



# HPTLC quantitative et lipidomique végétale

P. MOREAU - Laboratoire de Biogenèse Membranaire UMR 5200

## HPTLC / Quantification



**Métabolisme  
des PLs**

- **PC/PE Racines**
- **Transport de PS**

**Transport des Lipides /  
Morphologie des organelles**

- **Effet de la BFA**
- **Effet du Fenpropimorph**

**Domaines  
membranaires**

- **Voie sécrétoire**
- **Polarité cellulaire**



# HPTLC quantitative et lipidomique végétale

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## HPTLC / Lipidomique végétale

### Extraction des Lipides

#### Analytique

#### Préparatif

- Dépôts HPTLC en ligne (Linomats, ATS4)
- Solvants / Espèces lipidiques

- Colonnes (familles de lipides)
- HPTLC / Espèces lipidiques

#### Quantification en Masse

- Révélatons / Densitométrie
- Révélatons / CPG des AG

#### Quantification Radioactivité

(PhosphorImager)

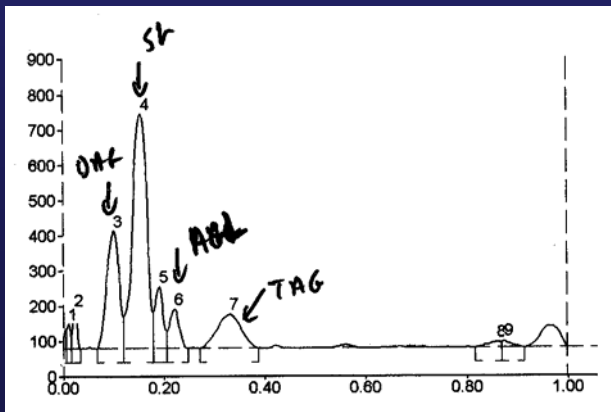
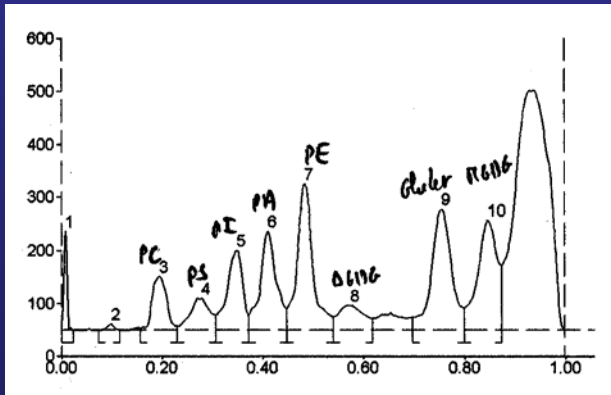
#### Identification et Quantification en Spectrométrie de masse

# HPTLC quantitative et lipidomique végétale

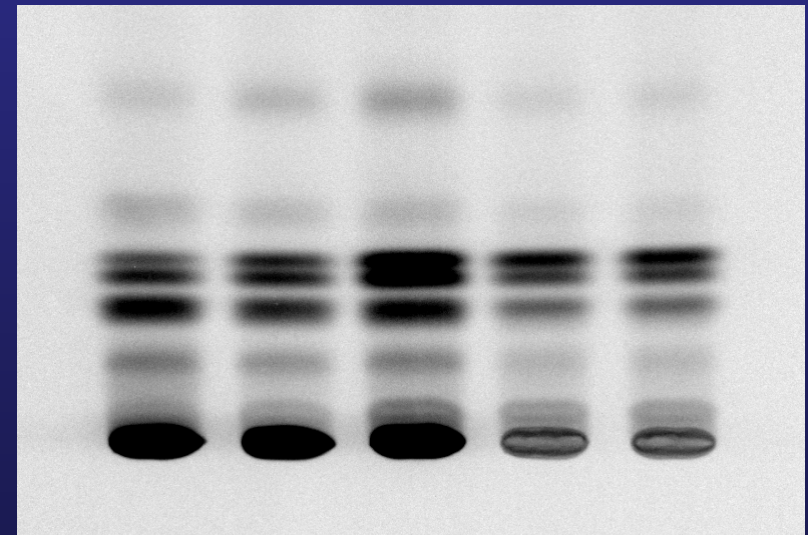
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## Quantification en Masse

- Révélations / Densitométrie (TLC Scanner III CAMAG)
- Révélations / CPG des AG



## Quantification Radioactivité (PhosphorImager)

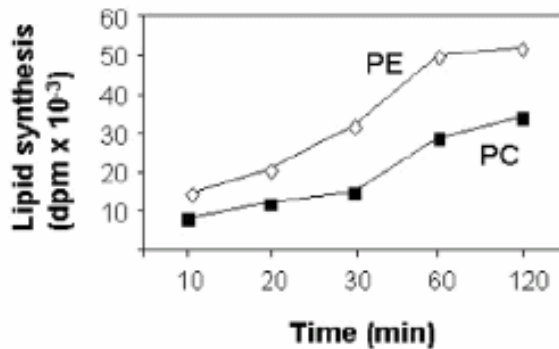


# Métabolisme des Phospholipides

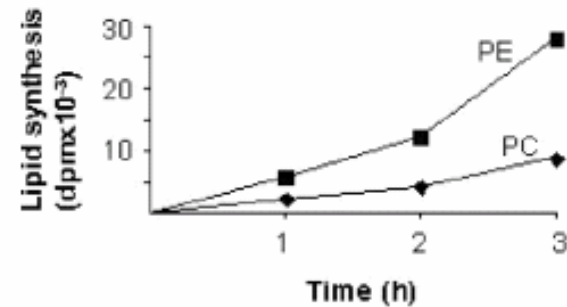
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## La synthèse de PC et PE dans les racines est différente

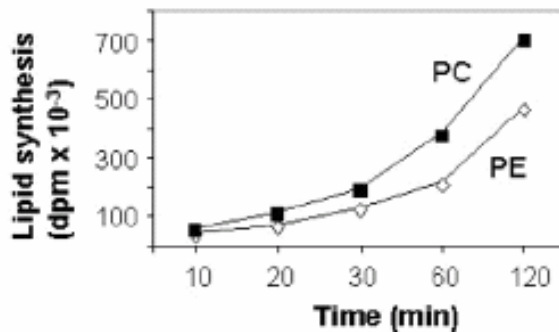
PC and PE synthesis in Leek roots



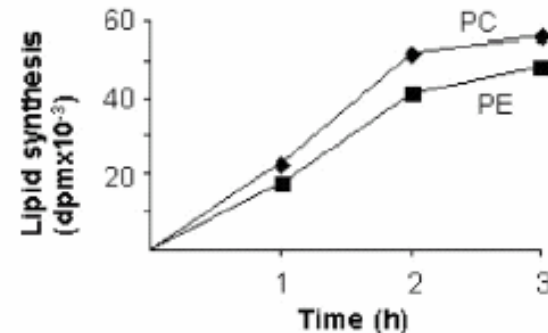
PC and PE synthesis in *A.thaliana* roots



PC and PE synthesis in Leek shoots



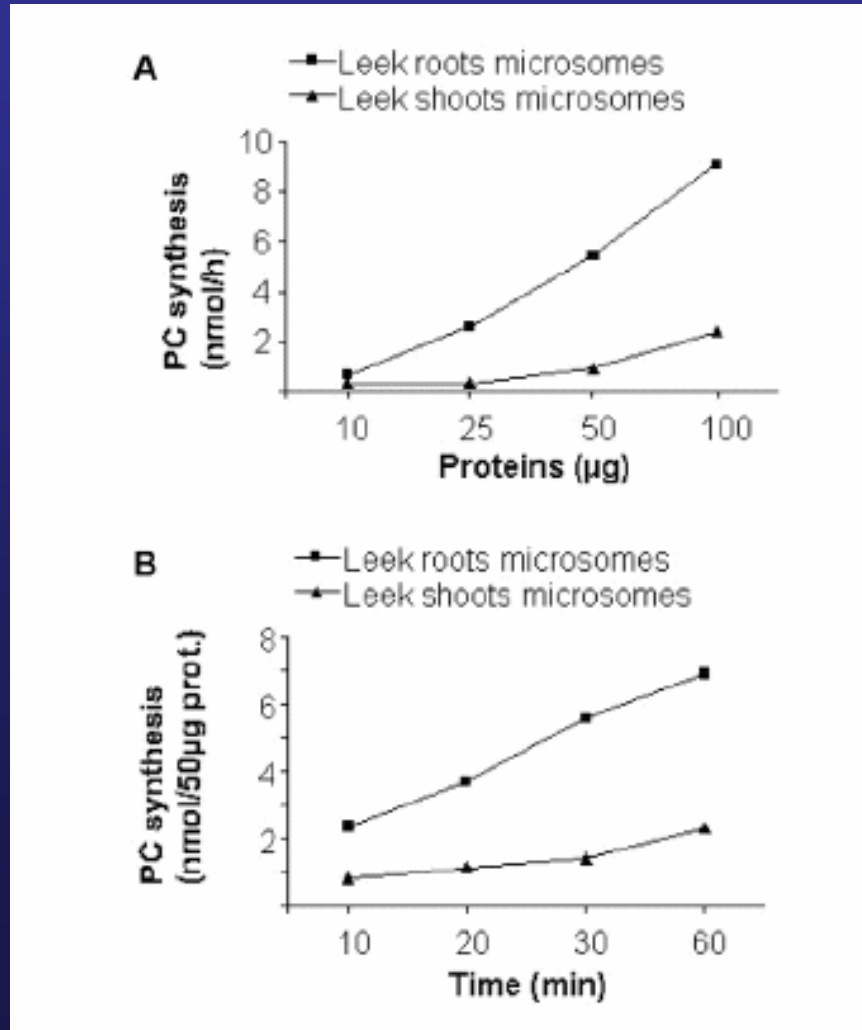
PC and PE synthesis in *A.thaliana* shoots



