Mecanismos de señalización celular mediados por fosfolípidos

PARTE II

“ Membranas biológicas: estudio de nivel estructural y funcional”

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Metabolismo de PA en el parásito *Trypanosoma cruzi*

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ORIGINAL ARTICLE

Regulation of Phosphatidic Acid Levels in *Trypanosoma cruzi*

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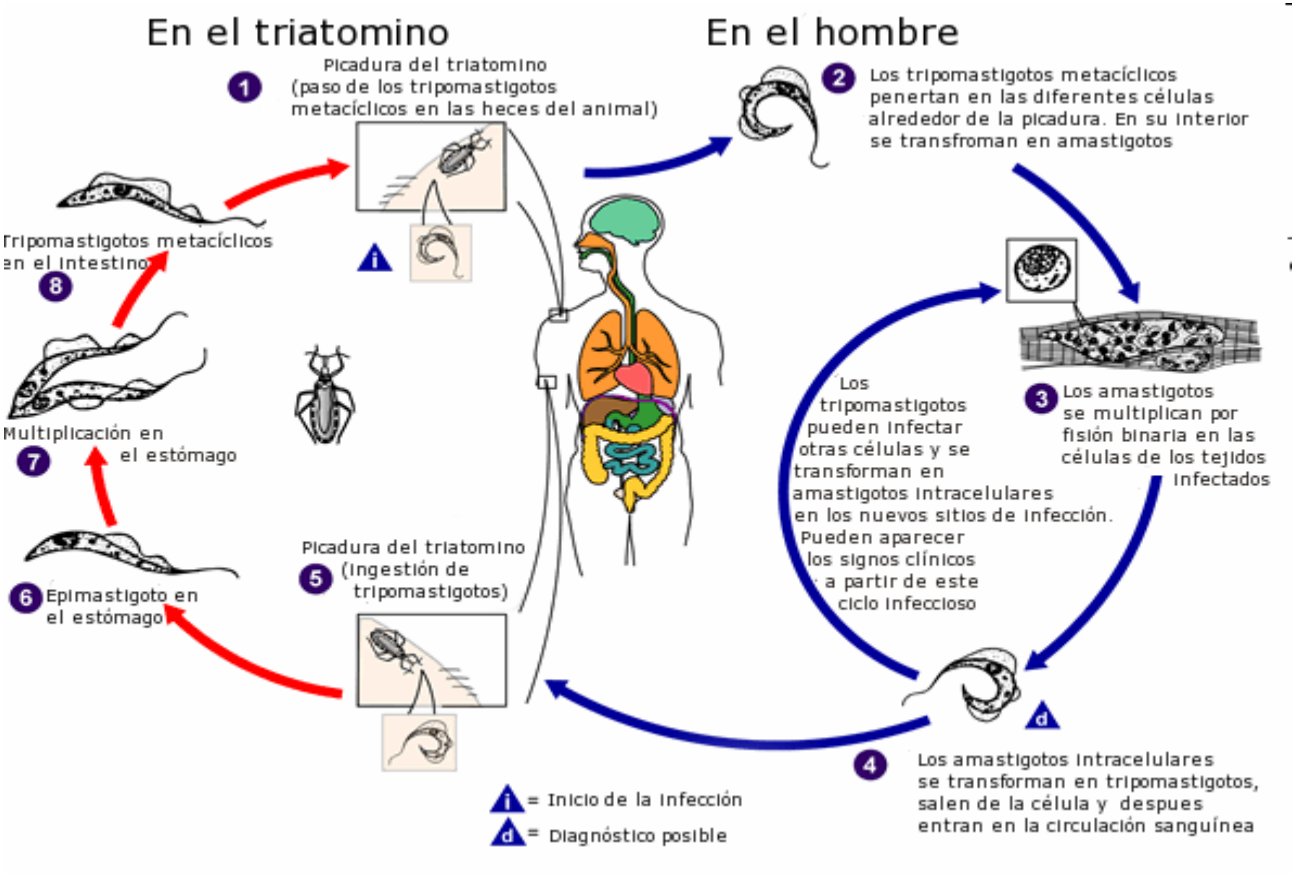
Ana L. Villasuso · Susana J. Pasquaré ·
Norma M. Giusto · Estela E. Machado

Formas intermedias-una forma preadaptativa

Metaciclogénesis

Aumenta la osmolaridad

Aumentan las formas intermedias



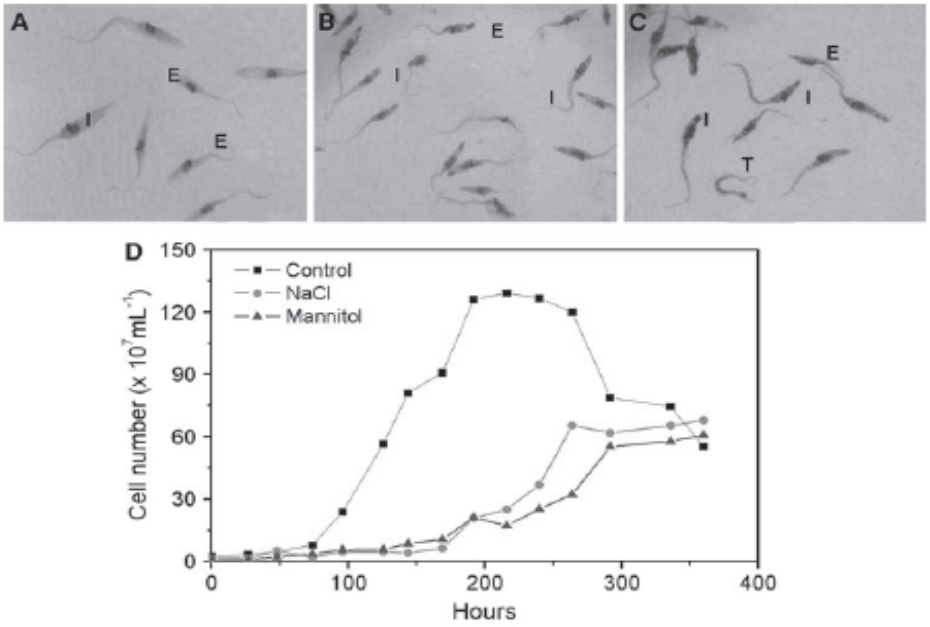
Estrés salino- formas intermedias del parásito

Antecedentes:

-Machado de Domenech EE, et al. (1992) Phospholipids of Trypanosoma cruzi: increase of polyphosphoinositides and phosphatidic acid after cholinergic stimulation. FEMS Microbiol Lett 95:267–270

-Marchesini N et al (1998) Diacylglycerol pyrophosphate: a novel metabolite in the Trypanosoma cruzi phosphatidic acid metabolism. FEBS Lett 436:377–381

-Santander V, Bollo M, Machado-Domenech E (2002) Lipid kinases and Ca2+ signaling in Trypanosoma cruzi stimulated by a synthetic peptide. Biochem Biophys Res Commun 293:314–320



