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STUDY OF LIPID-PROTEIN INTERACTION IN THE SECRETORY PATHWAY OF PLANT CELL BY RAISING AND USING ANTILIPID ANTIBODIES AGAINST PARICULAR LIPIDS AND PROTEINS IN *ARABIDOPSIS* AND TOBACCO PLANT

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Abstract

The lipids and proteins are transported through a well developed system between endoplasmic reticulum (ER) and Golgi and then to all other parts in the Eukaryotes. Lipid and protein based machineries for proteins transport are used in the secretory pathway that are rather well known in animal and yeast cells but far less in plant cells. SNAREs are proteins that interact with others lipids and proteins and are expected to be key regulators of membrane trafficking between ER and the Golgi. SNAREs proteins and their interaction with lipids such as acyl-CoAs was studied and found their presence and involvement in membrane fusion events in the secretory pathway of *Arabidopsis* comparing with tobacco plant. We produced recombinant proteins for 3 SNAREs and prepared antibodies against them. We purified, anti memb 11, anti Sec22 and anti sed5 IgGs from the sera and tested them in *Arabidopsis* to see if native proteins would be recognized. We had also prepared anti acyl-CoAs and tested and found that they can detect the acyl-CoAs when they are in interaction with some proteins, so these antibodies could help us to observe proteins interacting with acyl-CoAs. In fact, we used anti acyl-CoAs antibodies in immunoblot and found that they labeled some proteins in *Arabidopsis* and tobacco. One of these proteins was Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH).

Introduction

There is a well developed system of proteins transport in all the eukaryotes. Plants have also established their own unique system owing to the particular functions performed by the plant cells. The membranes of the cellular compartments are composed of lipids and proteins that are formed initially in the endoplasmic reticulum (ER) and then transported to all other compartments of the cell through the early secretory pathway. Machineries for protein transport in the secretory pathway are rather well known in animal and yeast cells (Robinson *et al*., 2007) but far less in plant cells (Watson & Stephen, 2005).

Eukaryotic cells are composed of numerous compartments that are separated from the cytosol by a lipid bilayer. Exchange of proteins, metabolites and lipids across the lipid bilayer can take place through integral membrane proteins as demonstrated for peroxisomes, chloroplasts or mitochondria. Within the Endo-membrane System, which include the endoplasmic reticulum (ER), the Golgi apparatus (GA), Endosomes and Lysosomes, vesicles transport cargo between organelles and mediate exchange of proteins and lipids. The movement of molecules between ER and GA is bidirectional and is associated with different types of molecular machineries. The movement of protein from ER to GA is the anterograde transport, generally thought to be mediated by COPII (Coatomer protein II) coating machineries and this type of transport has been found in plants. The movement from GA to ER is the retrograde transport that is mediated by COPI (Coatomer protein I) coated machineries. The path of a protein destined for secretion has its origins in the endoplasmic reticulum (ER) then proceed through the stacks of the Golgi apparatus (GA) and finally ends up in vesicles that fuse with the plasma membrane.

Generally, vesicle formation needed a conserved set of coat proteins that bind to cargo and induce membrane curvature. At the same time, a vesicle must incorporate proteins that target it to the right compartment and enable it to fuse with the target compartment. Thus, vesicle formation requires the uptake of targeting and fusion factors, including Rab-GTPases, tethering factors and SNAREs [soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptors]. Fusion seems to involve a cascade in which a Rab-GTPase, together with tethering factors, mediates membrane contact, which is followed by SNARE pairing and lipid bilayer mixing. SNAREs seem to operate at all stages (Moreau *et al*., 2007). They need to be incorporated into the right vesicle, interact with tethers, and are essential for tight membrane docking and lipid bilayer mixing. Although, in non plant systems, the central role of these proteins in membrane fusion is established but little is known about their role in the early secretory pathway of plant cells.

