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Central metabolic nodes for diverse biochemical production

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Central carbon metabolism is conserved among all organisms for cellular function and energy generation. The connectivity of this metabolic map gives rises to key metabolite nodes. Five of these nodes in particular, pyruvate, citric acid, tyrosine and aspartate, acetyl-CoA, serve as critical starting points for the generation of a broad class of relevant chemical molecules with ranging applications from fuels, pharmaceuticals and polymer precursors. This review highlights recent progress in converting these metabolite nodes into valuable products. In particular, acetyl-CoA, the most well-connected node, serves as the building block for several classes of molecules including fatty acids and terpenes. Systematic metabolic engineering efforts focused on these metabolic building blocks has enabled the production of industrially-relevant, biobased compounds.

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Introduction

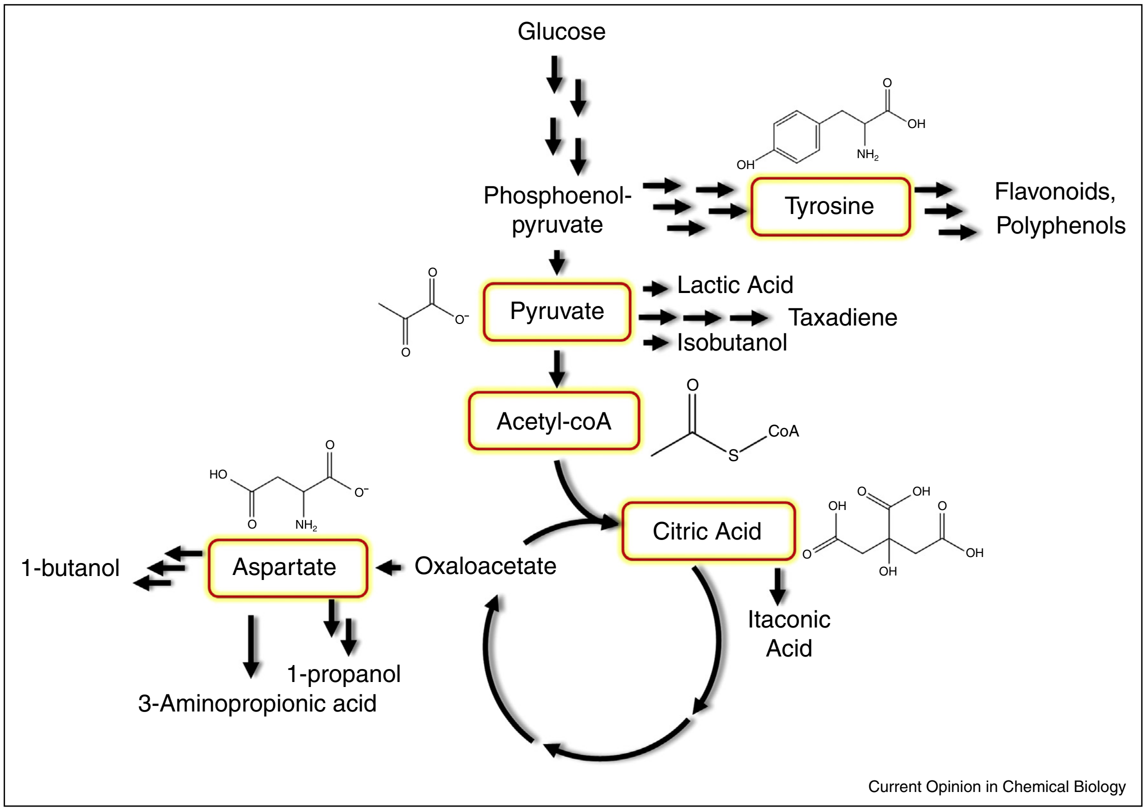
At first glance, the biochemical diversity of metabolism across all living species is immense and complex. Yet, most heterotrophic cellular systems are linked by a common thread — the ability to assimilate glucose into biological building blocks and energy. As a result, organisms have a highly conserved central carbon metabolism that embodies optimal pathways for energy and precursor generation. Toward this end, extensive network analysis of metabolism in *Escherichia coli* demonstrates a high interconnectivity of metabolites with minimum walks between key biomass precursors [1,2]. These interconnected metabolites provide the chemistry of life, but also enable routes to important biobased chemicals — including most in the 2004 US Department of Energy report of 'top value added chemicals from biomass' [3,4••]. The intersection of this list with key metabolites reveal pyruvate, citric acid, tyrosine, aspartate, and acetyl-CoA as critical metabolic nodes for bioproducts (Figure 1). Among these, the acetyl-CoA node has a central role in many biosynthetic pathways of study. This role is not surprisingly given that this molecule serves as a bridge between glycolysis and the tricarboxylic acid (TCA) cycle. Certainly each of these nodes have been the subject of systematic engineering for improved production of fuels, specialty chemical, and commodity chemicals [5•]. In this review, we seek to contextualize the myriad of biobased molecules and demonstrate how a select few precursors (specifically, pyruvate, citric acid tyrosine, aspartate and acetyl-CoA) can give rise to a wide variety of industrially-relevant, biobased compounds for fuels and biochemicals applications (Table 1). Specifically, we highlight a few important chemicals derived from each of these metabolites with a special focus on acetyl-CoA as it serves as the most fruitful node for producing important industrial biochemicals.

