43

Plant Genomics

Concept Outline

43.1 Genomic organization is much more varied in plants than in animals.

Overview of Plant Genomics. As agrarian societies formed, people began to select for desirable traits. Until relatively recently, plant biologists focused their research efforts on variation in chromosomes, but work is now shifting increasingly to the molecular level.

Organization of Plant Genomes. Plant genomes are more complex than those of other eukaryotic organisms due to the presence of multiple chromosome copies and extensive amounts of DNA with repetitive sequences.

Comparative Genome Mapping and Model Systems. RFLP and AFLP techniques are useful for mapping traits in plant genomes. Despite the technical success in sequencing the *Arabidopsis* genome and other genomes, we still don't know what most of these genes do and how the proteins they encode function in physiology and development.

43.2 Advances in plant tissue culture are revolutionizing agriculture.

Overview of Plant Tissue Culture. Because plants are totipotent, bits of tissue can be used to regenerate whole plants.

Types of Plant Tissue Cultures. Plant cells, tissues, and organs can be grown in an artificial culture medium, and some cells can be directed to generate whole plants.

Applications of Plant Tissue Culture. Plant tissue cultures can be used for the production of plant products, propagation of horticultural plants, and crop improvement.

43.3 Plant biotechnology now affects every aspect of agriculture.

World Population in Relation to Advances Made in Crop Production. It is uncertain whether advances made in crop production by improved farming practices and crop breeding can provide for an increasing world population.

Plant Biotechnology for Agricultural Improvement. Plants can be genetically engineered to have altered levels of oils and amino acids and to provide vaccines.

Methods of Plant Transformation. The genetic engineering of plants is based upon introduction of foreign DNA into plant cells.

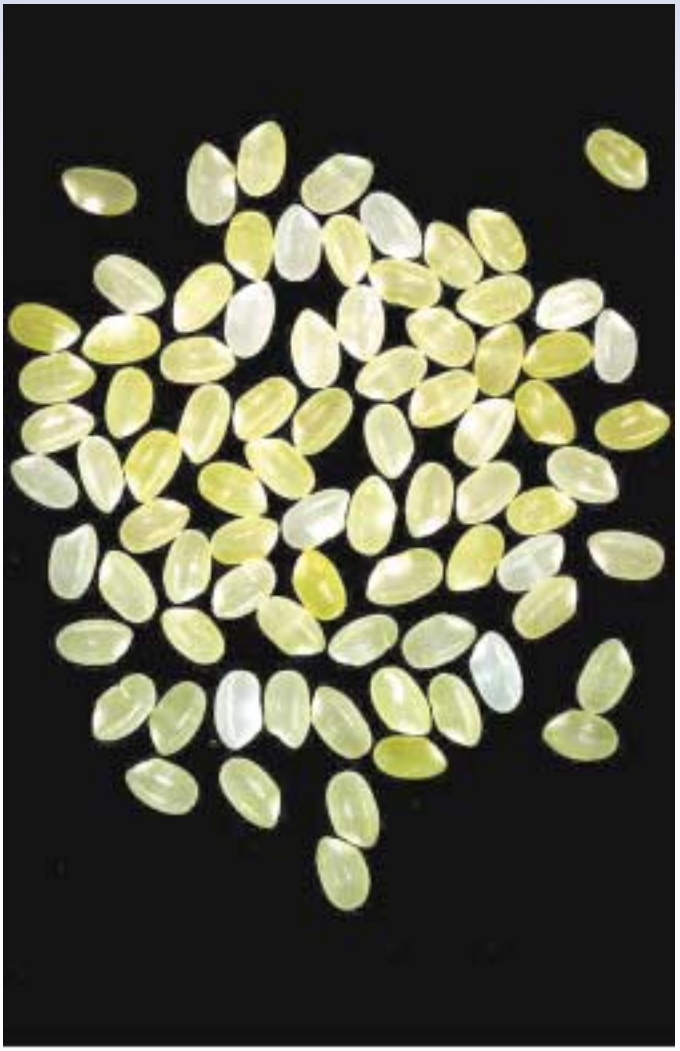

FIGURE 43.1
Golden rice. Rice is the dietary staple of almost half the world's population, but it lacks vitamin A. Vitamin A deficiency leads to vision and immunity problems. Genetically engineered rice that produces vitamin A has now been developed. The rice is golden because a biosynthetic pathway has been genetically modified to produce gold-colored beta-carotene, a precursor to vitamin A. Here, while rice is mixed in with golden rice. The intensity of golden color indicates the amount of pro-vitamin A present.

By selective breeding favoring desired traits, people have been genetically modifying plants since agrarian societies began. All of our key modern crops are the result of this long effort. Today, we have even more powerful tools, recombinant DNA technologies that are the subject of this chapter. This chapter looks ahead to the impact of these new technologies on the future of plants and our study of plant biology (figure 43.1). Both the *Arabidopsis* and rice genomes are essentially sequenced. Not only can we expect to learn much about the molecular basis of plant physiology and development from these rich databases; we will surely gain a far deeper understanding of plant evolution.

43.1 Genomic organization is much more varied in plants than in animals.

Overview of Plant Genomics

Early Approaches

While the term genetic engineering is commonly used to describe plants and animals modified using recombinant DNA technology, people have actually been genetic engineers for thousands of years. As agrarian societies formed, changes in the gene pool within crop species began. For example, seed dispersal was selected against in maize and wheat. Without the ability to disperse seed, these domesticated plants are completely dependent on humans for seed dispersal. Rice was converted from a perennial plant to an annual plant without the seed dormancy mechanisms present in wild rice. Parts of the plant that were of most dietary value to humans and domesticated animals have been selected for increased size. These include seeds, fruits, and storage organs like roots in the case of carrots. All of these changes were accomplished without knowledge of particular genes, by selecting and propagating individuals with the desired traits.

Breeding Strategies to Enhance Yield

At the beginning of the twentieth century, a growing understanding of genetics increased the rate of crop improvement. Among the most dramatic agricultural developments was the introduction of hybrid corn. As corn breeding progressed, highly inbred lines began to have decreased yield as deleterious recessive genes became homozygous. George Harrison Shull found that crossing two different inbred lines gave rise to offspring with "hybrid vigor." The yield increased fourfold! Hybrid corn now grows in almost all fields in the United States. Hybrid rice developed by the International Rice Research Institute in the Philippines has increased yield 20%.

Breeders have now turned to specific genes to optimize food quality (see figure 43.1). Only a small percentage of the genes and their function have been identified, but we start this century with technologically powerful new ways to understand genomes.

Studying Plant Genomes

Plant genomes are more complex than other eukaryotic genomes, and analysis reveals many evolutionary flips and turns of the DNA sequences over time. Plants show widely different chromosome numbers and varied ploidy levels (figure 43.2). Overall, the size of plant genomes (both number of chromosomes and total nucleotide base-pairs) exhibits the greatest variation of any kingdom in the biological world. For example, tulips contain over 170 times as much DNA as the small weed *Arabidopsis thaliana* (table 43.1). The DNA of plants, like animals, can also contain regions of sequence repeats, sequence inversions, or transposable element insertions, which further modify their genetic content. Traditionally, variation in chromosome inversions and ploidy has been used to build up a picture of how plant species have evolved (figure 43.3). Increasingly, researchers are turning to studying the organization of plant DNA sequences to obtain important information about the evolutionary history of a plant species.

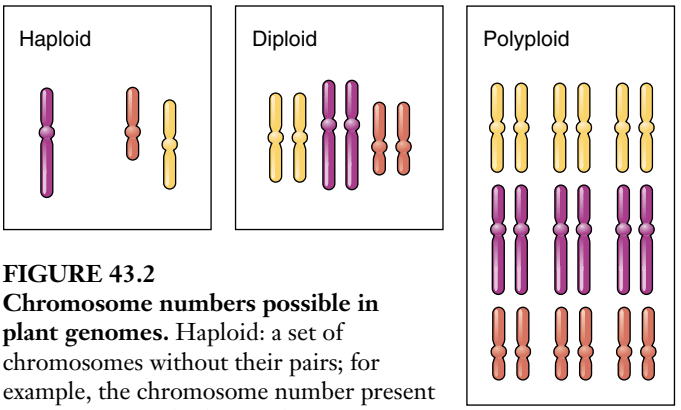

FIGURE 43.2
Chromosome numbers possible in plant genomes. Haploid: a set of chromosomes without their pairs; for example, the chromosome number present in a gamete. Diploid: a single set of chromosome pairs. Polyploid: multiple sets of chromosome pairs; for example, bananas have a triple set of chromosomes and are therefore polyploid.

Table 43.1 Genome Size of Plants

Scientific Name	Common Name	Genome Size (Millions of Base-Pairs)
Arabidopsis thaliana	Arabidopsis	145
Prunus persica	Peach	262
Ricinus communis	Castor bean	323
Citrus sinensis	Orange	367
Oryza sativa spp. *javanica*	Rice	424
Petunia parodii	Petunia	1,221
Pisum sativum	Garden pea	3,947
Avena sativa	Oats	11,315
Tulipa spp.	Garden tulip	24,704

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People have been genetically engineering plants for centuries by selecting for desired traits. Traditionally, biologists have examined variation among plants at the chromosome level; today, researchers are focusing more of their efforts at the DNA sequence level.

