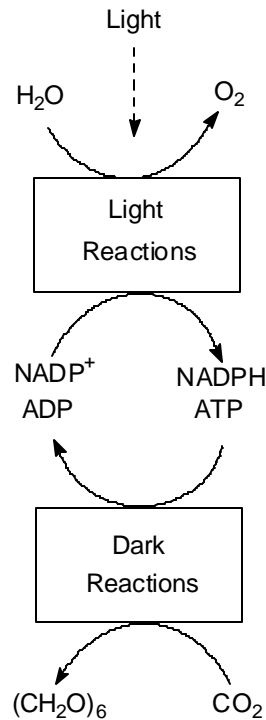


## Chapter 20 - The Calvin Cycle and the Pentose Phosphate Pathway

Pages: 550 - 555 + notes, 563 - 566, 568, 571 - 572.

### Dark Reactions - The Calvin Cycle

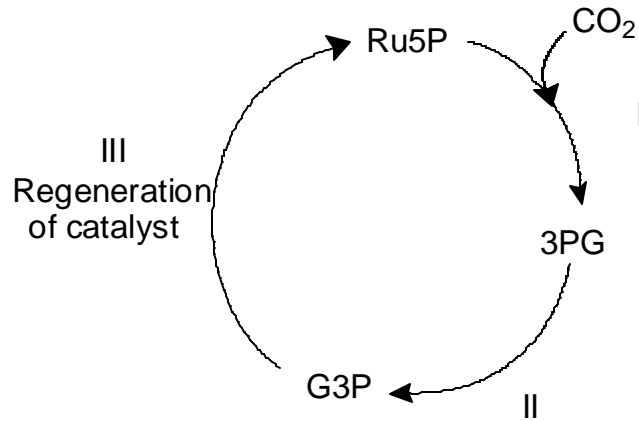
- Recall the relationship between the light and dark reactions of photosynthesis:



- The dark reactions actually take place predominantly during light hours, but do not directly require light. The biosynthesis of carbohydrate from  $CO_2$  is an energy-requiring, reductive process. These are provided by the light reactions in the form of ATP and NADPH. It is in the dark reactions that  $CO_2$  is incorporated into organic molecules.

- Calvin (early '50's), by adding radiolabeled  $CO_2$  to green algae, determined that the label initially wound up as a glycolytic intermediate, **3-phosphoglycerate (3PG)**. A search for a 2-Carbon precursor was unsuccessful. Eventually it was determined that  $CO_2$  incorporation involves a cyclic, *catalytic* process, and that the catalyst is a 5-Carbon molecules familiar to us from the nonoxidative branch of the pentose phosphate pathway, ribulose **5-phosphate (ru5P)**.

- The catalytic process can be thought of as occurring in three stages, I - III, all of which occur in the stroma of the chloroplast.

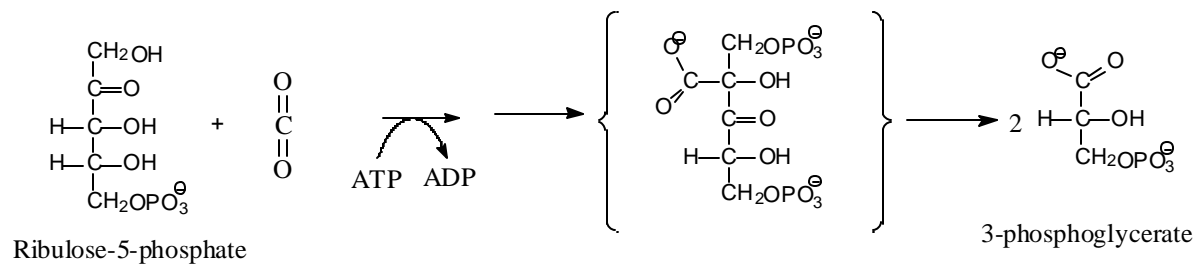


- Stage I corresponds to carbon fixation, Stage II to reduction of 3PG to glyceraldehyde 3-phosphate (G3P) in a series of reactions identical to the portions of gluconeogenesis, and Stage III, the regeneration of the catalyst, Ru5P. (Note that your text describes the dark reactions, combining stages I and II).

