

BCMB 8020
April 11,2006

Starch

Most significant **carbon reserve** in plants (i.e. amount made & distribution)

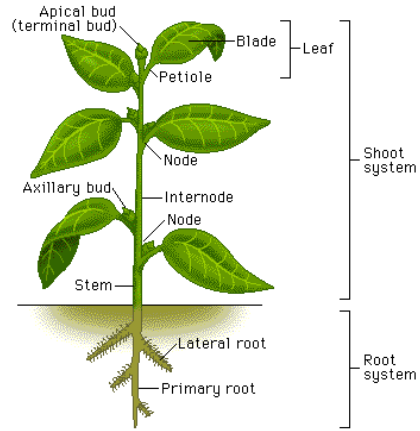
Major component of **crop plant yield** in the world

Major source of **calories in the human diet**



Important **raw material for industrial** processes (e.g. potato & maize)

Starch is synthesized in **plastids** in leaves from photosynthate during the day (**assimilation starch, a transitory reserve carbohydrate**) & mobilized at night largely to storage organs, seeds and fruits.



http://www.phschool.com/science/biology_place/biocoach/images/plants/plant.gif

Plant Cell

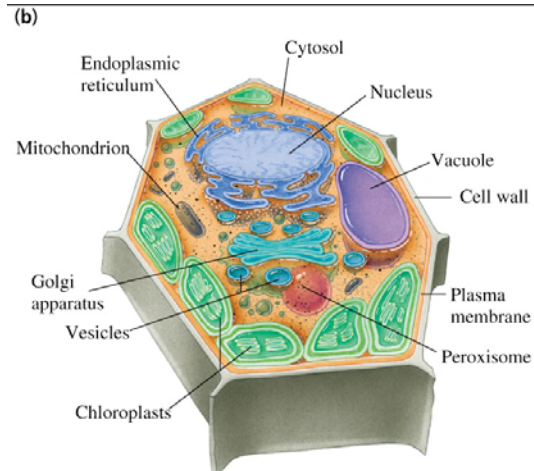


Fig 1.15(b), Principles of Biochemistry, Fourth Edition, Horton, H.R., Moran, L.A., Scrimgeour, K.G., Perry, M.D., Rawn, J.D. (2006)

Starch in **storage organs** (seeds, fruits, tubers, storage roots) is **synthesized** in plastids (amyloplasts) from **sucrose** that is **imported** from the rest of the plant.

The Seed

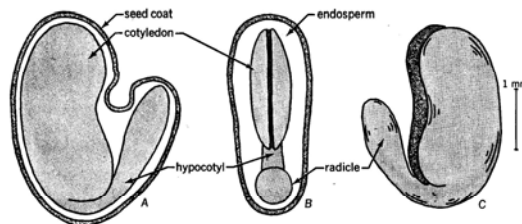
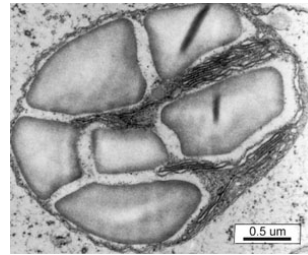
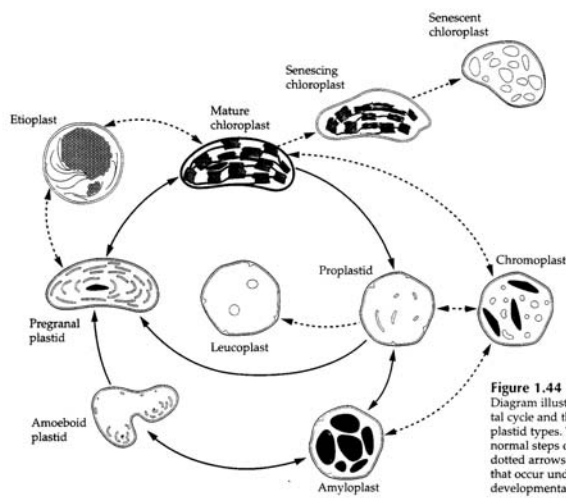


Figure 23.1 Diagrams of the seed of *Crotalaria intermedia* (Fabaceae) in longitudinal sections made parallel with (A) and perpendicular to (B) the plane of cotyledons, and of an embryo dissected from the seed (C). (Adapted from Miller.³¹)



Plastid from young strawberry that is intermediate between a chloroplast (presence of thylakoids) and an amyloplast (large starch grains).

<http://www.hcs.ohio-state.edu/hcs300/jpeg/THYLAK.JPG>

Figure 1.44 Diagram illustrating the plastid developmental cycle and the interconversion of various plastid types. The solid line arrows depict normal steps of chloroplast development; the dotted arrows show plastid interconversions that occur under certain environmental or developmental conditions.

