcriptos con aditividad, mientras que la heterosis de metabolitos estuvo explicada mayormente por transcriptos con sobredominancia. Los resultados presentados en este trabajo demuestran la posibilidad de obtener híbridos con heterosis y el impacto de seleccionar un cultivar como femenino o masculino en un cruzamiento. Este trabajo contribuye a una mayor comprensión de las bases moleculares de la heterosis y el RE, específicamente para metabolitos relacionados al sabor y composición nutricional del tomate.

Obtención de un chasis de *P. putida* auxótrofo para la asimilación de glifosato

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Resumen

Argentina, es uno de los principales países que utilizan herbicidas basados en Glifosato (GBH) para el control de malezas en cultivos agronómicos. El glifosato (GP) o N-fosfometilglicina (C3H8NO5P) es un miembro del grupo de compuestos químicos denominados ácidos fosfónicos o fosfonatos caracterizados por el enlace C-P. El uso excesivo del GP y su presencia generalizada en el ambiente, vuelven esencial contar con herramientas para desintoxicar esta molécula, si es necesario. En nuestro laboratorio, se han aislado bacterias capaces de degradar GP a partir de suelos de la provincia de Santa Fe con historia de repetidas aplicaciones con este herbicida y se han identificado molecularmente los aislados bacterianos de interés. Encontramos que la cepa *A. tumefaciens* CHLDO, fue capaz de degradar el GBH eficientemente y tiene una vía de degradación, denominada C-P liasa, codificada en el cluster *phnFGHIJKLO-duf1045-PhnMN* que está activa cuando se la crece en un medio mínimo con GBH como única fuente de fósforo.

En este trabajo mediante ingeniería metabólica se propuso obtener un chasis de *P. putida* derivado de EM42, auxótrofo para glicina, denominada SLTB7 (*P. putida* EM42 *ΔserA ΔltaE ΔThiO ΔBenABCD*, *attTn7::RNApolT7*), con el fin de tener una cepa de selección basada en el crecimiento para la expresión heteróloga de distintas variantes del cluster *phn* de *A.tumefaciens* CHLDO*.* La sobreexpresión del cluster *phnGHJIKLOCDE2E1-duf1045phnNM* demostró que esta cepa es capaz de tolerar más el efecto de inhibición del crecimiento dado por el GP al crecer en un medio con bajas concentraciones de Glicina. Sin embargo, la expresión de este cluster no es suficiente para revertir el fenotipo de auxotrofía. Por ello acudimos a la Evolución Aleatoria en el laboratorio, del inglés ALE, con el objetivo de optimizar paulatinamente la vía de degradación C-P liasa para que degrade eficientemente el herbicida GP. Al realizar este paso, encontramos un cuello de botella. La cepa SLTB7 revierte el fenotipo de auxotrofía para glicina y se pierde la presión de selección. En este contexto, nos planteamos un nuevo objetivo, el de modificar nuevamente el chasis SLTB7 e introducir nuevas mutaciones en la vía de asimilación de compuestos carbonados que permitan en una instancia final, generar una cepa que se pueda evolucionar de manera de utilizar al GP como única fuente de carbono. En este sentido, se generó una cepa mutante en AceA (que codifica para un isocitrato liasa), denominada SLTBA. También se sobreexpresaron enzimas relacionadas con el catabolismo de la glicina a piruvato (GlyA-I/II).

Los resultados de estos experimentos permitieron optimizar el chasis de *P. putida* auxótrofo para estudiar el mecanismo de la vía C-P liasa expresada heterólogamente, codificada en el cluster *phn de A. tumefaciens* CHLDO.

Mejoramiento de inoculantes bacterianos utilizados en cultivos de interés agronómico

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La microbiología aplicada al agro, ofrece ventajas metodológicas, posibilitando el aumento en los rendimientos, permitiendo la producción en zonas marginales, reduciendo los costos para los productores y lo más importante, haciendo sustentable la práctica agrícola.

Las bacterias del género *Rhizobium*, fijadoras de nitrógeno, establecen una relación simbiótica con la planta generando nódulos en las raíces de las mismas. A su vez, bacterias de otros géneros presentes en raíces, tallos u hojas tienen la capacidad de actuar como promotoras del crecimiento vegetal (PGPR, por sus siglas en inglés de *Plant Growth-Promoting Rhizobacteria*). Por otro lado, diferentes aislamientos bacterianos del género *Bacillus*, han demostrado poseer la capacidad de promover el crecimiento vegetal. Por lo tanto, estas bacterias también son de gran interés en la agricultura ya que mediante su aplicación posibilitan la recuperación de suelos, así como también el control de patógenos que afectan los cultivos. Específicamente, las bacterias utilizadas como inoculantes para leguminosas, habitualmente se utilizan en conjunto con productos antifúngicos. En nuestro grupo de trabajo se han aislado bacterias del género *Bacillus* las cuales mostraron capacidad antifúngica contra diversos hongos que afectan principalmente cultivos de interés agrícola. Sumado a esto, algunos aislados bacterianos han demostrado producción de ácido indol acético (IAA), una hormona promotora del crecimiento vegetal.

Considerando estas características, nos proponemos analizar el uso combinado de bacterias del género *Bacillus* con *Bradyrhizobium japonicum* E109 a fines de estudiar la posibilidad de obtener un co-inóculo que permita tanto la fijación de nitrógeno por parte de *B. japonicum* E109 así como también la capacidad de biocontrol antifúngico y de promoción del crecimiento vegetal por parte de las bacterias del género *Bacillus.*

CONSTRUCTION OF A BIOLUMINESCENT REPORTER STRAIN FOR SCREENING AND IDENTIFICATION OF NOVEL INHIBITORS OF FASR, A TRANSCRIPTIONAL REGULATOR ESSENTIAL FOR VIRULENCE OF *MYCOBACTERIUM TUBERCULOSIS*

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Mycobacterium tuberculosis (*Mtb*), the causal agent of tuberculosis (TB) in humans, has a very complex lifestyle. The flexibility in its metabolism allows it to adapt and survive in the infected host. During this process, lipid metabolism is affected. Despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *Mtb* envelope, little is known about the mechanisms that allow the bacteria to modulate and adapt the biosynthesis of the components of the cell wall in response to changes in environment. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *Mtb* represents a crucial step in the comprehension of the physiology of this pathogen, as well as to find potential drug targets and contribute to combat TB.

The biosynthesis of fatty acids in *Mtb* involves two different systems of fatty acid synthases (FAS I and FAS II). Both synthases are involved in the biosynthesis of membrane fatty acids and several lipid components of the cell wall, like mycolic acids (essential for viability and pathogenesis). The multi domain single protein FAS I catalyses *de novo* biosynthesis of acyl-CoAs, which are used as primers by the FAS II multiprotein system for the synthesis of mycolic acids. FasR is a TetR-like transcriptional regulator that plays a key role in this process. FasR activates transcription of the *fas-acpS* operon by binding to the *fas* (P*fas*) promoter in *Mtb*. The *fas* gene encodes the FAS I protein and the *acpS* gene is essential to produce functional ACP. We have previously shown that regulation of lipid biosynthesis mediated by FasR is critical for macrophage infection and essential for virulence *in vivo* using a mouse model of infection, suggesting that FasR would constitute an interesting drug target to identify anti-mycobacterial molecules with novel mechanisms of action.

In this work, a bioluminescent reporter strain was designed in order to search for compounds capable of inhibiting FasR activity. For this, we constructed the plasmid pSD2, that carries the *fas* gene promoter region (P*fas*) controlling the expression of the *lux* operon (*luxCDABE*). The construction of the reporter strain was carried out by electroporating the pSD2 integrative plasmid into *Mtb* H37Ra. In this strain, the expression of the *lux* operon is activated by FasR. We found that bioluminescence correlates with optical density (OD) in exponential phase growth and decreases in the presence of exogenously added fatty acids. We are currently performing assays in the presence of compound libraries available in our laboratory. We expect that those compounds that inhibit FasR activity will show reduced bioluminescence and will be further validated by electrophoretic mobility shift assays (EMSA). Thus, the high throughput screening of FasR inhibitors using our bioluminescent reporter strain will allow further validation of FasR as a new drug target for the design of new antimycobacterial agents.

