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Review

Tracking the metabolic pulse of plant lipid production with isotopic labeling and flux analyses: Past, present and future

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ABSTRACT

Metabolism is comprised of networks of chemical transformations, organized into integrated biochemical pathways that are the basis of cellular operation, and function to sustain life. Metabolism, and thus life, is not static. The rate of metabolites transitioning through biochemical pathways (i.e., flux) determines cellular phenotypes, and is constantly changing in response to genetic or environmental perturbations. Each change evokes a response in metabolic pathway flow, and the quantification of fluxes under varied conditions helps to elucidate major and minor routes, and regulatory aspects of metabolism. To measure fluxes requires experimental methods that assess the movements and transformations of metabolites without creating artifacts. Isotopic labeling fills this role and is a long-standing experimental approach to identify pathways and quantify their metabolic relevance in different tissues or under different conditions. The application of labeling techniques to plant science is however far from reaching it potential. In light of advances in genetics and molecular biology that provide a means to alter metabolism, and given recent improvements in instrumentation, computational tools and available isotopes, the use of isotopic labeling to probe metabolism is becoming more and more powerful. We review the principal analytical methods for isotopic labeling with a focus on seminal studies of pathways and fluxes in lipid metabolism and carbon partitioning through central metabolism. Central carbon metabolic steps are directly linked to lipid production by serving to generate the precursors for fatty acid biosynthesis and lipid assembly. Additionally some of the ideas for labeling techniques that may be most applicable for lipid metabolism in the future were originally developed to investigate other aspects of central metabolism. We conclude by describing recent advances that will play an important future role in quantifying flux and metabolic operation in plant tissues.

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1. Introduction

1.1. Network fluxes define cellular phenotype and are measured with isotope labeling

Plant cellular function is defined by networks of enzymatic reactions with substrates and products that are linked by mass and energy balances. Through developmental or environmental cues the expression of genes change the operating network and underscore the dynamic nature of metabolism. Fluxes establish the products of metabolism and can be measured through the rate of accumulation for end products (i.e., compounds that are not turned over such as storage protein or oil in a seed). However at metabolic steady state cellular intermediates do not accumulate and are both produced and consumed at rates that cannot be readily inferred from metabolite concentrations alone (i.e., metabolomics). Thus as a metabolic attribute, fluxes must be assessed through other means. Isotopes can serve as "tracers" that describe the rate of conversion between pools over time, by providing a change in metabolite molecular mass that occurs without perturbing metabolism. Therefore the isotopic "labeling" indicates formation and turnover through movement of an isotope from one metabolite to the next, leading to flux descriptions that are important to studies on metabolic operation, regulation and control [1].

1.2. Cellular roles for lipids in metabolism are diverse and complex

In biology few types of molecules serve as many diverse roles or are as poorly characterized as lipids. The Arabidopsis genome contains over 600 genes annotated with functions putatively tied to lipid and/or fatty acid catabolism and anabolism, however less than half of the genes have been characterized with *in vivo* studies of mutants that demonstrate a clear role in lipid metabolism [2,3]. In the most rigorously studied pathways many enzymes have multiple isoforms that conduct identical or very similar biochemical reactions, but reflect specialized cellular, subcellular or developmental activities for different aspects of lipid metabolism. Whereas some mechanisms of lipid metabolism are conserved between organisms, the subcellular descriptions of biosynthesis and degradation in plants are distinct from other species [4]. Thus textbook descriptions of lipid metabolism are not universal, and the operational network of metabolic reactions can vary between species, tissues, and cells; as well as across developmental and environmental conditions. Fluxes change to accommodate the different cellular demands for lipid production (e.g., membrane, surface, or storage lipids), as well as turnover (e.g., storage oil breakdown during germination) necessary to produce other metabolic precursors, energy, and to maintain homeostasis [5,6].

Lipids comprise membranes that are a defining feature of cell biology. Membrane lipids separate cells from their environment (i.e., plasma membranes) and establish subcellular organelles that compartmentalize metabolism in eukaryotes. Layered on surfaces, cutin and suberin provide a resistive, protective barrier to natural elements, while other lipids perform signaling functions or serve in an energy storage capacity. Triacylglycerols (TAG) in the seeds (e.g., soybean, rapeseed, sunflower) or fruit (e.g., olive, avocado, palm) of many plants are an energy dense storage form of biomass. Apart from pericarp tissues, stored oils are remobilized at germination providing carbon and ATP for plant growth until autotrophic metabolism can be sustained [7]. The biochemistry and genetics of storage lipid accumulation have received extensive attention, and this area of research remains an important focus in part because acyl chains are one of the most highly reduced forms of carbon (i.e., approximately twice the energy content per gram of dry weight than carbohydrates or storage protein) and can be used to supplant non-renewable petroleum in many applications. Our dependence on TAGs for food, fuel and chemicals contributes to an industry currently estimated at over $120 billion per year (http://lipidlibrary.aocs.org/market/prices.htm). Despite the many roles for lipids and intense research in plant lipid biochemistry and genetics over the past half century, significant gaps in our understanding remain, foremost among these are the identity and *in vivo* function of genes and enzymatic reaction networks involved in lipid metabolism [2,3].

1.3. The scope of opportunities for lipid production and scientific discovery

Given that oil content varies in plants from less than 1% of dry weight (e.g., lentils, potatoes) to approximately 70% (pecans, walnuts) and can exceed 88% in mesocarps (e.g., palm); there exists a large difference in metabolic operation among various cells that is an inviting prospect for engineering increased oil accumulation in plants. Tissues such as leaves are usually less than 5% lipid, however the abundance of leafy biomass *vs.* seed/mesocarp tissue in most plants has led to significant recent efforts to engineer lipid accumulation into vegetative tissues for biofuels [8–14]. To