OCCURRENCE OF STARCH

I. INTRODUCTION

Starch is a plant reserve polysaccharide, an end product of carbon fixation by photosynthesis, in which D-glucose residues are linked predominantly by α-(1,4) glucosidic bonds. It is present in most green plants and in practically every type of tissue: leaves, fruit, pollen grains, roots, shoots, and stems. Starch has a negligible osmotic pressure and thus allows plants to store large reserves of D-glucose without disturbing the water relations in the cell. All fruits contain starch, but in many of them only traces can be detected, and in most of them the starch is restricted to the chlorophyllous layers. Bananas and plantains have a relatively high starch content, especially before the onset of the climacteric, when nearly 90% of the dry weight of the fruit is starch. Starch present in pollen grains provides the energy required during germination and tube growth.

II. SEEDS

Members of the Gramineae (grasses) produce dry, one-seeded fruits, called caryopsis, commonly referred to as kernels or grains. The caryopsis (Fig. 1) consists of a fruit coat or pericarp, which surrounds the seed and adheres tightly to the seed coat. The seed consists of an embryo (or germ) and an endosperm enclosed by a nucellar epidermis and a seed coat. The main site of starch synthesis and accumulation is the endosperm, whose cells are packed with starch granules that form within the amyloplasts. Some starch is deposited in the embryo and pericarp early in development but later disappears. The starchy endosperm provides carbon skeletons and energy to the germinating embryo. Starch normally accounts for 65%–75% of the dry weight of the caryopsis in the mature, dry state. The embryo and the pericarp contain little starch, and values for the endosperm alone exceed 80%. The contents and cell walls of the endosperm make up the flour after the drying and processing of the grains. The baking properties of the flour are determined not only by the starch but also by the cell proteins that constitute the gluten.

