Carbon Metabolism, Carbohydrates & Sugars

1. Introduction: Overview of carbon flow in plant

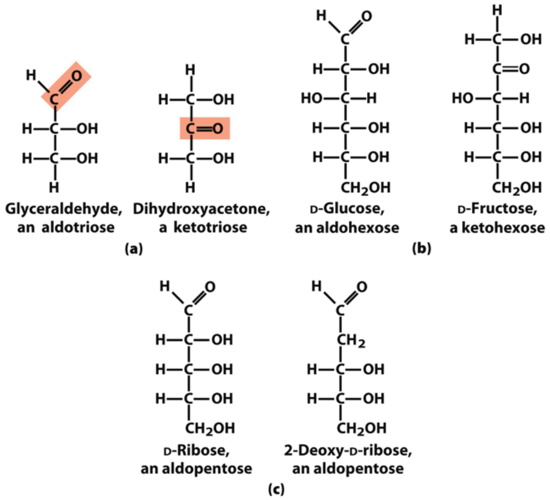
- carbon flow in metabolism: starch, sucrose, cell wall
- carbon flow in the plant: spatial organisation
- how is C allocation organized and regulated?

2. Background on carbohydrate and sugars (handout)

What is a **carbohydrate?**

= *polyhydroxyketones and polyhydroxyaldehydes*

- two basic types of sugars: **aldose** & **ketose**
- there are D & L stereoisomers (D is usual)
- ring formation in simple sugars leads to a or b **anomers**
  (isomers that differ only at the **anomeric carbon**)
- note numbering system in simple sugars
- in plants, 5C & 6C sugars are most common, but many structural isomers are possible



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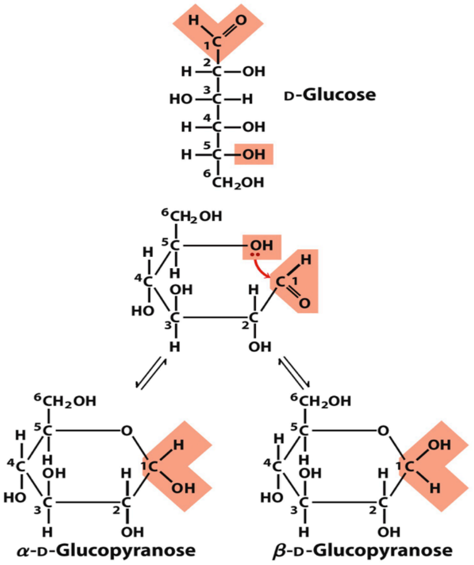

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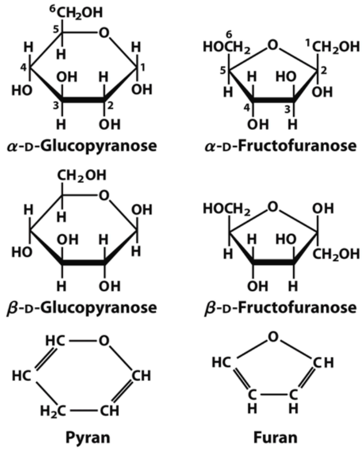

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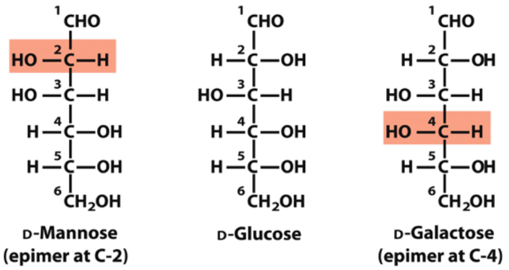

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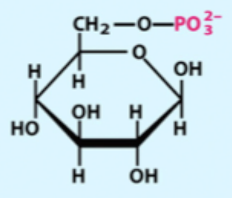
Reactions with simple sugars:

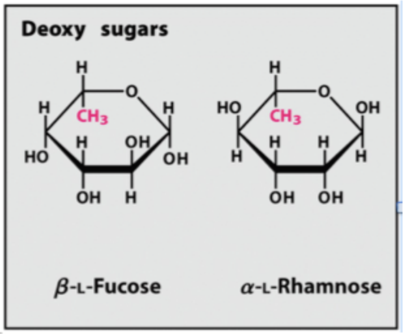
- the anomeric carbon is most reactive in forming bonds

