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Current Opinion in Chemical Biology

Carbon partitioning in photosynthesis

Anastasios Melis

The work seeks to raise awareness of a fundamental problem that impacts the renewable generation of fuels and chemicals via (photo)synthetic biology. At issue is regulation of the endogenous cellular carbon partitioning between different biosynthetic pathways, over which the living cell exerts stringent control. The regulation of carbon partitioning in photosynthesis is not understood. In plants, microalgae and cyanobacteria, methods need be devised to alter photosynthetic carbon partitioning between the sugar, terpenoid, and fatty acid biosynthetic pathways, to lower the prevalence of sugar biosynthesis and correspondingly upregulate terpenoid and fatty acid hydrocarbons production in the cell. Insight from unusual but naturally occurring carbon-partitioning processes can help in the design of blueprints for improved photosynthetic fuels and chemicals production.

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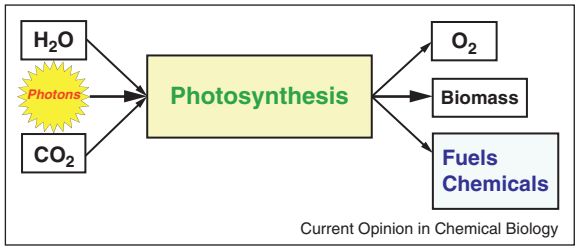
The discipline of (photo)synthetic biology seeks to apply cellular metabolic pathways toward product generation, typically fuels and chemicals. Application of this concept, regardless of the organism and final product, inevitably encounters the problem of carbon partitioning in the cellular metabolism. In simple terms, the living cell sets highly regulated priorities in carbon partitioning, which are directed toward biomass increase, cell propagation, and survival. Cellular metabolic priorities are thus inherently different from those required for renewable fuel and chemicals production. This dilemma is especially pronounced in the case of photosynthetic organisms, where photosynthesis is applied directly for the generation of renewable product. For example, the concept of *Photosynthetic Biofuels*, as applied in the lab of the author, entails a process whereby light-absorption, photosynthesis, and metabolism toward fuels and chemicals production all take place in one and the same organism. In this case, the same cell, or chloroplast, performs all the renewable product-related functions, beginning with light-absorption and utilization, including the steps of photosynthetic carbon fixation, and metabolic flux to specific fuels and chemicals (Figure 1).

Organisms of oxygenic photosynthesis can convert, with high efficiency [1], CO_2 into 3-phosphoglyceric acid (3-PGA) and glyceraldehyde-3-phosphate (G3P), leading to biosynthesis of sugars, but also products of the terpenoid and fatty acid biosynthetic pathways (Figure 2). The latter have attracted attention recently because they could be applied to generate fuels and chemicals [2••]. For example, the fatty acid biosynthetic pathway can be directed to yield 'biodiesel' type molecules [3], whereas the terpenoid biosynthetic pathway could yield terpene type molecules that serve as 'bio-gasoline' and 'jet-fuel', and also as feedstock for the synthetic chemistry industry [4–7,8•]. A paramount requirement for the commercially viable application of these pathways toward product development is, however, the ability to convert as large a proportion of photosynthetic carbon as possible into fuels and chemicals, which is opposite to the cellular priority of making sugars and biomass.

There is currently widespread interest in the use of microalgae and cyanobacteria, as well as land plants, for the generation of renewable fuels and chemicals. However, very few of these organisms constitutively synthesize and accumulate substantial amounts of lipids or hydrocarbons. Successful commercial application of a photosynthesis-to-fuels approach [2••] is hindered by low yields, which are due to a preference by the organism to direct photosynthetic carbon toward sugar biosynthesis and biomass accumulation, rather than terpenoid and fatty acid biosynthesis. A metabolic flux analysis conducted in the lab of the author [8•] showed that, during steady-state growth, the sugar biosynthetic pathway, leading to the synthesis of protein, cell wall, storage polysaccharide, nucleic acids, etc. (i.e., biomass) constitutively consumes more than 80% of the available photosynthate. The pathways of interest for the direct production of fuels and chemicals (i.e., the terpenoid and fatty acid biosynthetic pathways) are allocated only about 5% and 10% of photosynthetic carbon, respectively (Figure 2). This allocation is constitutive and is applied to satisfy the growth needs of the organism. Experience in the lab of the author has shown that the living cell stringently regulates this carbon partitioning and has often frustrated efforts to substantially alter carbon allocation ratios.