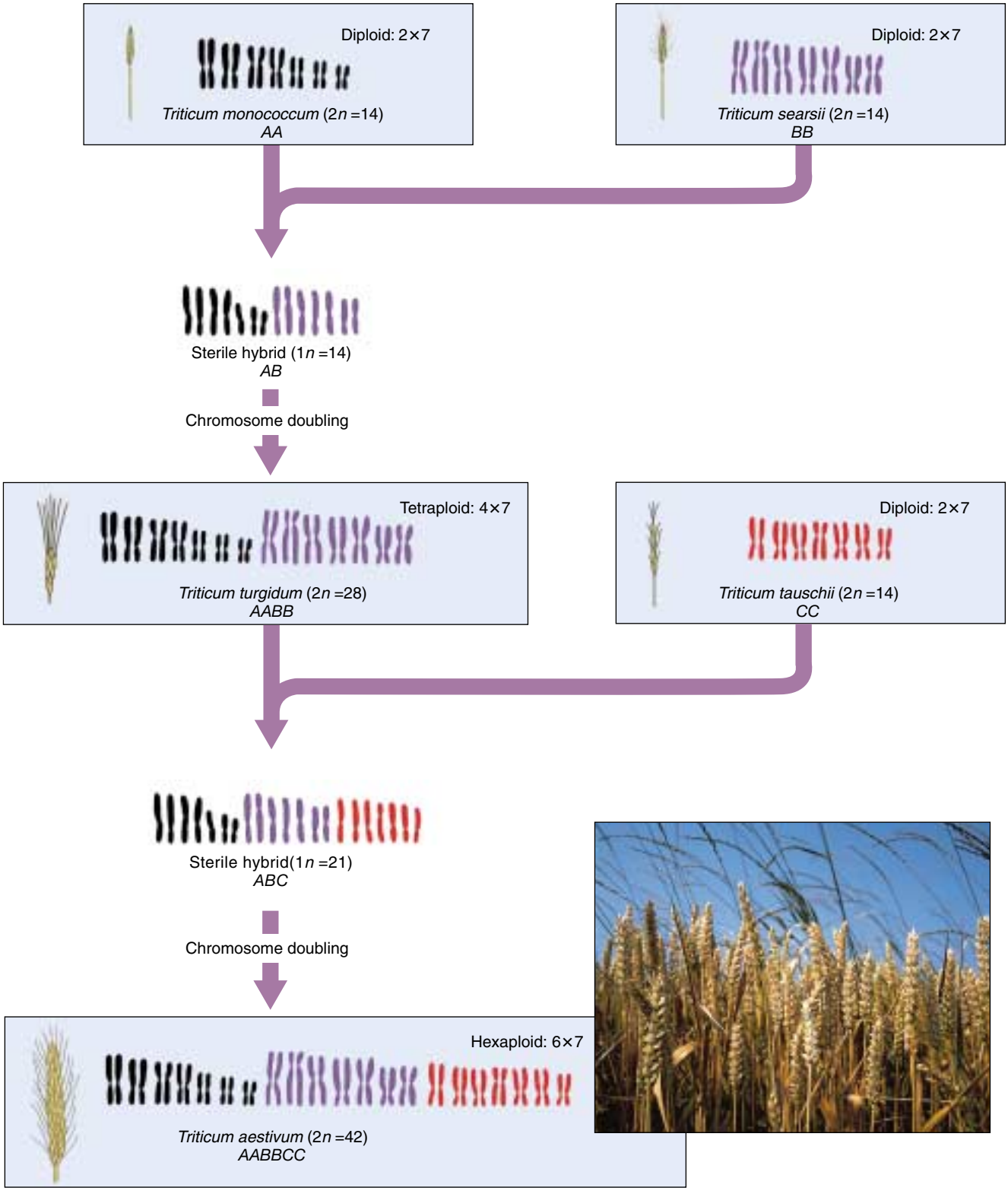


FIGURE 43.3
Evolutionary history of wheat. Domestic wheat arose in southwestern Asia in the hilly country of what is now Iraq. In this region, there is a rich assembly of grasses of the genus *Triticum*. Domestic wheat *(T. aestivum)* is a polyploid species of *Triticum* that arose through two so-called "allopolyploid" events. (1) Two different diploid species, *AA* and *BB*, hybridized to form an *AB* polyploid; the species were so different that *A* and *B* chromosomes could not pair in meiosis, so the *AB* polyploid was sterile. However, in some plants the chromosome number spontaneously doubled due to a failure of chromosomes to separate in meiosis, producing a fertile tetraploid species *AABB*. This wheat is used in the production of pasta. (2) In a similar fashion, the tetraploid species *AABB* hybridized with another diploid species *CC* to produce the hexaploid *T. aestivum*, *AABBCC*. This bread wheat is commonly used throughout the world.

Organization of Plant Genomes

Low-, Medium-, and High-Copy-Number DNA

Most seed plants contain quantities of DNA that greatly exceed their needs for coding and regulatory function. Hence, for plants, a very small percentage of the genome may actually encode genes involved in the production of protein. This portion of the genome which encodes most of the transcribed genes is often referred to as "low-copy-number DNA," because the DNA sequences comprising these genes are present in single or small numbers of copies. How do plants function with so much extra DNA inserted into the genome? It appears that most of these sequence alterations occur in noncoding regions.

"Medium-copy-number DNA" is composed largely of DNA sequences that encode ribosomal RNA (rRNA), a key element of the cellular machinery that translates transcribed messenger RNA (mRNA) into protein. In plant genomes, rRNA genes may be repeated several hundred to several thousand times. This is in contrast to animal cells, where only 100 to 200 rRNA genes are normally present. The extent of variability in plant genomes with respect to the number of rRNA genes and mutations in them has provided a useful tool for analyzing the evolutionary patterns of plant species.

Plant cells may also contain excess DNA in their genomes in the form of highly repetitive sequences, or "high-copy-number DNA." At present, the function of this high-copy-number DNA in plant genomes is unknown. Roughly half the maize genome is composed of such retroviral-like DNA. RNA retroviruses like HIV use their host genomes to make DNA copies that then insert into the host genome. Clearly, the effects of some retroviruses can be lethal. How maize came to tolerate such a large amount of this foreign DNA is an evolutionary mystery.

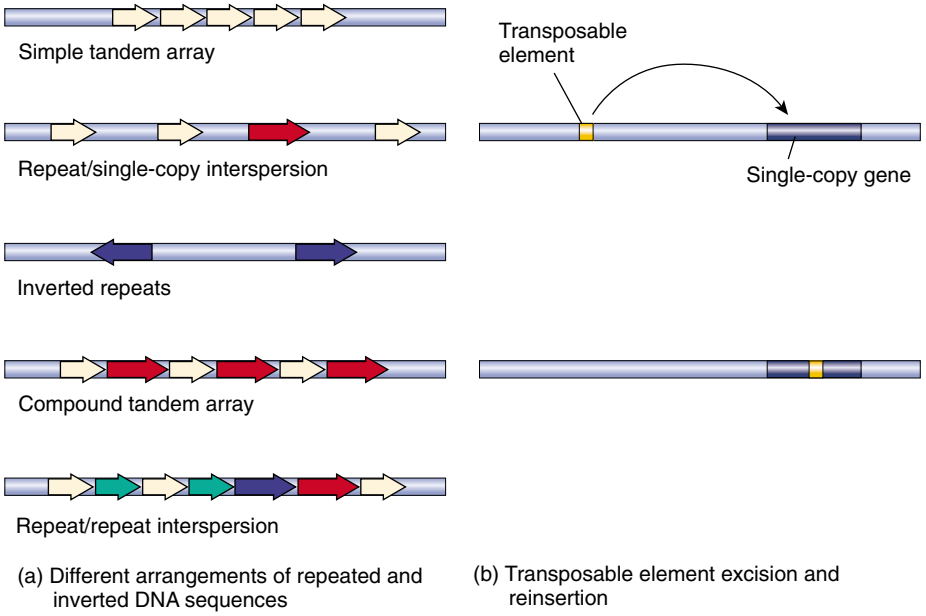


FIGURE 43.4
Organization of repeated DNA sequences and the mechanism of transposable elements in altering gene function. (*a*) Repeated DNA sequences can occur in plant genomes in several different arrangements. The arrows represent repeated DNA sequences. Arrows of the same size and color represent DNA sequences which are identical to each other. The direction of the arrowhead indicates the orientation of the DNA sequence. (*b*) Transposable elements can be a source of repetitive DNA that alters gene function. Following excision from its original location, a transposable element may reinsert in the single-copy DNA sequence comprising a gene and alter the gene's function.

Sequence Replication and Inversion

High-copy-number DNA sequences in the plant genome may be short, such as the nucleotide sequence "GAA," or much longer, involving up to several hundred nucleotides. Moreover, the number of copies of an individual high-copy repetitive DNA sequence can total from 10,000 to 100,000. There are several possibilities for how high-copy repetitive DNA sequences may be organized within a plant genome (figure 43.4*a*). Several copies of a single repetitive DNA sequence may be present together in the same orientation, in a pattern called "simple tandem array." Alternatively, repetitive DNA sequences can be dispersed among single-copy DNA in the same orientation ("repeat/single-copy interspersion") or the opposite orientation ("inverted repeats"). In addition, groups of repetitive DNA sequences can also occur together in plant genomes in a variety of possible arrangements, such as a "compound tandem array" or a "repeat/repeat interspersion." The presence of repetitive DNA can vastly increase the size of a plant genome, making it difficult to find and characterize individual single-copy genes. Characterizing single-copy genes can thus become a sort of "needle-in-the-haystack" hunt.

A variety of mechanisms can account for the presence of highly repetitive DNA sequences in plant genomes. Repetitive sequences can be generated by DNA sequence amplification in which multiple rounds of DNA replication occur for specific chromosomal regions. Unequal crossing over of the chromosomes during meiosis or mitosis (translocation) or the action of transposable elements (see next section) can also generate repetitive sequences.

Transposable Elements

Transposable elements, described in chapter 18, are special sequences of DNA with the ability to move from place to place in the genome. They can excise from one site at unpredictable times and reinsert in another site. For this reason, transposable elements have been called "jumping genes." Transposable elements often insert into coding regions or regulatory regions of a gene and so affect expression of that gene, resulting in a mutation that may or may not be detectable (figure 43.4*b*). Barbara McClintock won the Nobel Prize in 1983 for her work describing transposable elements in corn (see figure 18.23).

Due to their capacity to replicate independently and to move through the genome, transposable elements can also be involved in generating repetitive DNA sequences. This is believed to be the case with the extensive retroviral-like insertions in maize. Retention of the repetitive DNA sequence at a particular site in the genome would involve in each instance a mutation in the transposable element itself which removes its capacity to transpose.

Chloroplast Genome and Its Evolution

The chloroplast is a plant organelle that functions in photosynthesis, and it can independently replicate in the plant cell. Plant chloroplasts have their own specific DNA, which is separate from that present in the nucleus. This DNA is maternally inherited and encodes unique chloroplast proteins. Many of the proteins encoded by chloroplast DNA are involved in photosynthesis. Chloroplasts are thought to have originated from a photosynthetic prokaryote that became part of a plant cell by endosymbiosis. In support of this concept, research has shown that chloroplast DNA has many prokaryote-like features. Chloroplast DNA is present as circular loops of double-stranded DNA similar to prokaryotic chromosomal DNA. Moreover, chloroplast DNA contains genes for ribosomes that are very similar to those present in prokaryotes.