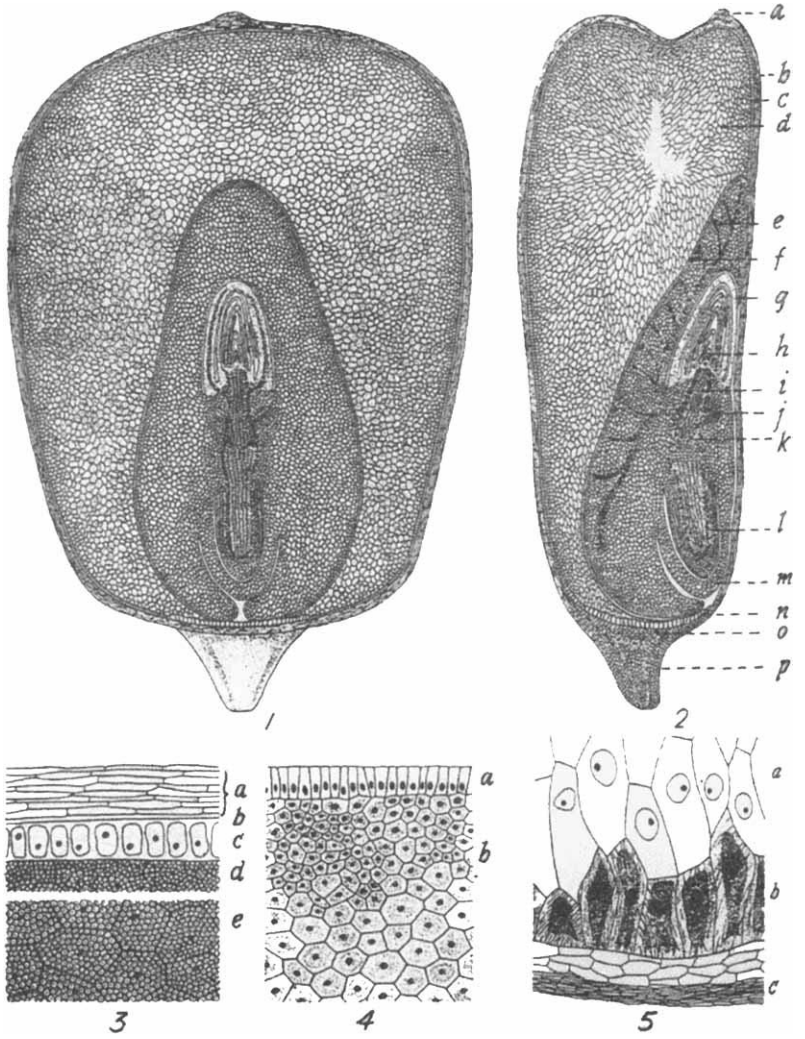

FIG. 1. The mature maize kernel. *1* and *2*, vertical sections in two planes of a mature kernel of dent corn, showing the arrangement of organs and tissues (magnification 7×); (*a*) silk (style) scar, (*b*) pericarp, (*c*) aleurone, (*d*) endosperm, (*e*) scutellum, (*f*) glandular layer of scutellum, (*g*) coleoptile, (*h*) plumule with stem and leaves, (*i*) first internode, (*j*) lateral seminal root, (*k*) scutellar node, (*l*) primary root, (*m*) coleorhiza, (*n*) lar node, (*o*) brown absission layer, (*p*) pedicel. *3.* Enlarged section through pericarp and endosperm (magnification 70×); (*a*) pericarp, (*b*) nucellar membrane, (*c*) aleurone, (*d*) marginal cell of endosperm, (*e*) inner endosperm cells. *4.* Enlarged section of scutellum (magnification 70×); (*a*) glandular layer, (*b*) inner cells. *5.* Vertical section of the basal region of endosperm (magnification 350×); (*a*) ordinary endosperm cell, (*b*) thick-walled conducting cells, (*c*) abcission layer. Figure reprinted with permission from Kiesselbach (1949).

The seeds of legumes have a lower percentage of starch than grass seeds: around 30% of dry weight for garden peas and 50% for cow peas. The study of the variations in seed morphology in maize and in peas, starting with Mendel, resulted in major contributions to the understanding of plant genetics. Some of these variations are caused by mutations affecting enzymes involved in the synthesis of starch and are discussed in the chapters corresponding to each enzyme.

III. STORAGE ROOTS AND TUBERS

Starch content in potato (*Solanum tuberosum*) tuber, in cocoyam corm (*Xanthosoma sagittifolium* and *Colocasia esculenta*), and in the roots of yam (*Dioscorea esculenta*), cassava (*Manihot esculenta*), and sweet potato (*Ipomea batatas*) ranges between 65 and 90% of the total dry matter, a result of a period of starch deposition that varies between 8 and 30 weeks. The dividing cells in newly initiated potato tubers, which are derived from stolons, contain little starch; however, once tuberization progresses, starch accumulation also progresses. Early in the development of the potato tubers, starch is distributed rather uniformly throughout the parenchyma. Later, two gradients of starch deposition appear and, as a result, the cortical parenchyma is richer in starch than the central part of the tuber, and the more mature, basal end of the tuber contains more starch than the younger distal tissues. Yams and cassava also display specific patterns of starch accumulation that are related to the particular pattern of differentiation of the organ.

IV. STARCH IN THE GRAVITATIONAL RESPONSE OF ROOTS AND STEMS

Sedimentation of amyloplasts within the cell has been correlated with the capacity of the plant to perceive gravity. The buoyant mass of amyloplasts present in specialized cells in the center of the root cap and in the stem (depending on the plant species, in the endodermis, the bundle sheath, or in the parenchyma to the inside of the vascular bundle) would allow the amyloplasts to sediment inside the cell, where the cytosol would have a relatively low viscosity. This sedimentation would translate into a signal of an unknown nature, maybe through pressure onto a sensitive part of the cell or acting as a mechano transducer, etc. Whatever the nature of the signal, it eventually results in the asymmetry of the organ and its curvature. The isolation of starchless mutants of *Arabidopsis thaliana* and *Nicotiana sylvestris* has made

it possible to compare the gravitational responses of plants differing only in the amount of starch, as plastids are present in both wild-type and starchless mutants. Although it was initially believed that the responses were identical (Caspar and Pickard, 1989), apparently the starchless mutants in both species are less sensitive to gravity (Sack and Kiss, 1989).

V. LEAVES

In leaves, starch is deposited in granules in the chloroplasts during active carbon dioxide fixation by photosynthesis throughout the day and is degraded by respiration at night. Starch remobilization ensures the constant availability of photosynthates to the whole plant. Mutants of *A. thaliana* that are able to synthesize sucrose but unable to synthesize starch grow at the same rate as the wild type in a continuous light regime, but growth rate is drastically reduced if they are grown in a day–night regime (Caspar *et al.*, 1986). The biosynthesis and degradation of starch in the leaf are, therefore, more dynamic than the metabolism in reserve tissues. Chloroplast starch granules are smaller than those in reserve tissues and their shapes are not species specific and are likely to be determined simply by the space available at the site where they are formed.

