How a first research experience had an impact on my scientific journey

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ABSTRACT As I look back to my scientific trajectory on the occasion of being the recipient of the E. B. Wilson Medal of the American Society for Cell Biology, I realize how much an early scientific experience had an impact on my research many years later. The major influence that the first research encounters can have in defining a scientist’s path makes the choice of the training environment so important for a future career.

During my scientific career I have worked on different topics. However, my scientific journey represents a progression of steps that build onto each other. Each experience, no matter how successful, had an impact on how I interpreted subsequent results, on how I assessed their implications, and on how I made subsequent programmatic decisions. My research at the interface of cell biology and neuroscience has spanned from membrane traffic and membrane remodeling, to phosphoinositide signaling, and more recently also to the role of membrane contact sites in the control of membrane lipid homeostasis in physiology and disease. While only partially related, all these topics are interconnected by an intellectual thread. As an example—which shows how defining our training directions many years later.

From the day I enrolled in medical school at the University of Milano I knew I wanted to be a scientist. While a medical student, I explored several research environments that would expose me to leading-edge science. Eventually I encountered a young scientist, Jacopo Meldolesi, who had just returned to Italy after studying the leading-edge science. I decided to join his lab to carry out the required short medical school thesis project. Jacopo was interested in the regulation of secretion and proposed that I explore the secretory pathway as a postdoc with Jim Jamieson and George Palade at Rockefeller University, and I decided to join his lab to carry out the required short medical school thesis project. Jacopo was interested in the regulation of secretion and proposed that I explore mechanisms underlying the so-called “PI (Phosphatidylinositol) effect” described by Hokin and Hokin in the 1950s (Hokin and Hokin, 1953). These investigators had found that stimulation of secretory cells resulted in the rapid cleavage of PI immediately followed by its resynthesis. It remained unclear whether these changes reflected a signaling reaction elicited by secretagogues or metabolic changes associated with membrane traffic reactions triggered by the stimulus. So, the idea was to determine in which membrane compartment this turnover occurred: selectively at the plasma membrane (thus supporting a role in signaling) or in membranes of the secretory pathway. We were puzzled by finding that at least under the rather poor time resolution of our experiments—incubation of pancreatic lobules for tens of minutes with radioactive inositol following stimulation with acetylcholine—newly synthesized PI was found at similar concentrations in all membranes. But then we became aware of the recently described proteins that transport lipids between membranes through the cytosol and independent of membrane traffic (Wirtz and Zilversmit, 1969). We thought that perhaps these proteins could contribute to rapidly equilibrating PI pools in different compartments, questioning the validity of our experimental approach to identify the site of PI resynthesis. I moved on to other projects, and I thought I would never again work with lipids.

In the following years the work of several labs established that the PI effect discovered by Hokin and Hokin mainly reflected agonist-stimulated phospholipase C (PLC)-dependent cleavage of the phosphoinositide PI(4,5)P2 at the plasma membrane to generate the second messengers diacylglycerol (DAG) and IP3 (phosphoinositides are the phosphorylated products of PI) (Lapetina and Michell, 1973; Inoue et al., 1977; Berridge et al., 1983). These findings became textbook knowledge, and the idea that PI(4,5)P2 had primarily a function in signal transduction at the cell surface became mainstream. The additional discovery of the PI(3,4)P2 and PI(3,4,5)P3 as mediators of growth factor actions added a new twist to the field by showing that not only metabolites generated by PI(4,5)P2 cleavage, but also phosphoinositides themselves, could have a signaling role (Tranyor-Kaplan et al., 1988; Whitman et al., 1988; Auger et al., 1989).
However, once again, this signaling appeared to be implicated in classical signal transduction, not in membrane traffic.

