In this thesis the question is raised if morphogenesis and cell differentiation during early seedling development of a higher plant are accompanied by corresponding changes in gene expression. In Chapter I a brief introduction is given in the currently favoured ideas about the molecular basis of cell differentiation and morphogenesis during development of eukaryotic organisms. The emphasis has been placed on the selective expression of genes as an important determinant in development, but this does not mean that it should be considered to be the only mechanism operative in development. Components of epigenetic regulatory systems (e.g. plant growth hormones, gradients of ions, self-assembly of cell components) may be of equal importance or may even singularly control certain aspects of development. It seems now well established however, that the expression of the nuclear genome into mRNAs is an important and perhaps the only means of communication between the stored hereditary information of a cell and the surrounding cytoplasm, where the expression of the genome into functional components is executed.

To detect genes, selectively expressed during development of plant seedlings, it was necessary to obtain an experimental system in which the formation of already differentiated but not yet functional plant organs could be studied with respect to the genes that are specifically transcribed into mRNA in these organs in the absence of environmental factors such as light. The pea plant was chosen as an experimental system because the seeds contain enough stored reserve food to sustain growth for a considerable time in the absence of an active photosynthetic system and in addition, can be easily cultured in large quantities under aseptical conditions. The latter was considered to be an important prerequisite to study mRNA populations in plant systems, since especially plant roots are very susceptible to contamination with micro-organisms (the rhizosphere is normally packed with bacteria and fungi).

The first experimental approach we used was a high-resolution two-dimensional separation of both in vivo synthesized polypeptides and polypeptides
synthesized as directed by the in vitro translation of isolated mRNA (Chapter II). Several parts of the developing seedling (roots, root meristem, root central cylinder, root cortex, shoot axis, plumule and cotyledons) were compared with respect to the mRNAs and proteins they synthesize. It became evident that the large majority of both in vivo and in vitro synthesized polypeptides visualized this way were present in all seedling parts studied. This result was remarkable since it showed that morphologically quite distinct plant parts are near identical when analyzed for the abundant mRNAs and proteins they synthesize. This was certainly not in accordance with the idea that different cell types and organs are characterized by a vast array of specifically expressed genes. Apart from quantitative differences of certain polypeptides between different seedling parts, the only differences that could be observed were some fifteen mRNAs specifically present in shoots but not in roots or cotyledons, irrespective of illumination. The most prominent "shoot-specific" mRNAs encoded six closely grouped in vitro translation products.

To investigate the effect of the transition of already differentiated but not yet functional plant organs to their functional status we also studied the mRNA populations synthesized by illuminated seedlings. Some twenty-five partly very abundant mRNAs were found that were not detectable in dark-grown seedlings. In addition, it was shown that illumination with red light resulted in the appearance of at least ten of these light-inducible mRNAs, suggestive of phytochrome control.

Since only abundant mRNAs could be visualized by in vitro translation and two-dimensional separation of the products, the possibility remained that a class of organ-specific mRNAs could be found among the mRNAs present in considerably lower concentration (rare sequences) that account for the majority of the mRNA sequence diversity in eukaryotic cells. Therefore we compared complexities of polysomal mRNAs present in roots and shoots of 4 d seedlings employing liquid hybridization of polysomal RNA with single copy DNA enriched for sequences transcribed into mRNA (mDNA) and with single copy DNA depleted of such sequences (null mDNA). The results of these experiments show that no significant class of organ-specific rare mRNAs is present among the approximately 20,000 different mRNAs synthesized in both roots and shoots (Chapter III). However, due to the method used regulation involving less than about 1,500 different mRNAs could not be detected. In addition it was shown that illumination of seedlings did not result in significant changes in the complexity of rare class mRNAs when compared to dark-grown seedlings. Reciprocal
complementary DNA/polysomal poly(A)RNA hybridizations showed that a number of abundant mRNA sequences becomes apparent upon illumination that were not present in dark-grown tissue, confirming the results obtained with in vitro translations.

The reassociation kinetics of total pea DNA and the isolation of single copy DNA tracers used in the hybridization studies is described in Chapter IV. It was shown that the calculation method as commonly applied in measurements of the total number of genes expressed into mRNA (determination of the sequence complexity of a mRNA population) should be modified when dealing with plant genomes that contain very large amounts of intricately interspersed repetitive DNA as is the case in pea. These calculations should be based on the fraction of single copy DNA actually isolated rather than the total fraction of DNA with single copy renaturation characteristics present, since the latter leads to gross overestimates of the number of genes expressed.

