Moore, D. (1998). Genetic and physiological nature of morphogenetic control during initiation and development of fungal fruit bodies. Special lecture during the Sixth International Symposium of the Mycological Society of Japan, Chiba University, Japan, 26-27 November, 1998. In: *Progress in Mycological Sciences in Japan and the United Kingdom, Proceedings of the Sixth International Symposium of the Mycological Society of Japan* (ed. Y. Kitamoto, Y. Harada & A. Suzuki), pp. 1-4. Mycological Society of Japan: Tokyo.

Genetic and physiological nature of morphogenetic control during initiation and development of fungal fruit bodies

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Summary

A fruit body carries out the functions of species propagation and, in most cases, this is coupled with the generation of genetic variation for evolutionary challenge. Classic genetic approaches have revealed several major genes which seem to be involved in defining parallel pathways. Some genetic factors enable fruiting in monokaryons, showing that dikaryosis and heterozygosity are not essential for fruit body formation. Selection of induced mutants in haploid fruiting strains has resulted in collections of gene defects in particular sub-pathways of development. Analyses of naturally-occurring fruit body polymorphisms imply that development is organised into a series of subroutines which define subsets of phenotype characters. These subroutines must be invoked correctly to obtain normal morphology, but the occurrence of polymorphisms shows that a level of imprecision can be tolerated and still permit spore formation and distribution (so reducing selection pressure against occurrence of the errors). Recent molecular analyses have revealed some fruit body structural genes whose function are to aggregate hyphae together to form the 3-D structure of the fruit body which, in most homobasidiomycetes serves to protect the spore dispersal system from rain. Overall, the genetic structure seems to be one in which relatively few genes acting as major regulators govern entry into integrated pathways to which a large number of other genes contribute. Regulation probably occurs both at transcription and translation. Although a fruit body is in most cases sexual, and genetic variation among progeny results from the pairing of two different nuclei followed by the well established pathway of meiotic recombination, random chromosomal segregation and migration of nuclei, there are still many unknowns. Recent work on *Volvariella volvacea* is an example. *V. volvacea* exhibits primary homothallism, but fruit bodies formed on mycelia grown from haploid, uninucleate basidiospores segregate physiological and DNA markers, suggesting that the organism has a mechanism (or mechanisms) to maintain variation despite inbreeding.

Introduction

Strands, rhizomorphs, sclerotia, conidia and other asexual spores make it clear that mycelium has a number of alternative developmental pathways open to it: continuation of hyphal growth, production of asexual structures, and progress into the sexual cycle. Sexual reproduction predominates over asexual in many strains collected from the wild (Croft & Jinks, 1977) although laboratory strains may carry mutations which shift the balance towards asexual reproduction.

Most research on sexual development in *Neurospora* has been aimed at understanding mating type structure and function. Johnson (1976) used heterokaryons as genetic mosaics to show that perithecia of *Neurospora* arise from an initiating population of 100 to 300 nuclei, and that the perithecium wall is composed of three developmentally distinct layers. Later, he identified 29 complementation groups (equivalent to functional genes) which were involved in perithecium development (Johnson, 1978), but this research has not been followed up. Ashby & Johnstone (1993) used genetically-engineered constructs with the *Escherichia coli* β-glucuronidase as a reporter gene to study development of *Pyrenopeziza brassicae* ascomata. They also found three tissue layers but the reporter gene revealed differential expression of the mating types. Both mating types were expressed in one of the layers, but the two mating types were expressed separately in each of the other two layers. The significance of this is unknown but it may be analogous with differential expression of genes in dikaryotic hyphae of *Schizophyllum commune* which is thought to depend on change in proximity of nuclei carrying the mating-type factors (Ásgeirsdóttir *et al.*, 1995).

What seems to be the first attempt to determine the genetic nature of a fruiting pathway was the study of Esser & Straub (1958) who used classical genetic approaches to establish a 'developmental pathway' for perithecium formation in *Sordaria*. These classic approaches are:

identification of variant strains,
application of complementation tests to establish functional cistrons,
construction of heterokaryons to determine dominance/recessive relationships
determination of epistatic relationships in heterokaryons (to indicate the sequence of gene expression).

