METABOLIC FLUX

Another title for this lecture might be "metabolism of photosynthesis" (the chapter title in Lawlor. Photosynthesis).

It encompasses two aspects.

One

One of these is the utilization of ATP & reducing equivalents for tasks other than photosynthate production in chloroplasts. Another is the utilization of photosynthate products in the cell.

Since, in a global sense, all metabolism relies on photosynthate products, the mandate is very broad.

Two

The other ~~spe~~ aspect focusses on "flux". That is, what is the throughput of photosynthesis, and what are the limiting factors? This is relevant in the context of bioengineering.

In toto, it is a complex interdependent system of multiple pathways.

Principal fluxes are summarized on the overhead.

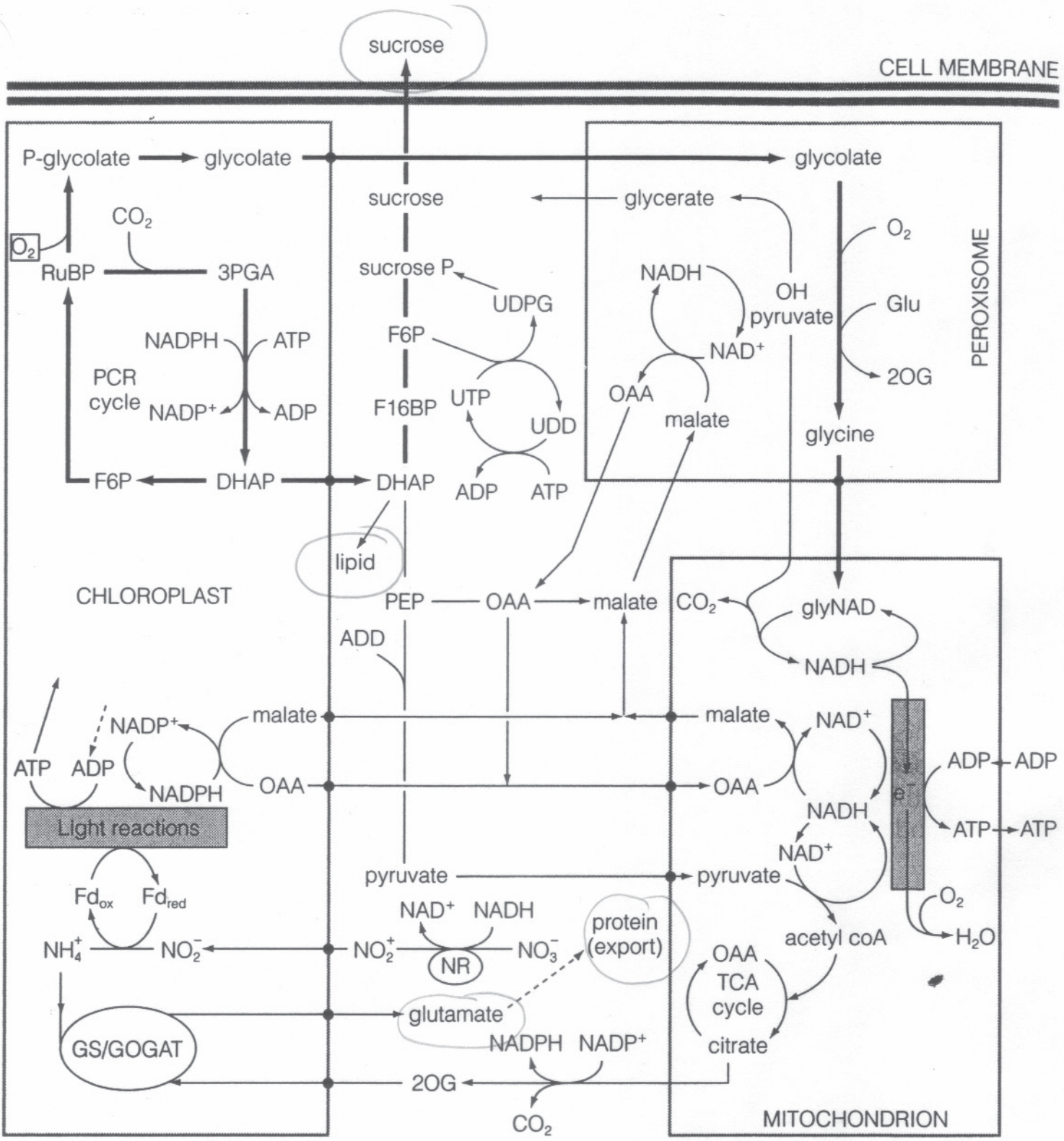


Figure 8.1. Principal fluxes of carbon and their relation to nitrogen assimilation in C3 plant leaf cells in the light

NITRATE REDUCTION

The best place to begin an exploration of metabolic fluxes is within the chloroplast.

REDUCING EQUIVALENTS

In the chloroplast reducing equivalents are used to convert N and S into metabolically-usable forms.

Nitrate Assimilation:

nitrogen is an essential component of proteins and nucleic acids (and other cellular constituents)

In plants, nitrogen is obtained as either nitrate (NO_3^-) or ammonia (NH_3) / ammonium (NH_4^+).* The sources are the soil, specifically via the nitrogen cycles in which bacteria play a key role. Another source is nitrogen fixation ($N_2 \rightarrow NH_3$) which occurs in specialized prokaryotic cells.

NH_4^+ can be used directly for assimilation into amino acids.

NO_3^- must first be reduced. Much of this reduction occurs in chloroplasts.

* $NH_{3\,(aq)} + H_2O \rightleftharpoons NH_4^+ + OH^-$ $\quad pK_b$ 4.75

$NH_4^+ \rightleftharpoons NH_3 + H^+$ $\quad pK_a$ 9.25

Thus at physiological pH, ammonia would be 99% protonated (ammonium ion: NH_4^+)

There are two major steps to reduce nitrate (NO_3^-)

The first is reduction to nitrite (NO_2^-):

$$NO_3^- + 2e^- + 2H^+ \underset{\text{nitrate reductase}}{\rightleftharpoons} NO_2^- + H_2O$$

The reductant is NADH. The enzyme has Mo and FAD co-factors, as well as a cytochrome. The reaction occurs in the cytoplasm.

The nitrite is transported into the chloroplast where it undergoes reduction to ammonia (NH_3)

$$NO_2^- + \overset{6Fd_{red}}{6e^-} + 7H^+ \underset{\text{nitrite reductase}}{\rightleftharpoons} NH_3 + 2H_2O \quad \overset{6Fd_{ox}}{} \quad \text{or } 8H^+ \ (NH_4^+)$$

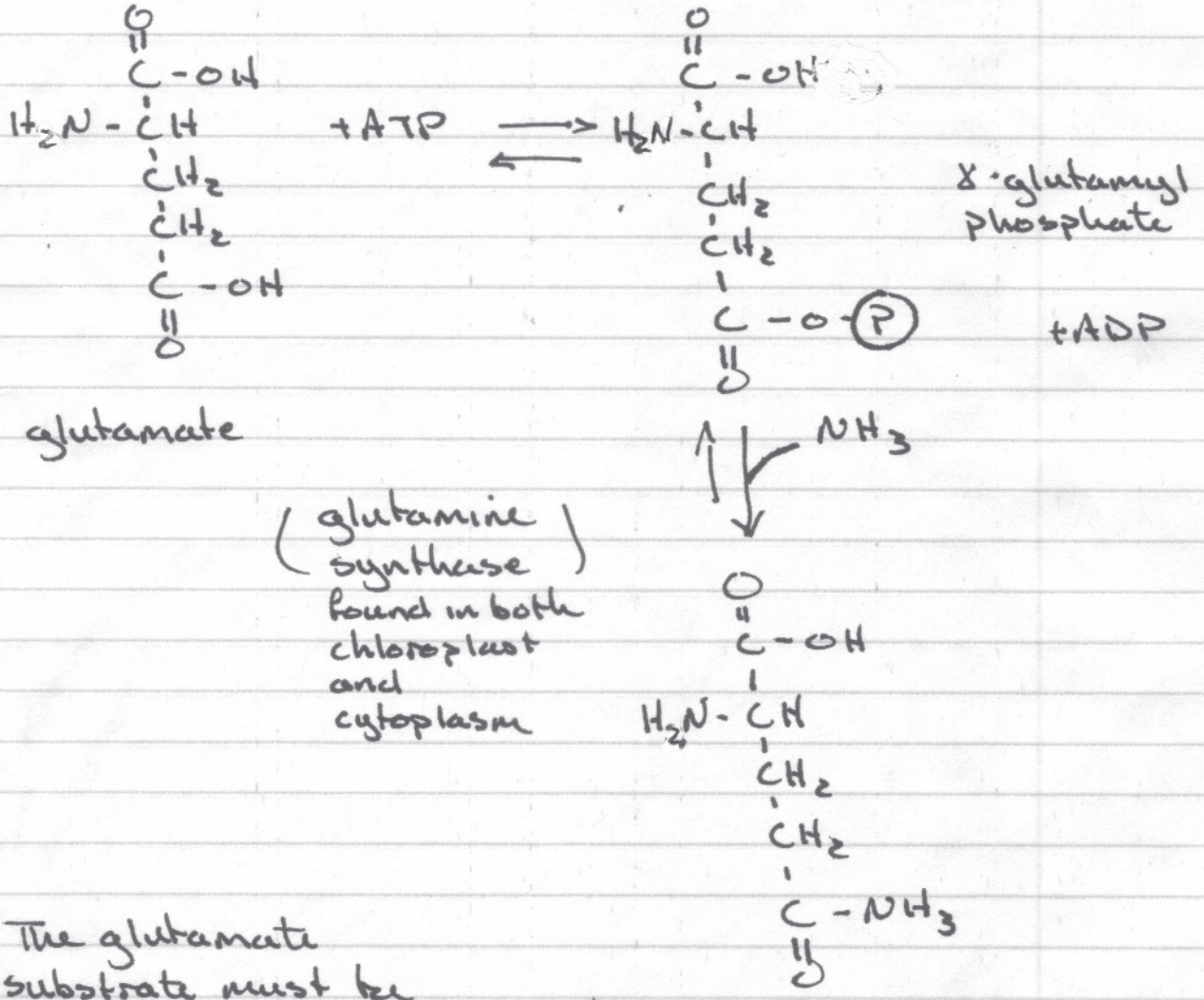
The reductant is ferredoxin (not NADPH). The enzyme includes a heme group and additional iron & sulfide groups.

