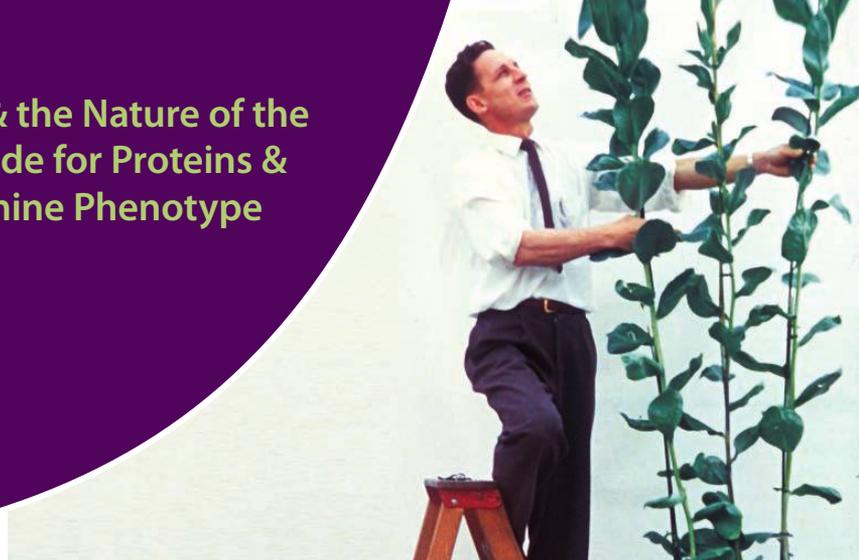


Mendel's Peas & the Nature of the Gene: Genes Code for Proteins & Proteins Determine Phenotype

● SUSAN OFFNER



ABSTRACT

We are beginning to understand the biochemical nature of the genes that Gregor Mendel studied in his classic experiments with garden peas. This paper shows where Mendel's genes are located on the pea chromosome map, discusses the mutations involved in some of these genes, and shows how they can be used to teach classical genetics and the nature of the gene.

Key Words: Gregor Mendel; garden peas; meiosis; genes code for proteins; enzymes determine phenotype; biochemical basis of Mendel's genes.

It has been nearly 150 years since Gregor Mendel published his work on the genetics of garden peas (Mendel, 1866). We can now look at this seminal work, available in English translation (Mendel, 1965), with 21st-century eyes and use it to teach about the nature of the gene, showing how genes code for proteins and how proteins determine phenotype.

Mendel studied seven genes in strains of peas he gathered from farmers in what is now Brno in the Czech Republic. These genes are shown in the linkage map of peas, Figure 1 (O'Brien, 1993). Because Mendel used seven genes and peas have seven pairs of chromosomes, it is tempting to think that each gene is on a different chromosome, especially given that Mendel reported that the genes were inherited independently of each other. But this is not true. The seven genes are on three of the chromosomes, numbers 1, 4, and 5. Mendel actually did a trihybrid cross in which two of the three genes were on the same chromosome. During meiosis, genes on the same chromosome are sorted together unless there is a crossover between them. Recombination is the result of crossing over. Genes that are more than 50 cM apart on a chromosome appear to be unlinked because crossing over occurs between them more than 50% of the time (Figure 2). This is why Mendel reported independent assortment among all the genes he studied even though two of them were widely spaced on the same chromosome. It is useful to teach meiosis at the beginning of the genetics unit so that students can see that Mendel's principles follow from the movement of chromosomes during meiosis.

A widespread student misconception is that dominant genes are more common than recessive genes.

The biochemical basis for three of Mendel's genes (yellow/green pea, tall/short plant, and round/wrinkled pea) has been identified. Each codes for a protein that functions as an enzyme; the reactions they catalyze determine the phenotype of the peas. We know something about the function of a fourth gene (purple/white flowers), although it is less well characterized. Enzymes speed up reactions about a billion times, thereby determining the reactions that occur in a cell (Dressler & Potter, 1991: p. 63). A billion seconds is about 33 years. Enzymes catalyze about 1000 reactions per second, and an uncatalyzed reaction would take about a million seconds, or 12 days. The cell would be dead before the reaction could happen.

