Photosynthesis 2: Carbon-Fixing Reactions – Teaching Guide

Overview – Photosynthesis in plants converts the energy in sunlight into chemical energy. Although photosynthesis involves many proteins and catalytic processes, it often is described as two sets of reactions, the "light-dependent reactions" and the "carbon-fixing reactions". In a separate Teaching Tool, *The Light-Dependent Reactions of Photosynthesis*, we describe how the energy of sunlight is converted to chemical energy and ultimately stored as ATP and NADPH. Here we describe how these energy-storing compounds are used in the reduction of carbon dioxide (CO_2) to form sugars, starting with the key enzyme of carbon fixation and one of the most intriguing of all enzymes, Ribulose-bisphosphate carboxylase/oxygenase, also known as Rubisco. Rubisco is a notoriously inefficient enzyme that only poorly discriminates between CO_2 and O_2. Its oxygenation reaction forms an inhibitory product that must be recycled through the process of photorespiration. This lesson introduces the core biochemistry of the carbon fixing reactions of photosynthesis, as well as its variations, C_4 and CAM. Finally, it addresses how and why plants are affected by rising atmospheric CO_2 levels and efforts to increase photosynthetic efficiency in current and future conditions.

Learning objectives

By the end of this lesson the student should be able to:

- Diagram the two reactions catalyzed by Rubisco and describe how each affects the plant's energy balance
- Summarize the function and key stages of the Calvin-Benson cycle
- Evaluate how the changing levels of atmospheric CO_2 and O_2 have affected the evolution of Rubisco and the evolution of carbon-concentrating mechanisms
- Interpret how heat and drought contribute to the balance between Rubisco's catalytic activities
- Assess the role of photorespiration on plant metabolism in terms of its benefits and and its costs
- Summarize and diagram three different carbon-concentrating mechanisms and identify the organisms in which they are found
- Diagram the steps upstream of Rubisco that occur in C_4 and CAM photosynthesis
- Compare and contrast C_3, C_4 and CAM in terms of water-use efficiency, nitrogen-use efficiency and photosynthetic efficiency (conversion of light into biomass)
- Summarize key features of environments that have favored the evolution of C_4 and CAM and identify economically important species that use each type of photosynthesis
- Compare and contrast anatomical traits associated with C_4 and strong CAM
- Evaluate efforts to introduce aspects of C_4 or CAM into C_3 crops
- Summarize the photosynthetic opportunities and threats conferred by rising levels of atmospheric CO_2

Study / exam questions (*understanding and comprehension*)

- Diagram the two reactions catalyzed by Rubisco (reactants and products). What factors determine the balance between the two reactions?
- For different forms of Rubisco, what is the relationship between Rubisco's maximum rate of carboxylation, specificity factor, and the environment in which the enzyme is found?
- Compare and contrast different carbon-concentrating mechanisms in terms of the proteins and structures / compartments involved and their efficiency.
- Diagram the possible fates of newly fixed CO_2 as it progresses through the Calvin-Benson cycle. When CO_2 is not limiting, how can the flow of CO_2 into fixed carbon be enhanced?
- What is photorespiration? What is the phenotype of photorespiratory-deficient mutants? What is the biochemical effect of preventing photorespiration?
- Diagram the flow of carbon in plant photorespiration. How does the photorespiratory bypass described in the text decrease the metabolic cost of photorespiration?
- What is the evidence to support the claim C_4 photosynthesis has evolved more than 60 times independently? Under what conditions is C_4 photosynthesis most beneficial?
- What is the role of carbonic anhydrase in C_4 photosynthesis?
- Diagram the flow of carbon in two-celled C_4-photosynthesis, starting with atmospheric CO_2 and ending with CO_2 carboxylation by Rubisco.
- For each element in C_4 plants, indicate if it is more abundant / active in bundle sheath cells, mesophyll cells, or is essentially the same in the two cell types:
    - PEPC
    - RUBISCO
    - NADP-ME, NAD-ME and PEPCK
    - Carbonic anhydrase
    - Malate / aspartate
    - PEP
- Why has the genus *Flaveria* been so useful for studies of C_4 photosynthesis?
- Draw the arrangement known as Kranz anatomy and indicate the biochemical processes that occur in each element of your diagram.
- What is the major factor that makes C_4 photosynthesis in a single cell risky? How does *Bienertia sinuspersici* get around this potential problem?
- Compare and contrast CAM and C_4 photosynthesis.
- Under what conditions is CAM particularly advantageous over C_4 or C_3 photosynthesis?
- About half of the species of orchids and bromeliads are CAM plants. Why is this trait so common in these plants?
- Many plants are described as facultative CAM plants. Under what conditions do such plants switch to CAM? What are the costs and benefits of facultative CAM versus CAM and versus C_3?
- Assess three ways that the study of CAM can contribute to improvements in plant productivity.
- Rising atmospheric CO_2 levels have diverse and sometimes antagonistic effects on plant growth. Identify three ways that rising CO_2 levels are