Note that the H^+ consumption, and the ammonia reaction with water will have a strong effect on chloroplast stroma pH, which must be regulated.

Having reduced nitrate to ammonia, the nitrogen must now be assimilated into amino acids.

The reactions for NH_3 assimilation start with incorporation into glutamate

glutamate + NH_3 + ATP ⇌ glutamine + ADP + P_i



The glutamate substrate must be regenerated:

glutamine + α-ketoglutarate + Fd_{red} ⇌ 2 glutamate + Fd_{ox}

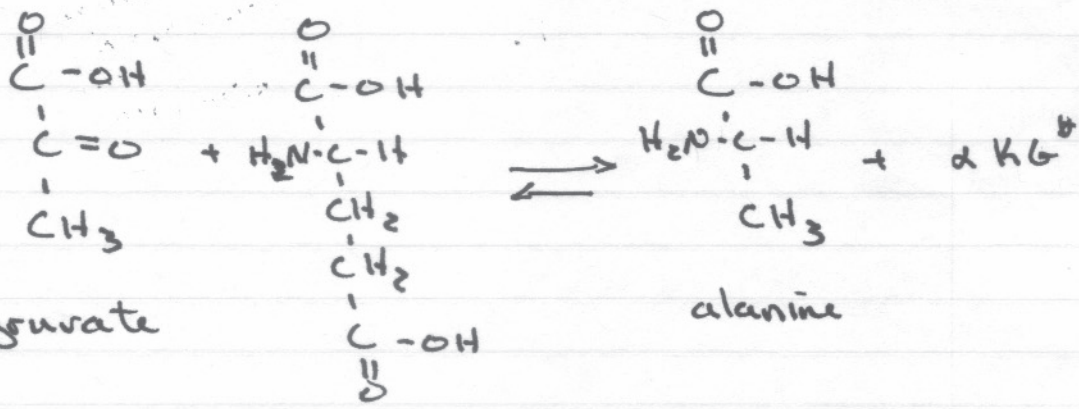
(can be supplied from mitochondria)

glutamate synthase (previously called GOGAT) found in chloroplast.

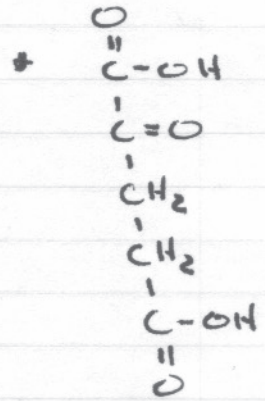
Chloroplasts do contain enzymes required for the synthesis of other amino acids, and are a ready supply of some of the carbon skeletons that are substrates in aminotransferase reactions.

$^{14}CO_2$ rapidly labels some amino acids. However, amino acid biosynthesis is probably "shared" with other organelles (for example, mitochondria supplying carbon skeletons from the Krebs cycle) and the cytoplasm.

For example: alanine can be synthesized:



aspartate is synthesized by transamination of oxaloacetic acid, then metabolized to produce lysine, threonine



serine can be produced by transamination of hydroxypyruvate.

etc.

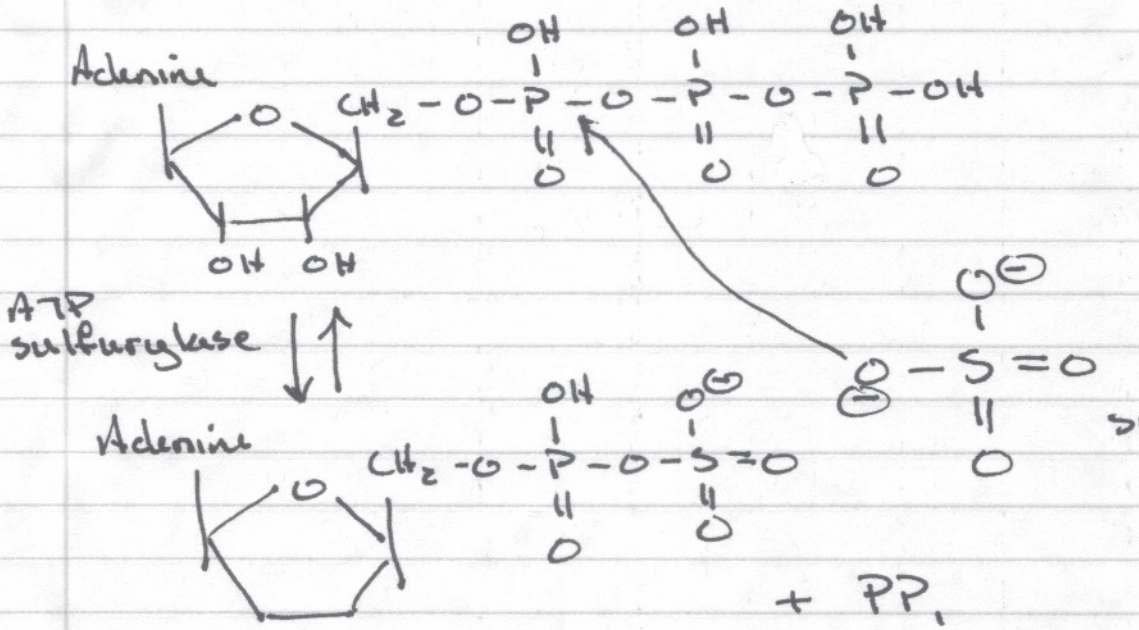
Sulfate Reduction

Sulfur is commonly available to the plant as the sulfate anion (SO_4^{2-}). Although required only in limited requirements, it is essential:

- for the amino acids methionine and cysteine
- as a part of Fe·S complexes in electron transport chains.
- glutathione (anti-oxidant)

Sulfate reduction, first to "sulfite" (SO_3^{2-}), then to sulfide (H_2S) occurs in the chloroplast.

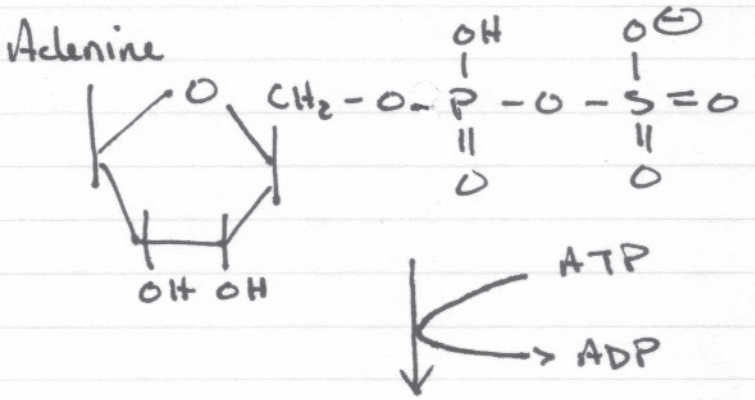
The sulfate is linked to AMP to form AMP sulfate (APS

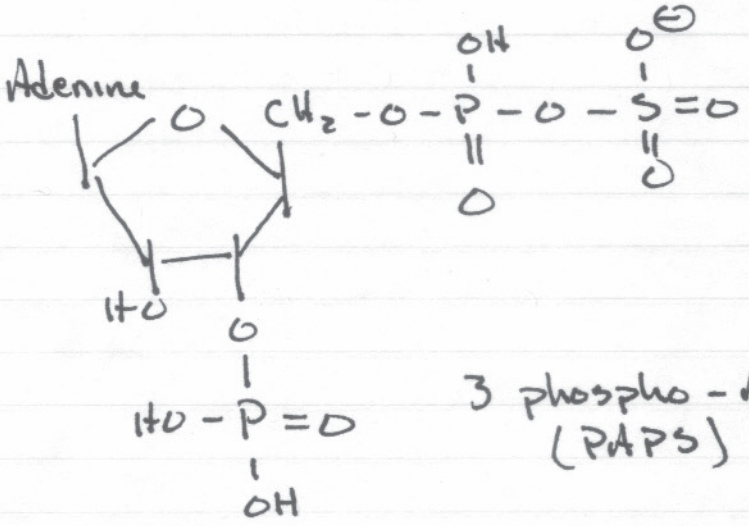


essential to "pull" the rxn to APS formation

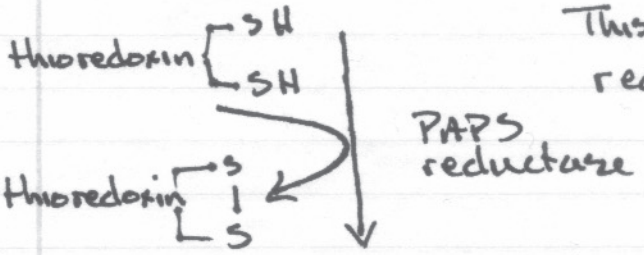
note bene: E_{mid} for SO_3^{2-}/SO_4^{2-} is -0.517 V, very high, which is why activation (APS) is required: $APS/SO_3^{2-} \approx 0.0$ V

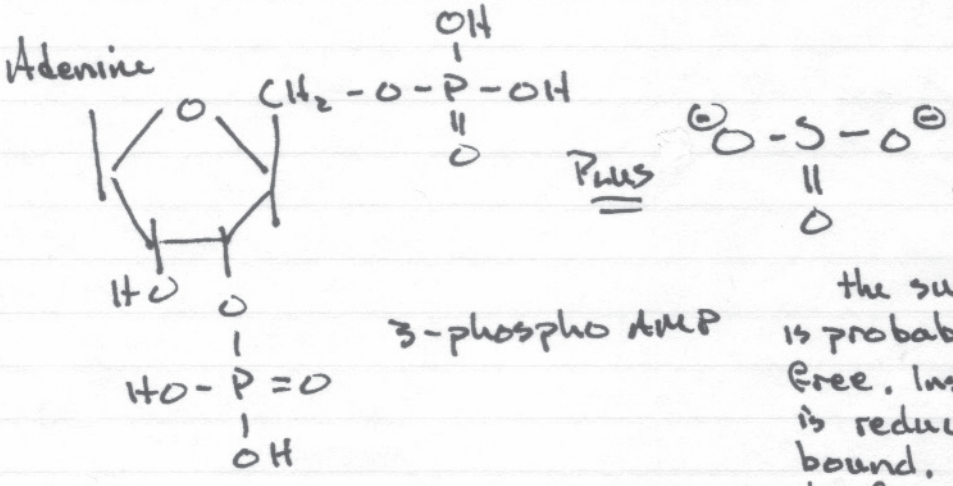
APS is then phosphorylated in the next potentiation.





