degenerated and malformed. Nevertheless, a number of basidia and subhymenial cells were alive and cytologically intact even on day 36. So even in severely senescent fruit bodies healthy, living cells were found and these are presumably the origin of an unusual phenomenon known as **renewed fruiting**.

Field-collected fruit body tissues of a mushroom usually generate abundant vegetative hyphae when inoculated onto nutrient agar plates. Such reversion from the fruiting stage into vegetative stage is not an abrupt process, rather there appears to be some sort of 'memory' of the differentiated state. Initial hyphal outgrowth from gill lamellae usually mimics the densely packed branching and intertwined hyphal pattern of the gill tissues at first, being quite unlike the pattern of normal vegetative hyphae in culture. The 'memory' could be no more than the residual expression of differentiation-specific genes before their products are diluted out by continued vegetative growth, but as we have shown above (see Section 12.14), there is considerable scope for **epigenetic marks** in fungi.

Renewed fruiting (the formation of fruit bodies directly on fruiting tissue) is not uncommon, and it can occur at various locations (cap, stem and/or gills) in improperly stored excised fruit bodies. Experiments *in vitro* show that numerous primordia can arise on excised fruit body tissues and can mature into normal, though miniature, fruit bodies. In comparison to vegetative cultures, the excised fruit body tissues form fruit bodies very rapidly. For example, in *Coprinopsis cinerea*, renewed fruiting occurred within four days, compared with cultures inoculated with vegetative dikaryon which, under the same conditions, formed fruit bodies in 10 to 14 days (Chiu & Moore, 1988a; Brunt & Moore, 1989; Bourne, Chiu & Moore, 1996). Renewed fruiting may have an important role in survival, consuming and immediately recycling the resource in the dying fruit body tissue to disperse further crops of spores.

Umar & Van Griensven (1997b, 1998) found that cell death is a common occurrence in various structures starting to differentiate, for example the formation of gill cavities in *Agaricus bisporus*. The authors point out that specific timing and positioning imply that cell death is part of the differentiation process. **Fungal PCD** could play a role at many stages in development of many species (Moore, 2013). Individual hyphal compartments can be sacrificed to trim hyphae to create particular tissue shaping. PCD is used, therefore, to sculpture the shape of the fruit body from the raw medium provided by the hyphal mass of the fruit body initial and primordium.

In several examples detailed by Umar & Van Griensven (1998) the programme leading to cell death involves the sacrificed cells over-producing mucilaginous materials which are released by cell lysis. Remember that in autolysing Ink Cap gills the cell contents released on death contain heightened activities of lytic enzymes. Evidently, in fungal PCD the cell contents released when the sacrificed cells die are specialised to particular functions too.

Fungal cultures suffer spontaneous degeneration through successive subculture on artificial media; the culture may stop growing or suffer loss of (or severe reduction of) asexual sporulation, sexuality, ability to fruit, or reduced production of secondary metabolites. Thus, fungal culture degeneration can be a significant economic problem for industrial production processes. It's a problem that applies as much to yeast cultures as to cultures of filamentous fungi. A budding yeast mother cell can produce a finite number of daughter cells before it stops dividing and dies, whereas filamentous fungal cultures frequently and spontaneously degenerate during ongoing culture maintenance, resulting in the formation of sterile and/or weakly-growing sectors in the colony. Senescence seems to result from a progressive decline in physiological function, including mitochondrial dysfunction. This physiological decline is linked to impairments of cellular machines and to the generation of reactive oxygen species (chemically reactive molecules containing oxygen) which arise during normal metabolism (e.g., in the respiratory chain), and serve essential functions, but can cause molecular damage if in excess.

In *Neurospora*, senescing strains usually contain ***mitochondrial plasmids***, which cause insertional mutagenesis when they integrate into the mitochondrial DNA. The functionally defective mitochondria replicate faster than the wild-type mitochondria and spread between hyphal cells (Maheshwari & Navaraj, 2008). Senescence can also be due to ***spontaneous*** lethal mutations, either in nuclear or mitochondrial genes. Ultimately the growth of a fungal colony ceases due to dysfunctional oxidative phosphorylation (Li *et al*., 2014; Wiemer *et al*., 2016). Although the detailed underlying processes may differ from species to species, this situation appears to be basically conserved between organisms and is a major cause of degenerative diseases in humans (Morales-González, 2013); yet another area of eukaryote biology in which research on fungi can contribute to human health.