Summary of the Calvin cycle (the reductive pentose phosphate cycle) of photosynthesis

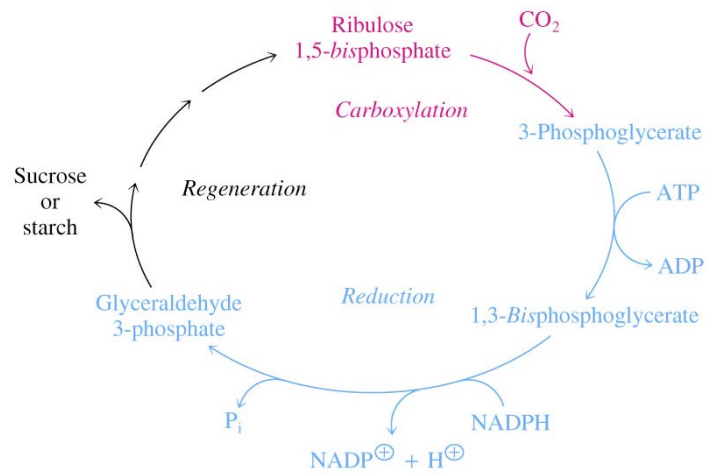
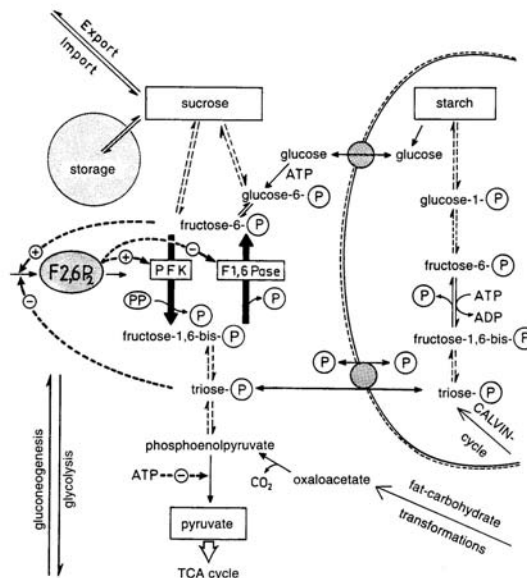
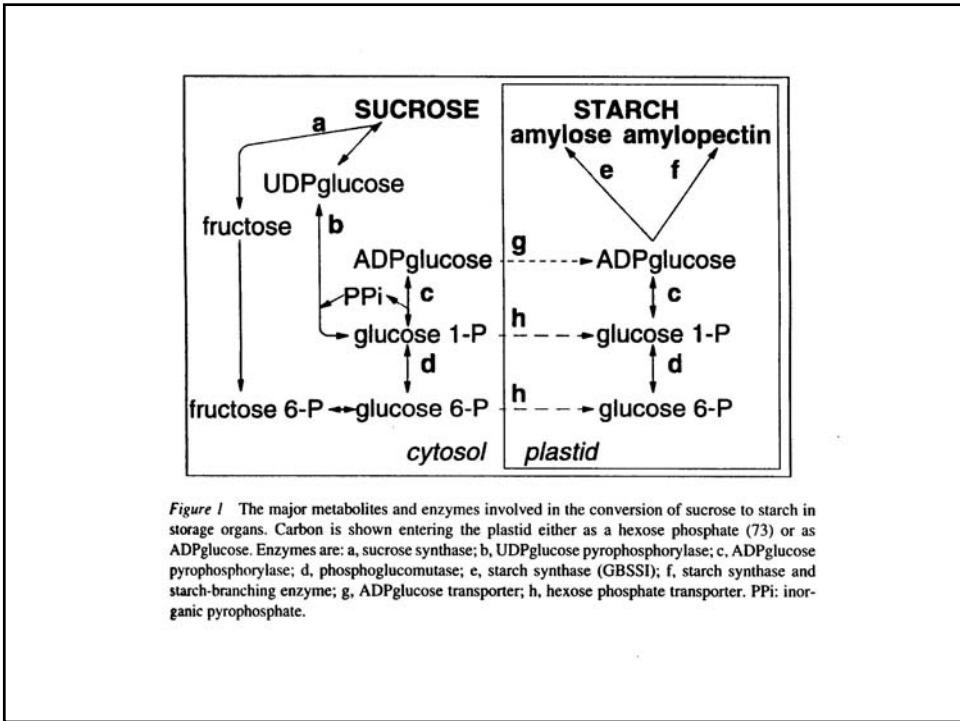
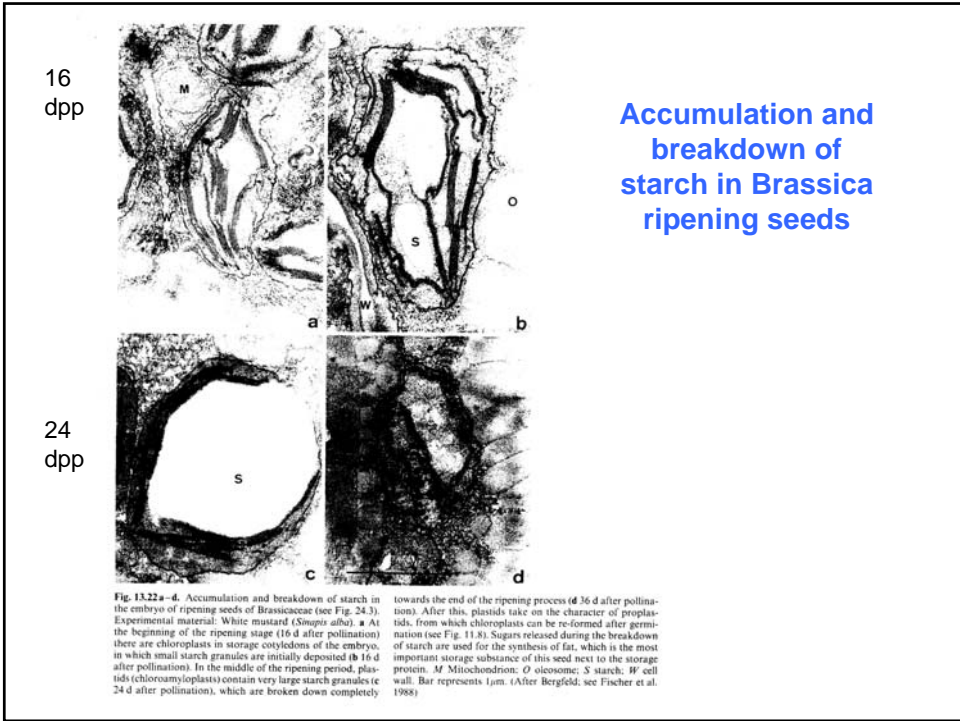