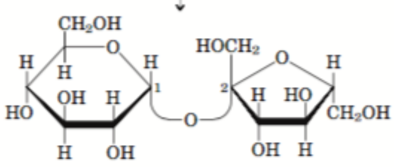
Glycosidic bond: *bond formed by reaction of anomeric carbon of one sugar with a hydroxyl of another sugar*

- there are mono-, di-, oligo-, poly-saccharides
- sugar phosphates are most common in cells ("activated")
- sugar nucleotides activate sugars, key roles in metabolism

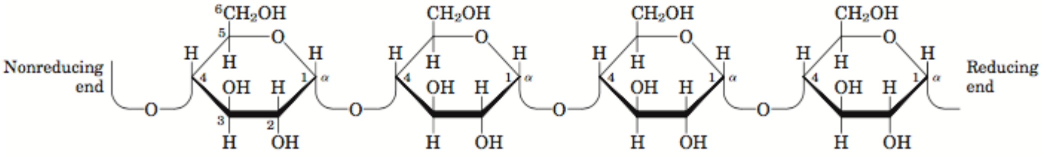


β-D-Glucose 6-phosphate





Sucrose



3. Starch synthesis: from triose-P to starch granules

Importance:

- the major form of storage carbohydrate in plants
- biggest source of calories for humans
- has many industrial uses (adhesive, gels, films, paper)

Features:

- polysaccharide (long chains of glucose)
- in granules (osmotically neutral!), reducing end in
- can act as **temporary** storage in chloroplasts (assimilatory starch), or **long-term storage** in amyloplasts (reserve starch)
- structure: **a(1,4) & a(1,6)** linkages, note branching
- components, differing in branching: amylose vs amylopectin

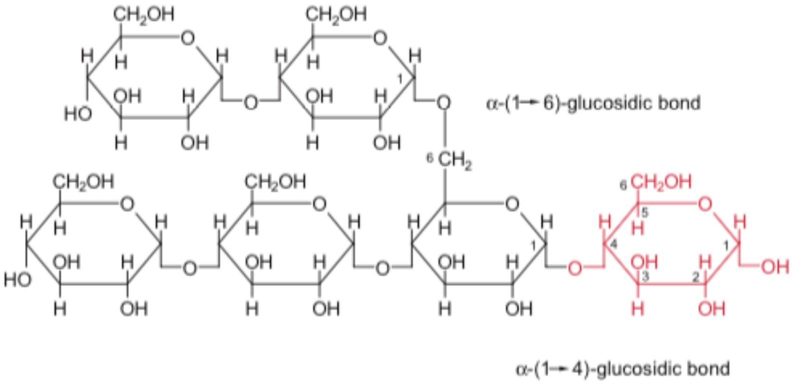


Figure 9.2 The glucose molecules in starch are connected by (α1→4)- and (α1→6)-glycosidic linkages to form a polyglucan. Only the glucose residue (colored red) contains a reducing C1-OH group.

Figure 9.3 Transitory starch in a chloroplast of a mesophyll cell of a tobacco leaf at the end of the day. The starch granule in chloroplasts appears as a large white area. (By D.G. Robinson, Heidelberg.)

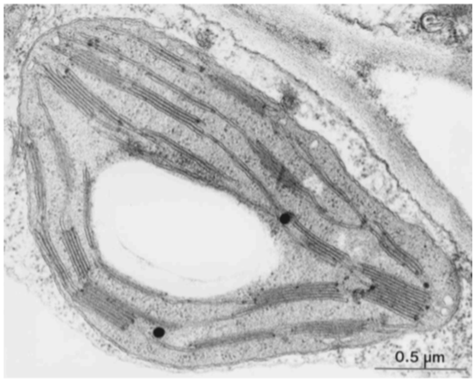


Table 9.1: Constituents of plant starch

	Number of glucose residues	Number of glucose residues per branching	Absorption maximum of the glucan iodine complex
Amylose	10^3		660 nm
Amylopectin	10^4–10^5	20–25	530–550 nm

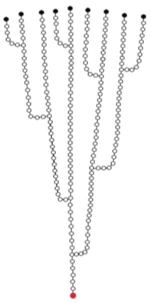


Figure 9.4 The polyglucan chains of amylopectin have a branch point at every 20 to 25 glucose residues. Neighboring chains are arranged in an ordered structure. The glucose residue, colored red at the beginning of the chain, contains a reducing group. The groups colored black at the end of the branches are the acceptors for the addition of further glucose residues catalyzed by starch synthase.