PROTEOMICS OF NEURAL STEM CELL DERIVED EXTRACELLULAR VESICLES

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Neural stem cells (NSC) have the capacity of regenerate the nervous tissue and represent a promising approach for the treatment of several neurodegenerative disorders. In addition, an alternative beneficial effect of stem cells is exerted by paracrine mediators, like extracellular vesicles (EVs). Extracellular vesicles are nanovesicles that mediate local and systemic cell-to-cell communication by transporting functional molecules such as proteins into target cells, thereby affecting the behavior of receptor cells. Despite their similar functions, extracellular vesicles from different origins present heterogeneous characteristics and components. In this study, extracellular vesicles secreted by NSCs (NSC-EVs) were isolated by ultracentrifugation and size exclusion chromatography. Results showed that extracellular vesicles, which have an average diameter expected for this vesicle, exhibit a cup-shaped morphology and express exosomal markers. By proteomics and using a label-free method, about 200 proteins were identified. Bioinformatic analysis revealed that exosomes carry a high number of proteins involved in important cellular processes. Gene Ontology analysis was performed and proteins were classified according cellular component, biological process and molecular function. The identified proteins might serve as potential therapeutic targets for some neurodegenerative diseases.

In vivo NMR characterization of unsaturated fatty acids in a *C. elegans* model of Parkinson Disease

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Alpha-Synuclein (aSyn) is an intrinsically disordered protein widely expressed throughout the human brain. Aggregates of aSyn are the primary protein component of Lewy bodies, amyloid deposits found in dopaminergic neurons of the *substantia nigra* of Parkinson Disease (PD) patients. While it's well known that misfolded aSyn has pathogenic properties that contribute to disease progression, the molecular mechanisms which cause aSyn to misfold are not clearly understood. Numerous studies showed that lipids can induce and/or accelerate the disease-associated misfolding of aSyn. Unsaturated fatty acids (UFAs), in particular, interact with aSyn *in vitro* and *in vivo* and this interaction may be implicated in PD pathogenesis. In fact, UFAs may also be regulated by aSyn in disease given that elevated levels of UFAs are observed in soluble lipid fractions of PD brain.

To gain insight about the interaction between UFAs and aSyn as well as its role in the onset of PD it is necessary to characterize this association with high resolution in a context that accurately recapitulates the biochemical changes that occur within an organism. Accordingly, we use novel *in vivo* multidimensional NMR methodologies in live *Caenorhabditis elegans* that overexpress human, tag-free aSyn. *C. elegans* shares over 80% genetic homology with humans including genes that are linked to disease and has been established as a useful model for PD. Also, its small size, high reproductive rate and low cost of maintenance are very advantageous. We have developed an efficient technique to enrich nematodes with NMR active isotopes and obtained high resolution multidimensional NMR spectra in control and aSyn overexpressing worms to study how the protein modifies the worm lipid profile at molecular resolution. This approach allows us to study the effects of aSyn on lipids composition and behavior within the native environment of a live animal.

CHARACTERIZATION OF A METALLO-β -LACTAMASE OF *PSEUDOMONAS* SP. ISOLATED FROM A BIOFILTER USED FOR PESTICIDE DECONTAMINATION

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β-lactam antibiotics are the most utilized drugs to treat bacterial infections in the clinic. Nonetheless, the efficacy of these life-saving drugs is being challenged continuously by the development and dissemination of different resistance mechanisms in bacteria. Consequently, antimicrobial resistance (AMR) represents one of the main concerns in public health. Available evidence has shown that many environmental microbes are drug resistant and the genes that make up this environmental resistome have the potential to be transferred to pathogens. The main mechanism of AMR is the expression of β-lactamases, which can hydrolyze and inactivate β-lactam compounds. Metallo-β-lactamases (MBL) can efficiently hydrolyze carbapenems β-lactam antibiotics (last resource drugs), representing a concerning class of β-lactamases. BioF is a new MBL discovered in a *Pseudomonas* sp. strain isolated from a biopurification system used on-farm to treat pesticide-polluted water. Accordingly to sequence alignment and *in silico* predicted protein structure, this enzyme belongs to the B2 MBL subclass. With kinetics studies, we determined that BioF has potent activity against carbapenems antibiotics, such as imipenem and meropenem, but no detectable activity against other classes of β-lactam antibiotics. Moreover, we demonstrated that in contrast with other MBLs belonging to B2 subclass such as CphA, BioF is not inhibited in presence of high concentrations of Zn(II). The apoprotein form of BioF was successfully obtained in order to perform Co(II) titration and study structural features of the active site and metal ligand identity. LMCT and ligand field bands were registered in electronic spectra between 340-700 nm so the geometry coordination and metal ligand were analyzed. Minimum inhibitory concentrations (MICs) were determined for imipenem and meropenem in *E. coli* expressing BioF. The presence of the new MBL B2 BioF conferred resistance phenotype against the tested carbapenems. Also, MICs were performed for imipenem and meropenem in the presence of increasing concentrations of Zn(II) and dipicolinc acid (DPA). In this way, we established that Zn(II) does not act as inhibitor of the BioF β-lactamase activity *in vivo*. In contrast, resistant phenotype was observed at high concentrations of Zn(II) (200 μM) and the phenotype was only disturbed at toxic concentrations. DPA presence decreased MICs values as its concentration increased, pointing that the sequestration of Zn(II) ions from the active site of the enzyme provokes the loss of activity, as expected for a metallo-β-lactamase. We also obtained a half-maximal inhibitory concentration (IC_{50}) of 0.13 mM with DPA using meropenem as substrate. This IC_{50} is relatively high for MBLs, since for the typical MBL B1 NDM-1 is under 0.003 mM. The finding and characterization of B2 MBL BioF as a carbapenemase from a bacterial isolate highlights the importance of studying antibiotic resistances present in the environmental microbiota.

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Structural and biochemical studies of the large glutamate dehydrogenase from Mycobaterium smegmatis

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Glutamate dehydrogenases (GDHs) are oligomeric enzymes that catalyze the oxidative deamination of L-glutamate in most living beings. They are classified into two subfamilies: small GDHs (S-GDHs) are hexameric, with monomers of 60 kDa, while large GDHs (L-GDHs) are tetrameric, with subunits of 115 or 180 kDa. S-GDHs have been the subject of diverse biochemical and structural studies while L-GDHs have been less studied.

The first structural model of a bacterial L-GDH_{180} was recently obtained by our group by X-ray protein crystallography and cryoEM (Lázaro *et al*, 2021, Commun. Biol.). The mycobacterial L-GDH_{180} (mL-GDH_{180}) consists of monomers that contrast with those of S-GDHs by containing long N- and C-terminal extensions flanking the catalytic domain. Such regions are modular and provide the surfaces for oligomerization. Based on features revealed by the 3D structure, it is possible to speculate about their possible role(s) in metabolic sensing. Our goal is to decipher the molecular basis of the activity and the regulation of mL-GDH_{180} and how it differs from that of S-GDHs.

A detailed structural comparison of the catalytic domain of mL-GDH_{180} from *M. smegmatis* with that of diverse S-GDHs revealed that secondary structure motifs involved in the oligomerization of S-GDHs are absent in mL-GDH_{180}, explaining the fact that the catalytic domain of mL-GDH_{180} is not necessary for the oligomerization of this enzyme. Moreover, several insertions were detected in mL-GDH_{180} as compared to S-GDHs, which could therefore constitute additional sites for allosteric regulation beyond the N- and C-terminal extensions.

To analyze the quaternary structure of mL-GDH_{180} in solution, the enzyme was produced recombinantly and a size-exclusion chromatography was carried out as the last purification step using buffers at different pHs. At pH 7.5 the protein eluted as a mixture of dimeric and tetrameric species while at pH 6.0 it behaved mainly as a tetramer. These observations suggested a modulation of the quaternary structure as a function of pH and is consistent with previous data obtained by single particle cryoEM.

Finally, we performed *in vitro* activity measurements with recombinant mL-GDH_{180}. We estimated, at neutral pH, a Michaelis constant of *ca.* 10 mM for glutamate, an order of magnitude lower than previously reported by other authors. We will discuss how these results bring us closer to a better understanding of the structural basis of the activity in the subfamily of L-GDHs.