The DNA in chloroplasts of all land plants has about the same number of genes (~100), and they are present in about the same order (figure 43.5). In contrast to the evolution of the DNA in the plant cell nucleus, chloroplast DNA has evolved at a more conservative pace, and therefore shows a more interpretable evolutionary pattern when scientists study DNA sequence similarities. Chloroplast DNA is also not subject to modification caused by transposable elements and mutations due to recombination. Over time, there appears to have been some genetic exchange between the nuclear and chloroplast genomes. For example, the key enzyme in the Calvin cycle of photosynthesis (RUBISCO) consists of a large and small subunit. The small subunit is encoded in the nuclear genome. The protein it encodes has a targeting sequence that allows it to enter the chloroplast and combine with large subunits. The evolutionary history of the localization of these genes is a puzzle.

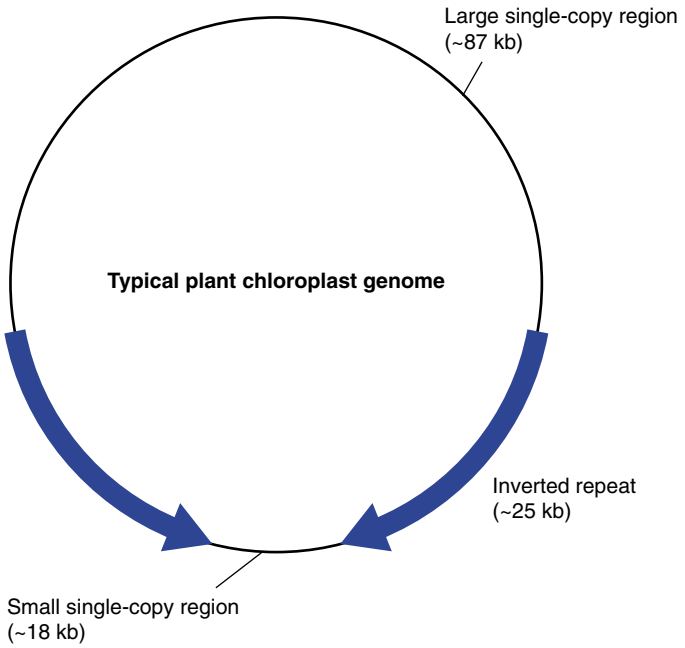


FIGURE 43.5
Chloroplast genome. A schematic drawing of a typical plant chloroplast genome indicates two regions containing single-copy genes, one containing about 87,000 nucleotides (87 kb) and another about 18 kb, and two symmetrical inverted repeats, each containing about 25 kb. Chloroplast DNA does not show recombination events that are common in the nuclear genome. It is thus a good subject for DNA phylogenetic analysis.

A characteristic feature of the chloroplast genome is the presence of two identical inverted repeats in the DNA sequence. Other DNA sequence inversions or deletions occur rarely, but when they do occur, they provide a character or a tool to analyze evolutionary relationships between plants. For instance, a large inversion in chloroplast DNA is found in the Asteraceae, or sunflower family, and not in other plant families. While previous work on the evolutionary relationships between plants has emphasized the comparative analysis of plant anatomy or morphology, there is increasing use of plant molecular data such as chloroplast DNA sequences. When considered together, morphological and molecular information can provide a clearer understanding of the evolutionary processes that govern biological diversity.

Plant nuclear genomes may contain large amounts of DNA in comparison to other eukaryotic organisms, but only a small amount of this DNA represents functional genes. Excess DNA in plant genomes can result from increased chromosome copy number (polyploidy), and DNA sequence repeats. Chloroplast genomes evolve more slowly than nuclear genomes and can provide important evolutionary information.

Comparative Genome Mapping and Model Systems

Knowledge of plant genomes has been growing with the advent of new techniques to study DNA sequences, such as gene mapping and chromosome synteny. An increased understanding of plant genomes can lead to better manipulation of genetic traits such as crop yield, disease resistance, growth abilities, nutritive qualities, or drought tolerance. Multiple genes could encode each of these traits. By genome mapping model plants, plant biologists can lay a foundation for future plant breeding and for an understanding of plant evolution at the genetic level. One such model system, rice, has been chosen because it has a high level of synteny with other grains. In a genomic sense, "rice is wheat." This provides a strong argument for rice as a model system. The other model system that has been selected in plants is *Arabidopsis*. This small weed that is a member of the mustard family has an unusually small genome with only 20% repetitive DNA (see table 43.1) which has made it possible to sequence the entire genome. Getting down to the level of individual base pairs is a stepwise process, as described below.

RFLP and AFLP as Tools to Map Genomes and Detect Polymorphisms

The classical approach to locating genes in linear order on chromosomes involves making crosses between plants with known genes identified by mutations. The frequency of recombination is used to calculate distance (see chapter 13). The result is a genetic or linkage map. This approach is limited to genes with alleles that can be phenotypically identified. Much more of the genome can be mapped using RFLPs (restriction fragment length polymorphisms) which need not have a macroscopic phenotype. This approach, described in detail in chapter 19 (see figures 19.2, 19.4, 19.9, and 19.10), involves analysis of the RFLP map, or the pattern of DNA fragments, produced when DNA is treated with restriction enzymes that cleave at specific sites. RFLP mapping can identify important regions of the genome at a glance, while sequence data require sophisticated computer-based searching and matching systems. A comparison of the RFLP maps of parents and progeny can give an indication of the heritability of gene traits and of heritable loci that are characteristic of traits. If the trait and the RFLP co-segregate, you have a direct link between the trait and the DNA sequence. Moreover, after full genomes are sequenced at the nucleotide level, the genetic identification of RFLP markers in regions of interest will be facilitated. Remember, RFLPs are chunks of DNA that may contain a part of one or more genes. Currently, the most dense RFLP map is in rice where 2000 DNA sequences have been mapped onto 12 chromosomes.

Another tool that utilizes sequence variability is AFLPs, or amplified fragment length polymorphisms.

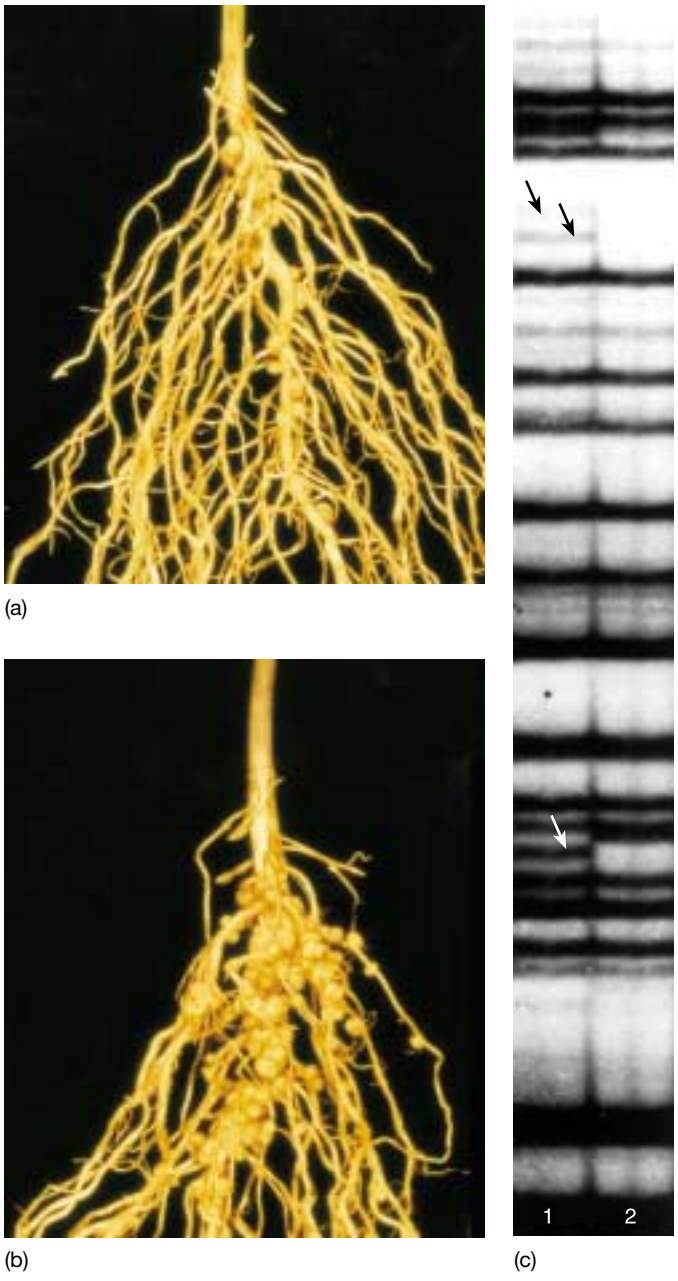


FIGURE 43.6
AFLP fingerprint pattern from normal and "hypernodulating" soybeans. It is still not known what determines the nodule number in (*a*) a normal soybean root versus (*b*) a "hypernodulating" mutant. The slight genetic differences between these plants can be evaluated by AFLP (*c*). The banding pattern changes indicate what genetic markers are linked to the "hypernodulation" mutation. Lane 1: normal soybean DNA; Lane 2: "hypernodulating" soybean DNA.