In the meantime, I had moved to Yale, first as a postdoc with Paul Greengard and then as a faculty in the Section of Cell Biology (which subsequently became the Department of Cell Biology). Following my postdoc with Greengard, I had become interested in the cell biology of synapses and in the molecular machinery underlying the exo-endocytic recycling of synaptic vesicles. In what turned out to be a seminal finding, in the mid-1990s Peter McPherson, a postdoc in my laboratory, identified a nerve terminal–enriched protein that shared some interactions with the GTPase dynamin, another abundant presynaptic protein studied in our lab (McPherson et al., 1994). As dynamin mediates the fission reaction of endocytosis by cutting the neck of endocytic buds to generate free vesicles (Takei et al., 1995; Roux et al., 2006; Ferguson and De Camilli, 2012), we speculated that this new protein would also have a role in endocytic traffic. Peter set out to clone it, and I vividly recall my surprise when, upon my returning from a summer vacation in Italy he reported to me that a domain of this protein, which we called synaptojanin, had homology to an inositol 5-phosphatase that dephosphorylates PI(4,5)P₂ (McPherson et al., 1996). A second domain of this protein was subsequently found to have inositol 4-phosphatase activity (Guo et al., 1999), so that synaptojanin can dephosphorylate PI(4,5)P₂ all the way to PI. Thus, I was back to lipids, specifically to inositol phospholipids, and to a potential role of these lipids not in the transduction of extracellular signals, but in membrane traffic. With a mind primed by my project in medical school, I delved with enthusiasm into this topic. PI(4,5)P₂ had been shown to interact with clathrin adaptors (Beck and Keen, 1991), but the physiological significance of these findings had remained unclear. Our studies led to a model in which the selective concentration of PI(4,5)P₂ in the plasma membrane is required for the recruitment of clathrin adaptors and other endocytic factors to this membrane, while the tight coupling of PI(4,5)P₂ dephosphorylation by synaptojanin to the dynamin-dependent fission reaction of endocytosis is required to allow the shedding of such factors once the vesicle has undergone separation from the plasma membrane (Cremona et al., 1999; Cremona and De Camilli, 2001; Wenk et al., 2001). We also discovered curvature-generating/sensing proteins (BAR domain–containing proteins, primarily endophilin) that are responsible for achieving this coupling by coordinating the formation of the narrow neck of endocytic buds with the recruitment of synaptojanin (Takei et al., 1999; Farsad et al., 2001; Frost et al., 2009; Wu et al., 2010; Milosevic et al., 2011). This model, which is encapsulated in Figure 1 and which is supported by genetic studies in mice and other model organisms, posits that a cycle of PI phosphorylation and dephosphorylation is nested within the synaptic vesicle cycle. The implication of a phosphoinositide phosphatase in membrane transport converged with the identification of a PI kinase (the PI 3-kinase Vps34) involved in “vacuolar protein sorting” in yeast (Schu et al., 1993). This raised the possibility that the phosphorylation and dephosphorylation of inositol phospholipids could have a general significance in membrane traffic (De Camilli et al., 1996).

The field grew very rapidly. It was found that reversible phosphorylation of phosphatidylinositol at the 3,4 and 5 positions of the inositol ring by a multiplicity of enzymes generates seven phosphoinositide species whose differential protein-binding specificities help control, often via a dual key mechanism (Wenk and De Camilli, 2004), membrane cytosol interfaces (Di Paolo and De Camilli, 2006; Vicinanza et al., 2008; Balla, 2013). Along with small GTPases (Behnia and Munro, 2005), phosphoinositides are key determinants of the identity of membranes or membrane subdomains and thus control the multiplicity of reactions that occur at membrane–cytosol interfaces, such as the recruitment and shedding of trafficking proteins, the function of integral membrane proteins, and the assembly and disassembly of signaling and cytoskeleton complexes. Importantly, it became clear that, as we had found for synaptojanin, interconversion of phosphoinositide species along the secretory and endocytic pathways functions as a switch to release membrane-associated factors that define one compartment and to recruit factors that define the next compartment (Odorizzi et al., 2000; Di Paolo and De Camilli, 2006; Zoncu et al., 2009). I think I would not have immediately grasped the significance of the identification of synaptojanin and then invested much of our work in this field had it not been for my early research experience in medical school.