Fig. 15.16 from Horton et al., 2006, Prentice Hall

Photosynthesis, storage starch, sucrose





Starch exists as **starch granules** (<1 to >100 μM diameter)

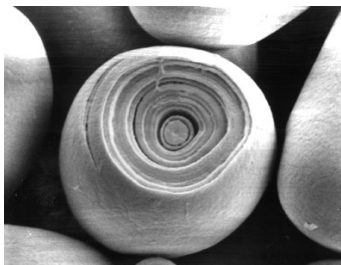
Glucose polymer arranged in 3D semicrystalline structure

Starch granules in leaves and storage organs differ in their macro structure.

Structural studies of starch are made on **storage organ starch** (i.e. not transient starch)

Once extracted, H-bonds form between linear regions of starch (amylose) to yield rigid gels (concentration, DP and temperature dependent).

starch granule



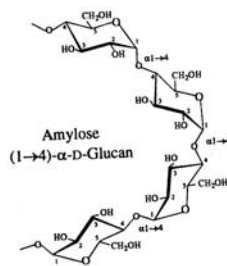
<http://mse.iastate.edu/images/microscopy/bol7.jpg>

Starch is made up of two types of polymers: **amylose** and **amylopectin**

Amylose is a linear homopolymer of α 1,4-linked glucose with a DP of \sim 1000

Amylose may have a low level of branching (\sim one branch per 1000 residues) with an α 1,6-linkage. Amylose makes up \sim 35% of starch (range of 11-36% depending on plant and organ). In solution amylose forms hydrogen bond with other amylose molecules to yield rigid gels.

Amylopectin is highly branched form of “amylose”. The linear α 1,4-linked glucose backbone is branched at every \sim 20 residues by an α 1,6-linkage which is extended by α 1,4-linked linkages. A single amylopectin molecule has one reducing end, contains branch clusters at every \sim 7-10 nm, is \sim 15 nm wide and \sim 200-400 nm long (i.e. has 20-40 clusters).



Amylopectin

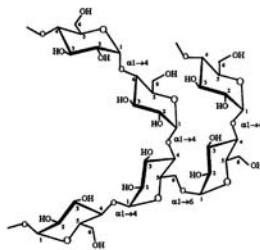
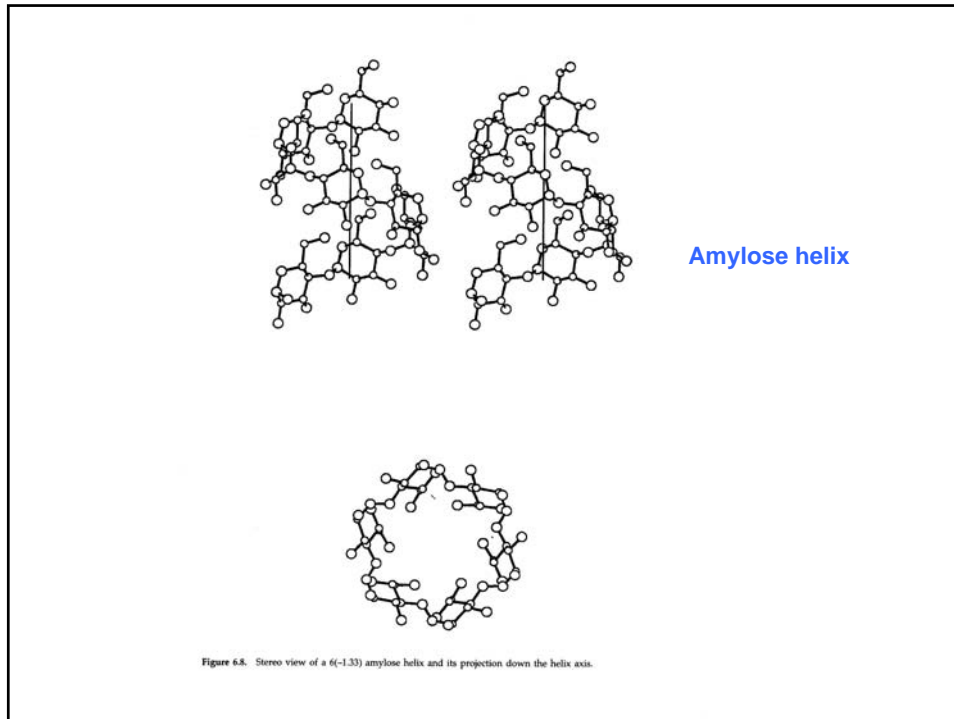
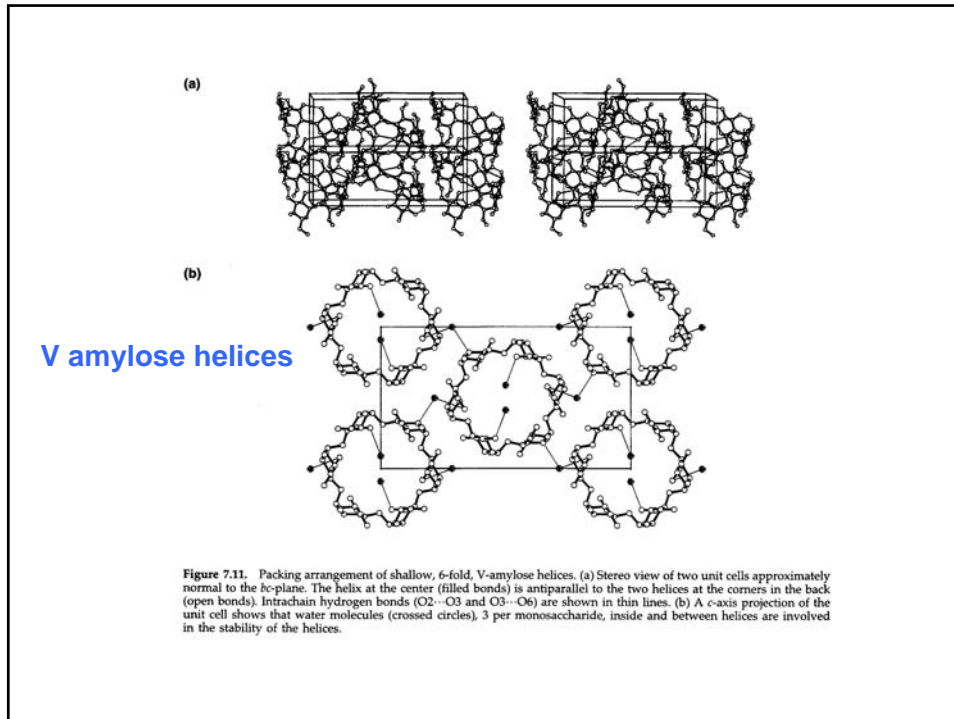