Unravelling the contribution of mesophyll and bundle sheath chloroplasts to increased stress tolerance

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Maize is the crop with largest global production, and its products are important as food and energy supply, being a major source of economic development. However, it is extremely susceptible to stress, which ultimately limits the corn yield. This specie was domesticated in the tropical regions of southern Mexico and has been spread to habitats with dramatically different environmental conditions, in many cases, adverse to its development. Given its economic relevance, improving stress tolerance in maize could represent a major achievement in agricultural terms. In maize, the CO_2 fixation is divided into two cell types (C4 photosynthesis). The primary assimilation occurs in the mesophyll (M) cells, where the chloroplasts generate ATP and NADPH principally by the linear electron flow. On the other hand, the classic C3 photosynthesis takes place in the chloroplasts of the bundle sheath (V) cells, performing only cyclic electron transport that produces only ATP, nor NADPH, the latter being generated by alternate enzymatic ways.

Previous research from our laboratory has demonstrated that the introduction of a constitutively expressed cyanobacterial flavodoxin (Fld) directed to chloroplasts resulted in the generation of tobacco plants with increased tolerance to multiple stress sources. The protective function of Fld has been associated to its interaction with the photosynthetic electron transport chain at photosystem I level, sharing redox properties with isofunctional ferredoxin. Application of Fld technology to a C4 specie is still uncharted territory.

In order to elucidate the contribution of each cell type to the stress tolerance and to determine if Fld can productively interact with cyclic and/or linear electron transport as it does in C3 chloroplasts, we generated transgenic maize plants expressing Fld specifically in the chloroplasts of V (Zmv-*pfld*, for Zea mays V plastidic Fld) or M cells (Zmm-*pfld*, for Zea mays M plastidic Fld).

We had already set up the conditions for the isolation of maize fractions enriched in V and M chloroplasts, demonstrating the presence of Fld expression in V and M chloroplasts in Zmv-*pfld* and Zmm-*pfld* genotypes, respectively, confirming tissue-specific location. Besides, application of oxidative conditions by paraquat,

which act as an alternative electron acceptor from photosystem I generating superoxide, showed lower electrolyte leakage for Zmm-*pfld* in comparison to control and Zmv-*pfld* genotypes. Furthermore, Zmm-*pfld* plants subjected to extreme drought in soil also exhibited an improved tolerance compare to *Zmv-pfld* an null segregants lines.

To sum up, our results indicate that presence of Fld in M chloroplasts provide an advantage facing adverse situations. We expect to determine if the lack of Fld effect when expressed in V chloroplasts is due to a null interaction with the cyclic electron transport, and in general to thoroughly establish the features of the conferred tolerance.

THE ATPNP-R1 RECEPTOR INTERACTS WITH PLANT (ATPNP-A) AND BACTERIAL (XACPNP) TYPE NATRIURETIC PEPTIDES.

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Natriuretic Peptide (NP) systems have been identified in many vertebrates and are associated with osmoregulatory homeostasis. In higher plants, PNPs are heterologous of animal NPs and elicit a number of responses that are essential in solute and solvent homeostasis and responses to biotrophic pathogens. The *Arabidopsis thaliana* PNP, AtPNP-A, interacts whit a leucine-rich repeat protein denominated AtPNP-R1. Through this interaction, is able to trigger different responses in plant, to adapt to changes in the environment and regulate internal homeostasis.
Xanthomonas citri subsp. *citri*, the biotrophic bacterial pathogen responsible for citrus canker, also has a PNP-like protein named XacPNP. Our previous results support the hypothesis that XacPNP mimics AtPNP-A in eliciting physiological responses in plants, such as stomatal opening, increases in net water flux and sustains photosynthesis during the early stage of pathogenic infection. We align the tertiary structures to AtPNP-A y XacPNP, obtained *in silico*, and we determined that these are similar even though their amino acid sequences have a percentage of identity of 49%. To mention is that amino acids are conserved within the active domain. This, together with previous results, is compatible with the hypothesis of horizontal gene transfer.
We were able to determined quantitatively that the receptor AtPNP-R1 is associated with the plasma membrane in plant epidermal cells and it moves along the membrane and also, intracellularly. For this, we expressed the construct AtPNP-R1-Venus, we labelled the plasma membrane with FM4-64 label and we performed colocalization analyses and plasmolysis assays. Also, we analysis the interactions between constructs AtPNP-Turquoise – AtPNP-R1-Venus and XacPNP-Turquoise – AtPNP-R1-Venus *in planta* using colocalization and FRET assays.

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The T2SS of *Serratia marcescens* promotes the elimination of microbial competitors

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Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical prevalence, factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. *S. marcescens* ability to adapt to and survive in either hostile or changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including chitinases, phospholipase, haemolysin, nuclease and proteases. The type II secretion system (T2SS) is a multiprotein secretion complex, present in a wide variety of organisms and frequently implicated in virulence. In our clinical RM66262 strain, we found the presence of a T2SS, which is chromosomally encoded in the majority of clinical isolates, but is absent from most non-clinical isolates including S. *marcescens* Db11, a reference strain. However, the substrates of the RM66262 T2SS, environmental signals and regulatory factors that modulate its expression are unknown. The objective of this work is to determine the role of T2SS in *S. marcescens* RM66262. We have assessed the regulation of T2SS using a *gfp*-containing reporter plasmid. Results showed that T2SS expression is induced during the stationary growth phase. One conspicuous defense of vertebrates against bacterial infections is nutrient deprivation, which prevents bacterial growth in a process termed nutritional immunity. The most significant form of nutritional immunity is the iron sequestration. We found that under iron-depleted conditions, the transcription levels of P*T2SS-gfp* is two-times increased than in iron-suppled medium. Performing killing assays between *S. marcescens* RM66262 and *E. coli*, *P. aeruginosa* or *S. marcescens* Db11, we have determined that T2SS contributes, together with T6SS, to inter-species and intra-species elimination of microbial competitors. In addition, we found that T2SS expression is five-times increased when *S. marcescens* RM66262 was challenged in competition assays using *Acinetobacter nosocomialis* as attacker. Taken together, our results suggest that in *S. marcescens* the regulated expression of T2SS would constitute a survival strategy in bacterial competition.

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HIGH THROUGHPUT SCREENING AND IDENTIFICATION OF INHIBITORS OF FASR, A KEY TRANSCRIPTIONAL REGULATOR OF CELL WALL SYNTHESIS IN *MYCOBACTERIUM TUBERCULOSIS*

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Mycobacterium tuberculosis (*Mtb*) has a complex lifestyle and its flexible metabolism allows it to adapt and survive in the infected host. During this process, one of the most affected pathways is lipid metabolism and despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *Mtb* envelope, little is known about the mechanisms that allow the bacteria to modulate and adapt the biosynthesis of the cell wall. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *Mtb* represents a crucial step in the comprehension of the physiology of this pathogen, as well as to find potential drug targets and contribute to combat tuberculosis.

The biosynthesis of fatty acids in *Mtb* involves two different systems of fatty acid synthases (FAS I and FAS II), both involved in the biosynthesis of mycolic acids, essential components for viability and pathogenesis. FAS I catalyses the *de novo* biosynthesis of long chain acyl-CoAs that are used by the FAS II for the synthesis of mycolic acids. The transcription factor that we study, FasR, plays a key role in this process by positively regulating the expression of *fas* and *acpS* genes. These genes, coding for FAS I and AcpS (essential to produce functional ACP), form a single operon in *Mtb*. FasR:DNA binding is regulated by long-chain acyl-CoAs (products of FAS I) which disrupt the interaction of FasR with its cognate DNA. Although FasR is not essential for in vitro growth, regulation of lipid biosynthesis mediated by FasR is critical for macrophage infection and essential for virulence *in vivo* using a mouse model of infection; that is why it could be an interesting drug target.

In this work, we obtained a set of 25 candidates through an *in silico* screening of a library of thousands of compounds, that fit into the FasR hydrophobic tunnel. The ability to uncouple FasR:DNA binding of this set of candidate compounds was tested *in vitro* using electrophoretic mobility shift assays (EMSA). We selected the best hits and the data obtained by docking these compounds in the crystal structure of FasR helped us to further refine the search and identify new compounds within the library. A second set of 25 candidates was generated through this *in silico* selection and then tested by EMSA. As results of both screenings, 18 active compounds were selected out of 50 candidates. The *in vivo* validation of these hits was carried out by testing the selected compounds against a *M. smegmatis* bioreporter strain, which has the *pfas* sequence (promoter region of the *fas-acpS* operon) fused with *lux* genes. We identified several compounds that showed a large drop in the luminescence signal, indicating the ability to uncouple FasR:DNA binding *in vivo*. Overall, these studies have provided relevant information on compound-protein key interactions that should allow the identification of more potent compounds.

