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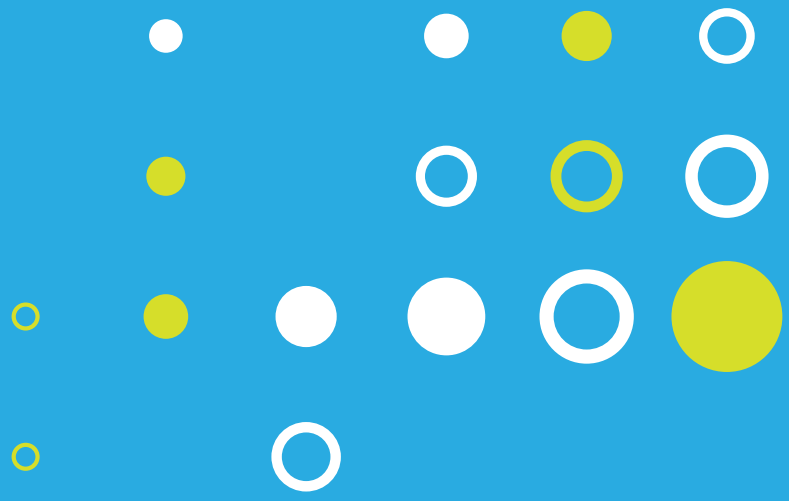

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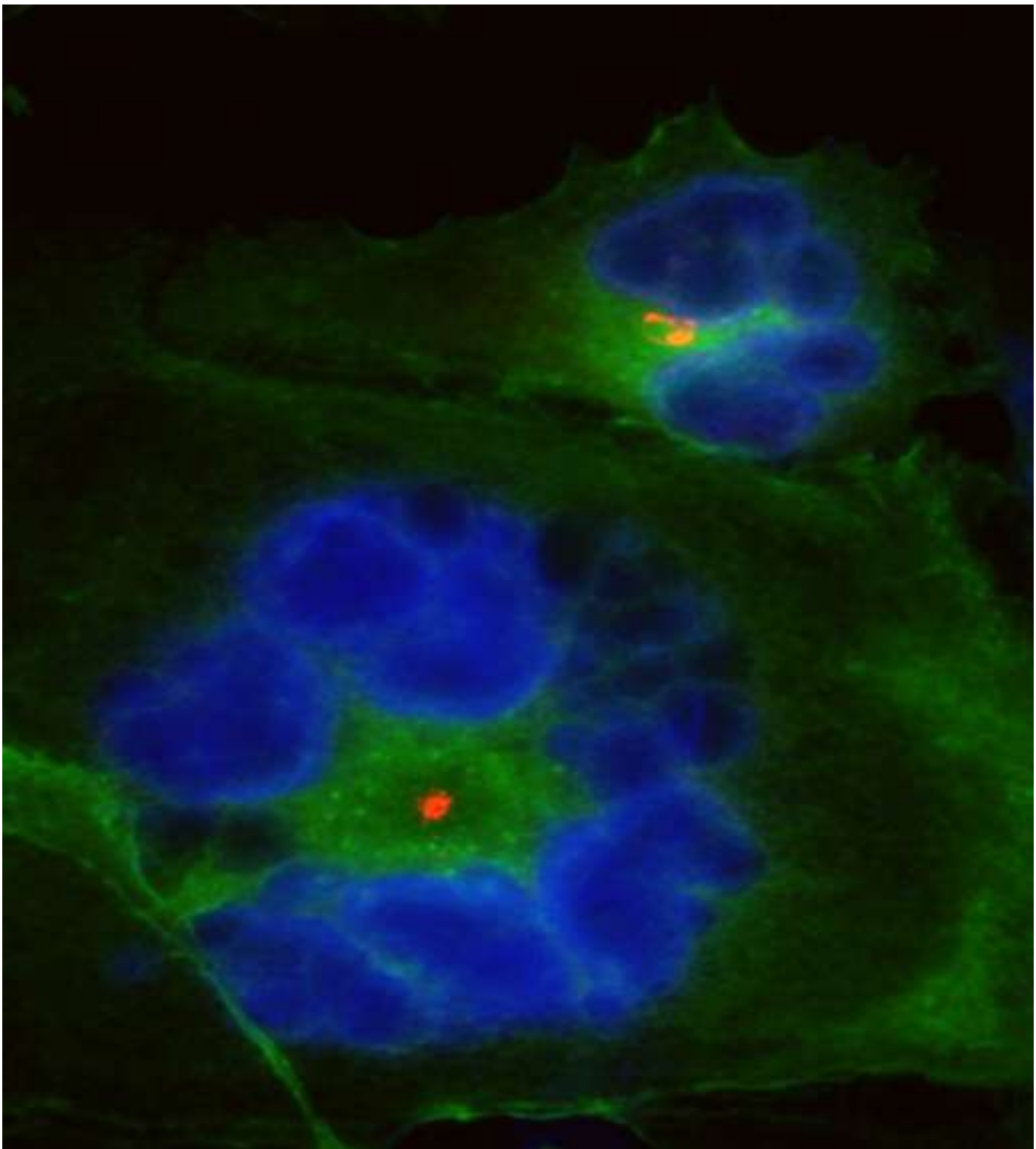

