AIM AND SCOPE

The aim of our project is to carry out studies to interpret the regulatory mechanisms controlling transcription and translation during plant embryo development. Such studies will not only broaden our understanding of a number of fundamental phenomenon in the life of a flowering plant, but also have useful applications in agriculture and horticulture. In the present study, some aspects of seed germination will be considered since germination is the continuation of the overall process of establishing another generation.

The germination of seed is the emergence of an embryo from rest and reflects a developmental shift from hypo-metabolism to 'optimal' or 'normal' metabolism and the resumption of growth. Biologically seed germination is the awakening of an embryo from a quasi-cryptobiotic state; it is the re-expression of the developmental genetic program. Unlike some other forms of rest (e.g. bud dormany) seed dormancy and quiescence are highly dessicated states and germination involves a rehydration of biochemically crucial interface. Germination transfers a metabolically inert embryo into a active state of growth and development. The germination of seeds offers a field of study in the biochemistry of development which is unparalleled in other living systems. Although we know a lot about these processes
we have only now begun to answer important questions concerning the control of nucleic acid metabolism and its implication in protein synthesis. There is yet much to be discovered. There are two important questions (i) what specific events are essential for visible germination to occur; (ii) what internal control if any are responsible for setting these events in motion? Unfortunately, definitive answers cannot yet be given to these fundamental questions but instead partial answers and some speculation must suffice. The aim of this project is to unravel the various aspects of differential gene expression during germination and particularly to study the process of transcription and translation occurring during the early phase of germination. Our aim is also to explain the biological phenomenon of germination in molecular terms, in the rise and fall of metabolites, of enzymes activities and to view finally in terms of differential gene expression and its regulation.

In the germination of dormant seeds, however, crucial metabolic intermediates are not readily available and specific trigger agents (e.g. chilling or photoblastic rhythms) are necessary to activate hydrolytic and subsequent oxidative pathways or to remedy early event metabolic deficiencies.
In addition, genetic transcription is highly repressed and germination agents require triggering to facilitate the activation of transcription and translation. In short, dormant seeds have to be brought into metabolic readiness to germinate.

One aspect of the biochemistry of seed formation concerns the characterization of storage proteins and aspects of their synthesis has received considerable attention. Another fascinating aspect of seed development deals with the general metabolic reversal that takes place in the nutrient storage tissue, i.e., endosperm or cotyledons, when germination begins. Cells that have been synthesizing enormous amounts of protein and carbohydrate or lipid reserve material during embryogenesis completely reverse this process and commence a very rapid hydrolysis of the same materials during germination. This reversal is generally not accompanied by cell division in these tissues and thus we have the very interesting phenomenon of a gross metabolic reversal taking place in an unchanging cell population. Obviously a certain amount of gene activation/deactivation may occur during the synthesis of all necessary cell constituents that accompanies cell division, this phenomenon becomes even more attractive to those interested in the molecular biology of development.
Detailed biochemical knowledge of embryogenesis has been emerging from the work of Dure and his colleagues with cotton. They have found that cotton embryos which have reached 60% of their maximum fresh weight synthesise mRNA for carboxypeptidase C and isocitritase. These enzymes are not normally synthesized until germination begins. Abscisic acid (ABA) which had been shown to be present in cotton fruit also inhibited enzyme synthesis. However, we have relatively little information about ABA levels in developing seeds. In 1975, Dure had proposed a model for cotton embryogenesis and for the role ABA plays in the process. He postulates that ABA is synthesized in the ovule and transported to the embryo at that stage of development in which mRNA is synthesized for the "germination enzymes". The presence of ABA inhibits the synthesis of these enzymes preventing the precocious germination of the embryo. This hypothesis was also supported by the observation that ABA inhibits germination of wheat as well as barley embryo (the present study). However, the precise mechanism by which ABA inhibits germination is not known.

Germination of seeds is characterized by a fairly reproducible and probably genetically determined sequence of events (1-7). The aim of our project is to unravel this
sequence in the case of barley seeds. There is considerable evidence for the existence of stored mRNA in higher plant seeds (8, 9). However, our knowledge of its properties and functions is still very poor. Stored mRNA seems to initiate protein synthesis as soon as the seed imbibes. It has been suggested that some stored mRNA is immature and requires polyadenylation during early germination before being translated (10). On the other hand, poly(A)-RNA has been isolated from a variety of dry seeds, indicating that at least part of the stored mRNA is translatable (11, 12, 13). Moreover, it has been reported that stored mRNA rapidly disappears following soaking (14). In the present study, we have shown that barley embryos contain stored mRNA which however, does not decay rapidly. Furthermore, our observations also show that new poly(A)-mRNA synthesis and polyadenylation of the conserved mRNA are entirely independent processes.

Considering these contradictory results, attempts were made to establish the complete sequence of events during barley embryo germination. In this work, we show that stable poly(A)-mRNA is present in the dormant embryo and that the protein synthesis which starts 15 min after the onset of germination is absolutely necessary for new RNA synthesis and for the germination of barley embryo. Although low DNA-
dependent RNA polymerase activity is present in dry barley embryo, RNA synthesis does not commence immediately after water imbibition. On the other hand, it is initiated only after 2 hr of germination and its synthesis requires the essential presence of early proteins. Furthermore, there is a progressive increase in the activity of RNA polymerase with increase in germination time and after 40 hr of germination, the activity of RNA polymerase is about 5-fold higher than in dry embryo. Further work is required to isolate the various RNA polymerases and study their properties. Moreover, it was found that cycloheximide blocks completely the enhanced activity of RNA polymerase, suggesting a role of early proteins in the initiation of new RNA synthesis in this developmental system. If the embryos are treated with cycloheximide for the first one hour after imbibition there is complete inhibition in root formation as well as RNA and protein synthesis supporting the view that very early proteins are essential for new RNA synthesis.