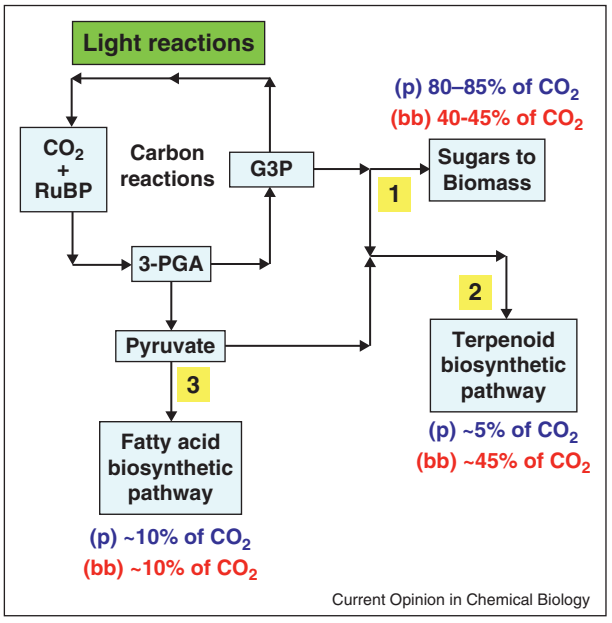
Constitutive partitioning of ~5% photosynthetic carbon into the terpenoid biosynthetic pathway and ~10%

Figure 1



Schematic of a direct photosynthesis to fuels and chemicals production process. The photosynthetic apparatus can serve as both catalyst and processor, absorbing sunlight, photosynthesizing, generating, and secreting ready-made duel and chemical-type molecules. The issue addressed in this opinion paper is how best to alter photosynthetic carbon partitioning and direct flux toward fuels and chemicals instead of metabolites leading to biomass propagation.

Figure 2



Photosynthetic carbon partitioning pathways in plants, algae, and cyanobacteria. In the vast majority of photosynthetic systems, carbon partitioning is primarily directed toward sugars (p = 80–85%) leading to biomass accumulation, with terpenoid (p = ~5%) and fatty acid biosynthesis (p = ~10%) consuming a minor aliquot of the photosynthetically generated organic substrate. Photosynthetic carbon partitioning in the colonial green microalgae *Botryococcus braunii* var Showa (the Berkeley strain) is naturally different with a diminished carbon flux toward sugars (bb = 40–45%) leading to slower rates of biomass accumulation, while terpenoid (bb = ~45%) and fatty acid biosynthesis (p = ~10%) together account for the majority of C-flux in the cell. *B. braunii*, offers example of a naturally occurring substantial alteration in chloroplast constitutive carbon partitioning.

photosynthetic carbon into the fatty acid biosynthetic pathway does not translate into 5% free terpene or 10% free fatty acid accumulation in the cell. Rather, chloroplasts and cells use this carbon flux to meet their own needs in carotenoid, phytol, quinone prenyl tail (terpenoids), and fatty acids needed for primarily diglyceride and some triglyceride formation. Little endogenous metabolic flux can thus be directed toward free fuel or chemical product accumulation. This was evidenced in recent work from this lab [7,8•], where heterologous transformation of cyanobacteria with the *Pueraria montana* (kudzu) isoprene synthase gene [9] succeeded in the constitutive production of isoprene. However, carbon-partitioning measurements showed that, without any further modification in metabolic flux, only about 0.8% of photosynthetic carbon was partitioned toward isoprene synthesis, with 4.2% (i.e., 84%) of the flux in the terpenoid biosynthetic pathway serving the growth needs of the organism. A similar conclusion was reached upon heterologous transformation of *Saccharomyces cerevisiae* (budding yeast) with the *Pueraria montana* (kudzu) isoprene synthase gene, where constitutive production of isoprene was achieved. Yields, however, were low, consistent with the notion of cellular terpenoid carbon partitioning toward biomass accumulation rather than isoprene synthesis [10].