Hybridizing DNA primers with genomic DNA fragments that have been cut with restriction enzymes, usually *Eco*RI and *Mse*I, and then subsequently amplified using the polymerase chain reaction (PCR) generates AFLP maps. The resulting PCR products, which represent each piece of DNA cut by a restriction enzyme, are separated by size

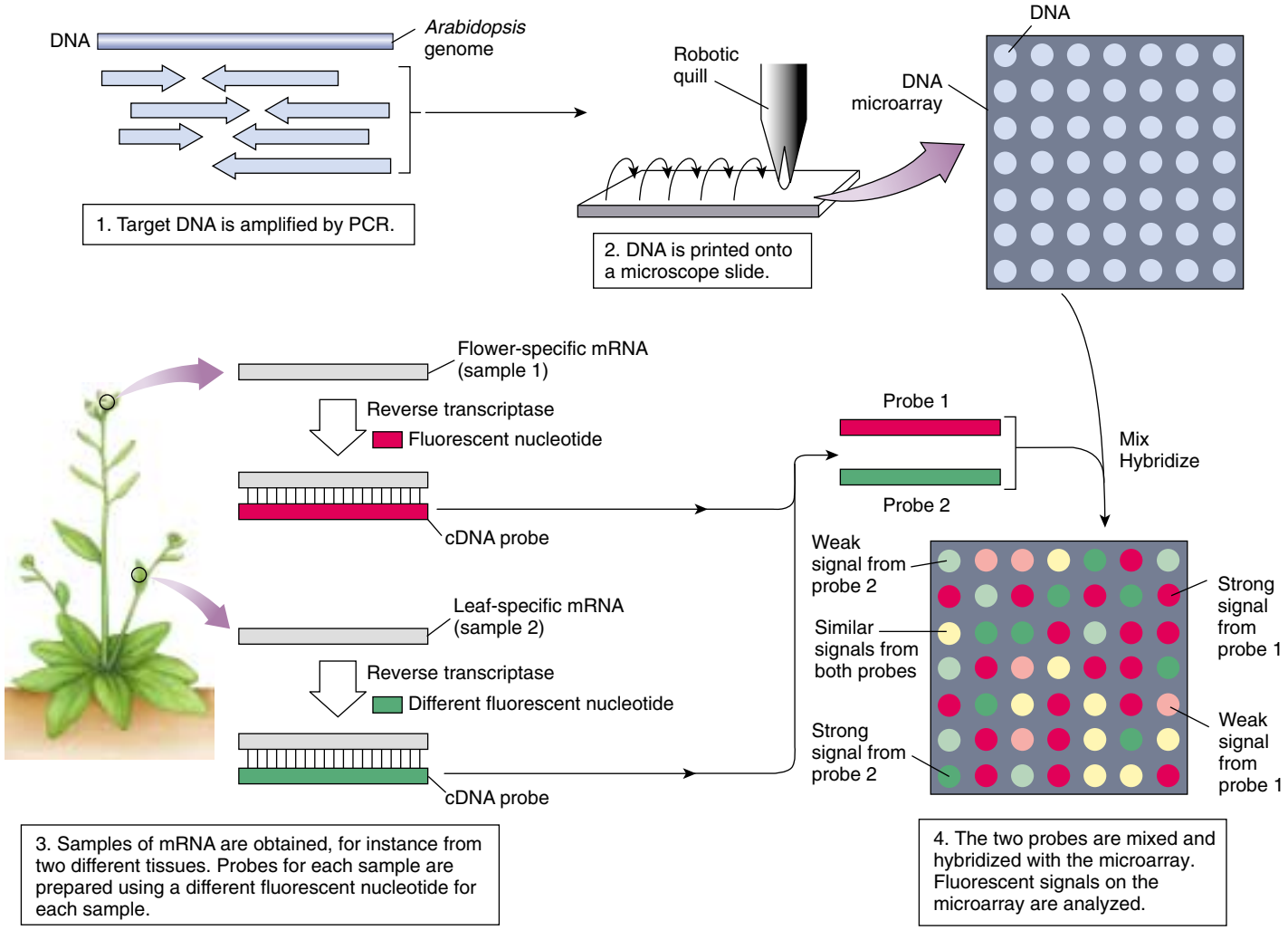


FIGURE 43.7
Microarrays. Microarrays are created by robotically placing DNA onto a microscope slide. The microarray can then be probed with RNA from tissues of interest to identify expressed DNA. The microarray with hybridized probes is analyzed and often displayed as a false-color image. If a gene is frequently expressed in one of the samples, the fluorescent signal will be strong (red or green) where the gene is located on the microarray. If a gene is rarely expressed in one of the samples, the signal will be weak (pink or light green). A yellow color indicates genes that are expressed at similar levels in each sample.

via gel electrophoresis. The band sizes on an AFLP gel tend to show more polymorphisms than those found with RFLP mapping because the entire genome is visible on the gel (figure 43.6). Both RFLPs and AFLPs (among many other tools for genome analysis) can provide markers of traits which are inherited from parents to progeny through crosses.

DNA Microarrays

How can DNA sequences be made available to researchers, other than as databases of electronic information? DNA microarrays are a way to link sequences with the study of gene function and make DNA sequences available to many. Also called biochips or "genes on chips," these convenient assays for the presence of a particular version of a gene were discussed in chapter 19. To prepare a particular DNA microarray, fragments of DNA are deposited on a microscope slide by a robot at indexed locations. Up to 10,000 spots can be displayed over an area of only 3.24 cm^2 (figure 43.7). The primary applications of microarrays are to determine which genes are expressed developmentally in certain tissues or in response to environmental factors. RNA from these tissues can be isolated and used as a probe for these microarrays. Only those sequences that are expressed in the tissues will be present to hybridize to the spot on the microarray.

Plant Genome Projects

The potential of having complete genomic sequences of plants is tremendous and about to be realized now that the *Arabidopsis* Genome Project is essentially complete. This project represents a new paradigm in the way biology is done. The international effort brought together research teams with the expertise and tenacity to apply new sequencing technology to an entire genome, rather than single genes. Powerful databases are being constructed to make this information accessible to all. The completely sequenced *Arabidopsis* genome will have far-reaching uses in agricultural breeding and evolutionary analysis. This information can be expected to help plant breeders in the future because the localization of genes in one plant species can help indicate where that gene might also be located in another species (figure 43.8). In plant genomes, local gene order seems to be more conserved than the nucleotide sequences of homologous genes. Thus, the complete genomic sequence of *Arabidopsis thaliana* will facilitate gene cloning from many plant species, using information on relative genomic location as well as similarity of sequences.

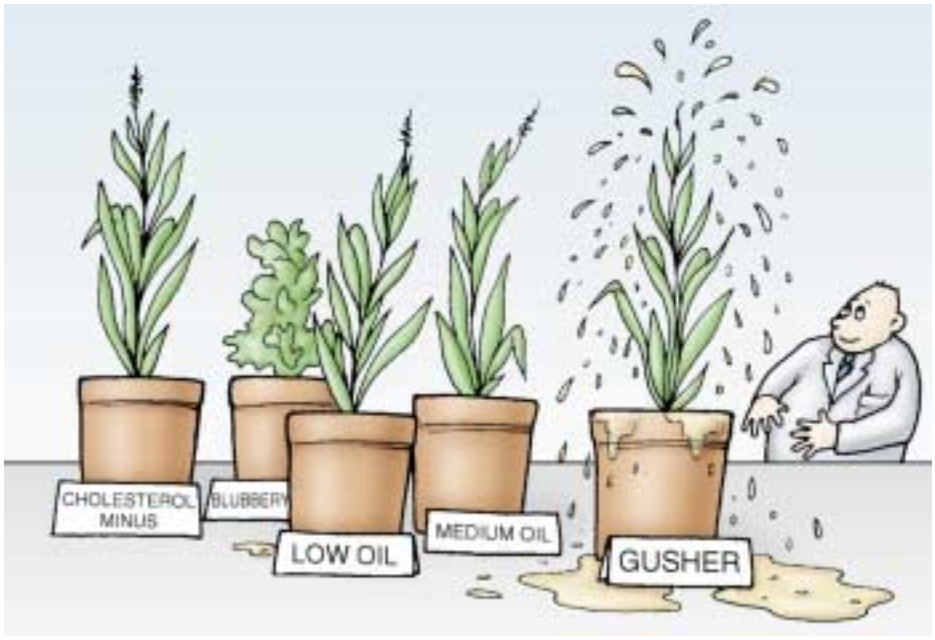


FIGURE 43.8
Future directions in the genetic engineering of vegetable oils?

Sequencing the rice genome provides a model for a small monocot genome. Rice was selected, in part, because its genome is 6, 10, and 40 times smaller than maize, barley, and wheat. These grains represent a major food source for humans. By understanding the rice genome at the level of its DNA sequence, it should be much easier to identify and isolate genes from grains with larger genomes. Even though these plants diverged more than 50 million years ago, the chromosomes of rice, corn, barley, wheat, and other grass crops show extensive conserved arrangements of segments (synteny) (figure 43.9). DNA sequence analysis of cereal grains will be important for identifying genes associated with disease resistance, crop yield, nutritional quality, and growth capacity. It will also be possible to construct an approximate map of the ancestral cereal genome.

Functional Genomics and Proteomics

Sequencing the *Arabidopsis* and rice genome represent major technological accomplishments. A new field of bioinformatics takes advantage of high-end computer technology to analyze the growing gene databases, look for relationships among genomes, and hypothesize functions of genes based on sequence. Genomics (the study of genomes) is now shifting gears and moving back to hypothesis-driven science. Again, an international community of researchers has come together with a plan to assign function to all of the 20,000 to 25,000 *Arabidopsis* genes by 2010 (Project 2010). In many ways, the goal is to ultimately answer the questions we have raised in chapters 37 through 42. One of the first steps is to determine when and where these genes are expressed. Each step beyond that will require additional enabling technology. Research will move from genomics to proteomics (the study of all proteins in an organism). Proteins are much more difficult to study because of posttranslational modification and formation of complexes of proteins. This information will be essential in understanding cell biology, physiology, development, and evolution. For example, how are similar genes used in different plants to create biochemically and morphologically distinct organisms? So, in many ways, we continue to ask the same questions that even Mendel asked, but at a much different level of organization.

Restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) represent important tools for mapping genetic traits in plant genomes. Due to its short life cycle, small size, and small genome, the mustard relative *Arabidopsis thaliana* is being used as a model plant for genetic studies. The genome of rice is also essentially sequenced and will be a valuable model for other monocot cereal grains such as wheat, barley, oats, and corn. Assigning function to these genes is the next challenge.