○ The Gene for Yellow Peas Codes for an Enzyme That Breaks Down Chlorophyll

Yellow peas (I) are dominant to green peas (i). The I gene is on chromosome 1. In all pea plants, immature peas are green. As part of ripening, chlorophyll in the immature peas is broken down in a pathway requiring four enzymes that are widely conserved in plants and several enzymes specific to different plant groups (Hörtensteiner, 2006). When the chlorophyll in the pea is broken down, the pea turns yellow. The third step in the chlorophyll degradation pathway is shown in Figure 3. This reaction is catalyzed by an enzyme called *Pheide a oxygenase* (PAO) (Hörtensteiner, 2006). Mendel's I gene, on chromosome 1, codes for this enzyme (Armstead et al., 2007). When this gene is mutated, no functional PAO is produced, the chlorophyll in the peas is not broken down, and the peas remain green. Botanists call this the "stay green" phenomenon. The PAO enzyme is conserved across a wide range of plants, both monocots and dicots, which implies that the gene coding for it was present early in plant evolution.

Typically, a heterozygote produces only half as much enzyme as a homozygote. Therefore, a heterozygous (Ii) plant will have half as much PAO as a homozygous (II) plant. Because the lower enzyme level is enough to break down all the chlorophyll, yellow is dominant to green. A dominant gene produces the homozygous phenotype

