

The Photosynthetic Dark Reactions Do Not Operate in the Dark

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A common misconception that has stubbornly persisted in high school and college-level introductory biology texts is that the “dark reactions” of the Calvin cycle actually occur in the dark. The reality of the situation is that several enzymes in the so-called “dark reactions” are, in fact, indirectly dependent on the presence of light for their activity. Evidence was available in the literature throughout the mid-to-late 70s that is irreconcilable with the commonly held view of the dark reactions (Buchanan 1980). Yet, despite these reports, most introductory texts and most high school teachers and college professors still hold to the position that the carbon fixation enzymes can and do operate in the dark, even though most college-level botany and plant physiology texts correctly present the story (Salisbury & Ross 1992; Taiz & Zeiger 1991).

There are three pathways by which plants can fix atmospheric CO₂ into carbohydrate. When discussing photosynthesis, we normally refer to plants that use the Calvin cycle enzymes to create two molecules of 3-phospho-glyceric acid, a three-carbon compound. Plants that begin photosynthesis in this manner are called C₃ plants, and the pathway is referred to as the C₃ pathway.

Another group of plants have, in addition to the C₃ pathway, a second pathway in which CO₂ is fixed by the enzyme phosphoenolpyruvate carboxylase (PEP carboxylase) to form malic acid. Throughout the day, the malate is metabolized and CO₂ is regenerated and supplements CO₂ entering the leaves through the stomatal pores. Plants having this additional CO₂ fixing pathway are called C₄ plants (malate is a four-carbon compound). The CO₂ generated by the C₄ pathway, as well as the atmospheric CO₂, is fixed by the C₃ pathway in the light and dramatically stimulates growth, making C₄ plants greater producers as compared to C₃ plants.

A third group of plants, the Crassulacean Acid Metabolism (CAM) plants, survive living in arid environments by opening their stomata in the dark instead of the light, as do C₃ and C₄ plants. CAM

plants fix CO₂ in the dark using PEP carboxylase. Like C₄ plants, CO₂ is generated in the light when the stomata are closed and is fixed by the C₃ pathway. What C₃, C₄ and CAM plants have in common is the Calvin cycle C₃ carbon fixing pathway that only operates in the light.

The idea that the Calvin cycle enzymes function in the dark is probably a historical holdover stemming from early *in vitro* experiments. In the 1950s, when the Calvin cycle was elucidated, the analysis of enzyme activity was a young field. It was possible to measure substrate disappearance or end product appearance for the carbon reduction enzymes for several minutes in the dark, provided that the concentrations of ATP and NADPH were maintained high. When these photosynthetic substrates were depleted, the enzyme reactions stopped. Because there was some residual enzyme activity in the dark, the pathway was commonly dubbed the “dark reactions.” But the fact of the matter is that the Calvin cycle enzymes cannot sustain activity in the dark. Several Calvin cycle enzymes exist in an active form in the light and an inactive form, or much less active form, in the dark.

When educators teach the topic of photosynthesis, we frequently ask the question, “What is the purpose of the light reactions?” The standard answer is that the light reactions provide the necessary ATP and NADPH (source of electrons) for the creation of sugars in the subsequent Calvin cycle reactions. It is logical to conclude that the Calvin reactions would not be operational in the absence of light because the light reactions provide two of the substrates (ATP and NADPH) required for the fixation of CO₂. What evolved is a set of two photosynthetic reactions—the light reactions and the Calvin cycle reactions—that are regulated and operate together in the light, but do not operate in the absence of light. Carbohydrate production is therefore shut off at night because of enzyme inactivity, closure of stomata preventing the diffusion of CO₂ into leaves, and a lack of ATP and NADPH.

Throughout the 1970s it became increasingly more obvious that several Calvin cycle enzymes and the PEP carboxylase of C₄ plants would only demonstrate maximal enzyme activity when light is present. The mechanisms for light regulation of these enzymes is