This reaction also 'pulls' the reaction to the final product



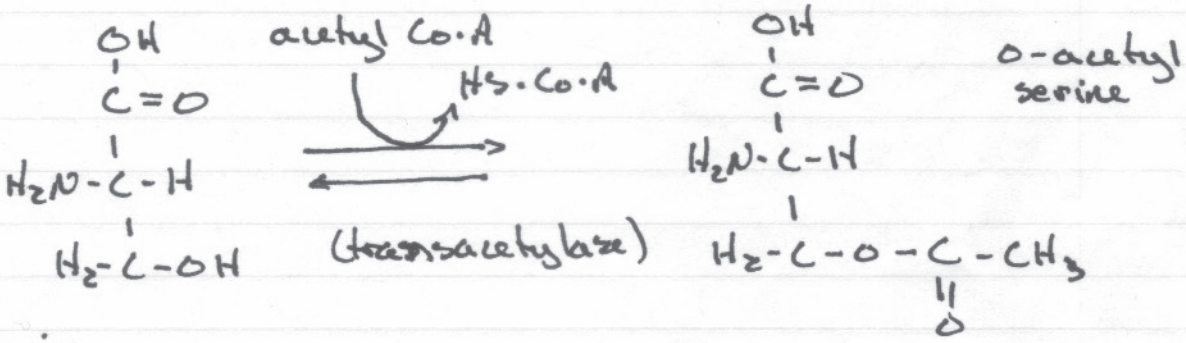


the sulfite is probably never free. Instead, it is reduced while bound. Then transferred to form cysteine.

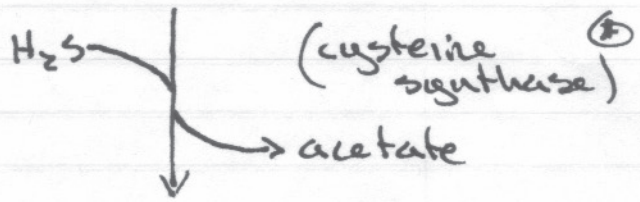
Sulfite reductase is similar to nitrite reductase.
It contains heme and a 4 Fe·S cluster.
Reducing equivalents are supplied by ferredoxin:

$$6\,Fd_{red} + SO_3^{2-} + 8H^+ \rightleftharpoons H_2S + 3H_2O + 6Fd_{ox}$$

The sulfhydryl group is transferred to O-acetyl serine to produce cysteine.



serine



Sources: Heldt: Plant Biochem & Molecular Biology
& Lawlor: Photosynthesis

cysteine

* catalyzes the overall reaction. the enzyme is abundant in chloroplasts

Since both nitrate & sulfate reduction require reducing equivalents from ferredoxin, it should be no surprise that the reductase reactions are regulated (activated) by light.

Although the major events are chloroplast-located (nitrite & sulfite reductase, and the enzymes required for 'fixation' in amino acids), regulation is multi-organ (e.g., roots can reduce nitrite) and multi-organellar (surplus NO_3^- and SO_4^{2-} can be stored in vacuoles).

nitrite, NO_2^-, is toxic and would not accumulate

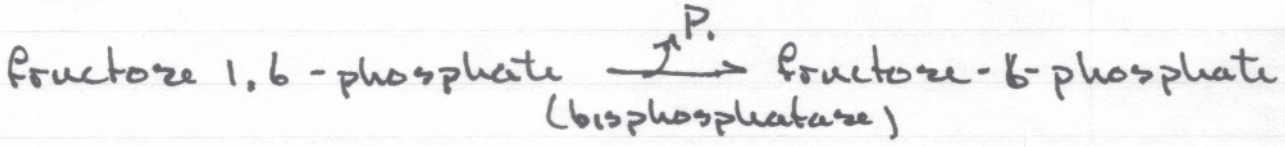
STARCH SYNTHESIS

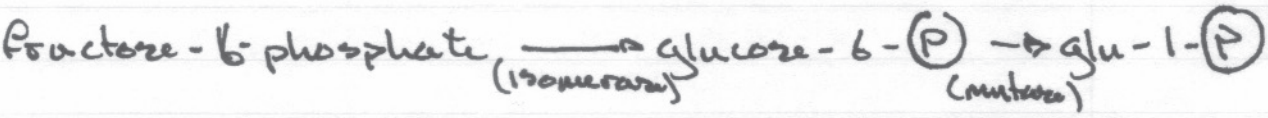
In the context of photosynthetic diversity. The photosynthate produced by the dark reactions can be stored in many forms. In general, in higher plants the short-term storage form is starch, localized in the chloroplast.

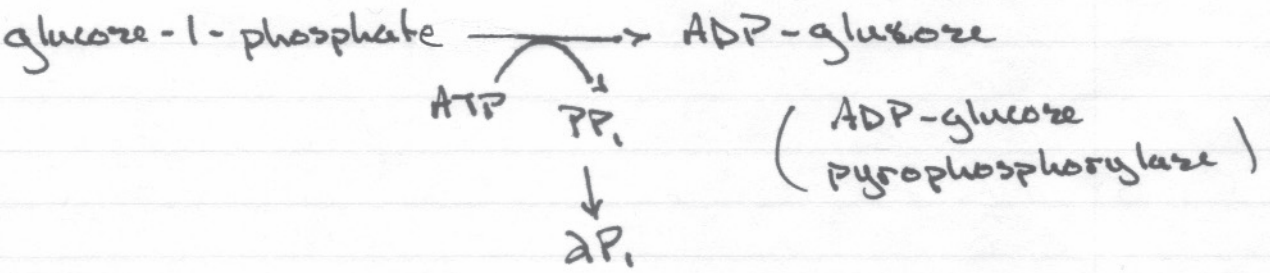
Starch synthesis in chloroplasts is tightly coupled with photosynthate export from the chloroplast. Export occurs by a triose-phosphate / phosphate exchanger.

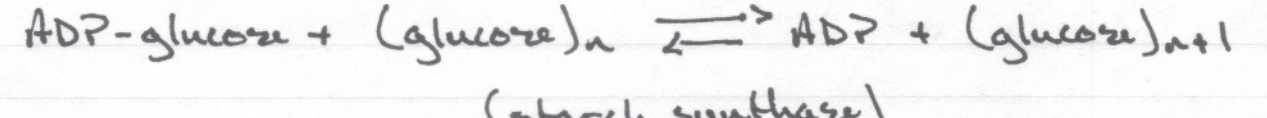
Decreasing expression of the triose-P / P translocator using anti-sense techniques prevents starch accumulation in chloroplasts.

The synthesis of starch starts with the Calvin Cycle intermediate fructose 1,6-phosphate.









Starch Synthesis.[1]

Starch is synthesized from fructose-6-phosphate supplied from the Calvin Cycle. It is first converted to glucose-6-phosphate, then to glucose-1-phosphate by hexose phosphate isomerase and phosphoglucomutase, respectively. The two reactions are freely reversible. The glucose is linked to ADP (ATP + glucose-1-phosphate <——> ADP–glucose + PP_i). Pyrophosphatase (PP_i <——> $2P_i$) 'pulls' the reaction in the direction of the ADP–glucose product. The glucose from ADP–glucose is then added to the glucose polymer (ADP–glucose + $(glucose)_n$ <——> $(glucose)_{n+1}$ + ADP). Starch consists of two glucose polymers: amylose (alpha-1,4 linkages) and amylopectin (alpha-1,4 linkages with alpha-1,6 branches).

