Plants: Novel Developmental Processes

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Plants represent a diverse group of organisms that have unique reproductive, developmental, and physiological processes. Although morphologically simple, plants have molecular genetic processes that are equivalent in complexity to those found in animals. Sophisticated gene transfer procedures, transposon mutagenesis in homologous and heterologous plants, and development of model organisms such as *Arabidopsis* permit almost any gene that is associated with an observable phenotype to be isolated and studied. These advances, coupled with general advances in molecular biology, now make it possible to dissect the molecular and cellular events responsible for controlling plant-specific processes.

THE USE OF PLANTS AS OBJECTS OF STUDY HAS YIELDED important new biological principles (*1*). Hooke's experiments in 1665 with cork, an epidermis-like tissue on the exterior of woody plant stems and roots, first demonstrated the existence of cells. Schleiden contributed to the formulation of the cell theory in 1838 by demonstrating that every plant tissue is composed of cells and that cells are the fundamental units of living organisms. Studies with pea plants enabled Mendel to discover the laws of heredity in 1866, and genetic crosses with corn by McClintock in the 1940s established the concept of transposons and a fluid eukaryotic genome. From a developmental perspective, Steward demonstrated in the late 1950s that differentiated carrot root cells retain the potential to undergo embryogenesis and develop into mature fertile plants; that is, plant cells are totipotent. At the biochemical level, Fraenkel-Conrat showed that tobacco mosaic virus particles can self-assemble in vitro and that RNA can function as the genetic material in the absence of DNA. Finally, experiments by Calvin, Hill, and others in the 1950s showed how carbon and light energy can enter the living world by photosynthesis in green plants.

During the last 5 years there has been a major refocusing on plants as a biological system. The renewed interest in plants arose partly from the realization that gene transfer technology could be used to introduce novel genetic traits into crop plants (*2*). There are now many examples of plants that have been genetically engineered for resistance to herbicides, insects, and viruses. Advances in plant biotechnology also stimulated new strategies to regenerate plants from single cells in culture, provided new approaches to transfer genes from one plant to another, and intensified research on physiological and biochemical phenomena at the molecular level (*3*). Current excitement about plants as experimental organisms also derives from the perception that plants represent a new biological frontier, ready for experimentation on novel aspects of plant biology such as photosynthesis, seed development, reproduction, nitrogen fixation, fruit ripening, pollination, and light control. Although these phenomena have been studied extensively for many years, new technology and the development of accessible plant systems now permit plant-specific problems to be explored with a degree of sophistication not possible a few years ago.

In this article I outline many biological processes that are specific to plants and highlight approaches that can be used to solve important problems of plant biology. I have focused on plant development because this area is under intense scrutiny and because

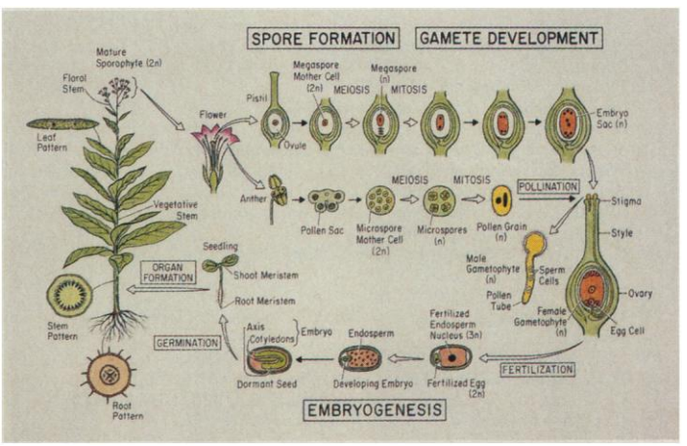

Fig. 1. Life cycle of a flowering plant.

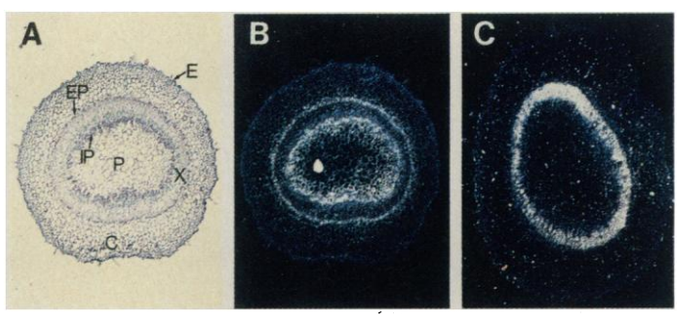

Fig. 2. Localization of mRNAs in the tobacco stem. Floral stems were fixed, embedded in paraffin, and hybridized in situ with single-stranded ^{32}S-labeled RNA probes (*15–17*). (**A**) Bright-field photograph. E, epidermis; C, cortex; P, pith; X, xylem; EP, external phloem; and IP, inner phloem. (**B**) Hybridization with the TS13 probe (*18*). TS13 represents a 0.7-kb mRNA that is present at high levels in the stem but is detectable at lower levels in heterologous organ systems (*19*). Photograph was taken by dark-field microscopy. The white grains represent regions containing RNA/RNA hybrids. (**C**) Hybridization with the TP7 probe (*18*). TP7 represents a 1.4-kb mRNA that is present at high levels in the stem and petal but is also detectable at lower concentrations in other organ systems (*20*). Dark-field micrograph as in (B). RNA/RNA hybrids are indicated by the white grains.