Analysis of the Arabidopsis (*Arabidopsis thaliana*) genome reveals 54 genes encoding SNARE proteins that have been divided into different kinds based on N-terminus (Jahn & Scheller, 2006), some of which are considered to be key regulators of membrane trafficking between the endoplasmic reticulum (ER) and the Golgi. *Arabidopsis* v-SNAREs Sec22, Memb11, Bet11, and the t-SNARE Sed5 are localized at the Golgi apparatus (Chatre *et al*., 2005). In addition, Sec22 was also distributed at the ER. Over expression of the v-SNAREs Sec22 and Memb11 but not of the other SNAREs induced collapse of Golgi membrane proteins into the ER. It is suggested that Sec22 and Memb11 are involved in anterograde protein trafficking at the ER-Golgi interface in tobacco leaf epidermal cells but their retrograde pathway role as reported for sec22p in yeast has to be determined. The simultaneous distribution

of Sec22 at the ER and possibly the Golgi apparatus suggested that this SNARE could be able to cycle between the 2 organelles. Genomic sequences encoding for several families of proteins, such as coat proteins, small GTPases, ATPases, and SNAREs that have been found to mediate secretory processes in animal and yeast cells. In particular, SNAREs interact through their coiled coil domains that lead to the formation of protein complexes between a vesicle and a target membrane prior to membrane fusion (Uemura *et al.*, 2004). Membrane trafficking is mainly controlled by close relationships between lipid-based and protein-based machineries (De Matteis & Godi, 2004). Recently, it is also reported that SNAREs proteins like Sec1 play complementary role in the membrane fusion (Thomas *et al.*, 2009).

Acyl-CoAs may interact with a large number of known proteins or yet unknown and these interactions could be predicted through the use of anti-acyl-CoA antibodies. Many proteins interact with acyl-CoAs for being acylated that influence the protein stability and aggregation and trafficking. It has been shown that anti-acyl-CoA antibodies can immunoprecipitate multi-protein complexes, principally related to vesicle trafficking and/or to membrane rafts (Diakou *et al*., 2006). So, directly or indirectly, they play important regulatory roles in many cellular events (Linder & Deschenes, 2007). Moreover, the acylation/de-acylation process of lipids plays a key role in changing membrane curvature during fusion or fission (Melia *et al.*, 2006). On the other hand, the case of a SNARE interacting with PS (phosphatidyl serine) has recently been reported in animal cells (Quetglas *et al.*, 2000). In rat liver membranes, Phosphatidyl serine (PS) was found to be targeted to ER-derived vesicles (Moreau *et al*., 1993) and also leek seedlings (Moreau *et al.*, 1998) and can be critical for membrane fusion (Moreau *et al*., 1993). In addition, Lipids have been shown to play an important role in pollen tube development (Monteiro *et al*., 2005). We have also shown that development of flower in tomato is perturbed upon phytoplasma infection (Ahmad *et al.*, 2012) and expression of defense related proteins involved in flower development were altered (Ahmad *et al*., 2011). Recently, it has been reported that some pollen specific R-SNAREs mediate the pollen tip growth indicating that vesicle recycling takes place in pollen tube formation (Guo *et al*., 2012).

Our objective was to study the specificity of the antibodies raised against 3 SNAREs (Memb11, Sed5 and Sec22) in order to use them for searching molecular partners involved in membrane fusion and search for proteins (SNAREs or others) interacting with acyl-CoAs (for example for protein acylation or lipid remodeling) in the ER to Golgi interface. We performed the experiments by considering the importance of above mentioned SNAREs proteins and their interaction with lipids such as acyl-CoAs, and found their presence and involvement in membrane fusion events in the secretory pathway of *Arabidopsis* comparing with tobacco plant. We produced recombinant proteins for these 3 SNAREs and prepared antibodies against them. We purified, anti memb11 and anti sed5 IgGs from the sera and tested them in *Arabidopsis* to see if native proteins would be recognized. We had also prepared anti acyl-CoAs and tested and found that they can recognize the acyl-CoAs when they are in interaction with some proteins, so these antibodies could help us to observe proteins interacting with acyl-CoAs. On the other hand, we can say that acyl-CoAs are involved in membrane fusion. In fact, we used anti acyl-CoAs antibodies in immunoblot and found that they labeled some proteins in *Arabidopsis* and tobacco. One of these proteins is Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH). We also planned to test the possible interactions of SNAREs or other proteins involved in membrane fusion with lipids by studying the interactions of those proteins with artificial membranes of known lipid compositions (ex Biacore) such as PC/PS vesicles with different composition of lipids. Anti-PS antibodies would help to improve this study as a positive control. By this technique, recombinant proteins can be produced and used for the transportation of some other important proteins such as insulin required for important biological function.