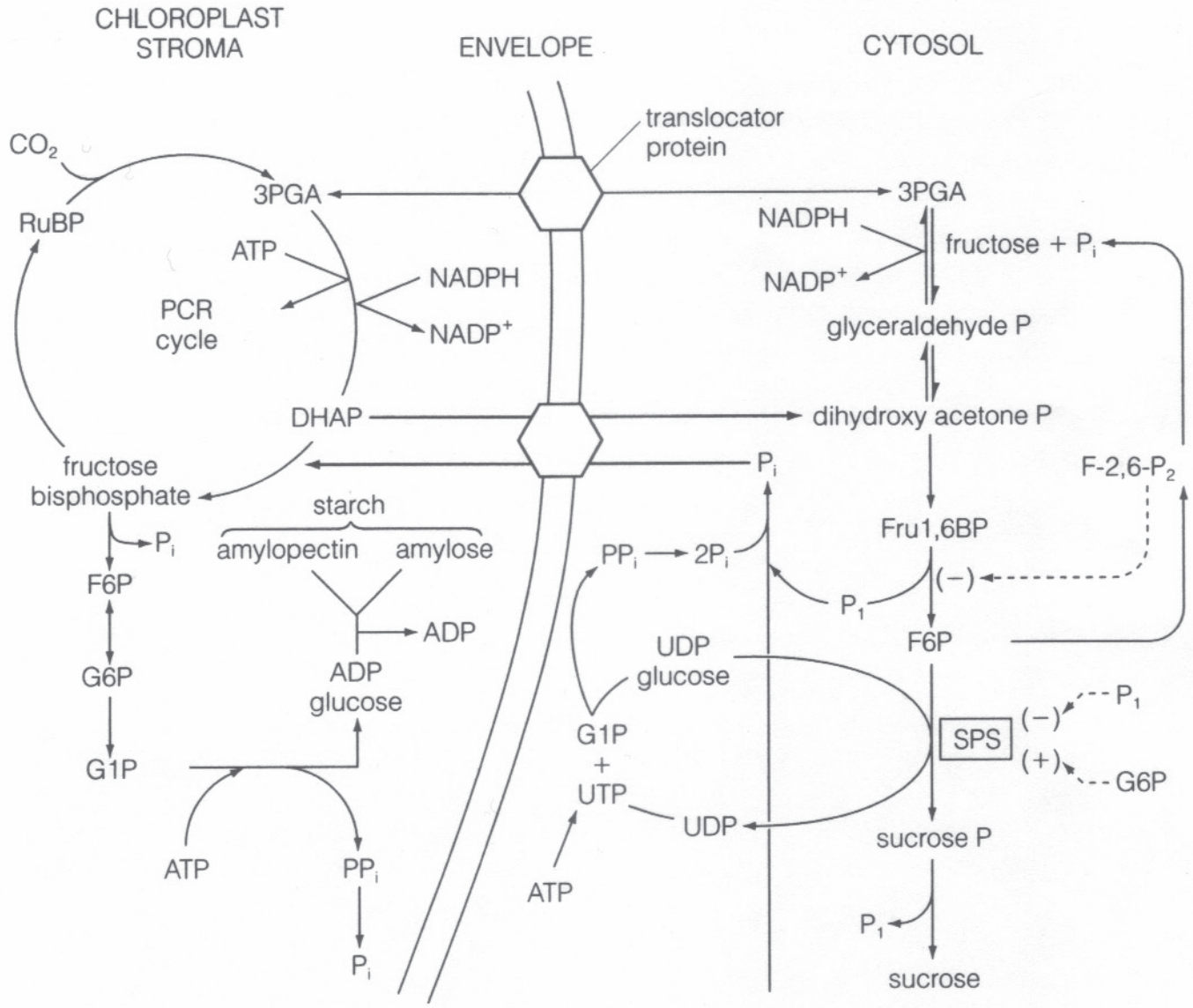


Figure 8.2. Scheme of carbon metabolism in the chloroplast and cytosol in the light, leading to starch and sucrose synthesis (see text for details)

[1] Source: DW Lawlor 2001 Photosynthesis. 3d edition.

The enzyme ADP-glucose pyrophosphorylase is an example of a heavily regulated enzyme.

3 phosphoglycerate and fructose-6-phosphate activate the enzyme. These accumulate in excess of that needed to regenerate RuDP

Inorganic phosphate (P_i) inhibits the enzyme. Elevated P_i can occur when there is insufficient ATP synthesis.

In general, phosphate concentrations are a strong determinant of starch accumulation.

During night, the starch is broken down to glucose by amylase, then exported to the cytoplasm by a glucose translocator.

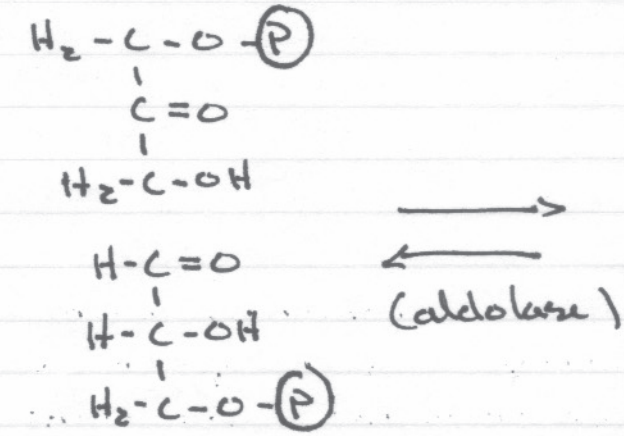
Starch breakdown also plays a crucial role in a specialized photosynthetic scheme called the Crassulacean acid metabolism (CAM) pathway. (page 10.14)

SUCROSE SYNTHESIS

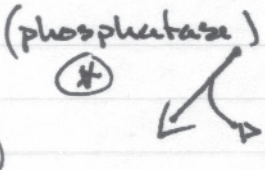
sucrose is synthesized in the cytoplasm. It is the major carbohydrate in plants. It is non-reactive, uncharged, and highly soluble, so it is readily transported to various organs of the plant, as required (for example, non-photosynthetic organs like roots and seeds, and to newly developing tissues like young leaves and growing shoot tips).

The 'progenitor' substrate is { glyceraldehyde 3-phosphate / dihydroxyacetone phosphate } exported from the chloroplast via the triose-phosphate / phosphate exchanger.

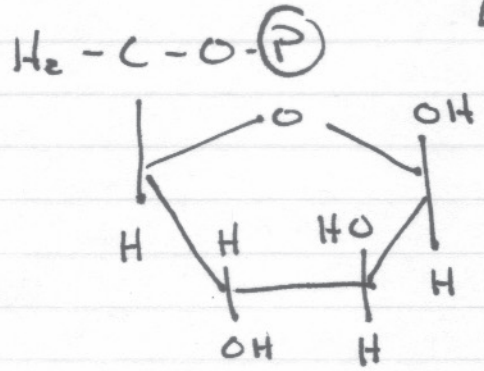
dihydroxyacetone phosphate



glyceraldehyde-3-phosphate



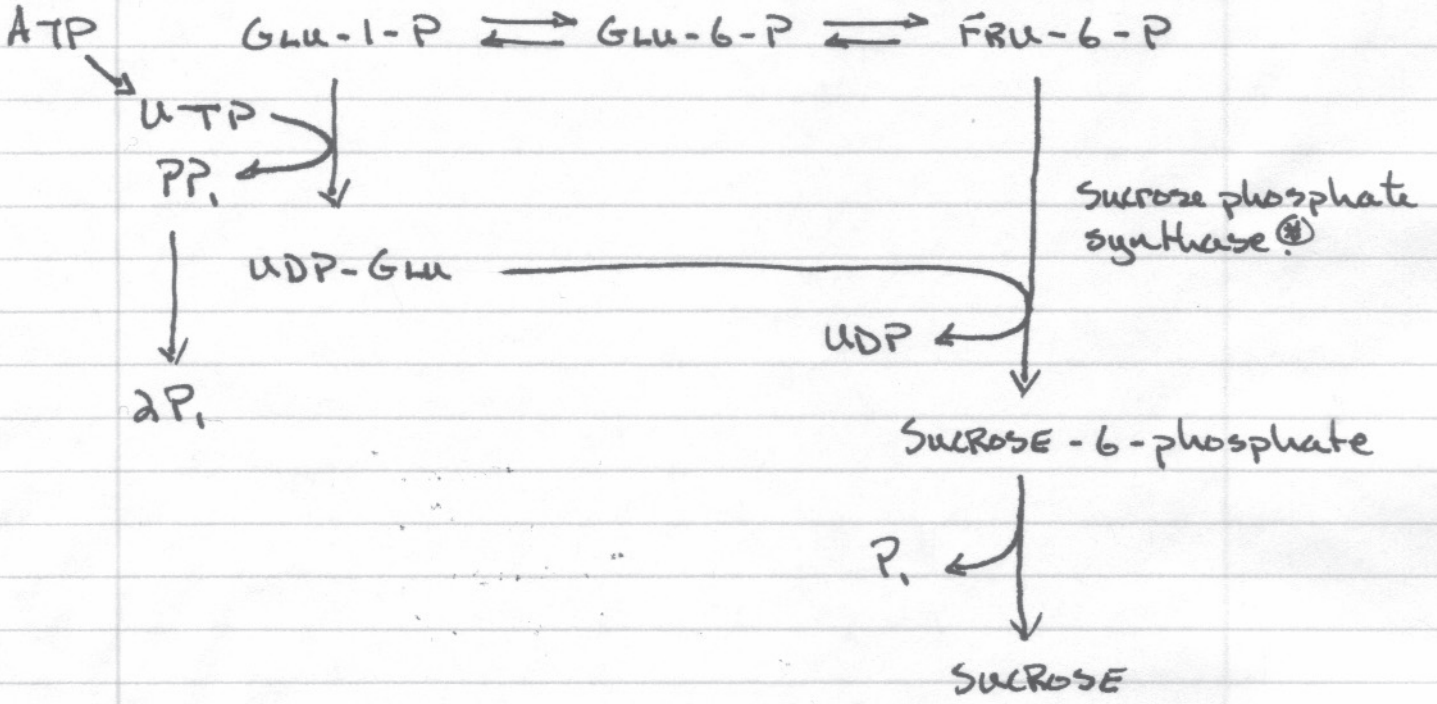
Fructose 1,6-diphosphate



Fructose-6-phosphate

inhibited by a "second messenger" Fructose-2,6-P =

The overall schema for sucrose synthesis from fructose-6-phosphate:



* sucrose phosphate synthase is regulated. The inactive form is phosphorylated by SPS kinase. High phosphate (P_i) levels inhibit the corresponding SPS phosphatase.

SPS phosphatase is light-activated.

So, activity depends upon 1) photosynthesis; and 2) sufficient triose phosphate export via the triose phosphate / P_i exchanger.

CRASSULACEAN ACID METABOLISM (CAM)

The CAM pathway is a C_4 mechanism, since CO_2 is fixed with the PEP carboxylase.

However PECase & RuBisCO activities are isolated temporally rather than spatially.

