Gene expression during fruit-body development in Schizophyllum commune

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This Thesis is concerned with the regulation of eight mRNAs presumed to have a role in fruit-body formation in *Schizophyllum commune* and with the localization and possible roles of these mRNAs in the developing system.

Regulation of "dikaryon-specific" mRNAs in relation to fruiting.

Mulder and Wessels (1986) have isolated eight cDNA clones corresponding to mRNAs abundantly expressed in dikaryons at the time of fruiting and concentrated in the fruit bodies. We extended this work (Chapter 2) by measuring the concentrations of these mRNAs in various heterokaryons and in homokaryons that carry constitutive mutations in the incompatibility genes. Only a heterokaryon with different alleles for both incompatibility genes and a homokaryon with mutations in both genes, both fruiting dikaryons, produced large amounts of these mRNAs. In dark-grown colonies of the dikaryon these dikaryon-specific mRNAs were virtually absent. After transfer of colonies to light these mRNAs and fruit bodies were concomitantly formed in approximately the same region of the colony. These results suggest that these abundant mRNAs are normally regulated by the concerted activities of the incompatibility genes and by light. They have no role in vegetative growth but are possibly required for fruiting.

In Chapter 2 it was also shown that a monokaryon (a homokaryon with wildtype incompatibility alleles) carrying so-called fruiting-alleles (*HF*) accumulated at least some of the "dikaryon-specific" mRNAs. This emphasized a possible role for these mRNAs in fruiting. Chapter 3 describes the isolation and properties of such monokaryotic fruiters, particularly so called FI fruiters. In the dikaryons formed by mating two FI-monokaryons the fruiting was also fast and took place in the dark, which is exceptional for *S. commune*. On the basis of morphological characteristics it was concluded that the alleles permitting the monokaryotic fruiting were originally present in the parental strain with weak monokaryotic fruiting, and their expression was enhanced by combination with the properties indirectly related to fruiting and originating from the non-fruiting parental strain. The strong influence of mating of FI monokaryons on the dikaryotic fruiting suggested that the same genes were involved in monokaryotic and dikaryotic fruiting. This was supported by detection in the FI fruiters of relatively high levels of the "dikaryon-

specific" mRNAs presumed to be fruiting specific.

Although the results reported in Chapter 3 clearly indicated that fruiting can sometimes occur in absolute darkness, in normal strains there is a (variable) requirement for light to initiate fruiting. Particularly UV-A light is effective (Yli-Mattila, 1985). In Chapter 4 is shown that a clear elevation of some mRNAs could be seen 6 h after the beginning of UV-A illumination. After 24 h the concentrations of six of the mRNAs were strongly increased. The concentration of a mRNA not related to fruiting was decreased within 24 h after transfer to UV-A light. These results suggest that light may control fruiting at the transcriptional level.

The results summarized above show that the presumed fruiting genes (coding for the probed mRNAs) are regulated by the incompatibility (mating) genes and environment, particularly by light. Similarly, sporulation in yeast is regulated by the mating type genes and environmental conditions. In both *Saccharomyces cerevisiae* (Kassir & Simchen, 1976; Hicks *et al.*, 1979, Mitchell & Herskowitz, 1986; Kassir *et al.*, 1988) and *Schizosaccharomyces pombe* (Beach, 1983) these controls have been extensively studied and well resolved due to the amenability of these organisms to methods of molecular genetics (availability of self-replicating plasmids, gene replacements etc, see Struhl, 1983). In *S. cerevisiae* the control of sporulation can be summarized as follows (See Fig 1): in a mating of two haploid yeast cells the mating-type genes *MAT*a and *MAT*α, cooperatively repress transcription of the *RME* gene. The product of the *RME* gene is an inhibitor of meiosis and sporulation and its role was originally discovered (Kassir *et al.*, 1976) because a mutation in this gene bypassed the requirement for presence of two different mating-type genes. Diploids homozygous for *rme* sporulated regardless of their mating type. Wessels (1987a) has provisionally likened the controls as operating in *S. cerevisiae* to those operating in *S. commune*. In such a scheme (Fig. 1) the *HF* genes themselves would normally be controlled by the mating-type genes (i.e. they would be active in the dikaryon only) and by environmental conditions such as light. In monokaryotic fruiters HF^+ alleles would be present which are no longer tightly controlled by the combined actions of mating-type genes and therefore would also acquire a different sensitivity to light. The *HF* genes would thus be similar to genes controlling sporulation in yeast; the *RME* gene which by itself is controlled by the mating-type genes (Mitchell and Herskowitz, 1986) or the *IME* gene (not

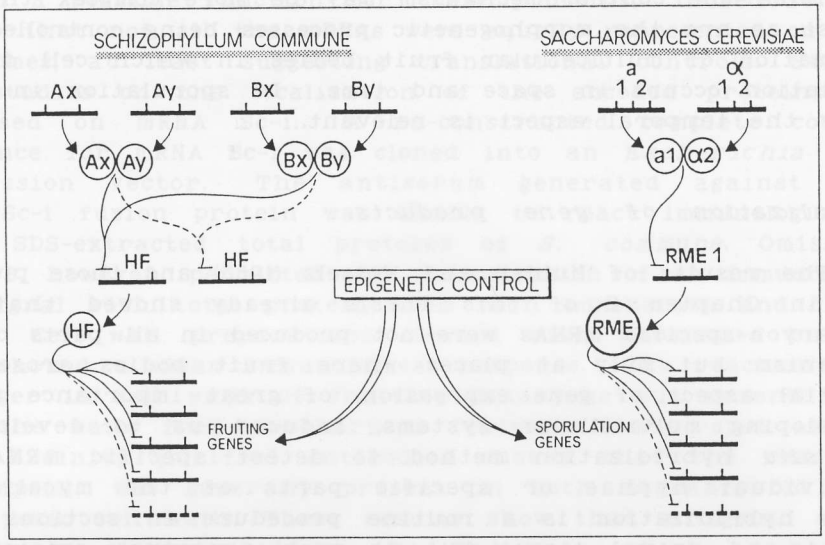

FIGURE 1. A model for genetic regulation of fruiting in *S. commune* (left) compared with the model for regulation of sporulation in *S. cerevisiae* as proposed by Mitchell and Herkowitz (1986)(right).