Actividades PLC y lipido quinasas en formas intermedias de Tc

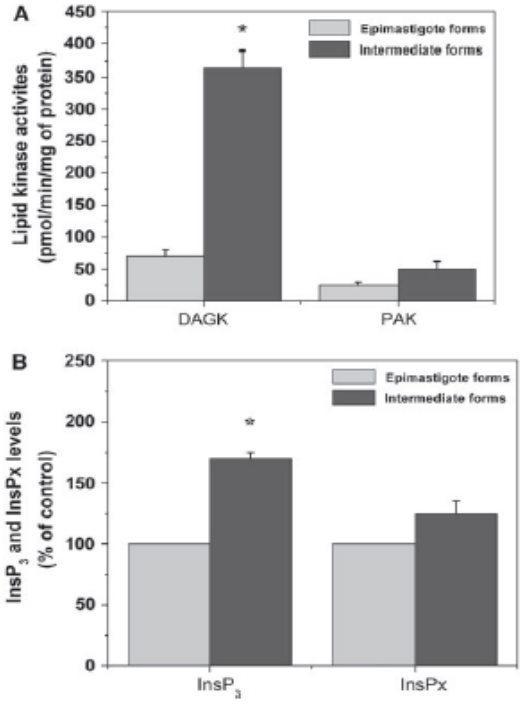
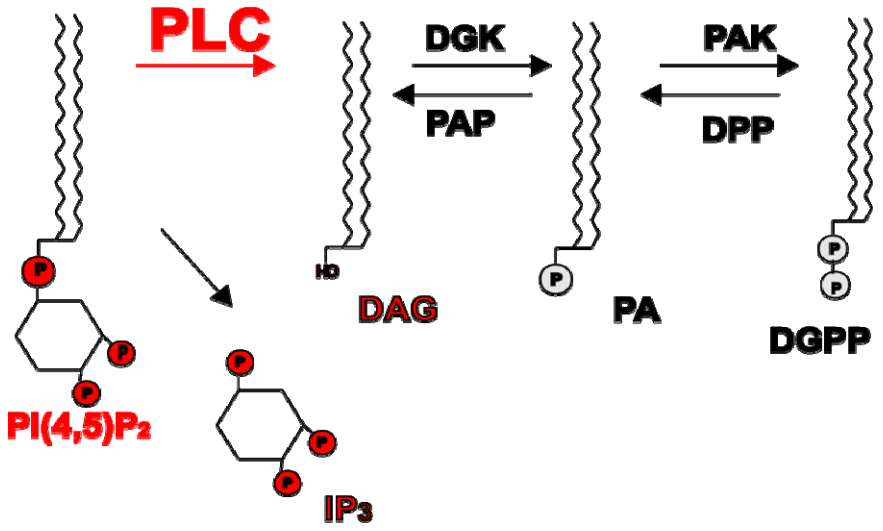


Fig. 2 Lipid kinase and phospholipase C activities. The enzymatic activities of phosphatidate kinase (PAK) and diacylglycerol kinase (DAGK) (a) were determined based on [γ-^{32}P] ATP phosphorylation of parasite membrane fractions. Values are mean ± standard error of the mean (SEM) from five independent experiments. [^{3}H] inositol-labeled cells were pre-incubated with 10 mM LiCl for 15 min. $InsP_3$ and InsPx levels (b) were analyzed as described in the "Materials and Methods" section. The results are expressed as the percentage of unstimulated control value (defined as 100%) ± SEM, $n = 4$. *$P < 0.05$



Fosfatidato fosfatasas en formas intermedias de Tc

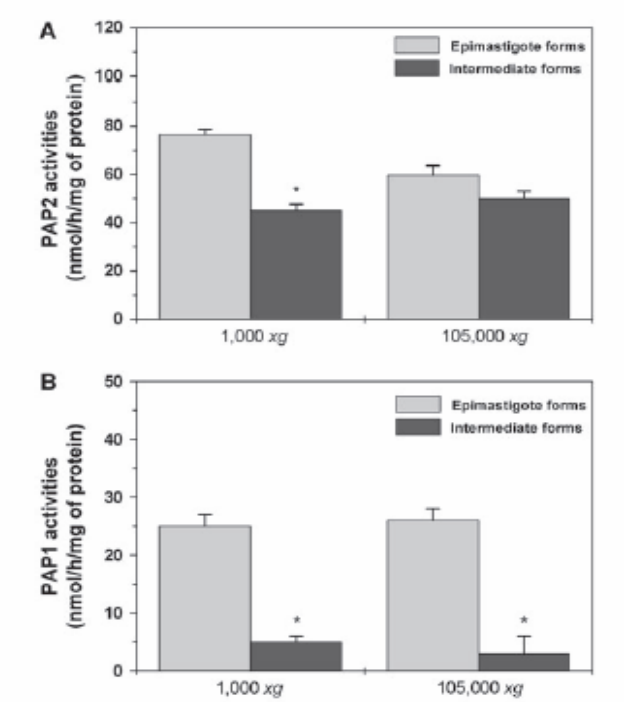
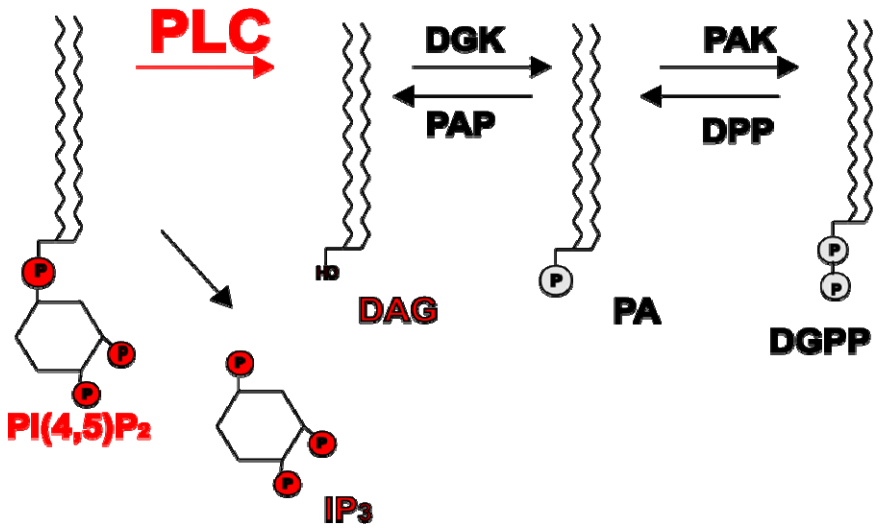


Fig. 3 The enzymatic activities of phosphatidate phosphatases type 2 (PAP2) (a) and phosphatidate phosphatases type 1 (PAP1) (b) were assayed using [^{3}H]-PtdOH (0.6 mM) plus dipalmitoyl PtdCho (0.4 mM) or [^{3}H]-PtdOH (0.6 mM) as substrates, respectively, and expressed as nmol [^{3}H]-DAG plus [^{3}H]-MAG/h/mg protein. Values are mean ± SEM, $n = 3$. $^*P < 0.05$



Diacilglicerol pirofosfato: estudios biofísicos

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

The surface organization of diacylglycerol pyrophosphate and its interaction with phosphatidic acid at the air–water interface