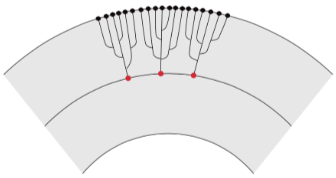
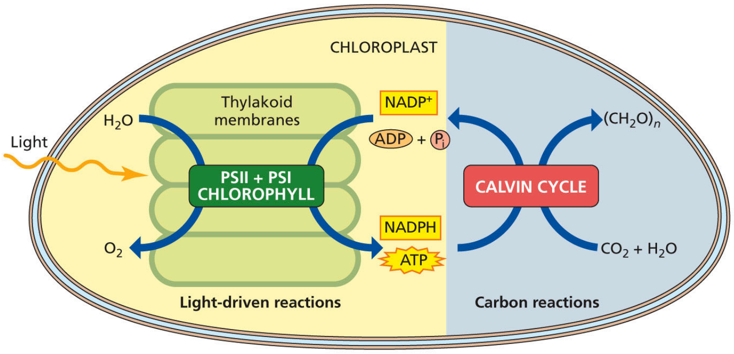


Figure 9.5 In a starch granule the amylopectin molecules are arranged in layers. Compare with Figure 9.4.

Starch synthesis is closely integrated with photosynthesis and Calvin cycle ('dark reactions', 'CO2 fixation')

- review of Calvin (C3) cycle (Fig. 6.3, 6.20)
- sucrose and starch building blocks are **triose-P**
- starch synthesis happens in chloroplasts



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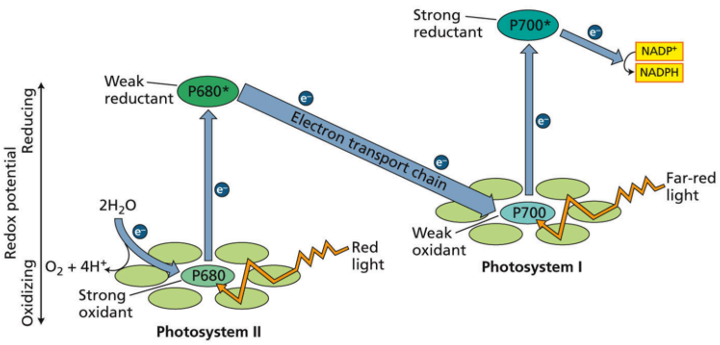

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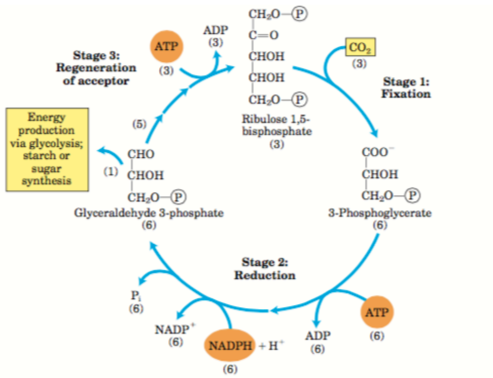


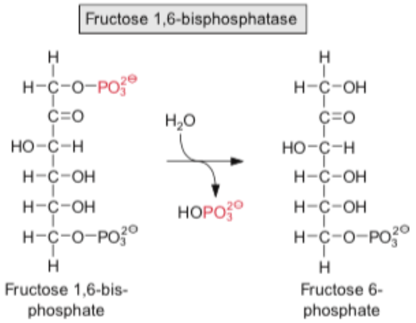
Biosynthesis in 4 steps

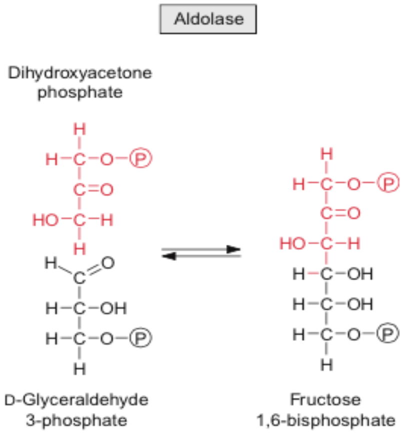
Step 1: [*make glucose-1-P*]

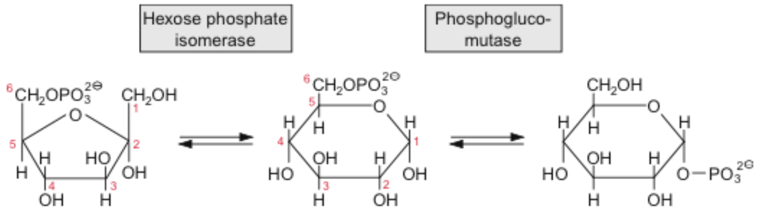
2 x triose ---> fructose-1,6-P ---> fructose-6-P ---> glucose-6-P ---> glucose-1-P

Enzymes: [in plastid, shared w. Calvin cycle]

aldolase, fructose-1,6-bisP phosphatase
hexose phosphate isomerase & phosphoglucomutase







Step 2. [*make ADP-glucose* - activates the anomeric C*]*

glucose-1-P + ATP ----> ADP-glucose + PP$_i$.