CHARACTERIZATION OF REPLICATION MODULES IN *Acinetobacter baumannii* RESISTANCE PLASMIDS

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The global spread of multidrug resistance (MDR), and in particular resistance to last-resource carbapenem β-lactams, among the clinical population of the healthcare-associated opportunistic pathogen *Acinetobacter baumannii* represents nowadays a major concern. The most frequent cause of carbapenem resistance among clinical *A. baumannii* strains is the horizontal acquisition of carbapenem-hydrolyzing class-D β-lactamases (CHDL), with the cognate bla_{OXA} genes being most frequently carried by plasmids endowed with replication modules carrying replication initiation protein genes (*repAci*) of the Rep_3 (PF01051) superfamily (Rep_3 plasmids). To date, 20 to 30 different Rep_3 *repAci* genes have been described, largely on the basis of sequence comparisons and phylogenetic analyses. Many *A. baumannii* Rep_3 plasmids contain more than one replicon, posing questions on their general functionality, incompatibility, and role(s), but few functional analyses have been conducted on these matters.
We have previously sequenced and characterized four different Rep_3 plasmids (pAb244_7, pAb242_9, pAb242_12 and pAb242_25) housed by three local MDR *A. baumannii* clinical strains of the CC15(P) clonal complex, two of them displaying additional carbapenem resistance (CRAB strains) (Cameranesi et al. 2018, 2020). pAb242_25, present only in CRAB strains, is a bi-replicon containing *repAci*23 and *repAci*22 genes and also carries a bla_{OXA-58}- and Tn*aphA6*-containing adaptive module conferring carbapenem and amikacin resistance. The other three plasmids contain only one replicon module each with the following *repAci* genes: *repAci*4 in both pAb244_7 and pAb242_9, and *repAci21* in pAb242_12. All contain the characteristic iteron repetitive sequences upstream of the corresponding *repAci* genes, indicating their mode of replication. Cloning of each of these replication modules in appropriate plasmid vectors indicated that all of them are functional in an *Acinetobacter* host, although some differences were observed concerning stability between replicons. Moreover, transformation with different combinations of the analyzed replication modules indicated that all of them could co-exist in the same host, suggesting their belonging to different incompatibility groups. qPCR assays indicated that they were present in copy numbers that ranged between 4 and 6 per *Acinetobacter* cell, suggesting that they represent medium-copy number replicons. The overall observations indicate that all of the four analyzed *A. baumannii* replicons were functional, represent medium-copy number replicons, and could also be assigned to different incompatibility groups. Moreover, they open significant questions on their cross-regulation in the *Acinetobacter* cell, in particular when co-existing in a multi-replicon plasmid.
Refs.: Cameranesi *et al.* Front Microbiol. 2018 9:66. doi: 10.3389/fmicb.2018.00066;
Cameranesi *et al.* Microb Genom. 2020 6(9):mgen000360. doi: 10.1099/mgen.0.000360.

HIGH RESOLUTION PROTEIN IN-CELL NMR IN ZEBRAFISH EMBRYOS

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Recently, In-cell NMR spectroscopy has emerged as a powerful tool to evaluate protein conformations and dynamics in the complex environment of live cells (1). Here we extend these studies to a multicellular organism. Zebrafish (*Danio rerio*) embryos are complex organisms with dynamic tissue organization. During the first few hours of development, the cytosol of embryonic cells is interconnected and soluble biomolecules are efficiently streamed to cells after microinjection. This provides the basis for NMR analysis of isotopically enriched proteins in zebrafish embryonic cells (2, 3). We used the intrinsically disordered proteins Alpha-synuclein (aSyn) as model. The intracellular properties of a-Syn were thoroughly evaluated in bacterial and mammalian cells, constituting benchmarks for comparisons and critical analysis of the benefits of our system. First, we microinjected the yolk of zebrafish embryos with FITC-aSyn adducts and confirmed fast and complete streaming to the embryonic cells. Western blot analysis of aSyn during the experiments ruled out protein degradation. High resolution 2D ^{1}H-^{15}N NMR showed that aSyn remains disordered inside embryonic cells and that the protein if efficiently N-α-acetylated at the terminal Met residue by zebrafish N-acetyl transferases, in line with previous evidences using cultured mammalian cell lines (4). Contacts between cellular components and different regions of aSyn were also preserved. Finally, a direct comparative analysis of gamma-synuclein (gSyn), a naturally occurring homolog of aSyn, in bacterial, mammalian and embryonic zebrafish cells showed specific interactions in zebrafish embryos that arise from specific cytosolic compositions and the sequence determinants at the C-terminus of the protein. Our results indicate that high resolution protein in-cell NMR is attainable in embryonic cells within the complex tissue context and biological activities of a multicellular organism.

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Inhibition of the assembly of amyloid curli fibers, the main structural components of *E. coli* biofilms, by a *B. subtilis* secondary metabolite

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Abstract

Biofilms are surface-associated multicellular communities that bacteria build by embedding themselves in an extracellular matrix (ECM) composed of polymeric fibers. Due to their high antibiotic tolerance, bacterial biofilms are involved in more than 50% of all chronic infections. An example of that are the urinary tract infections (UTI) caused by *Escherichia coli*, which frequently associate with the formation of biofilms on catheters and the bladder. Recognizing the need for solutions to combat biofilm-based infections in general, and of *E. coli* in particular, we focused on the search for compounds that can interfere with the production of curli, which are amyloid protein fibers that constitute the major structural component of *E. coli* biofilms. To do so, we explored interactions of *E. coli* with distinct microorganisms in agar-grown macrocolonies biofilms as a platform for the search of curli inhibitors. We found that *B. subtilis* NCIB 3610 is able to inhibit the production of curli amyloid fibers in macrocolonies of *E. coli* strains that produce them as the main ECM element. Curli inhibition was detected by the loss of staining with amyloid-specific dyes and by the absence of curli-dependent morphology of *E. coli* macrocolonies when they grew in close proximity to *B. subtilis* NCIB 3610 or in the presence of extracts derived from cell-free culture supernatants of this strain. This inhibitory action on curli was found to be mediated by a PKS metabolite whose synthesis in *B. subtilis* requires activation by the 4'-phosphopantetheinyl transferase (PPTase) associated with secondary metabolism. Analyses of expression of the *csgBAC* operon -which encodes the curli structural subunits CsgB and CsgA- independently of its natural promoter in the presence of the metabolite showed that the inhibitory effect occurs at post-transcriptional level. Further experimental evidences such as the detection of unpolymerized CsgA subunits in the agar underlying the macrocolony biofilms treated with the PKS metabolite and the inhibition of *in vitro* polymerization of CsgA-His-tagged in the presence of the purified metabolite demonstrated that the *B. subtilis* compound acts impeding the assembly of curli subunits into amyloid fibers. In sum, this work reveals a novel microbial compound that targets the major structural component of *E. coli* biofilms and provides molecular insights into its mode of action.

Emergence of differential spatial patterns of antibiotic tolerance in *Escherichia coli* biofilms. Role of the General Stress Response.

