The Study of Lipid Signaling. Multidisciplinary Approaches

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People say that success stems from persistence, effort and, above all, desire to succeed. But when it comes to success, there is always the chance to turn the cards on, because the only thing that matters is the value of things, not the things that have value. Which leads us to the key question: is success the key to happiness or is it the other way around? (News section, November 12, 2015: Master Publications List).

Our current research focuses primarily on the lipid signaling enzymes phospholipase A$_2$ and phosphatidate phosphohydrolase (phosphatidic acid-specific phospholipase C; lipin). The latter is a key enzyme in the de novo pathway for glycerolipid biosynthesis, providing an excellent example that enzymes involved in this pathway may also act to initiate intracellular signaling. General events that we are interested in include (i) the spatiotemporal regulation of these phospholipases in a cellular context, which we study utilizing advanced microscopy techniques, (ii) pharmacological manipulation of enzymatic activity both in intact cells and in vitro, (iii) analysis of lipid metabolite production by state-of-the-art mass spectrometry (lipidomics & metabolipidomics), and (iv) the physiological functioning of phospholipases in animal models.

Ongoing studies in our labs focus on the localization and stimulus-driven translocation of different members of the phospholipase A$_2$ and lipin families. Phospholipase A$_2$s cleave the fatty acid at the sn-2 position of phospholipids and thus constitute the earliest regulatory point of the eicosanoid biosynthetic cascade. Lipins dephosphorylate phosphatidic acid to form diacylglycerol, which can be used for the biosynthesis of glycerophospholipids and triacylglycerol, and may function as intracellular signalers as well. Current studies are being carried out by transfecting chimeric constructs of green fluorescent protein (GFP) (or any of its colored varieties) with the appropriate phospholipase. GFP is placed at either the N- or C-termini of the enzymes. These constructs provide a very useful tool to visualize the intracellular movements of the enzymes in response to the different stimuli. Mutagenesis studies are also being conducted to pinpoint the specific amino acids of the phospholipase A$_2$s and lipins that are implicated in the movement among intracellular compartments.

Another of our goals is to apply a lipidomics approach to the study of the mechanisms governing the availability and oxidative metabolism of free arachidonic acid during activation of macrophages by stimuli of the innate immune response. Availability of free arachidonate is a limiting step for the synthesis of eicosanoids. While the pathways of fatty acid uptake, incorporation and remodeling in glycerolipids are well documented, the individual lipid species in which arachidonate is stored and released from have not been identified. This is so because of the impossibility of traditional methods for lipid separation (i.e. thin-layer chromatography, liquid chromatography) to differentiate among individual lipids within various classes and subclasses. This is now possible with the advent of electrospray mass spectrometry (ESI-MS). Application of this technology to the field of lipid biochemistry has been a major breakthrough in profiling the lipidomes of cells and tissues in physiological and pathophysiological conditions. We are conducting lipidomic analyses of all the lipid molecular species involved in arachidonic acid homeostasis, from those that act as acceptors of the fatty acid to
those from which the fatty acid is liberated for subsequent eicosanoid synthesis, and including as well a full survey of arachidonate-derived oxygenated metabolites.

In the context of these studies, we have described a number of novel arachidonate-containing lipids, the levels of which increase during cell activation. We are currently investigating the metabolic pathways involved in their biosynthesis as well possible biological processes mediated by these species. (From Results section, retrieved Nov 14, 2015: Master Publications List).

REFERENCES