expected to affect food production, and evaluate their impact on rice (a C_3 plant), corn (a C_4 plant) and soybean (a C_3 legume).
- Design and evaluate three different methods to engineer greater carbon-fixing efficiency into a C_3 plant. Which do you think has the most risk? Which do you think has the most potential benefit?
- With rising atmospheric CO_2 levels, we can anticipate higher temperatures and changes in rainfall pattern leading to increased periods of water stress. Focusing on photosynthesis, identify three approaches to engineer greater climate resiliency into plants.

Discussion questions (*engagement and connections*)
- Rubisco action in photosynthetic carbon fixation is not the only way for inorganic carbon to enter the biosphere. What organisms fix carbon independently of photosynthesis, and how do they do this? How can studying these organisms benefit plant science? (Here are some references to help you think about this: Berg et al., 2010. Autotrophic carbon fixation in archaea. Nat. Rev. Microbiol. 8: 447 – 460. doi:10.1038/nrmicro2365. Fuchs, 2011. Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? Annu. Rev. Microbiol. 65: 631 – 658. doi: 10.1146/annurev-micro-090110-102801. Braakman and Smith 2012. The emergence and early evolution of biological carbon-fixation. PLOS Comput. Biol. 8(4): e1002455. doi: 10.1371/journal.pcbi.1002455).
- For many years it was thought that there is a trade-off between Rubisco's maximum rates of carboxylation and its specificity for CO_2 vs O_2, but a recent paper suggests that this is not entirely true. What do these new data suggest about the trade-offs between investment in Rubisco and investment in carbon-concentrating mechanisms? (Young et al., 2016. Large variation in the Rubisco kinetics of diatoms reveals diversity amount their carbon-concentrating mechanisms. J. Exp. Bot. 67: 3445 – 3456. doi: 10.1093/jxb/erw163).
- Photorespiration increases when plants are water stressed and at high temperatures. Explain how water stress and heat affect the ratio between RuBP carboxylation and RuBP oxygenation.
- Investigate the ways that researchers have tried to improve Rubisco. How have these studies been carried out and how successful have they been? What conclusions have been drawn about their success or lack of success?
- Rubisco activity is regulated by many factors that can fine tune it to environmental conditions. Investigate the mechanisms and outcomes of Rubisco regulation. (See for examples Parry, M.A.J., Andralojc, P.J., Scales, J.C., Salvucci, M.E., Carmo-Silva, A.E., Alonso, H. and Whitney, S.M. (2013). Rubisco activity and regulation as targets for crop improvement. J. Exp. Bot. 64: 717-730, doi: 10.1093/jxb/ers336, and Carmo-Silva, E., Scales, J.C., Madgwick, P.J. and Parry, M.A.J. (2015). Optimizing Rubisco and its regulation for greater resource use efficiency. Plant Cell Environ. 38: 1817-1832, doi: 10.1111/pce.12425).
- In 2016, the Journal of Experimental Botany produced a special issue on Photorespiration (http://jxb.oxfordjournals.org/content/67/10.toc). Select one of these papers to read carefully and summarize in a one-page synopsis.
- The June 2016 issue of Current Opinion in Plant Biology includes a series of articles on the theme of *CO_2 concentrating mechanisms in photosynthetic organisms: evolution, efficiency and significance for crop improvement* (http://www.sciencedirect.com/science/journal/13695266/31). By exploring these and other articles as resources, how feasible do you think it would be do introduce a carbon-concentrating mechanism such as a pyrenoid or carboxysome into C_3 plants to decrease the frequency of RuBP oxygenation?