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Within self-organized communities known as biofilms, bacteria can tolerate and survive antibiotic treatments, which ultimately facilitates the persistence of the infections, as frequently occurs with those caused by *Escherichia coli*. Due to the structural complexity of biofilms, it has remained challenging to reveal in which internal zones the bacteria better tolerate the antibiotics and which molecular mechanisms they deploy to tolerate the treatments. Taking advantage of microscopic approaches that allow examining *E. coli* macrocolony biofilms at single-cell resolution, we previously revealed that cells within these communities physiologically differentiate giving rise to two strata (upper and lower) that include subzones where cells exhibit either vegetative growth or stationary phase physiology. This complex physiological stratification results from the differential activation of cellular responses -such as the General Stress Response (GSR) mediated by the stationary-phase sigma factor RpoS- that integrates nutrient and oxygen gradients generated across the biofilms. Based on this knowledge, we performed studies that combined antibiotic (aminoglycosides) treatment of *E. coli* macrocolony biofilms, differential labelling of non-viable/viable cells, thin-sectioning of the biofilms and microscopy with the aim of revealing the spatial patterns of survival/death of individual cells within treated biofilms and examining the role of the RpoS-mediated GSR in cell survival. Our studies showed that in young biofilm regions the aminoglycosides killed those bacteria located at the outermost colony border, which is consistent with this region being commonly occupied by actively growing cells that are hence likely to be susceptible to the antibiotics. Remarkably, in more mature biofilm regions (towards the center of the treated macrocolonies) we observed that each stratum exhibits a zone where cells effectively die due to the bactericidal action of the aminoglycosides and a zone where cells survive the treatments (here referred to as "susceptibility zones" and "tolerance zones", respectively). Susceptibility zones spatially coincide with areas of vegetative growth, whereas tolerance zones, which are the outer zone of the upper stratum (at the interface with the air) and the inner zone of the lower stratum, coincide with areas where cells exhibit active RpoS expression. Deletion of *rpoS* rendered cells in the outer zone of the upper macrocolony stratum highly susceptible to the aminoglycosides, supporting a role for RpoS, and hence for the GSR, in promoting antibiotic tolerance of cells located in that zone of the biofilms. Overall, our studies revealed for the first time the existence of distinct zones of antibiotic tolerance inside *E. coli* macrocolony biofilms that could serve as independent reservoirs of surviving cells -including persisters- and demonstrated the involvement of the GSR in the emergence of one such zone.

Keywords: biofilms, antibiotic tolerance, stress responses

UREA REGULATION IN SERRATIA MARCESCENS AND ITS ROLE IN QUORUM SENSING AND INTRA-BACTERIAL COMPETITION

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Serratia marcescens belongs to the family of Enterobacteriaceae and could be isolated from a wide variety of environmental niches, from water and soil to air. In addition to its ecological ubiquity, *S. marcescens* is an emerging health-threatening nosocomial pathogen. In recent years, numerous outbreaks of strains carrying multidrug resistance and a high incidence have been reported. In 2017, the World Health Organization declared *S. marcescens*, along with other Enterobacteriaceae, a priority research target to develop alternative antimicrobial strategies given the high frequency of clinical isolates resistant to carbapenems. Our laboratory study model is the *S. marcescens* RM66262 strain, a non-pigmented clinical isolate from a patient with urinary tract infection (UTI) from a hospital in Rosario, Argentina.
The major component of urine is urea, which has been shown to repress the detection of quorum sensing in *Pseudomonas aeruginosa*. In our laboratory, we carried out a transcriptional analysis of *S. marcescens* exposed to urea. The RNA-seq analysis showed that urea is a regulatory signal that affects the expression of numerous genes in *Serratia*, including a subset related to the metabolism and detection of quorum sensing molecules. Among these genes, the expression of a putative lactonase was increased by the presence of urea in the culture medium. Phenotypic assays confirmed that this gene encodes for a quorum quenching protein with cytoplasmic activity, and has the ability to degrade acyl-homoserine lactones (AHL) from *Serratia* and other bacteria such as *P. aeruginosa*. Furthermore, our results indicate that *S. marcescens* produced AHL under static growth conditions (quorum sensing), while lactonase activity occurred under shaking conditions (quorum quenching) when exposed to urea treatment. The integrity of the *luxR* gene and AHL are necessary for urea-mediated induction to be verified.
Finally, inter-bacterial competition assays between *Serratia* and *P. aeruginosa* show that our strain behaves as a more aggressive attacker in the presence of urea (*Serratia* was able to diminish 3-fold *P. aeruginosa* CFU count). We can conclude that urea is a signal that modulates the expression of quorum sensing molecules, as well as inter-bacterial competition capacity.

DYNAMIC REGULATION OF EXTRACELLULAR ADENOSINE TRIPHOSPHATE IN SERRATIA MARCESCENS

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Serratia marcescens is a highly ubiquitous Gram-negative enteric bacterium that can be isolated from most abiotic environmental sources, as well as from plants, insects, and nematodes. In the clinical setting, *S. marcescens* is the cause of urinary tract, respiratory, wound, ocular, cardiac, bloodstream, and surgical infections, mostly affecting intensive care unit patients. In 2017, the World Health Organization declared *S. marcescens*, along with other Enterobacteriaceae, a priority research target to develop alternative antimicrobial strategies given the high frequency of clinical isolates resistant to carbapenems.

In our previous work, we have demonstrated that *S. marcescens* is able to be internalized by nonphagocytic cells. We showed that, once inside the cell, *Serratia* is able to inhabit and proliferate inside large membrane-bound compartments. These vesicles exhibit autophagic-like features, as they acquire markers typically recruited throughout the progression of autophagosome biogenesis in the antibacterial process. *Serratia* maneuvers the normal progression of host cell traffic, and this contribute to explaining the potential for *Serratia* to establish infection and persist in the host. In addition, ShlA, a pore-forming toxin, is responsible for inducing autophagy in nonphagocytic CHO epithelial cells, previous to the internalization process.

In this study we seek to analyze the role of extracellular ATP (eATP), on *Serratia* dependent autophagy of CHO cells, a mammalian cell model. The CHO cell possesses two families of nucleotide receptors, metabotropic (P2Y) and ionotropic (P2X), with various subtypes displaying high affinity for eATP. Preincubation of CHO cells with an excess of apyrase (an ATP diphosphohydrolase, to remove eATP) or with the P receptor blocker suramin inhibited by ~ 50% the autophagic response induced by ShlA. P2X blockers did not have any effect, indicating that a potential effect of eATP might be P2Y mediated.

To estimate ATP release from CHO cells, the kinetics of eATP accumulation was quantified by real time luminometry. CHO cells were exposed to wild-type S. *marcescens*, to the *shlA* mutant strain, and to a *E. coli* overexpressing the toxin. Exposure of CHO cells to wild-type *S. marcescens*, as well as to *E. coli*-ShlA, promoted a 3-fold increments of [eATP], while no ATP release was detected in the *shlA* mutant strain. Hydrolysis of eATP by nucleotidases of CHO cells and those of *S. marcescens* and *E. coli* were very low and therefore did not affect the experimental eATP kinetics. Results indicate that trigger of the autophagy response depends on *Serratia* ShlA, which promotes ATP release from CHO cells, and the resulting eATP acting on P2Y receptors of the target cell.

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Bioinformatic study of large glutamate dehydrogenases in Actinobacteria

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ABSTRACT

Glutamate dehydrogenases (GDHs) are ubiquitous oligomeric enzymes that play key roles in amino acid homeostasis. The subfamily of large GDHs (L-GDHs) includes L-GDHs$_{115}$, composed of 115 kDa monomers, and L-GDHs$_{180}$, composed of 180 kDa subunits. We have recently solved the 3D structure of the mycobacterial L-GDH$_{180}$ (mL-GDH$_{180}$), the first experimental structure available for a L-GDH (Lázaro *et al*, 2021, *Commun. Biol.*). It was found that mL-GDH$_{180}$ adopts a unique quaternary architecture, which is radically different from that of related low molecular weight enzymes. Contacts between mL-GDH$_{180}$ subunits are provided by a C-terminal domain that adopts a new fold and a flexible N-terminal segment that includes domains possibly involved in the direct sensing of metabolic signals. On the other hand, L-GDHs$_{180}$ from *Mycobacterium* species are modulated by the regulator GarA in response to nutrients available in the extracellular medium. In this way, the tubercle bacillus could integrate intracellular and host metabolic information through mL-GDH$_{180}$.

In this work we identified 21 L-GDHs$_{115}$ and 140 L-GDH$_{180}$ in a database of 247 complete and referenced genomes available for Actinobacteria. These sequences were grouped based on the conservation of the primary structure and a set of 33 representative L-GDHs was selected to perform structural predictions. These calculations were carried out using *ab initio* algorithms that use machine learning to infer contacts between residues from patterns of coevolution. Altogether, the results obtained indicate that, despite the low conservation of the N- and C-terminal regions in L-GDHs from distant species, the tertiary structure of the modules that make up monomers is maintained as well as the location of the residues involved in the stabilization of the quaternary structure. Consequently, this work contributes to support the hypothesis that the structural characteristics found for mL-GDH$_{180}$ constitute a common theme in the L-GDHs subfamily.