The molecular cloning of complementary DNA molecules corresponding to the shoot-specific mRNAs found in pea is described in Chapter V. Since these mRNAs, although belonging to the abundant class of mRNAs, were present in relatively small amounts in shoots we employed a complementary DNA enrichment procedure before cloning. In this procedure we used heterologous hybridization of complementary DNA made on shoot mRNA with mercurated root mRNA. The complementary DNA sequences corresponding to mRNAs present in both roots and shoots could then be removed by selective binding on thiol-Sepharose columns. This resulted in a complementary DNA population highly enriched for the shoot-specific mRNAs. Clones containing sequences corresponding to the shoot-specific mRNAs could then be selected quite easily by differential colony hybridization screening and were identified by in vitro translation of hybridization selected mRNAs followed by two-dimensional separation of the products. This yielded the same pattern of closely grouped spots of Mr 27,500 and iso-electric point of 5 that was also observable in two-dimensional patterns of total in vitro translated RNA from shoots. Apparently the cloned sequence selected slightly different but related mRNAs from the total population, indicating the occurrence of microheterogeneity of these mRNAs. In addition, clones were obtained that corresponded to the major light-induced and nuclear-encoded mRNAs, the small subunit of the ribulose 1,5 diphasphate carboxylase (ssRuBPCase) and a component of the light-harvesting chlorophyll a/b complex (LHCP) that were identified by immunoprecipitation.

In Chapter VI experiments are described aimed to learn more about the
regulation and identity of the shoot-specific sequence. The results of Northern blottings of RNA extracted from various organs of young pea seedlings and adult plants grown under different light conditions revealed that:

1. The level of the shoot-specific mRNA decreases slightly upon illumination with white light, whereas the level of both ssRuBPCase and LHCP mRNA is greatly increased,
2. the shoot-specific mRNA is found predominantly in stem tissue and not in (incipient) leaves,
3. the level of the shoot-specific mRNA is slightly increased upon red light illumination, suggestive of phytochrome control,
4. the presence of the shoot-specific mRNA is not an integral property of shoot tissue since the mRNA is first detected 2 days after the start of germination concomitant with the beginning of shoot development,
5. the shoot-specific sequence is only found in pea and not in other legumes or cereals.

In conclusion, several observations have been made in this thesis that are relevant to the question which role selective expression of genes plays in the development of higher plants. One of the surprising findings was that in pea not only morphologically very different organs (e.g. roots, shoots and cotyledons) contain very similar populations of both abundant and rare mRNAs but that also differentiated tissues contain almost the same abundant mRNAs as the meristem from which they originate (e.g. cortex, central cylinder and meristem in roots). This may be a reflection of the observed totipotency that differentiated plant cells possess or, alternatively, may point to the importance of nuclear gene regulation in this process.
tance of epigenetic regulatory mechanisms not involving the expression of the nuclear genome to a large extent in development. Apart from the work presented in this thesis, only in tobacco a similar investigation of mRNA populations present in different (adult) plant organs has been conducted (see Chapter III for references). In that study it was shown that a considerable part (about 70%) of the whole mRNA sequence diversity was regulated in a organ-specific pattern. Therefore it does not seem appropriate to speculate about the absence of significant regulation of rare mRNA sequences during plant development in general and it would now be of great interest to include more plant species in this type of experiments. However, it is now becoming increasingly clear that cell differentiation and morphogenesis may take place without the activity of large numbers of selectively expressed genes. This has been amply demonstrated by for instance recent results from this laboratory where it was shown that the development of fruit-body initials of the fungus Schizophyllum commune involves only the expression of a very limited number of genes coding for (unidentified) abundant polypeptides. In addition, although not directly applicable to the system described in this thesis, it is of interest to mention the recent discovery of cellular oncogenes, the activity of which is associated with the development of tumor cells. In some cases the normal cell division control mechanism seems to be completely disrupted by the intracellular raise of the level of a single, otherwise normally functioning, protein.

Although we did not find evidence for the existence of genes that may determine the formation of a given plant organ or cell type in this organ, the attractive but speculative possibility remains that the shoot-specific polypeptide described in this thesis is involved in some (unknown) way in growth of pea stem tissue. It would now be of great importance to learn more about the encoded protein and its localization in the different stem tissues as well as its subcellular localization. Answers to these questions must however await the availability of specific antibodies to the shoot-specific polypeptide. In addition, it would be of interest to compare the level of regulation with that of other pea genes and to look for possible regulatory regions in the DNA surrounding the gene and compare these to similar regions of other (e.g. light-inducible) pea genes.