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an understanding of the molecular processes controlling gene expression during plant development should emerge in the not too distant future.

Plants Are a Diverse Group of Organisms

Plants are generally thought of as flowering species such as ornamentals, trees, shrubs, and vegetable crops. Although flowering plants constitute more than 90% of the 275,000 known plant species and are the focus of most plant research, the plant kingdom contains diverse groups of nonflowering plants with unique biological characteristics (*4*). In many cases, nonflowering plants offer experimental advantages over flowering plants for the study of common plant-specific processes.

The plant kingdom contains both nonvascular and vascular species. There are two broad categories of plants (Table 1). Nonvascular plants, such as mosses, do not have specialized water-conducting and food-conducting tissues and, therefore, lack true leaves, stems, and roots. This severely limits the size and habitat of nonvascular plants. By contrast, vascular seedless and seed-producing plants have highly differentiated xylem and phloem cells that conduct water and food over great distances. Both plant groups are thought to have evolved from a common green multicellular alga more than 450 million years ago (*4*). Support for this idea derives from the high degree of similarity in chloroplast genome organization in a flowering plant (tobacco) and a bryophytic nonvascular plant (*Marchantia*) (*5*).

What characteristics do nonvascular and vascular plants share that place them in the same kingdom? First, both are photosynthetic multicellular eukaryotic organisms that are highly adapted for growth and reproduction on land. Second, each has an alternation of a diploid spore-producing generation (sporophytic) and a haploid gamete-producing generation (gametophytic) in its life cycle (Fig. 1). Meiosis in all plant species, unlike that in animals, yields haploid spores rather than gametes (*4*). In vascular plants, the sporophytic phase dominates and the gametophytic phase is dependent on the sporophyte for nutrition and support. By contrast, the gametophyte is free-living in nonvascular plants and dominates the life cycle (Table 1). This characteristic makes nonvascular plants ideally suited for studying processes that are dominant in the gametophyte; for example, gamete production. Third, cellulose is the major polysaccharide found in nonvascular and vascular plant cell walls, and both groups form a cell plate during cell division. Finally, vascular and nonvascular plants store glucose as starch in their chloroplasts and utilize both chlorophyll a and chlorophyll b as light receptors.

Mosses and other nonvascular plants can provide insight into many plant-specific problems. Nonvascular plants offer opportunities to study plant processes that have not yet been fully realized. Because these plants are haploid for most of their life cycle, large numbers of mutants can be obtained for the genetic dissection of physiological and developmental events (*6*). One notable example is the moss *Physcomitrella patens* (*7*). This organism has been developed extensively as a model plant genetic system. *Physcomitrella* plants can be regenerated from protoplasts, and protoplast fusion permits complementation analysis to be carried out. Chemical mutagenesis has been used to induce auxotrophic, morphogenetic, hormonal, phototropic, and gravitropic mutants. Although transformation of *Physcomitrella* has not yet been reported, it should be possible to combine genetic and molecular approaches to identify and study genes controlling important plant processes.

Algae are being used to study specialized plant processes. Although not plants, many protistans and bacteria with plant-like processes are being used for the study of specific plant problems. Blue-green algae (*Cyanobacteria*) are providing new insight into photosynthesis and nitrogen fixation because these processes are analogous to those that occur in plants and, in contrast to the situation with plants, gene replacement studies can be easily carried out (*8*). For example, deletion strains of *Cyanobacteria* have been constructed that lack critical photosynthesis genes and that permit functional dissection of both plant and blue-green algal genes important in photosynthesis (*9*). Similarly, the unicellular green alga *Chlamydomonas* has been used to dissect genetically photosynthetic reaction centers and chloroplast biogenesis (*10*). Uniparental genetics have permitted many mutants that are defective in important photosynthetic proteins to be induced (*11, 12*). For example, mutants containing defective photosystem II proteins have been used to localize amino acids required for interaction with electron acceptors in the photosystem II complex (*11, 12*). Because *Chlamydomonas* contains only one chloroplast and because numerous chloroplast mutants exist, the recent development of a chloroplast transformation system in this organism (*13*) should permit a functional dissection of genes and proteins critical for light reception and harvesting.

Finally, the multicellular brown alga *Fucus* has been used extensively for studying egg cell development and the establishment of embryonic form (*14*). Unlike higher plant egg cells that reside within the sporophyte and cannot be readily isolated (Fig. 1), *Fucus* eggs can be purified in large numbers, fertilized in vitro, and radioactively labeled (*14*). Although few molecular studies have been carried out and DNA transformation has not yet been reported, *Fucus* is a valuable model for studying how plant eggs become polarized after fertilization and how the embryonic axis is established in the absence of cell movement.

Higher Plants Have Novel Developmental Processes

Flowering plants are the most complex and intensely investigated organisms within the plant kingdom and have all of the features specific to plants as a biological system. These plants evolved from nonflowering seed-producing relatives at least 120 million years ago, and within a short time interval (60 million years) became the

Table 1. Diversity of plants. Taxonomic groupings and time of first appearance were taken from (*4*); mya, million years ago.

