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IMPLICACIÓN DE LOS FILAMENTOS DE ACTINA EN LA ARQUITECTURA, HOMEOSTASIS Y TRÁFICO DE SALIDA DEL APARATO DE GOLGI
Y
ESTUDIO DE LA FORMACIÓN Y DEGRADACIÓN DE UN AGRESOMA DE ACTINA

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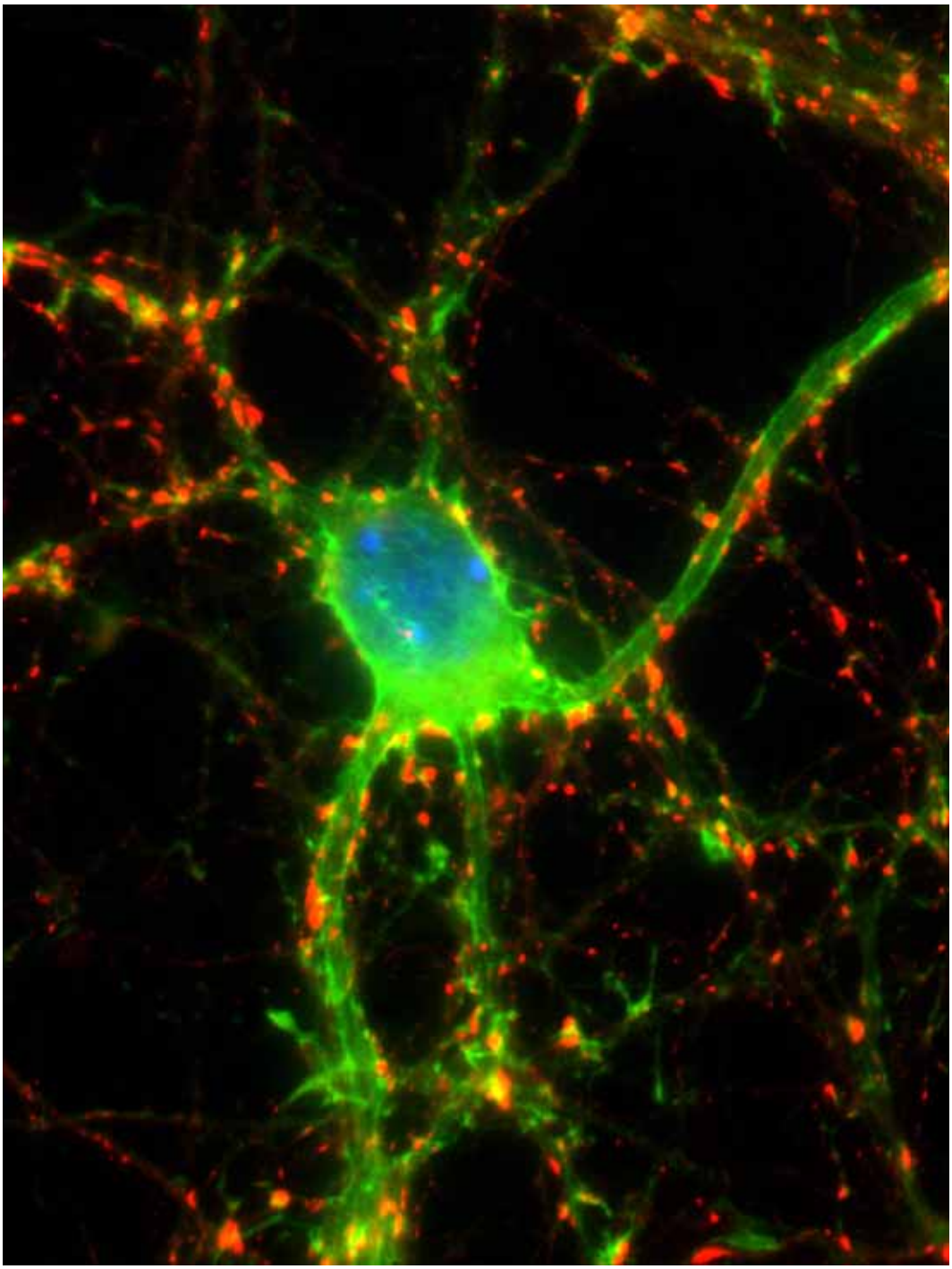

- ANEXO -

OTRAS PUBLICACIONES EN LAS QUE HE PARTICIPADO DURANTE EL PERIODO DE LA TESIS

Tomas, M., **F.Lazaro-Dieguez**, J.M.Duran, P.Marin, J.Renau-Piqueras, and G.Egea. 2003. Protective effects of lysophosphatidic acid (LPA) on chronic ethanol-induced injuries to the cytoskeleton and on glucose uptake in rat astrocytes. *J. Neurochem.* 87:220-229.