# Métabolisme des Phospholipides

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## La synthèse de PC est opérationnelle dans les racines

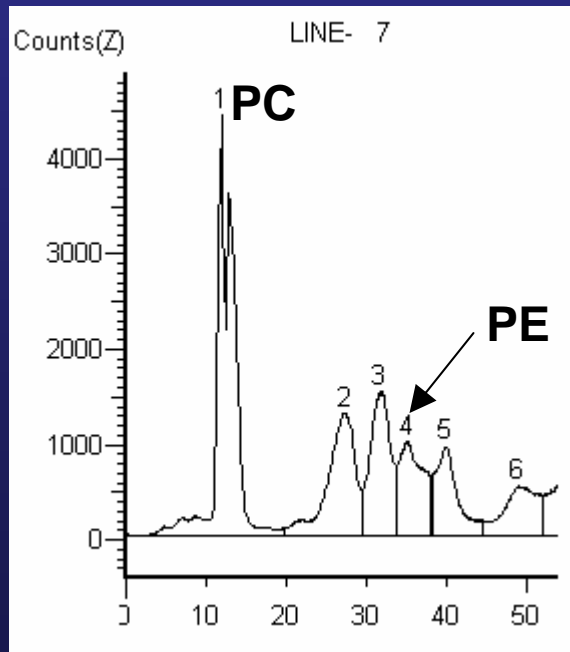


# Métabolisme des Phospholipides

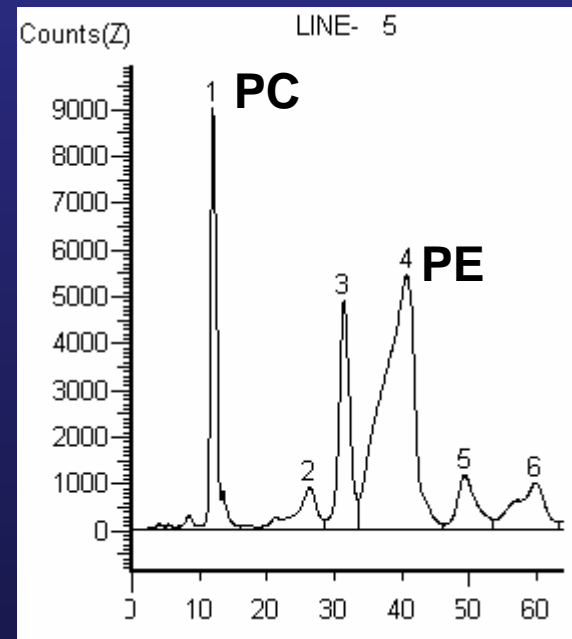
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## Forte acylation de PE dans les racines

*A. Thaliana* shoots



*A. Thaliana* roots





# Transport des Lipides

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## Composition Lipidique des Membranes de la Voie Sécrétoire

	PA	PC	PE	PG	PI	PS
<b>ER</b>	<b>4.0</b>	<b>35.3</b>	<b>21.3</b>	<b>5.1</b>	<b>4.4</b>	<b>2.4</b>
<b>Golgi</b>	<b>4-9</b>	<b>18.9</b>	<b>16.7</b>	<b>3.9</b>	<b>7.3</b>	<b>2.8</b>
<b>Plasma Membr.</b>	<b>0-8</b>	<b>15.8-18.4</b>	<b>14.2-21.2</b>	<b>0.8-5.3</b>	<b>1.9-4.6</b>	<b>2.7-4.8</b>

**Table 1.** Sterol composition of membrane fractions isolated from 7-d-old etiolated leek seedlings

The values are from two independent lipid analyses.

Membrane Fraction	Phospholipids $\mu\text{g} \cdot \text{mg}^{-1} \text{ protein}$	Sterols	Molar Ratio of Sterols to Phospholipids	Relative Sterol Compositions <sup>a</sup>				
				Ch	24-M	St	Si	Is
ER	580	8.8	0.025	10.5	8.5	5.0	69.5	6.5
Golgi	500	18.9	0.065	8.5	8.0	3.0	66.5	14.0
PM	420	45.5	0.18	5.0	8.0	3.0	61.5	17.0

<sup>a</sup> Ch, cholesterol; 24-M, 24-methylcholesterol; St, stigmasterol; Si, sitosterol; Is, isofucoesterol.



# Transport des Lipides

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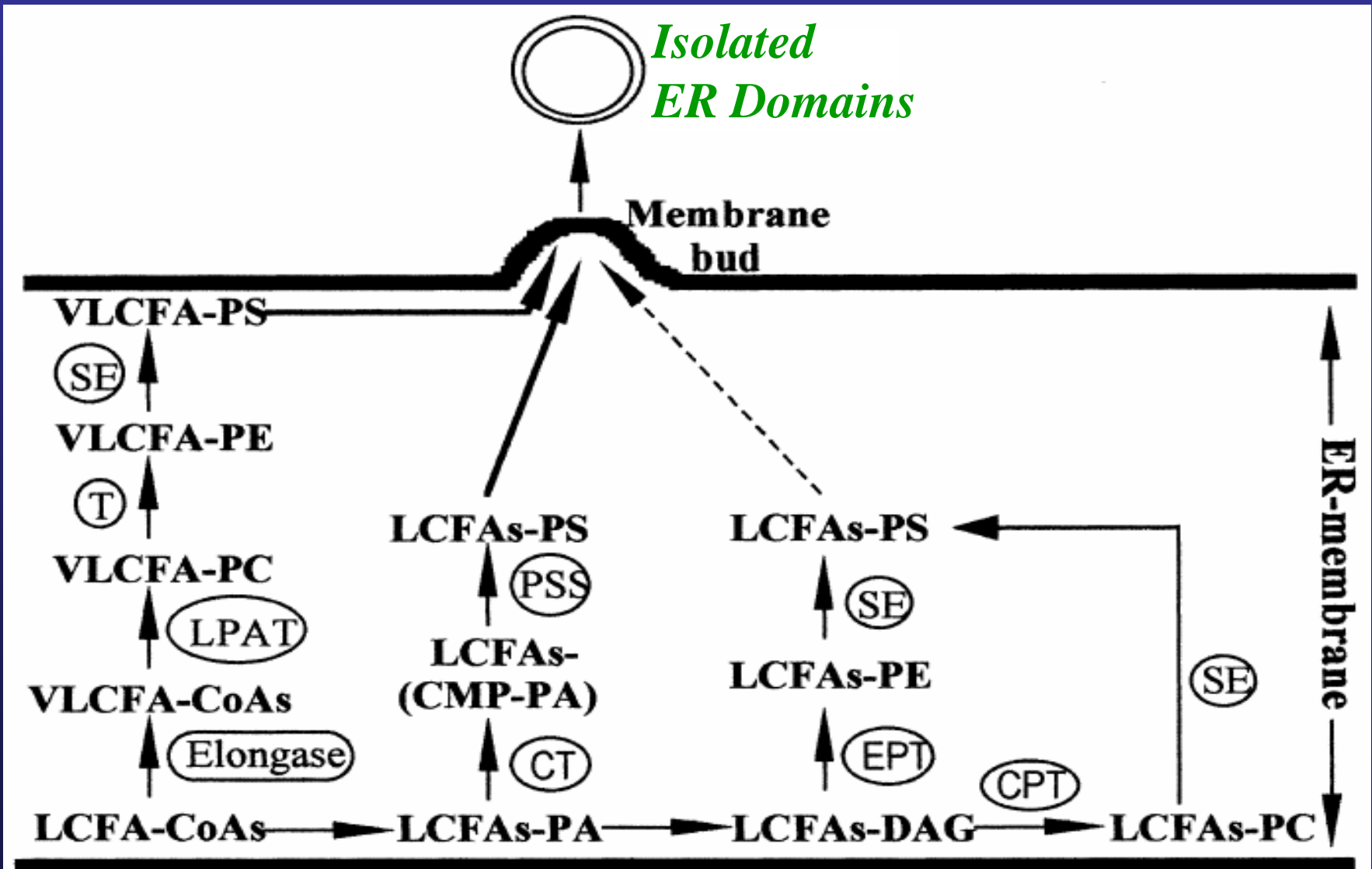
## VLCFA-PS in ER-derived vesicles

Phospholipid and Fatty Acid (Carbon Chain Length)	ER	TV(+)
	<i>% of total</i>	
PC		
16	22.7 ± 0.7	24.5 ± 3.6
18	74.0 ± 1.4	71.6 ± 4.4
VLCFA	3.3 ± 0.8	3.9 ± 2.4
PE		
16	34.1 ± 1.3	37.0 ± 2.3
18	59.7 ± 1.4	58.1 ± 2.8
VLCFA	6.2 ± 2.4	4.9 ± 3.3
PS		
16	28.0 ± 4.2	20.8 ± 2.5
18	58.4 ± 3.5	45.1 ± 3.4
VLCFA	13.6 ± 4.1	<b>34.1 ± 4.4</b>



# Transport des Lipides

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# Effet de la BFA sur le Golgi