For the fruit-bodies of basidiomycetes, which include mushrooms, toadstools, bracket fungi, puff-balls, stinkhorns and bird's nest fungi, classical genetic approaches reveal a more complex picture. These basidiomycete fruit bodies are normally formed by heterokaryotic secondary mycelia. Occurrence of two (or more) nuclei (= two or more genotypes) makes it difficult to study the genetics of development by conventional means. Fruiting by monokaryotic mycelia has been recorded in a number of basidiomycetes (Stahl & Esser, 1976; Elliott, 1985), however, and these strains have been particularly useful for study of the genetic control of fruit body development.

Genetics of monokaryotic fruiting in basidiomycetes

Several genera produce monokaryons able to fruit. Ullrich (1973) found that 27% of *Sistotrema* isolates formed monokaryotic fruit bodies, but only 7% of *Schizophyllum* strains did so (Raper & Krongelb, 1958), and just one of 16 monokaryons of *Coprinus cinereus* tested by Uno & Ishikawa (1971). The fact that most monokaryotic fruit bodies are abnormal structures, usually incomplete, sterile or both, raises the question of whether genes which influence fruiting in monokaryons are relevant to the normal process of dikaryotic fruiting.

Conventional genetic crosses between monokaryotic fruiting strains of *Polyporus ciliatus* identified three unlinked genes involved in monokaryotic fruiting (Stahl & Esser, 1976). These were: fi^+, which was thought to initiate monokaryotic fruiting, fb^+, regarded as being responsible for 'moulding' the structure of the fruit initiated by fi^+ into a fruit body; whilst the third gene, mod^+, appeared to direct development into a futile pathway leading to formation of non-fruiting mycelial masses called stromata. In the dikaryon, mod^+ inhibited fruiting, but neither fi^+ nor fb^+ showed any expression in the dikaryon even when homozygous.

A very similar genetic system was found in analogous experiments with the agaric *Agrocybe aegerita* (Esser & Meinhardt, 1977). Again, fi^+ was identified as being responsible for initiation of monokaryotic fruiting, and a second, fb^+, was considered to be responsible for modelling the initiated structures into fruit bodies. In contrast to the situation in the polypore *Polyporus*, the *Agrocybe* genes affected fruiting in the dikaryon as well. Fertile fruit bodies were produced only by dikaryons carrying at least one allele of both fi^+ and fb^+.

A number of monokaryotic fruiting strains of *Schizophyllum commune* have been studied. Raper & Krongelb (1958) examined some called *hap* and found no correlation between monokaryotic and dikaryotic fruiting. They suggested that monokaryotic fruiting was probably under polygenic control. Later, Esser *et al.* (1979) identified four genes which controlled monokaryotic fruiting. There were two 'fruiting initiation genes' (fi-1^+ and fi-2^+, either of which alone allowed differentiation into fruit body 'initials' of about 2 to 3 mm in size; when both were present, fruit body stems 6 to 8 mm long were formed. A third gene (fb^+) allowed formation of complete monokaryotic fruit bodies. The fourth gene (st^+) prevented expression of the others. Any monokaryon carrying st^+ produced only stromata and a homozygous st^+/st^+ dikaryon did not fruit. The other three genes had no effect on formation of fruit bodies by the dikaryon but did influence how quickly the dikaryon fruited. Dikaryons homozygous for all three monokaryotic fruiter genes fruited most rapidly. Dikaryons which did not carry any of the monokaryotic fruiter alleles fruited most slowly. Although slow to fruit, dikaryons lacking monokaryon fruiting alleles did form fruiting bodies and this clearly implies a major difference in the impact which these genes have on the fruit body development pathway in the two types of mycelium. Barnett & Lilly (1949) also reported increased frequency of fruiting in dikaryons made from monokaryotic fruiters in *Lenzites trabea*.

Monokaryons of *Schizophyllum commune* initiate fruiting bodies in response to mechanical and chemical injuries. Leslie & Leonard (1979a, b) identified 8 genes in four distinct genetic pathways involved in this. These genes were thought to operate at a stage prior to formation of aggregations of hyphae without defined shape (Leslie & Leonard, 1979b). They may be distinct from those identified by Esser *et al.* (1979), which produce structures with a recognisable (stem-like) shape. However, subjective descriptions of morphology are the only basis for judgement about relationships between these systems. The range of genetic factors involved in monokaryotic fruiting parallels the range of physiological conditions which are able to promote such fruiting. A particularly peculiar report is that dikaryotic fruiting bodies were induced on monokaryotic cultures of *Coprinus cinereus* when the latter were subjected to nutritional stress for several weeks to several months (Verrinder Gibbins & Lu, 1984). The authors= explanation was that nutritional stress triggers a mating type switch which results in a conventional dikaryon being established. This would result in four mating types segregating from fruit bodies formed on initially monokaryotic cultures. Unfortunately, the progeny spores were not subjected to mating type analysis.

