This thesis presents an analysis of the structure and regulation of genes from the basidiomycete Schizophyllum commune presumably involved in fruiting and emerged growth in general. This analysis led to assignment of functions to some of these genes in development. To study the transcriptional regulation of these genes by the mating-type genes and other regulatory elements, a gene-reporter system was developed based on truncated homologous genes.

The molecular structure and nucleotide sequence of one of the fruiting genes, Sc1, was previously determined (Dons et al., 1984b). The present research started with the analysis of the Sc4 gene, abundantly expressed during fruiting in the dikaryon (up to 3.5% of total mRNA mass), and the Sc3 gene which is abundantly expressed during formation of aerial hyphae both in monokaryons and dikaryons (up to 1% of total mRNA mass; Mulder and Wessels, 1986; Ruiters et al., 1988). As described in Chapter 2, unexpectedly all three genes turned out to be homologous both in gene structure and deduced amino acid sequence. The Sc1 and Sc4 genes contain three introns exactly conserved with respect to their positions while two of the four introns of the Sc3 gene are also conserved at similar positions. All three encoded proteins contain leader sequences for secretion, they are very hydrophobic with hydrophobicity patterns clearly resembling each other and all contain eight cysteines at conserved positions.

Although the proteins, called hydrophobins, encoded by these genes were expected to be present at high concentrations because of the abundance of their mRNAs, they could not be detected in hot-SDS extracts of the fungus. However, antibodies raised against synthetic peptides based on the nucleotide sequences determined for the Sc1 and Sc4 genes indicated the presence of these proteins in the cell walls and in the culture medium (S.A. Ásgeirsdóttir, unpublished results). Because of the high cysteine content of these proteins, 35S-sulphate labeling experiments of several co-isogenic wild-type and mutant strains, showing different expression patterns of the hydrophobin genes, were performed to see which proteins followed these expression patterns (Chapter 3). Proteins behaving according to this criterion could only be visualized after extraction of walls with concentrated formic acid and oxidizing the formic-acid released proteins with performic acid. Performic acid oxidation also revealed the abundant presence of these proteins in the culture medium. N-terminal amino acid sequence analysis confirmed the identity of the Sc3 and Sc4 encoded proteins in these extracts. It was shown that the hydrophobins excreted into the medium form high molecular weight insoluble complexes in the walls of emergent hyphae. These complexes, which are insoluble in hot SDS, only dissociate by treatment with formic acid/performic acid or trifluoroacetic acid. The complexes are therefore probably due to hydrophobic interactions between the hydrophobin proteins. We suspect these hydrophobins to be necessary for the emergence of aerial structures in S. commune; pSc3 providing the hydrophobic surface of aerial hyphae, pSc1 and pSc4 involved in the aggregation of fruit-body hyphae. A rodlet layer which has been assumed to confer hydrophobicity to the wall surface has been observed on the surface of aerial hyphal walls of S. commune (Wessels et al., 1972; H.A.B. Wosten, unpublished results) and
preliminary observations have shown that heat-precipitated material from the culture medium containing Sc3 protein can form rodlet-like structures in vitro (H.A.B. Wosten, unpublished results). The recent finding that the rodA gene of the ascomycete Aspergillus nidulans, implicated in the formation of the hydrophobic rodlet layer on conidiospores of this fungus, shows homology to the S. commune hydrophobin gene family (Stringer et al., 1991) suggests that this class of proteins is of general occurrence in fungi and mediates the development of aerial structures.

Two other fruiting genes, Sc7 and Sc14, were also analyzed in detail (Chapter 4). These genes are closely linked (within 6-kb) and they are also homologous both in gene structure and predicted amino acid sequence. They contain five introns, four of which are in identical positions. The encoded proteins contain leader sequences for secretion but, in contrast to the hydrophobins, they are hydrophilic and rich in aromatic amino acids which are largely conserved with respect to their positions. Comparison with sequences in data banks showed similarities to pathogenesis related proteins group I (PRI) from plants, to testis-specific proteins from mammals and to a venom allergen from an insect. Unfortunately, all these proteins are of unknown function. However, the similarity to the PRI proteins from plants is intriguing. These PRI proteins are induced after infection by a plant virus or a fungal pathogen. One could speculate that these PRI proteins somehow interfere with functions of the invading fungus encoded by homologues of the Sc7/Sc14 genes. Future research aimed at elucidation of the location and function of the Sc7/Sc14 proteins may reveal whether there is some truth in this speculation.

