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Programmed cell death is not involved in initiation of the gill cavity of *Coprinus cinereus*: A study using morphological mutants

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ABSTRACT: Mushroom development results from a co-ordination of independent genetic and physiological subroutines. The nature of the subroutines involved and how and when they are invoked creates the spatial and temporal morphological pathway that is the taxonomic characteristic of a species. Morphogenesis can require the removal of tissue as well as tissue growth. The cell death responsible for this removal must be controlled in time and position as part of the differentiation process. This, called programmed cell death (PCD), could form a developmental subroutine able to sculpture tissue blocks from the hyphal mass which makes up the fruit body initial and primordium. PCD is highly organized in animals, though it occurs in plants, too. It is important to appreciate that the apoptotic cell death that occurs in vertebrates is a specialized form of PCD in which most of the process is kept inside an intact cell membrane to avoid leakage of antigens and consequent autoimmunity. Fungal PCD can, therefore, be very different from animal PCD because the immune reaction is not an issue. Indeed, fungal PCD appears to involve lysis of hyphal compartments. Recent work suggests that cell death is involved in formation of gill cavities in *Agaricus bisporus*. However, no sign has been found of cell disintegration/cell death during gill formation in the mushroom of *Coprinus cinereus*. Gill formation in this mushroom has been interpreted as featuring branches of determinate growth being organized into opposing palisade cell plates, forming an incipient fracture plane. This plane being opened out into a cavity when expansion of underlying tissue put tension across the 'fracture' and pulled the palisades apart. Morphological mutants have been used very effectively to dissect morphogenetic pathways in *Coprinus cinereus*. A hymenophoreless mutant is interesting in this context as the hymenophore bearing gills must arise within the fruit body cap. So a mutant unable to make hymenophores enables us to determine whether programmed cell death is involved in defining the pathway and architecture of a hymenophore. We find that in hymenophoreless mutants of *C. cinereus*, cap expansion and sufficient consequential mechanical stress to cause cell disruption and create space definitely occur. However, no gills are formed. In contrast, the *revoluta* mutant (in which the cap does not enclose the stem) ***is*** able to make mature gills but no cell death is involved. In neither sort of mutants is open space a committing step for gill cavity formation. This leads us to conclude that the key is the patterning of hyphal tips into the palisades which become opposing hymenia, and not the formation of a primordial gill space. Thus, a gill organizer must be present to make gills in *Coprinus*.

1 INTRODUCTION

A mushroom fruit body results from co-ordinated tissue development (Chiu & Moore 1996; Moore 1998). The spatial and temporal morphological pathway is a taxonomic characteristic of a species (Singer 1986; Clémençon 1997) although developmental and genetic plasticity has been reported in many species too (Watling 1985; Chiu 1988; Chiu & Moore 1990b, 1996, 1999a, b; Moore 1998). Morphogenesis can require the removal of tissues as well as tissue growth. The cell death responsible for this removal must be controlled in time and position.

This is programmed cell death (Lockshin, Zakeri & Tilly 1998). In the animal system, the programmed cell death called apoptosis has the following characteristics: the cell membrane stays intact but loses its asymmetry and shows the phenomenon of blebbing (in contrast to necrosis where the cell membrane becomes leaky). Also, in animal and plant apoptosis, the chromatin condenses and the DNA is degraded into multiple oligonucleotide fragments (Lockshin *et al.* 1998).

Morphological mutants have been used to dissect morphogenetic pathways in *Coprinus cinereus* (Kanda & Ishikawa 1986; Chiu & Moore 1990a, 1996; Muraguchi & Kamada 1998; Muraguchi, Takemaru & Kamada 1999). A hymenophore-less mutation which is to be reported here is the most interesting as the hymenophore (which can be in one of the following forms: gills, pores, teeth, etc.) is the tissue subroutine differentiated in a fruit body for the final production of sexual cells for species propagation.

In fungal taxonomy, there are three major modes of development of the hymenial initials (Singer 1986; Watling 1985; Clémençon 1997):

a. Levhymenial type: a smooth, uninterrupted hymenial palisade producing gills whose edges never touch the underlying trama and whose hymenium is therefore never interrupted (always covering the gill edge) (e.g. *Volvariella* species, Chiu & Moore 1990b);
b. Rupthymenial type: there is never a continuous hymenial palisade, as the gills take shape by the formation of radial slits in the pileus trama that remains connected with the stipe trama. Later, the gills separate from the stipe surface, but the gill edge usually remains free of basidia.
c. Schizohymenial type: radial gill cavities are formed by lysis of pileus trama and the cavities become lined with a hymenial layer (e.g. *Amanita*, Singer 1986).