As mentioned above, some of the genes identified in monokaryons do show expression in the dikaryon (Stahl & Esser, 1976; Esser & Meinhardt, 1977; Esser *et al.*, 1979) but the role which they might play is obscure. The only molecular observation made on this phenomenon is that Horton & Raper (1991) identified a DNA sequence which induced monokaryotic fruiting in strains of *Schizophyllum commune* into which it was introduced by transformation. This gene (*Frt1*) may be a component of a fruit body production pathway and is being used as the basis of a molecular search strategy for other sequences involved (Horton, 1998).

Genetics of dikaryotic fruiting

The only organism in which any concerted attempt has been made to apply conventional genetic approaches to study of fruit body formation by the dikaryon is the ink-cap fungus, *Coprinus cinereus*. Dikaryons of *C. cinereus* can form sclerotia and mushroom fruit bodies, monokaryons may also form sclerotia but normally do not fruit. The earliest stages in the development of both sclerotia and fruit bodies have been described separately and the descriptions are remarkably similar (Matthews & Niederpruem, 1972; Waters *et al.* 1975a). Development of both structures from the mycelium involves similar patterns of hyphal aggregation so there may be a shared initial pathway of development. This was proved with the aid of monokaryons unable to form sclerotia, a phenotype which segregated in crosses as though controlled by a single major gene. Four *scl* (sclerotium-negative) genes were found; one, *scl*-4, caused developing fruit body primordia to abort even when paired in the dikaryon with a wild type nucleus. The other *scl* genes behaved as recessive alleles in such heteroallelic dikaryons and were mapped to existing linkage groups (Waters *et al.*, 1975a, b). Later, Moore (1981a) showed that dikaryons in which both nuclei carried the same *scl* allele (homoallelic dikaryons) were unable to form either sclerotia or fruit bodies. Since these single genetic defects blocked development of both dikaryon structures it was concluded that sclerotia and basidiomata share a common developmental pathway in the initial stages, which is governed by the *scl* genes. When *scl* genes mutate they are usually recessive, so the pathway can proceed only in the heteroallelic dikaryon where the missing *scl* function is provided by the nucleus from the normal parent.

Takemaru & Kamada (1971, 1972) searched for developmental abnormalities among the survivors of mutagen-treated fragments of dikaryotic mycelium of *Coprinus cinereus* (under the name *C. macrorhizus*). Including spontaneous mutations, a total of 1594 were identified out of 10641 dikaryotic survivors tested and were classified into categories on the basis of the phenotype of the fruit body produced. The categories were:

> *knotless,* no hyphal aggregations were formed,
> *primordiumless,* aggregations were formed but did not develop further,
> *maturationless,* primordia were produced which failed to mature,
> *elongationless,* stem failed to elongate but cap development was normal,
> *expansionless,* stem elongation normal but cap failed to open,
> *sporeless,* few or no spores were formed in what were otherwise normal fruit bodies.

In these experiments the mutagen survivors were dikaryotic, so the genetic defects identified were all dominant. *Elongationless* mutants have been used to study stem elongation (Kamada & Takemaru, 1977a & b, 1983), and *sporeless* mutants have been used to study sporulation (Miyake *et al.*, 1980a, b). The regular categories into which these mutant phenotypes can be classified suggest that fruit body development is organized into different pathways which are genetically separate. For example, prevention of meiosis (the *sporeless* phenotype) still permits the fruit body to develop normally, demonstrating that meiosis and spore formation are entirely separate from construction of the spore-bearing structure. Similarly, the significance of separate classes of mutants with defects in either cap expansion or stem elongation is that it shows that these events can also be separated genetically. Both processes depend on enormous cell inflation, and the fact that they can be separated by mutation indicates that the same result (increase in cell volume) is achieved by different means. Moore *et al.* (1979) discuss physiological features which lead to the same conclusion.