Chemicals derived from key nodes in metabolism

Pyruvate-derived molecules

Situated at the end of glycolysis, pyruvate is one of the most connected metabolites — it serves as a precursor to critical amino acids (valine, leucine, and isoleucine) and can interconvert in either direction of the TCA cycle. A long-standing bioproduct derived from this node is lactic acid, generated through dehydrogenation using redox equivalents similar to ethanol production. Polymerization of lactic acid into the bioplastic polylactic acid has growing interest especially for applications in 3D printing [6]. Alternatively, lactic acid can be polymerization to poly(lactate-*co*-glycolate), a biodegradable and nontoxic polymer for biomedical applications, which has been achieved through dual uptake of glucose and xylose in *Escherichia coli* [7]. High level production of lactic acid was achieved in *Saccharomyces cerevisiae* by introducing lactate dehydrogenase in addition to cytosolic modulation of NADH redox to obtain 117 g/L in a 2 L bioreactor [8]. For further improved titers, immobilization of *Rhizopus oryzae* in a sponge-like cubic particle led to 231 g/L lactic acid from glucose [9]. Pyruvate also serves as the gateway to the isoprenoid and terpene pathways via condensation with the glycolysis metabolite glyceraldehyde-3-phosphate. In a recent example, optimization of the isoprene pathway led to production of 1020 mg/L of taxadiene, an important intermediate to the anticancer

Figure 1



Central nodes in carbon metabolism as precursors to relevant biochemical. A simplified schematic of metabolism is provided with an emphasis on the core metabolites discussed in this review. Each arrow corresponds to two enzymatic steps when more than one step occurs.

drug known as Taxol or paclitaxel [10]. Likewise, the biofuel isobutanol has been produced from pyruvate as part of the valine pathway with titers of 22 g/L achieved in *E. coli* [11]. Finally, in conjunction with acetyl-CoA (see section below), pyruvate can serve as a precursor backbone for short and medium chain fatty acid ester production. As examples, isobutyl acetate titers in *E. coli* of 19.7 g/L were obtained in a recent study with further demonstrations that the longer chain isobutyl butyrate was also possible with a similar reaction, albeit with a lower initial titer (27.1 mg/L) [12,13]. Utilizing these pathways, longer chain esters (from C12–C18 fatty acid backbones) were obtained with titers of 25.4 mg/L in *S. cerevisiae* and just over 1 g/L in *E. coli* [14,15]. High level production from the pyruvate branch highlights the importance of this central metabolite for bioproducts.

Citric acid-derived molecules

Citric acid, an important organic acid in the TCA cycle, serves as a critical node where acetyl-CoA enters for energy generation. Citric acid itself also has applications as a preservative used in cosmetics, food and beverage industries. This compound has historically been produced through submerged fermentation of *Aspergillus niger*; however, recent efforts have created an alternative with the oleaginous yeast *Yarrowia lipolytica*. Specifically, introduction of an inulinase enzyme to degrade inulin as a feedstock led to the production of nearly 78 g/L of citric acid in shake flasks [16]. Due to its metabolic positioning, citric acid can be converted through short pathways to important precursors and monomers such as itaconic acid [17••]. In a two-step reaction, itaconic acid can be generated from citric acid through a cis-aconitic acid decarboxylase resulting in a *Y. lipolytica* strain producing 4.6 g/L [18]. These examples demonstrate the importance and variety of industries that make use of the citric acid node.

Tyrosine-derived molecules

The aromatic amino acids (specifically those linked with tyrosine) serve as a rich branch of chemistry with the functionality of the aromatic ring. These molecules are produced via the shikimate pathway by combining erythrose-4-phosphate and phosphoenolpyruvate. After seven enzymatic conversions, chorismate serves as the branch point between tryptophan, phenylalanine and tyrosine. Chorismate is also a metabolite with one of the shortest mean path lengths upon network analysis with downstream pathways leading to the bioproduction