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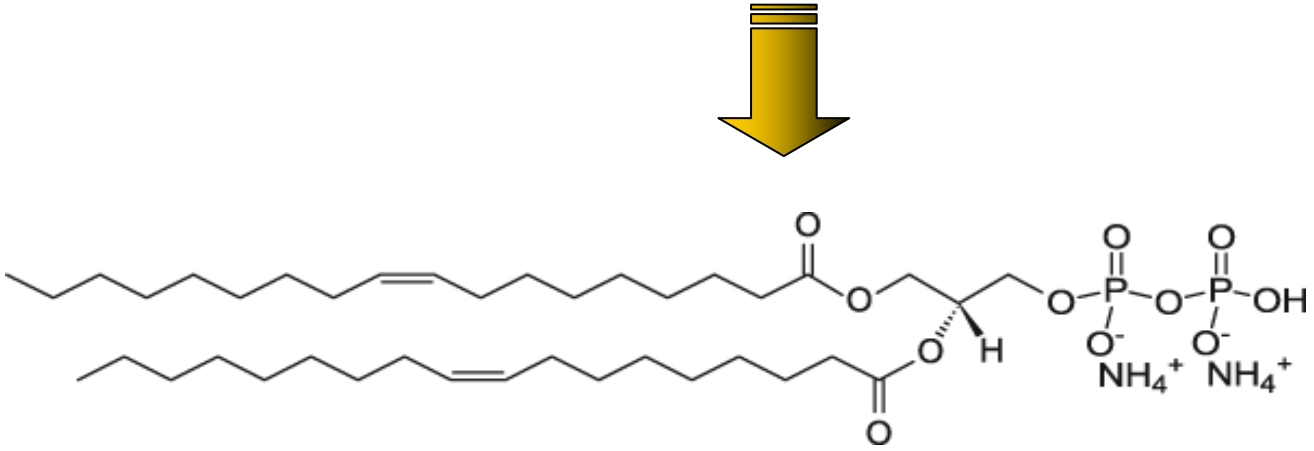
Diacilglicerol pirofosfato: estructura

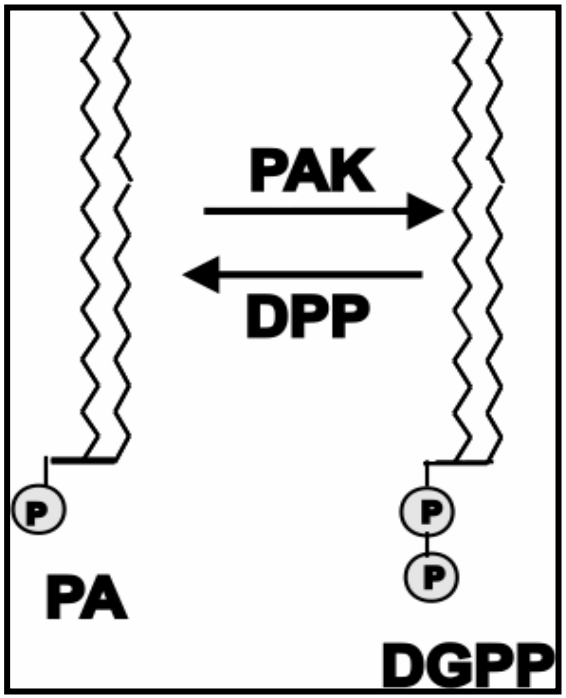
Diacylglycerol pyrophoshate:

Diacylglycerol pyrophosphate (DGPP) is a novel phospholipid found in biological membranes, with a relatively simple chemical structure within the glycerophospholipid family



Diacilglicerol pirofosfato: metabolismo

DGPP is synthesized from PA and ATP via the reaction catalyzed by PAK and dephosphorylated to PA by the enzyme DGPP phosphatase



Diacilglicerol pirofosfato: ¿dónde estás?

Both enzymes were identified in several plants, bacteria and eukaryotic microbes but in mammalian cells such phospholipid has not been identified to date.

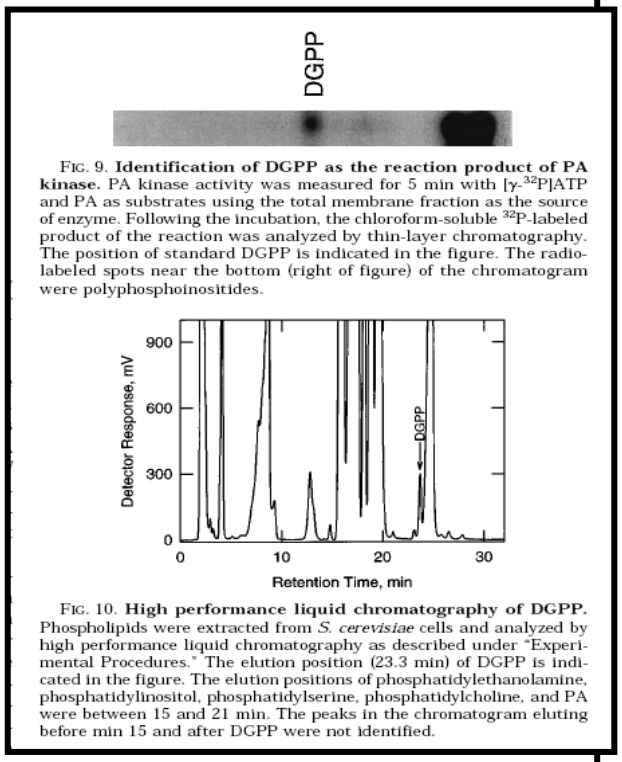
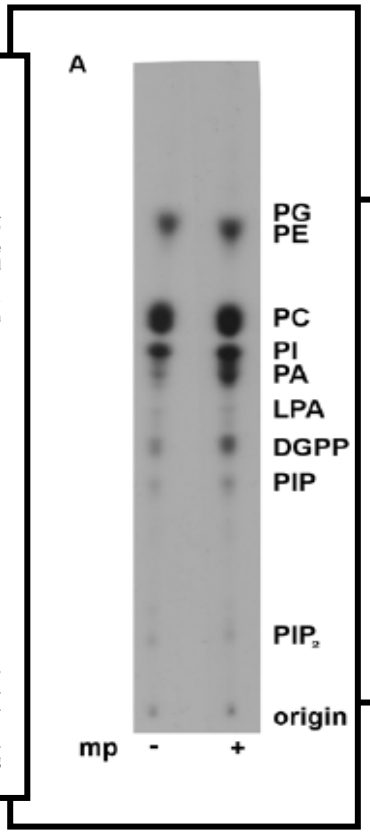


FIG. 9. **Identification of DGPP as the reaction product of PA kinase.** PA kinase activity was measured for 5 min with [γ-^{32}P]ATP and PA as substrates using the total membrane fraction as the source of enzyme. Following the incubation, the chloroform-soluble ^{32}P-labeled product of the reaction was analyzed by thin-layer chromatography. The position of standard DGPP is indicated in the figure. The radiolabeled spots near the bottom (right of figure) of the chromatogram were polyphosphoinositides.

FIG. 10. **High performance liquid chromatography of DGPP.** Phospholipids were extracted from *S. cerevisiae* cells and analyzed by high performance liquid chromatography as described under "Experimental Procedures." The elution position (23.3 min) of DGPP is indicated in the figure. The elution positions of phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, and PA were between 15 and 21 min. The peaks in the chromatogram eluting before min 15 and after DGPP were not identified.