The roles of phosphoinositides in membrane identity imply the existence of mechanisms to tightly control their localizations and also to ensure availability of PI in membranes—primarily the plasma membrane—where the backbone of this lipid is consumed by PLC. Surprisingly, genetic evidence suggested that one mechanism to control PI4P levels in membranes is via their direct contacts with the endoplasmic reticulum (ER), where a main PI4P phosphatase is localized (Foti et al., 2001; Stefan et al., 2011; Mesmin et al., 2013). Concerning availability of PI, DAG generated by PLC and its phosphorylated downstream product phosphatidic acid must be returned to the ER for metabolic recycling by the ER-localized phosphatidylinositol synthase, and newly synthesized PI then needs to be rapidly transported to the plasma membrane to replace its depleted pool. Strong evidence indicates that these reactions are mediated at least in part by lipid transfer proteins, and we have now learned that much of this protein-mediated lipid transport occurs at membrane

FIGURE 1: Schematic cartoon depicting the occurrence of a cycle of phosphatidylinositol phosphorylation–dephosphorylation nested within the exo-endocytic recycling of synaptic vesicles at synapses. PI(4,5)P₂ in the plasma membrane helps define this membrane as the acceptor for the fusion of synaptic vesicles and as a coreceptor for endocytic factors responsible for their reinternalization. PI(4,5)P₂ dephosphorylation by synaptojanin allows the shedding of endocytic factors and the reutilization of vesicles for a new cycle of secretion. Recruitment of synaptojanin is coupled to dynamin-dependent fission via its binding to the BAR domain–containing protein endophilin, a curvature-generating/sensing protein that also binds dynamin.
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REFERENCES

Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC (1989). PDGF-dependent tyrosine phosphorylation stimulates production of novel phosphoinositides in intact cells. Cell 57, 167–175.

Ball T (2013). Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev 93, 1019–1137.

Beck KA, Keen JH (1991). Interaction of phosphoinositide cycle intermediates with plasma-membrane-associated clathrin assembly protein AP-2. J Biol Chem 266, 4442–4447.

Behnia R, Munro S (2003). Organelle identity and the signposts for membrane traffic. Nature 438, 597–604.

Berridge MJ, Dawson RM, Downes CP, Hesslop JP, Irvine RF (1983). Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem J 212, 473–482.

Bishop RE (2019). Ratcheting up lipopolysaccharide transport. Nature 567, 471–472.

Chung J, Torta F, Masai K, Lucusta L, Czaplka H, Tanner LB, Narayanaswamy P, Wenk MR, Nakatsu F, De Camilli P (2015). Intracellular transport. PI4P-phosphatidylycerine countertransporter at ORPs- and ORP8-mediated ER-plasma membrane contacts. Science 349, 428–432.

Cockcroft S, Raghu P (2018). Phospholipid transport protein function at organelle contact sites. Curr Opin Cell Biol 53, 52–60.

Cremona O, De Camilli P (2001). Phosphoinositides in membrane traffic at the synapse. J Cell Sci 114, 1041–1052.

Cremona O, Di Paolo G, Wenk MR, Luthi A, Kim WT, Takei K, Daniell L, Nemo Y, Shears SB, Flavell RA, et al. (1999). Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell 99, 179–188.

De Camilli P, Emr SD, McPherson PS, Novick P (1996). Phosphoinositides as regulators in membrane traffic. Science 271, 1533–1539.

Di Paolo G, De Camilli P (2006). Phosphoinositides in cell regulation and membrane dynamics. Nature 443, 451–457.

Dong R, Saheki Y, Swarup S, Lucast L, Harper JW, De Camilli P (2016). Endosome-ER contacts control actin nucleation and retromer function through VAP-dependent regulation of PI4P. Cell 166, 408–423.

Farsad K, Ringstad N, Takei K, Floyd SR, Rose K, De Camilli P (2001). Generation of high curvature membranes mediated by direct endophilin-bilayer interactions. J Cell Biol 155, 193–200.

Ferguson SM, De Camilli P (2012). Dynamin, a membrane-remodelling GTPase. Nat Rev Mol Cell Biol 13, 75–88.

Foti M, Audhya A, Emr SD (2001). Sac1 lipid phosphatase and Stt4 phosphatidylinositol 4-kinase regulate a pool of phosphatidylinositol 4-phosphate that functions in the control of the actin cytoskeleton and vacuole morphology. Mol Biol Cell 12, 2396–2411.