- The metabolic pathway known as C_3-C_4 intermediate or C_2 photosynthesis has been proposed as a possible intermediate stage in the evolution of C_4 photosynthesis from C_3. Diagram the flow of carbon in this pathway and indicate how it can be beneficial as compared to C_3 photosynthesis.
- An international consortium is working to introduce C_4 photosynthesis into rice. What are the potential benefits of this project? Summarize the two key goals that need to be met to achieve this objective, and summarize the specific tasks that must be completed to meet these goals. Which of the two goals do you think is most interesting, and which is the most challenging? Justify your answer.
- CAM depends on tight temporal control of PEPC and Rubisco. Investigate how plants achieve this.
- CAM is widely considered to be a water-conserving strategy, yet some aquatic plants use CAM. How can these seemingly contradictory statements be reconciled?
- Read one of the first two papers on CAM plant genomes and summarize what was learned from it about the evolution of CAM (Ming et al., 2015. The pineapple genome and the evolution of CAM photosynthesis. Nat. Genet. 47: 1435 – 1442 or Cai et al., 2015. The genome sequence of the orchid *Phalaenopsis equestris*. Nat. Genet. 47: 65 – 72.)
- Describe and interpret the effects of various anatomical traits associated with strong CAM.
- There is some flexibility to the CAM cycle, including CAM cycling and CAM idling. How do these variations compare to CAM and under what conditions are they beneficial?
- Identify the roles of transporter proteins involved in carbon-fixing reactions and propose a strategy to alter some of their properties to increase the efficiency of CO_2 fixation.
- Leakey and Lau (2012) draw on an evolutionary perspective to understand how plants will respond to future atmospheric CO_2 levels. What are their key conclusions and how do they propose we can use these insights to engineer crops for improved performance in present and future conditions? (Leakey, A.D.B. and Lau, J.A. (2012). Evolutionary context for understanding and manipulating plant responses to past, present and future atmospheric [CO_2]. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 367: 613-629. doi: 10.1098/rstb.2011.0248).

Lecture synopsis

Overview and introduction (1 - 7)

Photosynthesis captures light energy as reduced carbon. Photosynthesis is two sets of connected reactions. The ***light-dependent reactions*** capture light energy and produce O_2, ATP and NADPH. The ***carbon-fixing*** reactions use that stored energy to produce high-energy, reduced carbon in the form of sugars by way of the Calvin-Benson cycle. CO_2 assimilation into organic form is initiated by the enzyme Rubisco. Some plants have additional carbon-uptake steps that serve to concentrate CO_2 to enhance the efficiency of photosynthesis and the plant's water use efficiency.

Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes competing carboxylation and oxygenation reactions (8 - 21)

Rubisco's carboxylation reaction joins CO_2 to ribulose bisphosphate (RuBP) to form an unstable compound that splits into two identical molecules of 3-phosphoglyerate (3-PGA), which are used to regenerate RuBP or assimilated into other organic compounds. Rubisco's oxygenation reaction joins O_2 to RuBP, forming one molecule of 3-PGA and one of 2-phosphyglycolate (2-PG). 2-PG is partially reassimilated through the reactions of photorespiration (below). Different organisms have different forms of Rubisco with different catalytic properties. In plants, Rubisco is assembled from four subunits each of the large- and small-subunits, and its activity is regulated at many levels.