VI. GREEN ALGAE

The presence of starch has been demonstrated in several species of green algae (*Chlorophyceae*). Starch content in four genera of green algae studied by Love *et al.* (1963) contained about 1% starch. The viscosity of algal starch solutions was lower than that of potato starch, indicating a lower degree of polymerization, but the percentage of amylose was not very different. Extraction of algal starch is complicated by the presence of a large amount of other polysaccharides, especially sulfated ones.

Algae lack differentiated organs and one would expect the role of starch and its structure to resemble those of leaf starch rather than those of reserve tissues. In this decade, a green algae, *Chlamydomonas reinhardtii,* has become a system of choice for the study of starch synthesis. Ball and his collaborators (1990) studied this algae under sets of conditions that favor accumulation of "storage" starch (N depletion, dark, carbon, and energy supplied as acetate) or "photosynthetic" starch (light, complete nutrient solution). The structure and site of accumulation within the cells vary according to the growth conditions.

VII. OTHER RESERVE POLYSACCHARIDES

Starch is not the only storage polysaccharide found in plants. A storage substance is one that can be broken down rapidly to provide energy and/or building blocks for new growth by respiration. Reserve polysaccharides are stored in plastids (as in the case of starch), in the cell vacuole, or outside the plasmalemma, in the cell-wall region. The presence in the plant of enzymes capable of degrading the substance is a good indicator of its role as reserve. This definition can be applied with ease to starch in higher plants or to glycogen in cyanobacteria, but for other polysaccharides found in some algae, the role is less clear (Percival and McDowell, 1985). For example, xylans—polymers of xylose present in Rhodophyta, the red algae, and in Chlorophyta, the green algae—may fulfill more than a single function in the same algae (i.e., as reserve and as part of the cell-wall structure). Cell-wall polysaccharides in some senescent tissues, such as ripening fruits, can be turned over and the monosaccharides produced can be incorporated into polysaccharides. An arabinogalactan mucilage present within the style canal of *Lilium* acts as a source of carbohydrate precursor for the growing pollen cell wall (Loewus and Labarca, 1973).

Laminarin, a linear glucan containing mainly β-D-(1→3) linked glucose, with some β-D-(1→6) branching points, is found in *Laminaria,* a brown seaweed.

Mannans, in which mannose units are linked predominantly in β-D-(1→4) bonds, are found in the red seaweed *Porphyra umbilicalis,* in the seed of the tagua palm (*Phytelephas macrocarpa*) in the form of massive thickening of the cell walls of the endosperm, and in the endosperm of members of the Umbelliferae and of the Compositae (e.g., lettuce seed).

Other reserve glucans have been described (Meier and Reid, 1982), but in higher plants only starch and fructan, a water-soluble polymer of D-fructose that is osmotically active, are widespread. Hendry (1987) estimated that fructans are present in about 12% of vascular plants, many of them from temperate climates. It has been proposed that fructans, which are located in the cell vacuole and are osmotically active, can decrease the freezing point of the cell sap, slow the rate of freeze-dehydration, and afford frost hardiness to the plants that store them. Long-term storage of fructan can occur in specialized organs (e.g., the tubers of the Jerusalem artichoke) (Jefford and Edelman, 1961), in the stems and developing inflorescences of temperate grasses and cereals during periods of reproductive development (Archbold, 1940), and in the seeds of some Gramineae during the early stages of grain development, before starch synthesis begins. Pollock and Chatterton (1988) discussed the possible advantages afforded to plants by fructan accumulation in leaves as compared to starch.

Floridean starch containing α-D(1→4), α-D-(1→6), and possibly some α-D-(1→3) bonds is the characteristic reserve polysaccharide in the Rhodophyceae (red algae) and is present as granules in the cytosol. The presence of α-D-(1→3) bonds, if confirmed, would clearly differentiate floridean starch from both starch and glycogen, but they could be an artifact. Floridean starch has been detected in many species of red algae (Meeuse *et al.*, 1960) but has been characterized in only a few cases. In its viscosity and molecular weight (MW) of approximately 10^8, it resembles amylopectin (Greenwood and Thomson, 1961), but in other respects, (e.g., average chain length) it resembles glycogen (although chain lengths can vary from about 10 to 18).

Glycogen, an α-1,4-glucan with α-1,6 branching points, is the storage polysaccharide for cyanobacteria (blue-green algae). Cyanobacteria are prokaryotes and, although they are photosynthetic, they have no plastids and their glycogen is present as small granules in the cytosol. In thin sections seen under the electron microscope, they appear as spheres of 25 to 30-nm-diameter or rods (31 by 65 nm in *Nostoc*) that stain densely with lead citrate and are often located between the thylakoids and are more prominent in nitrogen-limited photosynthesizing cells (Shively, 1988).