Frost A, Unger VM, De Camilli P (2009). The BAR domain superfamily: membrane-molding macromolecules. Cell 137, 191–196.

Giordano F, Saheki Y, Idevall-Hagen O, Colombo SF, Pirruccello M, Milosevic I, Gracheva EO, Bagriantsev SN, Borgese N, De Camilli P (2013). Ph(4,5)(P)(2)-dependent and Ca(2+)-regulated ER-PM interactions mediated by the extended synaptotagmins. Cell 153, 1494–1509.

Guillen-Samander A, Leonzino M, Hanina MG, Tang N, Shen H, De Camilli P (2021). VPS13D bridges the ER to mitochondria and peroxisomes via Miro. J Cell Biol 220, e202010004.

Guo S, Stolz LE, Lemrow SM, York JD (1999). SAC1-like domains of yeast SAC1, INPS2, and INPS3 and of human synaptopjanin encode polyphosphoinositide phosphatases. J Biol Chem 274, 12990–12995.

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REFERENCES

Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC (1989). PDGF-dependent tyrosine phosphorylation stimulates production of novel phosphoinositides in intact cells. Cell 57, 167–175.

Ball T (2013). Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev 93, 1019–1137.

Beck KA, Keen JH (1991). Interaction of phosphoinositide cycle intermediates with plasma-membrane-associated clathrin assembly protein AP-2. J Biol Chem 266, 4442–4447.

Behnia R, Munro S (2003). Organelle identity and the signposts for membrane traffic. Nature 438, 597–604.
Hokin MR, Hokin LE (1953). Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. J Biol Chem 203, 967–977.

Inoue M, Kishimoto A, Takai Y, Nishizuka Y (1977). Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain. J Biol Chem 252, 7610–7616.

Kumar N, Leonzino M, Hancock-Cerutti W, Horenkamp FA, Li P, Lees JA, Wheeler H, Reinsch KM, De Camilli P (2018). VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. J Cell Biol 217, 3625–3639.

Lang AB, John Peter AT, Walter P, Kornmann B (2015). ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. J Cell Biol 210, 883–890.

Lapetina EG, Michell RH (1973). A membrane-bound activity catalysing phosphatidylinositol breakdown to 1,2-diacylglycerol, D-myoinositol 1:2-cyclic phosphate and D-myoinositol 1-phosphate. Properties and subcellular distribution in rat cerebral cortex. Biochem J 131, 433–442.

Leonzino M, Reinsch KM, De Camilli P (2021). Insights into VPS13 properties and function reveal a new mechanism of eukaryotic lipid transport. Biochim Biophys Acta Mol 1866, 159003.

Li P, Lees JA, Lusk CP, Reinsch KM (2020). Cryo-EM reconstruction of a VPS13 fragment reveals a long groove to channel lipids between membranes. J Cell Biol 219, e202001161.

McPherson PS, Garcia EP, Slepnev VI, David C, Zhang X, Grabs D, Sossin WS, Bauerfeind R, Nemoto Y, De Camilli P (1996). A presynaptic inositol-5-phosphatase. Nature 379, 353–357.

McPherson PS, Takei K, Schmid SL, De Camilli P (1994). p145, a major Grb2-biding protein in brain, is co-localized with dynamin in nerve terminals where it undergoes activity-dependent dephosphorylation. J Biol Chem 269, 30132–30139.

Mesmin B, Bigay J, Moser von Filsseck J, Lacas-Gervais S, Drin G, Antony B (2013). A four-step cycle driven by P(4)P hydrolysis directs sterol/P(4)P exchange by the ER-Golgi tether OSBP. Cell 155, 830–843.

Milosevic I, Grováš S, Lou X, Raimondi A, Collesi C, Shen H, Paradise S, O’Toole E, Ferguson S, Cremona Q, De Camilli P (2011). Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoupling after fusion. Neuron 72, 587–601.

Odorizzi G, Babst M, Emr SD (2000). Phosphoinositide signaling and the regulation of membrane trafficking in yeast. Trends Biochem Sci 25, 229–235.