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MYO1C, MYO6 AND MYO18A ARE NECESSARY FOR *CHLAMYDIA TRACHOMATIS* DEVELOPMENT

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Chlamydia trachomatis (CT) is an obligate intracellular bacterium and the most frequent bacterial agent of sexually transmitted infections. The latest research has placed CT as a risk factor of cellular transformation, which could lead to cervical or ovarian cancer development. This bacterium induces several alterations in the host cell such as inhibition of apoptosis and cytokinesis, a decrease in molecules involved in cell adhesion, and loss of front–rear polarity in migrating cells. The cytoskeleton and the associated proteins are the main factors that ensure polarized trafficking and a correct cell division. Particularly, myosins stand out, not only because of their role in actin cytoskeleton arrangement but also because of their implication in vesicular transport. By confocal microscopy, we observed that MYO1C is recruited to the chlamydial inclusion at 24 h post-infection. We recently published that MYO1C stabilizes actin at the Golgi apparatus facilitating the arrival of incoming transport carriers at this organelle. Strikingly, CT establishes a close relationship with the Golgi apparatus, receiving from this organelle a continuous supply of vesicles loaded with essential nutrients. Thus, CT could recruit this myosin as a strategy to ensure the arrival of post-Golgi vesicles. Interestingly, the knockdown of MYO1C impairs the CT development, assessed by flow cytometry and confocal microscopy. Moreover, this function could be shared with MYO6 and MYO18A that are also necessary for the normal chlamydial development and function of the Golgi apparatus. Our results suggest that MYO1C, MYO6, and MYO18A are manipulated by CT to ensure its development.

CB-P11
CHLAMYDIA TRACHOMATIS CAUSES THE INTRACELLULAR REDISTRIBUTION OF MHC-I AND IMPAIRS ANTIGEN PRESENTATION IN DENDRITIC CELLS

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Chlamydia trachomatis (CT) is the most frequent bacterial cause of sexually transmitted infections worldwide. This highly adapted intracellular bacterium has evolved multiple strategies to hide inside cells. However, little is known about the molecular mechanisms underlying CT evasion of the immune response. Dendritic cells (DCs) are the most efficient antigen-presenting cells of the immune system and an essential link between innate and adaptive immunity. Therefore, DCs could play a key role in CT´s clearance. In this study, we analyzed in CT-infected DCs the process of cross-presentation, in which exogenous antigens are associated with MHC-I molecules to activate CD8+ T lymphocytes. By confocal microscopy and flow cytometry-based approaches, we observed, after chlamydial infection, a decrease in MHC-I molecules exposed at the plasma membrane while they are redistributed intracellularly. However, the total amount of MHC-I molecules did not change after infection, as assessed by western blot analysis. Finally, we found that CT-infected DCs were less efficient than non-infected ones to cross-present the model antigen Ovalbumin, as measured colorimetrically by the activation of the antigen-specific CD8+ T cell hybrid called B3Z. Altogether, these findings indicate that CT infection impairs antigen-cross presentation in DCs through the disturbance of MHC-I transport.

CB-P12
NOVEL CAFFEINE ANALOGS AS POTENTIAL LEADERS ON THE CHOLINERGIC SYSTEM

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Cholinergic deficit is regarded as an important factor responsible for Alzheimer's disease symptoms. Two molecular targets for the treatment of this disease are acetylcholinesterase (AChE) and nicotinic receptor (nAChR). We previously demonstrated that caffeine has a dual effect on muscle and a7 nAChRs, behaving as a weak agonist at low concentrations and as a negative modulator at high concentrations. Furthermore, it is well-known that caffeine also acts as an inhibitor of AChE. The aim of this work was to synthesize more potent caffeine analogs with a dual effect on the cholinergic system by inhibiting AChE and potentiating nAChRs. With this objective, a theophylline fragment, resembling the caffeine chemical structure, was connected with a pyrrole fragment, which is present in the nicotinic chemical structure, through homologation from 3 to 6 carbon atoms (C_n). We first tested the capacity of the different compounds to inhibit the AChE. We found that whether theophylline alone inhibited the enzyme, pyrrolidine did not. With respect to C_n, they all can inhibit the AChE at concentrations of 100, 200, and 400 µM, having C6 the strongest effect. We then explored if theophylline, pyrrolidine, and C_n influence the nAChR conformational state. To this end, we used the AChR conformational-sensitive fluorescence probe crystal violet (CrV) and AChR-rich membranes from *Torpedo californica*. We found that whether pyrrolidine induced changes in the K_D values of CrV taking the nAChR to a state close to the desensitized one at concentrations of 200 and 400 µM, theophylline did not show a significant change in the K_D value. The combined analogs also produced changes in the K_D values of CrV. This effect was dependent on the length of homologation, being C5 and C6 the most potent analogs with effect at concentrations lower than 50 nM. To understand the molecular mechanism underlying the conformational changes of the nAChR, we expressed adult muscle nAChR in HEK293 cells and performed single-channel recordings with different C_n concentrations. We found that C5 activated muscle nAChR at very low concentrations (from 0.01 pM). At the highest tested concentration (30 µM), we observed a decrease in the mean open duration, which suggests that C5 also acts as an open channel blocker. As a partial conclusion, we can say that we have synthesized more potent caffeine analogs through the combination of caffeine and nicotinic structures. The effect of theophylline on AChE, the effect of pyrrole on AChR, and the effect of C_n on

both proteins suggest different pharmacophore profiles for these target molecules. Our results bring new information about the mechanism of modulation of pharmacologic targets for the design of new therapies for the intervention in neurological diseases.