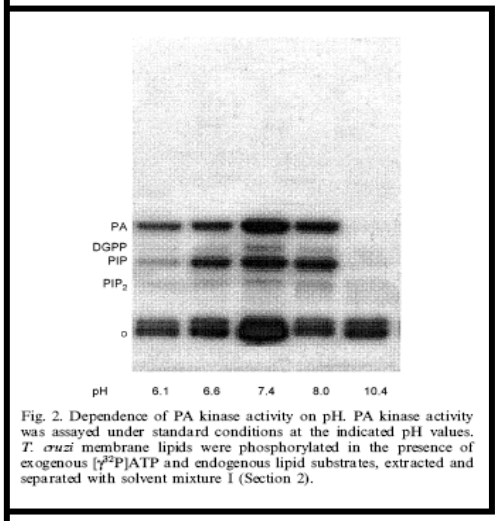


Fig. 2. Dependence of PA kinase activity on pH. PA kinase activity was assayed under standard conditions at the indicated pH values. *T. cruzi* membrane lipids were phosphorylated in the presence of exogenous [γ^{32}P]ATP and endogenous lipid substrates, extracted and separated with solvent mixture 1 (Section 2).

Diacilglicerol pirofosfato: implicancia en el estrés

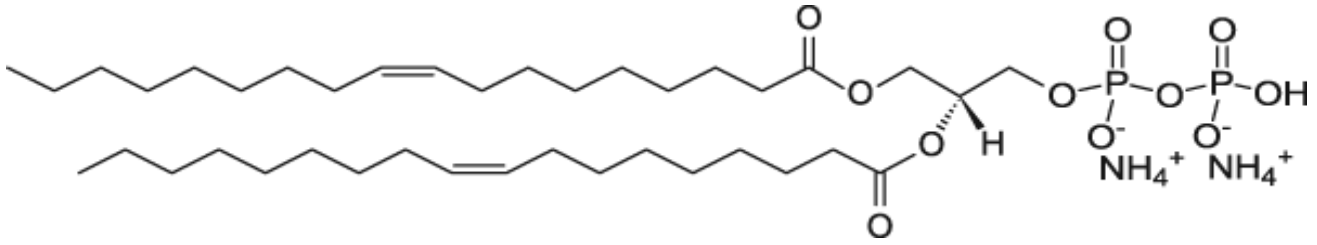
The average concentration of DGPP in cell membranes is usually very low but recent evidence suggests that DGPP may act as a novel second messenger with important roles in diverse cellular processes related to drought and osmotic **stress or salinity.**

Table 2. DGPP formation in response to different stresses

Stress	Species	Reference
Hyperosmotic	*Chlamydomonas moewusii*	[30] [19]
	Craterostigma plantagineum	[19]
	Lycopersicon esculentum suspension cells	[19]
	Medicago sativa suspension cells	[19]
	Arabidopsis thaliana suspension cells	[16]
Elicitors	*Lycopersicon esculentum* suspension cells	[21]
	Nicotiana tabacum suspension cells expressing the *Cladosporium fulvum* Cf-4^{+} resistance gene	[32]
Nod factors	*Vicia sativa* seedlings	[23]
	Medicago sativa suspension cells	[22]
Drought	*Craterostigma plantagineum*	[19]
ABA	*Arabidopsis thaliana* suspension cells	[17]
	Arabidopsis thaliana seeds	[18]
	Hordeum vulgare aleurone cells	[20]

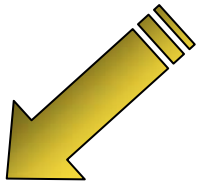
Diacilglicerol pirofosfato: mecanismo de acción

DGPP is an anionic phospholipid with a pyrophosphate group attached to diacylglycerol. It has been suggested that, depending on the pH, the pyrophosphate moiety of DGPP could display 2 or 3 negative charges making it a highly polar molecule. Although it has not been demonstrated *in vivo*, it has also been suggested that the pyrophosphate group may play an important role for electrostatic interactions between DGPP and proteins as well as bivalent cations like Zn2+ and Ca2+.



Diacilglicerol pirofosfato: al menos dos alternativas

Mechanism of DGPP action in diverse processes is not yet clear, although two possibilities are likely.



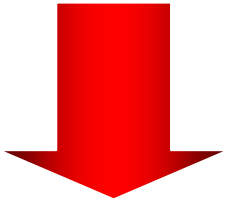
DGPP may function through the activation/recruitment of effector proteins by direct interaction.

and/or by a modulation of membrane properties such as packing, curvature and electrostatics.

Hence, it is of great importance to first understand the interfacial packing and electrostatic behavior of this bioactive lipid as well as the interaction of this molecule with its precursor.

Diacilglicerol pirofosfato: estudios biofísicos

Little is known about the interaction of PA with DGPP and if these lipids can molecularly mix, with or without interactions that may modify their individual properties.



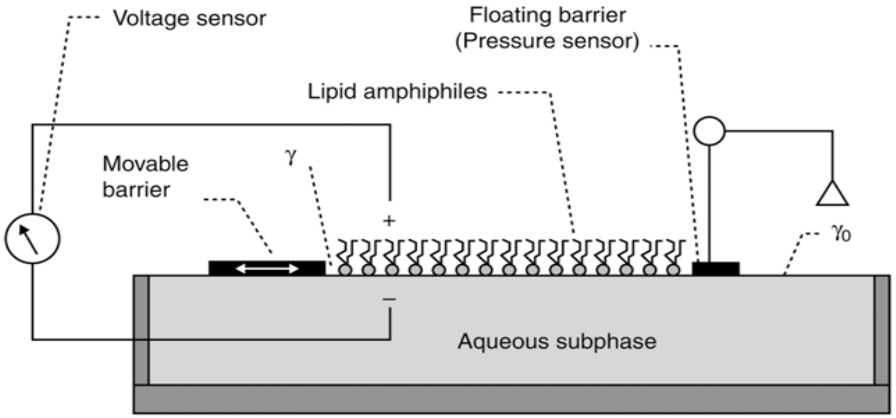
Molecular packing, in-plane elasticity, and surface electrostatic of films of pure DGPP, and on the variation of those properties that occur as a consequence of intermolecular interactions between DGPP and PA in mixed monolayers at the air-water.

Diacilglicerol pirofosfato: carga del grupo pirofosfato

Taking the pyrophosphoric acid pKa's (The pyrophosphoric acid exhibits four pKa's values: pKa1= 0.91, pKa2= 2.10, pKa3= 6.70, pKa4= 9.32 (Lide, 2005).) in consideration, we performed compression isotherms on subphases at pH 5 and at pH 8 with the aim of analyzing the effect of charge on the molecular packing behavior.