Takemaru & Kamada (1972) observed a very high frequency of dominant mutations (more than 1 in 10 of the mutagen survivors) and over 72% of the mutants belong to just two phenotypes (595 maturationless and 582 sporeless isolates in the total of 1582 mutants). The original authors suggested that genes involved in development may be easy to mutate, but there are no reports of mutation at such frequencies in other *C. cinereus* dikaryons so this explanation is unlikely. An alternative interpretation (Moore, 1981a) is that the genes which were being caused to mutate were not those involved directly in development, but rather genes which modify the dominance (or penetrance) of developmental variants which already existed in the dikaryon used by Takemaru & Kamada (1972). This interpretation was arrived at following work showing that the penetrance of *scl* genes in heteroallelic dikaryons depended on the segregation of modifiers (Moore, 1981c). Differentiation in basidiomycetes clearly involves extensive protein processing (Zantinge *et al.* 1979; de Vries *et al.* 1980; Moore & Jirjis, 1981). If the >penetrance modifiers= are actually components involved in processing signal sequences of structural proteins, then a mechanism for this activity is evident. In the presence of particular modifier alleles (those which cause the change in penetrance), signal processing might lead to normal structural proteins failing to reach their correct destination, or to abnormal proteins being partially corrected so that they do reach the target site, despite being defective. In either case, the processing modification will affect gene expression.

Isolation of strains of *C. cinereus* which have mutations in both mating type factors (*Amut Bmut* strains) has enabled new studies of the genetics of morphogenesis in this organism. *Amut Bmut* strains are homokaryotic phenocopies of the dikaryon. They are similar to the dikaryon in that their hyphae have binucleate compartments and extend by conjugate nuclear division with formation of clamp connections. Also, the cultures can produce apparently normal fruit bodies. On the other hand they are homokaryons, and contain only one (haploid) genetic complement (Swamy *et al.*, 1984). This last feature allows expression of recessive developmental mutations and these strains have been used to study a number of developmental mutants (Kanda & Ishikawa, 1986) especially in meiosis and spore formation (Zolan *et al.*, 1988; Kanda *et al.*, 1989a, 1990; Kamada *et al.*, 1989) and in the formation of fruit body primordia (Kanda *et al.*, 1989b). No overall fruit body developmental pathway has yet emerged, nor has any information about major regulators other than mating type factors, but the molecular methods of analysis which are now being applied (De Groot *et al.*, 1997; Van Griensven & Visser, 1998) seem likely to increase enormously our knowledge of this topic in the next few years.

Expression of fruiting genes in higher fungi

Classical genetic approaches give no guidance about the way in which genes causing developmental variants cause their effects. Possibly the first enzymes identified as having an important role in morphogenesis were glucanases involved in wall degradation. The idea that cell wall components are re-cycled during morphogenesis originated with studies made by Wessels with *Schizophyllum commune* (Wessels, 1965; Wessels & Niederpruem, 1967; Wessels & Sietsma, 1979), and has received support from work with fruit bodies of *Flammulina velutipes* (Kitamoto & Gruen, 1976) and *Coprinus congregatus* (Robert, 1977a & b) among basidiomycetes, as well as *Aspergillus nidulans* cleistothecia (Zonneveld, 1977). The latter example is important because a mutant of *A. nidulans* which lacked α-163 glucan was unable to form cleistothecia (Polacheck & Rosenberger, 1977) and mutants deficient in either cleistothecial formation or conidiation or both, confirmed the close correlation between the presence of α-163 glucan, depletion of glucose, synthesis of α-163 glucanase and cleistothecial formation (Zonneveld, 1974). Another important reason for referring to these studies on *A. nidulans* cleistothecium development is that they emphasise the flexibility of the

developmental process by showing that if glucan reserves are low, proteins may be utilized for cleistothecium formation. As in many fungal developmental processes, cleistothecium production is triggered by nutrient limitation, but the exact nature of that limitation determines whether glucans or proteins are used during morphogenesis. When nutritional circumstances demand, specific >developmental= glucanases can be replaced by specific proteinase activity (Zonneveld, 1980). This sort of flexible integration of enzyme activities to suit the prevailing conditions is an example of developmental plasticity (= tolerance of imprecision).