Organisms of oxygenic photosynthesis operate the prokaryotic deoxy-D-xylulose-phosphate/methyl-erythritol phosphate (DXP/MEP) pathway for the generation of cellular terpenoids (Figure 2) [11–13], whereas archaea and eukaryotes employ the substantially different mevalonic acid (MVA) pathway [14,15•]. Observations above, showing the severe rate-limiting and yield-limiting aspects of the DXP/MEP pathway in photosynthetic systems, and of the MVA pathway in eukaryotic systems are consistent with the notion of cell-imposed limitation in flux toward terpenoids, which are independent of the prokaryotic DXP/MEP or eukaryotic MVA pathways.

A technoeconomic analysis (Melis unpublished) suggested that a constitutive product-to-biomass carbon partitioning ratio of 20%, or greater, is needed with a high productivity photosynthetic system to make a photosynthetic biofuels process commercially viable. Product to biomass carbon partitioning ratios greater than 20%, when achieved, would favorably impact the overall economics of the process. It is thus evident that achieving commercially viable fuels and chemicals production demands a substantially different constitutive photosynthetic carbon partitioning in the cell, such that bio-product yield is enhanced at the expense of biomass accumulation. Precursor flux amplification, coupled with enhancement in the rate of the terminal step [16,17,18•] and removal of the final desirable product [15•] has succeeded to improve yields in model bacterial systems, such as *Escherichia coli*. Such improvements, however, have not yet been demonstrated with photosynthetic systems.

There is, however, a naturally-occurring process, whereby a 45% terpenoid-to-biomass carbon-partitioning ratio

is constitutively achieved (Figure 2). This has been documented in the colonial green microalgae *Botryococcus braunii*, race-B, and especially so in the strain Showa (also known as the Berkeley strain; [19,20]). It is of interest to note that in Showa, as in all other photosynthetic systems, 5% of the flux through the terpenoid biosynthetic pathway is directed toward the synthesis of intracellular carotenoids, phytol, quinone prenyl tails and other endogenous cell-required compounds. The additional 40% of the flux through the terpenoid biosynthetic pathway is specifically directed toward the synthesis of the aberrant $C_{30}H_{48}$ triterpene known as 'botryococcene' along with a small complement of the carotenoid 'botryoxanthin' [21], both of which form intracellular lipophilic vesicles, followed by vesicle excretion from the cell, ending up in the extracellular space. The cell biology of botryococcene synthesis, intracellular sequestration, and eventual excretion and accumulation in the extracellular space of the colonial microalgae constitute concept validation in support of the notion of constitutive synthesis and accumulation of fuels and chemicals, emanating directly from the photosynthesis of cyanobacteria, algae, and plants.

The above discussion and analysis pertains to constitutive growth and fuels–chemicals production processes. There are examples of inducible processes in which terpenoid and fatty acid-based molecules accumulate under conditions of no biomass increase or in the stationary phase of growth. Selective overexpression of the terpenoid biosynthetic pathway for product accumulation is known to occur in certain unicellular green algae, for example, *Dunaliella bardawil* and *Haematococcus pluvialis*, under a variety of environmental stress conditions [22]. Nutrient deprivation and irradiance stress bring about a prompt down regulation of sugar biosynthesis and growth in these green microalgae, and concomitant enhancement of endogenous carbon flux through the terpenoid biosynthetic pathway. Such physiologically occurring alterations in metabolic flux are not mechanistically or genetically understood. Nevertheless, they result in a >30-fold increase in the cellular content of β-carotene in *Dunaliella* and astaxanthin in *Haematococcus*, respectively [23,24]. Similarly, nutrient (nitrogen) deprivation in combination with irradiance stress causes certain microalgae, for example, typically of the genus *Chlorella* and *Nannochloropsis*, to accumulate high levels of triacylglycerols, rich in polyunsaturated fatty acids [25,26]. This phenomenon offers an example of a naturally occurring stress-regulated alteration in metabolic carbon flux and regulated increase in the activity of the terpenoid and fatty acid biosynthetic pathways, respectively. It offers concept validation for the proposed constitutive or inducible over-expression of terpenoid and fatty acid hydrocarbons, emanating directly from the photosynthesis of cyanobacteria, algae, and plants.