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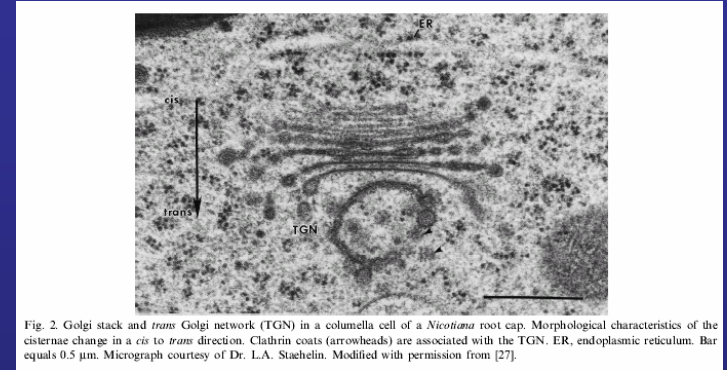
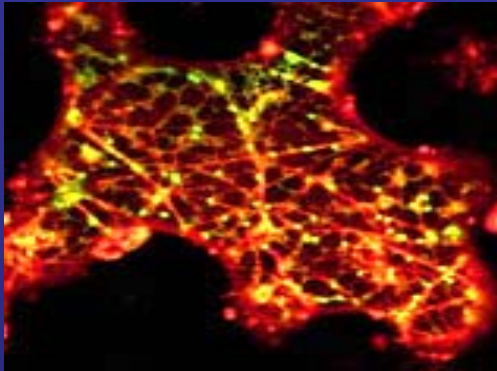
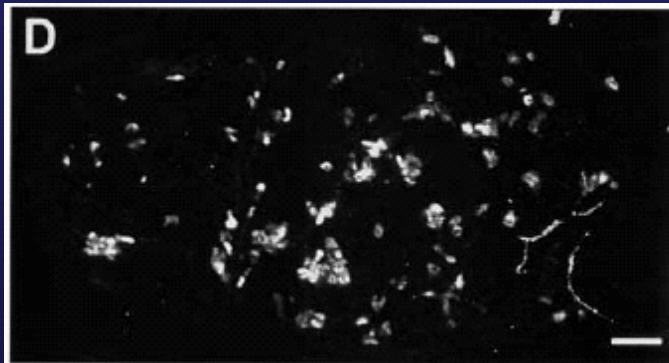
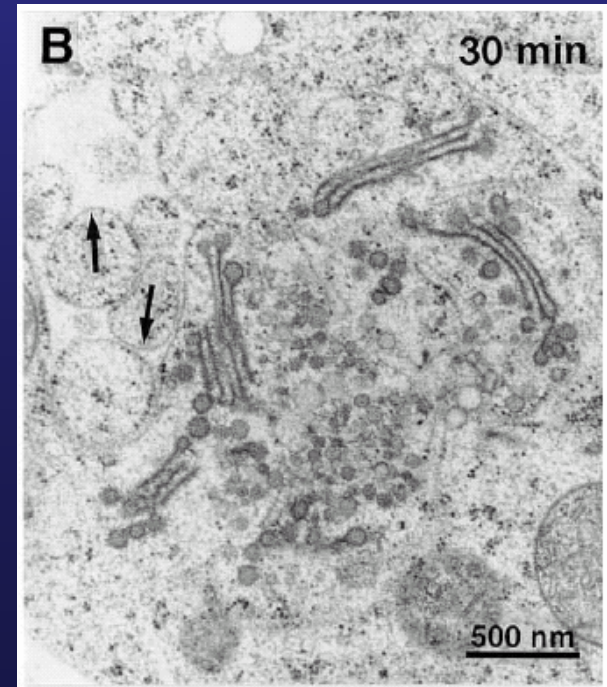
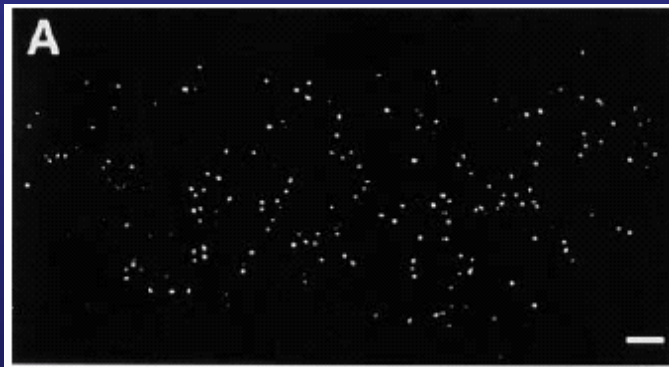


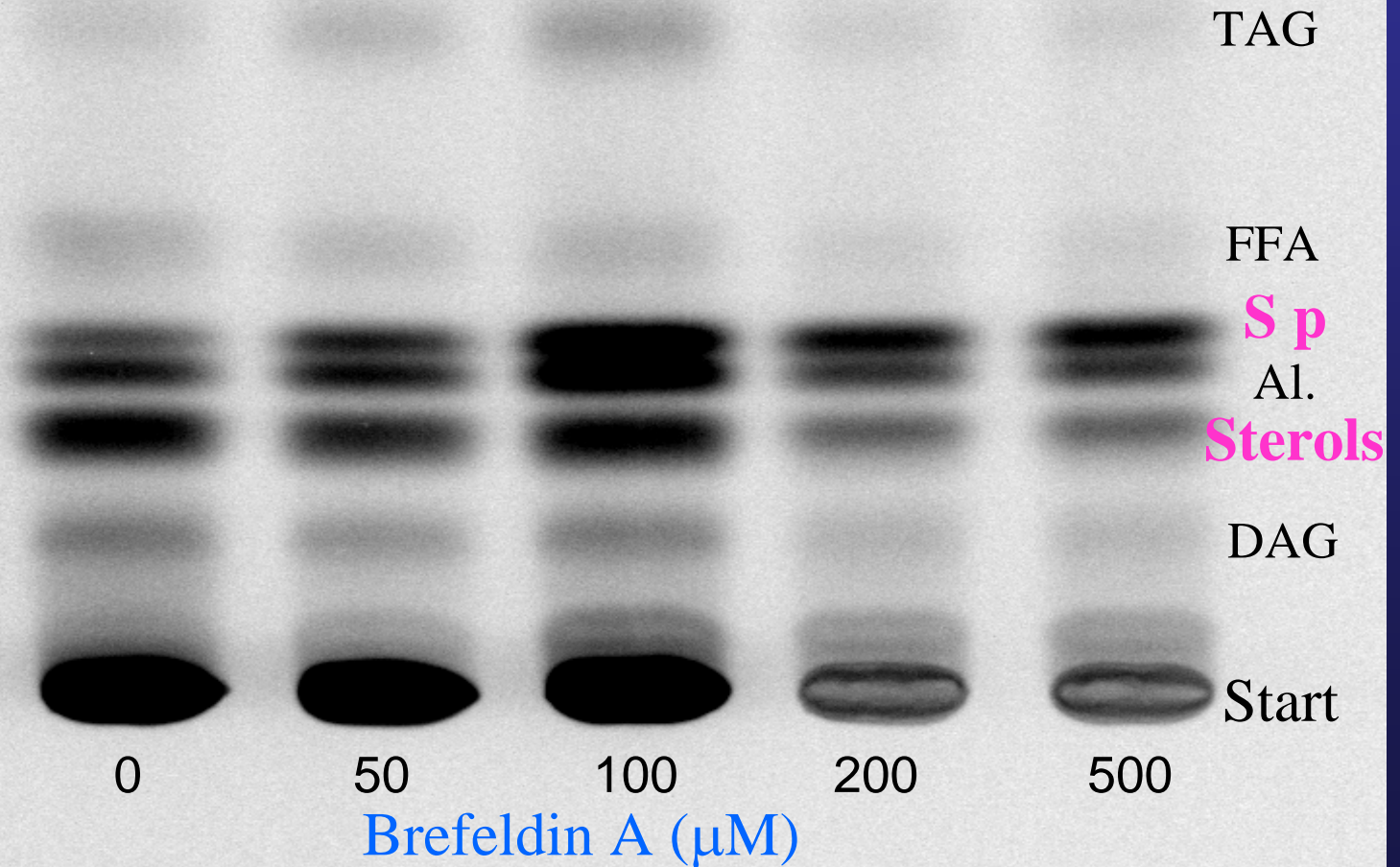
Fig. 2. Golgi stack and *trans* Golgi network (TGN) in a columella cell of a *Nicotiana* root cap. Morphological characteristics of the cisternae change in a *cis* to *trans* direction. Clathrin coats (arrowheads) are associated with the TGN. ER, endoplasmic reticulum. Bar equals 0.5  $\mu$ m. Micrograph courtesy of Dr. L.A. Staehelin. Modified with permission from [27].