Figure 6.4. Schematic representation of a section of the branched polymer, amylopectin.



Amylose forms complexes with iodine, butanol & nitro compounds: designated **V amylose**

V amylose in the crystalline state is a left-handed helix of 13 Å diameter, pitch of 8 Å, and 6 residues per turn (i.e. $n = 6$; h (rise) = 1.33 Å). Thus, it is a 6(-1.33) helix.



Amylopectin has a regular clustering of double helices formed by twisting of short chains: 11-13 glucans in **Starch A** (cereals) ; 16-18 glucans in **Starch B** (tubers). Structures based on electron diffraction data from crystals of Starch A and B are left-handed helices of 6(-3.55) and 6(-3.47) respectively.

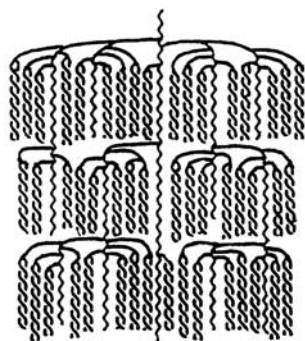


Figure 6.11. Schematic model of amylopectin molecule from A-type starch granule. (Modified from Perez *et al.* (1990).

Amylopectin Starch A

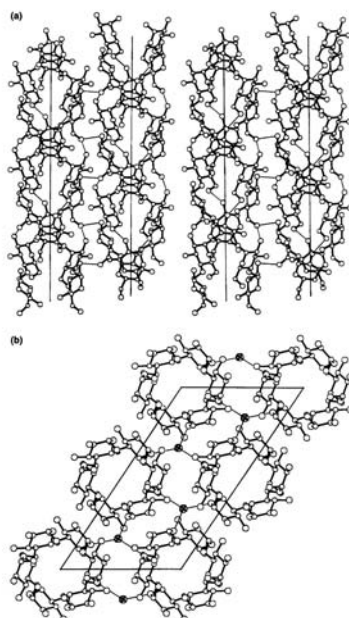
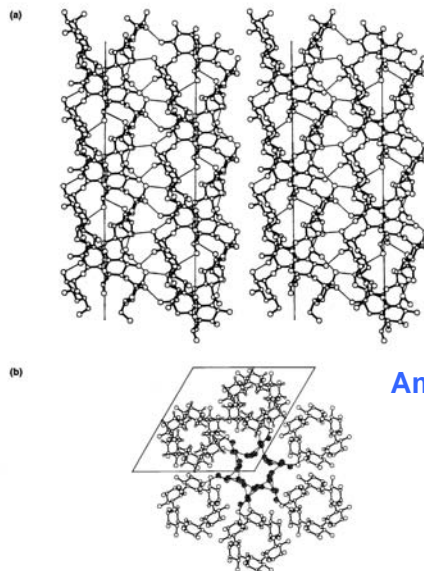
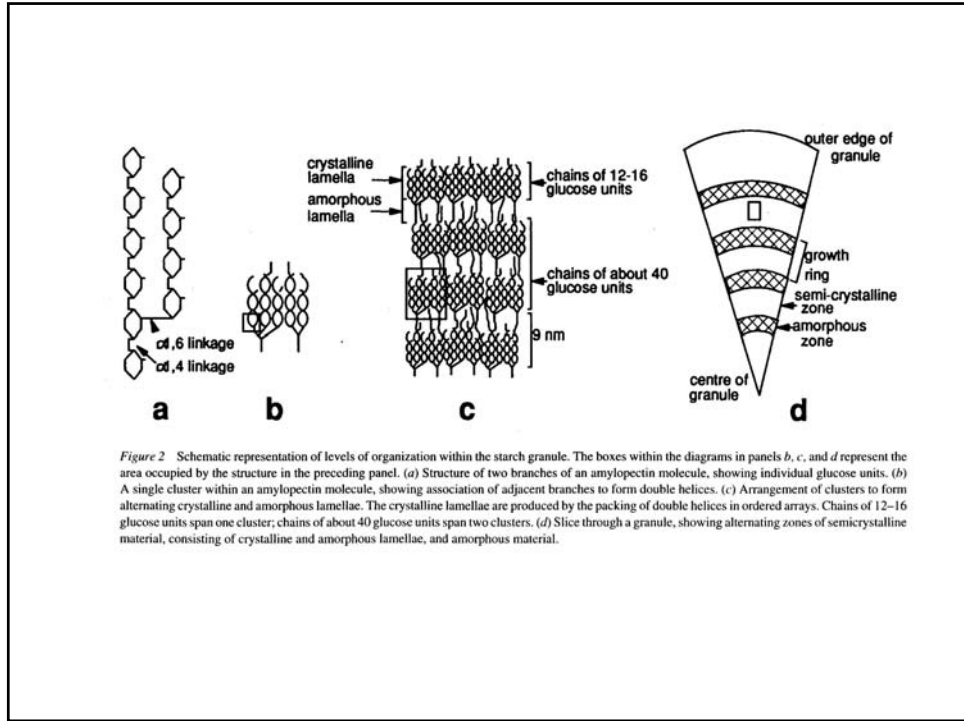


Figure 7.8. Parallel packing arrangement of 6-fold, A-amylose molecules. (a) Stereo side view of less than 2 turns of a pair of double helices 11.5 Å ($\approx \pi/2$) apart. The two strands in each helix are distinguished by open and filled bonds, and the helix axis is also drawn, for