It is currently unknown how any biological system regulates carbon partitioning among the various biosynthetic pathways in the cell; either constitutively or inducibly, when cellular metabolic activity is altered as a result of stress. The fact that photosynthetic and nonphotosynthetic systems allot a rather small fraction of endogenous carbon toward the two biosynthetic pathways (terpenoid and fatty acid), which are of interest in the generation of fuels and chemicals, constitutes a barrier to commercial exploitation. Realization of this shortcoming and the analysis presented in this paper is a call to action, to help overcome this flux barrier in the commercial generation of biofuels and renewable chemicals.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Melis A: **Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antenna to maximize efficiency**. *Plant Sci* 2009, **177**:272-280.

2. •• Melis A: **Photosynthesis-to-fuels: from sunlight to hydrogen, isoprene, and botryococcene production**. *Energy Environ Sci* 2012, **5**:5531-5539.
Advances in metabolic engineering and sunlight utilization efficiency have improved the performance of photosynthesis and diverted cellular metabolic flux toward alternative high-impact fuels and chemicals.

3. Blatti JL, Beld J, Behnke CA, Mendez M, Mayfield SP, Burkart MD: **Manipulating fatty acid biosynthesis in microalgae for biofuel through protein–protein interactions**. *PLoS One* 2012, **7** http://dx.doi.org/10.1371/journal.pone.0042949 Article Number: e42949.

4. Hillen L, Pollard G, Wake LV, White N: **Hydro cracking of the oils of *Botryococcus braunii* into transport fuels**. *Biotechnol Bioeng* 1982, **24**:193-205.

5. Lichtenthaler HK: **Biosynthesis, accumulation and emission of carotenoids, α-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance**. *Photosynth Res* 2007, **92**:163-179.

6. Lichtenthaler HK: **Biosynthesis and emission of isoprene, methylbutanol and other volatile plant isoprenoids**. In *The Chemistry and Biology of Volatiles.* Edited by Andreas Herrmann. John Wiley & Sons, Ltd.; 2010:11-47.

7. Bentley FK, Melis A: **Diffusion-based process for carbon dioxide uptake and isoprene emission in gaseous/aqueous two-phase photobioreactors by photosynthetic microorganisms**. *Biotechnol Bioeng* 2012, **109**:100-109.

8. • Lindberg P, Park S, Melis A: **Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *Synechocystis* as the model organism**. *Metabol Eng* 2010, **12**:70-79.
The concept of 'photosynthetic biofuels' envisions application of a single organism, acting both as photo-catalyst and producer of ready-made fuel. This concept was applied in this work upon genetic engineering of the cyanobacterium Synechocystis, conferring the ability to generate volatile isoprene hydrocarbons from CO_2 and H_2O.

9. Sharkey TD, Yeh S, Wiberley AE, Falbel TG, Gong D, Fernandez DE: **Evolution of the isoprene biosynthetic pathway in kudzu**. *Plant Physiol* 2005, **137**:700-712.

10. Hong S-Y, Zurbriggen A, Melis A: **Isoprene hydrocarbons production upon heterologous transformation of *Saccharomyces cerevisiae***. *J Appl Microbiol* 2012, **113**:52-65.