### Stage I

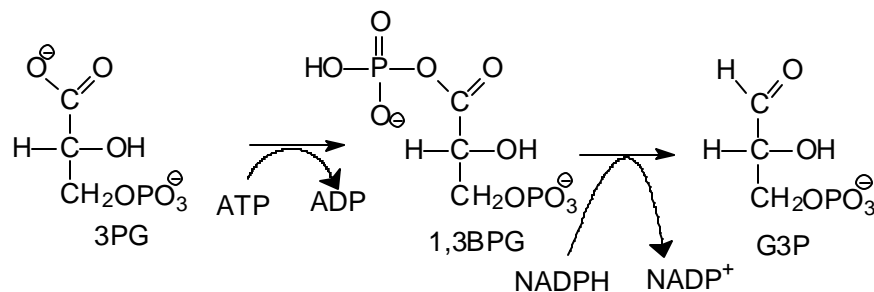
- CO<sub>2</sub> fixation actually involves ribulose-1,5-bisphosphate (RuBP). The enzyme involved in incorporating CO<sub>2</sub> into RuBP is RuBP carboxylase, one of the world's most important enzymes. Approximately 10<sup>11</sup> tons of carbon are incorporated into biomolecules in this way annually.

- Beginning with the catalyst, Ru5P, stage I proceeds as follows:



### Stage II

- This reductive stage consists of several reactions identical to those in gluconeogenesis:





The three-carbon G3P (and DHAP - precursors) and five-carbon Ru5P (products) are underlined.

### Regulation

- The Calvin Cycle is energy-requiring. Glycolysis, on the other hand is energy-yielding. Thus, if both the Calvin Cycle and glycolysis were both active at the same time, a wasteful futile cycling would result. Hence the need for regulation. The light and dark reactions are active during daylight; at night glycolysis, and also the pentose phosphate pathway, must supply ATP and the reducing power of NADPH.

- The light reactions result in both a pH gradient across the thylakoid membrane separating stroma from lumen (as protons are pumped into the lumen via photo-electron transport, the pH in the stroma increases as it becomes increasingly basic. Ru5P carboxylase has a pH optimum of about 8. Thus the activity of this enzyme increases during daylight hours.

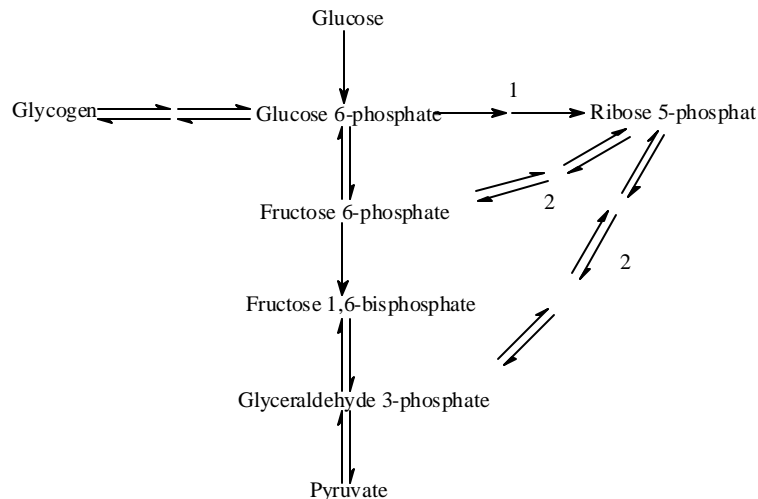
- As  $H^+$  are pumped into the lumen,  $Mg^{++}$  are pumped out (antiport).  $Mg^{++}$  stimulates Ru5P carboxylase.

- Sunlight also results in photooxidation, thus producing strong reducing agents, in this case Photosystem I. Photooxidized Photosystem I indirectly reduces a disulfide linkage in both fructose biphosphatase and sedoheptulose biphosphatase, thereby increasing their activity.

### Pentose Phosphate Pathway (PPP)

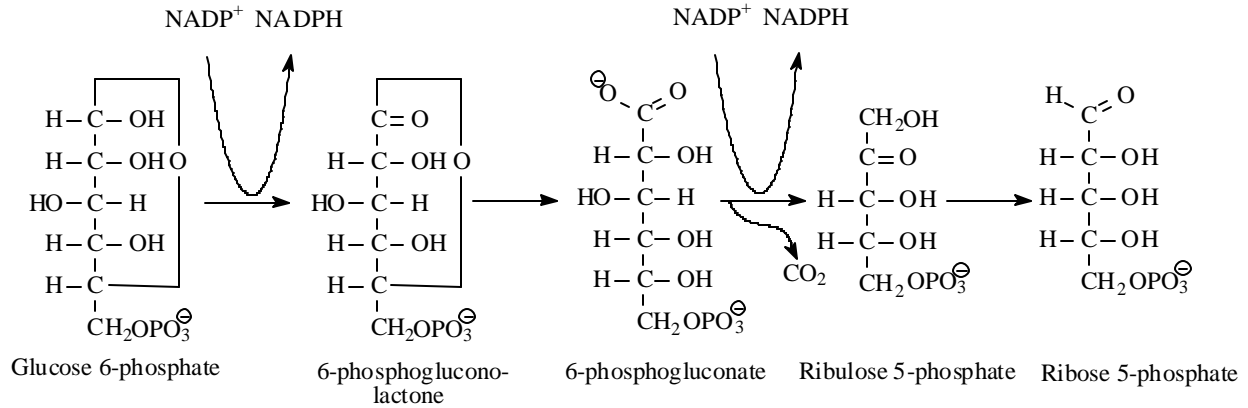
-Metabolic goals are to provide a) reducing power in the form of NADPH (compare to NADH) for biosynthesis and b) ribose 5-phosphate, a five-carbon sugar for nucleic acid and other biosynthetic processes.