Additionally, a coevolution analysis of L-GDH$_{180}$/GarA pairs was performed to predict possible zones of interaction between these proteins. A possible interaction zone between L-GDH$_{180}$ and GarA variants was detected, which would involve a region of the catalytic domain of the L-GDH$_{180}$ and two segments of the FHA domain in GarA.

Structural and functional characterization of the sensor/transducer MecR1 protein of *Staphylococcus aureus*

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen that poses a worldwide threat. MecR1 is involved in the resistance to β-lactams in MRSA. We are interested in unveiling how the presence of β-lactams activate this metalloprotease, resulting in manifestation of resistance. To date, no high-resolution model of full-length MecR1 is available.

To structurally and functionally characterize full-length MecR1, we have purified full-length MecR1 (E205A mutant) as a fusion to Mistic. SEC-MALS and AUC experiments showed two oligomerization states in detergent micelles: a monomer (major species) and a dimer. Both species bind the fluorescent penicillin Bocillin-FL. The monomer fraction increased upon incubation of MecR1 in membranes with β-lactams, suggesting that the monomer is the physiologically relevant species. Cryogenic electron microscopy (cryo-EM) images of Mistic.MecR1 monomer sample were obtained. The 2D classes revealed some features and shapes. However, the original images had a low signal-to-noise ratio and, in 3D, the *ab initio* model was difficult to refine, producing a low-resolution map (in the range of 10 to 15 Å).

We used another strategy to characterize the conformational changes in MecR1 that involved nitroxide labeling at Cys residues to obtain nanometric distance restrictions by Paramagnetic Electronic Resonance Spectroscopy (EPR). To this end, the accessibility of Cys residues was evaluated and we corroborated appropriate detection of nitroxide labels in Mistic-MecR1 samples by EPR.

We concluded that Mistic-MecR1 is not a homogeneous sample as it is in equilibrium between monomer and dimer, being the monomer the most abundant species after binding of β-lactams in the membrane. We obtained good preliminary cryo-EM data, that justifies optimization of sample and grids. Mutagenesis of the native Cys residues in MecR1 is required before we can use nitroxide labeling and EPR to monitor the conformational changes in MecR1.

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Estudio de los efectos de la sobreexpresión de la proteína TcHMGB sobre la estructura de la cromatina en epimastigotes de *T. cruzi* por U-ExM

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Las proteínas High Mobility Group B (HMGB) son factores nucleares involucrados en la arquitectura de cromatina y en procesos nucleares claves tales como el control transcripcional, la replicación del ADN, la recombinación y la reparación. Anteriormente, hemos demostrado que la proteína High Mobility Group B de *Trypanosoma cruzi* (TcHMGB) se une al ADN como sus ortólogas de mamíferos. Para el estudio de la proteína contamos con parásitos capaces de sobreexpresar TcHMGB por inducción con tetraciclina. En estos parásitos, en el estadio epimastigote, se evidenció una disminución en el crecimiento y alteraciones en el ciclo celular. Mediante microscopía electrónica de transmisión, se observó en la estructura nuclear de los parásitos sobreexpresantes, un aumento del área total del núcleo y un incremento de la relación eucromatina:heterocromatina acompañada de una reducción de la región granular del nucléolo [(1)]. Estas observaciones sugieren que los niveles de TcHMGB en el núcleo deben ser regulados para el correcto funcionamiento de los procesos nucleares en *T. cruzi,* como la transcripción.

Recientemente, en nuestro laboratorio, se ha validado el uso de microscopía de expansión de ultraestructura (U-ExM) en *Trypanosoma cruzi*[(2)]. Decidimos evaluar si esta técnica es útil para el estudio de proteínas nucleares como TcHMGB y si es posible evidenciar cambios en la cromatina en parásitos sobreexpresantes de la proteína por fluorescencia. Para esto, expandimos cultivos de epimastigotes de *T. cruzi* Dm28c que contienen la construcción pTcINDEXGW-TcHMGB:HA inducidos con tetraciclina durante 24 hs, seguido de inmunomarcación con anticuerpos específicos de TcHMGB. Finalmente, analizamos por microscopía confocal.

La proteína pudo ser hallada en los núcleos de los parásitos expandidos, por lo que la técnica de U-ExM puede ser utilizada perfectamente para análisis proteínas en este compartimento subcelular. Por otro lado, encontramos diferencias en la estructura de la cromatina que esperamos poder examinar por análisis de imágenes de fluorescencia utilizando diferentes enfoques. En base a estos resultados, concluimos que la U-ExM es una gran herramienta para el estudio de la biología celular de *T. cruzi* y en nuestro caso particular, su estructura nuclear. Estos análisis de la estructura de la cromatina se integrarán con estudios de transcripción global y RNA-Seq de los parásitos sobreexpresantes, que nos encontramos realizando en la actualidad, y esperamos así poder dilucidar el rol de TcHMGB en la transcripción de genes.

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(2) Tavernelli, L. E., Motta, M. C., Silva Gonçalves, C., Santos da Silva, M., Elias, M. C., Alonso, V. L., . . . Cribb, P. (2019). Overexpression of Trypanosoma cruzi High Mobility Group B protein (TcHMGB) alters the nuclear (TcHMGB) alters the nuclear reduces the parasite infectivity. *Nature. Scientific Reports, 9*, 192.

IN VITRO AND *IN SILICO* ANALYSIS OF TWO *STREPTOMYCES SP.* ISOLATED FROM SOYBEAN PLANTS WITH POTENTIAL AS PLANT GROWTH-PROMOTING BACTERIA

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Nowadays, there is a need to develop sustainable and eco-friendly strategies for crop production, with the aim of minimizing the use of agrochemicals. The utilization of soil beneficial microbes as plant growth-promoters has been proved to be one of the main alternatives. Bacteria belonging to the genus *Streptomyces* have been considered a promising group to improve plant growth and protect them from several phytopathogens. However, their role as plant-growth promoter bacteria (PGPB) is not deeply understood. The main objective of this work was to characterize strains of *Streptomyces sp.* isolated from soybean [*Glycine max* (L.) Merr] plants and its rizosphere for their potential application as plant growth promoters and biocontrol agents. Thus, 78 actinobacteria, mainly belonging to the genus *Streptomyces*, were isolated from soybean plants cultivated in the core productive area of Argentina. First, their antagonistic effect against some of the principal phytopathogenic fungi that threaten this crop was tested. Only 12 (15%) showed good *in vitro* antifungal activities. Then, in order to select the strains with the best PGPB properties, they were further analyzed through different *in vitro* assays. The ability to produce chitinases, siderophores, indole-3 acetic acid (IAA) and to solubilize inorganic phosphate, allowed the selection of the two most promising strains as PGPB. In order to deeply understand the mechanisms involved, their genomes were sequenced and the sets of genes contributing to these plant-beneficial functions were analyzed. Bioinformatic analysis revealed the presence of genes encoding putative alkaline and acid phosphatases possibly implicated in the solubilization of inorganic phosphate. Also exopolyphosphatases and polyphosphate kinases were detected, two key enzymes involved in the accumulation of polyphosphates in microbes. Six chitinase-encoding genes were found, one of them belonging to the GH-19 family, a group of enzymes implicated in the plant defense response against fungal pathogens invasions. Despite the *in vitro* production of IAA, none of the conventional biosynthetic pathways could be completely reconstructed *in silico*. A group of genes related to the iron acquisition and metabolism and the biosynthesis of different polyamines were identified. Moreover, *in planta* experiments under greenhouse conditions have shown a significant promotion of germination and emergence process as well as high protection indexes against *Diaporthe aspalathi*, causal agent of Stem Canker in soybean. Finally, mutagenesis of different identified genes will be performed in order to validate the role of each one in the PGPB properties of these *Streptomyces sp.*

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Tipo: Póster | Tópico: Proteins: structure, dynamics and function

Binding Mechanisms in the QLQ-SNH complex from the intrinsically disorder proteins GRF and GIF.

Proteínas: estructura, dinámica y función.