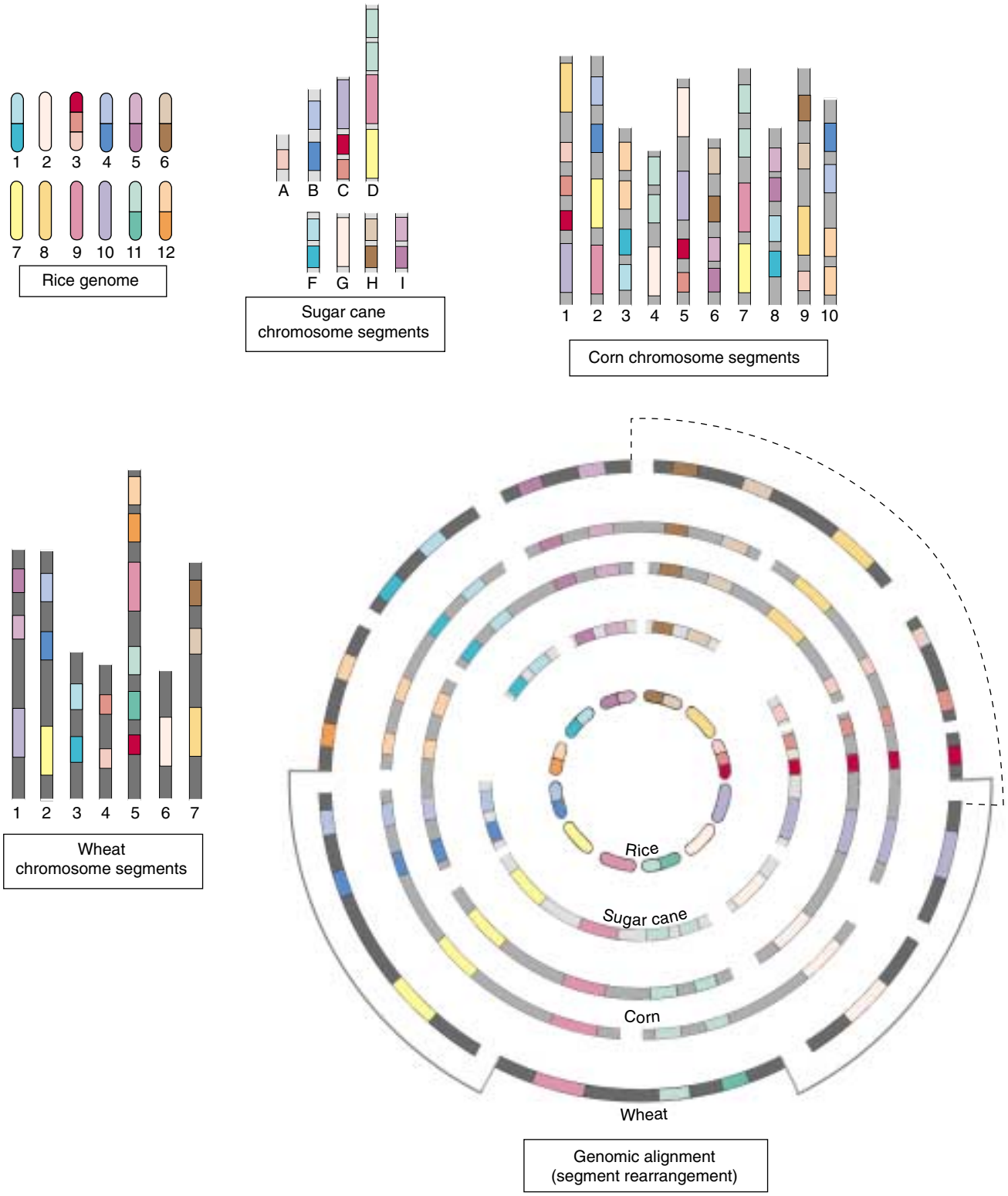


FIGURE 43.9
Grain genomes are rearrangements of similar chromosome segments. Shades of the same color represent pieces of DNA that are conserved among the different species but have been rearranged. By splitting the individual chromosomes of major grass species into segments, and rearranging the segments, researchers have found that the genome components of rice, sugar cane, corn, and wheat are highly conserved. This implies that the order of the segments in the ancestral grass genome has been rearranged by recombination as the grasses have evolved. Data: G. Moore, K. M. Devos, Z. Wang, and M. D. Gale: "Grasses, line up and form a circle," *Current Biology* 1995, vol. 5, pp. 737-739.

43.2 Advances in plant tissue culture are revolutionizing agriculture.

Overview of Plant Tissue Culture

One of the major hopes for the plant genome projects is using newly identified genes for biotechnology, and advances in tissue culture are facilitating this. Having an agriculturally valuable gene in hand is just the beginning. With methods discussed in chapter 19 and below, desirable genes can be introduced into plants, yielding **transgenic** cells and tissues. Whole plants can than be regenerated using tissue culture. While animals can now be cloned, the process is much simpler in plants. Many somatic (not germ-line) plant cells are totipotent, which means they can express portions of their previously unexpressed genes and develop into whole plants under the right conditions.

The successful culture of plant cells, tissues, or organs requires utilizing the proper plant starting material, appropriate nutrient medium, and timing of hormonal treatments to maximize growth potential and drive differentiation (figure 43.10). Most plant tissue cultures are initiated from *explants*, or small sections of tissue removed from an intact plant under sterile conditions. After being placed on a sterile growth medium containing nutrients, vitamins, and combinations of plant growth regulators, cells present in the explant will begin to divide and proliferate. Under appropriate culture conditions, plant cells can multiply and form organs (roots, shoots, embryos, leaf primordia, and so on) and can even regenerate a whole plant. The regeneration of a whole plant from tissue-cultured plant cells represents an important step in the production of genetically engineered plants. Using plant tissue cultures, genetic manipulation can be conducted at the level of single cells in culture, and whole plants can then be produced bearing the introduced genetic trait.

Plant cells growing in culture can also be used for the mass production of genetically identical plants (clones) with valuable inheritable traits. For example, this approach of clonal propagation using plant tissue culture is commonly used in the commercial production of many ornamental plants such as chrysanthemums and ferns. As we will describe next, different types of cultures can be generated based on the initial type of plant tissue used for the explant and on the composition of the growth medium.

It is often possible to regenerate an entire plant from one or a few cells. Depending on the plant tissue used and the growth medium selected, different types of cultures may be produced.

(a)

(b)

(c)

FIGURE 43.10
Culture of orchid plants. While the natural maturation of a single orchid plant may take up to seven years, commercial growers can produce thousands of cultured orchids in a relatively short time. (*a*) The apical meristem is removed from an orchid plant. (*b*) The meristematic tissue is grown in flasks containing hormone-enhanced media, and roots and shoots begin to form. (*c*) The plantlets are then separated and grown to maturity.

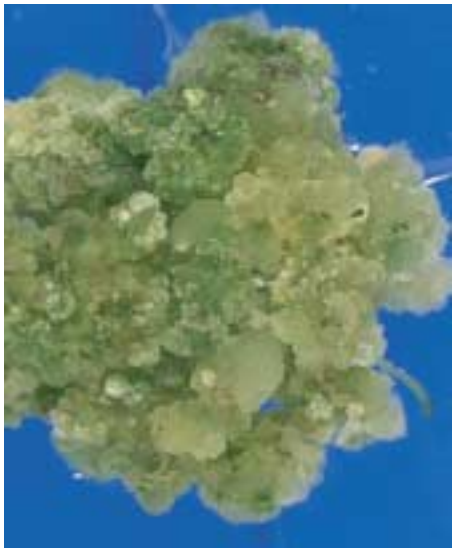
Types of Plant Tissue Cultures

Depending on the type of plant tissue used as the explant and the composition of the growth medium, a variety of different types of plant tissue cultures can be generated. These different types of plant tissue cultures have applications both in basic plant research and commercial plant production.

Callus Culture

Callus culture refers to the growth of unorganized masses of plant cells in culture. To generate a callus culture, an explant, usually containing a region of meristematic cells, is incubated on a growth medium containing certain plant growth regulators such as auxin and cytokinin (figure 43.11). The cells grow from the explant and divide to form an undifferentiated mass of cells called a callus. This unorganized mass of growing cells is analogous to a plant tumor. Cells can proliferate indefinitely if they are periodically transferred to fresh growth media. However, if the callus cells are transferred to a growth medium containing a different combination of plant growth regulators, the cells can be directed to differentiate into roots and/or shoots. This process of converting unorganized growth into the production of shoots and roots is called **organogenesis,** and it represents one means by which a whole plant can be regenerated from tissue culture cells. When a plantlet produced by organogenesis is large enough, it can be transferred to a large container with nutrients or soil and grown to maturity.

(a)



(b)

(c)

(d)

FIGURE 43.11
Callus culture. (*a*) An explant is incubated on growth media. (*b*) The cells grow and divide and form a callus. (*c*) The callus cells, grown on media containing new plant growth regulators, differentiate into plant parts. (*d*) After the plantlet is large enough, it is grown to maturity in soil.

Cell Suspension Culture

Plant cell suspension culture involves the growth of single or small groups of plant cells in a liquid growth medium. Cell suspension cultures are usually initiated by the transfer of plant callus cells into a liquid medium containing a combination of plant growth regulators and chemicals that promote the disaggregation of the cells into single cells or small clumps of cells (figure 43.12). Continued cell growth requires that the liquid cultures be shaken at low speed to promote aeration and chemical exchange with the medium. Suspension cell cultures are often used in research applications where access to single cells is important. The suspension bath can provide an efficient means for selecting out cells with desirable traits such as herbicide tolerance or salt tolerance because the bath is in uniform contact with all the cells at once. This differs from callus culture, where only those cells in contact with the solid medium can be selected by chemical additions to the medium. Suspension cultures can also provide a convenient means for producing and collecting the plant chemicals cells secrete. These can include important plant metabolites, such as food products, oils, and medicinal chemicals. In addition, plant suspension cell cultures can often be used to produce whole plants via a process known as **somatic cell embryogenesis** (figure 43.13). For some plants, this provides a more convenient means of regenerating a whole plant after genetic engineering takes place at the single-cell level. In somatic cell embryogenesis, plant suspension culture cells are transferred to a medium containing a combination of growth regulators that drive differentiation and organization of the cells to form individual embryos. Under a dissection microscope, these embryos can be isolated and transferred to a new growth medium, where they grow into individual plants.

FIGURE 43.12
Cell suspension culture. Plant cells can be grown as individual cells or small groups of cells in a liquid culture medium. Liquid suspension culture of plant cells ensures that most cells are in contact with the growth medium.