Park JS, Thorsness MK, Policastro R, McGoldrick LL, Hollingsworth NM, Thorsness PE, Neiman AM (2016). Yeast Vps13 promotes mitochondrial function and is localized at membrane contact sites. Mol Biol Cell 27, 2435–2449.

Pemberton JG, Kim YJ, Balla T (2020). Integrated regulation of the phosphatidylinositol cycle and phosphoinositol-driven lipid transport at ER-PM contact sites. Traffic 21, 200–219.

Roux A, Uyhazi K, Frost A, De Camilli P (2006). GTP-dependent twisting of dynamin implicates constriction and tension in membrane fission. Nature 441, 528–531.

Saheki Y, Bian X, Schauder CM, Sawaki Y, Surma MA, Klose C, Pincet F, Reinsch KM, De Camilli P (2016). Control of plasma membrane lipid homeostasis by the extended synaptotagmins. Nat Cell Biol 18, 504–515.

Schauer CM, Wu X, Saheki Y, Narayanaswamy P, Torta F, Wenk MR, De Camilli P, Reinsch KM (2014). Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. Nature 510, 552–555.

Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD (1993). Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. Science 260, 88–91.

Stefan CJ, Manford AG, Baird D, Yamada-Hanff J, Mao Y, Emr SD (2011). Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. Cell 144, 389–401.

Takei K, McPherson PS, Schmid SL, De Camilli P (1995). Tubular membrane invaginations coated by dynamin rings are induced by GTP-gamma-S in nerve terminals. Nature 374, 186–190.

Takei K, Slepnev VI, Haucke V, De Camilli P (1999). Functional partnership between amphipathin and dynamin in clathrin-mediated endocytosis. Nat Cell Biol 1, 33–39.

Traynor-Kaplan AE, Harris AL, Thompson BL, Taylor P, Sklar LA (1988). An inositol tetrakisphosphate-containing phospholipid in activated neutrophils. Nature 334, 353–356.

Ugur B, Hancock-Cerutti W, Leonzino M, De Camilli P (2020). Role of VPS13, a protein with similarity to ATG2, in physiology and disease. Curr Opin Genet Dev 65, 61–68.

Valverde DP, Yu S, Boggavarapu V, Kumar N, Lees JA, Walz T, Reinsch KM, Melia TJ (2019). ATG2 transports lipids to promote autophagosome biogenesis. J Cell Biol 218, 1787–1798.

Vicinanza M, D’Angelo G, Di Campli A, De Matteis MA (2008). Phosphoinositides as regulators of membrane trafficking in health and disease. Cell Mol Life Sci 65, 2833–2841.

Wenk MR, De Camilli P (2004). Protein-lipid interactions and phosphoinositide metabolism in membrane traffic: insights from vesicle recycling in nerve terminals. Proc Natl Acad Sci USA 101, 8262–8269.

Wenk MR, Pellegrini L, Kielenin VA, Di Paolo G, Chang S, Daniell L, Arioka M, Martin TF, De Camilli P (2001). PIP kinase Igamma is the major P(4,5)P(2) synthetase enzyme at the synapse. Neuron 32, 79–88.

Whitman M, Downes CP, Keeler M, Keller T, Cantley L (1988). Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature 332, 644–646.

Wirtz KW, Zilversmit DB (1969). Participation of soluble liver proteins in extended synaptotagmin indicates a role in lipid transfer. Nature 374, 186–190.

Wong LH, Copic A, Levine TP (2017). Advances on the transfer of lipids by lipid transfer proteins. Trends Biochem Sci 42, 516–530.

Wu M, Huang B, Graham M, Raimondi A, Heuser JE, Zhuang X, De Camilli P (2010). Coupling between clathrin-dependent endocytic budding and F-BAR-dependent tubulation in a cell-free system. Nat Cell Biol 12, 902–908.

Zoncu R, Perera RM, Balkin DM, Pirrucello M, Toomre D, De Camilli P (2009). A phosphoinositide switch controls the maturation and signaling properties of APPL endosomes. Cell 136, 1110–1121.