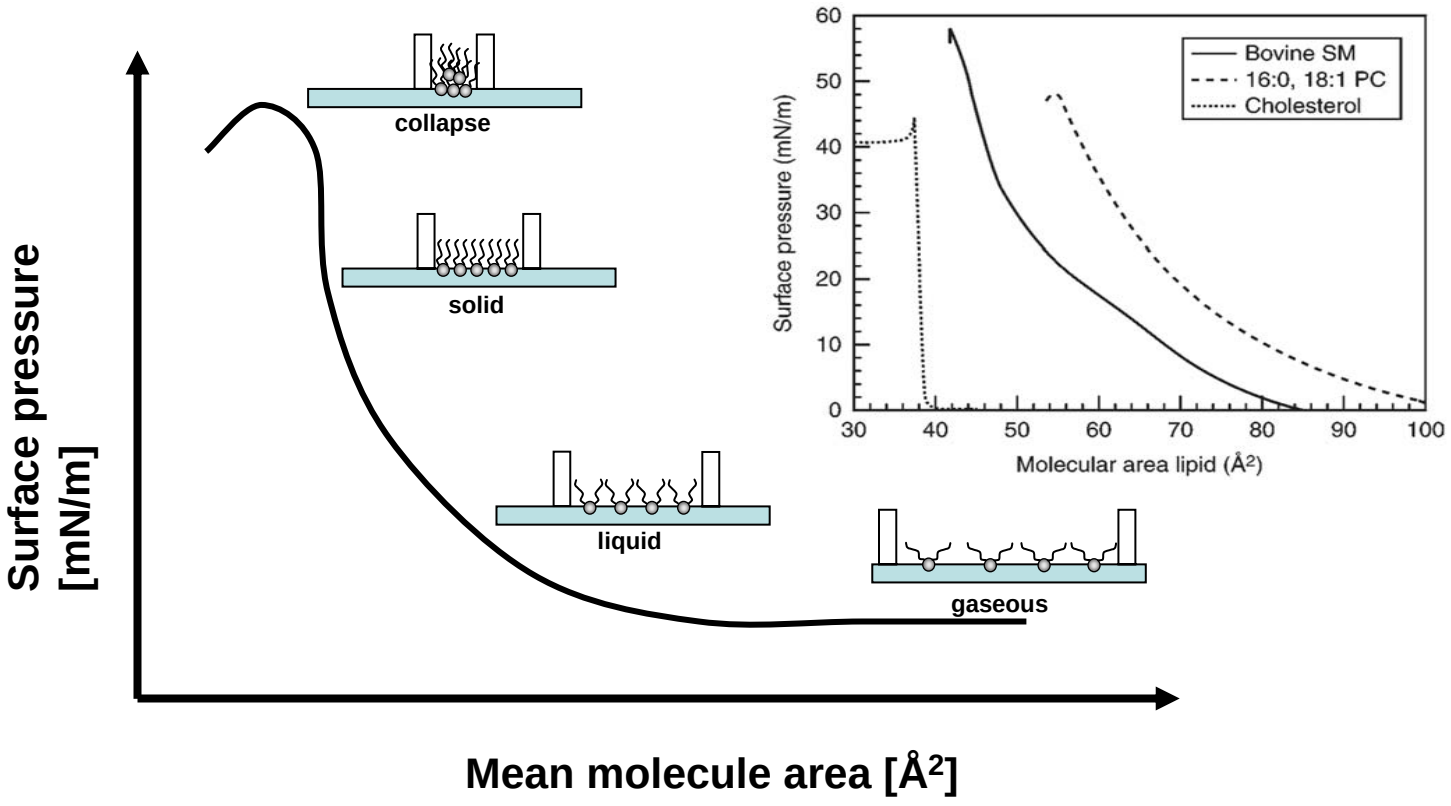
Diacilglicerol pirofosfato: Langmuir film

Langmuir-type surface balance

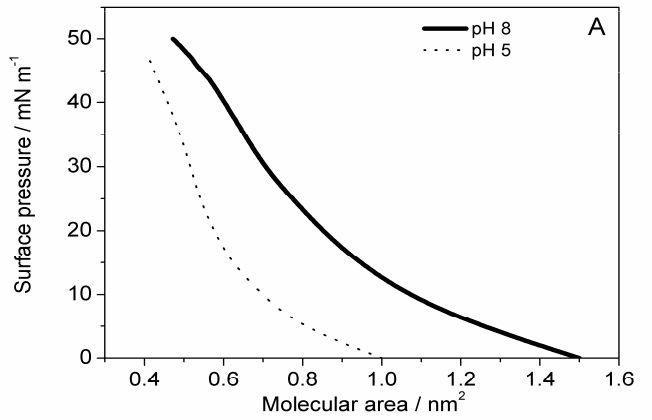


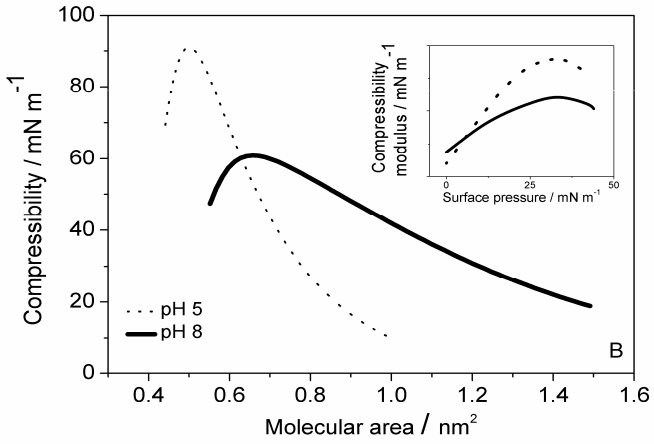
Langmuir-type surface balance for determining the surface pressure and surface potential as a function of average cross-sectional molecular area. γ is the surface tension of surface occupied by lipid amphiphile and γ0 is the surface tension of clean aqueous subphase.

Isoterma


collapse
solid
liquid
gaseous
Surface pressure [mN/m]
Mean molecule area [Å²]
Surface pressure (mN/m)
Molecular area lipid (Å²)
Bovine SM
16:0, 18:1 PC
Cholesterol
0
10
20
30
40
50
60
30
40
50
60
70
80
90
100

Compression isotherms for monolayers of diacylglycerol pyrophosphate on subphases at the indicated pHs.





- **DGPP forms liquid-expanded monolayers with no detectable pressure-area reorganization during the compression.**

- **The lateral pressure increases monotonically with compressibility values from 20 mN m-1 to 60 mN m-1 (Fig. B). The monolayer collapses at 43 mN m-1 and 0.57 nm2 and the lift off area is 1.5 nm2.**

- **Lowering the subphase pH causes a diminution in the average molecular area (Fig. A) and an increase of the compressibility modulus (Fig. B) at lateral pressures higher than 5-10 mN m-1 (see inset in Fig. 2 B), with a slightly reduced collapse pressure (collapse point: 40 mN m-1 at 0.46 nm2). The lift off area at this pH is 1 nm2.**

- **The compressibility modulus ranges from 10 mN m-1 to 90 mN m-1, also indicating a liquid-expanded behavior (Davies and Rideal, 1963) that becomes more condensed under compression above 5-10 mN m-1, compared to the behavior at pH 8.**

pH versus comportamiento interfacial

The compression isotherms of monolayers for a particular lipid species depends on:

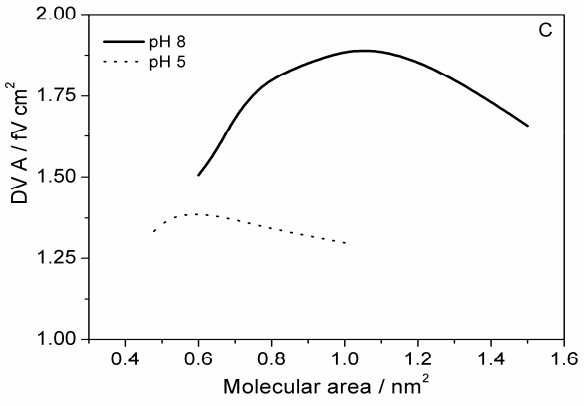
- the length and unsaturation of the hydrocarbon chain
- on the bulkiness
- charge of the polar headgroup.