- The PPP intersects glycolysis on the metabolic road map:

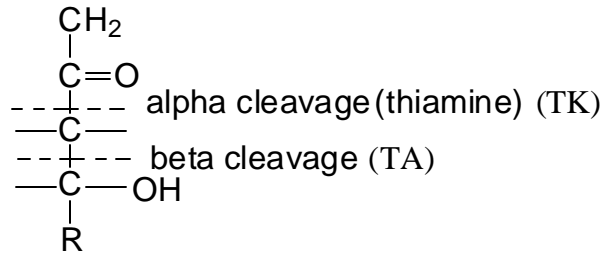


- The PPP consists of 1) the oxidative branch and 2) the non-oxidative branch. Both metabolic goals are accomplished in the oxidative branch. The non-oxidative branch allows the cell to accomplish either/or both of these goals.

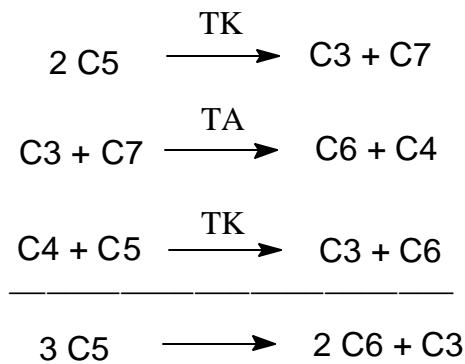
- The oxidative branch consists of familiar reactions:



- The non-oxidative branch utilizes two enzymes, transaldolase (TA), which catalyzes a beta-cleavage, and transketolase (TK), which catalyzes an alpha-cleavage, thus requiring thiamine).



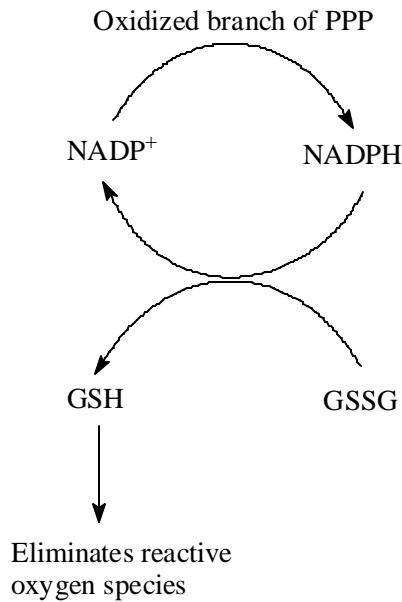
- The combined action of TA and TK converts the C5 ribose into C3 and C6 glycolytic intermediates.



- Regulation occurs via the first step of the oxidative branch, catalyzed by glucose 6-phosphate dehydrogenase. The most important regulatory factor is the level of NADP<sup>+</sup>.

- Oxidative metabolism constantly results in the generation of reactive oxygen species, largely by the incomplete transfer of electrons to oxygen during electron transport discussed previously. As we have seen the combined actions of superoxide dismutase and catalase convert the superoxide

radical, one such reactive species into water and oxygen. The NADPH produced in the oxidative branch of the pentose phosphate is also instrumental in combating oxidative stress. Also involved in this process is glutathione, a tripeptide containing a free sulfhydryl group (SH). Glutathione exists in both an oxidized (GSSG) and reduced (GSH) form. The reduced form is required to eliminate reactive oxygen species. The NADPH formed in the oxidative branch of the PPP is necessary to maintain glutathione in its reduced, active form:



- An antimalarial drug, pamaquine, induces a hemolytic anemia in individuals heterozygous for a defective gene encoding glucose 6-phosphate dehydrogenase. The story is similar to the sickle cell anemia story in that the heterozygous conditions confers a built-in protection from malaria to Africans (red cells need the NADPH from the PPP). The parasite that causes this disease invades red cells during their life cycle. These cells are more fragile and lyse in heterozygous individuals because their PPP activity is compromised. However, the drug also increases red cell fragility, leading to onset of symptoms in some cases.

- Problems: 14