Material and Methods

Purification of IgGs by column protein G affinity chromatography: Rabbit polyclonal immunoglobulins G (IgGs) or pre-immune IgGs were purified by protein G affinity Chromatography column from the different sera obtained in the laboratory. Serum (0.5mL) was diluted 10 times in 20mM phosphate buffer pH 7.2 and loaded onto the protein G column. Column was washed with the same buffer, and then IgGs were eluted with 0.1M glycine buffer pH 2.5 and neutralized with 1M Tris buffer pH 9. IgGs were aliquoted and stored at -20°C.

Separation of the proteins through SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis): SDS-PAGE was carried out on 12% polyacrylamide gels. Fractions to test were mixed with 1 volume of laemmli buffer and heated at 90°C for 5 minutes. They were loaded on the gel together with molecular size markers kit for proteins (118, 85, 47, 36, 26, 20 kDa). Electrophoresis was carried out on 200V at room temperature in 0.025 M Tris, 0.192 M glycine, 0.1% SDS buffer pH 8.3. After SDS-PAGE, the part of the gel for staining was cut and put into the colorant for 2 hours and then in decolorant solution.

Immunoblots: After electrophoresis, immunoblots were carried out when necessary. Gels were blotted on a PVDF membrane (Perkin Elmer) by transverse electrophoresis at 100V at 4°C, in 0.025 M Tris base, 0.192 mM glycine buffer. The immune-labeling was carried out at room temperature. The PVDF membranes were sequentially incubated, with the saturation solution (PBS Tween20 0.05%, 1% BSA) for 1 hour. Then membranes were washed four times rapidly with PBS Tween20 0.05%. PVDF membranes were incubated for 2 hours with Anti-Sed5 IgG 1/2000, or Anti-Sec22 1/6000, or Anti-Memb11 1/4000, or anti-acyl-CoAs 1/4000, each diluted with PBS Tween20 0.05% buffer. Membrane was washed again with PBS Tween20 0.05%, (1x15min and 4x5min). Membrane was incubated with anti rabbit-IgG 1/50000 in PBS Tween20 0.05% for 30 min. Membrane was washed again with PBS Tween20 0.05%, (1x15min and 4x5min) and labeling was revealed by chemiluminescent reagent

kit (Perkin Elmer) in dark room. The Membranes were then exposed to Kodak Biomax Light films.

Immuno-precipitation of the proteins involved in membrane fusion: Immuno-precipitation of fractions from *Arabidopsis* was carried as follows. 100 grams of hydroponic *Arabidopsis* 21-day-old plants were homogenized on ice in 200 ml of extraction buffer (50 mM Hepes-KOH, pH 6.5. 10 mM potassium acetate 100 mM sodium chloride 5 mM EDTA 0.4 M sucrose) with a protease inhibitor mixture such as cocktail 1 tablet/10ml extraction buffer (Sigma). The homogenate was passed through Miracloth (Calbiochem) to remove debris. This extract was also used for Western blot analysis after adding an equal volume of laemmli buffer. The extract was centrifuged at 13,000*g*/30 min to generate a membrane pellet 20 ml. The supernatant was re-centrifuged at 100,000g/ 1 hour to obtain the pellet 7ml. To solubilise the membrane proteins, the pellets was re-suspended in 7 ml of extraction buffer containing 1% (vol/vol) Triton X-100 and protease inhibitor mixture and incubated at 4°C for 2 h. Insoluble material was repelleted at 10,000*g*/10 min. The supernatant (total protein extract) was incubated 1h30 with 3 µL of IgGs and then with 10 µL of protein G-Sepharose beads. Beads were washed in extraction buffer. Equal volumes of immune-precipitate, adding the laemmli buffer, were separated by SDS-PAGE followed by immune-blotting with appropriate antibodies or by mass spectrometry analysis (plateforme genomique fonctionnelle).