shown in Fig 1) which is controlled by the mating-type genes via *RME* and by environmental signals (Kassir,*et al.*, 1988). If an *HF* gene was to form an activator of fruiting genes in the dikaryon after light induction, an HF^+ fruiting allele may be represent a variant which is less stringently controlled by the mating-type genes and produces the activator in a monokaryon after light induction. In a dikaryon such relaxed HF^+ alleles, further stimulated by the presence of unlike mating-type genes, might produce enough activator to allow for fruiting in darkness. An analogous reasoning could apply in the case that an *HF* gene was to produce a repressor of fruiting genes.

As pointed out by Wessels (1987a), however, there are important differences. In contrast to the yeast system, there are not two but *many different alleles of the mating genes* which are stably inherited. A transposon mediated switch as in yeast (Hicks *et al.*, 1979; Beach, 1983) is very unlikely (Ullrich *et al.*, 1985). Also there are no reasons to believe that the incompatibility genes of *S. commune* specify any properties of the interacting monokaryons, e.g. the

formation of pheromones or pheromone-receptors. Also in *S. commune* the control mechanism may be more complex than in yeast as are the morphogenetic processes being controlled; the formation of multicellular fruit bodies in which cell differentiation occurs in space and time. In sporulation in yeast only the temporal aspect is relevant.

Localization of gene products.

The results of Mulder and Wessels (1986) and those presented in Chapter 2 of this Thesis already showed that the dikaryon-specific mRNAs were not produced in all parts of the organism but only at places where fruit bodies arose. The spatial aspect of gene expression, of great importance in all developing multicellular systems, induced us to develop an *in situ* hybridization method to detect specific mRNAs in individual hyphae or specific parts of the mycelia. *In situ* hybridization is a routine procedure in sections from plant and animal tissue but no such procedure existed for fungal tissues where the special hyphal structures makes it necessary for probes to traverse the cell wall. A treatment with an RNase depleted wall-lytic enzyme preparation (Chapter 7) served to permeabilize the walls after which nucleic acid probes with radioactive or biotin labels could be used to localize RNAs in freeze microtome sections (Chapter 5) or whole colonies growing on membranes (Chapter 6). An 18S-rDNA clone was isolated (Chapter 8) to serve as a probe for total RNA.

Using biotinylated probes and alkaline phosphatase reaction to visualize hybridized probes showed that the dikaryon-specific RNAs were concentrated in the developing fruit bodies and that each mRNA followed a somewhat specific pattern of distribution during development without being confined to any particular cell type (Chapter 5). *In situ* hybridizations of whole colonies with [^{32}P]-labeled cDNA clones showed that the specific mRNAs were only formed in parts of the colony where fruit-body initials arose. At later stages these mRNAs disappeared from abortive fruit bodies but remained high in fruit bodies continuing development.

The rather general distribution of the dikaryon-specific mRNAs does not exclude a more limited distribution of the ultimate gene products, the encoded proteins. Translational controls may exist superimposed on transcriptional controls to distribute the proteins more precisely within the develop-

ing system. In addition localization of the proteins may reveal a possible function for these proteins. Indeed it was found that not all the mRNAs were equally represented on the polysomes, at least suggesting translational controls. Starting studies on the localization of the encoded proteins we focussed on mRNA Sc-1. A re-constructed complete coding sequence for mRNA Sc-1 was cloned into an *Escherichia coli* expression vector. The antiserum generated against the lac-Z::Sc-1 fusion protein was unable to react immunologically with SDS-extracted total proteins of *S. commune*. Omission of SDS, however, permitted the demonstration of an immunological signal with total protein from both monokaryon and dikaryon. Cell wall preparations cleaned with phosphate buffer also showed a clear immunological response. The reaction was stronger in cell walls of fruiting dikaryons but nevertheless also the walls from monokaryons reacted. Immuno-spot assays with affinity purified antibodies showed that the reacting epithope(s) was generally present in both monokaryotic and dikaryotic cell walls. These results show that antigenic sites similar to those coded by the Sc-1 gene, which is highly expressed in the dikaryon, were also present in the wall and soluble proteins of the monokaryon.

In retrospect a result as above might have been expected in view of the extensive amino acid sequence homology between the proteins encoded by the Sc-1, Sc-4 and, to a lesser degree, Sc-3 mRNAs which was recently found (F.H.J. Schuren). Therefore it will now be necessary to raise antibodies against known different epithopes of the proteins. However, if the antibodies raised against the fusion protein do react with a number of proteins encoded by the mRNAs studied in this Thesis, it may be that these proteins fulfill similar functions. Although this could reduce the number of different functions to be assigned, it would complicate functional analysis by gene disruptions which are now possible, in principle, by employing the recently developed method for molecular transformation of *S. commune* (Munoz-Rivas *et al.*, 1986; Froelinger *et al.*, 1987).

The incompatibility genes of *S. commune* are now being cloned (Ullrich personal communication) and this may eventually also be possible for the haploid fruiting genes described in this Thesis. If *S. commune* yields to some of the molecular methodologies now applicable to yeast, this developmental system may eventually solve some of the problems which now still riddle multicellular development in eukaryotes.