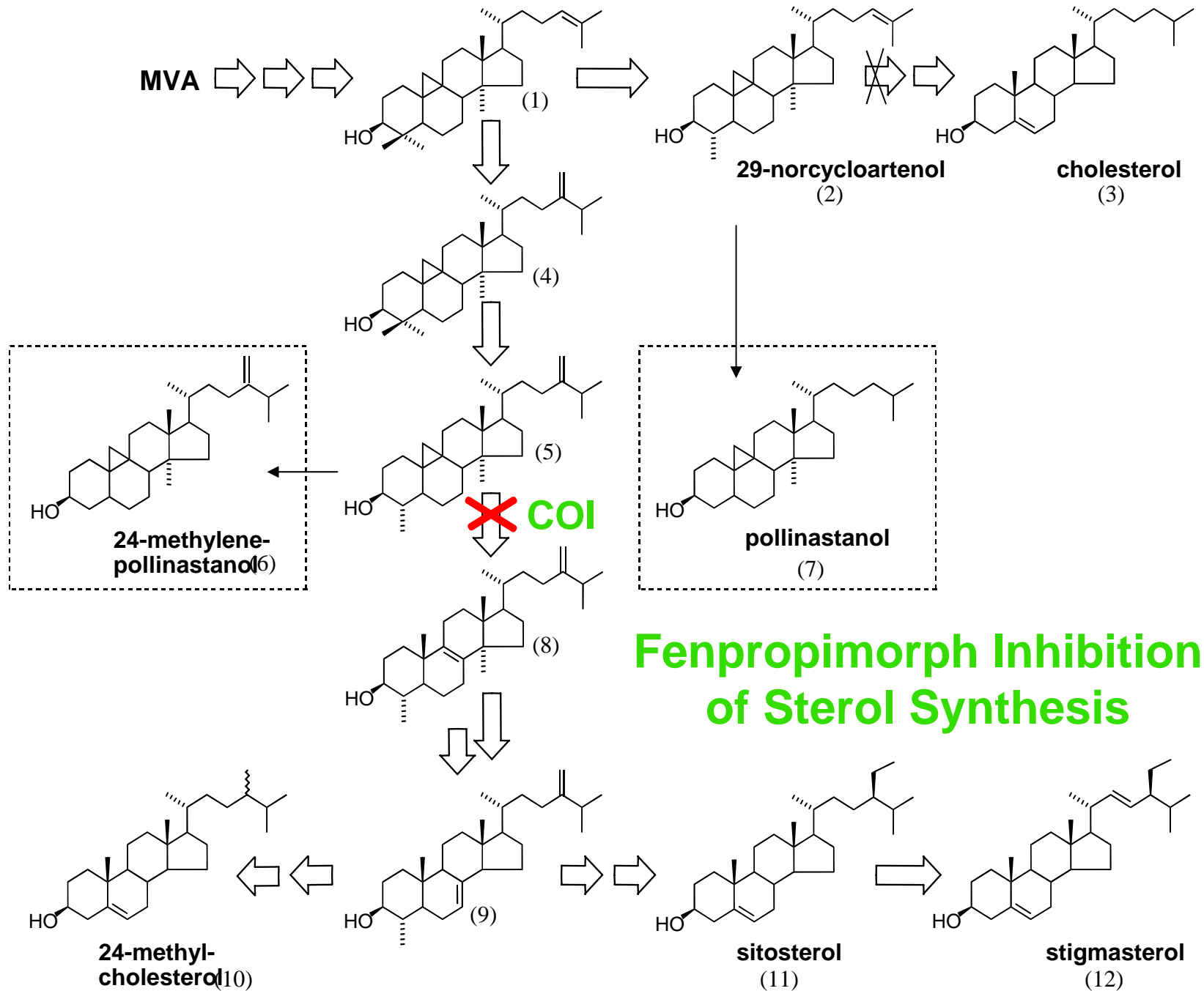


# BFA and Sterol Metabolism in Maize Root Cells

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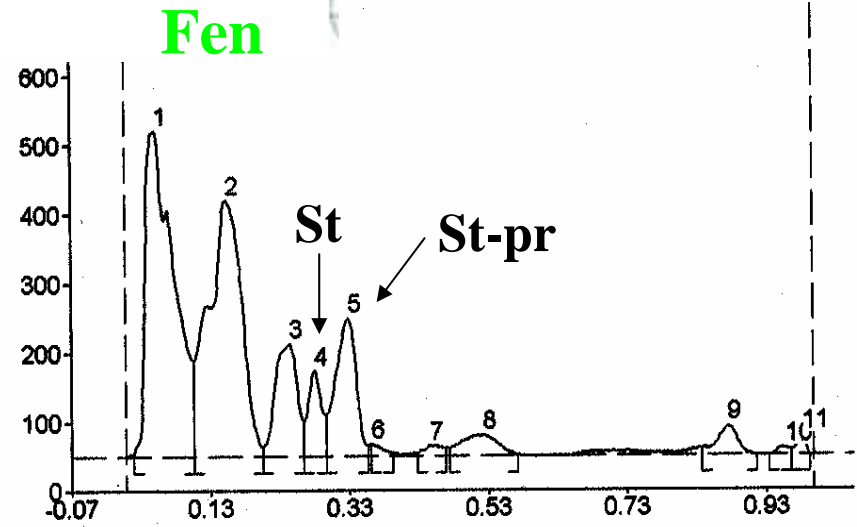
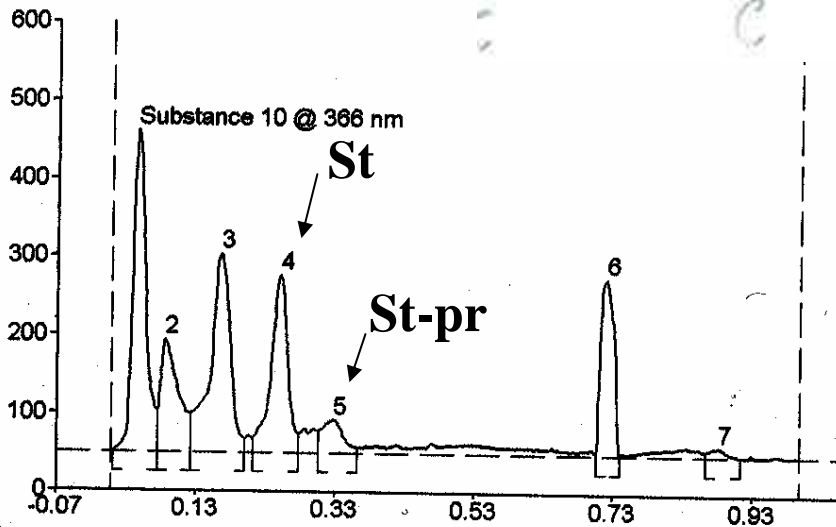
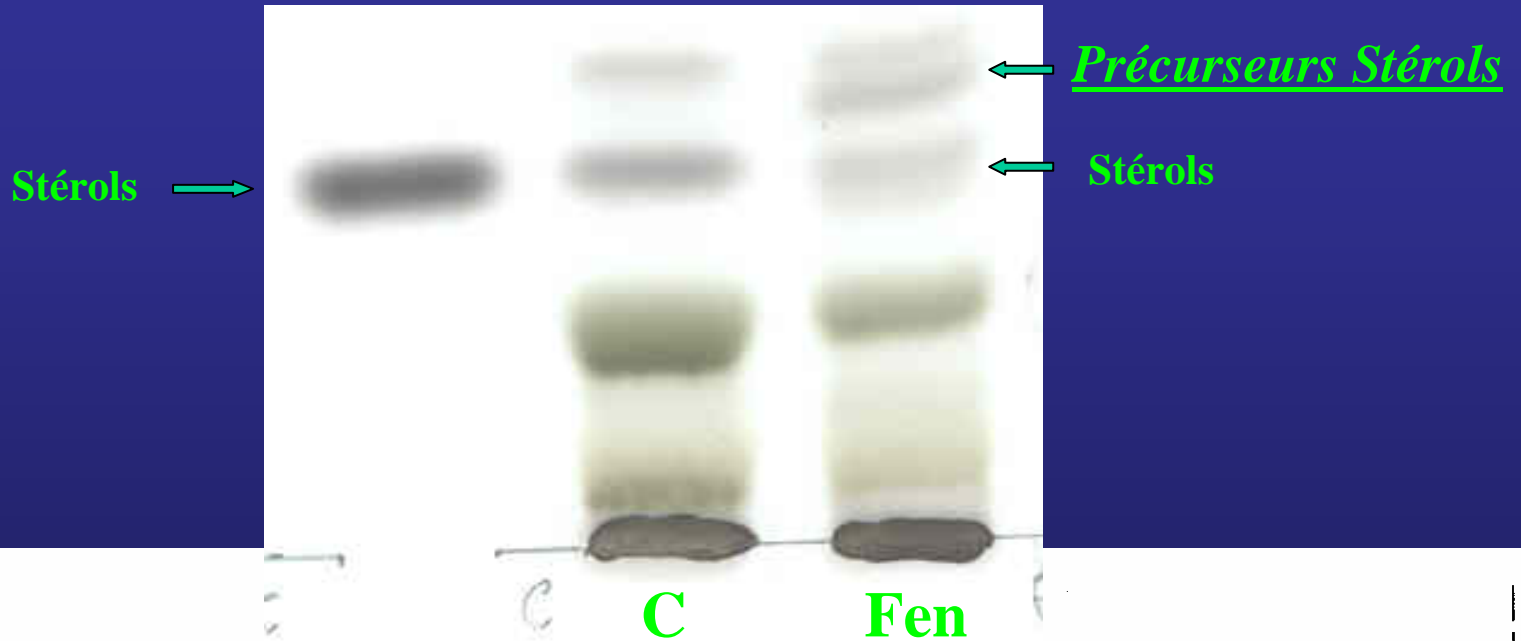
S p: Sterol precursors