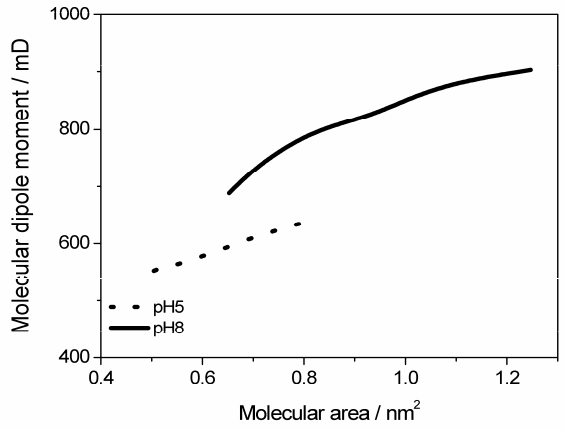
•Long and saturated hydrocarbon chains would interact through Van der Waals attractions, promoting more condensed monolayers.

•By contrast, ionization of the lipid head groups should result in repulsive interactions, leading to loosely packed monolayers (Brown and Brockman, 2007).

Thus, the observed compression isotherms of DGPP at pH 5 and 8 are in agreement with an increased electrostatic repulsion at pH 8 where a higher net charge (about -2.2 at pH 8 and about -1.8 at pH 5) is expected, according to the pKa's values for the pyrophosphate acid.

Surface potential density as a function of the average molecular area.

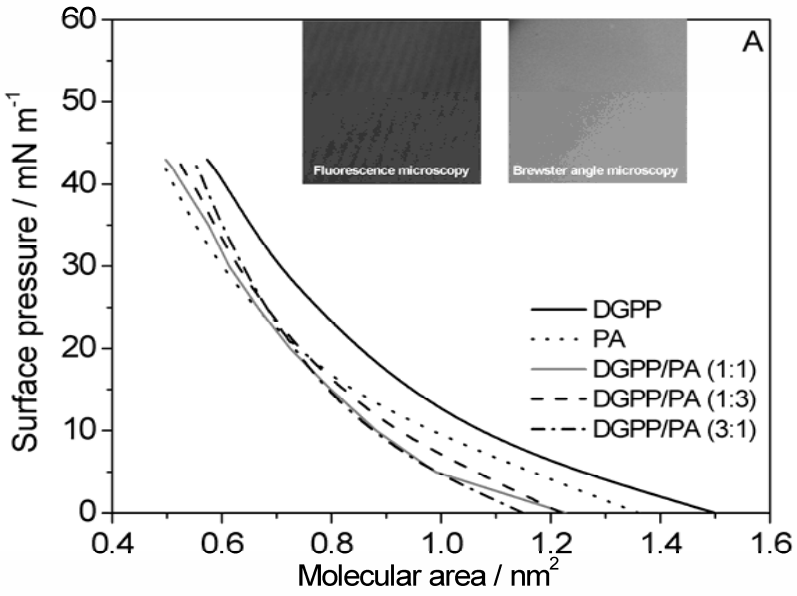




Resultant perpendicular dipole moment as a function of the mean molecular area at different pHs.

In biological membranes, DGPP formation after stimulus takes place after a transient increase of the PA levels (Munnik et al., 1995). As a consequence, a temporary and local accumulation of DGPP and its precursor in the membrane is expected. This may affect the phospholipid packing during signaling processes. Since the monolayer packing properties are affected by the interactions with neighboring lipids (Maggio, 2004).

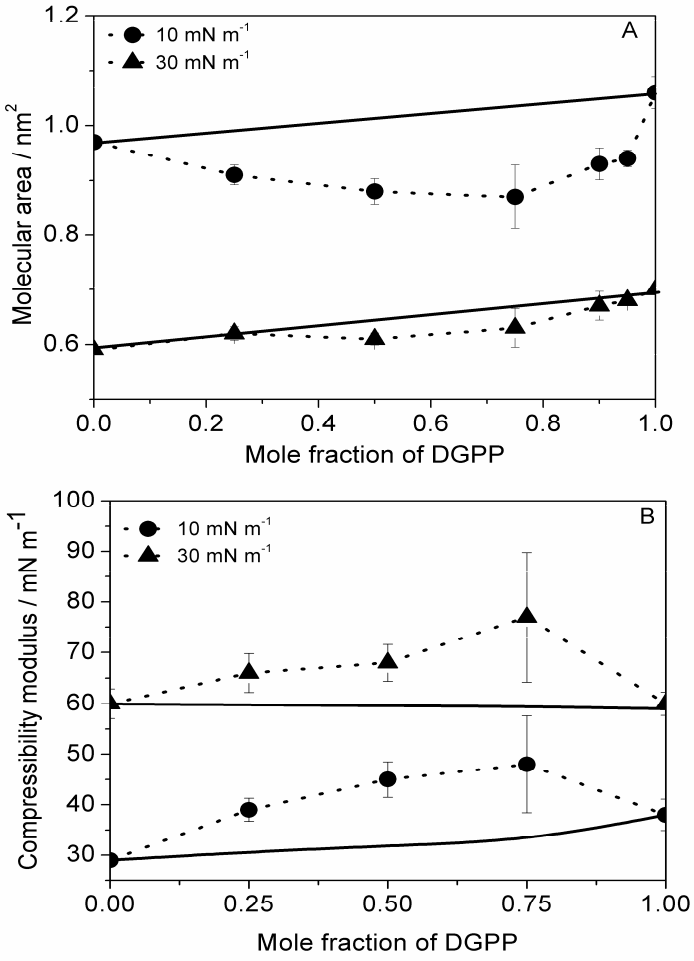
Compression isotherms for monolayers of DGPP, PA and their mixtures on subphases at pH 8



• The mixtures are homogeneous on the micron scale (0.25 μm2), as revealed by either FM or BAM indicating that there is no formation of microscopic phase-segregated domains.

•Mixed films of DGPP with its precursor PA also form liquid-expanded films

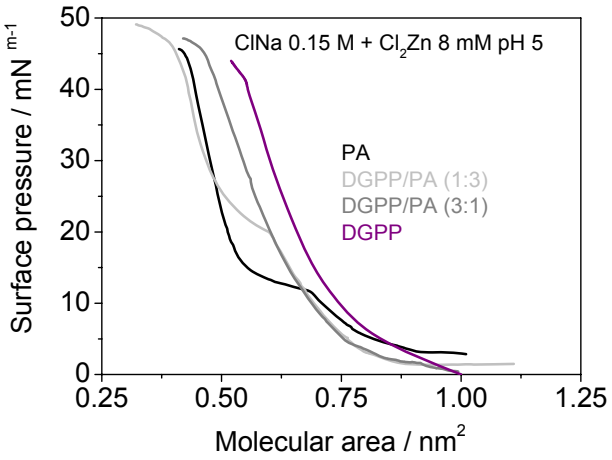
Compression isotherms for monolayers of DGPP, PA and their mixtures on subphases at pH 8