Category	Division	Examples	Dominant generation	Time of appearance (mya)
Nonvascular	Bryophyta	Mosses, liverworts	Gametophyte	430
Vascular				430
Seedless	Psilophyta	Psilatum	Sporophyte	
	Lycophyta	Club mosses	Sporophyte	
	Sphenophyta	Horsetails	Sporophyte	
	Pterophyta	Ferns	Sporophyte	
Seeds				360
Nonflowering	Cycadophyta	Cycads	Sporophyte	
	Ginkgophyta	Maidenhair tree	Sporophyte	
	Coniferophyta	Pines, firs	Sporophyte	
	Gnetophyta	Genetum, welwitschia	Sporophyte	
Flowering	Anthophyta	Soybean, corn	Sporophyte	125

pre-eminent plant group (*4*). Flowering plants undergo many unique developmental events during their life cycle (Fig. 1). Events such as spore and gamete formation, seed development, and organogenesis from meristems are shared with one or more other plant groups. However, the presence of a flower, which functions exclusively in reproduction, adds additional developmental complexity and can be studied only by using flowering plants as experimental organisms. Developmental processes unique to higher plants include regeneration potential of single cells, reproduction, seed development, indeterminate growth patterns, morphogenesis in the absence of cell movement, and environmental induction of specific developmental states.

Plants are morphologically simple organisms. A striking feature of higher plants is their apparent morphological simplicity. This contrasts greatly with the situation in higher animal forms such as vertebrates. During the life cycle of the flowering plant, only three vegetative organ systems (leaf, stem, and root) and three reproductive organ systems (petal, stamen, and pistil) are formed (Fig. 1). Additional organs, such as the fruit (a mature ovary) and embryonic cotyledon (seed leaf), form during specific developmental periods and are equivalent to vegetative and floral organ systems in structural complexity.

Each plant organ system is highly specialized to carry out specific biological functions such as photosynthesis (leaf), support and water conduction (stem), water absorption (root), spore formation (pistil and stamen), and food reserve synthesis (cotyledons). Irrespective of their specialized nature, plant organ systems are composed of anatomically similar cells and tissues. Collectively, only ten basic tissue types containing approximately 15 structurally diverse cells are represented in the organ systems of higher plants (*4*). For example, five prominent tissues can be visualized in the cross section of a tobacco floral stem (Fig. 2A): epidermis, cortex, xylem, phloem, and pith. With the exception of the cortex and pith, each tissue is structurally unique and contains specialized cell types. The pith and cortex both contain storage parenchyma cells and are similar tissues that differ only by their position relative to the vascular cylinder. In situ hybridization studies (*15–17*) indicate that many mRNAs are nonrandomly distributed within the stem and are preferentially localized within specific tissues (*18–20*). A stem mRNA, designated TS13 (*19*), is highly concentrated within the inner and outer phloem cells, as shown in Fig. 2B. TS13 mRNA sequences are not detectable in other floral stem tissues, although they may be present at concentrations below those observable with the in situ hybridization procedure. By contrast, Fig. 2C shows that a nonhomologous stem mRNA, designated TP7 (*20*), is localized preferentially within the xylem and is not detectable in phloem or other stem tissues. These findings indicate that plant tissues with distinct functions can be distinguished at the molecular level.

From an anatomical point of view, many tissues and cells in plant organ systems appear to be identical. Major differences in plant organ system morphology depend primarily on how the various tissues are organized into spatial patterns rather than apparent differences in tissues and cell types (Fig. 1). Thus, the leaf and root have tissues analogous to those shown in Fig. 2A for the floral stem. Because each organ system carries out unique functions, the morphological similarity must mask inherent differences at the biochemical level. Support for this notion derives from a comparison of the nuclear RNA and messenger RNA sequence sets in all tobacco organ systems (*21, 22*) (Fig. 3). First, each floral and vegetative organ system has a nuclear RNA complexity (1.2×10^5 kb) and an mRNA complexity (3.5×10^4 kb) equivalent to those observed for complex animal organ systems such as the liver and kidney (*23*). Thus, despite their apparent morphological simplicity, a very complex genetic program is expressed in each organ system. Second, each organ system has a large gene set that is not detectably expressed at the mRNA level in heterologous organs. For example, the stem has approximately 6000 diverse mRNA species—representing 25% of the stem mRNA complexity—that are not detectable on the polysomes of other vegetative and floral organ systems (*21, 22*). Finally, in situ hybridization studies with the TS13 stem mRNA (Fig. 2B) did not detect this sequence in the phloem of the root and petal, suggesting that there is a molecular diversification of similar tissues in distinct plant organ systems (*18, 19*). A major unsolved problem of plant biology is the identity of proteins and processes that confer uniqueness on plant cells; that is, what is the nature of gene products expressed in specific plant cells and tissues, and how are these products integrated into processes that are unique to each organ system?

Plant cells are totipotent. A remarkable property that distinguishes plant cells from their animal counterparts is the capacity to regenerate into mature fertile plants (*24, 25*). Although not all plant cells retain their regenerative capacity, this property is the basis for the success of gene transfer technology in producing genetically engineered higher plant species (*2, 3*). Many plant cells have the capacity to reorganize into embryos and undergo somatic embryogenesis analogous to that which occurs after fertilization (*24, 25*). Alternatively, under the proper conditions, somatic cells can establish meristematic foci and are able to regenerate into mature plants through an organogenesis pathway (*24*). The regenerative capacity is not confined to diploid sporophytic cells, because microspores also have the potential to regenerate into haploid plants. The totipotency property of plant cells implies that they have a complete unrearranged set of genes and that this gene set retains the potential to establish and maintain all differentiated higher plant states.