convenience. Interchain hydrogen bonds are marked in thin lines. Note that atom O6 mediates both intra- and inter-double helix hydrogen bonds. (b) A projection of the unit cell contents along the *c*-axis, with α down and β across the page. A water molecule (crossed circle) per trisaccharide bridges three surrounding helices.



Amylopectin Starch B

Figure 7.10. Parallel packing arrangement of 6-fold, B-amylose molecules. (a) Stereo side view of slightly less than 2 turns of a pair of double helices 10.7 Å apart along the long diagonal of the *ab*-plane. The two strands in each helix are distinguished by open and filled bonds, and the helix axis is also drawn, for convenience. Interchain hydrogen bonds are marked in thin lines. Note that atom O6 mediates both intra- and inter-double helix hydrogen bonds. (b) A view of the unit cell contents down the *c*-axis and remaining four helices which surround a cluster of 6 water molecules (crossed circles) per disaccharide in the middle.



Biosynthesis of starch in the chloroplast

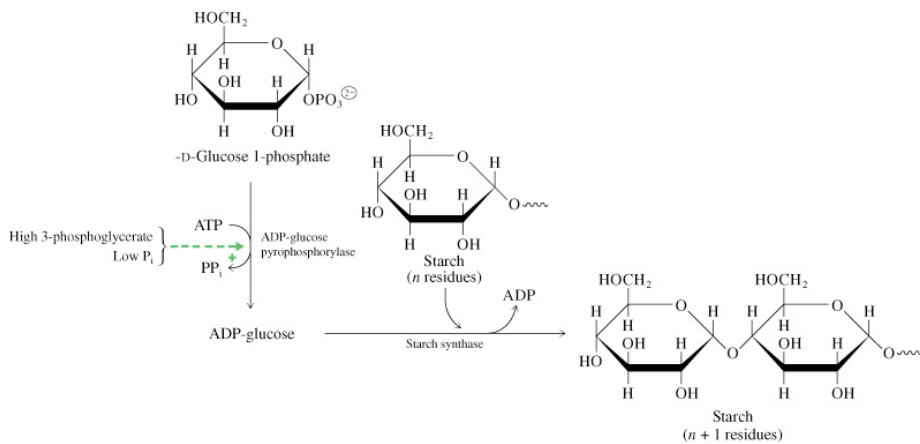


Fig. 15.16 from Horton et al., 2006, Prentice Hall

Three enzymes are directly required for **Starch Biosynthesis**. A fourth enzyme has been proposed to play a major role.

Biosynthesis occurs in the plastid.