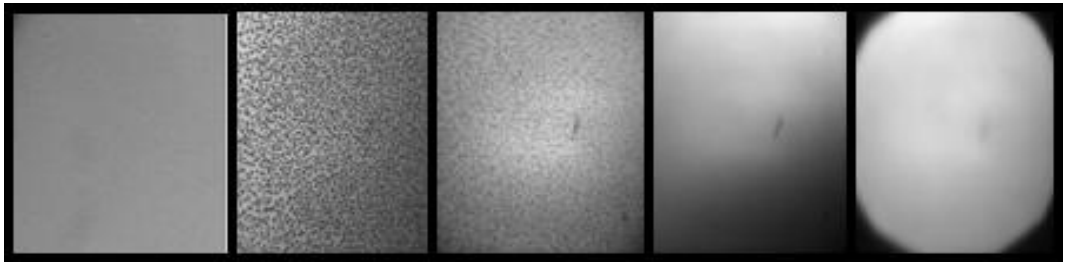


Mixed films of DGPP with PA form liquid expanded films (Fig. 4A). However, the compression isotherms are shifted to lower average molecular areas, compared to either of the pure lipids at lateral pressure below 20mNm−1. At higher lateral pressures, the mean molecular areas of the mixed films approach to those of DGPP and are higher than those of PA. This leads to an increased compressibility modulus of the mixtures compared to films of the pure lipids. The packing behavior of the mixed films is non ideal at low lateral pressures.

DGPP/ PA mezclas a pH 5 en presencia de un catión

Zn2**+** induces on monolayers of POPA a phase transition from a liquid-expanded to liquid-condensed at pH 5. The phase transition pressure increases as DGPP is added to the mixture and becomes less noticeable until it is no longer observed (DGPP mole% higher than 50%).





POPA (subphase without Zn2+) **POPA** **DGPP/POPA 1:3** **DGPP/POPA 3:1** **DGPP**

Surface pressure 20 mN m-1

A) Monolayer of DGPP and their mixture with POPA in presence of ZnCl2 at pH 5. B) Representative fluorescent microscopy photos for monolayers on ZnCl2 pH 5 formed by mixtures of POPA with 0.25 and 0.75 mole % of DGPP. 20 mN m−1 during compression. Photos size: 200×200 μm.

Conclusiones finales

Our results indicate that the surface behavior of the individual lipids can be modified by changes of the relative lipid proportions, indicating their inherent capability for transducing membrane events through dynamic variations of molecular packing, in-plane elasticity, electrostatic interactions and compositional changes.

All these effects can constitute structural-electrostatic signaling events involving DGPP that may be sensed both along themembrane surface and into the surrounding aqueous environment whereupon regulate the recognition and activity of bioactive ligands.

ABA y PAP2 en Arabidopsis thaliana

Plant Physiology and Biochemistry xxx (2011) 1–6

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Short communication

Arabidopsis thaliana lipid phosphate phosphatase 2 is involved in abscisic acid signalling in leaves

Sophie Paradis [a], Ana Laura Villasuso [b], Susana Saez Aguayo [a,1], Régis Maldiney [a], Yvette Habricot [a], Christine Zalejski [a,2], Estela Machado [a], Bruno Sotta [a], Emile Miginiac [a], Emmanuelle Jeannette [a,*]

[a] *Université Pierre et Marie Curie, Laboratoire de Physiologie Cellulaire et Moléculaire des Plantes, Unité de Recherche 5 - Equipe d'Accueil 7180 du Centre National de la Recherche Scientifique, case 156, 4 place Jussieu, 75252 Paris cedex 05, France*

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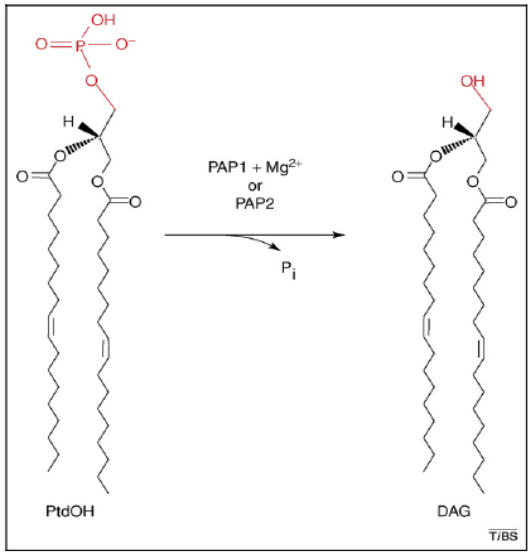
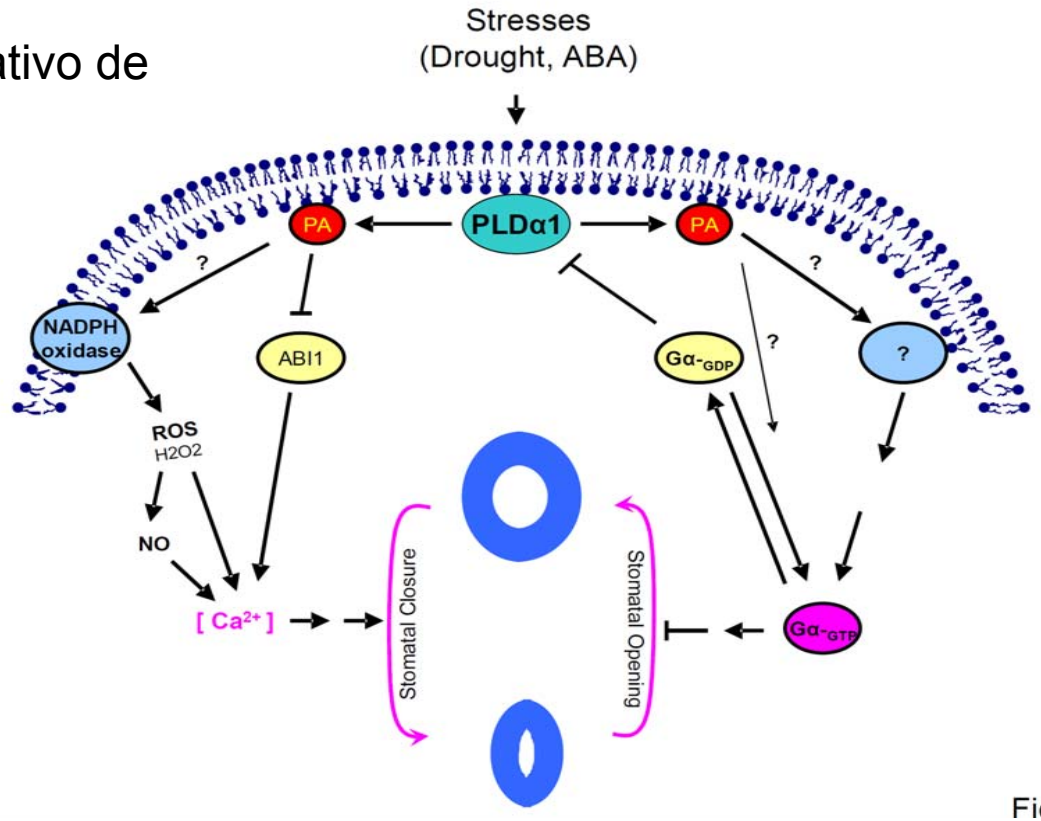
ABA y PAP2 en Arabidopsis thaliana