Only a small fraction of the genome is specific to morphogenesis, and correspondingly few morphogenesis-specific polypeptides have been identified. A development-specific protein has been identified in sclerotia of *Sclerotinia sclerotiorum* (Russo *et al*., 1982) and a polypeptide specific to fruit body (ascomatal) development has been detected in *Neurospora tetrasperma* (Nasrallah & Srb, 1973, 1977) and localized to the mucilaginous matrix surrounding the asci and paraphyses (Nasrallah & Srb, 1978). In *Sordaria brevicollis*, 17 out of over 200 polypeptides detected after pulse-labelling were found in perithecia after crossing (Broxholme *et al*., 1991). De Vries & Wessels (1984) found only 15 polypeptides specifically expressed in fruit body primordia of *Schizophyllum commune*. Analysis of specifically-transcribed RNA also suggest that expression of only a small proportion of the genome is devoted to morphogenesis in both *S. commune* (Zantinge *et al*., 1979; de Vries *et al*., 1980) and *Coprinus cinereus* (Yashar & Pukkila, 1985; Pukkila & Casselton, 1991). In *C. cinereus*, Kanda *et al*. (1986) found only four so-called 'cap proteins' which were abundant in cap cells but rare in the stem.

In situ hybridisation has been used to demonstrate reallocation of ribosomal-RNA between fruit bodies and their parental vegetative mycelium in *S. commune* (Ruiters & Wessels, 1989a) and accumulation of fruiting-specific RNAs in the fruit body has also been demonstrated (Mulder & Wessels, 1986; Ruiters & Wessels, 1989b). Dons *et al*. (1984) cloned a gene from among the fruiting-specific sequences which belongs to a family of sequences encoding hydrophobins. These are cysteine-rich polypeptides which are excreted into the culture medium but polymerise on the wall of aerial hyphae as they emerge into the air (to form fruit body initials, for example) and invest them with a hydrophobic coating (Wessels, 1992, 1996). In *S. commune*, some hydrophobin genes are under control of the mating-type genes (Ásgeírsdottir *et al*., 1995), and sequences coding for hydrophobins have been found in *Agaricus bisporus* (Lugones *et al*., 1996; De Groot *et al*., 1996), one of which specifically accumulates in the outer layers of mushroom caps (the 'peel' tissue) during fruit body development (De Groot *et al*., 1996). Hydrophobins have been very widely encountered in fungi, however (de Vries *et al*., 1993). About 20 have been recognised by gene sequence homology. They are two-domain proteins (one domain hydrophilic, the other hydrophobic) capable of self-assembly at hydrophilic-hydrophobic interfaces (= interfacial self-assembly) into amphipathic films which may be very insoluble (protein films formed by *S. commune* SC3 are insoluble in most aqueous and organic solvents). The hydrophobins are a large and diverse family of proteins which contribute to the non-specific interactions which assist microorganisms to attach to surfaces (Marshall, 1991). As such, they have been suggested to have roles in spore dispersal and adhesion (particularly in pathogens) as well as during morphogenesis (Hazen, 1990; Stringer *et al*., 1991; Wessels *et al*., 1991; Wessels, 1992; St Leger *et al*., 1992; Talbot *et al*., 1993; Bidochka *et al*., 1995a, b).

For morphogenesis, it is important to remember that there are numerous hydrophobins. The different ones may function differently and at different times. The *S. commune* SC3 hydrophobin coats aerial hyphae and hyphae at the surface of fruit bodies, the SC4

hydrophobin coats voids (air channels?) within solid fruit body tissues (Wessels, 1994). Both confer hydrophobicity to these surfaces, but since hydrophobins form amphipathic layers, they can also make hydrophobic surfaces wettable. Teflon sheets immersed in SC3 hydrophobin become coated with a strongly adhering protein film that makes the plastic surface wettable (Wösten *et al.*, 1994). The hydrophobins alone suggest mechanisms which may be responsible for adherence of hyphae to each other and to other surfaces. More generally, they indicate that the surface properties of the hypha can be controlled and manipulated to serve particular morphogenetic purposes as a result of specific gene expression. In *Lentinula edodes*, a fruit body specific gene product, *mfbAc* (not detected in vegetative mycelia or primordia but only in the immature and mature fruit body stages) has a motif (Arg-Gly-Asp) with demonstrable adhesion properties. Thus it may be secreted to function as a cell adhesion protein involved in maturation of fruit bodies (Yasuda *et al.*, 1997). Galectins (lectins which bind galactose and related sugars) are similarly thought to contribute to development of multicellular structures in *Coprinus cinereus* because they are localised to the hyphal wall and produced particularly in sclerotia and fruit bodies (Boulianne *et al.*, 1998).

Genes directly involved in morphogenesis are presumably ultimately controlled in some way by the transcriptional regulators produced by the mating type factors. Certainly, most of the recognisable developmental-specific genes seem to be transcriptionally regulated (de Vries *et al.*, 1980; Huang & Staples, 1982; Lee & Dean, 1993; Schuren *et al.*, 1993). It is important to remember, though, that the >text-book= heterothallic fungi (*Neurospora crassa*, *Coprinus cinereus*, *Schizophyllum commune*) are not representative of all fungi, so fungal morphogenesis cannot depend on mating type factor activity.