Results and Discussion

Separation of the proteins through SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis): The proteins in the samples of the *Arabidopsis thaliana* and tobacco plants were separated through the SDS-PAGE electrophoresis. The proteins are separated on the gels according to their molecular mass as mentioned in Chen *et al.,* (2011). The staining of the gels indicated the position of the proteins in the samples of both plants. Among these proteins, some are involved in the membrane fusion events in the early secretory pathway of the plant cells between endoplasmic reticulum and Golgi apparatus. Three proteins of the SNAREs family, Sed5, Sec22, and Memb11 play an important role to fuse the two apposed lipid bi-layers during the transport of the proteins between ER and GA. These SNAREs proteins have been shown on the staining gels in the Fig. 1. Sed5 is t-SNAREs protein found on the target membranes particularly is distributed on the GA. This protein formed the band in the range of 40-kDa. Sec22 and Memb 11 are v-SNAREs proteins and are found on the vesicles that transport the other proteins. Sec22 and Memb11 formed the band on their separation through SDS-PAGE within the range of 40-kDa and 34-kDa respectively. We had three recombinant proteins of Arabidopsis Sed5, Memb11, Sec22 against which we prepared antibodies anti sed5, anti memb11 and anti sec22 respectively. We purified and tested the IgGs against these recombinant proteins in immune-blot experiments for *Arabidopsis* and tobacco samples and found that IgGs have shown positive results in *Arabidopsis* without cross reaction for proteins in tobacco. The confirmation of their presence can be made through the western blots by using the IgGs against them.

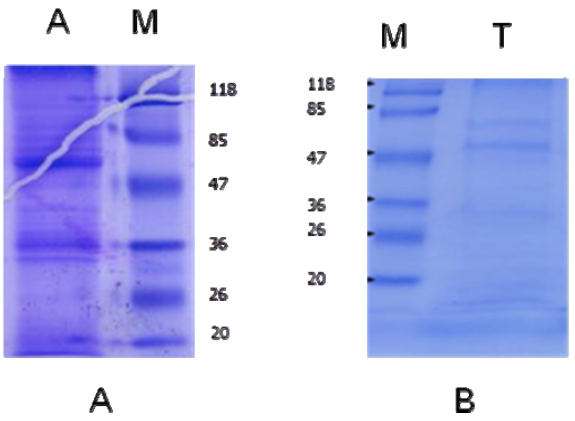

Fig. 1. Electrophoresis of the proteins from the lysates of *Aarabidopsis* (A) and *tobaccum* (T). A. Electrophosis of *Arabidopsis* proteins (10µL per lane). The molecular size markers (kDa) are indicated in the right. B. Electrophosis of *tobaccum* proteins (10µL per lane). The molecular size markers (kDa) are indicated in the left.

Anti-Sed5 antibodies recognize Sed5 SNAREs recombinant proteins in *Arabidopsis thaliana:* SNAREs reside either on the vesicle membrane or the target membrane. The SNAREs that are present on target membrane are called T-SNAREs. According to another classification, they are also called Q-SNAREs. Sed5 is very important target membrane SNAREs of Golgi apparatus that help in the fusion of the target membrane with vesicles. We had recombinant t-SNAREs Sed5 in the laboratory against which anti-Sed5 antibodies were prepared and purified to test its specificity against recombinant protein of *Arabidopsis* in western blot by separating the proteins through SDS-PAGE.

Anti-sed5 IgGs has labeled the protein in *Arabidopsis* around 40-kDa as it has been indicated on staining gel Fig. 1A. These results of immunoblots showed that the IgGs against recombinant proteins can identify and label the native proteins in *Arabidopsis* but not in tobacco (result not shown here) suggesting that antibody is specific for Sed 5 shown in Fig. 2B. IgGs were prepared not of full length of Sed5 protein but for the specific peptide of that protein. Immunobloting of tobaco has not been indicated here. On the basis of sequence similarity with their animal and yeast counterparts (as syntaxin), it can be expected that t-SNAREs Sed5 was putative candidate for the anterograde ER-Golgi pathway. The role of Sed5 SNAREs along with Sec22 and memb11 is not fully understood in plants. In protoplast from *Arabidopsis* suspension cultured cell, Uemura *et al*., (2004) found Sed5 in the Golgi bodies. The sub-cellular distribution of Sed5 in Arabidopsis appears to be different than tobacco. Sed5 is located in Golgi with wider distribution in tobacco leaf epidermal cells than in Arabidopsis cultured cells (Chatre *et al*., 2005) and it has been shown in yeast that sed5 is a Golgi syntaxin involved in ER-Golgi transport. The precise location of SNAREs protein Sed5 and complex formation with other SNAREs for membrane fusion during transportation can be easily understand by using the anti-bodies against SNAREs and their effect on Golgi secretory markers.