12.16 Basic principles of fungal developmental biology

The main features of fungal developmental biology have been summarised as a set of principles by Moore (2005). The list (see below) combines most of the observations made in the ***pregenomics era*** discussed above.

It starts with the reminder that in many fungi hyphae differentiate from the vegetative form that ordinarily composes a mycelium and aggregate to form tissues of multihyphal structures; which may be linear organs that emphasise parallel arrangements of hyphae, or globose masses that emphasise interweaving of hyphae, such as sclerotia and fruit of the larger Ascomycota and Basidiomycota.

The series of principles on which fungal morphogenesis is suggested to depend, most of which differ from both animals and plants, are as follows:

- **Principle 1**: the fundamental cell biology of fungi on which development depends is that hyphae extend only at their apex, and cross walls form only at right angles to the long axis of the hypha.
- **Principle 2**: fungal morphogenesis depends on the placement of hyphal branches. Increasing the number of growing tips by hyphal branching is the equivalent of cell proliferation in animals and plants. To proliferate, the hypha must branch, and to form an organised tissue the position of branch emergence and its direction of growth must be controlled.
- **Principle 3**: the molecular biology of the management of cell-to-cell interactions in fungi is completely different from that found in animals and plants; comprehensive genome surveys found no evidence in fungal genomes sequences which are crucial animal regulators, nor of plant control sequences.
- **Principle 4**: fungal morphogenetic programmes are organised into developmental subroutines, which are integrated collections of genetic information that contribute to individual isolated features of the programme. Execution of all the developmental subroutines at the right time and in the right place results in a normal structure. Developmental subroutines are equivalent to the transcriptional clusters, transcriptional waves of differential gene expression and differentially expressed gene sets detected in the transcriptional programme of the genome.
- **Principle 5**: because hyphae grow only at their apex, global change to tropic reactions of all the hyphal tips in a structure is sufficient to generate basic fruit body shapes.
- **Principle 6**: over localised spatial scales co-ordination is achieved by an inducer hypha regulating the behaviour of a surrounding knot of hyphae and/or branches. These are called hyphal knots and they have two fates, becoming either sclerotia or fruit body initials depending on temperature and illumination (high temperature and darkness favour sclerotia; light and lowered temperature favour the fruit body pathway).

- **Principle 7**: the response of tissues to tropic signals and the response of hyphal knots to their inducer hyphae, coupled with the absence of lateral contacts between fungal hyphae analogous to the plasmodesmata, gap junctions and cell processes that interconnect neighbouring cells in plant and animal tissues suggest that development in fungi is regulated by morphogens communicated mainly through the extracellular environment. Up-regulation of genomic transcripts in the 'extracellular matrix' category corresponds with what is known about the importance of the extracellular matrix to formation of hyphal knot which exude fluid droplets as they mature and become more condensed.
- **Principle 8**: fungi can show extremes of cell differentiation in adjacent hyphal compartments even when pores in the cross wall appear to be open (as judged by transmission electron microscopy).
- **Principle 9**: meiocytes appear to be the only hyphal cells that become committed to their developmental fate. Other highly differentiated cells retain totipotency - the ability to generate vegetative hyphal tips that grow out of the differentiated cell to re-establish a vegetative mycelium. Genomic analysis demonstrates that fungi, in general, have numerous genes involved in creating epigenetic marks by DNA methylation.
- **Principle 10**: in arriving at a morphogenetic structure and/or a state of differentiation, fungi are tolerant of considerable imprecision (= expression of fuzzy logic), which results in even the most abnormal fruit bodies (caused by errors in execution of the developmental subroutines) being still able to distribute viable spores, and poorly (or wrongly-) differentiated cells still serving a useful function.
- **Principle 11**: mechanical interactions influence the form and shape of the whole fruit body as it inflates and matures, and often generate the shape with which we are most familiar.

These **Principles** form the warp and weft of the canvas upon which fungal developmental biology has been built by the cell and molecular biologists of the pre-genomics era. It is now up to the ***genomic systems analysts*** to paint the rest of the individual details of the stories on that canvas (Kües & Navarro-González, 2015; Kües *et al*., 2018; Halbwachs *et al*., 2016; Pelkmans *et al*., 2016; Hibbett *et al*., 2017; Stajich, 2017).

Chapter 12.17 References and further reading

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