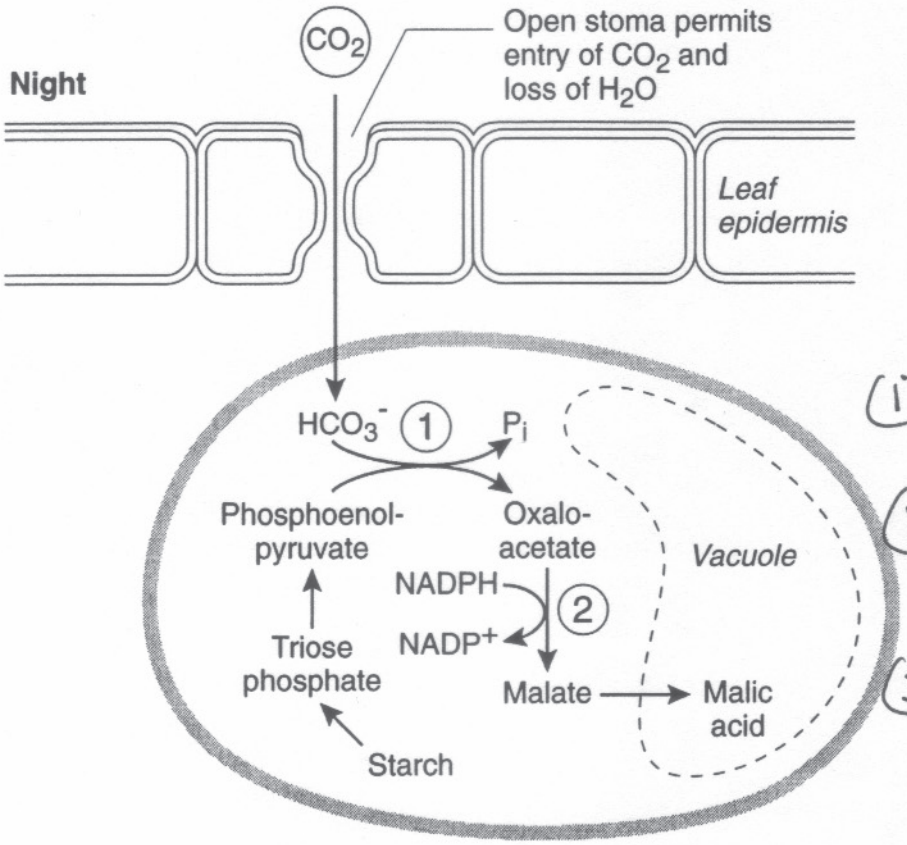
To avoid water loss during the day, stomata (through which CO_2 enters the leaf) are closed.

At night, stomates are open, CO_2 is fixed by PEPCase, then the product oxaloacetate is reduced to malate, which is stored in the vacuole.
The phosphoenolpyruvate substrate is produced from starch.

The starch is synthesized during the day from CO_2 released from malate.

(see overhead; Blankenship Fig. 9.14).

CAM plants are usually succulents (e.g. cacti) found in hot dry conditions.
Orchids (probably epiphytes) can use CAM photosynthesis.

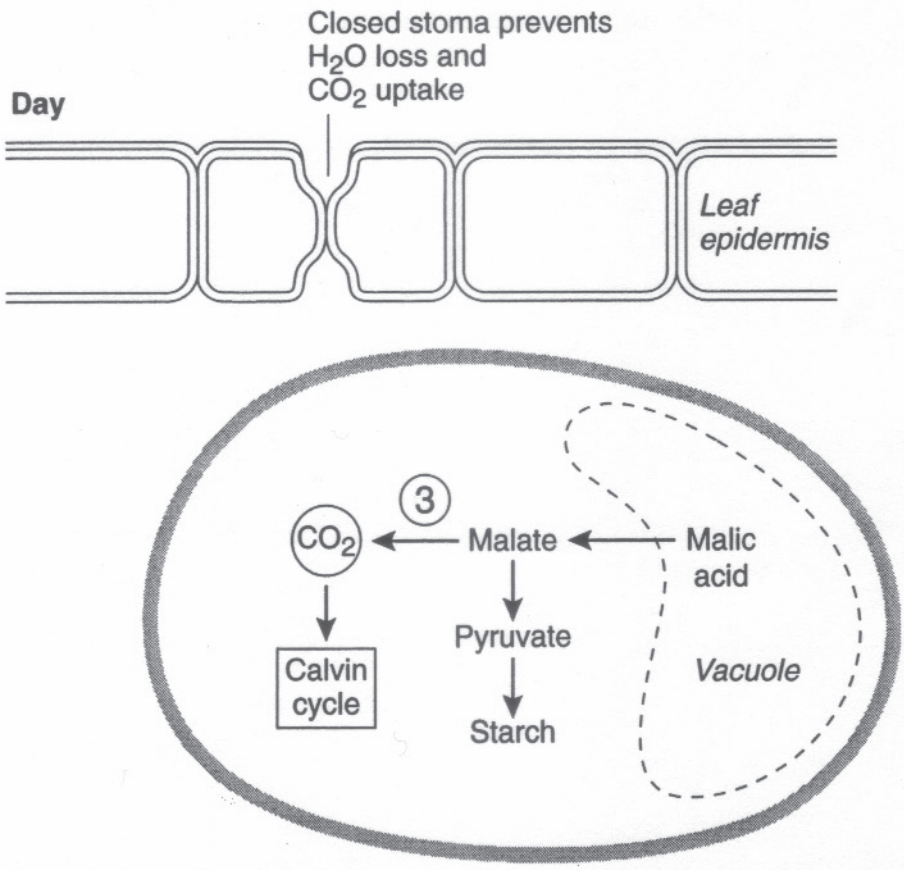


(1) PEP carboxylase

(2) NADP malate dehydrogenase

(3) NADP malic enzyme

(malate + $NADP^+$ ⇌ pyruvate + CO_2 + NADPH)



CHLOROPLAST LIPID BIOSYNTHESIS

In the context of plant biochemistry, lipid synthesis is cell-specific. Unlike water soluble carbohydrates and amino acids, lipids are not easily transported.

Their synthesis occurs in plastids and the endomembrane system (endoplasmic reticulum and Golgi bodies).

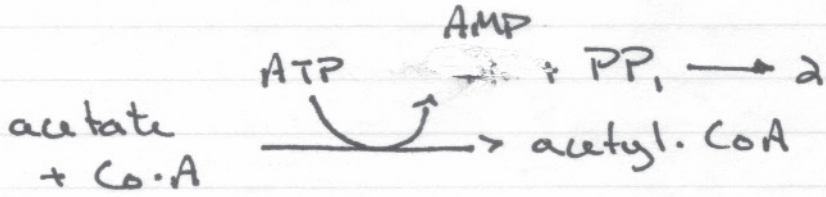
In photosynthetic cells, much of lipid biosynthesis occurs in chloroplasts. Indeed, lipids represent ~ 30% of chloroplast dry weight, and many lipids are 'unique' to chloroplasts (mono- and digalactosyl diacylglycerols are comprise about 75% of total acyl lipid content.

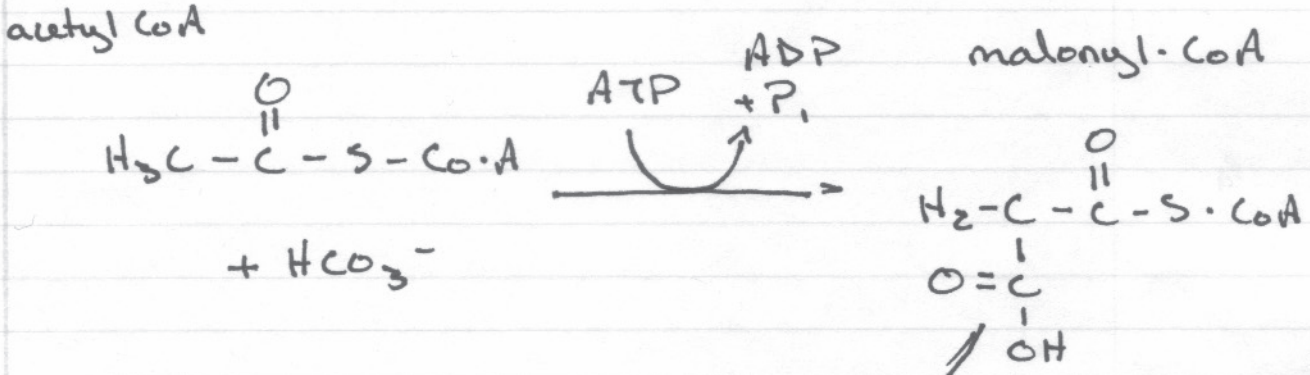
Glycerolipid	chloroplast thylakoid membs	mitochondria	plasma membrane
monogalactosyl diacylglyceride MGDG	51%	0	0
digalactosyldiacylglyceride DGDG	26%	0	0
sulfoquinovosyl diacylglyceride SL	7	0	0
phosphatidylcholine PC	3	27	32
serine PS	0	25	0
ethanolamine PE	0	29	46
glycerol PG	9	0	0
inositol PI	1	0	19
cardiolipin CL	0	20	0

source: Heldt Pl. Biochem & Molec. Biol pp 322-324

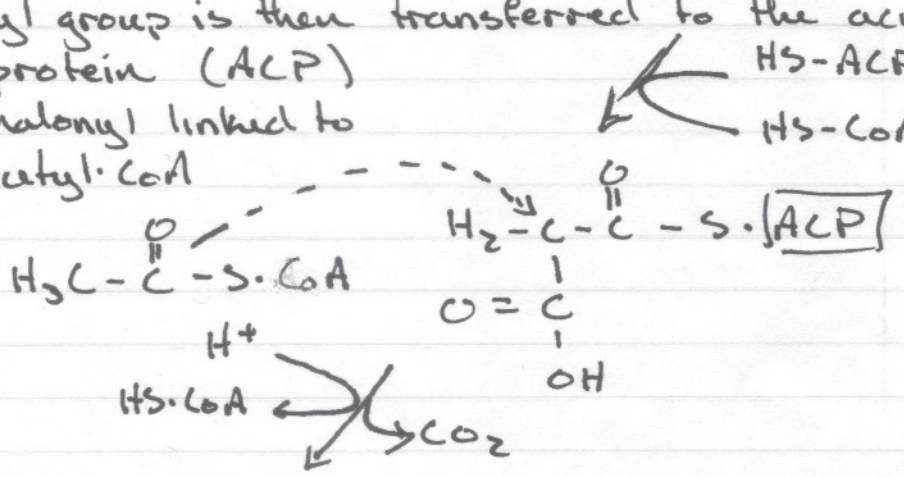
There are two pre-cursors of lipid synthesis:
acetate (acetyl CoA) and bicarbonate (HCO_3^-)

