whether or not geneticists should continue to use the traditional methods of inducing and recovering mutants in whole, complicated plants. The answer will depend on what sort of mutant is desired and the ability of the biological assay system to permit recognition of it. When the phenomenon to benefit from mutational analysis involves sequential action of molecules compartmentalized in space or time, or all but the most simple molecular interactions, classical genetic technology is the cutting edge. For example, there is little question that classical genetics would have the greater chance of finding a mutant altering the anaerobic gene program itself. Since we know so little about the rules or mechanism governing how development controls groups of genes, it is wise to allow the organism maximum freedom to give us clues. Once particular DNA sequences are defined, then *in vitro* genetics becomes the approach of choice.

Michael Freeling, Associate Professor, Genetics, U.C., Berkeley.

Leaf protein synthesis

William C. Taylor
Timothy Nelson
Mark Harpster
Lino Fragoso
Belinda Martineau
Steven Mayfield
Judy Yamaguchi

The most abundant proteins in the leaves of higher plants perform specialized functions in photosynthesis. Many of these proteins are located within the chloroplast. Some are encoded by the chloroplast genome, and some by the nuclear genome. The synthesis of several of these proteins has been shown to be controlled by light.

We are interested in how synthesis of these proteins is regulated during leaf development. Our studies have shown that environmental factors interact with the leaf's developmental program to influence the quantity and timing of synthesis of several abundant proteins. We have also discovered that synthesis of the same protein is regulated in very different ways in two different plants.

The most abundant protein in green leaves of the bean plant (*Phaseolus vulgaris* 'Red Kidney') is ribulose-1, 5-bisphosphate carboxylase (RuBPCase). This is the enzyme responsible for fixing atmospheric carbon dioxide, thereby providing a carbon source for photosynthesis. It is found in the chloroplast and consists of eight small subunit polypeptides encoded by a gene in the nucleus and eight large subunit polypeptides encoded by a gene in the chloroplast. It has been known for some time that light influences the amount of RuBPCase in developing leaves of many plants. We have isolated synthetic DNA copies of small subunit mRNA (cloned cDNA) using recombinant DNA technology. This cloned cDNA gives us a very sensitive probe with which we can detect small quantities of small subunit mRNA and measure changes in its relative concentration within the cell. We were unable to detect small subunit mRNA when seedlings were grown in the complete absence of light. However, when seedlings that had been grown in the dark were shifted to continuous illumination, there was a rapid and dramatic accumulation of small subunit mRNA. Light induces a greater than 1,000-fold stimulation of small mRNA synthesis in bean primary leaves.

Fixation of atmospheric carbon dioxide is more complex in corn (*Zea mays*, inbred line B73, donated by Pioneer Seed Company, Des Moines, Iowa). The process involves two different enzymes located in different cell types. RuBPCase is restricted to the chloroplasts of vascular bundle sheath cells; phosphoenolpyruvate carboxylase (PEPCase) is found only in the cytoplasm of mesophyll cells. We have measured the accumulation of both proteins under a variety of dark and light growth regimens using sensitive antibody probes. Synthesis of both proteins begins about four or five days after germination. This time of synthesis initiation is independent of light. Accumulation of both carboxylases proceeds rapidly, even in the absence of light. The only measurable effect of light is to increase the rate of accumulation of both enzymes.

Contrasted to this light-independent regulation of PEPCase and RuBPCase during corn leaf development is the regulation of the chlorophyll a/b binding protein (chl a/b protein). This protein forms a complex with chlorophylls a and b and is responsible for harvesting the light energy that drives photosynthesis. Our preliminary studies indicate that light regulates synthesis of chl a/b protein in corn. In the absence of light there is no immunologically detectable chl a/b protein.

Our studies of leaf protein synthesis in corn and in beans demonstrate that genes encoding the same protein can be regulated in different ways in different plants. This fact, while interesting to the plant developmental biologist, has profound implications for the genetic engineer. Studies by plant physiologists indicate that, in a number of instances, efficiency of carbon dioxide fixation could be a limiting factor in crop yield. Transformation of a plant with a gene encoding a more efficient RuBPCase might therefore improve crop yield. Once the host plant has been transformed with a cloned gene, however, it is necessary that this new gene be expressed in the correct quantity in the correct leaf cells. Problems may arise if the cloned gene is regulated by a different set of signals than those employed in the host plant. It is clear that attention must be paid to the details of regulation of a gene when it is passed into the genome of a different plant.