11. Lichtenthaler HK: **The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants**. *Annu Rev Plant Physiol Plant Mol Biol* 1999, **50**:47-65.

12. Lichtenthaler HK: **Non-mevalonate isoprenoid biosynthesis: enzymes, genes and inhibitors**. *Biochem Soc Trans* 2000, **28**:785-789.

13. Cunningham FX, Gantt E: **Genes and enzymes of carotenoid biosynthesis in plants**. *Ann Rev Plant Physiol Plant Mol Biol* 1998, **49**:557-583.

14. McGarvey D, Croteau R: **Terpenoid biosynthesis**. *Plant Cell* 1995, **7**:1015-1026.

15. • Zurbriggen A, Kirst H, Melis A: **Isoprene production via the mevalonic acid pathway in *Escherichia coli* (Bacteria)**. *Bioenergy Res* 2012, **5**:814-828.
A useful strategy to improve the outcome of metabolic engineering is introduction of specific ribosome-binding site and nucleotide spacers between individual genes in a superoperon structure. This approach enabled maximal expression of critical genes in the terpenoid biosynthetic pathway and translated into an improved flux of endogenous metabolites to isoprene hydrocarbons production.

16. Martin VJ, Pitera DJ, Withers ST, Newman JD, Keasling JD: **Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids**. *Nat Biotechnol* 2003, **21**:796-802.

17. Wu S, Schalk M, Clark A, Miles RB, Coates R, Chappell J: **Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants**. *Nat Biotechnol* 2006, **24**:1441-1447.
Terpenes constitute a distinct class of natural products (1) that attract insects (2), defend against phytopathogenic microbes (3) and combat human diseases (4). However, like most natural products, they are usually made by plants and microbes in small amounts and as complex mixtures.

18. • Leonard E, Ajikumara PK, Thayer K, Xiaoa W-H, Moa JD, Tidor B, Stephanopoulos G, Prather KLJ: **Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control**. *Proc Natl Acad Sci U S A* 2010, **107**:13654-13659.
A common strategy of metabolic engineering is to increase the endogenous supply of precursor metabolites to improve pathway productivity. The ability to further enhance heterologous production of a desired compound may be limited by the inherent capacity of the imported pathway to accommodate high precursor supply. Work in this paper highlighted the importance of engineering proteins along with pathways as a key strategy in achieving microbial biosynthesis and overproduction of pharmaceutical and chemical products.

19. Eroglu E, Melis A: **Extracellular terpenoid hydrocarbon extraction and quantitation from the green microalgae *Botryococcus braunii* var, Showa**. *Bioresour Technol* 2010, **101**:2359-2366.

20. Eroglu E, Okada S, Melis A: **Hydrocarbon productivities in different *Botryococcus* strains: comparative methods in product quantification**. *J Appl Phycol* 2011, **23**:763-775.

21. Okada S, Matsuda H, Murakami M, Yamaguchi K: **Botryoxanthin A, a member of a new class of carotenoids from the green microalga *Botryococcus braunii* Berkeley**. *Tetrahedron Lett* 1996, **37**:1065-1068.

22. Ben-Amotz A, Katz A, Avron M: **Accumulation of beta-carotene in halotolerant algae — purification and characterization of beta-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae)**. *J Phycol* 1982, **18**:529-537.

23. Lamers PP, Janssen M, De Vos RCH, Bino RJ, Wijffels RH: **Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications**. *Trends Biotechnol* 2008, **26**:631-638.

24. Misawa N: **Pathway engineering of plants toward astaxanthin production**. *Plant Biotechnol* 2009, **26**:93-99.

25. Anandarajah K, Mahendraperumal G, Sommerfeld M, Hu Q: **Characterization of microalga *Nannochloropsis* sp. mutants for improved production of biofuels**. *Appl Energy* 2012, **96**:371-377.

26. Griffiths MJ, van Hille RP, Harrison STL: **Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions**. *J Appl Phycol* 2012, **24**:989-1001.