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Abstract

The control of gene expression is essential for plant growth and development. It is subject to exquisite regulation at different levels, including the formation of complexes between transcription factors and transcriptional co-regulators. Despite the vital role of these proteins, information on the physical interaction remains scarce. Growth-regulating factors (GRF) and GRF-interacting factors (GIF), are part of a complex regulatory module that affects key biological processes such as cellular proliferation, leaf longevity and organ development. The GRF family shares a conserved architecture, predicted to be mostly disordered, featuring two N-terminal domains QLQ and WRC. GIF proteins are also mostly disordered and present a short, conserved domain called SNH in its N- terminal region. The QLQ domain is also present in essential core ATPase subunits of the chromatin remodeling complex of the BRAHMA (BRM) family. Previously, we showed that SNH domains are loose molten globules and binding to QLQ domain results in a tight complex, with low dissociation constant, stable to both thermal and urea unfolding, and without formation of ternary complex. In this work, we introduce the structure of the QLQ-SNH complexes and describe the interaction interface. We found differences in the surfaces exposed in BRM and GRF complexes, located in the second QLQ´s alpha helix and the first SNH´s alpha helix. It is likely that these dissimilarities could explain the hierarchy of interaction propensities between the QLQ and SNH in A. thaliana. In order to understand their conformational behavior and molecular function, we investigate their concerted binding and folding mechanism by means of equilibrium and rapid kinetic measurements.

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Functional characterization of the *mec* system and development of a crosslinking-strategy to study the signal transduction mechanism

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In methicillin-resistant *Staphylococcus aureus*, the *mec* system confers resistance to all classes of β-lactams antibiotics through the production of an accessory transpeptidase with low affinity for these antibiotics. The expression of PBP2a is inducible and it was historically proposed that, due to its structural homology, the *mec* system would function like the better characterized, *bla* operon. The intramolecular events that lead to the activation of MecR1/BlaR1 sensor proteins are yet to be elucidated.

On one side, we studied the molecular events that give rise to the activation of the *mec* system using a *S. aureus* RN4220 reporter strain and RT-qPCR. β-Lactam-induced activation of transcription/translation did not result in manifestation of resistance. Nevertheless, we confirmed the inducible expression of PBP2a in membranes of the reporter strain through labeling with the fluorescent antibiotic Bocillin-FL and mass spectrometry. Based on published results, we hypothesize that the lipid composition of the *S. aureus* RN4220 membrane prevents activation of PBP2a. UV-Vis spectra of staphyloxanthins extracted from the reporter strain and clinical strains showed differences in the carotenoid content, which can explain the absence of resistance in the reporter strain. Overall, our results reveal significant differences in the mechanism of regulation of the resistance determinants by the *bla* and *mec* systems.

On the other side, in order to unveil the conformational changes involved in activation of the sensor/transducer protein MecR1, we incorporated the unnatural photoactivatable amino acid *p*-azido-phenylalanine (*p*AzF). After expressing and purifying the irradiated mutant proteins in the presence and absence of antibiotic, they were subjected to in-gel trypsin digestion and analyzed by ESI-MS/MS to map intramolecular interactions. We found no evidence of intramolecular crosslinking, which poise a reevaluation of the crosslinking strategy and/or the proposed MecR1 model.

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ANPCyT and CONICET for the PhD fellowships awarded to CF.
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Characterization of "*in vitro*" models of Parkinson's disease: a rescue effect of phosphatidylcholine

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Parkinson´s disease is the second most prevalent neurodegenerative disease in the world. It is caused by death of dopaminergic neurons in the "*substantia nigra*", and characterized by the aggregation of the α-Synuclein protein in cytoplasmic inclusions called Lewy bodies, mitochondrial dysfunction and generation of reactive oxygen species. The present study aimed to establish *in vitro* models of Parkinson's disease. For this, SHSY5Y neuroblastoma cells were transfected with plasmids designed to overexpress the wild type α-Synuclein or the mutant A53T (mutation of adenine by threonine in amino acid 53 that increases protein aggregation), or cells were treated with 6-hydroxydopamine, a drug that induces mitochondrial deficits and stimulates several pro-apoptosis molecular factors. To validate the models, survival was analyzed and cellular death was identified and quantified by biochemical and morphological methods. We have previously demonstrated that liposomes of phosphatidylcholine exert a neuroprotective effect under inflammatory conditions by increasing neuronal plasticity and differentiation. Thus, we evaluated the effect of this phospholipid in the established models of Parkinson's disease to further characterize its neuroprotective effect.

[*] These authors contributed equally to this work

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Nowadays, there is a need to develop sustainable and eco-friendly strategies for crop production, with the aim of minimizing the use of agrochemicals. The utilization of soil beneficial microbes as plant growth-promoters has been proved to be one of the main alternatives. Bacteria belonging to the genus *Streptomyces* have been considered a promising group to improve plant growth and protect them from several phytopathogens. However, their role as plant-growth promoter bacteria (PGPB) is not deeply understood. The main objective of this work was to characterize strains of *Streptomyces sp.* isolated from soybean [*Glycine max* (L.) Merr] plants and its rizosphere for their potential application as plant growth promoters and biocontrol agents. Thus, 78 actinobacteria, mainly belonging to the genus *Streptomyces*, were isolated from soybean plants cultivated in the core productive area of Argentina. First, their antagonistic effect against some of the principal phytopathogenic fungi that threaten this crop was tested. Only 12 (15%) showed good *in vitro* antifungal activities. Then, in order to select the strains with the best PGPB properties, they were further analyzed through different *in vitro* assays. The ability to produce chitinases, siderophores, indole-3 acetic acid (IAA) and to solubilize inorganic phosphate, allowed the selection of the two most promising strains as PGPB. In order to deeply understand the mechanisms involved, their genomes were sequenced and the sets of genes contributing to these plant-beneficial functions were analyzed. Bioinformatic analysis revealed the presence of genes encoding putative alkaline and acid phosphatases possibly implicated in the solubilization of inorganic phosphate. Also exopolyphosphatases and polyphosphate kinases were detected, two key enzymes involved in the accumulation of polyphosphates in microbes. Six chitinase-encoding genes were found, one of them belonging to the GH-19 family, a group of enzymes implicated in the plant defense response against fungal pathogens invasions. Despite the *in vitro* production of IAA, none of the conventional biosynthetic pathways could be completely reconstructed *in silico*. A group of genes related to the iron acquisition and metabolism and the biosynthesis of different polyamines were identified. Moreover, *in planta* experiments under greenhouse conditions have shown a significant promotion of germination and emergence process as well as high protection indexes against *Diaporthe aspalathi*, causal agent of Stem Canker in soybean. Finally, mutagenesis of different identified genes will be performed in order to validate the role of each one in the PGPB properties of these *Streptomyces sp.*

Construcción y análisis de una cepa mutante nula en el gen FabH de *Mycobacterium tuberculosis* H37Ra: estudio proteómico e impacto en la síntesis de lípidos.

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La tuberculosis es un grave problema de salud a nivel mundial, siendo la novena causa de muerte global y la primera causa de muerte por un único agente infeccioso. El agente causal de la tuberculosis es el bacilo *Mycobacterium tuberculosis*, junto con otras micobacterias comparten la característica de poseer una extensa membrana externa formada principalmente por ácidos micólicos, los cuales son esenciales para la viabilidad y patogénesis. Esta compleja membrana externa se correlaciona con la particularidad de estas bacterias de poseer dos sintasas de ácidos grasos, FAS-I y FAS-II, encargados de la síntesis de ácidos grasos y del precursor principal para la síntesis de ácidos micólicos, respectivamente. Los sistemas FAS están conectados por una enzima condensante denominada FabH, la cual cataliza una condensación de malonil-ACP con los C_{16-18}-CoAs producidos por FAS-I, dando lugar a un β-cetoacil-ACP. Este producto es el sustrato inicial del sistema FAS-II. Nuestra hipótesis de trabajo consiste en que la regulación coordinada de los sistemas FAS I y FAS II es fundamental para la viabilidad y el normal desarrollo del metabolismo de las micobacterias. Si bien hay mucha información sobre la estructura y función biológica de los lípidos presentes en la envoltura de las micobacterias, aún existen ciertas inconsistencias respecto al modelo planteado para la síntesis de ácidos micólicos. Por ejemplo, la no esencialidad que muestra la enzima FabH en los experimentos por trasposición a gran escala (TraSH)[1]. Con el fin de caracterizar el rol fisiológico de la enzima FabH en micobacterias y analizar su impacto sobre la síntesis de lípidos y viabilidad, nos propusimos construir una cepa mutante nula en el gen FabH en *Mycobacterium tuberculosis* H37Ra (ΔFabH).