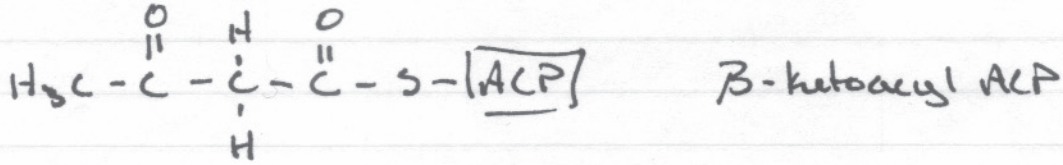
acetate (*) is provided from mitochondria and acetyl·CoA synthesized by Acetyl CoA synthetase:





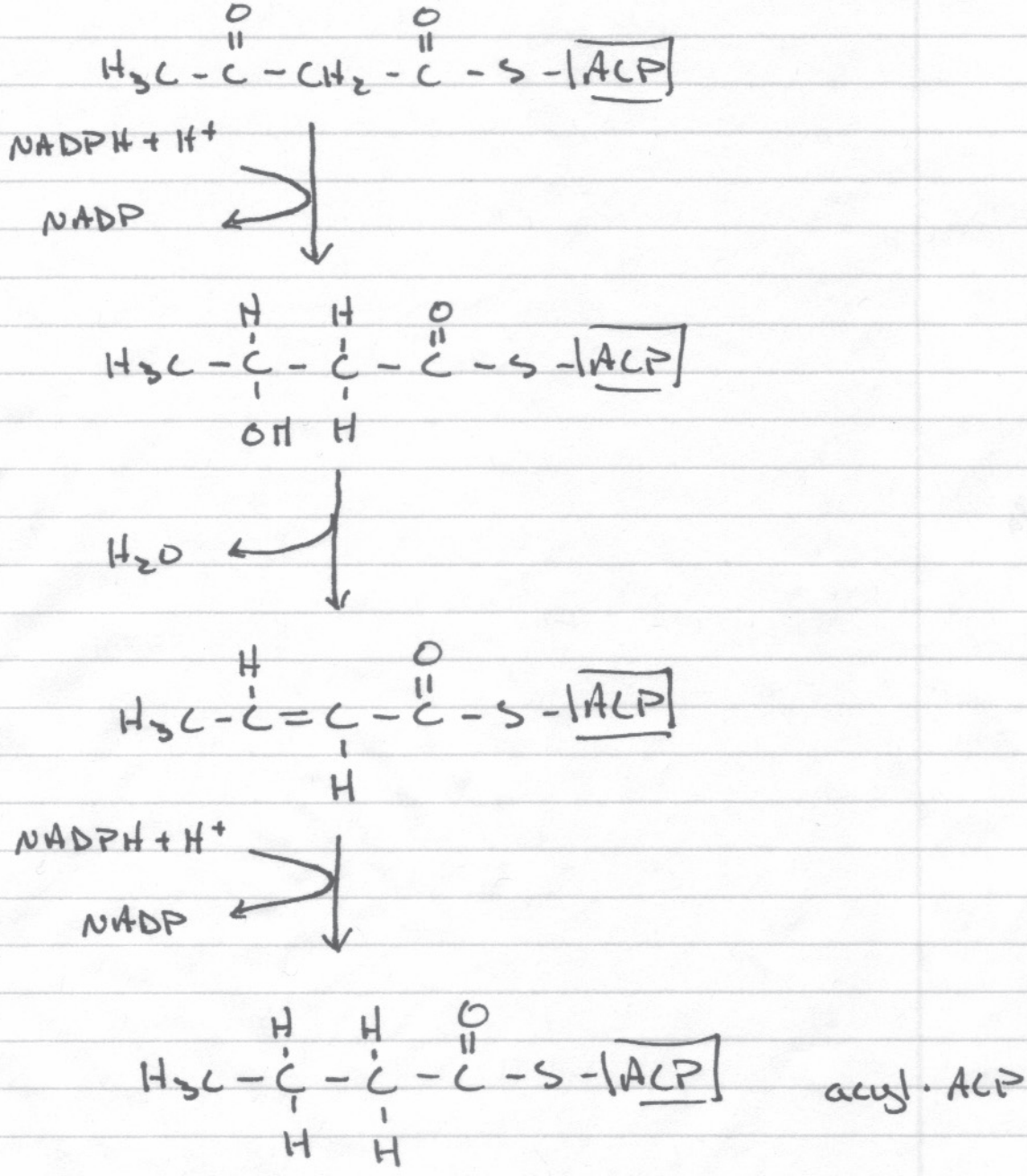
The malonyl group is then transferred to the acyl carrier protein (ACP) and the malonyl linked to another acetyl·CoA





(*) In photosynthetic tissues dihydroxyacetonephosphate would leave the chlt, enter the mitochondria, be decarboxylated to produce acetyl CoA. but acetate would be imported into chlt.
~~be converted to pyruvate~~

Next, there are a series of reductions & dehydrations

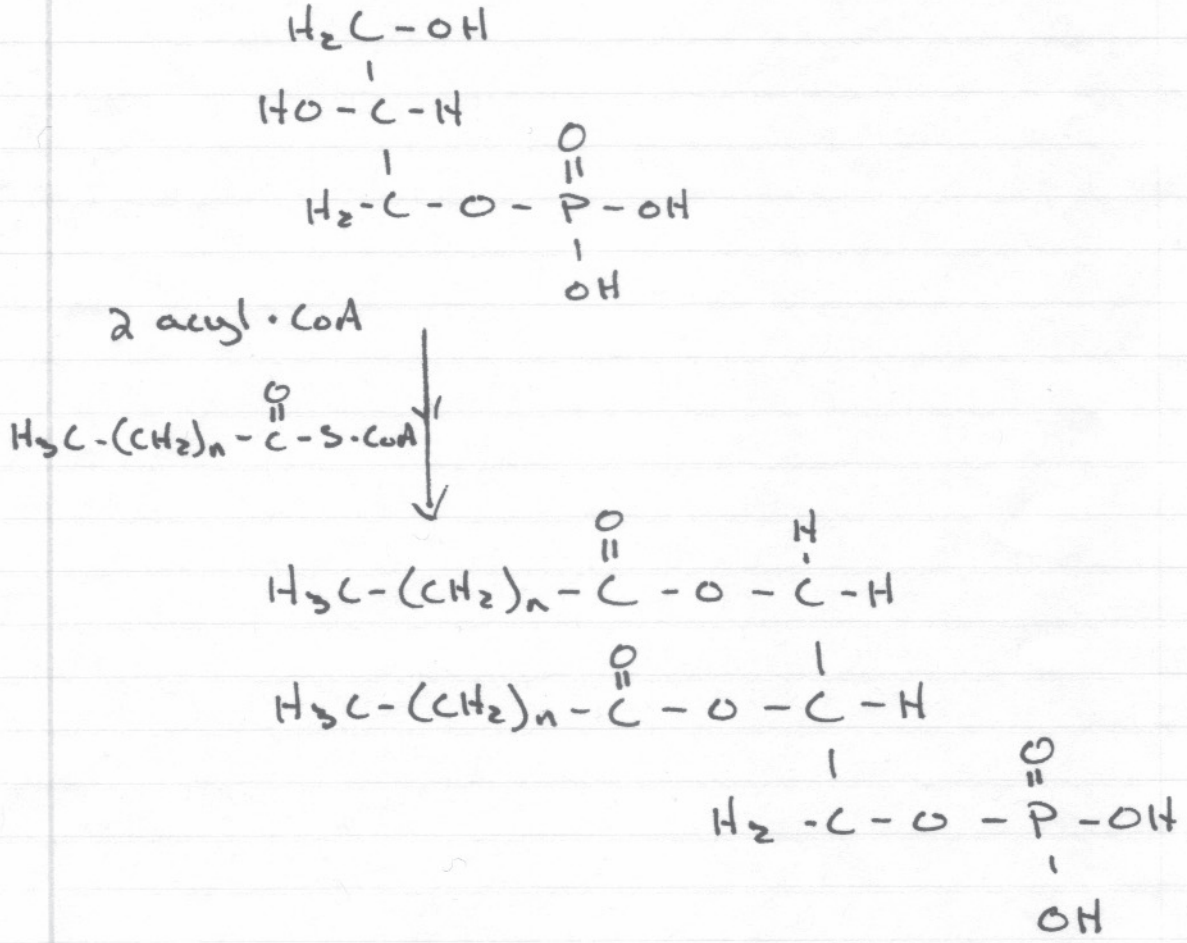


Additional carbon units (2 carbons at a time) are supplied by malonyl·ACP

Acyl chain lengths of C16 & C18 are common, with varying levels of saturation.

The acyl groups would be cleaved from the ACP and exported into the cytoplasm as fatty acids. Once in the cytoplasm, they would be converted back to acyl·CoA and processed as required.