Coprinus initiates the first rank of gills within the fruit body cap using the rupthymenial mode of gill initiation (Reijnders 1963, 1979; Rosin & Moore 1985). Also, the *revoluta* mutant is interesting as it lacks this genus-specific feature but retain the other modes of gill formation as with other fleshy mushrooms (Chiu 1988; Chiu & Moore 1990a, b). Thus in the present study, both types of mutants were examined to detect the relationship of programmed cell death and gill cavity in *Coprinus cinereus*.

2 ORIGIN & CHARACTERISATION OF THE MORPHOLOGICAL MUTANTS

Two hymenophore-less mutants (cw2 and cw20) were obtained after mutagenesis. They had nonparental colony morphology, nonparental fruit body morphology, and differed in five DNA fingerprints generated by polymerase chain reaction using random or arbitrary primers (RAPD and AP-PCR) (Chiu & Moore 1999b). Similarly, the *revoluta* strain is an AmBm *paba*-1 auxotrophic mutant.

When crossed with the wild type, heterozygous dikaryons made with these mutants produced normal fruit bodies, indicating that the mutations are recessive.

3 FRUIT BODY MORPHOLOGY

3.1 *Hymenophore-less strains*

Both hymenophore-less mutants showed highly similar fruit body morphology. Primordia of these mutants were spherical to ovoid, sometimes with a groove in the apical centre. Both mutants bore a well-defined 'knob' delimiting the apex of the fruit body cap. In larger fruits, veils cells were less obvious than normal (not swollen, nor elongated) and were not deciduous. In addition, no pileipellis was found. The upper portion of the presumptive cap region expanded and caused random cleavage around the intact apex, giving a 'crown-like' appearance. Gills, however, were never found. The lower portion of the presumptive stem was short, slender and sometimes curved; it failed to elongate fully (Figure 1). When the plate cultures or compost cultures were placed in darkness, etiolated fruit bodies were produced.

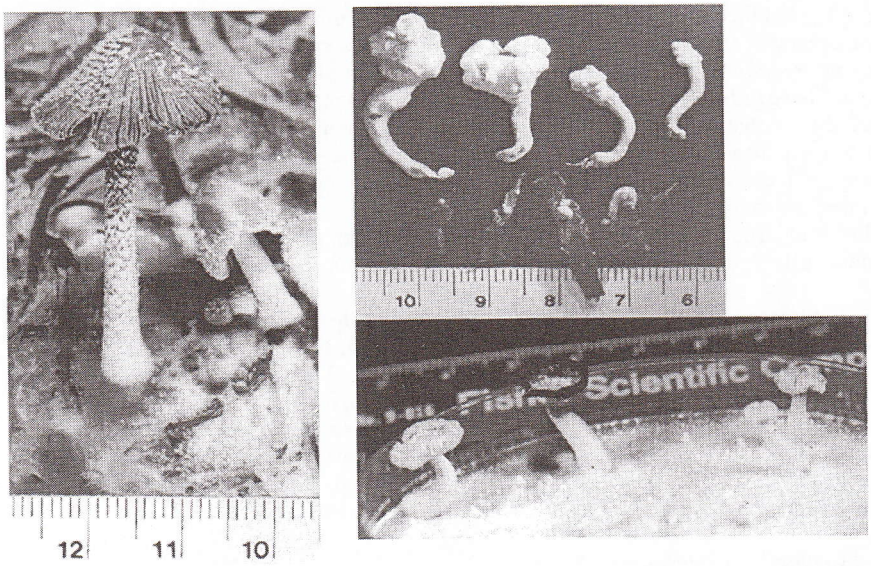

Figure 1. Fruit body morphologies of the parent (left), hymenophoreless mutant (top right) and revoluta mutant (bottom right).

3.2 *The revoluta mutant*

The *revoluta* mutant fruited much more slowly, taken more than 20 days. After primordia appeared, they grew in size but the stem did not reach normal height. This short and solid stem makes the mutant look like a fleshy mushroom, rather than the coprinoid type. Stem, cap and hymenophore initiation did not occur at the same time as in the wild type (Rejinders 1963, 1979; Moore, Elhiti & Butler 1979). Rather, cap differentiated out from the apical stem. Veils cells differentiated to cover the cap. When the cap expanded to create space between the stem and the lower surface of cap, gills then appeared. These first formed gills were convolute and the free edge of the cap was not connected to the central stem but finally revoluted (curved upwards) away from the stem. Sporulation was normal and four-spored basidia were observed. Then final stage was the autolysis of gills and spore dispersal. As the gills were formed not via rupthymenial mode, all the gills of revoluta mutant had entire margins.