Enzyme: *ADP-glucose pyrophosphorylase (* regulatory)*

*(*also *pyrophosphatase drives reaction forwards)*

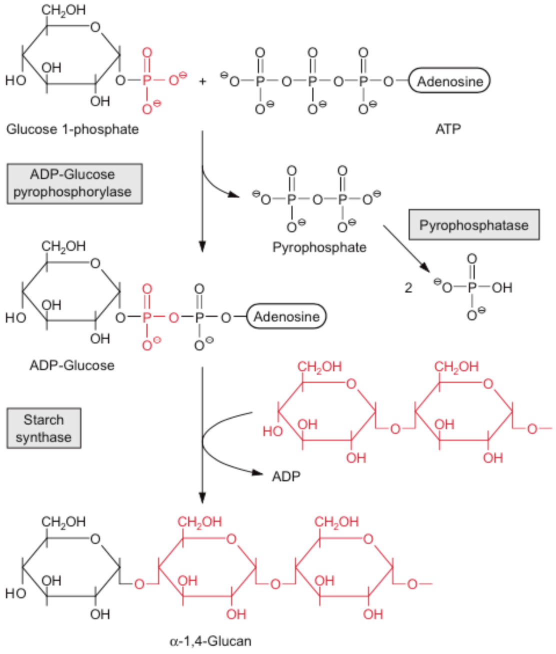


Figure 9.7 Biosynthesis of starch. Glucose 1-phosphate reacts with ATP to synthesize ADP-glucose. The pyrophosphate is hydrolyzed by pyrophosphatase and in this way the formation of ADP-glucose becomes irreversible. The activated ADP-glucose is transferred by starch synthase to a terminal glucose residue of a glucan chain.

Step 3. [polymerise ADP-glucose]

$(\text{glucose})_n$ + ADP-glucose ---> $(\text{glucose})_{n+1}$ + ADP

Enzyme: *Starch synthase, SS*

- requires a primer, continues to add **a1,4** linkage

Note: there are granule-bound vs soluble forms of SS - important for structure and ratio of amylose : amylopectin

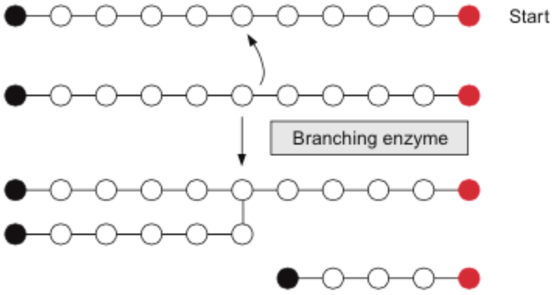
- *waxy maize mutant has no amylose: specialty uses.*

Step 4. [create branches in polymer

- formation of **a** (1,6) linkages]

Enzyme*: SBE, starch branching enzyme [amylo(***a** *1,4->1,6) transglucosylase]*

Note: important for starch structure and seed filling - Mendel's wrinkly pea mutant is deficient in SBE!



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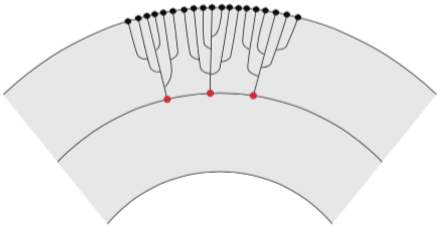