The Calvin-Benson cycle regenerates ribulose 1,5-bisphosphate (22 – 26)

Rubisco's 3-PGA product enters the Calvin-Benson cycle. Here, the carbon can be directed into various organic carbon products including sugars. Much of the role of the Calvin-Benson cycle is to regenerate RuBP. When CO_2 levels are not limiting, RuBP regeneration can become limiting; studies have suggested that increasing the expression levels of some Calvin-Benson cycle enzymes can increase the efficiency of photosynthesis in some conditions.

Photorespiration recycles products of Rubisco's oxygenation reaction (27 – 34)

Photorespiration is a term used for a set of reactions that recycle 2-PG (the product of RuBP oxygenation) to the Calvin-Benson cycle. In plants, photorespiration is a multi-organellar process that involves enzymes in the chloroplast, peroxisome and mitochondrion, and transporter to move intermediates between them. For every two molecules of 2-PG, one 3-PGA is produced and one molecule of CO_2 released. The reactions also involve the deamination of glycine to release NH_3, which must be reassimilated. Due to the cost associated with reassimilating CO_2 and NH_3, photorespiration is a metabolically costly process, yet it is essential. Mutants deficient in photorespiration are lethal unless grown in a high CO_2 environment that suppresses RuBP oxygenation.

Carbon-concentrating mechanisms in bacteria, algae and plants (35 – 41)
Due to the high metabolic cost associated with RuBP oxygenation, many organisms have evolved mechanisms to concentrate CO_2. Cyanobacteria produce a protein-enclosed structure known as a carboxysome that efficiently concentrates Rubisco and CO_2. Many green algae produce pyrenoids that consist of tightly packed Rubisco that CO_2 is delivered to (initially in the form of HCO_3^-). Some plants use the enzyme phosphoenolpyruvate carboxylase (PEPC) to concentrate CO_2 in organic form by first attaching it to phosphoenolpyruvate (PEP), producing a four-carbon compound.

PEPC-based carboxylation reactions in plants: C_4 and CAM (42 - 86)
Two different types of PEPC-based strategies are found in plants. In C_4 photosynthesis, CO_2 is initially assimilated by PEPC in one cell type (the mesophyll cell), then transported to another cell type (the bundle sheath cell) where it is released by decarboxylation and fixed by Rubisco (although in a very small number of species these two reactions can occur within a single cell). In CAM, PEPC is active at night and the assimilated CO_2 is stored in the vacuole as an organic acid, resulting in nighttime assimilation. During the day, CO_2 is released from these stored acids and fixed by Rubisco.

C_4 photosynthesis (43 - 72)
About 8000 species use C_4 photosynthesis, which has evolved repeatedly more than 60 times. These plants are typically found in hot, dry, sunny regions where C_4 is most advantageous. Many C_4 plants exhibit Kranz anatomy in which the arrangement of bundle sheath cells and mesophyll cells form a concentric arrangement reminiscent of a wreath. There is considerable interest in identifying the factors controlling the compartmentalization of C_4 biochemistry and the development of Kranz anatomy, due to the potential to engineer C_4 into C_3 crops such as rice.

Crassulacean acid metabolism (CAM) (73 – 86)
CAM resembles C_4 except that PEPC is active at night and its product stored in the photosynthetic cells' vacuoles. CAM's advantage is that it allows CO_2 uptake to occur at night, when the temperature is lower and the humidity higher. By opening their stomata for CO_2 uptake at night, CAM plants can minimize evapotranspiration, so this trait is most common in environments where water is a major limitation. CAM plants are less photosynthetically efficient than C_3 or C_4 plants because the amount of CO_2 they can store as acid is not large. Many plants are facultatively CAM and only switch to nighttime CO_2 uptake during periods of drought or other stress.

Effects of rising CO_2 levels on plants (87 - 93)
Because of the cost associated with photorespiration, many C_3 plants benefit from increased levels of CO_2. By contrast, rising CO_2 levels have essentially no benefit for C_4 plants. However, as atmospheric CO_2 levels rise so do global temperatures, and

rainfall patterns become more irregular. The benefits of elevated CO_2 are to a large extent offset by these other effects.