Glucose-1-P + ATP → ADP-Glc + PPi

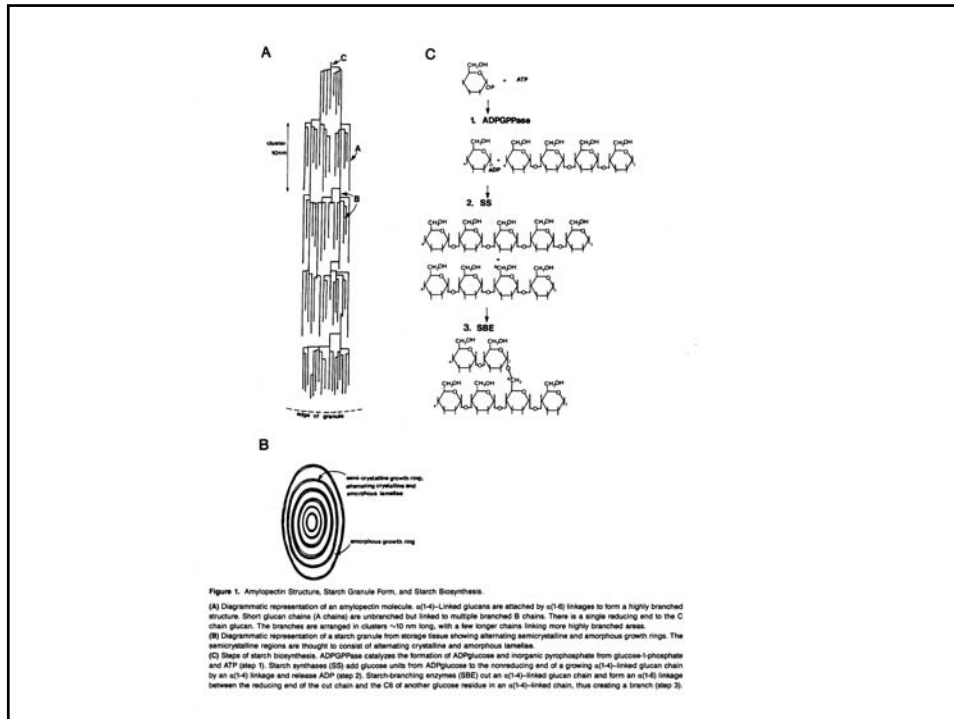
ADPglucose pyrophosphorylase (ADPGPPase)

ADP-Glc + amylose(n) → ADP + amylose(n+1)
starch synthase (SS)

Amylose + Amylose → amylopectin + amylose
starch branching enzyme (SBE)

Starch debranching enzyme (isoamylase; glycogen 6-glucohydrolase)

All species studied have multiple isoforms for each of the starch biosynthetic enzymes. Multiple genes also exist.



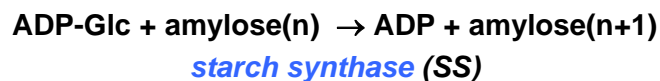
ADPglucose pyrophosphorylase (ADPGPPase)

ADP-Glc synthesized in the plastid, although some *ADPGPPase* may also act cytosolically in some tissues/plants (e.g. maize & barley endosperm). *ADPGPPase* is (+) allosterically regulated by 3-phosphoglycerate and (-) regulated by PO_4^- .

ADPGPPase is a heterotetramer of 2 large (54-60KD) and 2 small (51-55 kd) subunits. cDNA's, isolated for both subunits, show sequence homologies & suggest they arose from a common ancestor gene. Multiple genes encode the large subunit (differ in tissue specific expression), and multiple genes can encode the small subunit. Both subunits required for activity. Small subunit thought to be main catalytic activity, large subunit is regulatory (although it also has activity).

Large subunit mutants: maize *shrunk2* (sh2) (endosperm);
pea *rugosusb* (rb)

Small subunit mutants: *brittle2* (bt2) (endosperm)



Starch Synthase catalyzes α 1,4-linkage between non-reducing end of glucan chain & Glc from ADP-Glc. **SS** can use both amylose and amylopectin as acceptors. Priming event not known: some evidence for protein primer, some evidence for de novo synthesis.

Both granule bound **SS (GBSSI)** and **soluble SS** are found in amyloplasts. **GBSSI** makes amylose *in planta* and has low activity *in vitro*. **GBSSII** (which may be granule bound or soluble) has high activity *in vivo*. Any plant species probably has several active soluble SS.

GBSSI mutant: *waxy (wx)* mutants in maize, rice sorghum & Amaranthus; potato *amylose free (amf)*, pea *LAM*: mutant make little of no amylose. Thus **GBSSI** probably important for amylose synthesis, and in synthesis of long amylose-like stretches of amylopectin.

SSII mutant: pea *RUG5*: mutant has few chains of 15-45 residues but rather has many very short chains (DP < 15) and many very long chains. Concluded that **SSII** elongates very short chains to create the chains that form the basis of the clusters in amylopectin

Soluble SS mutant: Chlamydomonas *STA3*: mutant has more short chains (DP 2-7) and fewer long chains

SSIII also present in some plants/tissues

SS isoforms have conserved C-terminal region of ~60kD similar to glycogen synthase from bacteria

Amylose + Amylose → amylopectin + amylose
starch branching enzyme (SBE)

SBE hydrolyzes α 1,4-linkage in glucan chain in stable double helical conformation & catalyzes formation of α 1,6-linkage between reducing end of “cut” chain and glc in another chain.