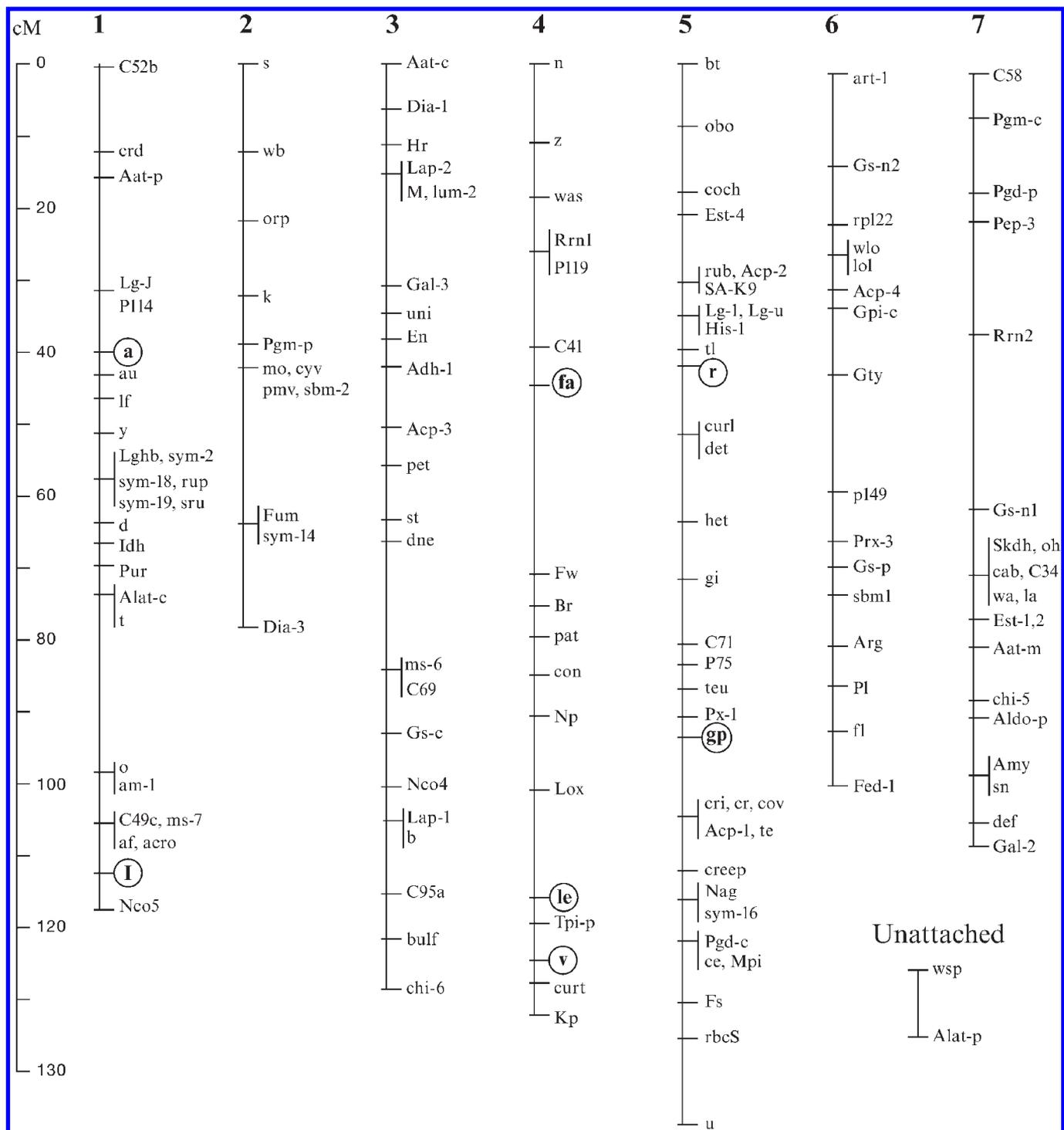


Figure 1. Linkage map of peas. This map of garden peas (*Pisum sativum*) shows the seven linkage groups that correspond to the seven different chromosomes of the pea genome. Genes are placed on a linkage map by how often they recombine during meiosis. The closer two genes are on a chromosome, the less frequently they will recombine, because there is less chance of a crossover occurring between them. The line on the left is chromosome distance measured in centiMorgans (cM) in honor of fruit-fly geneticist Thomas Hunt Morgan: 1 cM = 1% recombination. Mendel studied the following genes (the dominant trait is given first). Chromosome 1: a, - Purple/white flowers; I, Yellow/green peas. Chromosome 4: fa, Axial/terminal flowers; le, Tall/short plant; v, - Inflated/constricted pods. Chromosome 5: r, Round/wrinkled peas; gp, green/yellow pods. Linkage map is modified from O'Brien (1993) and reprinted with permission from Cold Spring Harbor Press.

in a heterozygote because half of the protein is enough to get the job done. In practice, we cannot tell whether a gene is dominant or recessive unless we do the cross. For each trait, dominance depends on the details of what the enzyme the gene codes for actually does.

A widespread student misconception is that dominant genes are more common than recessive genes. This trait illustrates why this is not true. Most of us eat green peas, not yellow peas. This is because green peas were selected by farmers to be the food crop and therefore green is

MENDEL'S DIHYBRID CROSSES

- Round Seed *r*#5 Yellow Pea *I*#1 x wrinkled seed green pea
- Violet-red Flowers *a*#1 Tall Plant *le*#4 x white flowers short plant

MENDEL'S TRIHYBRID CROSS

Round Seed *r*#5 Yellow Pea *I*#1 Gray Seed Coat *a*#1 x wrinkled seed green pea white seed coat

(110 cM) (40 cM)

The *I* and *a* genes are both on chromosome 1, but are 70 cM apart.

Figure 2. Mendel's dihybrid and trihybrid crosses. Mendel used these crosses to establish the principle of independent assortment. The gene and the chromosome it is on are shown beneath the dominant allele in each cross. These are the only dihybrid and trihybrid crosses that Mendel reported. In both dihybrid crosses, Mendel used genes on different chromosomes; as expected, these genes assorted independently. In the trihybrid cross, Mendel used three genes. One of these, *r*, is on chromosome 5 and assorts independently of the other traits. The other genes, *I* and *a*, are both on chromosome 1 but are located at 110 cM and 40 cM, respectively. Since they are 70 cM apart, there is 70% crossing-over between them, so they assort independently even though they are on the same chromosome. Note: "Gray Seed Coat" and "Violet-red Flowers" refer to the *a* locus, the same locus as Purple Flowers. This is an example of pleiotropy. The *A* allele results in purple (red-violet) flowers and gray seed coat, whereas the *a* allele results in white flowers and white seed coat.