William C. Taylor, Assistant Professor of Genetics and Assistant Geneticist; Timothy Nelson, American Cancer Society Postdoctoral Fellow; Mark Harpster, Lino Fragoso, and Belinda Martineau, graduate students, Genetics; Judy Yamaguchi, Staff Research Associate, Genetics; and Stephen Mayfield, graduate student, Plant Physiology. All are at U.C., Berkeley.

Storage protein genes

R. William Breidenbach
Robert B. Goldberg
Scott E. Taylor

Interest in the developmental and molecular biology of the proteins that accumulate as reserves in seeds has become keen in recent years. Although most plant cells contain large numbers of different proteins, each present only in small quantities, food chemists, using criteria of size and solubility, long ago found that most of the protein in seeds of the soybean and other legumes appears to be composed of only a few different kinds. In the late 1960s we recognized that, if this were true, it was likely that a correspondingly small number of different kinds of messenger RNA (mRNA) molecules encoded to direct the synthesis of the storage proteins would also be present in higher concentrations in seed tissue cells. Higher concentrations of specific mRNA's would make it much easier to learn how these intermediaries between genes and their protein products are modulated and, in turn, how they control the rates of protein production.

These two aspects of the regulation of gene expression are among the most important unanswered questions in biology and are fundamental to the practical problems of plant improvement by either conventional plant breeding or molecular genetic engineering. To learn how to move genes from one organism to another and have them usefully ex-

pressed, we must have model genes to work with. The products of model genes must be recognized easily in the recipient if they are expressed. Soybean storage proteins seem to be well suited to this purpose. They can be recognized by: (1) specific antibody reactions; (2) their electrophoretic mobilities on gels; (3) peptide maps; (4) specific amino acid sequences. We also have recombinant DNA probes to detect their expression at the mRNA level.

Although not quite as simple as the food chemists had thought, it is still true that a relatively small number of storage globulins are synthesized very rapidly during embryo (seed) development. Moreover, they are not synthesized in detectable amounts in any other tissue at any other time in the life cycle of the plant. In other words, their expression is under strong control. Beginning with the fertilized egg, cell division is very rapid, and virtually every cell present in the mature seed is laid down. This period ends with the formation of the tiny globular heart-stage embryo. Very little if any storage protein is present in the embryos at this stage. Rapid growth of the embryo by cell expansion follows, accompanied by rapid synthesis and accumulation of the storage proteins known as the globulins.