# Effets du Fenpropimorph

## Accumulation de Stérols-précurseurs (cyclopropylstérols)





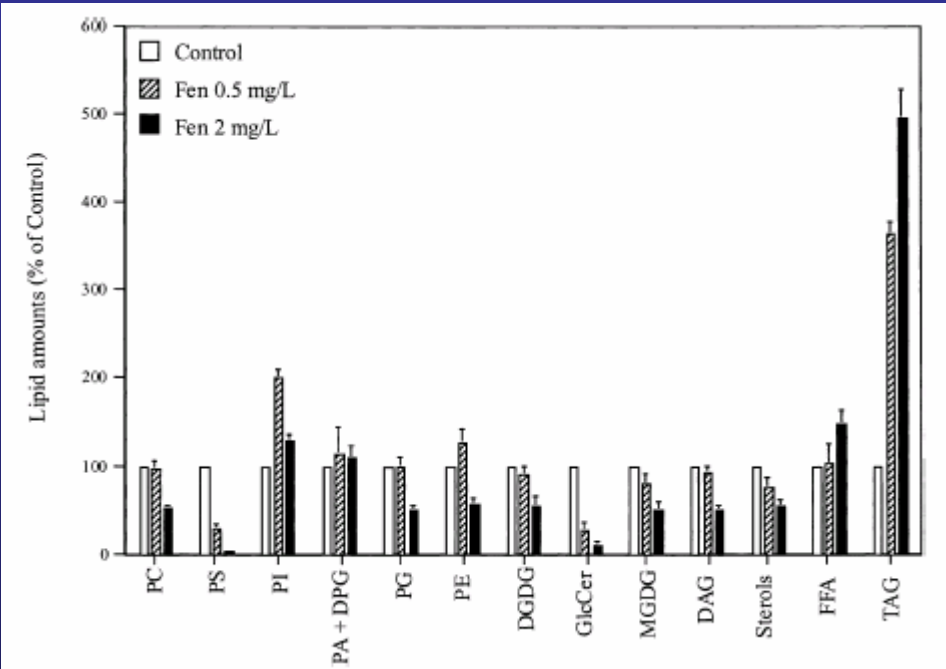
# Effets du Fenpropimorph

Laboratoire de Biogenèse Membranaire UMR 5200

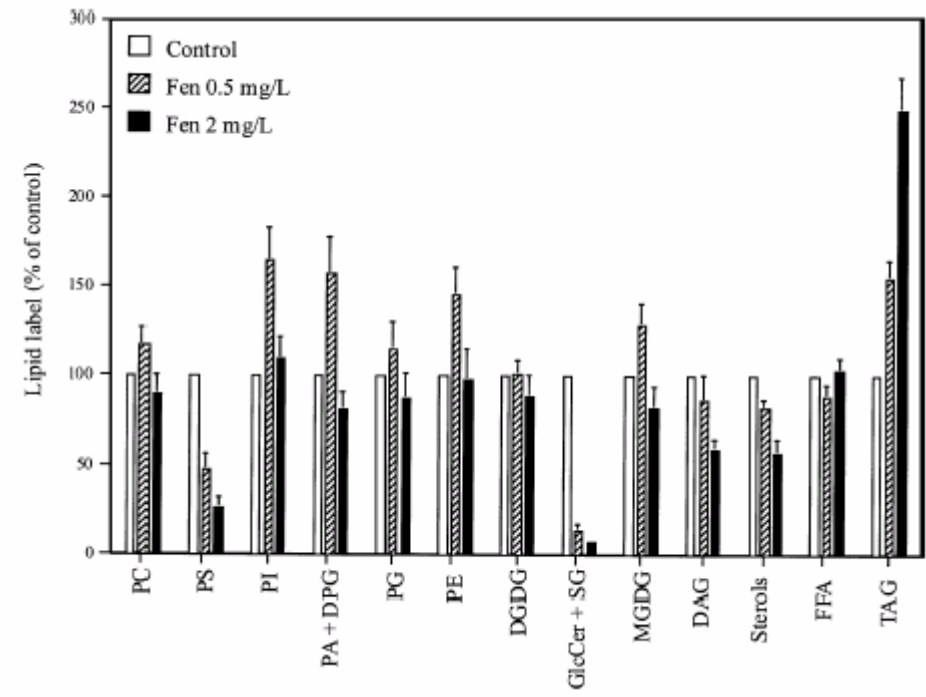
## Analyse en Masse

L'inhibition des stérols s'accompagne d'une forte diminution de PS, GluCer et SG

## Analyse en Radioactivité



(Stérols = St + St-pr)



# Effets du Fenpropimorph

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L'inhibition des stérols s'accompagne d'une augmentation de PA

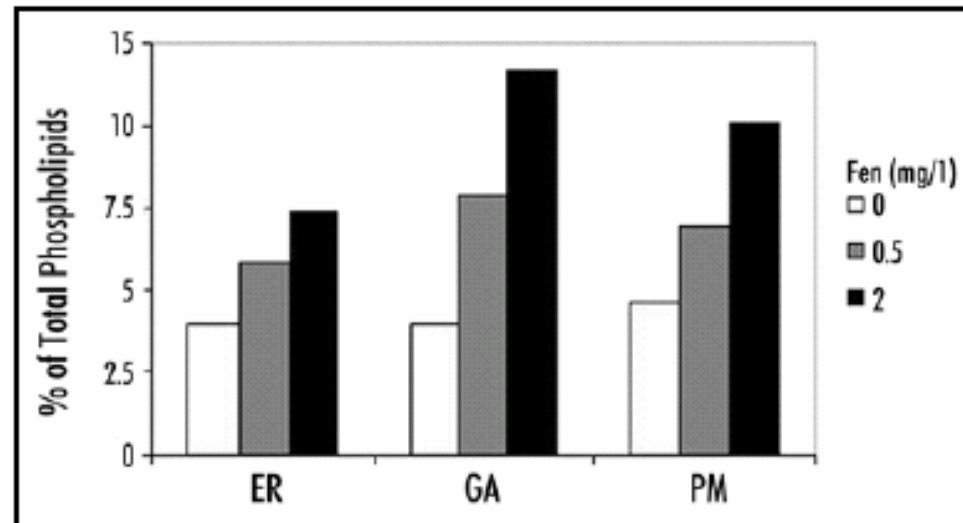
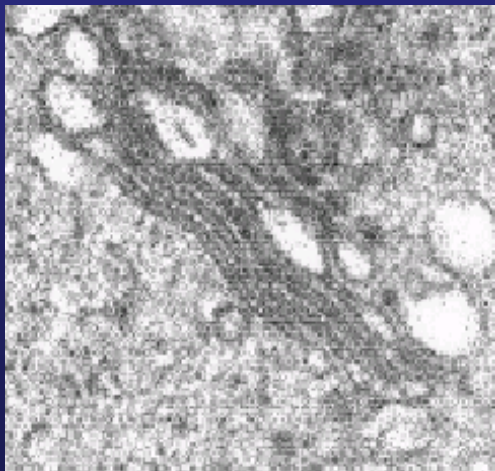


Figure 1. Phosphatidic acid level in endomembranes of *A. porrum* seedlings treated with fenpropimorph. ER, endoplasmic reticulum; GA, Golgi membranes; PM, plasma membranes.

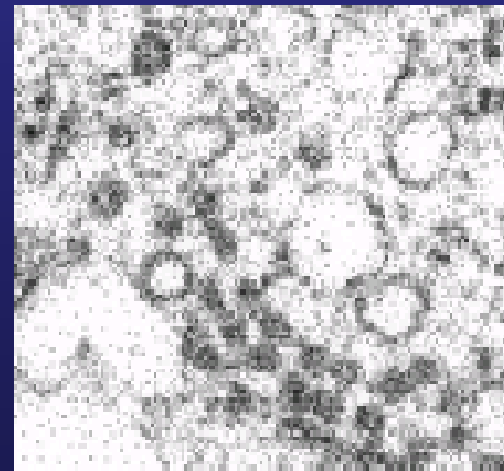
# Effets du Fenpropimorph

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## Morphologie du Golgi et Métabolisme des stérols: une relation?