The underlying cellular and molecular processes that enable plants to regenerate via embryogenic or meristematic pathways are not yet understood. In some cases, the inability to regenerate routinely from single cells commercially important varieties of many plants (for example, corn and soybean) has been an impediment to producing agriculturally superior crops by gene engineering technology (*2, 25*). The fact that differentiated plant cells with restricted functions (for example, microspore and parenchyma storage cells) can regenerate into fully mature plants raises two important questions regarding plant development. First, what is the meaning of determination in higher plants? Although a root cell or a microspore is determined within the context of the normal plant life cycle, these cells retain a flexible capacity to serve as foci for the differentiation of an entire multicellular plant. Clearly, this is very different from the situation in animals. Second, because many plant cells are capable of reorganizing into embryos, the role of the maternal or egg gene expression program on subsequent embryogenesis is unclear. That is, are maternal agents or factors important in establishing embryo-specific gene expression programs early in development? Either the egg cell plays a minor role in embryonic morphogenetic events, or somatic plant cells can be induced to express a maternal gene expression program before embarking on an embryogenetic pathway. Whatever the answer, the molecular basis of plant cell totipotency and the identification of cellular reorganization processes that must occur to establish a regenerative potential remain major unresolved problems.

Plants reproduce using both spores and gametes. As pointed out earlier, plants undergo an alternation of spore- and gamete-forming generations (Fig. 1). Sporogenous cells within the anther and ovary of the flower undergo meiosis and give rise to haploid microspores and megaspores, respectively. The haploid spores divide mitotically and differentiate into a three-celled male gametophyte (pollen grain) and a seven-celled female gametophyte (embryo sac). The pollen grain contains two sperm cells, whereas the embryo sac contains one

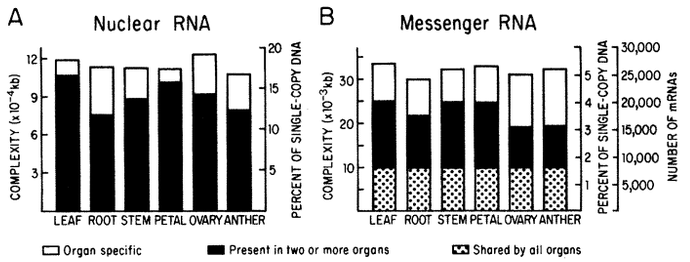

Fig. 3. Regulation of gene expression in flowering plants. Data represent the results of solution RNA–excess hybridization experiments with tobacco single-copy DNA and were taken from Kamalay and Goldberg (*21, 22*). Complexity values were calculated by using a tobacco single-copy DNA complexity of 6.4×10^5 kb (*21*). (**A**) Hybridization with nuclear RNA populations. Total nuclear RNA [poly(A)$^+$ and poly(A)$^-$] was used for these experiments (*22*). In general, the nuclear RNA/mRNA complexity ratio for any given organ system is approximately 3.6. (**B**) Hybridization with mRNA populations. Experiments were performed with total polysomal RNA [poly(A)$^+$ and poly(A)$^-$] that was released from polysomes with EDTA (*21*). The number of diverse mRNA species in each mRNA population was calculated by assuming an average transcript size of 1.2 kb (*21*). Leaf and petal mRNA sets are qualitatively indistinguishable from each other in population hybridization experiments (*21*). Leaf and petal white bar areas represent mRNA species that are present in both the leaf and petal but are undetectable in heterologous organ system mRNAs. Recent experiments with petal cDNA clones indicate that there are quantitative differences with respect to specific mRNA species in the leaf and petal (*20*).

egg cell. By contrast with gametogenesis in animals, the gametes are produced mitotically from cells that are not present in the embryo. Thus, plants do not have a specialized germ line that is set aside during embryogenesis.

The molecular events leading to gamete formation in higher plants are largely unexplored. Male gametophyte development has been investigated to a limited extent because developing pollen grains can be isolated and studied (*26*). Expression of both the diploid anther genome and the haploid microspore genome is required to produce a mature pollen grain with functional sperm cells (*26*). Gene expression studies have shown that there are approximately 25,000 diverse polyadenylated [poly(A)] RNAs present in the mature pollen grain and that many of these RNAs are represented in the sporophyte as well (*26*). The distribution of these RNAs within the three male gametophytic cells is unknown. Sperm cell isolation procedures (*27*) coupled with in situ hybridization studies (Fig. 2) should permit the cellular processes leading to sperm cell differentiation to be studied in more detail.

By contrast, egg cell development has been studied only with cytological procedures (*24*), and nothing is known about the gene expression programs that occur during egg cell formation. This differs greatly from the situation in animals for which there is a large body of information on the molecular biology of egg development (*28*). Because the higher plant egg cell is one of seven cells in the female gametophyte, and because the female gametophyte is buried within several layers of ovary and ovule cells (Fig. 1), it has not yet been possible to isolate egg cells in order to carry out molecular studies. Thus, as already pointed out, the contribution of maternal gene products to early embryonic events is largely unknown in plants. In the absence of techniques that permit easy access to large numbers of egg cells, plant egg cell development may be better studied by using a nonplant model such as *Fucus* (*14*). A conceptual understanding of plant development will require a functional characterization of the gene products and regulatory events required to differentiate haploid spores into male and female gametophytes. This information may suggest novel ways to create male-sterile and female-sterile plants useful for hybrid crop production (*2*).