At contiene 5 genes PAP1 ortologos a los de cianobacterias y 4 genes PAP2/LPP

LPP2-2 participa en control negativo de la germinación por ABA

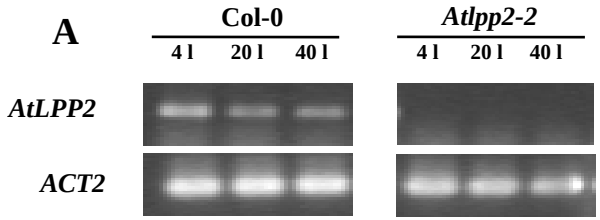




Fi

ABA y PAP2 en *Arabidopsis thaliana*

Objetivo: estudiar el papel de LPP2 en la respuesta a ABA mediante una mutante Atlpp2-2 que contiene una T-DNA inserción en el gen de AtLPP2



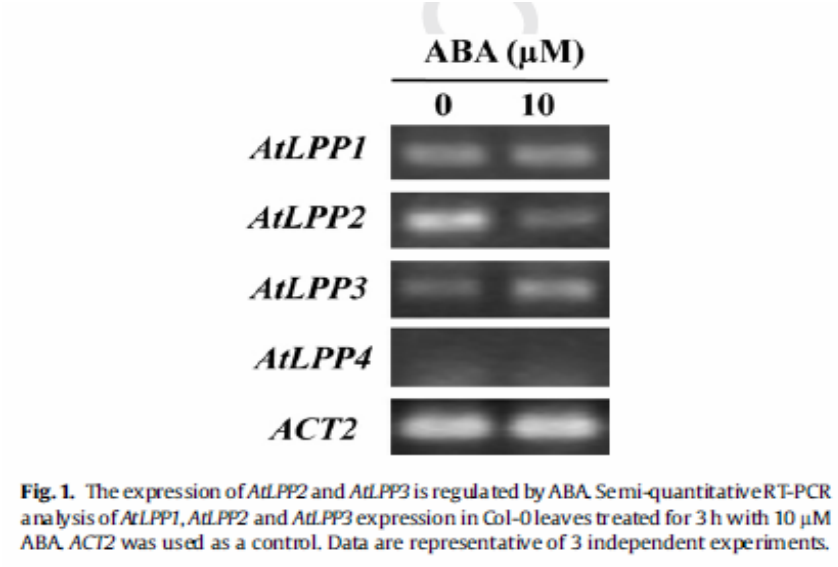
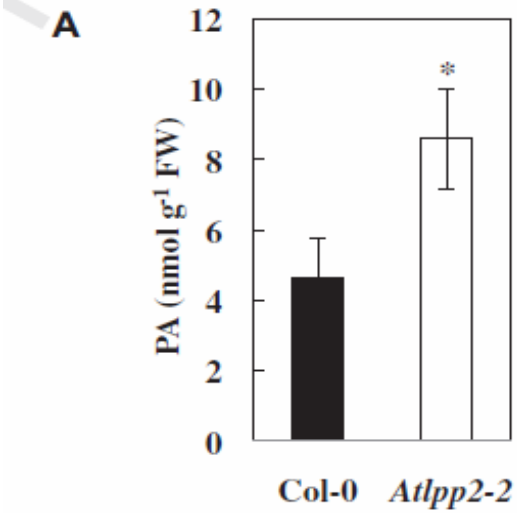
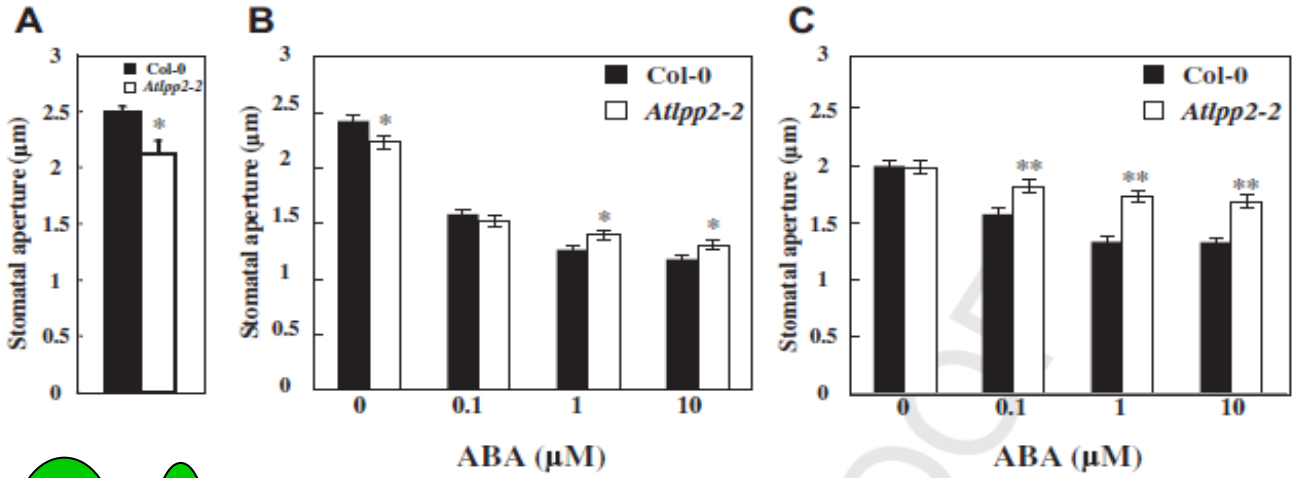
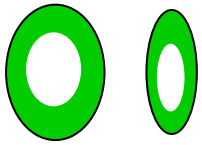


Fig. 1. The expression of *AtLPP2* and *AtLPP3* is regulated by ABA. Semi-quantitative RT-PCR analysis of *AtLPP1*, *AtLPP2* and *AtLPP3* expression in Col-0 leaves treated for 3 h with 10 µM ABA. *ACT2* was used as a control. Data are representative of 3 independent experiments.

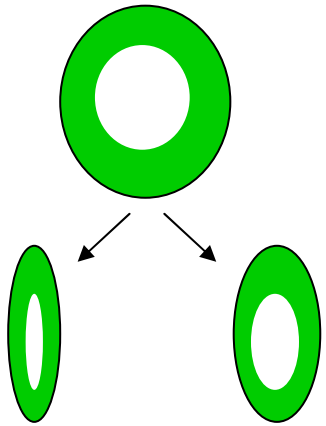


Respuesta al ABA en la célula de la guarda

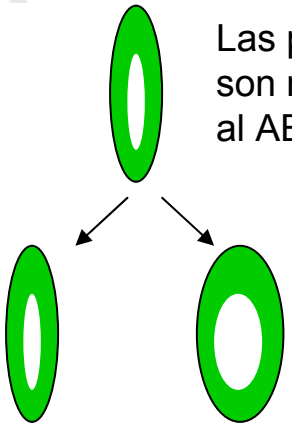




Células de la guarda



Col-0 *LPP2-2*



Las plantas Atlpp2-2 son menos sensibles al ABA

Col-0 *LPP2-2*

Steve Jobs

Tu tiempo es limitado, de modo que no lo malgastes viviendo la vida de alguien distinto.

No quedes atrapado en el dogma, que es vivir como otros piensan que deberías vivir.

No dejes que los ruidos de las opiniones de los demás acallen tu propia voz interior.

Y, lo que es más importante, ten el coraje para hacer lo que te dicen tu corazón y tu intuición.