**Control**



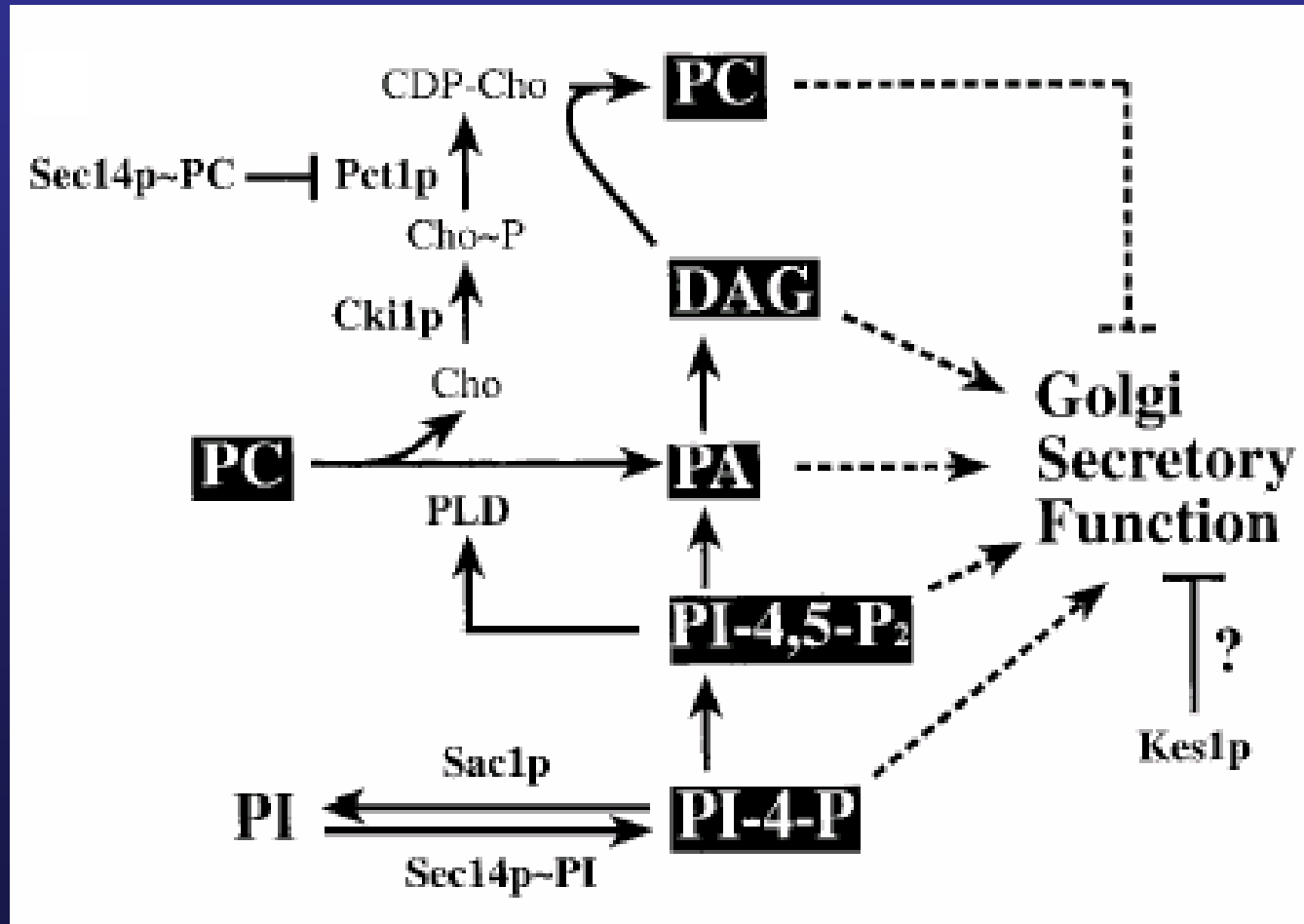
**Fen 2mg/l**



# Relation métabolisme/morphologie/sécrétion

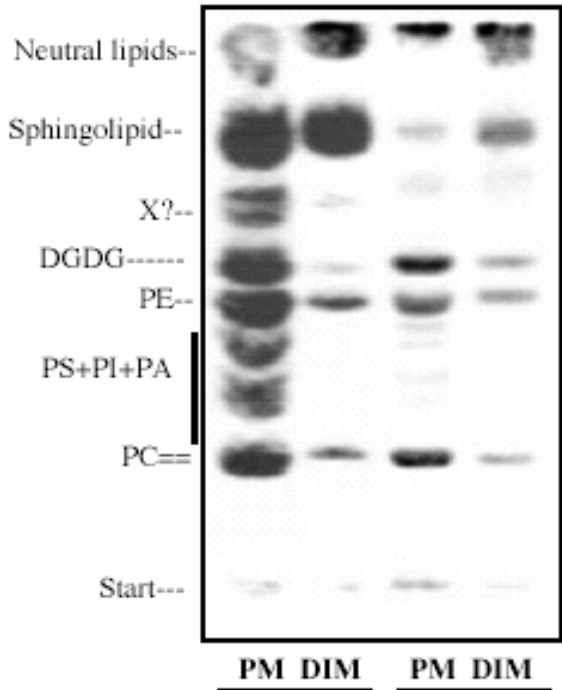
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## Sec14p et la fonction du Golgi chez la levure

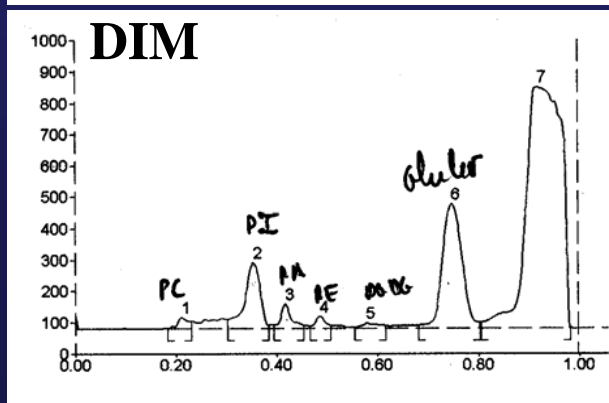
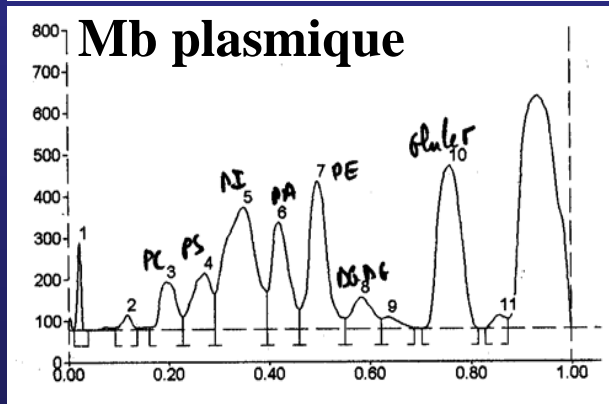
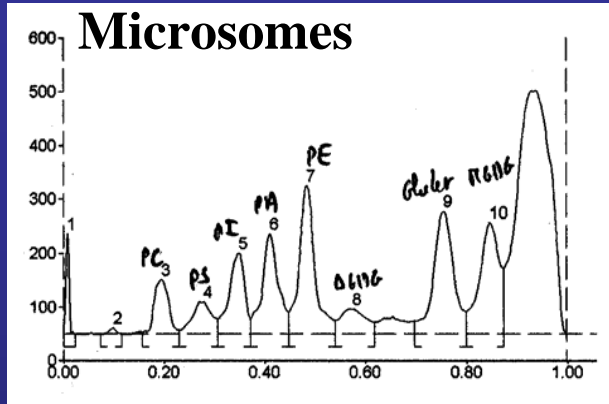


# Les microdomaines membranaires

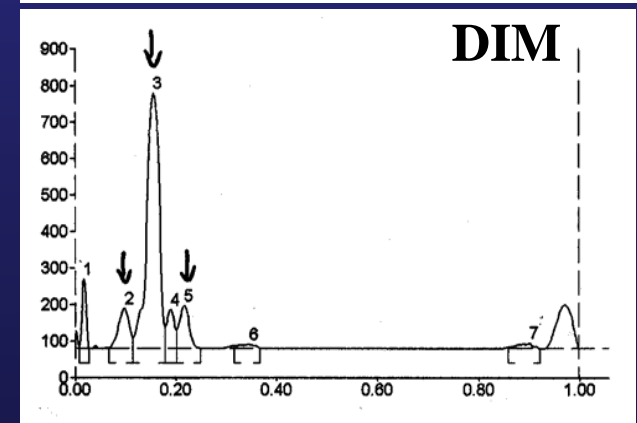
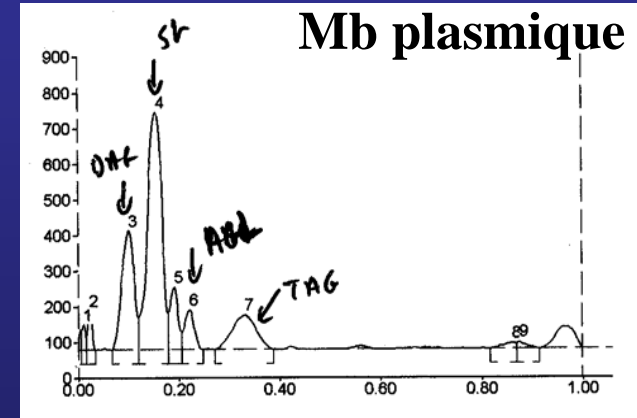
**A**



**Tabac**  
**S. Mongrand**



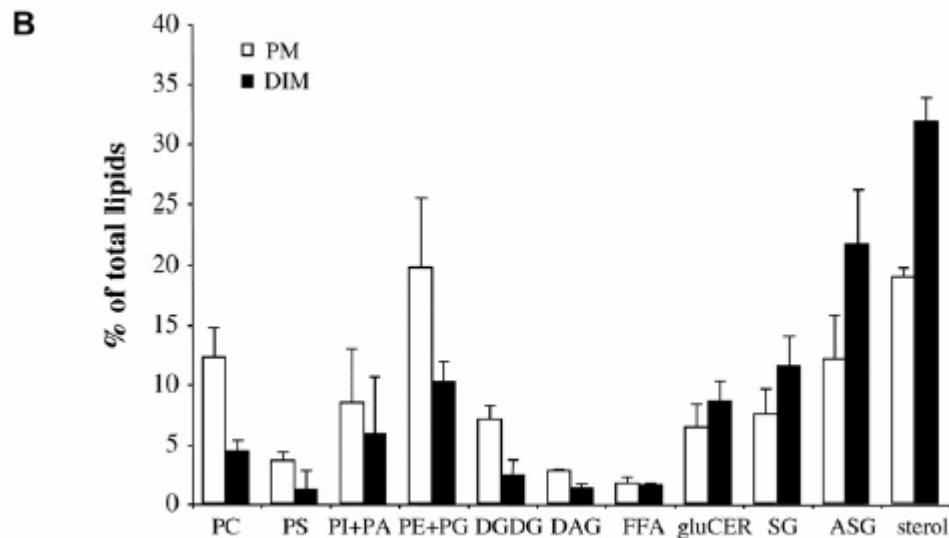
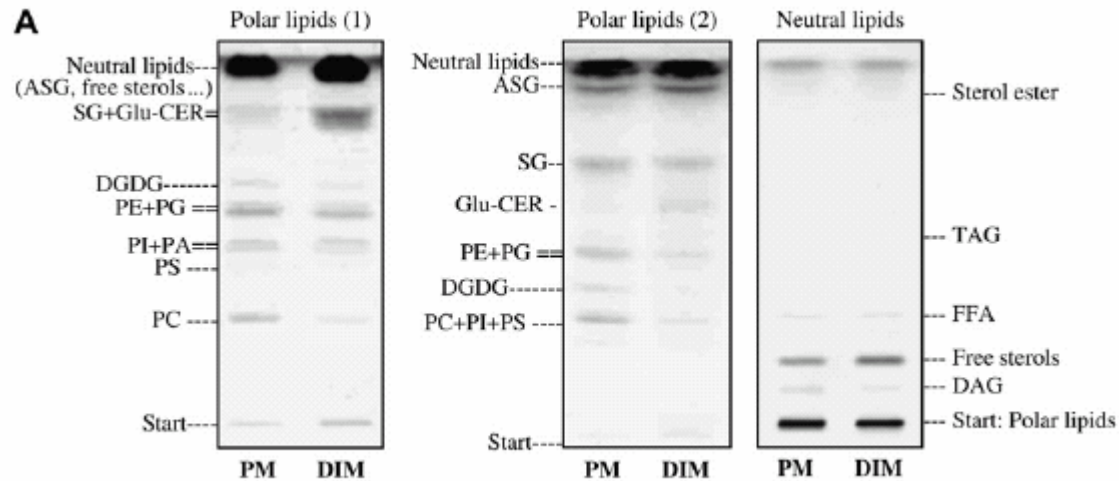
**Plantules  
d'Allium porrum**