VIII. EXPERIMENTAL SYSTEMS IN THE STUDY OF STARCH METABOLISM

The model experimental systems mentioned more frequently in this book are the kernels of maize and rice, the potato tuber, the pea seed, the aerial parts (leaves and stem) of *Arabidopsis thaliana,* and the alga *Chlamydomonas reinhardtii.* Some of these systems (e.g., rice, potato) have been chosen by researchers for their economic importance, whereas other plants have been chosen because many mutant lines are available for study (e.g., pea) or because they are particularly amenable to genetic studies (*Arabidopsis*). It should not be expected, however, that these few species represent "perfect" models (if such a thing exists) of how starch synthesis operates in plants in general, and one should be cautious when extrapolating to other species the information obtained using one system. For example, potato and maize have been selected for centuries in the search of high starch production, and we could expect that breeding has introduced some peculiar characteristics leading to high starch accumulation that may not be typical of what the species was before domestication. However, *Arabidopsis* is a good system in the sense that it has not been subject to selective pressure, but the plant is very small, making biochemical studies difficult and limited mostly to the leaves.

It is worth noting that bread wheat (*Triticum aestivum*), one of the most important world crops, is far from an ideal experimental system. Wheat is a natural allopolyploid. It has 21 pairs of chromosomes, which represent three sets of chromosomes that come from three different wild relatives, possibly *T. monococcum, T. searsii,* and *T. tauschii.* The bread wheat as we know it is the result of a combination of naturally arising mutations, such as the gene *Ph* that allows the coexistence of the three related sets of chromosomes, and cultivation by humans for more than 10,000 years. Breeding has resulted in a very high harvest index; that is, a gradual increase in the proportion of above-ground assimilates going to the grains, the harvested sink organs. The molecular bases for this ever-increasing harvest index are probably related to increased starch synthesis selected by breeding. However, the hexaploidy of wheat makes genetic manipulation complicated, and biochemical study of the kernel enzymes is also difficult.

A. MAIZE

Maize (*Zea mays*) is a cross-pollinated plant that has evolved (with great help from humans) into thousands of varieties or races that are composed of a great deal of genetic variability; the wild relatives of maize are teosinte (*Zea mexicana*) and *Tripsacum.* The maize cultivated in commercial agriculture represents a very small fraction of this genetic variability and consist of a few hybrids obtained by the systematic crossing of a few inbred lines. Besides its commercial importance, another reason why maize is frequently used as a model system is that it bears male and female flowers on separate structures (Fig. 2). This characteristic facilitates controlled pollinations and genetic studies, and also the outcrossing responsible in part for the enormous genetic variability of the species. Maize produces a large ear with 500 or more individual kernels (the main site of starch deposition), each containing a prominent endosperm and a large embryo, facilitating biochemical studies. There is also a large amount of data available on the physiology of the whole plant and its ultrastructure, and maize is the most extensively characterized flowering plant from a genetic and cytogenetic point of view.

The development of the kernel following fertilization takes 40–50 days and is accompanied by a 1400-fold increase in the volume of the embryo sac; the growth of the embryo and accumulation of food reserves in the endosperm is completed by about day 40. A mature kernel has three parts: pericarp, endosperm, and embryo (Fig. 1). The pericarp, the tough, transparent, outer layer of the kernel, is derived from the ovary wall and is, therefore, genetically identical to the maternal parent; the endosperm and embryo represent the next generation.