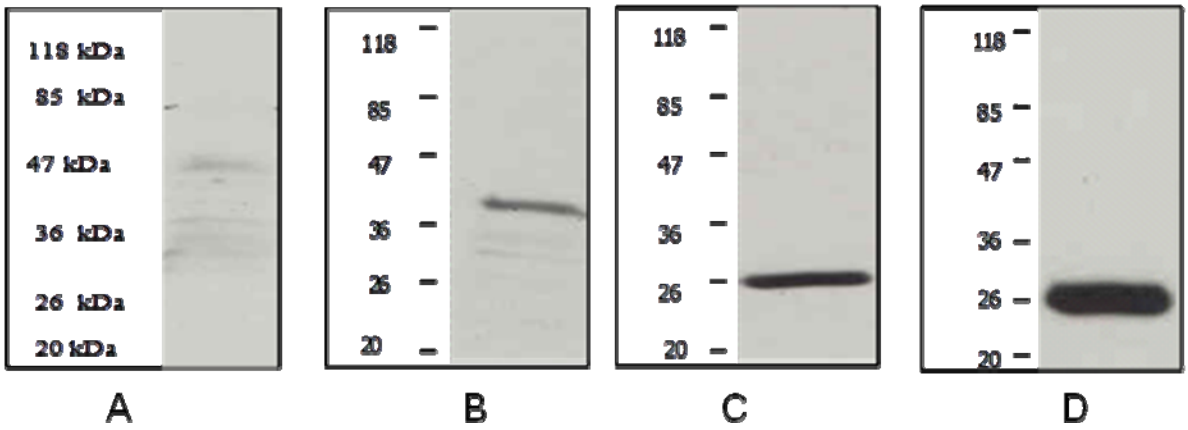

Fig. 2. Immunoblots followed by Electrophoresis on the PVDF membrane from the lysates of *Arabidopsis*: (A) with Anti sec 22 antibodies (3µL antibodies per membrane), (B) with anti Sed5 antibodies (6µL antibodies per membrane), (C) with anti memb11 antibodies (3 µl of anti Memb11 antibodies per membrane). (D) Electrophoresis of the protein immunoprecipitated from the lysates of *Arabidopsis* (A) with anti memb11 antibodies (I mg of lysate for 5 µg of antibodies). Electrophoresis was followed by -MS analysis. The molecular size markers (kDa) are indicated in the left.

Anti-Sec22 antibodies detected a SNAREs Sec22 recombinant proteins in *Arabidopsis thaliana:* Sec22 is a vesicle SNAREs and also known as R-SNAREs that is predominantly found in both ER and mobile punctate structures corresponding either to endoplasmic reticulum export cites (ERES, site of vesicle formation on ER) and /or *cis*-Golgi cisternae while others SNAREs like memb11 and Sed5 are found in mobile structures in tobacco leaf epidermal cells (Chatre *et al*., 2005). The presence of Sec22 in both ER and GA suggest that Sec22 SNAREs recycle between these two organelles and is involved in the transportation of other proteins. This role of Sec22 has been confirmed in tobacco leaf epidermal cells, when cells were treated with Brefeldin A (BFA), all the sec22 protein in Golgi redistributed in ER. It is difficult to distinguish the location of Sec22 in ERES and Golgi, so anti Sec22 antibodies may help us to know the exact location and other SNAREs interaction. So proteins in the samples of *Arabidopsis* and tobacco were separated in electrophoresis and then were blotted to the PVDF membrane and incubated with anti-Sec22 antibodies in immunoblots Fig. 2A. As antibodies has recognized the Sec22 in *Arabidopsis* in the range of 40-45 kDa also consisted with the staining gel without cross reaction with tobacco showing the specificity of anti bodies as shown in Fig. 2A. Labeling by anti Sec22 in *Arabidopsis* is very weak and the result of immunoblot in tobacco is not shown here. Our result also supports the recent finding that SNARE proteins and Sec1like has role in the membrane fusion of vesicle and target membrane (Thomas *et al*., 2009).