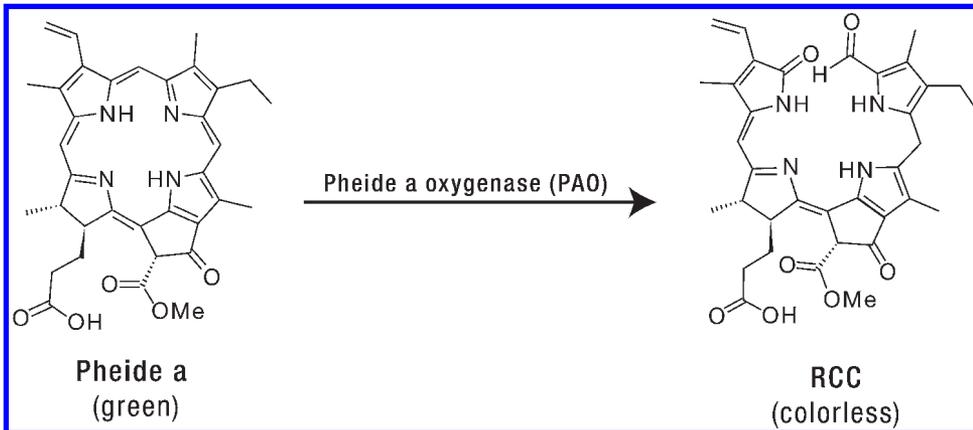


Figure 3. One step in the breakdown of chlorophyll. Mendel's *I* gene (yellow peas) codes for the enzyme PAO. Modified from Hörttensteiner (2006) and reprinted with permission from Annual Reviews, Inc.

more common than yellow, even though green is recessive. An example in humans is Huntington's disease, the devastating inherited neurological disorder that took the life of folk singer Woody Guthrie. Huntington's disease is caused by a dominant gene which, mercifully, is rare.

○ The Gene for Tall Peas Codes for an Enzyme Required to Make Gibberellin

Tall pea plants, over 6 feet tall (*Le*), are dominant to short plants, less than 1 foot tall (*le*). The *le* gene is located on chromosome 4.

To illustrate this, I show my students a picture of five genetically short cabbage plants. Gibberellin has been added to the three plants on the right (Figure 4). Students can see how these plants, all genetically identical, grow taller when gibberellin is added. Gibberellin is a plant hormone that promotes stem elongation. Gibberellin is produced in a series of reactions, each requiring an enzyme coded for by a gene. Short plants have a mutation in the gene that codes for GA 3-beta-hydroxylase, the enzyme for the last step of the pathway (Figure 5; Lester et al., 1997). Short is recessive: heterozygous plants, despite making only half as much of this enzyme, produce enough gibberellin that they are over 6 feet tall.

At the molecular level, the difference between *Le* (tall plant) and *le* (short plant) is a single amino acid substitution in the enzyme GA 3-beta-hydroxylase, a protein 374 amino acids long. In this protein, the 229th amino acid in the active protein (*Le*) is alanine; in the inactive protein (*le*) it is threonine. The order of amino acids in a protein determines how the protein will fold (its tertiary structure); this single amino acid substitution causes enough of a change in the tertiary structure of the enzyme to render it inactive. This is another example of how changing one



Figure 4. The five cabbage plants in this photograph are genetically identical. They are dwarf, like Mendel's short peas, and unable to produce active gibberellin. Gibberellin has been added to the three plants on the right, causing them to be tall. The man in this picture is Dr. Sylvan Wittwer. The picture was taken in the early 1950s, when he was a Professor of Horticulture at Michigan State University studying the properties of gibberellins as growth activators. Reprinted with permission from Sylvan Wittwer/Visuals Unlimited, Hollis, NH 03049.

amino acid in a protein, presumably the result of changing one base pair in the DNA coding for the protein, can inactivate the protein, causing a significant phenotypic change. In humans, a single-base-pair change in the beta-hemoglobin gene changes one amino acid in the beta-hemoglobin protein, resulting in sickle cell anemia.