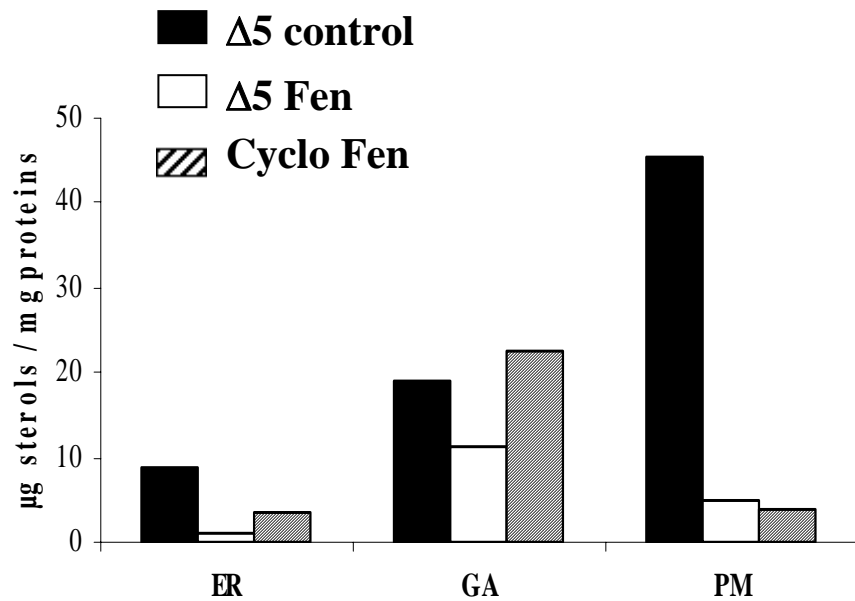
# Les microdomaines membranaires

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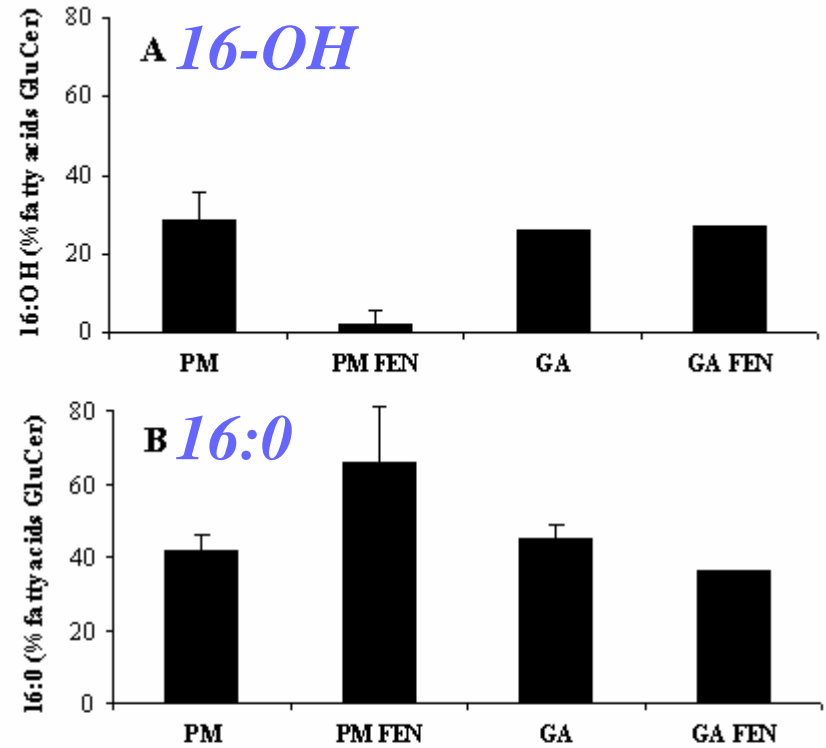
## Racines de Médicago



# Effect of Fenpropimorph on lipid distribution between membrane fractions from etiolated leek seedlings

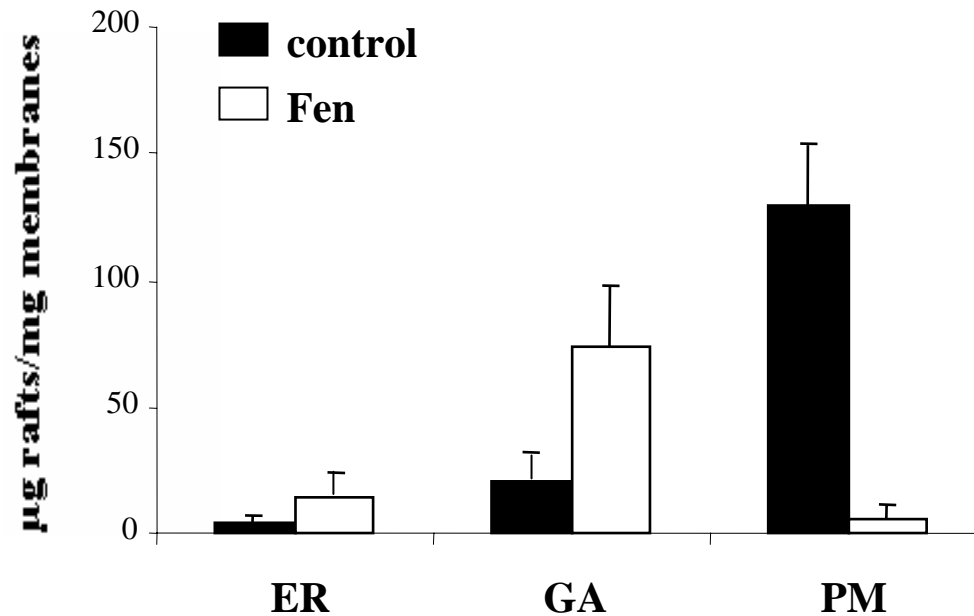


Sterols



GluCer

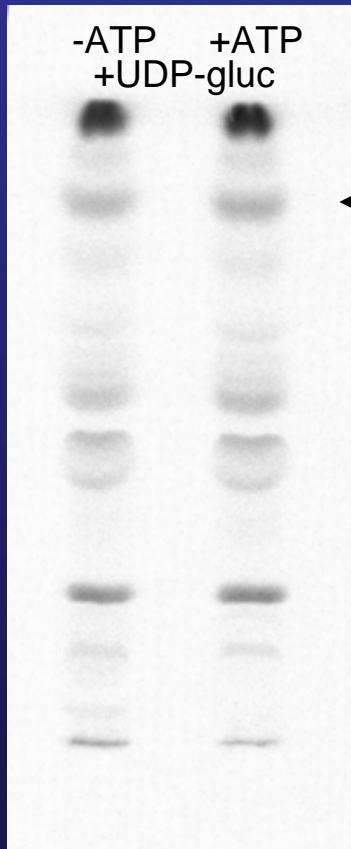
# Effect of Fenpropimorph on the distribution of lipid rafts between membrane fractions from etiolated leek seedlings



# Etude la Glucosyltransférase (GCS)

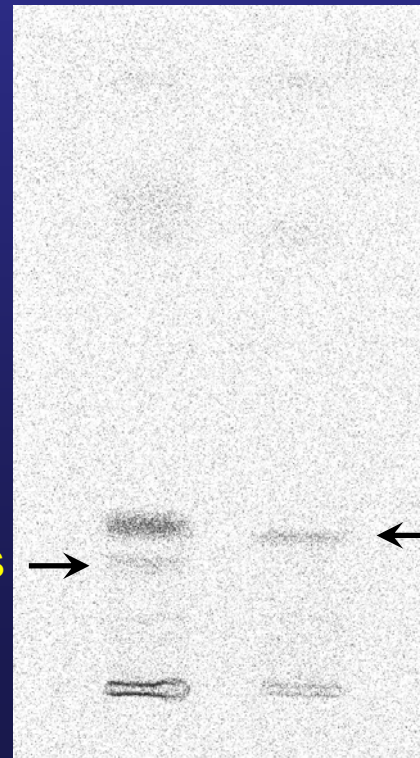
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## Incubation acetate [<sup>14</sup>C]