3.3 *Implications of the Morphological Mutations*

3.3.1 *Hymenophore formation is not initiated by cell death*

Umar & Van Griensven (1997, 1998) suggested that cell death is involved in the formation of the first gill spaces in *Agaricus bisporus* and other fungi. Lu (1974, 1991) claimed that gill cavities in *Coprinus* arise as a result of cell disintegration process he called programmed cell death. Neither Reijnders (1963, 1979) nor Rosin & Moore (1985) found any sign of cell disintegration or cell death during gill formation in this mushroom.

Cap expansion and sufficient mechanical stress to cause severance of hyphae and create space by cell disruption definitely occur in hymenophore-less mutants, but no gills were formed. Thus formation of gills of the first rank in *Coprinus* does not involve programmed cell death.

The interpretation of gill formation of Rosin & Moore (1985) features branches of determinate growth being organized into opposing palisade cell plates, forming an incipient fracture plane. This plane can be opened out into a cavity when expansion of underlying tissue puts tension across the 'fracture' and pulls the palisades apart (Moore 1994). The key process is the

patterning of hyphal tips into a fracture plane by some sort of gill organizer.

Tensions generated from cap expansion lead to appearance of cavities in the fruit body. As revealed by the *revoluta* strain and wild types, even for gills of the higher ranks, cell death is not involved. These gills of the higher ranks are formed through bifurcation of the existing gills by localized differentiation into space created by cap expansion within a pre-existing gill, and by folding of the palisade when the cap circumference increases (Chiu & Moore 1990a, 1996). These spaces are created mechanically as the cystidia connect opposing gills to each other, and the cap continues to expand.

Thus, this study supports the concept that a gill organizer must be present (Moore 1994; 1998) to make gills, and in these hymenophore-less mutants, its expression is impaired.

3.3.2 *Pleiotropic aspects of these mutations: effect on stem elongation*

Numerous studies seem to imply that extracts or diffusates of the cap can stimulate growth of the stem (Novak Frazer 1996) and the gill is usually considered to be the source of these active agents (Moore 1998). In the hymenophore-less mutants, as there is no hymenophore formation, growth factors or hormones were presumably not produced to stimulate stem growth. Consequently, these fruits have shortened, slender and curved stems. The *revoluta* strain showed a comparatively short but solid stem resembling the basal region of a normal stem, implying that insufficient stimulation of stem elongation occurred in these abnormal fruit bodies.

3.3.3 *The hymenophore subroutine*

Reijnders (1963, 1979) emphasized the contribution of the veil and pileipellis (the 'epidermis' of the cap) to the form and shape of a mature fruit body. The *revoluta* strain shows a normal (wild type) veil and pileipellis. In hymenophore-less mutants, there was no pileipellis and veil cells were rudimentary. So veil and pileipellis are secondary characteristics of a hymenophore subroutine.

3.4 *Other Related Mutants*

Recently, a spontaneous recessive allele giving rise to a similar phenotype to hymenophore-less was recovered from a field isolate in Japan (Muraguchi & Kamada 1998). The defect in the Japanese isolate was traced to a deletion of the promoter extending into the 5' region of a gene named *ich*1 which encodes a novel protein containing nuclear targeting signals. In normal fruit body development, the transcript was specific for the cap and abundance of the transcript decreased as basidiospores were produced (Muraguchi & Kamada 1998).

Specific primer sets for detecting the promoter (primer set: 1 and 2), the 5' region (primer set: 3 and 4) and middle region (primer set 5 and 6) of the structural *ich*1 gene were used for polymerase chain reaction. The *cw*2 mutant was amplified with the primer sets (3 and 4) and (5 and 6). Thus this mutant is also a deletion mutant but the deficiency region locates at the promoter only. In contrast, the *cw*20 mutants yielded amplification products of the expected sizes for the detected regions. Unfortunately, a cross between *cw*2 and *cw*20 mutants has so far failed to fruit and the resultant colony showed signs of autolysis and senescence. This is in contrast to the report of viable homozygous *ich* dikaryons (Muraguchi & Kamada 1998).