○ The Gene for Round Peas Codes for an Enzyme Involved in the Branching of Starch

Round pea (R) is dominant to wrinkled pea (r). The r gene is on chromosome 5. Amylose, a form of starch, consists of glucose molecules that are linked to each other in long, straight chains (Figure 6). Amylopectin is another form of starch similar to amylose, but branched. Glucose molecules in amylose are joined by 1,4 linkages, bonds between the number 1 carbon atom of one glucose and the number 4 carbon atom of the next glucose. The six carbon atoms in glucose are numbered 1 to 6, with the number 1 carbon containing the aldehyde group. The branches in amylopectin are 1,6 linkages, bonds between the number 1 carbon atom of one glucose and the number 6 carbon of the adjacent glucose. A specific enzyme SBEI (starch-branching enzyme isoform) is required to form these 1,6 linkages. The R gene codes for this enzyme (Bhattacharyya et al., 1990). A mutated R gene does not code for an active enzyme, so wrinkled peas do not produce amylopectin. Cells of wrinkled peas contain amylose and very high levels of sucrose, a disaccharide consisting of glucose plus fructose. Their overall starch concentration is lower than in round peas. During development, wrinkled peas have a higher solute concentration because of the extra sucrose and retain a lot of water. When the peas mature, they lose the extra water and dry out, causing the wrinkled phenotype. The r mutant is common among cultivated peas because the extra sucrose makes the wrinkled peas very sweet.

At the molecular level, the r gene has an 800-base-pair insertion that is not present in the R gene coding for the starch branching enzyme SBEI. This insertion results in an early stop codon, so the last 61 amino acids of the normal SBEI protein are not present in the enzyme in the wrinkled peas.

○ The Gene for Purple Flowers Codes for a Protein Involved in Regulating the Production of an Enzyme Required for Anthocyanin Synthesis

Purple flowers (A) are dominant to white flowers (a). The a gene is on chromosome 1. Purple color is due to the presence of anthocyanin, a purple molecule, in flower petals. Anthocyanin is produced in a series of reactions, each of which requires an enzyme. The first reaction in this series requires an enzyme called *chalcone synthase*. Mendel's A gene does not code for this enzyme. It codes for a protein required for the expression of the gene coding for this enzyme (Harker et al., 1990). If the A gene is mutated, this protein is not produced; as a result, the chalcone synthase enzyme is not produced, anthocyanin is not made, and the flowers are white. Proteins like this are called *transcription factors*. By binding

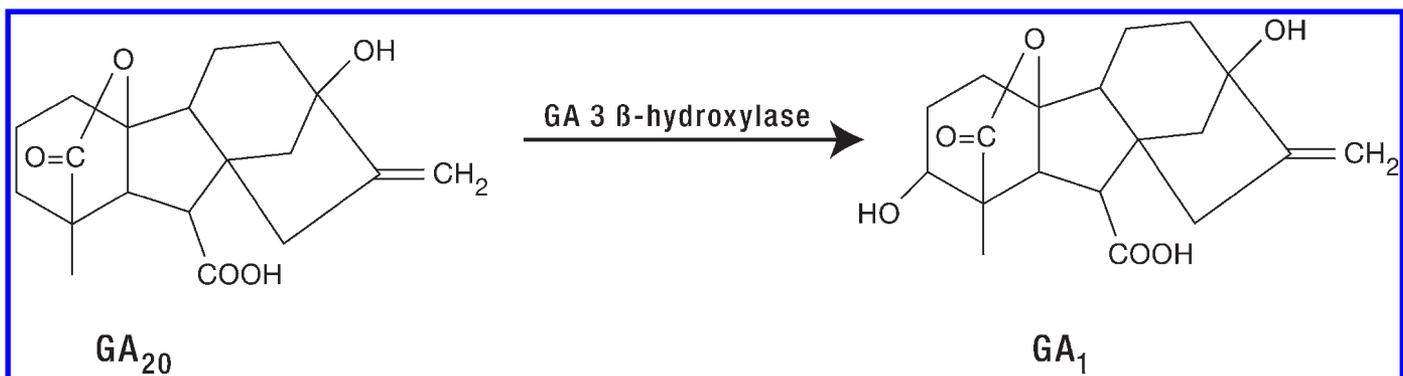


Figure 5. The last step in the reaction pathway for the production of gibberellin. GA_1 is the active form of gibberellin. GA = gibberellic acid. GA structures from Koning (1994).

to specific DNA sequences in regulatory regions of DNA, they turn genes on or off.