Dual fertilization processes occur in higher plants. In addition to an alternation of spore- and gamete-forming generations, flowering plant reproduction requires two independent fertilization events to trigger sporophytic development (Fig. 1). Because the male and female gametophytes reside in different sporophytic organ systems, a pollination process is required to transfer sperm cells within the male gametophyte to the pistil where the egg cell resides (Fig. 1). Pollination and fertilization represent major examples of the small number of developmental processes in higher plants that require specific cell-cell recognition events to occur, in contrast to the situation in animals.

Specific pollen grain–pistil interactions must occur to ensure pollination of homologous plant species. Molecular studies suggest that specific proteins must be deposited on the pollen wall and on the surface of pollen receptor cells on the pistil (the stigma) (*29*). Similarly, the sperm cells must recognize two of the seven female gametophyte cells. One sperm cell fertilizes the egg to form a diploid zygote that will develop into the new sporophyte. The other unites with two previously fused nuclei (central cell nucleus) to form a triploid endosperm (Fig. 1). The endosperm develops into a terminally differentiated, nonembryonic tissue that is responsible for supplying nutrients to the embryo during embryogenesis or to the developing sporophyte after seed germination (Fig. 1). Sperm cells appear to be differentiated prior to fertilization and unite with specific female gametophytic cells (*30*)—that is, the double fertilization process is directed and not random. The molecular basis of sperm cell specification and the determinants that enable specific sperm cell–female gametophyte interactions to take place are not yet understood and require critical investigation.

Plant embryogenesis terminates with the formation of a dormant seed. In contrast to the situation in animal development (*28*), most major ontogenetic events in higher plants occur postembryonically. Plant embryogenesis terminates with the formation of a dormant embryo that is packaged within a seed (Fig. 1). Plant embryogenesis, unlike the development of many animal species (*28*), does not lead to the production of an organism that resembles a mature plant (Fig. 1). Rather, after seed dormancy ends, the sporophyte develops from meristematic tissues that are specified during embryogenesis.

The seed permits the embryo to persist for long periods of dormancy and is present within a fruit that is responsible for dispersing the embryo over large distances (*4*). Genetically controlled events cause the ovary to differentiate into a fruit with an elaborate structure designed for a particular dispersal process (*4*). What cellular signals trigger the differentiation of an ovule into a seed (Fig. 1), and what molecular processes program the development of an ovary into a fruit, remain unresolved plant-specific problems.

Higher plant embryos form two organ systems with different developmental fates. The embryo differentiates during embryogenesis into two organ systems—the axis and the cotyledon—that have different developmental fates. The cotyledon is a terminally differentiated organ system that senesces after germination and synthesizes highly specialized food reserves that are used by the germinating seedling. By contrast, the axis contains the root and shoot meristems that will give rise to sporophytic organ systems through the life cycle. Figure 4 shows the development of a soybean embryo and a tobacco embryo (*31*). In both cases an embryo can be visualized that is contained within the developing seed and that is embedded within nonembryonic endosperm tissue. A striking event in plant embryogenesis is the change from a globular embryo with radial symmetry (Fig. 4, A, B, and H) to a heart-shaped embryo with bilateral symmetry (Fig. 4, C, I, and J). At this embryogenic stage the cotyledons begin to differentiate, the plant body becomes polarized, and the root-shoot axis forms (Fig. 4, C and J). In analogy to the situation with the egg cell, the plant embryo is embedded deep

within the developing fruit (Fig. 1), making it difficult to isolate large numbers of globular and heart-shaped embryos for molecular studies. As a result, the gene expression programs that occur during the very early stages of plant embryogenesis, as well as the molecular and cellular processes that direct embryo polarization and specify the cotyledon and axis cell lineages, are not known. The use of cultured somatic embryos (*32*) and embryo-lethal mutants (*33*) may provide a novel opportunity to investigate critical genetic events that take place during early embryogenesis.

Approximately 20,000 diverse genes are expressed by both the axis and the cotyledon during embryogenesis (*34*). Most of these genes encode rare class mRNAs of unknown function. A small gene set, however, directs the synthesis of highly prevalent mRNAs that encode seed proteins that are packaged preferentially into cotyledon cell storage bodies (*35*). Seed protein genes are highly regulated during the plant life cycle. These genes encode mRNAs that accumulate and decay during embryogenesis and are either absent from or present at low concentrations in mature plant organ systems (*34–38*). One example of a cell-specific seed protein gene expression program is shown in Fig. 4G (*31*). In situ hybridization shows that soybean lectin mRNA (*36*) is highly localized within cotyledon parenchyma cells and is not detectable within the vascular tissue (*31*). Soybean lectin mRNA is localized within analogous cells in transformed tobacco plants (*31, 37*) (Fig. 4M), indicating that the regulatory machinery controlling seed protein gene expression is highly conserved in divergent plant species. Recent studies have begun to identify the cis elements and trans factors that control seed protein gene expression (*38*). The precise regulatory network responsible for controlling seed protein gene expression is not yet known.