Applications 1: Engineering more efficient C_3 plants (94 – 95)
The metabolic costs (and accompanying yield losses) associated with photorespiration are substantial, so many scientists are exploring avenues through with C_3 photosynthesis can be made more efficient. These include modifications to CO_2 or HCO_3^- transporters, the introduction of C_4 or CAM-like traits, and the introduction of structural carbon-concentrating mechanisms such as carboxysomes and pyrenoids. One of the most ambitious projects is the multinational C_4 rice project, which seeks to engineer C_4 photosynthesis into rice, one of our most important crop plants.

Applications 2: Engineering plants for a future with elevated CO_2 (96 – 98)
Other scientists are addressing the challenges that plants will face due to rising atmospheric CO_2 levels, particularly drought and high temperatures. Although climate change is impacting all aspects of plant physiology, development and biotic interactions, in this article we specifically addressed how it can detrimentally impact photosynthesis. For example, as some regions become drier, CAM will become more beneficial, so efforts are underway to introduce facultative CAM into plants to help them better cope with prolonged drought.

Summary and future directions (99)
Carbon fuels life. Photosynthetic carbon fixation is the entry point into the biosphere of nearly all of life's carbon. The food and energy needs of more than 7 billion people (soon to be 9 billion) depend on the flow of the Sun's energy into organic carbon compounds through the process of photosynthesis. For the past 70 years scientists have been assembling a detailed picture of this most crucial process, and are making strides towards being able to redesign and optimize photosynthesis for diverse environments and conditions. Many questions remain, and as scientists fill in the gaps of our knowledge they are also seeking to use their insights to increase the efficiency and resiliency of the plants upon which we depend.

Slide concepts

Slides	Table of contents – concepts
1	**Carbon-fixing reactions of photosynthesis**
2	Lesson outline
3	**Overview: Photosynthesis captures light energy as reduced carbon**
4	Photosynthesis is two sets of connected reactions
5	Light reactions produce O_2, ATP and NADPH
6	Through the Calvin-Benson cycle, ATP and NADPH are used to fix CO_2
7	Some plants have additional, CO_2-concentrating steps
8	**Rubisco catalyzes both carboxylation and oxygenation reactions**
9	Rubisco carboxylates RuBP, producing two molecules of 3-PGA
10	Rubisco oxygenates RuBP, producing one 3-PGA and one 2-PG
11	Rubisco does not bind directly to CO_2 or O_2, making discrimination difficult
12	Rubisco is found in a variety of forms
13	Evolutionary timeline and phylogenetic distribution of Rubisco
14	Different forms of Rubisco have different catalytic properties
15	Rubisco's catalytic inefficiency means that plants invest hugely in Rubisco
16	Rubisco activity is regulated at many levels including by light and metabolites
17	In plants Rubisco assembles from large and small subunits in the chloroplast
18	A lysine in the active site must be carbamylated for activity
19	Rubisco is subject to inactivation which can be reversed by Rubisco activase
20	Rubisco activase is heat labile. Heat-stabilized RA can confer thermotolerance
21	Carbon fixation is regulated and controlled by many other factors
22, 23	**The Calvin-Benson cycle regenerates ribulose 1,5-bisphosphate**
24, 25	The Calvin-Benson cycle provides carbon skeletons for growth too
26	Different factors limit the rate of photosynthesis under various conditions
27	**Photorespiration recycles products of Rubisco's oxygenation reaction**
28	Alternative pathways for glyoxylate conversion in cyanobacteria
29	Photorespiration is a multi-organellar process
30	Photorespiration releases CO_2 and NH_3 from organic form
31, 32	Photorespiration: Glycine decarboxylase complex disassembles glycine
33	Photorespiratory bypasses can minimize energetic costs of photorespiration
34	**Summary: Rubisco, Calvin-Benson cycle and photorespiration**
35	**Carbon-concentrating mechanisms in bacteria, algae and plants**
36	Pyrenoids, possibly carboxysomes, evolved long after plastid endosymbiosis
37	The bacterial carboxysome sequesters Rubisco and concentrates CO_2
38	Pyrenoids concentrate carbon in some photosynthetic eukaryotes