A v-SNAREs Memb11 is recognized by the anti-Memb11 antibodies in *Arabidopsis thaliana*: Like sec22, Memb11 is v-SNAREs that interact with other SNAREs proteins through their Coiled coil domain that contained the Arg or Gln in the central position of the HELIX bundle (Fasshauer *et al*., 1998). Thus they form protein complex between a vesicle and a target membrane prior to membrane fusion; mem11 SNAREs have their putative SNAREs candidates in yeast and mammals (Bos1 in yeast and Membrin in mammals). Their role in yeast and mammals is well known but very little work is done in plants. Chatre *et al.*, (2005) have found that Memb 11 is involved in anterograde protein trafficking at the ER-Golgi interface in tobacco leaf epidermal cells. We are developing new technique to find the interacting partners by producing the specific anti SNAREs antibodies. Anti-memb11 IgGs has labeled the protein in *Arabidopsis* around 26-kDa as it has been indicated on staining gel Fig. 1A. These results of immunoblots showed that the IgGs against recombinant proteins can identify and label the native proteins in *Arabidopsis* but not in tobacco suggesting that antibody is specific for memb11 shown in Fig. 2C. Immunobloting of tobaco has not been indicated here.

Immuno-precipitation of a SNARE protein Memb11: In order to identify the protein interacting with the anti-memb11 antibodies, we have carried out immunoprecipitation experiments in view of mass spectrometry analysis. For immunoprecipitation, *Arabidopsis* lysates were used. A representative result is presented in Fig. 2D. The MS analysis of the immunoprecipitate confirmed that the antibodies raised against a recombinant protein can recognize native Memb11 from *Arabidopsis*. Their use in future experiments may confirm the implications of Memb11 in vesicle budding that have been reported to be related to vesicle trafficking, fusion or fission and/or to membrane microdomains-rafts.

Anti-Acyl CoAs antibodies detect the Acyl CoAs together with interacting protein partners: Acyl- CoAs are present in the cell either as free molecules or, more probably, in interaction with other lipids or proteins. Many proteins interact with acyl-CoAs for being acylated, influencing the protein trafficking, stability and aggregation. Acyl-CoAs can be implicated in many

biological processes involving proteins other than those metabolizing or transporting them (Linder & Deschenes, 2007). However, the presence of acyl-CoAs can only be studied indirectly, by biochemical methods or by studying some proteins known to bind acyl-CoAs. It is very difficult to estimate the acyl- CoA content of a sub cellular membrane or cytosolic fraction because during extraction, acyl-CoAs spontaneously partition between the membranes and the aqueous phase. Therefore, only immunocytochemical methods would allow localization in situ, provided that specific antibodies are available We have developed antibodies directed against long chain acyl-CoAs and demonstrated in vitro that they specifically recognize acyl-CoAs, particularly with long fatty acyl chains (Maneta-Peyret *et al*., 1998). We separated the proteins present in the samples of Arabidopsis and tobacco plants through the SDS-PAGE and carried out immunoblot exsperiments with the anti-acyl-CoA antibodies. Different proteins were labeled. In tobacco samples the protein bands were more clearer than *Arabidopsis* Fig. 3.

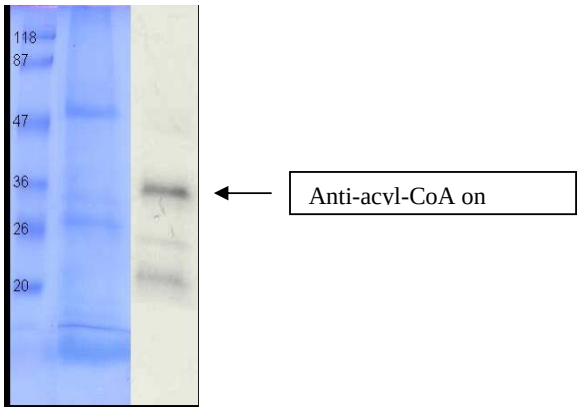

Fig. 3. Electrophoresis and immunoblots with lysates of *tobaccum* . Left lane,Molecular size marker (kDa) shown are same for electrophoresis and blots. Central lane, Electrophoresis (revealed by Coomassie blue) with lysates of *tobaccum* (30 µL with 1 volume of laemli. Right lane, Immunoblot with tobacco lysates and anti-acyl-CoAs antibodies.