Although detailed information about the molecular mechanisms of gene expression in S. commune development is not yet available, a number of trans-acting regulatory elements are known. It was shown earlier that fruiting mRNAs are regulated by the mating-type genes. They accumulate in A ≠ B ≠ heterokaryons and in Acon Bcon homokaryons but not in A = B ≠ and A ≠ B = heterokaryons nor in Ax Bx, Ax Bcon and Acon Bx homokaryons (Ruiters et al., 1988). This clearly shows that both mating-type genes are involved in the regulation of these genes. Two other genes, FBF and THN, that have been shown to regulate the formation of aerial structures, also regulate the expression of these genes. The fbf mutation suppresses the formation of fruit bodies in the Acon Bcon homokaryon and, if present in a double dose, in the A ≠ B ≠ heterokaryon. At the same time it prevents the accumulation of all fruiting mRNAs (Springer and Wessels, 1989). The thn mutation suppresses the formation of aerial mycelium in the homokaryon and, if present in a double dose, of aerial mycelium and fruit bodies in the heterokaryon. This is accompanied by the absence of Sc3 mRNA in monokaryon and dikaryon and the absence of all fruiting mRNAs in the dikaryon (Wessels et al., 1991a).

From this information a regulatory model can be inferred (Fig. 1). In this model, which only shows the structural genes characterized in the present study (Sc1, Sc4, Sc7, Sc14 and Sc3), the FBF gene acts as a secondary regulatory gene, activated by the mating-type genes. However, FBF could also be expressed constitutively and only be able to activate fruiting-associated gene expression in interaction with the products of the mating-type genes. In this alternative the mating-type genes would directly influence the expression of the fruiting genes. The THN gene product is also necessary for the expression of the fruiting genes but, in addition it is necessary for the expression of the Sc3 gene.

To begin to study the regulation of the expression of fruiting genes in more detail, it
rial from the culture
in vitro (H.A.B. Wösten, Aspergillus
Aspergillus
family (Stringer et al., 1981), but in fungi and mediates
in detail (Chapter 4). The Sc7/Sc14 homologous both in gene
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with sequences in data bases (PR1) from plants, to
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first had to be established whether specific RNA accumulation patterns were caused by
differential transcription or by differences in transcript processing or mRNA stability. Run-on
transcription assays with isolated nuclei showed that the expression of most
fruiting genes is clearly regulated at the transcriptional level (Chapter 5). This justifies
studies on the nature of cis-regulatory sequences, for instance by using footprinting
techniques or reporter genes.

Because the expression signals of the glyceraldehyde-3-phosphate dehydrogenase
(GPD) gene of A. nidulans could not be used in S. commute to obtain constitutive
expression of a foreign reporter gene, the GPD gene of S. commute was isolated and
analyzed (Chapter 6). Comparison of the S. commute GPD gene with GPD genes from
the basidiomycetes Phanerochaete chrysosporium and Agaricus bisporus isolated and
analyzed in our laboratory (S.M. Moucha and M.C. Harmsen, unpublished results) and
with published GPD sequences from filamentous ascomycetes showed that GPD genes
from these two groups are clearly distinct from each other and both differed considerably
from the GPD genes of ascomycetous yeasts.

Because of the apparent differences in the GPD genes (for details see Chapters 6) a
more general comparison was made between gene structure in filamentous ascomycetes
and homobasidiomycetes (Chapter 7). Although the structure of genes in these two groups
of fungi showed many similarities, some differences were found which may influence
gene expression. Especially demands on the position and sequence of the TATA element
seem to be more stringent in basidiomycetes than in ascomycetes. Alternatively, it is
possible that heterologous DNA is inactivated in homobasidiomycetes as was found by
Mooibroek et al. (1990), who showed methylation of heterologous sequences in S.
commune.

Because of the problems encountered in expressing heterologous (reporter) genes in S.
commune, we decided to develop a reporter-gene system using homologous genes (Chapter 8). Internal deletions were made in the highly expressed fruiting gene Sc4 and in the constitutively expressed GPD gene. These deletions resulted in shorter transcripts which could be observed on Northern blots. The expression of the deleted reporter genes was similar to that of the endogenous genes. The Sc4 reporter gene, containing 1150 bp upstream sequence, was only expressed in dikaryons at the time of fruiting, indicating regulation by both the mating-type genes and developmental control. The GPD reporter gene, containing 1300 bp of upstream sequences, was expressed in both monokaryons and dikaryons at levels similar to that of the endogenous gene. This indicates that the regulatory sequences present in both reporter constructs were sufficient for normal gene expression. This allows for further analysis of these regulatory sequences. As far as we know this is a novel technique to study cis-regulatory sequences which offers the additional advantage of monitoring the activities of the endogenous gene and the introduced reporter gene at the same time. Furthermore, the GPD reporter construct will be used for studying the effects of inserted heterologous sequences on gene expression in S. commune. Perhaps this will reveal why it is so difficult to express heterologous genes in homobasidiomycetes.