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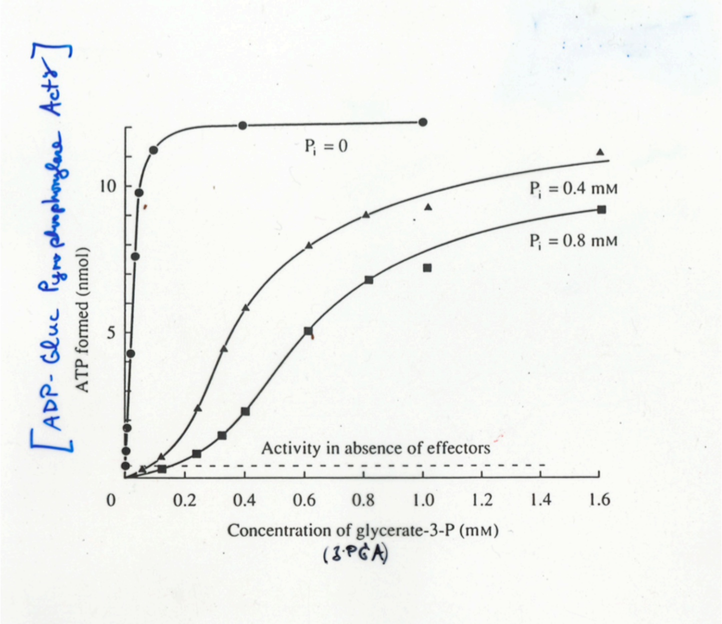
4. Starch synthesis in leaves is tightly regulated

Why?: - starch synthesis needs to be in step with Calvin cycle (plastid) and sucrose synthesis (cytosol)



Note: Ratio of *P_i (inorganic phosphate)* : 3-P-glycerate is key

Major regulator: *ADP-glucose pyrophosphorylase*
- allosteric regulation (see extra figure)
- P_i inhibits (= negative effector)
- 3-P-glycerate (3-PGA) stimulates (= positive effector)

- linkage to sucrose synthesis via the Triose-P Phosphate translocator (TPT) and P_i
  --> reduced sucrose synthesis --> low [P_i] in plastid--> releases *ADP-glucose pyrophosphorylase* from inhibition

- linked to Calvin cycle by 3-PGA
  --> low photosynthesis leads to reduced [3-PGA] --> this inhibits *ADP-glucose pyrophosphorylase* and starch synthesis

- *note the crucial role played by the* ***Triose phosphate - phosphate translocator (TPT)*** *in linking chloroplast and cytosolic carbohydrate metabolism*

5. Starch Degradation (at night, export / metabolism)

- liberated triose-P is used for sucrose -> export to other organs
- triggered by low triose-P, high [Pi], low ATP/ADP ratio

(glucose)$_n$ polymer is converted to glucose monomers

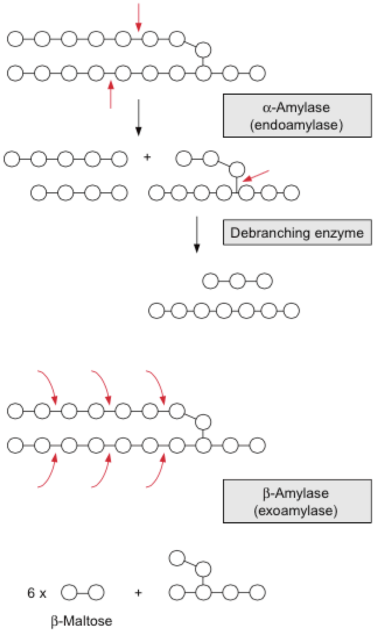
a-amylase**: =* **a(1,4)* , an endoamylase
- a hydrolase, uses H2O to cleave glycolytic linkages
- cleaves randomly within starch polymer (endo-amylase)
- usually required

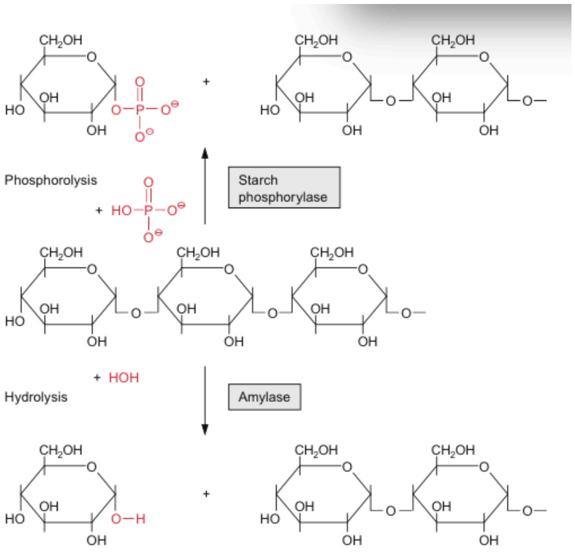
ß-amylase: an exoamylase that releases **maltose** units (**a**1,4 linked glucose dissaccharide)