Abstract: Ethanol induces severe alterations in membrane trafficking in hepatocytes and astrocytes, the molecular basis of which is unclear. One of the main candidates is the cytoskeleton and the molecular components that regulate its organization and dynamics. Here, we examine the effect of chronic exposure to ethanol on the organization and dynamics of actin and microtubule cytoskeletons and glucose uptake in rat astrocytes. Ethanol-treated cells cultured in either the presence or absence of fetal calf serum showed a significant increase in 2-deoxyglucose uptake. Ethanol also caused alterations in actin organization, consisting of the dissolution of stress fibres and the appearance of circular filaments beneath the plasma membrane. When lysophosphatidic acid (LPA), which is a normal constituent of serum and a potent intercellular lipid mediator with growth factor and actin rearrangement activities, was added to ethanol-treated astrocytes cultured without fetal calf serum, it induced the re-appearance of actin stress fibres and the normalization of 2-deoxyglucose uptake. Furthermore, ethanol also perturbed the microtubule dynamics, which delayed the recovery of the normal microtubule organization following removal of the microtubule-disrupting agent nocodazole. Again, pre-treatment with LPA prevented this alteration. Ethanol-treated rodent fibroblast NIH3T3 cells that constitutively express an activated Rho mutant protein (GTP-bound form) were insensitive to ethanol, as they showed no alteration either in actin stress-fibre organization or in 2-deoxyglucose uptake. We discuss the putative signalling targets by which ethanol could alter the cytoskeleton and hexose uptake and the cytoprotective effect of LPA against ethanol-induced damages. The latter opens the possibility that LPA or a similar non-hydrolysable lipid derivative could be used as a cytoprotective agent against the noxious effects of ethanol

Tomas, M., J.M.Duran, **F.Lazaro-Dieguez**, T.Babia, J.Renau-Piqueras, and G.Egea. 2004. Fluorescent analogues of plasma membrane sphingolipids are sorted to different intracellular compartments in astrocytes; Harmful effects of chronic ethanol exposure on sphingolipid trafficking and metabolism. *FEBS Lett.* 563:59-65.

Abstract: Sphingolipids are basic constituents of cellular membranes and are essential for numerous functions such as intracellular signalling. They are transported along the exocytic and endocytic pathways in eukaryotic cells. After endocytosis, fluorescent-labelled sphingolipids are sorted to distinct intracellular organelles prior to recycling (via early/recycling endosomes) or degradation (late endosomes/lysosomes). Here we examine, in primary cultures of rat astrocytes, the internalisation routes followed by C(6)-NBD-glucosylceramide (NBD-GlcCer) and C(6)-NBD-sphingomyelin (NBD-SM) and the effects of ethanol on their endocytic trafficking. Endocytosed plasma membrane

NBD-GlcCer and NBD-SM are diverted to the Golgi apparatus and lysosomes, respectively. These different internalisation pathways are maintained regardless of the differentiation stage of astrocytes. Chronic ethanol exposure did not alter this endocytic sorting, but delayed the internalisation of both NBD-sphingolipids. Moreover, ethanol also stimulated the in situ metabolism of NBD-ceramide to NBD-GlcCer and NBD-SM. We conclude that in rat astrocytes internalised plasma membrane NBD-sphingolipids are sorted to different subcellular compartments. The exposure to chronic ethanol perturbed the lipid endocytic process and stimulated the de novo synthesis of NBD-sphingolipids, shifting the balance of sphingolipid metabolism in favour of the sphingomyelin pathway

Azorin, I., M.Portoles, P.Marin, **F.Lazaro-Dieguez**, L.Megias, G.Egea, and J.Renau-Piqueras. 2004. Prenatal ethanol exposure alters the cytoskeleton and induces glycoprotein microheterogeneity in rat newborn hepatocytes. *Alcohol Alcohol* 39:203-212.

Abstract: AIMS: Prenatal ethanol exposure (PEA) increases both liver weight and total protein content in the Golgi complex and alters its morphological and functional properties. As PEA-induced protein retention could be the synergetic consequence of alterations in the cytoskeleton and in the glycan biosynthesis, and there are no data that in liver PEA perturbs the cytoskeleton, we examined in hepatocytes whether PEA affects the main cytoskeleton elements. We also analysed whether ethanol induces glycoprotein microheterogeneity by altering the sugar composition of glycoproteins. METHODS: Livers from 0-day newborn control and PEA rats were used. The carbohydrate moiety of glycoproteins was determined by lectin blotting. The content and intracellular distribution of cytoskeleton proteins was analysed using immunoblotting, immunofluorescence and immunogold. RESULTS: PEA delayed the post-Golgi transport of albumin but not of transferrin. PEA also increased the levels of cytokeratin and tubulin, but it decreased the amount of tubulin capable of assembling into functional microtubules. PEA perturbed the distribution of cytokeratin and tubulin and induced microheterogeneity in several glycoproteins. CONCLUSIONS: PEA-induced retention of proteins in fetal hepatocytes could be the result of an alteration of glycoprotein biosynthesis and cytoskeleton-mediated transport

Martinez, S.E., **F.Lazaro-Dieguez**, J.Selva, F.Calvo, J.R.Piqueras, P.Crespo, E.Claro, and G.Egea. 2007. Lysophosphatidic acid rescues RhoA activation and phosphoinositides levels in astrocytes exposed to ethanol. *J. Neurochem.* 102:1044-1052.