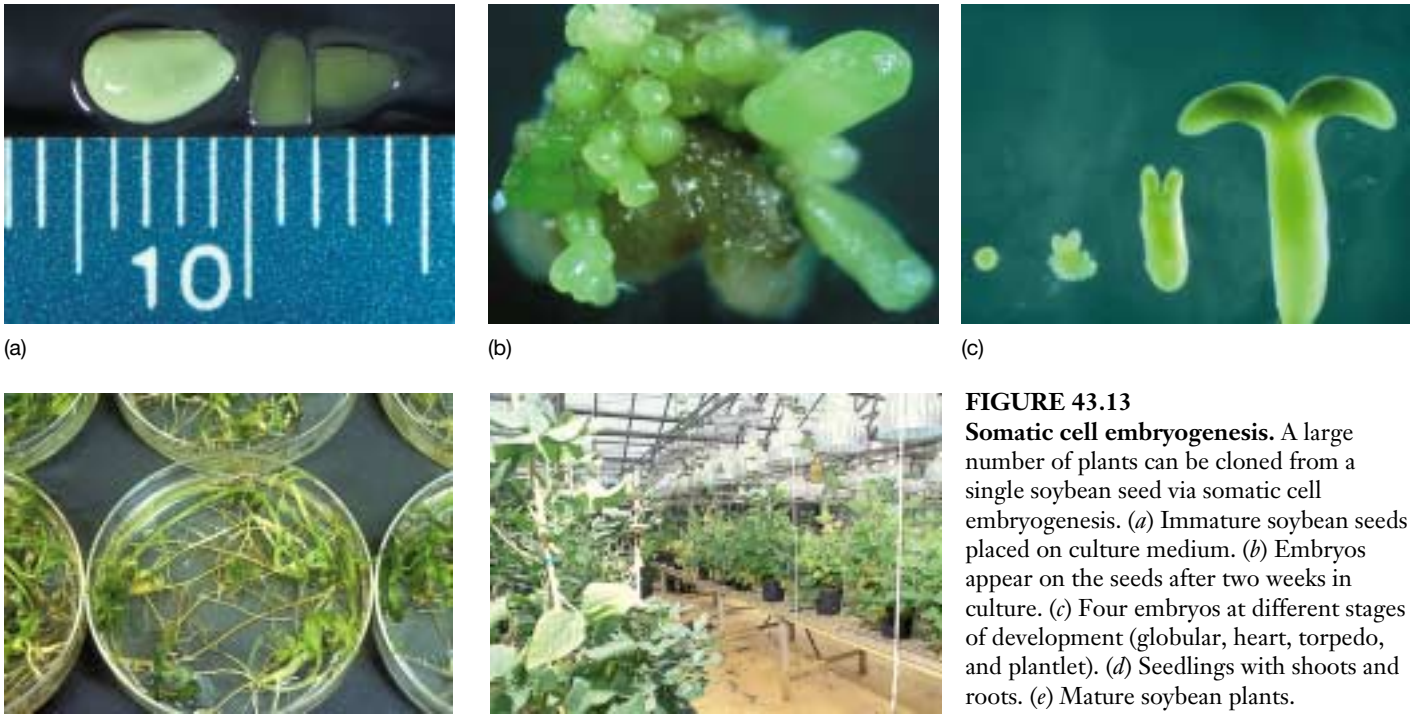


FIGURE 43.13
Somatic cell embryogenesis. A large number of plants can be cloned from a single soybean seed via somatic cell embryogenesis. (*a*) Immature soybean seeds placed on culture medium. (*b*) Embryos appear on the seeds after two weeks in culture. (*c*) Four embryos at different stages of development (globular, heart, torpedo, and plantlet). (*d*) Seedlings with shoots and roots. (*e*) Mature soybean plants.

Protoplast Isolation and Culture

Protoplasts are plant cells that have had their thick cell walls removed by an enzymatic process, leaving behind a plant cell enclosed only by the plasma membrane. Plant protoplasts have been extremely useful in research on the plant plasma membrane, a structure normally inaccessible due to its close association with the cell wall. Within hours of their isolation, plant protoplasts usually begin to resynthesize cell walls, so this process has also been useful in studies on cell wall production in plants. Plant protoplasts are also more easily transformed with foreign DNA using approaches such as electroporation (see the subsequent section). In addition, protoplasts isolated from different plants can be forced to fuse together to form a hybrid. If they are regenerated into whole plants, these hybrids formed from protoplast fusion can represent genetic combinations that would never occur in nature. Hence, protoplast fusion can provide an additional means of genetic engineering, allowing beneficial traits from one plant to be incorporated into another plant despite broad differences between the species. When either single or fused protoplasts are transferred to a culture growth medium, cell wall regeneration takes place, followed by cell division to form a callus (figure 43.14). Once a callus is formed, whole plants can be produced either by organogenesis or by somatic cell embryogenesis in culture.

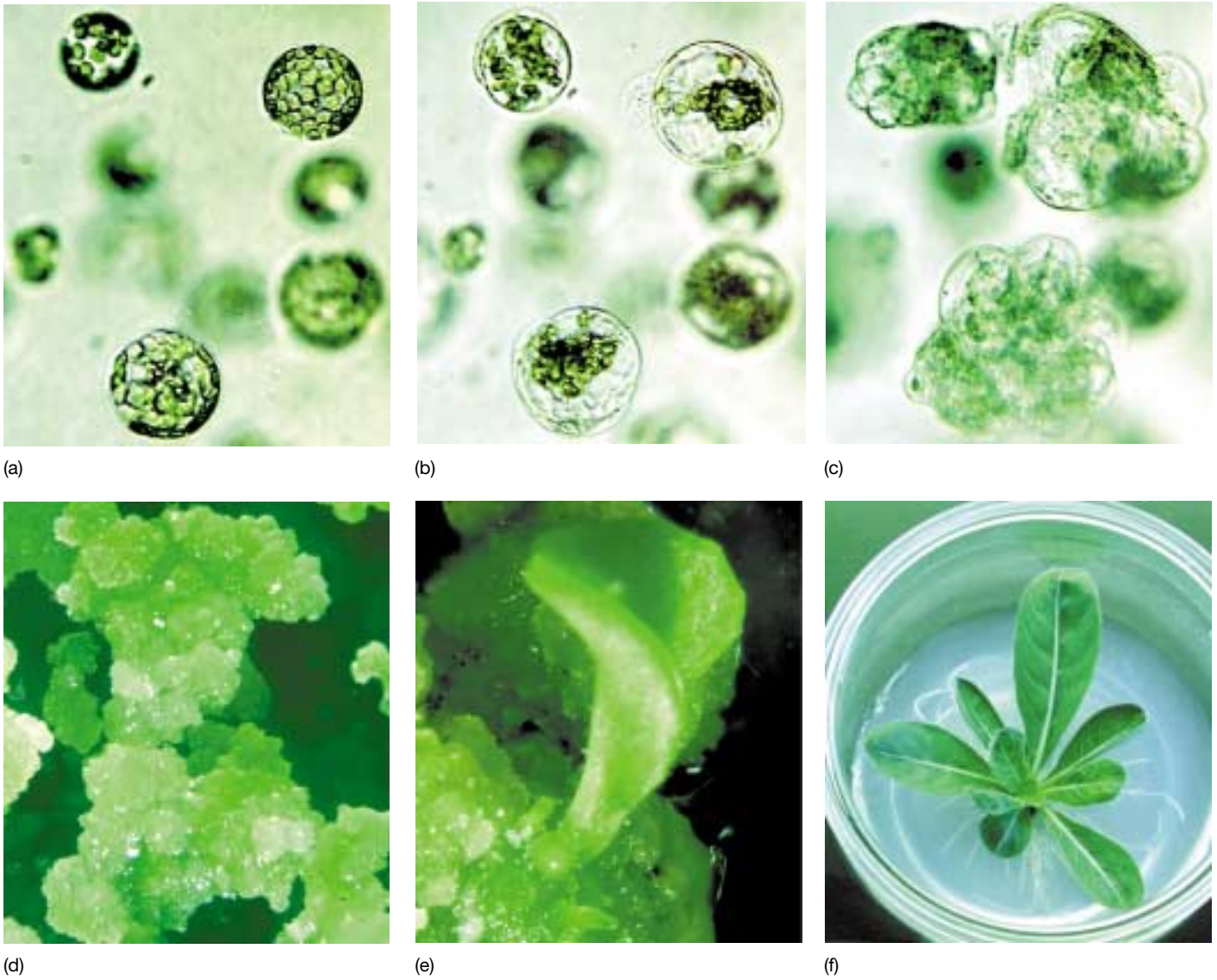


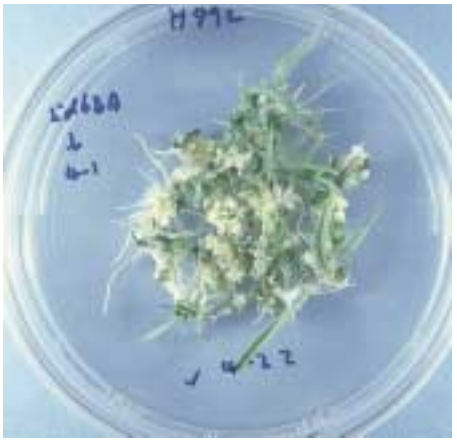
FIGURE 43.14
Protoplast regeneration. Different stages in the recovery of intact plants from single plant protoplasts of evening primrose. (*a*) Individual plant protoplasts. (*b*) Regeneration of the cell wall and the beginning of cell division. (*c*, *d*) Aggregates of plant cells resulting from cell division which can form a callus. (*e*) Production of somatic cell embryos from the callus. (*f*) Recovery of a plantlet from the somatic cell embryo through the process described in figure 43.13.

Anther/Pollen Culture

In flowers, the anthers are the anatomical structures that contain the pollen. In normal flower development, the anthers mature and open to allow pollen dispersal. In anther culture, anthers are excised from the flowers of a plant and then transferred to an appropriate growth medium. After a short period of time, pollen cells can be manipulated to form individual plantlets, which can be grown in culture and used to produce mature plants. The development of these plantlets usually proceeds through the formation of embryos (figure 43.15). Plants produced by anther/pollen culture can be haploid because they were originally derived from pollen cells that have undergone meiosis. However, these plants may be sterile and thus not useful for breeding or genetic manipulation. On the other hand, plants derived from anther/pollen culture can be treated at an early stage with chemical agents such as colchicine, which allows chromosome duplication. Chromosome duplication results in the conversion of sterile haploid plants into fertile diploid organisms. Under these conditions, plants can be produced that are homozygous for every single trait, even those which tend to be recessive traits. On a cautionary note, not every cell exposed to colchicine becomes diploid. Some have unusual ploidy levels, and they can be screened for chromosome number. The homozygous plants are useful tools, allowing breeders to introduce a normally recessive trait.

(a)

(b)



(c)

FIGURE 43.15
Anther culture. Callus formation from maize pollen. Anthers containing pollen can be regenerated on tissue culture medium. The pollen in the anthers contain a haploid set of chromosomes, which can be doubled to form a homozygous diploid cell. Regenerated homozygous diploid plants are important for plant breeding purposes. (*a*) Maize anthers in culture medium. (*b*) Callus formation from pollen. (*c*) Callus and shoot formation.