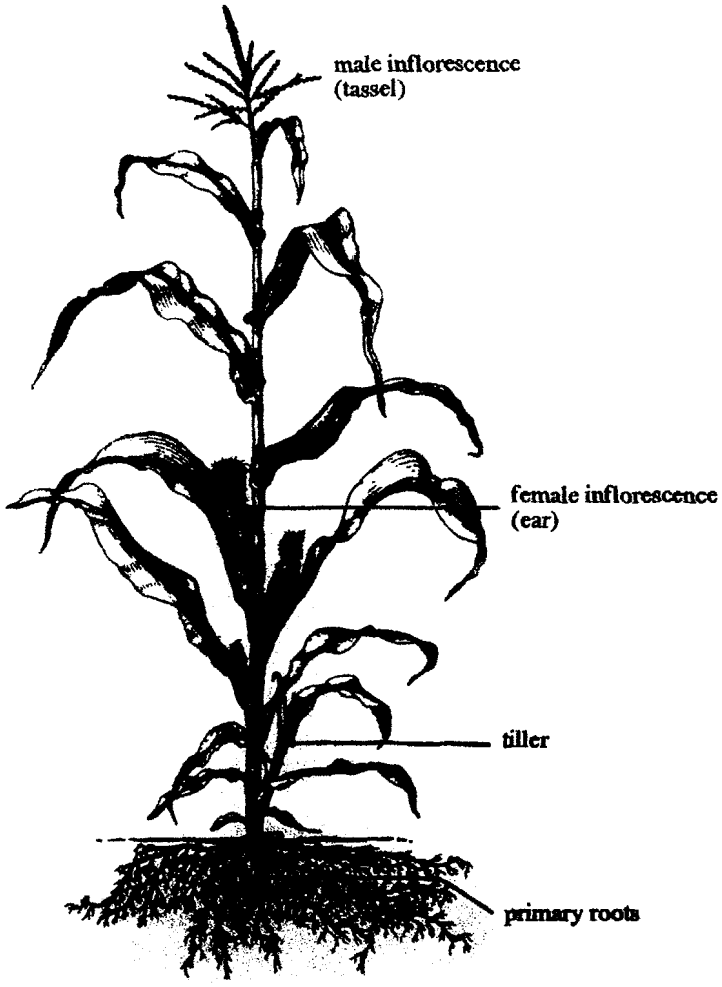

FIG. 2. The maize plant. (Classic drawing by W. C. Galinat.)

Besides the usual forms of genetic change present in other plants (i.e., gene mutation and recombination), transposable genetic elements, also called *jumping genes,* are an additional source of genetic variation in maize. These are genetic elements that can occasionally move (transpose) from one position in the chromosome to another position in the same chromosome or in a different chromosome. Transposable elements can mediate chromosomal rearrangements, and were first discovered in maize by M. Rhoades,

where they manifested themselves as unstable mutant alleles, i.e. alleles for which reverse mutation occurs at a very high rate. In the 1950s, Barbara McClintock found a genetic factor *Ds* (*Dissociation*) that causes a high tendency towards chromosome breakage at the location in which it appears. Controlling elements in maize can inactivate the gene in which they reside, cause chromosome breaks, and transpose to other locations within the genome. Complete elements can perform these functions unaided; other forms with partial deletions can only transpose with the aid of a complete element located elsewhere in the genome.

One locus related to starch synthesis, *waxy,* has been the object of intense study on the effects of the *Ds* element. The *Ds* element can move into a gene making it into an unstable mutant dependent on the other element, Ac. The *wx* locus is one example and was studied in detail by Oliver Nelson, who paired many different unstable wx alleles in the absence of the *Ac* mutation. He then screened the heterozygotes for the rare wild-type recombinants by staining the pollen with iodine reagent (*Wx* pollen, containing normal starch, stains black; *wx* pollen, lacking amylose, stains red) and, by counting the frequency of the wild-type recombinants, he obtained a fine structure map of the waxy gene. Nelson also showed that the different mutable waxy mutant alleles were caused by the insertion of the *Ds* element in different positions within the *waxy* gene.

Maize is a particularly favorable material for the investigation of the biochemical effects of genetic lesions because of the large size of its seeds and because of the translucent pericarp, which allows detection of any deviation from normal development. The substantial background of genetic information is also very helpful. Some of the mutants available for study are *amylose extender, dull, sugary 2,* and *waxy,* all of which affect the ratio of amylopectin to amylose. The *shrunken-1, shrunken-2,* and *brittle-2* mutations reduce starch content of the endosperm. The *sugary-1* mutant is unique in that the principal storage polysaccharide is not starch but the highly branched and water soluble phytoglycogen. Besides the mutants that have been biochemically characterized, O. Nelson (1985) mentions many more mutants (not allelic to those mentioned previously) that even now are awaiting identification.

B. POTATO

The potato plant (*Solanum tuberosum*) is bushy, sprawling, and dark green, with compound leaves that resemble those of a close species, the tomato. The leaves are arranged in a spiral around the stem, and the flowers are arranged in clusters. They are about 1-inch wide and 5-petaled, and range in color from white to pale blue to purple. The plant is completely

poisonous except for the tubers; indeed, all plant members of the nightshade family, which includes potatoes, tomatoes, and eggplants, contain the poisonous alkaloid called solanine, a natural defense against its many predators.