Abstract: Long-term ethanol treatment substantially impairs glycosylation and membrane trafficking in primary cultures of rat astrocytes. Our previous studies indicated that these effects were attributable to a primary alteration in the dynamics and organization of the actin cytoskeleton, although the molecular mechanism(s) remains to be elucidated. As small Rho GTPases and phosphoinositides are involved in the actin cytoskeleton organization, we now explore the effects of chronic ethanol treatment on these pathways. We show that chronic ethanol treatment of rat astrocytes specifically reduced endogenous levels of active RhoA as a result of the increase of in the RhoGAP

activity. Furthermore, ethanol-treated astrocytes showed reduced phosphoinositides levels. When lysophosphatidic acid was added to ethanol-treated astrocytes, it rapidly reverted actin cytoskeleton reorganization and raised active RhoA levels and phosphoinositides content to those observed in untreated astrocytes. Overall, our results indicate that the harmful effects of chronic exposure to ethanol on a variety of actin dynamics-associated cellular events are primarily because of alterations of activated RhoA and phosphoinositides pools.

Fernandez-Ulibarri, I., M.Vilella, **F.Lazaro-Dieguez**, E.Sarri, S.E.Martinez, N.Jimenez, E.Claro, I.Merida, K.N.Burger, and G.Egea. 2007. Diacylglycerol is required for the formation of COPI vesicles in the Golgi-to-ER transport pathway. *Mol. Biol. Cell* 18:3250-3263.

Abstract: Diacylglycerol is necessary for trans-Golgi network (TGN) to cell surface transport, but its functional relevance in the early secretory pathway is unclear. Although depletion of diacylglycerol did not affect ER-to-Golgi transport, it led to a redistribution of the KDEL receptor to the Golgi, indicating that Golgi-to-ER transport was perturbed. Electron microscopy revealed an accumulation of COPI-coated membrane profiles close to the Golgi cisternae. Electron tomography showed that the majority of these membrane profiles originate from coated buds, indicating a block in membrane fission. Under these conditions the Golgi-associated pool of ARFGAP1 was reduced, but there was no effect on the binding of coatomer or the membrane fission protein CtBP3/BARS to the Golgi. The addition of 1,2-dioctanoyl-sn-glycerol or the diacylglycerol analogue phorbol 12,13-dibutyrate reversed the effects of endogenous diacylglycerol depletion. Our findings implicate diacylglycerol in the retrograde transport of proteins from Golgi to the ER and suggest that it plays a critical role at a late stage of COPI vesicle formation.

Calvo-Garrido, J., S. Carilla-Latorre, **F. Lazaro-Dieguez**, G. Egea,and R. Escalante. 2008. Vacuole membrane protein 1 is an endoplasmic reticulum protein required for organelle biogenesis, protein secretion and development. *Mol. Biol. Cell (en revision)*

Abstract: Vacuole membrane protein 1 (Vmp1) is a putative membrane protein of unknown function that has been associated to different unrelated pathological situations such as pancreatitis and cancer. We describe for the first time the disruption of vmp1 in a model system. *Dictyostelium discoideum* expresses a *vmp1*-related gene previously identified in a functional genomic study and the loss-of-function of this gene leads to a severe phenotype that compromises growth and development. The expression of a mammalian *Vmp1* in *Dictyostelium* mutant complemented the phenotype, suggesting a functional conservation among these evolutionary distant species and highlights *Dictyostelium* as a valid experimental system to address the function of this gene. We found that *Dictyostelium Vmp1* is a protein necessary for the integrity of the endoplasmic reticulum. As a result, the mutant cells display pleiotropic defects in the secretory pathway and organelle biogenesis. The contractile vacuole, which is necessary to survive under hypoosmotic conditions, is not functional in the mutant. The structure of the Golgi apparatus, the function of the endocytic pathway and conventional protein secretion are also affected.

Macroautophagy is induced in the mutant but maturation of the phagosomes seems to be challenged as suggested by the accumulation of undigested material. In addition to these defects observed at the vegetative stage, the onset of *Dictyostelium* multicellular development is also compromised as a consequence of defective gene expression.