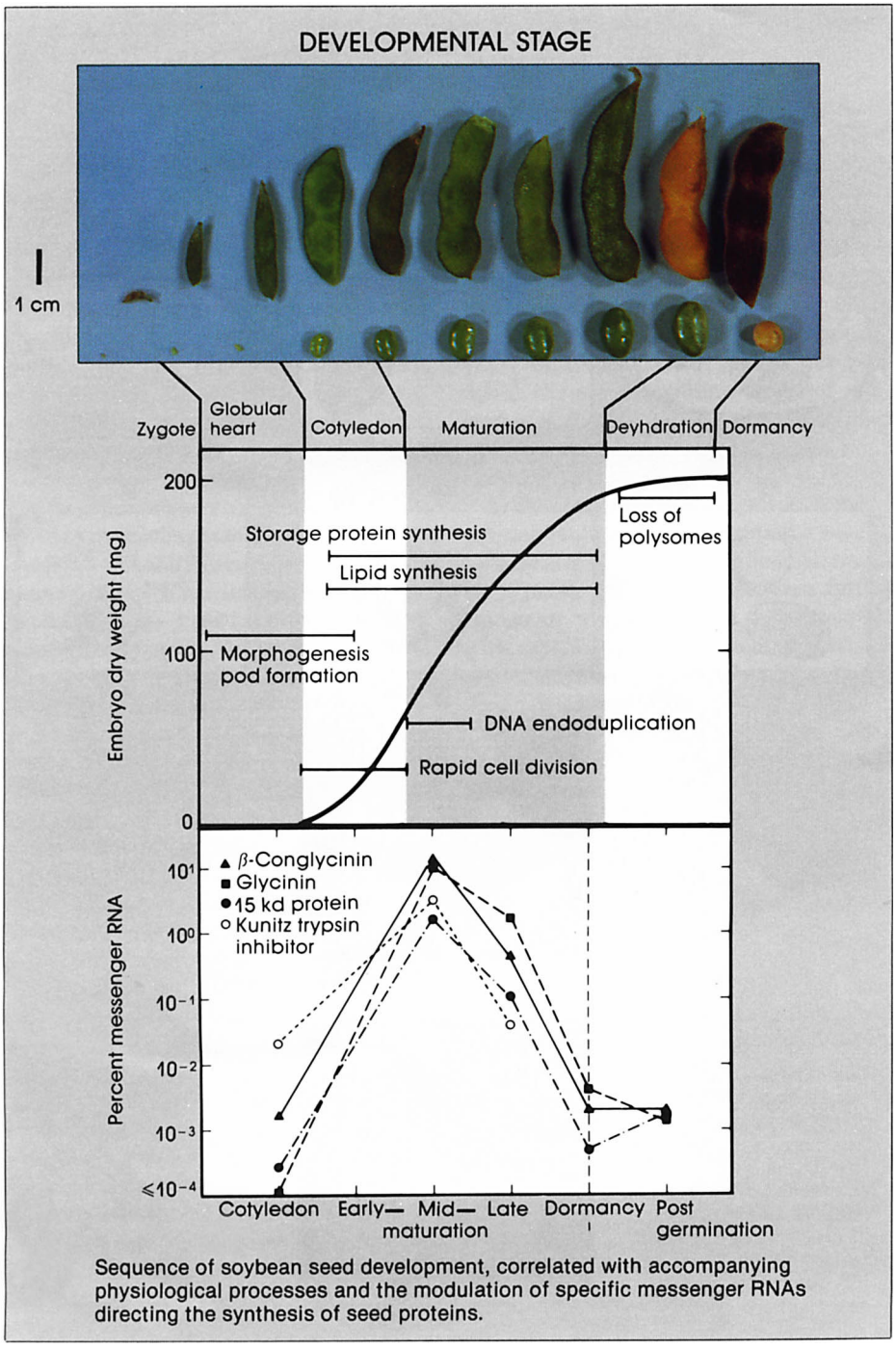
There are two major storage globulin families, the conglycinins and the glycinins. Conglycinins are an assemblage of three polypeptide subunits, each believed to be one from three sets of slightly different polypeptides. Each set is coded for by a small number of genes that arose by duplication from an ancestral gene during evolution. After duplication, the genes underwent some slight changes in sequence. In fact, the three sets of genes coding for the three subunits probably represent three more divergent branches, all derived from one original DNA sequence. Together, they constitute what is called a multigene family.

The other major storage protein, glycinin, is thought to consist of assemblages of six large and six small subunits, all encoded in the genes of a multigene family. Each gene in the family directs the synthesis of one polypeptide that includes both a large and a small subunit arranged in tandem. These polypeptides are subsequently cleaved and assembled into glycinin molecules.

During soybean seed development, other recognizable gene products accumulate in smaller amounts. Some of these products are known by their biological activities as protease inhibitors (the Kunitz trypsin inihibitor on the graph), red blood cell agglutinin, and the like. Others, such as the 15 kd protein, are known only by their electrophoretic mobility.

Synthesis and accumulation of the storage proteins and the other embryo-specific proteins are accompanied by an increasing amount of mRNA. The amount of mRNA directing the synthesis of these proteins in the tissue can be measured by titration with cloned cDNA, that is, with DNA that has been enzymatically synthesized using mRNA molecules as a template, then cloned, selected, and amplified in bacteria. The amount of each mRNA is modulated slightly differently during soybean seed development; all reach a maximum at the time of most rapid protein synthesis, and all decline as the embryo matures.

Since the recombinant DNA probes will hybridize with homologous nuclear DNA from plant cells, they can be used to study the organization of genes in the soybean genome and how it may control expression. They can also be used to detect specific DNA sequences (such as the soybean storage protein genes) introduced into other species in genetic engineering experiments.



Sequence of soybean seed development, correlated with accompanying physiological processes and the modulation of specific messenger RNAs directing the synthesis of seed proteins.

R. William Breidenbach, Agronomist, Plant Growth Laboratory, Agronomy and Range Science, U.C., Davis; Robert B. Goldberg, Associate Professor, Biology, U.C., Los Angeles; Scott E. Taylor, Post Graduate Research Plant Physiologist, Plant and Soil Biology, U.C., Berkeley.