Regardless of the genetic mechanism of these mutations, the present study indicates that neither programmed cell death nor necrosis could be a cause for initiation of the gill cavity in *Coprinus cinereus*.

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REFERENCES

Chiu, S. W. 1988. *Basidiome Morphogenesis in Two Basidiomycetes,* Coprinus cinereus and Volvariella bombycina. PhD Thesis, The University of Manchester, UK.

Chiu, S. W. & D. Moore 1990a. A mechanism for gill pattern formation in *Coprinus cinereus. Mycol. Res.* 94: 320-326.

Chiu, S. W. & D. Moore 1990b. Development of the basidiome of *Volvariella bombycina. Mycol. Res.* 94: 327-337.

Chiu, S. W. & D. Moore 1996. *Patterns in Fungal Development.* Cambridge, UK: Cambridge University Press.

Chiu, S. W. & D. Moore 1999a. *Sexual development in higher fungi.* In R. P. Oliver & M. Schweizer (eds) *Molecular Fungal Biology*: 231-271. Cambridge, UK: Cambridge University Press.

Chiu, S. W. & D. Moore 1999b. Segregation of genotypically diverse progeny from self-fertilized haploids of the Chinese straw mushroom, *Volvariella volvacea. Mycol. Res.* 103: 1335-1345.

Clémençon, H. 1997. *Anatomy of the Hymenomycetes.* Teufen, Switzerland: Flück-Wirth Verlag.

Kanda, T. & T. Ishikawa 1986. Isolation of recessive developmental mutants in the basidiomycete *Coprinus cinereus. J. Gen. Appl. Microbiol.* 32: 541-543.

Lockshin, R. A., Z. Zakeri & J. L. Tilly 1998. *When Cells Die: a Comprehensive Evaluation of Apoptosis and Programmed Cell Death.* New York: Wiley–Liss.

Lu, B. C. 1974. Meiosis in *Coprinus*. V. The role of light on basidiocarp initiation, mitosis, and hymenium differentiation in *Coprinus lagopus. Can. J. Bot.* 52: 299-305.

Lu, B. C. 1991. Cell degeneration and gill remodelling during basidiocarp development in the fungus *Coprinus cinereus. Can. J. Bot.* 69: 1161-1169.

Moore, D. 1994. Tissue formation. In N. A. R. Gow & G. M. Gadd (eds) *The Growing Fungus*: 423-465. London: Chapman & Hall

Moore, D. 1998. *Fungal Morphogenesis*. New York: Cambridge University Press.

Moore, D., M. M. Y. Elhiti, & R. D. Butler 1979. Morphogenesis of the carpophore of *Coprinus cinereus*. *New Phytol.* 83: 695-722.

Muraguchi, H. & T. Kamada 1998. A developmental mutation which blocks pileus formation in fruiting of *Coprinus cinereus. Development* 125: 3133-3141.

Muraguchi, H., T. Takemaru & T. Kamada 1999. Isolation and characterization of developmental variants in fruiting using a homokaryotic fruiting strain of *Coprinus cinereus. Mycoscience* 40: 227-234.

Novak Frazer, L. 1996. Control of growth and patterning in the fungal fruiting structure. A case for the involvement of hormones. In S. W. Chiu & D. Moore (eds) *Patterns in Fungal Development*:156-181. Cambridge, UK: Cambridge University Press.

Reijnders, A. F. M. 1963. *Les problèmes du développement des carpophores des Agaricales et de quelques groupes voisins*. The Hague: Dr W. Junk.

Reijnders, A. F. M. 1979. Developmental anatomy of *Coprinus. Persoonia* 10: 383-424.

Rosin, I. V. & D. Moore 1985. Origin of the hymenophore and establishment of major tissue domains during fruit body development in *Coprinus cinereus. Trans. Br. Mycol. Soc.* 84: 609-619.

Singer, R. 1986. *The Agaricales in Modern Taxonomy*. Koenigstein: Koeltz Scientific Books:

Umar, M. H. & L. J. L. D. Van Griensven 1997. Morphogenetic cell death in developing primordia of *Agaricus bisporus*. *Mycologia* 89: 274-277.

Umar, M. H. & L. J. L. D. Van Griensven 1998. The role of morphogenetic cell death in the histogenesis of the mycelial cord of *Agaricus bisporus* and in the development of macrofungi. *Mycol. Res.* 102: 719-735.

Watling, R. 1985. Developmental characters of agarics. In D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland (eds) *Developmental Biology of Higher Fungi*: 281-310. Cambridge, UK: Cambridge University Press.

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