Within the chloroplast, the fate of the acyl chains is linkage to glycerol (or, more accurately, glycerol 3 phosphate)



To synthesize monogalactosyl diacylglyceride
& di ~ ~ ~

The phosphate group is removed (product is diacylglycerol)

and, sugar groups (galactose) transferred from UDP-sugar (UDP-gal)

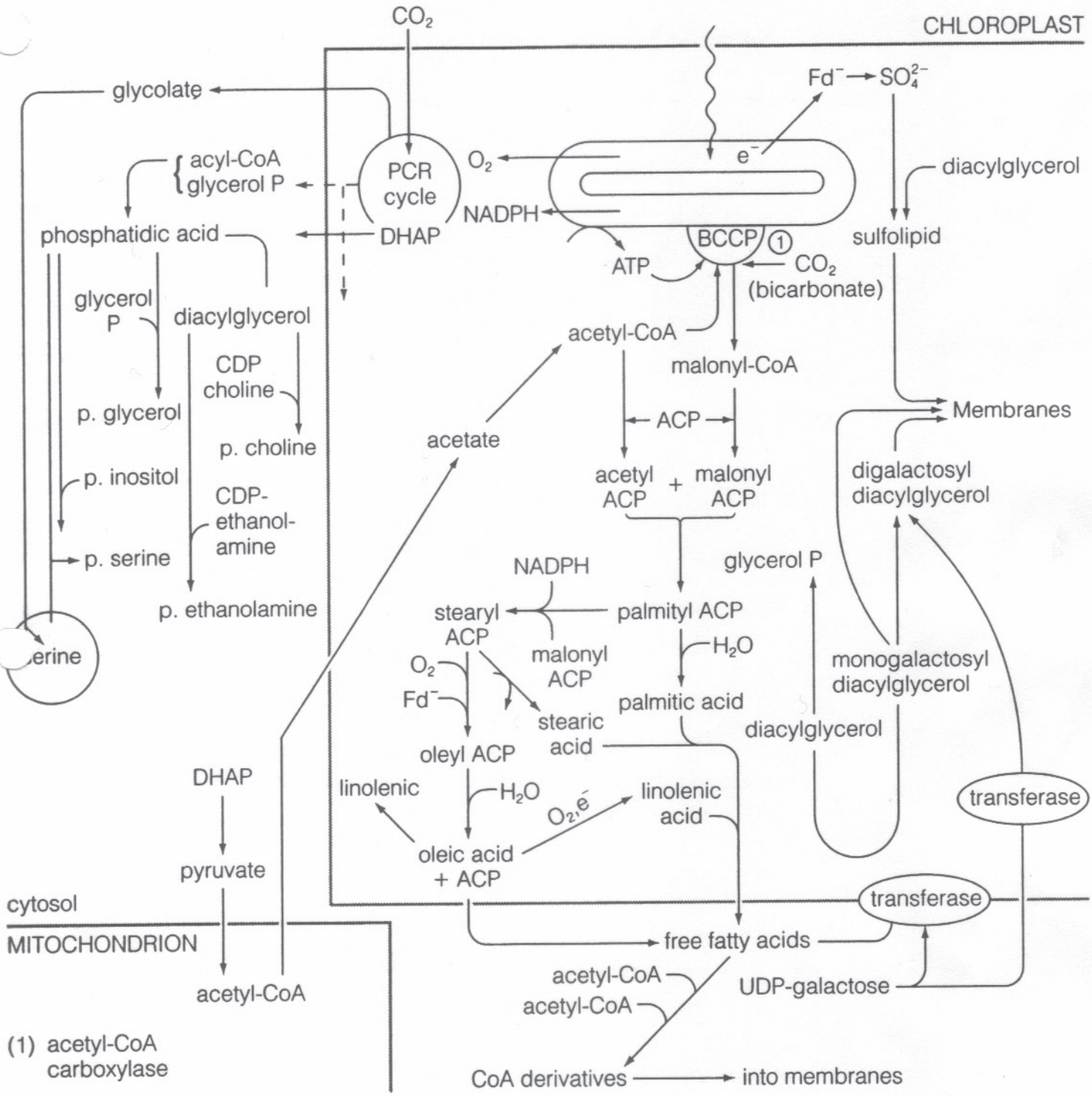


Figure 8.4. Fatty acid synthesis in leaf cells requires products of the light reactions and the PCR cycle and cooperation between organelles (see text). p, phosphatidyl

C3 PATHWAY BIOENGINEERING.

There is a diverse complexity associated with the C3 pathway. "Diverse" in the sense that enzymatic activity is tightly regulated, primarily through the light-modulation thioredoxin, but also through "normal" allosteric regulators (see example: energy charge*). But in addition there is compartmental regulation since end-products may be synthesized elsewhere in the cell.

At the level of the individual enzymes, catalyzing the generalized reaction:

$$A + B \rightleftharpoons C + D$$

* energy charge

$$EC = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

The change in free energy exerts control over the direction of the reactions:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$$

normally, reactions are characterized as near-equilibrium & far from "

But this over-simplifies the situation, since the rate of reaction will depend upon the catalytic capacity, a function of the specific activity of the reaction, and the amount of enzyme:

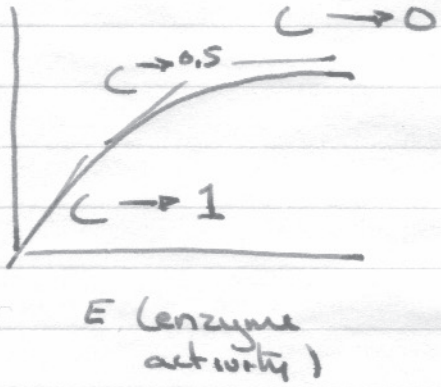
$$\text{capacity} = \left(\begin{matrix}\text{specific} \\ \text{activity}\end{matrix} \cdot \text{amount}\right)$$

Both energetics and capacity will affect the reaction flux and may exert control (as a limiting factor) on overall metabolic flux in the C3 pathway.

The relation between metabolic flux through the C3 pathway (C3 flux) and enzyme activity is complex. It can, will, vary depending upon environmental pathways. At low light levels, enzymatic activities are unlikely to be a limiting factor. At high light intensities, enzymatic activity can limit C3 flux, the effect varies with the specific properties of the enzyme.

This dependency (between enzymatic activity and C3 flux) is described by a "flux control coefficient":

$$C = \frac{\left(\frac{dJ}{J}\right)}{\frac{\partial E}{E}}$$



C will vary from 1, where enzymatic activity exerts complete control, to 0, where enzymatic activity is surfeit & exerts no control.

For C3 flux, the flux control coefficients of all the enzymatic activities must sum to 1.

This is the theoretical background, a very useful underpinning for experiments that modulate enzymatic activity for selected enzymes, and examine the effect on C3 flux.

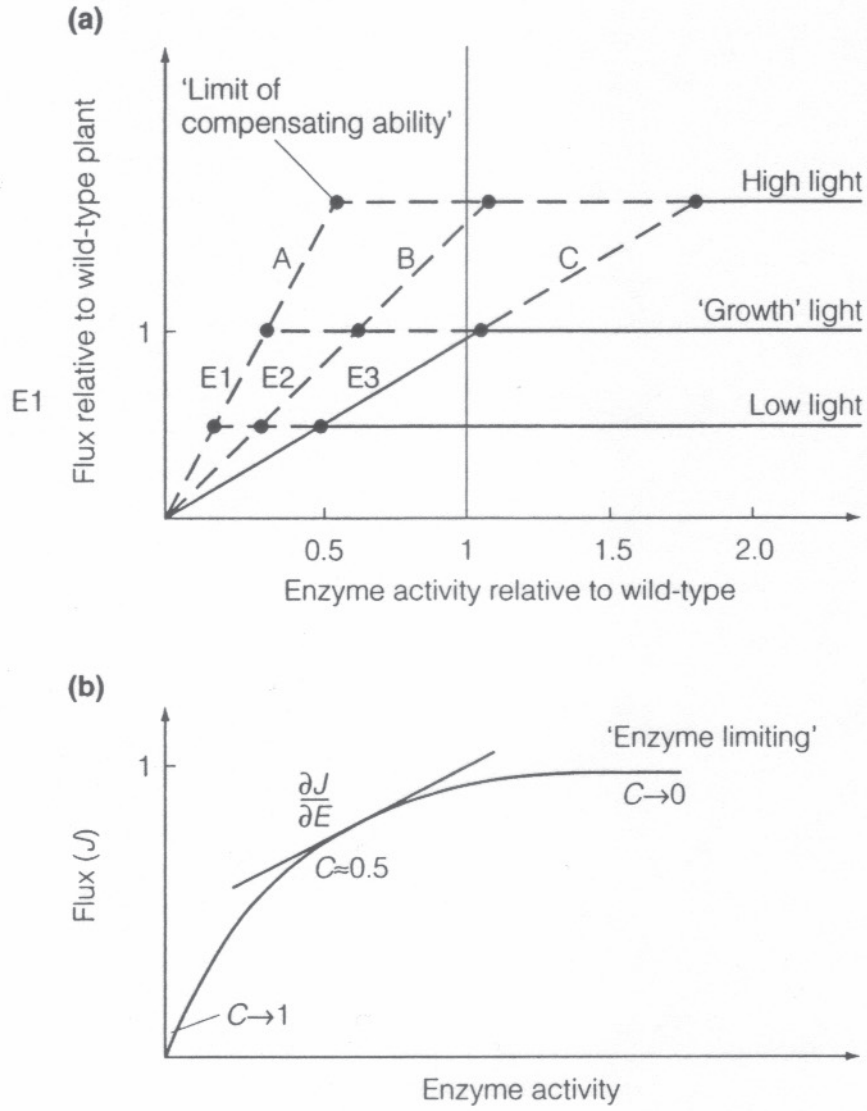


Figure 7.6. Illustration of the response of the relation between flux and enzyme activity for a metabolic pathway. (a) Dependence of flux, relative to the flux under growth conditions, on enzyme activity (relative to wild-type activity). The 'limit of compensating ability' (LCA) is indicated for three enzymes, E1, E2 and E3, differing in control over the flux. E1 has little control, E2 more and E3 full control, shown by the progressively larger LCA values. Increasing the activity would not increase the flux. In light levels below the growth conditions the enzymes' LCAs become smaller and other factors increase in importance. With much higher light intensity the LCAs increase compared to the standard condition. In the case of E3 the flux could not increase unless the activity increased (see Fell, 1997, and Fridlyand *et al.*, 1999 for details). (b) shows the response of flux through an enzyme to activity of the enzyme in a system. The flux control coefficient, $C = \partial J/\partial E$, where J is the flux and E the enzyme activity. Approximate values of C are given, showing how it differs between the theoretical limits of 0 and 1. Flux control is not generally dependent on a single step in multicomponent systems and is distributed so that the limiting values may only be obtained under extreme circumstances (see text)

To explore the role of individual enzymes in C3 metabolic flux, plants are transformed with either anti-sense or sense sequences of the enzyme. Note that either plastid or cytoplasmically-located gene expression may can be modified. Anti-sense causes lower enzyme levels. Sense elevates the level. (These generalizations must be confirmed experimentally).