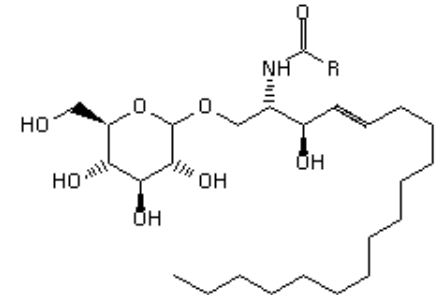


TLC polar lipids

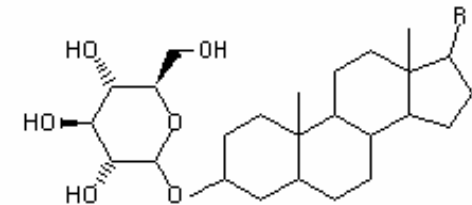
← GC spot  
Acid hydrolysis  
→



TLC neutral lipids



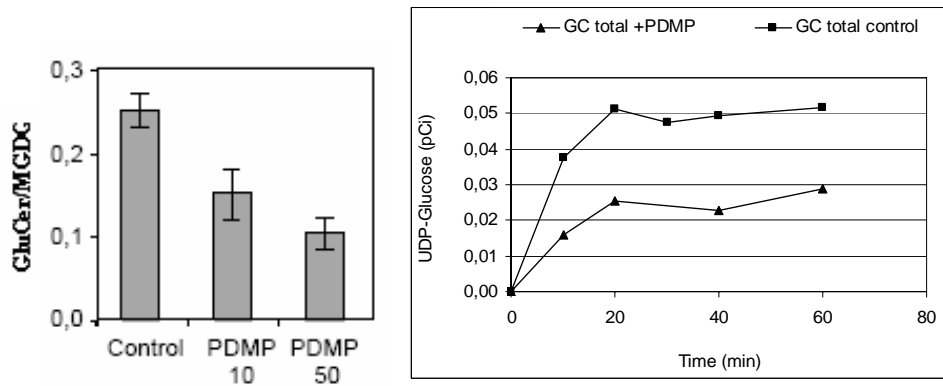
Glucosyl Ceramide



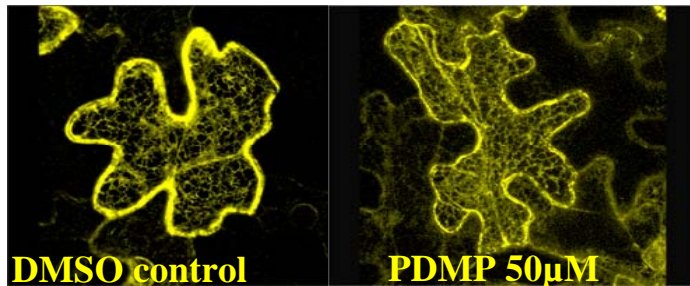
Sterol Glucoside

Fatty acids

## Approche Inhibiteur (*Epiderme de feuilles de tabac*)

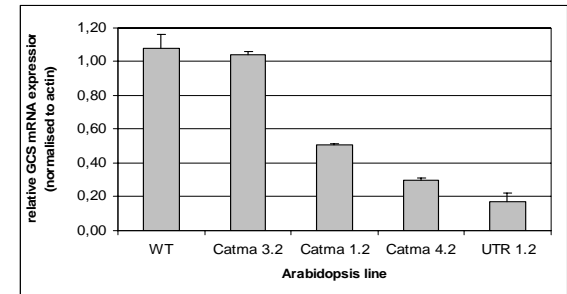


### Inhibition de la GCS

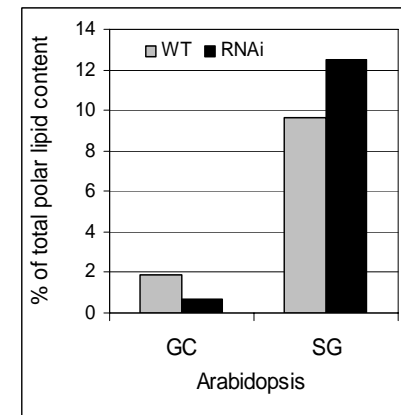


### Inhibition du transport de nSec-YFP

## Approche Mutants (*A. thaliana*)

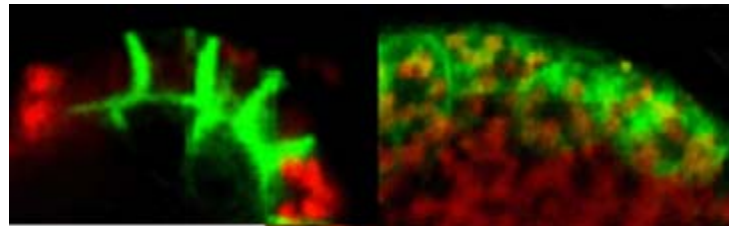
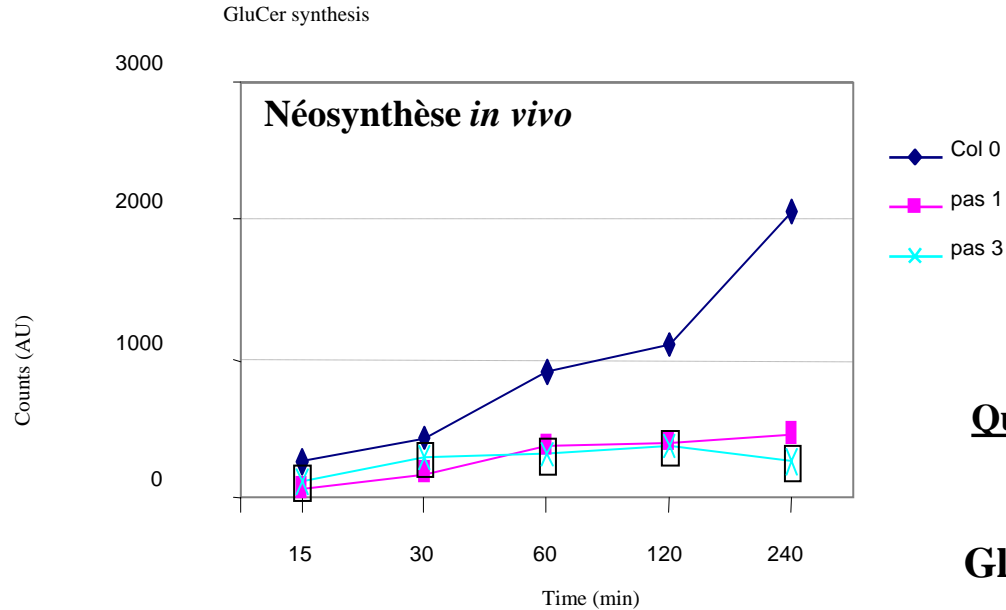


Réduction 75-85% de l'expression de la GCS



↘ GluCer et ↗ SG

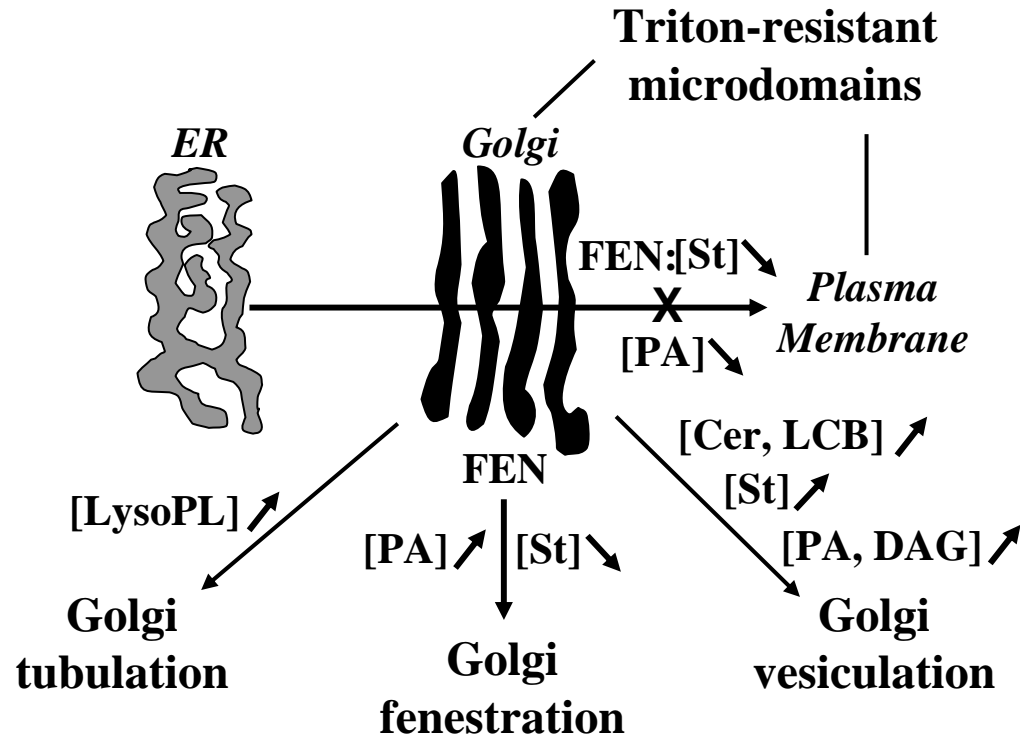
## La synthèse du GluCer est modifiée dans les mutants *pas*



La mutation *pas1* conduit à une dépolarisation de PIN1



# Les lipides acteurs de la voie sécrétoire



**Métabolisme des Lipides - Dynamique des endomembranes  
Chez les eukaryotes**



# Remerciements

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**B. Sturbois**

**P. Vincent**

**S. Melser**

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**V. Wattelet-Boyer (TCN CNRS)**

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**B. Satiat-Jeunemaitre (Gif)**

**A. Zachowski (Univ. Paris VI)**