Plant Organ Culture

Plant organs can also be grown under culture conditions, and this has provided a useful tool in the study of plant organ development. For example, pollinated flowers of a plant such as a tomato can be excised and transferred to a culture flask containing an appropriate medium. Over time, the ovular portion of the plant will develop into a tomato fruit that will eventually turn red and ripen. Sections of plant roots can also be excised and transferred to a liquid growth medium. In this medium, the roots can proliferate extensively, forming both primary and secondary root branches (figure 43.16).

FIGURE 43.16
Plant organ culture. Plant roots growing in a liquid culture medium. From small excised sections of plant roots, the roots will grow and proliferate with extensive lateral root formation (branching).

Many plant cells are totipotent; a whole plant may be regenerated from a single plant cell. Depending upon the explant type, culture medium, and combinations of plant growth regulators, it is possible to grow plant cells, tissues, or organs in sterile cultures.

Applications of Plant Tissue Culture

In addition to the applications already described, plant tissue cultures have a variety of uses both in agriculture and in industry.

Suspension Cultures as Biological Factories

An important industrial application of plant tissue culture involves the use of plant cells as biological factories. Large-scale suspension cultures can be grown to produce antimicrobial compounds, antitumor alkaloids, vitamins, insecticides, and food flavors. Plant roots can also be grown in liquid culture, creating a mesh of roots that can produce a number of useful plant compounds.

Horticultural Uses

Plants with valuable traits can be mass propagated through tissue-culture cloning. In this application of plant tissue culture, hundreds or even thousands of genetically identical plants can be produced by vegetative asexual propagation from one plant source. This has been extensively used in the flower industry where genetically identical plants can be produced from a superior parent plant. Propagation of plant tissue in the sterile environment of the growth medium can also help in the production disease-free plants, such as those cultured from the meristematic (apical dome) tissue untouched by viruses or other diseases because it is new growth. This approach has been particularly useful in the culture of disease-free orchids and raspberries.

(a)

(b)

(c)

FIGURE 43.17 Somaclonal variation. Regeneration of plants from tissue culture can produce plants that are not similar to their parents due to chromosomal alterations. This variability can be used to select plants with altered traits. These maize plants show evidence of somaclonal variation. (*a*) Yellow leaf stripe. (*b*) Dwarf maize. (*c*) Yellow leaf tip.

Somaclonal Variation

Plant tissue culture also has a problematic side effect that can be used as an asset under certain conditions. During periods of extended growth of plant cells in callus or suspension cell culture, various parts of the plant genome may become more or less "active" due to a release of control over gene expression. Transposable elements may also become more active, and chromosomal rearrangements may occur. Sometimes you end up with unusual numbers of chromosomes. This altered control provides a new source of genetic diversity that can result in novel traits which were not even present in the original plant material used as the explant to start the cultures (figure 43.17). This increased genetic diversity following extended time in tissue culture is called **somaclonal variation.** It can be problematic if the desired goal is the propagation or production of identical plant clones. However, somaclonal variation, induced by intentionally growing plant cells in tissue over a longer time period, can be very useful to generate novel plants with traits not currently present in a given gene pool. These traits can be identified either at the tissue culture stage (for example, disease resistance or heat tolerance) or following the regeneration of whole plants by either organogenesis or embryogenesis (plant size, photosynthetic rates, and so forth).

Plant cell, tissue, and organ cultures have important applications in agriculture and industry.

43.3 Plant biotechnology now affects every aspect of agriculture.

Plant biotechnology provides an efficient means to produce an array of novel products and tools for use by our global society. Agricultural biotechnology has the potential to increase farming revenue, lower the cost of raw materials, and improve environmental quality. Plant genetic engineering is becoming a key tool for improving crop production.

World Population in Relation to Advances Made in Crop Production

Due in a large part to scientific advances in crop breeding and farming techniques, world food production has doubled since 1960. Moreover, productivity of agricultural land and water usage has tripled over this time period. While major genetic improvements have been made in crops through crop breeding, this can be a slow process. Furthermore, most crops grown in the United States produce less than 50% of their genetic potential. These shortfalls in yield are due in large part to the inability of crops to tolerate or adapt to environmental stresses (salt, water, and temperature), pests, and disease (figure 43.18).

The world now farms an area the size of South America, but without the scientific advances of the past 30 years, farmland equaling the entire western hemisphere would be required to feed the world. Nevertheless, the world population is expected to double to 12 billion by the first half of this century, and it is not clear whether current levels of food production can keep pace with this rate of population growth. Many believe the exploitation of conventional crop breeding programs may have reached their limit. The question is how best to feed billions of additional people without destroying much of the planet in the process. In this respect, the disappearance of tropical rain forests, wetlands, and other vital habitats will accelerate unless agriculture becomes more productive and less taxing to the environment. Advances in our understanding of plant reproduction from the molecular to the ecosystems levels are providing tools to further protect natural environments by preventing the spread of modified genes to wild populations.

Although improved farming practices and crop breeding have increased crop yields, it is uncertain whether these approaches can keep pace with the food demands of an ever-increasing world population.

FIGURE 43.18
Corn crop productivity well below its genetic potential due to drought stress. Corn production can be limited by water deficiencies due to drought during the growing season in dry climates.

Plant Biotechnology for Agricultural Improvement

It seems certain that plant genetic engineering will play a major role in resolving the problem of feeding an increasing world population. The nutritional quality of crop plants is being improved by increasing the levels of nutrients they contain, such as beta-carotene and vitamins A, C, and E, which may protect people from health problems such as cancer and heart disease. Biotechnology is now being employed to improve the quality of seed grains, increase protein levels in forage crops, and transform plants to improve their resistance to disease, insects, herbicides, and viruses. Other stresses on plants, such as heat or salt, can be improved by engineering higher tolerance levels.

Compared with approaches that rely on plant breeding, genetic engineering can compress the time frame required for the development of improved crop varieties. Moreover, in genetic engineering, genetic barriers, such as pollen compatibility with the pistil, no longer limit the introduction of advantageous traits from diverse species into crop plants. Once a useful trait has been identified at the level of individual genes and their DNA sequences, the incorporation of this trait into a crop plant requires only the introduction of the DNA bearing these genes into the crop plant genome. The process of incorporating foreign DNA into an existing plant genome is called **plant transformation.** At present, there are several approaches for plant transformation; the use of *Agrobacterium tumefaciens* in this process was described in chapter 19. This approach works best if the plant being transformed is a dicot. However, many food crops, such as the cereal grains (rice, wheat, corn, barley, oats, and so on) are monocots. Two additional plant transformation methods that can be used with both dicots and monocots are discussed in the next section.

Useful Traits That Can Be Introduced into Plants

Although plant transformation represents a relatively new technology, extensive efforts are underway to utilize this approach to develop plants and food products with beneficial characteristics. We discussed a variety of biotechnological applications for crop improvement in chapter 19. Further applications of this approach involve modifications of nutritional quality of foods, phytoremediation, production of plastics, and using plants as "edible vaccines."

Improved Nutritional Quality of Food Crops. Approximately 75% of the world's production of oils and fats come from plant sources. For medical and dietary reasons, there is a trend away from the use of animal fats and toward the use of high-quality vegetable oils. Genetic engineering has allowed researchers to modify seed oil biochemistry to produce "designer oils" for edible and nonedible products. One technique modifies canola oil to replace cocoa butter as a source of saturated fatty acids; others modify the enzyme ACP desaturase for the creation of monounsaturated fatty acids in transgenic plants. High-lauric acid canola has been planted in several countries and used in both foods and soaps.

Attempts are also underway to modify the amino acid contents of various plant seeds to present a more complete nutritional diet to the consumer. A high-lysine corn seed is being developed; this would cut down on the need for lysine supplements that are currently added to livestock feed. Biotechnology has the potential to make plant foods healthier and more nutritious for human consumption. Fruits and vegetables, such as tomatoes, may be engineered to contain increased levels of vitamins A and C and beta-carotene, which, when included in the human diet, may help protect against chronic diseases.

Phytoremediation. Cleaning up environmental toxins to reclaim polluted land is an ongoing challenge. Genetically modified plants offer an enticing solution. Work is progressing on plants that accumulate heavy metals at high concentrations. These plants can then be harvested. Because most of their biomass is water, the dried plants allow for the collection of the metals in a small area. Organic compounds that pose hazards to human health have the potential to be taken up by plants and broken down into harmless components. Modified biochemical pathways are being used to break down toxic substances. Modified poplars, for example, have been engineered to break down TNT.

Plants Bearing Vaccines for Human Diseases. Another very interesting application of plant genetic engineering includes the introduction of "vaccine genes" into edible plants. Here, genes encoding the antigen (for example, a viral coat protein) for a particular human pathogen is introduced into the genome of an edible plant such as a banana, tomato, or apple via plant transformation. This antigen protein would then be present in the cells of the edible plant, and a human individual that consumed the plant would develop antibodies against the pathogenic organism. Currently, researchers are trying to develop such edible vaccines for a coat protein of hepatitis B, an enterotoxin B of *E. coli*, and a viral capsid protein of the Norwalk virus. The measles gene has been introduced into tobacco as a model system and is now being introduced into lettuce and rice. This is a terrific advance for tropical areas where it is difficult to keep the traditional vaccine cold (remember that proteins degrade rapidly as the temperature increases).

Genetic engineering of crop plants has allowed researchers to alter the oil content, amino acid composition, and vitamin content of food crops. Genetic engineering may also allow the production of food crops bearing "edible vaccines."