At least 2 **SBE** gene families have been identified in maize, rice and pea. They share sequence similarity with bacterial glycogen-branching enzymes. **Family A** has lower affinity for amylose than Family B, & uses shorter glucan chains during branch formation → shorter branch lengths for **Family A-synthesized** amylopectin. Expression of **Family A SBE** in *E. coli* mutant w/o glycogen-branching enzyme gives glycogen-like polymer with more short chains (DP 6-9) and less long chains (DP >14) than expression of Family B **SBE** in *E. coli*. Function *in planta* controversial.

Structure of **SBE** have been modeled based on structure of α -amylase which has a central ($\alpha\beta$)8 barrel structure involved in hydrolysis. ** Since the loop between β -strand 8 and α -helix 8 is similar in all Family A isoforms & distinct from Family B isoforms in length & sequence, it is thought to be involved in determining branch length.

SBE Family A mutants: *pea rugosus (r)*; *maize amylose extender (ae)*

A fourth enzyme that may influence starch formation is:

Starch debranching enzyme (isoamylase; glycogen 6-glucanohydrolase)

Starch debranching enzyme (DBE) mutants: maize & rice sugary (*su1*) have reduced starch levels but contain a highly branched, soluble $\alpha(1-4)/\alpha(1-6)$ -linked glucan polymer referred to as phytoglycogen (structure reminiscent of glycogen).

DBE Chlamydomonas mutant (STA7): produce phytoglycogen-like starch

Current hypothesis is that the final structure of amylopectin is determined by a balance between the activities of debranching and branching enzymes (see Ball et al. review, 1996).

Complication: multiple DBEs exist in plants. Some prefer pullulan (pullanase- or limit-dextrinase-like DBEs); some prefer amylopectin (isoamylase-like DBEs)

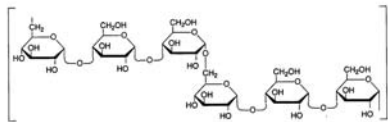


FIG. 1 Structure of pullulan.

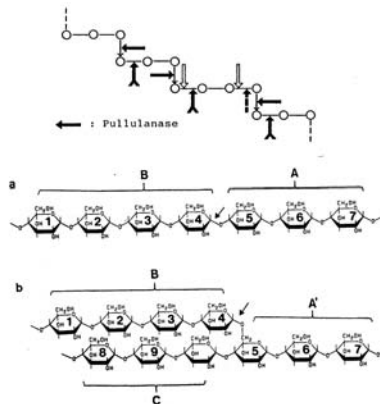
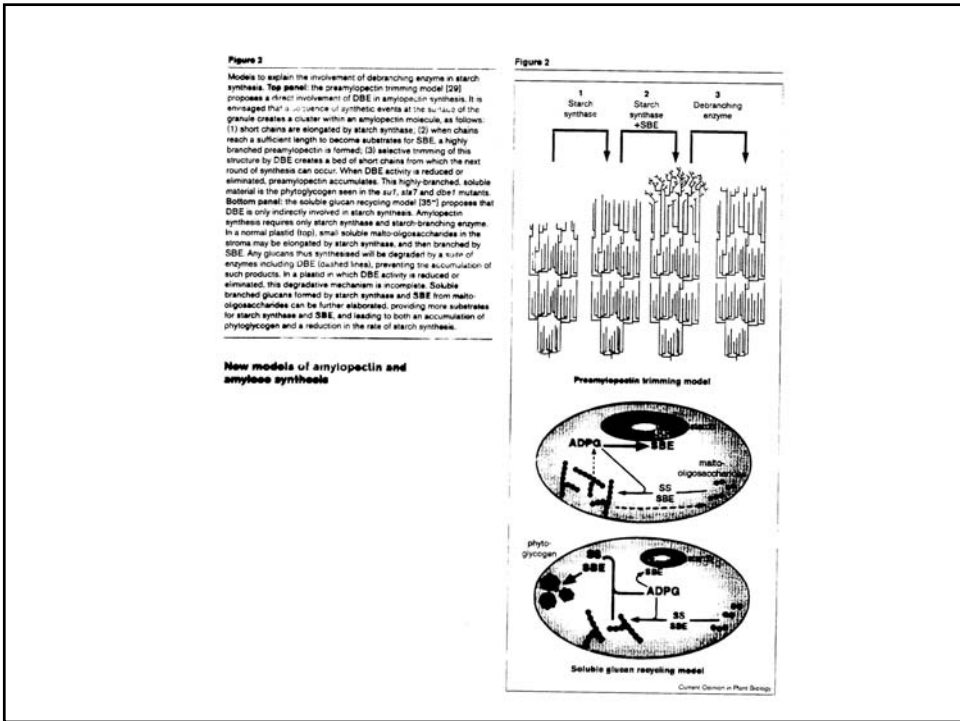
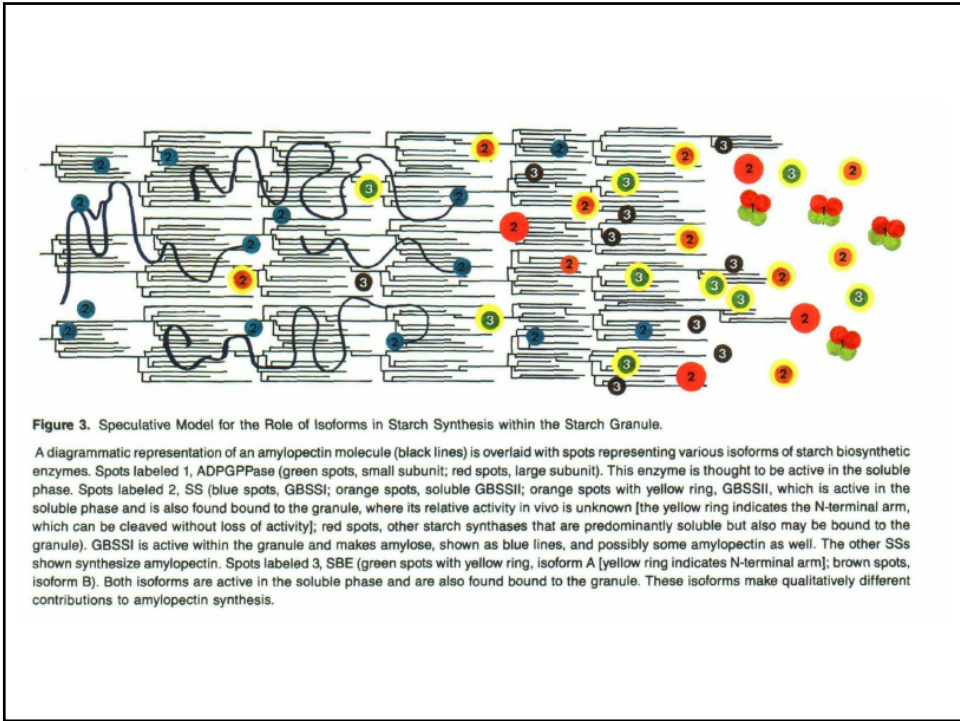