○ We Still Don't Know the Basis for the Inflated/Constricted Pod, Axial/Terminal Flower, & Green/Yellow Pod Traits

It is a measure of how difficult this work is that the first of Mendel's genes (round/wrinkled) was identified at the biochemical level in 1990, 125 years after the publication of his paper. Even today, the genes for inflated/constricted pod, axial/terminal flowers, and green/yellow pod where green is dominant to yellow remain uncharacterized, and the gene for purple/white flowers is incompletely understood.

○ Mendel's Life Is Also Informative

Mendel's life and work provide an opportunity to discuss the nature of science. Mendel joined the monastery in Brno as a young adult. Contrary to our image of Mendel as a lone genius, this monastery was a center of scientific learning; the experimental garden in which Mendel worked existed long before Mendel arrived. In his paper, Mendel named and paid tribute to "numerous careful observers... [who] have devoted a part of their lives with inexhaustible perseverance" to the

study of genetics (Mendel, 1965). He did, indeed, stand on the shoulders of giants.

It is nice to show students Mendel's actual numbers, which are widely available in textbooks. Mendel counted more than 30,000 peas and pea plants. He took a course in probability, which in the mid-1800s was a new science. He realized that one needed large numbers to get reliable ratios and was the first person to apply probability to genetics. Mendel was also a careful experimenter. After obtaining pure breeding strains of peas from farmers, he spent 2 years crossing them to make sure that they were indeed pure breeding. Mendel spent 8 years on his work; afterwards, he became the abbot of the monastery, a position he held for the rest of his life. His work was not recognized in his lifetime; it was rediscovered in 1900, 16 years after he died.

In conclusion, the new research on Mendel's peas provides us with an excellent chance to explain the nature of the gene to our students; in particular, how genes code for proteins and proteins determine phenotype, using as examples some of the best-known traits in biology.

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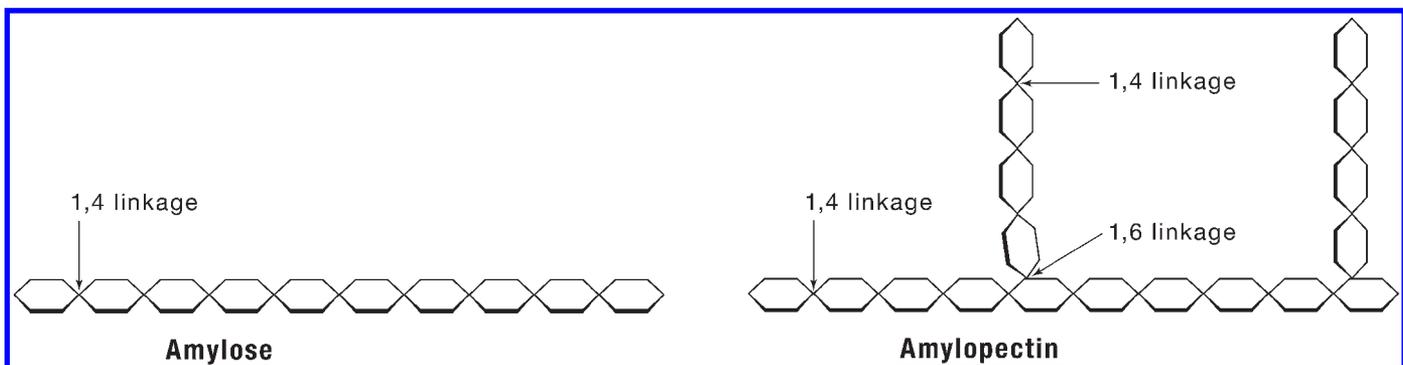


Figure 6. Amylose and amylopectin, showing 1,4 and 1,6 linkages. The SBEI gene codes for the enzyme that makes the 1,6 linkages. A different enzyme makes the 1,4 linkages. Each hexagon represents one glucose molecule.

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