Nuestros resultados confirman lo observado en los experimentos de TraSH, es decir, que el gen FabH no es esencial para el crecimiento de *Mycobacterium tuberculosis* H37Ra. Mediante TLC observamos que la síntesis de ácidos micólicos se encuentra parcialmente disminuida en la cepa mutante ΔFabH. Lo llamativo es que no hay reportes de enzimas o vías alternativas que puedan generar derivados de acil-ACPs para iniciar la síntesis de ácidos micólicos en el sistema FAS-II. Con el objetivo de identificar enzimas redundantes en su función a FabH, realizamos un experimento de proteómica “bottom-up”. Bajo la hipótesis de que en ausencia de la enzima FabH, los niveles de alguna enzima alternativa podrían estar aumentados en la cepa mutante. Los resultados de la proteómica muestran un reordenamiento en la síntesis de lípidos, sobre todo en la β-oxidación, lípidos complejos de la membrana y en la síntesis de TAGs[2]. Además de otros reordenamientos metabólicos, por ejemplo, de las subunidades proteicas del ribosoma. Además, una proteína de función desconocida (EchA6), postulada como un punto de unión entre la síntesis de ácidos micólicos y la β-oxidación se encuentra aumentada en la cepa mutante. Creemos que la enzima EchA6 podría ser la encargada de bypasear a la enzima FabH, lo que explicaría la capacidad de seguir sintetizando ácidos micólicos de esta cepa mutante. En paralelo a estos experimentos, nos encontramos poniendo a punto una lipidómica mediante infusión directa[3]. Esta técnica nos serviría para profundizar los resultados observados en las TLC, y asignar de manera más precisa cual es el rol fisiológico de la enzima FabH.

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Structure-based drug discovery in the lipoic acid salvage pathway against MRSA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare-related infection worldwide. The increasing emergence of multidrug resistant strains urgently requires novel therapeutic approaches in order to keep the drug discovery pipeline filled. Lipoic acid (LA) is a universally conserved sulfur-containing cofactor required for intermediary metabolism, that is either synthesized de novo or acquired from environmental sources. In the model Gram-positive bacterium *Bacillus subtilis* LA synthesis involves four protein activities, instead of the two enzymes necessary in the Gram-negative bacterium *Escherichia coli*. First, the octanoyl-acyl carrier protein (ACP): protein-N-octanoyltransferase, LipM, transfers the octanoyl moieties to GcvH, the H subunit of the glycine cleavage system. Then, the lipoate synthase LipA inserts sulfur atoms into C6 and C8 of the octanoyl moieties. Finally, the amidotransferase LipL transfers the lipoyl side chain from GcvH to the E2 subunits of dehydrogenase complexes. Since lipoate ligase, LplJ, can only transfer exogenous lipoate to GcvH and E2o (the lipoylable subunit of oxoglutarate dehydrogenase), LipL is also required for modification of the remaining E2s. The bacterial pathogen *S. aureus* also employs this "lipoyl-relay" pathway for de novo biosynthesis and salvage. Furthermore, it encodes two additional proteins: a secondary lipoate ligase, LplA2, and a protein similar to GcvH, named GcvH-L, that are sufficient for LA salvage during infection. Due to its essentiality for cell viability and virulence, interfering with LA synthesis represents a promising approach for treating *S. aureus* infections. In this work, we performed a phenotypic screen of different molecules that were identified by a virtual screen against *S. aureus* enzymes involved in LA salvage, LplA1 and LplA2. We selected a compound, lpl-004, that caused a marked growth inhibition of the WT strain. This effect was less severe in Δ*lplA1* or Δ*lplA2* single mutants. Furthermore, growth of the double mutant Δ*lplA1* Δ*lplA2* was not affected in the presence of the compound. Similar results were obtained using the LA analogue selenolipoate, a compound reported to block dehydrogenase activity in *E. coli*. Using protein extracts of different mutants of *S. aureus*, deficient in LA synthesis and uptake, we determined that lpl-004 would be bound to E2s. We can conclude that, by the sequential action of staphylococcal lipoate ligase and amidotransferase, lpl-004 would be accepted as a substrate and transferred to E2s, eventually impairing dehydrogenase activity. This compound would be useful for further drug development against this pathogenic bacterium.

METAGENOMICA VIRAL DE EPITELIOS CUTANEOS: EVALUACION DE METODOS PARA ESTUDIOS DE VIROMA

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Introducción: La flora microbiana de la piel juega un rol crucial en el mantenimiento de la integridad cutánea y funciona como una barrera externa crítica. Distintas investigaciones han demostrado que la disbiosis del microbioma cutáneo se encuentra relacionada con el desarrollo de diversas patologías de la piel, incluyendo la psoriasis. Los virus tienen un gran potencial para modular el estado de salud de la piel; sin embargo, el conocimiento sobre las comunidades virales cutáneas y las relaciones con sus huéspedes es escaso.
Objetivos: Establecer una metodología adecuada de metagenómica viral para el estudio del viroma cutáneo en salud y enfermedad.
Metodología: Se evaluaron 3 protocolos para el procesamiento de muestras de piel: A) purificación de ADN total; B) filtración + ultracentrifugación + purificación de ADN; y C) purificación de partículas virales. Para ello, se prepararon 3 pooles de hisopados de piel recolectados de 30 individuos sin patologías cutáneas y cada pool fue procesado con los protocolos A, B o C; el ADN extraído en cada caso fue sometido a secuenciación de alto rendimiento (Hiseq 2000, Illumina). Mediante diversas herramientas bioinformáticas, las lecturas obtenidas fueron sometidas a análisis de calidad y clasificación taxonómica con bases de datos virales.
Resultados: En total se obtuvieron 1.376.217 lecturas (A: 540.546; B: 349.762; C: 485.909) con parámetros de calidad adecuados, de las cuales 116.265 (A: 20.418; B: 95.621; C: 226) mapearon con 36 familias virales diferentes (A: 28; B: 22; C: 10). El método B fue el más efectivo para obtener la mayor cantidad de secuencias virales. Las familias *Papillomaviridae, Genomoviridae, Poxviridae y Arteriviridae* fueron las más frecuentemente encontradas entre las familias de virus que infectan vertebrados.
Conclusión: Este trabajo aporta información relevante para la selección de protocolos adecuados de procesamientos de muestras cutáneas para estudios orientados a conocer el rol del viroma en patologías de la piel como la psoriasis.
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A premetazoan origin of plasmalogen biosynthesis in animals

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Plasmalogens are glycerophospholipids with a vinyl ether bond at *sn*-1 position, which have unique physical-chemical properties. They are involved in the regulation of membrane functions and in important signaling pathways. Its synthesis evolved first in anaerobic bacteria but did not persist in facultative and aerobic bacteria that appeared after the rise of oxygen in the primitive Earth's atmosphere. Plasmalogen biosynthesis, now requiring molecular oxygen, later reappeared in animals (vertebrates and invertebrates), some protists and mixobacteria, but not in plants and fungi. The final and key step in plasmalogen synthesis is catalyzed by a 1´alkyl desaturase (PDES1): This, till very recently orphan enzyme, was shown to be encoded by the *CarF* and *TMEM189* genes in mixobacteria and vertebrates, respectively. *Capsaspora owczarzaki* belongs to the clade Filozoa (Filasterea, Choanoflagellata and Metazoa), and is one of the closest unicellular relatives of animals. Its life cycle alternates between filopodial (adherent), aggregative and cystic stages. In this work we analyzed the fatty acid profile of each stage. This analysis also showed a progressive increase in the content of plasmalogen-derived aldehydes from the filopodial to the cystic stage. This is the first report of plasmalogens in a non-metazoan Filozoa. We found a highly reliable ortholog of PDES1 in *Capsaspora* but not in other members of Opisthokonta. A phylogenetic analysis revealed that the *Capsaspora* desaturase clusters together with TMEM189, indicating a single and unicellular origin for the reappearance of plasmalogen in animals, probably in the common ancestor of Filozoa. The finding of CarF/TMEM189-like desaturases and plasmalogen synthesis in Amoebozoa and Excavata protists should be ascribed to an independent acquisition.