39	Pyrenoids are highly packaged Rubisco, interspersed with thylakoid membranes
40	An abundant repeat protein, EPYC1, links Rubisco to form pyrenoids
41	CO_2 is concentrated in pyrenoids via transporters and carbonic anhydrase
42	**PEPC-based carboxylation reactions in plants: C_4 and CAM**
43	**C_4 photosynthesis has evolved more than 60 times, mainly in hot, dry regions**
44	C_4 photosynthesis is advantageous in dry, hot, sunny regions (1)
45	C_4 photosynthesis is advantageous in dry, hot, sunny regions (2)
46	Photoinhibition dramatically limits C_3 photosynthesis at higher temperatures
47, 48	History: Hatch and Slack, 1966. “A new carboxylation reaction”
49	PEPC and Rubisco discriminate differently between $^{13}CO_2$ and $^{12}CO_2$
50	Isotope discrimination is the sum of many processes and reactions
51	C_4: Phylogeny and distribution
52	Economically important C_4 species
53	C_4 photosynthesis separates carbon assimilation and carbon fixation
54	Most C_4 plants have Kranz (“wreath”) anatomy revealing specialized functions
55	In most C_4 plants, CO_2 is fixed first in mesophyll cells, then bundle sheath cells
56 - 58	HCO_3^- fixed in mesophyll cells is moved into bundle sheath cells in three ways
59	Plants use a combination of biochemical subtypes in C_4 photosynthesis
60	All enzymes required for the C_4 pathway are present in C_3 plants
61	*Setaria viridis* (green foxtail) and *Setaria italica* (foxtail millet) are new models for C_4 photosynthesis
62	How did C_4 evolve repeatedly from C_3?
63	*Flaveria* includes C_3 and C_4 species: A good system to study C_4 evolution
64	C_2 photosynthesis concentrates photorespiratory CO_2 in BS cells
65	C_2 photosynthesis: Metabolite transfer to specialized bundle sheath cells
66	How did C_4 evolve repeatedly from C_3?
67	C_4 plants vary in their anatomy as well as their biochemistry
68	There are several different types of Kranz anatomy in flat and round leaves
69	Developmental onset of C_4 can be studied in a maize leaf
70	The transition to C_4 can be studied inducible-C_4 plants
71	Two chloroplast populations within a single-cell suffice for C_4 in some species
72	**C_4 Summary**
73	**Crassulacean Acid Metabolism (CAM): HCO_3^- fixed by PEPC at night**
74	In CAM, organic acids accumulate at night and are metabolized during the day
75	Classically, the CAM cycle is represented as four phases

76	CAM splits carbon fixing reactions into day and night reactions
77	Like C_4, CAM appearance is associated with periods of low CO_2
78	About 6% of angiosperms are CAM plants, which are found in many families
79	Economically important CAM plants
80	Important models for studying CAM
81	Many plants are “facultative CAM” meaning they use CAM when stressed
82	CAM cycling and CAM idling involve reassimilation of respiratory CO_2
83	CAM is associated with low intercellular air spaces and air/mesophyll interfaces
84	Is there a fitness cost associated with C_3-CAM indeterminacy?
85	Moving forward with CAM research
86	**CAM summary**
87	**Effects of rising CO_2 levels on plants**
88	C_3 plants are expected to benefit more than C_4 or CAM plants from elevated CO_2
89	Physiology is integrated: Elevated CO_2 levels also affect water-use efficiency
90	Increasing atmospheric [CO_2] also impacts temperature, rainfall, pests
91	The combination of drought and heat is particularly challenging
92	Plants grown in elevated CO_2 often show decreased protein and nutrient content
93	Free-air CO_2 enrichment (FACE) studies reveal plant responses in situ
94	**Applications 1: Engineering more efficient C_3 plants**
95	Turbocharging rice: The C_4 rice project
96	**Applications 2: Engineering photosynthesis for a CO_2-elevated future**
97	Strategies to improve C_4 photosynthesis
98	Engineering CAM to improve water-use efficiency
99	**Summary and Future Directions**