Plants have indeterminate developmental programs. As pointed out above, most higher plant developmental events occur after seed germination from meristems contained within the embryonic axis (Fig. 1). Both the shoot and root meristems are determined by the end of embryogenesis and are committed to pursue a given developmental pathway. Plant meristems consist of continuously dividing cells that regenerate themselves and are committed to produce specific organ systems. Meristematic cells are analogous to animal stem cells because they yield a new meristem upon division. However, in contrast with animal stem cells, plant meristems lead to the formation of complex organ systems. In addition, each meristem differentiates into three primary meristems: protoderm, ground meristem, and provascular cambium (*4*). In the case of the stem (Fig. 2), the primary meristems give rise to the epidermis, cortex and pith, and xylem and phloem tissues, respectively. The cellular processes responsible for specifying the meristems during embryogenesis are not yet understood.

The conversion of a vegetative shoot meristem into a flower-producing, floral meristem breaks the continuous development cycle. A floral meristem does not regenerate itself and leads to determinate growth and development of the flower. Diffusible substances, designated as florigens, trigger the conversion of a vegetative meristem to a floral meristem (*39*). What these flower-inducing agents are and how they mediate a pattern reorganization within the shoot meristem are not known.

Floral organ systems express highly regulated gene sets, and each organ of the flower has a unique mRNA collection that is not detectable in heterologous vegetative and floral organ system (*21, 22*) (Fig. 3). Experiments with individual floral mRNAs indicate that gene expression is regulated both temporally and spatially during flower development (*40–42*). Figure 5 shows the development of a tobacco flower, and the differentiation of the pistil and anther (*40*). The developmental dot blots (Fig. 5) indicate that two anther-specific mRNAs, designated TA13 and TA29, accumulate early in anther development and then decay prior to anther dehiscence and pollen grain release. By contrast, two different anther mRNAs, TA20 and TA25, persist throughout anther development. Localization of the TA20 and TA29 mRNAs within floral organ systems is shown in Fig. 6 (*41*). The TA20 mRNA is highly concentrated in the cell layer that connects the ovule to the ovary, as well as in specific ovary wall regions (*41*) (Fig. 6, A and B). Within the anther the TA20 mRNA is localized in all tissues except the filament and the tapetum (*41*). By contrast, the TA29 mRNA is present within the anther tapetum cell layer, and is not detectable in other anther tissues (Fig. 6, C and D). The decay of the TA29 mRNA parallels the degeneration of the tapetal cell layer late in anther development (*26, 41*). These data indicate that different cell-specific and time-specific gene expression programs occur during flower development. The molecular processes responsible for establishing flower-specific gene expression programs are not yet understood.

Morphogenesis in plants occurs in the absence of cell movement. A major feature that distinguishes plant and animal cells is the presence of a plant cell wall. Because plant cells are glued together after division, morphogenesis must occur in the absence of cell movement. Distinct morphological patterns are produced by a combination of asymmetric cell division, division in distinct planes, differential growth after cell division, and different division rates (*4*).

As pointed out in the previous section, the morphological pattern that gives rise to each plant organ system is an intrinsic property of the meristem. How patterns are established within the root, shoot, and floral meristems to give rise to organs with specific morphologies is not yet known. Clonal analysis has provided some insight into the fate of specific meristematic cells (*43*). However, what causes the meristem to become organized and committed to a specific organ system pattern remains a central problem of plant development (*44*). As in *Drosophila*, homeotic mutants exist in plants that convert one floral organ system into another (for example, anther to pistil) (*45*). In addition, mutants exist that alter flower morphology (*46*). These mutants may prove useful for dissecting the genetic and cellular events required to establish unique plant morphological forms.

Environmental factors play a major role in plant development. Finally, in contrast with animals, plants are highly dependent on their environment and use extrinsic cues such as light, water, and temperature to trigger specific developmental events (*4*). For example, seed germination depends on temperature to break embryonic dormancy and on water to reinitiate cell division and metabolic processes (*4*). Physical forces such as gravity also play major roles in plant development. Much research has been carried out in these areas (*47*), particularly with light control (*48*). Photoreceptor proteins such as phytochrome have been identified and shown to participate in light-regulated gene expression programs (*47, 48*). However, the exact mechanisms by which light and other environmental signals are perceived by plant cells and converted into molecular genetic information are not yet understood.

Higher Plants Have Complex Genetic Processes

As outlined above, plant developmental events are different from those found in the animal kingdom and are simpler in many cases. Paradoxically, gene expression processes that ultimately control and guide plant development are similar to those found in animal cells and are equally complicated. Plant genomes are as large and complex as those observed in the animal kingdom (*49*). The corn plant, for example, has a genetic potential equal to that of humans despite much less apparent biological complexity (*50*). In contrast with