Perhaps surprising is that modifying RuBISCO levels had no effect on C3 metabolic flux, unless saturating light and CO_2 levels were used. Thus, under "normal" ambient levels, RuBisCO is not a significant controlling factor. The flux control coefficient was lower than 0.2.

It was also unexpected that aldolase exerted significant control when endogenous levels were decreased below 50%. Unexpected because the reactions it catalyzes: flux control coefficient of 0.18 to 0.32 ambient high CO_2 & light

dihydroxyacetone-P + glyceraldehyde 3-P ⟶ fructose 1,6 di-phosphate

dihydroxyacetone-P + erythrose-4-P ⟶ sedoheptulose 1,7 diphosphate.

are freely reversible ($\Delta G \approx 0$).

Source: Raines, CA 2003
The Calvin cycle revisited.
Photosyn. Res. 75: 1-10.

Transketolase, another freely reversible reaction:

Sedoheptulose · 7 · P + Glyceraldehyde · 3 · P → xylulose · 5 · P + ribose · 5 · P.

also exerts more control on the C3 metabolic flux than expected, notable under high irradiance (flux control coefficient of 0.32, compared to 0.07 at low light intensity).

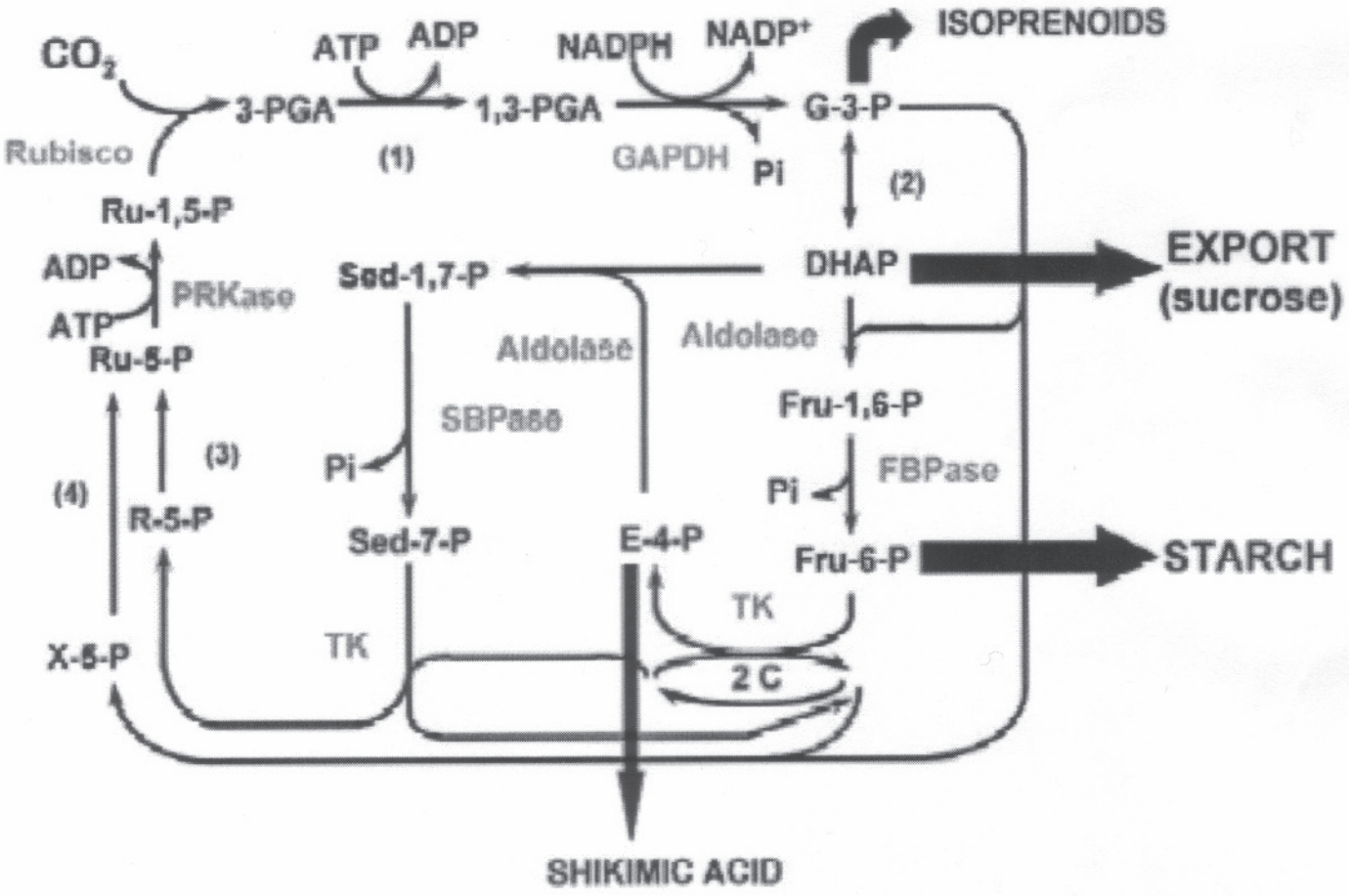
Anti-sense - induced low enzyme levels caused a shift in partitioning: to higher starch and lower sucrose export. Only with transketolase levels ≤ 75% control, sucrose levels declined to ≥ 25% ≤ control.

On the basis of energetics, glyceraldehyde 3 phosphate dehydrogenase, fructose 1,6 - bisphosphatase, and phosphoribulokinase would all be expected control points, but in fact anti-sense experiments reveal they exert little control.

Sedoheptulose - 1,7 · bisphosphatase turns out to be an important control point. (≤ control coefficient 0.3 to 0.5 under ambient conditions)
Overexpression causes larger plants (Overhead, from Raines 2003).

Calvin Cycle BioEngineering[1]

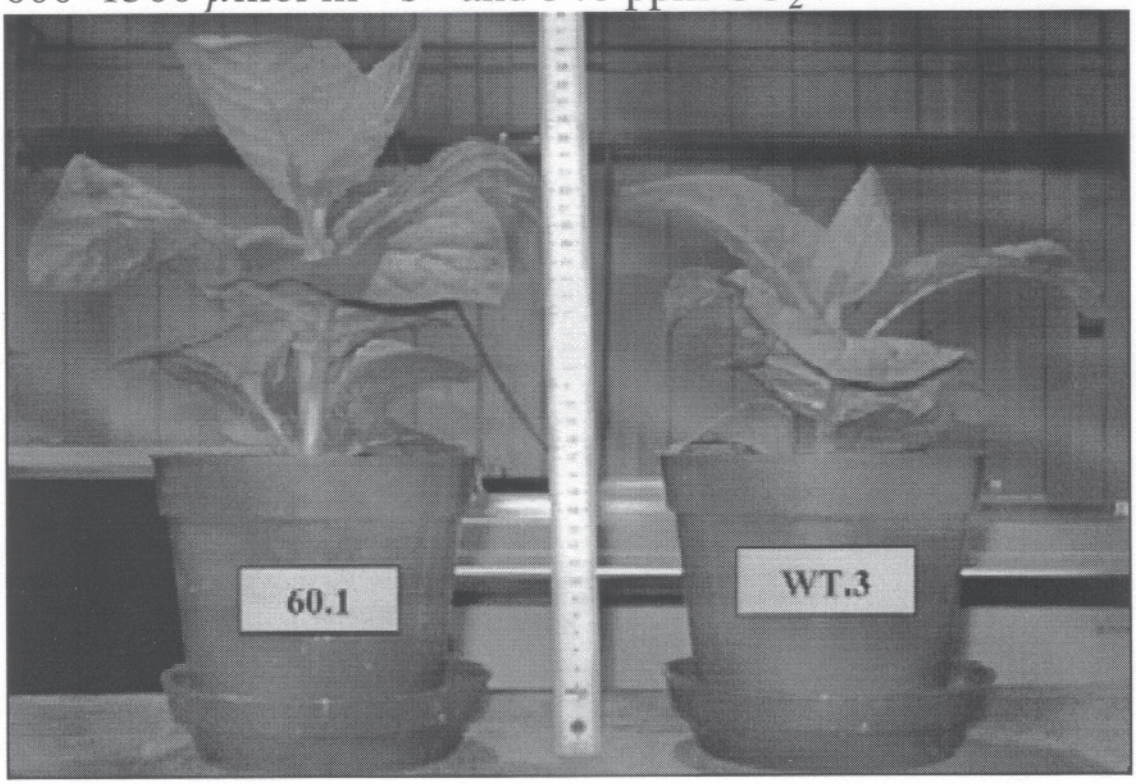
The Calvin cycle showing the intermediates from the first stable carbon compound, 3-PGA, to the cabon dioxide acceptor molecule, ribulose-1,5-bisphosphate and the exit points form the cycle into the pathways of sucrose, starch, isoprenoids and shikimic acid . The reactions catalysed by the enzymes whose levels have been manipulated in transgenic plants, are shown in grey. The site of function of the enzymes (1) 3-phosphoglycerate kinase (2) triose phosphate isomerase (3) ribose-5-phosphate isomerase and (4) ribulose-5-phosphate epimerase are also indicated:



[1] Source: Christine A. Raines (2003) The Calvin cycle revisited. Photosynthesis Research 75:1–10.

C3 Pathway Bioengineering.[1]

Growth of wild type and transgenic *Nicotiana tabacum* plants over-expressing Arabidopsis SBPase. Wild type and T1 progeny were analysed after 6 weeks of growth in soil in greenhouse conditions, at an irradiance of between 600–1500 μmol m^{-2} s^{-1} and 340 ppm CO_2 .



The results indicate that over expressing specific enzymes of the C3 pathway can have an effect on plant growth and development.

[1] Source: Christine A. Raines 2003 The Calvin cycle revisited. Photosynthesis Research 75:1–10.