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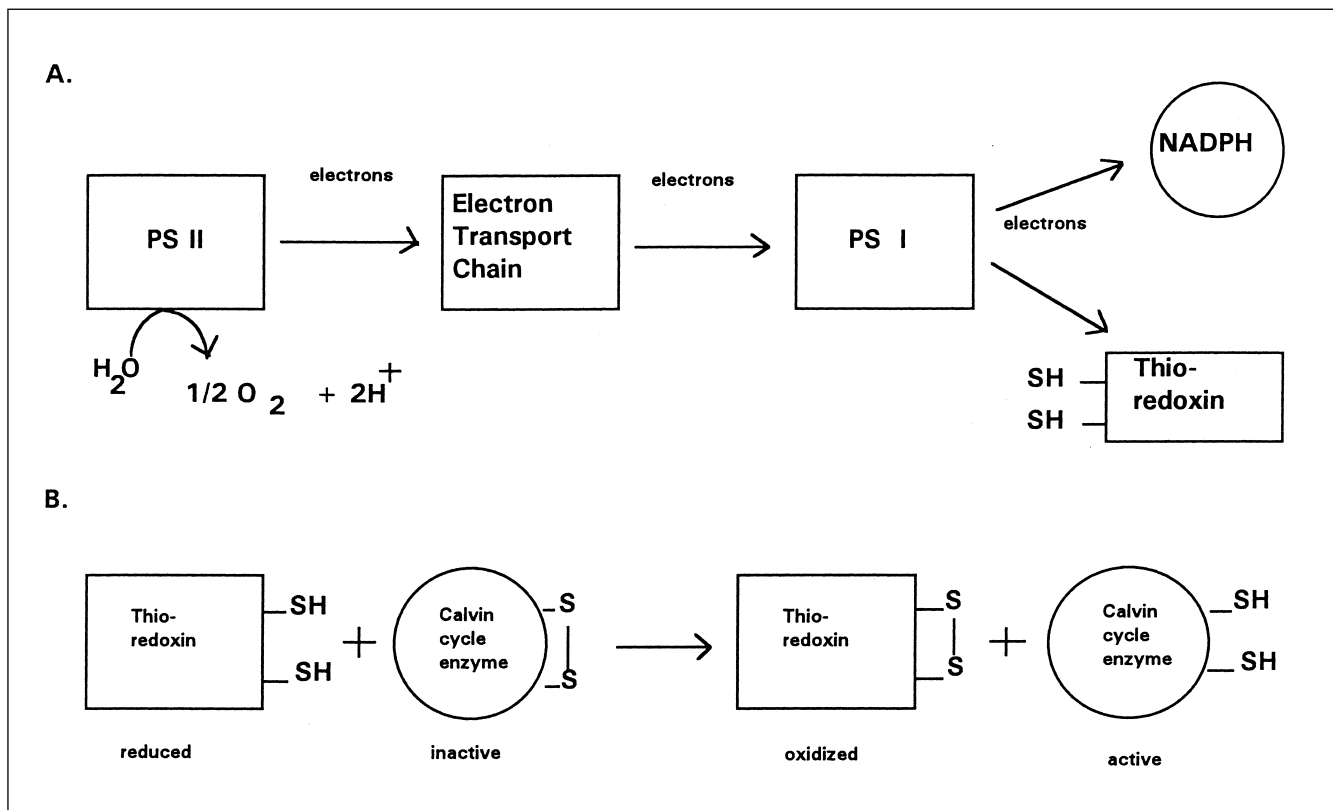


Figure 1. (A) The photosynthetic light reactions can pass electrons from water to either NADPH or to the protein thioredoxin which is loosely attached to the surface of the thylakoid membrane. Reduction of thioredoxin causes disulfide bonds to become reduced (-SH). (B) The reduced thioredoxin reduces the inactive Calvin cycle enzymes, which breaks their disulfide bonds and activates the enzymes. The thioredoxin is oxidized and can be reduced again at the surface of the thylakoid membrane.

not direct, in that light energy is not absorbed by the enzymes. Instead, the enzymes are activated by electrons generated during the light reactions, or by kinase enzymes that are only active in the light. Five enzymes in C_3 plants are regulated by light: ribulose-1,5-bisphosphate carboxylase (also called Rubisco), 3-phosphoglycerate dehydrogenase, fructose-1,6-bisphosphate phosphatase, sedoheptulose-1,7-bisphosphate phosphatase, and ribulose-5-phosphate kinase as well as three additional enzymes in C_4 plants: PEP carboxylase, $NADP^+$ malate dehydrogenase and pyruvate, phosphate dikinase (Buchanan 1980). Three separate mechanisms have been identified for these enzymes.