CB-P13
DISSECTING THE INTRACELLULAR CHOLESTEROL TRANSPORT IN *TETRAHYMENA THERMOPHILA*

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The ciliated protozoa *Tetrahymena thermophila* has not sterol requirements, but when available, they are incorporated and converted to 7,22-*bis* dehydrocholesterol, replacing the endogenous sterol surrogate, tetrahymanol, in the cell membranes. In this process, genes involved in sterol bioconversion, such as sterol C22 desaturase (*Des22*), are induced whereas tetrahymanol synthesis genes, like squalene synthase (*SqS*), are repressed. Although phagocytosis through the oral apparatus is the main route for sterol uptake, we have previously found that there is at least a second internalization pathway, and furthermore, some genes responded differently depending on the entry mechanism. However, the details of the intracellular traffic of sterols in *T. thermophila* and the associated signaling pathways involved in the regulation of gene expression have not yet been established. Using radiolabeled cholesterol and following its incorporation and esterification by thin-layer chromatography, we have previously observed that U18666A, an inhibitor of the export of sterols from lysosomes to the endoplasmic reticulum, impaired cholesterol esterification. This effect was not due to inhibition of ACAT activity since no differences in cholesteryl esters levels were detected in assays with cell-free extracts, therefore indicating that sterol transport to endoplasmic reticulum was indeed affected. In addition, the time-course analysis of gene expression upon treatment with U18666A revealed a delay in the cholesterol-induced upregulation of *Des22* and an absence of *SqS* repression. When cells were treated with the Golgi disrupting agent Brefeldin A neither cholesterol uptake nor its esterification were affected, but, interestingly, RT-qPCR data showed higher levels of *Des22* mRNA and a further downregulation of *SqS*. Together, these results suggest that cholesterol reaches the endoplasmic reticulum where it is modified and from where it triggers signaling pathways leading to changes in gene expression.

CB-P14
PALMITOYLATION OF THE CYSTM FAMILY OF PROTEINS IN YEAST

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A superfamily of proteins called CYSTM proteins was identified using a bioinformatics approach and found to be widely distributed among eukaryotes. These proteins are in general small, ranging from 60 to 120 aminoacids. The family is characterized by the presence of a conserved motif at the C-terminal region, which is rich in cysteine and that has been annotated as a transmembrane helix. High-throughput studies suggest that members of this family are localized to the plasma membrane. Orthologues of these proteins are involved in resistance to pathogens and they might be involved in resistance to different kinds of stress, including that caused by heavy metals. However, no thorough experimental analysis of this family of proteins has been carried out. In yeast, the family comprises the genes YDL012C, YBR016W, YDR034W-B, YDR210W and the recently characterized Mnc1 (YBR056W-A) Manganese-chelating protein 1. We became interested in these proteins because the CYSTM module could be the substrate of palmitoylation and if so, might not be able to form a TMD as predicted. Moreover, YBR016W was suggested to be palmitoylated in a high-throughput study. Protein S-acylation, commonly known as palmitoylation, is a post-translational modification (PTM) that consists of the addition of long-chain lipids on cysteine residues. This modification plays some critical roles in the regulation of many cellular processes. Palmitoylation is mediated by a family of transmembrane palmitoyltransferases (PATs), which are defined by the presence of a conserved Asp-His-His-Cys (DHHC) catalytic domain. *Saccharomyces cerevisiae* has seven members of this family in its genome. YBR016W, YDR034W-B, YDR210W were fused to GFP and we confirmed that they are indeed localized to the plasma membrane, although in polarized fashion. This polarity is achieved by endocytic cycling since it is lost in the endocytosis mutant *sla1Δ* and the proteins are retained in inner structures in a recycling mutant *ric1Δ*. Acyl-biotin exchange (ABE) experiments indicate that these proteins are indeed palmitoylated. Expression of YBR016W in strains lacking each of the yeast PATs showed that the plasma membrane localization, and most of the fluorescence, is lost in the strain that lacks the PAT Akr1. This degradation was confirmed by Western blot. ABE-PEG indicates that although the protein is degraded, palmitoylation is not completely lost suggesting that other PAT must be modifying it. Finally, treatment with the palmitoylation inhibitor 2-bromopalmitate results in loss of fluorescence from the plasma membrane, suggesting that these proteins are indeed bound to the membrane via palmitates and that the CYSTM module is, in fact, a palmitoylation motif.

CB-P15