Text of an invited opening **keynote lecture** at a symposium organised jointly by the British Mycological Society and the Mycological Society of Japan, 25th and 26th November, 1998, in Chiba University Japan.

Moore, D. (1998). Genetic and physiological nature of morphogenetic control during initiation and development of fungal fruit bodies. In: *Progress in Mycological Sciences in Japan and the United Kingdom, Proceedings of the Sixth International Symposium of the Mycological Society of Japan* (Y. Kitamoto, Y. Harada & A. Suzuki, eds), 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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Like 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. 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.

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.

Classical genetic approaches to establishing a developmental pathway for fruit body formation involve:

- 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 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.

The most successful work was that of Takemaru & Kamada (1971, 1972) who 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. 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. Physiological features lead to the same conclusion.

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. This last feature allows expression of recessive developmental mutations and these strains have been used to study a number of developmental mutations especially in meiosis and spore formation and in the formation of fruit body primordia. 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 seem likely to increase enormously our knowledge of this topic in the next few years.

Only a small fraction of the genome is specific to morphogenesis, and correspondingly few morphogenesis-specific polypeptides have been identified. In *Sordaria brevicollis*, 17 out of over 200 polypeptides detected after pulse-labelling were found in perithecia after crossing and only 15 polypeptides were 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 and *Coprinus cinereus*.

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. 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, the "textbook standards" 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 not 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.

Attempts have been made to simplify many of these genetic observations into a traditional pathway, 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 and can span generic and even wider taxonomic boundaries. Consideration of these fruit body polymorphisms has led to the suggestion that normal morphogenesis may be an assemblage of distinct developmental subroutines. 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; Moore, 1998).

Moore, D. (1998). *Fungal Morphogenesis*. Volume 35 in the Developmental and Cell Biology series. Cambridge University Press: New York.

Takemaru, T. & Kamada, T. (1971). Gene control of basidiocarp development in *Coprinus macrorhizus*. *Reports of the Tottori Mycological Institute (Japan)* **9**, 21-35.

Takemaru, T. & Kamada, T. (1972). Basidiocarp development in *Coprinus macrorhizus*. I. Induction of developmental variations. *Botanical Magazine (Tokyo)* **85**, 51-57.

Timberlake, W. E. (1993). Translational triggering and feedback fixation in the control of fungal development. *Plant Cell* **5**, 1453-1460.

Watling, R. & Moore, D. (1994). Moulding moulds into mushrooms: shape and form in the higher fungi. In *Shape and Form in Plants and Fungi* (ed. D. S. Ingram & A. Hudson), pp. 270-290. Academic Press: London.