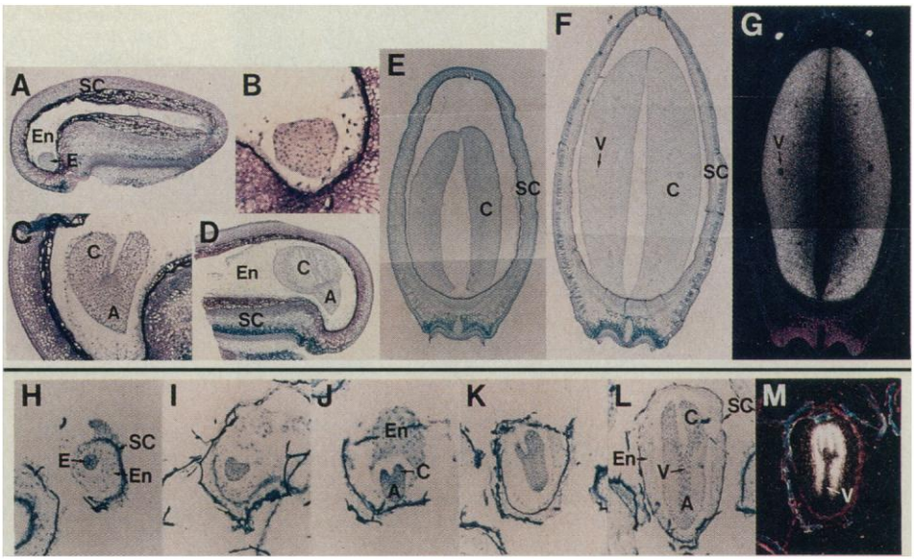

Fig. 4. Localization of seed protein mRNA in soybean and transformed tobacco seeds. (A–F) Bright-field photographs of soybean seed development (*31*). (**A** and **B**) Globular embryo, (**C**) heart stage embryo, (**D**) cotyledon stage embryo, (**E** and **F**) maturation-stage embryos. (**G**) In situ hybridization of a labeled single-stranded lectin RNA probe with a longitudinal section of a maturation stage soybean embryo (*16, 17*). The photograph was taken by dark-field microscopy, and the white grains represent regions with RNA/RNA hybrids. (H to L) Bright-field photographs of tobacco seed development. Tobacco seed development was characterized by Barker and Goldberg (*16*). (**H**) Globular stage embryo, (**I** and **J**) heart-stage embryos, (**K** and **L**) torpedo or maturation-stage embryos. (**M**) In situ hybridization of a labeled lectin mRNA probe with a longitudinal section of a transformed tobacco seed containing a soybean lectin gene (*37*). The photograph was taken by dark-field microscopy (*16, 17*). Abbreviations: E, embryo; En, endosperm; SC, seed coat; C, cotyledon; A, axis; and V, vascular tissue.

simple eukaryotes such as fungi (*51*), plants have complex nuclear RNA populations (*22*). The nuclear RNA complexity of each organ system is approximately four times that observed in the cytoplasm (Fig. 3), and reflects, in part, transcription of introns contained within plant genes (*52*). DNA sequencing studies and functional analysis of plant genes in transformed plants indicate that they have developmental control elements, splice junctions, promoters, and poly(A) addition sequences analogous to those found in the animal kingdom (*3, 52*). A large number of genes are expressed in plant organ systems, and plant gene expression is highly regulated (Fig. 3) (*21, 22*). Finally, both transcriptional and post-transcriptional events program plant gene expression (*21, 22, 53*). Experiments designed to unravel the molecular basis of plant gene expression will have to deal with relatively large genomes that express complex gene sets. Even the small *Arabidopsis* genome has 70,000 kb of diverse single-copy sequence (*45*). Ultimately, the major differences in plant and animal gene expression programs will involve the nature of signals that activate and repress specific genes—that is, how do unique plant physiological processes interface with gene expression events to trigger novel plant developmental states?

Higher Plants Have Many Advantages as an Experimental System

In the preceding sections I outlined many problems specific to plants. How difficult is it to study these problems at the molecular level? First, plant gene transfer technology is highly developed, easy to use, and applicable to a large number of plant species (*2, 3*). Plant cells can be transformed by microinjection, by use of *Agrobacterium* Ti plasmid vectors, or by direct uptake of exogenous DNA (*2, 3*); for example, it is not uncommon to obtain a 20% plant cell transformation frequency with Ti plasmid vectors (*54*). In addition, a single transformed tobacco cell can produce at least 250,000 seeds (*55*). Thus, investigating a large number of transformed plants is relatively easy. There are now many examples of development-specific and constitutively expressed genes that have been investigated in either transformed plants or protoplast cultures (*2, 3*). Second, because plant cells are totipotent, expression of modified genes can be studied throughout the plant life cycle and in any cell type that can be visualized by in situ hybridization or marker gene localization procedures (Fig. 4) (*2, 3, 15*). Transformation experiments have been carried out with genes expressed during embryogenesis (Fig. 4), as well as with those active in the mature sporophyte (*3*). Third, genetically defined transposon systems in corn, snapdragon, and petunia are available for the identification of specific plant genes by transposon tagging (*56–58*). Several corn regulatory loci have been isolated by this procedure. These include the cI gene that regulates