Methods of Plant Transformation

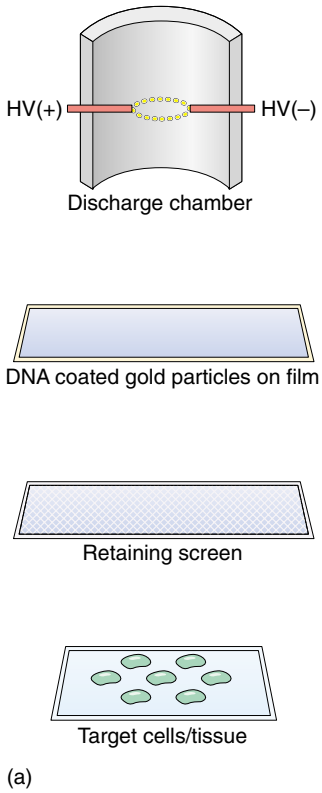
Plant Transformation Using the Particle Gun

Using a "gun" to blast plant cells does not seem like a suitable method for introducing foreign DNA into a plant genome. However, it works, and many whole plants have been regenerated after foreign DNA is shot through the cell wall and then integrated into the plant genome. The *particle gun* utilizes microscopic gold particles coated with the foreign DNA, shooting these particles into plant cells at high velocity. Acceleration of the particles to a sufficient velocity to pass through the plant cell wall can be achieved by a burst of high-pressure helium gas or an electrical discharge (figure 43.19*a*). Only a few cells actually receive the foreign DNA and survive this treatment. These cells are identified with the help of a **selectable marker** also present on the foreign DNA. The selectable marker allows only those cells receiving the foreign DNA to survive on a particular growth medium (figure 43.20). The selectable markers include genes for resistance to a herbicide or antibiotic. Plant cells which survive growth in the selection medium are then tested for the presence of the foreign gene(s) of interest.

Plant Transformation Using Electroporation

Foreign DNA can also be "shocked" into cells that lack a cell wall, such as the plant protoplasts described earlier. A pulse of high-voltage electricity in a solution containing plant protoplasts and DNA briefly opens up small pores in the protoplasts' plasma membranes, allowing the foreign DNA to enter the cell (figure 43.19*b*). Ideally, the DNA incorporates into one of the plant's chromosomes. Following electroporation, the protoplasts are transferred to a growth medium for cell wall regeneration, cell division, and, eventually, the regeneration of whole plants. As with the use of the particle gun, a selectable marker is typically present in the foreign DNA, and protoplasts containing foreign DNA are selected based upon their ability to survive and proliferate in a growth medium containing the selection treatment (antibiotic or herbicide). Once regenerated from electroporated protoplasts, whole plants can then be evaluated for the presence of the beneficial trait.

Plant biotechnology may play an important role in the further improvement of crop plants. The particle gun and electroporation are useful methods for introducing foreign DNA into plants.



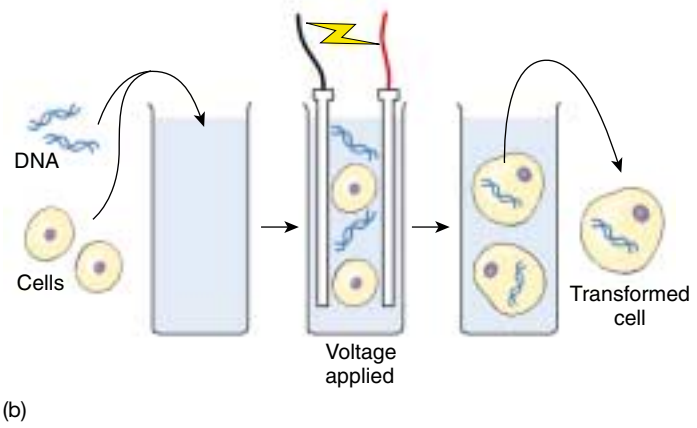
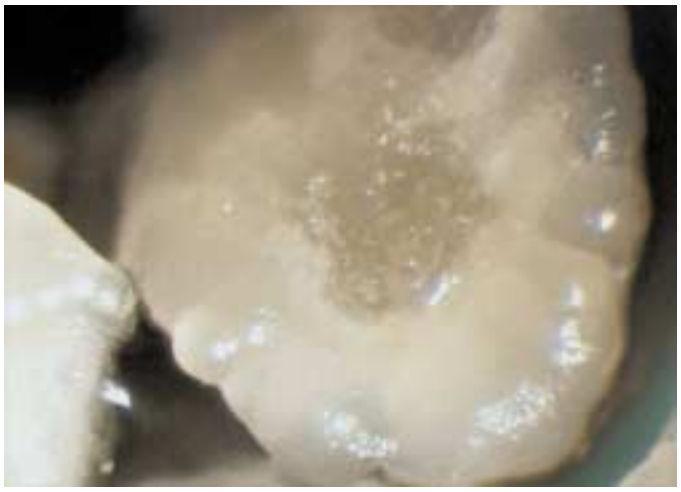


FIGURE 43.19
Methods for plant transformation. (*a*) The particle gun is one method for introducing foreign DNA into plant cells. Here an electrical discharge propels DNA-coated gold particles into plant cells or tissue. A retaining screen reduces cellular damage associated with bombardment by only allowing the DNA-coated particles to pass and retaining fragments of the mounting film. (*b*) Foreign DNA can also be introduced into plant protoplasts by electroporation. A brief pulse of electricity generates pores in the plasma membrane, allowing DNA to enter the cells.



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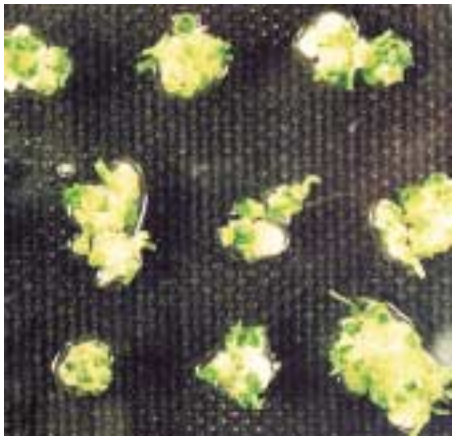

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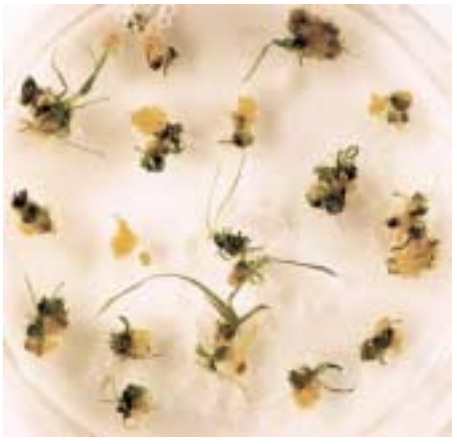

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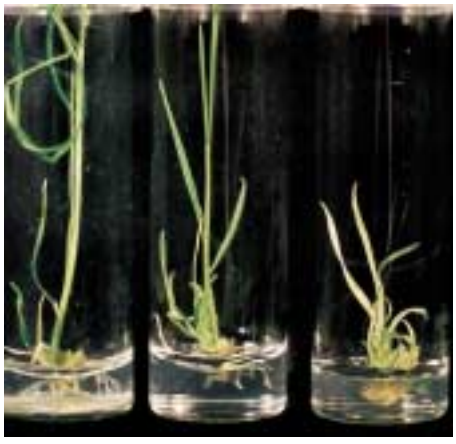

(e)

(f)

FIGURE 43.20
Regeneration after transformation with the use of a selectable marker. Stages in the recovery of a plant containing foreign DNA introduced by the "particle gun" method for plant cell transformation. A selectable marker, in this case a gene for resistance to herbicide, aids in the identification and recovery of plants containing the DNA insert. (*a*) Embryonic callus just prior to particle gun bombardment. (*b*) Following bombardment, callus cells containing the foreign DNA are indicated by color from the *gus* gene used as a tag or label on the foreign DNA. (*c*) Shoot formation in the transformed plants growing on a selective medium. Here, the gene for herbicide resistance in the transformed plants allows growth on the selective medium containing the herbicide. Nontransformed plants do not contain the herbicide resistance gene and do not grow well. (*d*) Production of plantlets from transformed plants growing on the selective medium. (*e*) Comparison of growth on the selection medium for transformed plants bearing the herbicide resistance gene (*left*) and a nontransformed plant (*right*). (*f*) Mature transgenic plants resulting from this process.

Chapter 43

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Summary | Questions | Media Resources

43.1 Genomic organization is much more varied in plants than in animals.

- Plant genomes are very large in comparison to other eukaryotes, mainly due to a high amount of repetitive DNA.
- Plant genomes can be compared with one another by mapping the locations of certain genes or gene traits in various plants. RFLPs and AFLPs can be used to map plant DNA.
- *Arabidopsis thaliana* has a small genome, for a plant. This complete genome is essentially sequenced, so all genes and their positions are known.
- The molecular maps of the genomes of rice and other grains demonstrate remarkable similarity.
- Functional genomics and proteomics will allow us to understand and utilize the information in fully sequenced plant genomes.

1. Describe mechanisms for the generation of highly repetitive DNA in plants.

2. What characteristics of *Arabidopsis thaliana* make it useful as a model system in genetic studies and for the sequencing of its entire genome? Why is rice useful as a model system for the analysis of the genome of a monocot plant?

3. Why will microarrays be useful in functional genomics?

4. What type of questions can be asked now that the *Arabidopsis* and rice genomes are essentially sequenced?

- Scientists on Science: Plant Biotechnology

43.2 Advances in plant tissue culture are revolutionizing agriculture.

- With the addition of appropriate combinations of plant growth regulators (auxin, cytokinin), plant cells in culture can be directed to form organs, embryos, or whole plants.
- Anther cultures can produce haploid plants or plants that are homozygous for all traits.
- Plant tissue culture has a number of practical applications, including the industrial production of plant chemicals, clonal propagation of horticultural plants, and the generation of disease-free plants.
- Growth of plant cells in tissue culture over extended time results in an increase in genetic variation called somaclonal variation. This variation can extend beyond the traits present in the gene pool and can generate novel genetic variations in breeding studies.

5. Describe how whole plants can be regenerated from tissue-cultured plant cells using either organogenesis or somatic cell embryogenesis. Which approach requires the use of suspension cell cultures?

6. How are plant protoplasts generated, and what is protoplast fusion? How can plant protoplasts be used to generate hybrid plants that would not occur in nature?

43.3 Plant biotechnology now affects every aspect of agriculture.

- Genetic engineering and biotechnology can be utilized to improve the quality of food crops, increase disease resistance, and improve the tolerance of crops to environmental stress.
- A key aspect of plant genetic engineering is the introduction of foreign DNA into plant cells. This can be achieved using a particle gun or electroporation.

7. Describe how the particle gun and electroporation can be used to introduce foreign DNA into plant cells. Which approach requires the use of plant protoplasts? Why?

8. How can a plant be "engineered" to produce an edible vaccine ?

- Student Research: Plant Crop Protection