Fig. 2. Substrates for α -amylases, debranching and branching enzymes. (a) Substrate for enzymes acting on α -1,4-glycosidic bonds. (b) Substrate for enzymes acting on α -1,6-glycosidic bonds. (A, A') α -1,4-Glucan chain on reducing side of bond to be hydrolyzed (bond marked 1). (B) α -1,4-Glucan chain on nonreducing side of bond to be hydrolyzed. (C) In a branched substrate, section of α -1,4 glucan main chain on nonreducing side of glucose residue 5.



Starch Catabolic enzymes in plants

Endoglycanases

α -amylase: hydrolyzes $\alpha(1,4)$ glucan linkages within the glucan chain

Exoglycanases

β -amylase (seeds, cotyledons, leaves, tubers): cleaves off maltose units (Glc- α -1,4-Glc) from the non-reducing end of the glucan chain

starch phosphorylase (located in chloroplast): phosphoryolytic cleavage of glucan chains from the non-reducing end

$\alpha(1-6)$ -glucosidase: cleaves $\alpha(1-6)$ -branch point in amylopectin (or glycogen)

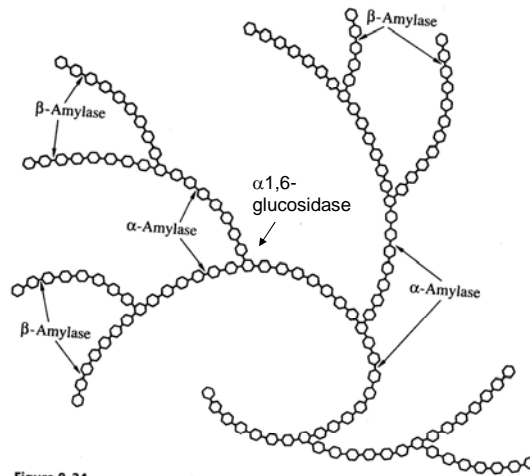
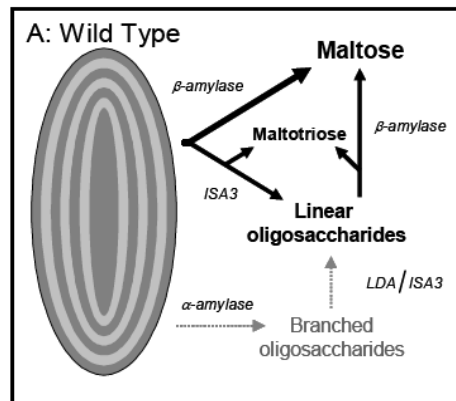


Figure 8-24
 Action of α -amylase and β -amylase on amylopectin. α -Amylase catalyzes random hydrolysis of internal α -(1 \rightarrow 4) glycosidic bonds; β -amylase acts on the nonreducing ends. Each hexagon represents a glucose residue; the single reducing end of the branched polymer is red. (An actual amylopectin molecule contains many more glucose residues than shown here.)

Starch breakdown in Arabidopsis leaves

In Arabidopsis exoamylases (β -amylases) release maltosyl residues from the surface of the granule to expose a β -limit dextrin structure with short external chains or "stubs" (with DP3 or larger). The stubs are removed by the action of ISA3 (isoamylase 3) and any linear chains longer than DP3 are degraded further by β -amylases to yield maltose and maltotriose.

from Delatte et al., 2006 JBC



Starch Catabolic enzymes in animals

Endoglycanases

Salivary α -amylase

Pancreatic α -amylase → maltose, maltotriose (DP3), limit dextrin

Exoglycanases

Debranching enzyme: α (1-6)-glucosidase

Maltase (small intestine): hydrolyzes maltose to glucose