Activation of Enzymes by Reduced Thioredoxin

All of the enzymes named above, except for Rubisco and PEP carboxylase in C_4 plants, are activated by electrons originating from the electron transport chain of the light reactions. These enzymes each have disulfide bonds (S-S) that are reduced and broken in the light to form two sulfhydryl groups (-SH and -SH). The breakage of the disulfide bonds modifies enzyme structure, causing the enzyme reactions to

occur at a faster rate. The electrons used to reduce the sulfhydryl groups are derived from the water-splitting reactions associated with photosystem II (PSII). The electrons pass through the electron transport chain to photosystem I, but instead of reducing NADP to NADPH, some of the electrons move to the protein called thioredoxin, which is thought to be loosely attached to the outer surface of the thylakoid membrane. Thioredoxin itself contains disulfide bonds that are reduced by the electrons from photosystem I. The reduced thioredoxin dissociates from the thylakoid surface and then reduces the enzymes, thus activating the enzymes (Figure 1). The enzymes are inactivated in the dark by an O_2 -dependent oxidation, presumably by a reversal of the steps diagramed in Figure 1 (Anderson et al. 1978; Buchanan 1980).

Activation of Rubisco by pH & Mg^{2+}

In the light, H^+ are pumped from the stroma of the chloroplast into the thylakoid membrane. The movement of the protons results in an elevation of pH in the stroma, the location of the Calvin cycle enzymes, from pH 7 to 8. Rubisco is more active at pH 8 than 7. In the light, Mg^{2+} is also transported out of the thylakoid into the stroma. The transfer is

part of the mechanism used to balance electrical charge during the proton translocation. Rubisco requires Mg^{2+} for maximal activity (Figure 2). Therefore, Rubisco is activated by the change in pH as well as the concentration of Mg^{2+} that occurs when the light reactions start (Ben-Hayyim 1978; Mizioro & Lorimer 1983; Seeman et al. 1985). In addition, a six-carbon compound called 2-carboxyarabinitol-1-

phosphate (CA1P) has been identified as a strong, naturally occurring inhibitor of Rubisco. CA1P levels reach a peak at night, thereby inhibiting the activity of Rubisco. CA1P is degraded in the daylight by a phosphatase enzyme that removes the phosphate group from carbon 1 (Hug et al. 1980). How the light causes the phosphatase to become activated is not yet known.