The life cycle of the potato plants cultivated today is completely asexual (i.e., tuber to sprout to plant to tuber). When rapid leaf growth slows down, the plant begins to form flowers, and underground stems (stolons) begin to branch out and swell at their tips. Sucrose is sent from the mature leaves, the sources, to the rest of the plant and the stolons, the sinks. The starch is deposited at the ends of the stolons, forming tubers.

C. ARABIDOPSIS THALIANA

The cruciferous weed *Arabidopsis thaliana* has become a model system for the study of an unusually wide variety of aspects of plant biology. *Arabidopsis thaliana* is a small weed, related to the mustards, and possesses a number of characteristics that make it an ideal object of genetic study. It has a rapid life cycle, passing from germination to flowering and setting of seeds in about 5 weeks; the plant may be self- or cross-pollinated, facilitating genetic analysis. The small size of the plants facilitates its cultivation of large numbers in laboratory conditions and the screening for relevant mutants after chemical mutagenesis. Another advantage is that it is relatively easy to transform some lines of *Arabidopsis thaliana* using the *Agrobacterium* Ti plasmid. The *Arabidopsis* genome is relatively small, with about 10^8 bp of DNA, and most of this genetic material is single copy sequences, facilitating the development of a very detailed genetic map.

D. ANTIRRHYNUM MAJUS

Antirrhynum majus is a common cultured garden plant, the snapdragon. The normal typus or wild type of *A. majus* is defined to be the Sippe 50 strain.

Gene inactivations and reactivations caused by the insertion and excision of transposable elements of the kind first discovered in maize, also appear in *Antirrhynum*, facilitating the identification and molecular analysis of genes involved in flower development and organ identity. Although in *Antirrhynum* the best studied genes are those involved in the synthesis of pigments and in flowering, it is now being used in the investigation of the mechanism of starch biosynthesis by Romero and colleagues.

Gene disruption is an experimental tool used for "reverse genetics," in which a gene is specifically inactivated, as pioneered in yeast, so that the precise function of the gene may be determined. A "cryptic" DNA or protein sequence is used to discover the normal role of the gene at the

phenotypic level. Another gene with a selectable function can be inserted into the middle of a wild-type allele of the gene of interest carried on a plasmid. A linear derivative of such a construct would insert itself specifically at the wild-type locus, automatically disrupting it and at the same time allowing the selection of the recombinant via the selectable gene. In the case of starch biosynthesis, study is still limited to the specific effects of the relevant genes for which mutants have been obtained, but the use of gene disruption in plants such as *Antirrhynum* would greatly expand the options available to the biochemist in search of the role of enzymes of starch metabolism and multiforms in the final architecture of the starch granule.

E. CHLAMYDOMONAS REINHARDTII

Although cyanobacteria (also called *blue–green algae*) are often used as a model system for plants because they are photosynthetic, they are prokaryotic and more similar to bacteria than to plants in many ways. Cyanobacteria, for example, accumulate glycogen rather than starch and have no organelles. Conversely, *Chlamydomonas reinhardtii,* a unicellular organism used since 1990 in the study of starch synthesis is a green algae, is a better system to study the effect of mutations in the relevant enzymes on starch structure. *Chlamydomonas* is a large genus of green flagellates; more than 600 species have been described worldwide from marine and freshwaters, soil, and even snow. Until the 1970s, *Chlamydomonas* was considered by many to be the most ancient of the green plants, but according to the current opinion they are considered nonancestral members of the chlorophyte lineage (Chlorophyceae) of green algae. Several species of *Chlamydomonas* have become important experimental organisms in fields such as cell and molecular biology, genetics, plant physiology, and biotechnology.

Swimming cells have a single nucleus and two flagella inserted into a minute papilla at the anterior end of the cell; the cell wall is thin. Most of the cell volume is occupied by one or more grass-green chloroplasts. In the most frequently used species, *C. reinhardtii,* only one cup-shaped chloroplast is present; one or more pyrenoids are present within the chloroplast; starch grains surround the pyrenoid.

Vegetative cells are usually haploid, and reproduce asexually by division into two, or some small multiple of two, progeny cells. Under certain conditions, usually involving induction of vegetative cell growth under nitrogen limitation, vegetative cells divide to form gametes. Gametes look like vegetative cells, but have differentiated mating structures near their apices. Cysts are usually diploid, formed by fusion of gametes. Meiosis in the cysts

usually yields four vegetative cells. The life cycle of *Chlamydomonas* is easy to manipulate under controlled culture conditions.

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

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