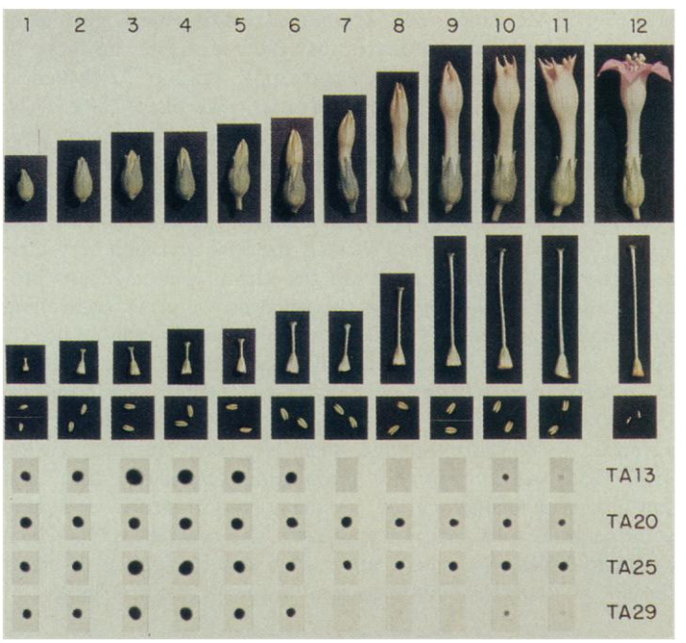

Fig. 5. Regulation of gene expression during tobacco flower development. Tobacco flower development was divided into 12 stages, with floral bud and petal lengths as developmental markers (*40*). In general, the transition from stage 1 to stage 12 occurred over a 1- to 2-week period depending on the time of year. Stage 1 and stage 12 flowers averaged 0.8 cm and 4.6 cm in length, respectively. Anther mRNAs were isolated from 12 stages of flower development, spotted onto Nytran membranes, and hybridized with labeled anther cDNA clones (*40*). TA13 and TA29 cDNA clones represent divergent mRNAs of the same gene family (*40*). By contrast, TA20 and TA25 cDNA clones represent unique anther mRNAs. At their peak levels, TA13, TA29, TA20, and TA25 mRNAs represent 0.42%, 0.26%, 0.48%, and 0.23% of the anther mRNA mass, respectively (*40*). TA13, TA25, and TA29 mRNAs are not detectable in heterologous vegetative and floral organ system mRNA populations. TA20 mRNA is represented at lower prevalences in pistil and petal mRNAs.

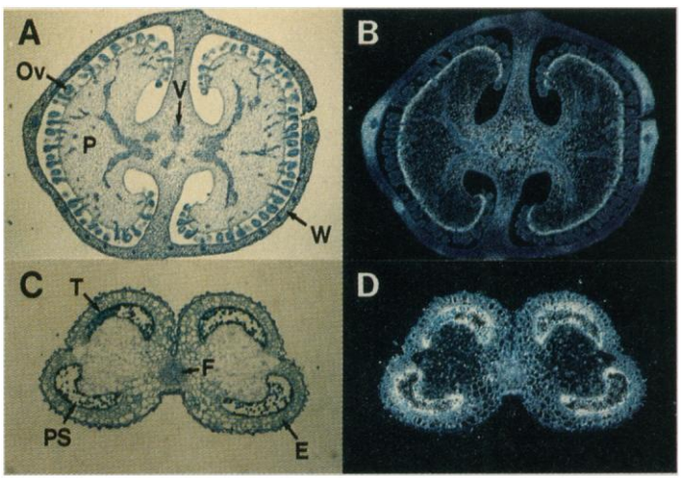

Fig. 6. Localization of mRNAs in the tobacco anther and ovary. Stage 6 ovaries and stage 4 anthers (Fig. 5) were fixed, embedded in paraffin, and hybridized in situ with single-stranded ^{35}S-labeled RNA probes (*15–17*). (**A** and **B**) Hybridization of an ovary cross section with the TA20 probe (Fig. 5). The TA20 mRNA is approximately 20% less prevalent in the pistil than in the anther (*40, 41*). (A) Bright-field photograph. (B) Dark-field photograph. White grains represent regions containing RNA/RNA hybrids. (**C** and **D**) Hybridization of an anther cross section with the TA29 probe (Fig. 5). (C) Bright-field photograph. (D) Dark-field photograph. White grains represent regions with RNA/RNA hybrids. Abbreviations: Ov, ovule; P, placenta; V, vascular tissue; W, ovary wall; PS, pollen sac; T, tapetum; and E, endothecium.

anthocyanin biosynthesis (*57*) and the opaque-2 gene that controls seed protein deposition (*58*). Transformation of heterologous plant species by transposons indicates that these elements can move in foreign cell environments (*59*). For example, the corn Ac element is transposable in tobacco, carrot, and *Arabidopsis* plants (*59*). Thus, insertional mutagenesis schemes can be devised to isolate any gene that produces a scorable phenotype. Fourth, the *Arabidopsis* plant provides an accessible organism with which to study plant-specific processes (*45*). Because *Arabidopsis* has a small genome (70,000 kb), a short life cycle (6 weeks), a small size (30 cm), and very little repetitive DNA, large transposon and chemical mutagenesis programs can be carried out for the selection of genes controlling specific plant processes (*45*). Genes with chemically induced defects can be isolated, in principle, by complementation with wild-type DNA segments that flank restriction fragment length polymorphism (RFLP) markers linked to mutant genes (*45*). Finally, general advances in molecular biology, such as protein microsequencing (*60*), DNA binding protein purification procedures (*61*), and antisense gene technology (*62*) are being used to identify and study plant genes by a biochemical approach. The relative ease by which both molecular and genetic approaches can be applied in plants should facilitate the solution to many plant-specific problems.

Conclusions

Plants offer a large number of exciting and unexplored biological questions that cannot be examined in other organisms. Insight into plant-specific processes may suggest novel ways to produce superior crops by gene engineering technology. Despite the difficulties in studying certain aspects of the higher plant life cycle of higher plants and the long generation time in many plant species, there is an unprecedented opportunity to study plants at the molecular and cellular level.

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