starch phosphorylase:
- sequentially removes glucose from non-reducing end, releasing glucose-1-P
- glucose(n) --> glucose(n-1) + glucose-1-P

debranching enzyme = ("*D-enzyme*") = a *<u>transglycosylase</u>*:

glycosidase ("R- enzyme") to remove **a**(1, 6) linkages





Further breakdown and export from plastids

glucose-1-P --> glu-6-P --->fru-6-P--->fru-1,6-bisP ---> triose-P

- pretty much the reverse of glucose-1-P synthesis (except phosphofructokinase step)
- export to cytoplasm as trioses (via the **PT**)
- (export also as glucose-6-P)

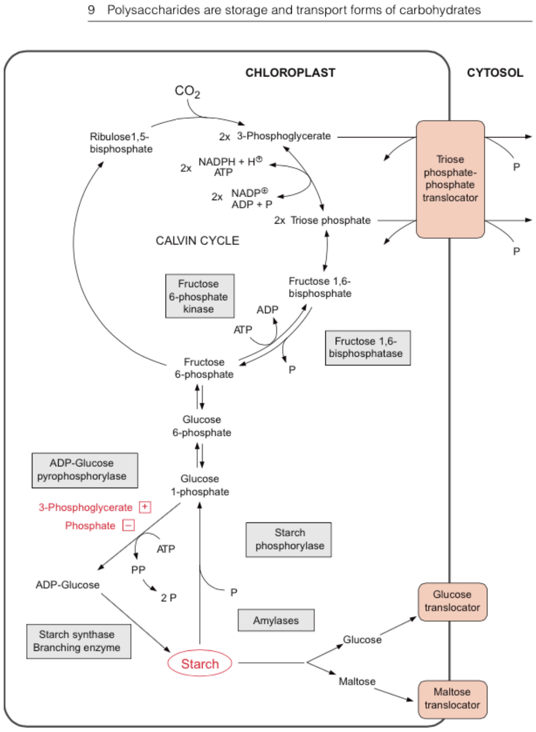
6. Reserve starch (long-term, in starchy seeds and tubers)

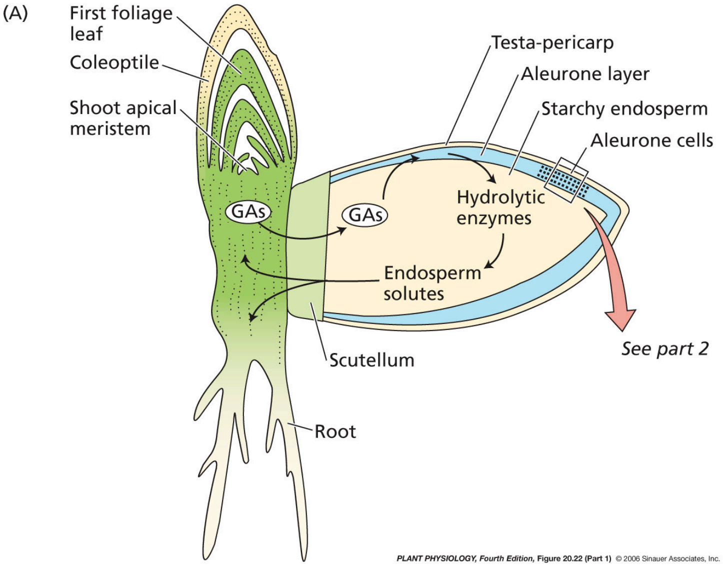
Synthesis is as before except:

- may begin w. glucose-1-P (from translocated sucrose)
- forms very large granules (starch grains)
- same enzymes (or isozymes) as above but less tightly regulated (*why*?)

Breakdown of reserve starch:

- mixture of enzymes, same as chloroplasts. Cereals contain ***maltase***, the enzyme which splits maltose into two glucose





7. Why seed starch degradation is important - beer brewing

Molecular controls for seed starch degradation:

- plant need tight control, starting with <u>gibberellins</u> from embryo
- mechanisms: gene expression: *a*-amylase, protease
  - proteolytic activation of *ß*-amylase)

Steps in making malt:

- germinate barley 5-9 days (seeds make enzymes)
- dry in kiln to caramelize and stop germination (80% maltose)
- 'mash' at 70 C (gelatinize starch for enzyme access) -> 'wort'
- boil wort with hops, sterilize, add yeast.

Why is (good) beer made from barley?