Indeed, they are least representative of commercial fungi. *Agaricus bisporus* is secondarily homothallic, but the most extreme example is *Volvariella volvacea*, which exhibits primary homothallism. In *V. volvacea*, fruit bodies formed on mycelia grown from haploid, uninucleate basidiospores frequently segregate self-fertile and self-sterile progeny in a 1:1 ratio. This segregation has been shown to persist through three successive generations of self-fertilization. Progenies of self-fertilized fruit bodies also regularly segregate a range of mycelial morphological variants and DNA markers generated by arbitrarily-primed PCR also reveal a high level of variation in self-fertilized F_1 and F_2 progenies. Clearly, in this organism morphogenesis is independent of mating type factors and there are mechanisms (presently poorly understood) which are able to generate genetic variation at the DNA level (rather than the chromosomal level) so that this haploid homothallic fungus can maintain diversity through periods of inbreeding.

Overview

Recessive mutations can lead both to loss and gain of the ability to form multicellular structures. As examples we can cite the *scl* mutants of *C. cinereus* which are involved in fruit body initiation and which have ***lost*** the ability to form sclerotia. Contrast these with the *fis* mutants and the *roc* gene which ***cause*** monokaryotic fruiting or stromatic proliferations (Uno & Ishikawa, 1971; Nyunoya & Ishikawa, 1979) in *C. cinereus*. The *hap*, *fi* and *fb* genes in *Schizophyllum* also enable the monokaryon to form a fruit body, a phenotype which is normally a character of the dikaryon. Attempts have been made to simplify many of these observations into a single developmental pathway (Esser & Hoffman, 1977; Esser *et al.*, 1977; Meinhardt & Esser, 1983), but the evidence more strongly points to there being a number of discrete partial pathways which can run in parallel. This appears to be reflected in the fact that variation in fruit body morphology is common in higher fungi (Watling, 1971; Chiu *et al.*,

1989) and can span generic (Bougher *et al*., 1993) and even wider taxonomic boundaries (Watling & Moore, 1994). Consideration of these fruit body polymorphisms has led to the suggestion that normal morphogenesis may be an assemblage of distinct developmental subroutines (Chiu *et al*., 1989). This concept views the genetic control of overall morphogenesis as being compartmentalised into distinct segments which can be put into operation independently of one another. This model postulates subroutines for hymenophore, hymenium, stem, cap, etc., which in normal development appear to be under separate genetic control. In any one species they are thought to be invoked in a specific sequence which generates the >normal= morphology of that species. Stress (nutritional, environmental, genetic) may cause the same subroutines may be invoked in a different sequence to form an abnormal (= polymorphic) fruit body. The model provides a unifying theme for categorising fruit body ontogeny and for clarifying phylogenetic and taxonomic relationships (Watling & Moore, 1994).

As in other eukaryotes, fungal genes are controlled by regulatory proteins which can bind to short sequences upstream of the start of transcription, and there is a good deal of evidence that transcriptional control is an important gene regulation mechanism during fungal morphogenesis. It is not the only mechanism, however. The translational regulation observed in *Aspergillus* conidiation is a powerful means of relating entry into a developmental pathway to nutritional status and to other extracellular signals. Timberlake (1993) described activation of the conidiation pathway in *A. nidulans* as 'translational triggering' because the messenger RNA encoding the regulators is already present but the regulatory open reading frames (ORFs) are untranslated. They remain untranslated because translation of an upstream reading frame takes precedence and prevents ribosomes attaching to the regulatory reading frames. Physiological stress represses translation of the upstream >trigger-ORF= and this enables the downstream regulators to be translated. Timberlake (1993) suggests that the translational trigger may be a way of making development sensitive to the nutritional status of the hypha, as nitrogen limitation (and other common environmental signals for initiation of differentiation) could reduce aminoacyl-tRNA pools and disturb translational regulation by trigger-ORF.

Given the prevalence of data which indicate that hyphae (i) need to develop a state of competence before they are able to undertake a developmental pathway, and (ii) can be precipitated into a developmental pathway by a variety of environmental signals, it is very likely that translational triggering is widely used as a regulatory mechanism throughout the higher fungi.

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