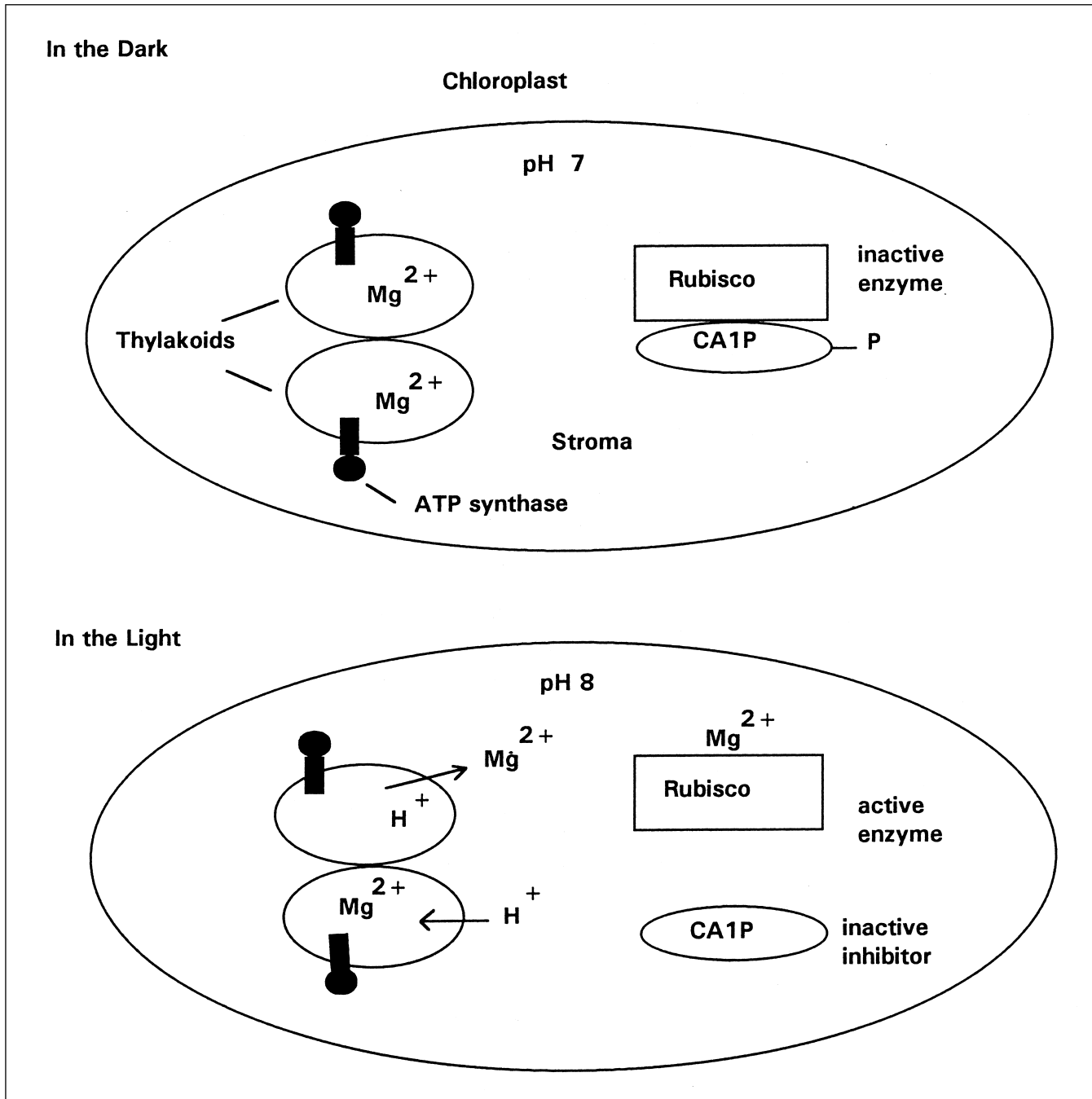


Figure 2. Upper panel: In the dark, the pH of the chloroplast stroma is 7, magnesium is stored inside the thylakoid sacs and Rubisco is inactive, in part because the inhibitor CA1P is bound to the enzyme in its phosphorylated form. Lower panel: In the light, magnesium ions move out of the thylakoid sacs while protons are transported into the sacs, thereby elevating the pH to about 8. In the light, the CA1P inhibitor is inactivated by removal of the phosphate. Rubisco is activated by the release of the inhibitor, the binding of magnesium and the change in pH.

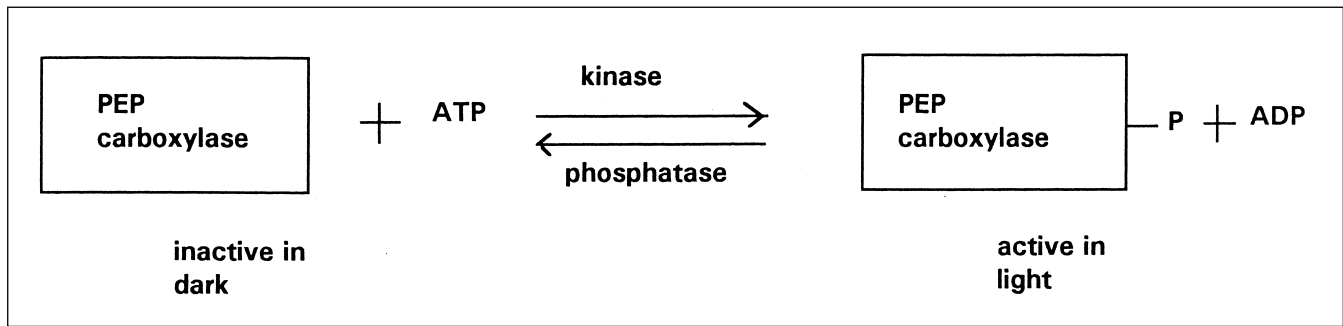


Figure 3. PEP carboxylase, found in C₄ plants, is inactive in the dark. In the light, a kinase uses the terminal phosphate from an ATP molecule and phosphorylates the enzyme, thereby activating it. In the dark, a phosphatase removes the phosphate inactivating the enzyme.

Activation by Kinase & Phosphatase Activity

PEP carboxylase is activated in mesophyll cells during the daylight and is inactive at night, preventing CO₂ fixation in C₄ plants. Evidence suggests that PEP carboxylase is phosphorylated in the light by a kinase. In this type of reaction, the terminal phosphate from an ATP molecule is transferred by the kinase enzyme onto one of the amino acids of the protein.

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Phosphorylation of the enzyme increases its activity. At night, the phosphate group is removed by a phosphatase enzyme and the enzyme activity decreases (Figure 3) (Bennett 1979). The mechanism by which the kinase and phosphatase enzymes are regulated is uncertain.

In view of the fact that the Calvin cycle reactions are not functional in the dark, the statement that these reactions are "light-independent" should be abandoned. A more appropriate description is that the Calvin cycle pathway is indirectly regulated at several steps by the presence of light. Such regulation insures that the activity of the two related pathways is coordinated to occur at the same time.

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