We also carried out immunoprecipitation experiments with the anti-acyl-CoA antibodies in order to identify the proteins labeled by the antibodies thus interacting with acyl-CoAs. Among these proteins, identified by mass spectrometry analysis, there was GAPDH that we immunoprecipitated from both *Arabidopsis* and Tobacco plants. GAPDH exists as a tetramer containing identical 37-kDa subunits. Although this abundant protein (10- 20% of total cellular protein) is commonly known as a key enzyme in glycolysis. A number of intriguing intracellular roles have been reported for GAPDH, including modulation of the cytoskeleton, phosphotransferase/kinase activity. GAPDH is a multi-functional protein that binds to DNA, causing activation of transcription as well as DNA replication. It has also been reported that GAPDH induces membrane fusion when acylated, this means that GAPDH interacts with acyl-CoAs for being acylated and this could explain why it is labeled by our anti-acyl-CoA antibodies. It has also been reported that GAPDH shows a strict requirement for phosphatidyl- serine (PS) for vesicle fusion. The most rapid GAPDH-induced membrane fusion occurred when the vesicles contained 6 mol% of PS suggesting that GAPDH interacts with membrane phospholipids. So our Anti-Acyl CoAs antibodies allowed us to identify a protein that could be involved in the ER to Golgi membrane fusion: GAPDH and we will further study some more results.

Conclusion

The first step of secretion in the secretory pathway of eukaryotic cells is the transport of soluble and membrane cargo from the ER to the GA. The movement of these molecules between ER and GA is bidirectional and is associated with COP I and COPII coated vesicles. In the Endo-membrane System, vesicular transports of proteins have been observed. The vesicle formation and its proper targeting and fusion with the right targeting membrane is required to recruit the essential components of the transport machinery such as COPs proteins for the binding, Rab-GTPases for membrane contact and SNAREs for lipid bilayer mixing and fusion of vesicles with the target membrane. Among these, SNAREs are involved at all stages and very important for tight membrane docking and lipd bilayer mixing by interacting with other partners involved in transportation.

From the literature, it has been proved that 3 important SNAREs (Sed5, Sec22 and Memb11) are involved in membrane fusion events. Sub-cellular localization of these 3 SNAREs have been determined by the Chatre *et al.*, (2005). They found that *Arabidopsis* v-SNAREs Sec22, Memb11, Bet11 and t-SNAREs Sed5 are localized in GA but Sec22 was also found in ER suggesting that Sec22 and Mem11 are involved in anterograde protein trafficking at ER-GA interface. In this article so we identified the presence of these SNAREs in *Arabidopsis* with the help of anti SNAREs antibodies. For this purpose, we purified the antibodies prepared against these 3 recombinant SNAREs proteins from the sera obtained in the Laboratory of Biogenése Membranaire UMR5200. These antibodies were specific so we tested the specificity against these SNAREs in *Arabidopsis* through immunoblot and found that they labeled the recombinant proteins in *Arabidopsis* but not in tobacco because of the abscence of possible homologues of these SNAREs in tobacco. This result shows the specificity of antibodies and can be very helpful to study the SNAREs sub-cellular location, their transportation and to find all possible other partners involved in SNAREs complex formation during fusion of vesicles with target membrane. Lipids and proteins also play very important role in development of floral parts and for the transportation of proteins. It is suggested that when cellular events or pathways are perturbed then transportation of proteins and lipids is greatly affected. For example, we have indicated by studying the expression of some proteins genes that upon infection by stolbur PO phytoplasma, there is malformation of floral parts and alteration in the expression of proteins genes (Ahmad *et al.*, 2011) suggesting that functionality of possible SNAREs was affected during infection. Membranes properties are highly dependent on lipid protein interactions so membranes structure and membrane trafficking is controlled by close relationship

between protein based and lipid based machineries (Hashem *et al.,* 2012). Many proteins interact with lipids for being acylated and deacylated and play a key role to control the membrane curvature. Long chain Acyl-CoAs may interact with a large number of proteins and considers a key modulator for cellular function (Rai *et al*., 2011). We used anti-acyl CoAs antibody to know the possible interacting protein partners with Acyl CoAs in *Arabidopsis* and tobacco and found that acyl CoAs have a great interaction with GAPDH protein and immune-precipitated from the *Arabidopsis* and tobacco. Anti acyl CoAs antibodies can immune-precipitate even protein complex, hence is a better tool to find the protein partners involved in trafficking. We also purified anti-PS antibodies and tested against various ratios of PC/PS composed vesicles through the system of BIA core in which vesicles interact with the membrane of known lipid composition and still working. Anti SNAREs antibodies and Anti-lipid antibodies, concluding my discussion, are the greatest tools of research to know the lipid protein interaction at any level in the field of science. In future, by using this technique, we will be able to identify and use some important proteins and lipids by improving the transportation system in secretory pathway of plant as well as in animal and human being which will be very helpful to cure many diseases caused by the improper proteins transportation.

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