Biology of Fungi, Lecture 5: Fungal Development and Differentiation

Mold-Yeast Dimorphism

- Some fungi have the ability to alternate between a mold form and a that of a yeast form - dimorphic fungi
- Several pathogens of humans exhibit dimorphism
  - *Candida albicans*
  - *Histoplasma capsulatum*
- Dimorphism occurs in response to environmental factors, of which no one common factor regulates the morphological switch in all dimorphic fungi [Table 5.1, Deacon]
  - e.g., *Histoplasma capsulatum* - mold at 25°C, yeast at 37°C
  - e.g., *Mucor rouxii* - mold with oxygen, yeast in the absence of oxygen
- What is clear is that there is a change in polarity in terms of growth, thereby making study of the cell cycle a significant focal point
- To help identify the control of dimorphic growth, the mold and yeast phases of a fungus are compared - typically differences in biochemistry, physiology, and gene expression are noted
- Two fundamental questions on the observed differences:
  - Are the differences the cause of dimorphism?
  - Did the dimorphic switch cause the differences?
- Examples of differences:
  - Cell wall composition
  - Cellular signaling and regulatory factors
    - Calcium, calcium-binding proteins, cAMP, pH, and protein phosphorylation have all been shown to fluctuate depending upon the growth form of a dimorphic fungus
    - Not clear is some or all or any directly impact the changes in cell morphology
  - Gene expression differences
    - Measurement of mRNA production
    - Again, no clear cut answer as to an obligatory role of a gene in dimorphism
  - Possible unifying theme - the Vesicle Supply Center (VSC; Spitzenkörper)
    - Using computer generated models, Bartnicki-Garcia has postulated that the VSC is the key element in morphogenesis
    - The VSC ‘bombards’ the cell membrane with vesicles to direct wall biosynthesis
    - The VSC can change direction or even split to generate growth in two different directions simultaneously

Infection Structures

- ◆ Plant pathogens (and by analogy, insect pathogens) infect a host using a specialized pre-penetration structure
  - ∗ Swelling of germ tube tip - appressorium
  - ∗ Short lateral swelling of hyphal branch - hyphopodium
  - ∗ Several points of attack from a complex structure termed an infection cushion
- ◆ All of these structures serve as an anchor for release of enzymes (e.g., cutinase) followed by full penetration by an infection peg
- ◆ Penetration pegs push into material via turgor pressure formed by the conversion of stored glycogen into osmotically active compounds
- ◆ The appressoria produce an adhesive compound attachment and their cell walls contain melanin
  - ∗ Melanin helps appressoria resist deformation due to turgor pressure, re-directing the pressure to the infection peg
  - ∗ Melanin helps appressoria survive on surface by helping the fungus resist dessication and the effects of UV light
- ◆ Triggering mechanism for differentiation of infection structures relies on two types of contact-sensing:
  - ∗ Nontopographical - response is merely to the presence of a hard surface leading to localized secretion of adhesives and wall-degrading enzymes
  - ∗ Topographical -
  - ∗ Topographical - more specific response to ridges or grooves of particular heights/depths on the host surface
    - ● Hyphae grow randomly on surface until a groove is found
    - ● Growth then occurs vertically to this groove
    - ● When hyphae sense a stomatal ridge, they form an appressorium and begin to penetrate the surface via the stomatal opening
    - ● Involves stretch-activated ion channels leading to the influx of ions into the fungal cell
- ◆ Once penetration occurs, the fungus forms a huastorium within the tissue that absorbs nutrients from the host

Other Specialized Structures

- ◆ Sclerotia
  - ∗ Hyphal bodies involved in survival by dormancy
  - ∗ Structurally consist of repeated, localized hyphal branching that anastomose

* ∗ Germinate to form either
    * ● Hyphae (myceliogenic)
    * ● Sexual fruiting body (carpogenic)
* ∗ Triggering mechanisms:
    * ● Formation - nutrient depletion
    * ● Germination - nutrient favorable conditions

◆ Nutrient-translocating organs - formed due to lack of nutrients
* ∗ Mycelial cords - consolidated hyphae with non-specific structure
* ∗ Rhizomorphs - more defined structure than mycelial cord

Asexual Reproduction

◆ Two fundamentally different processes lead to the development of two distinct types of mitospores:
* ∗ Sporangiospores
* ∗ Conidia (conidiospores)

◆ Sporangiospores
* ∗ Formed by the cleavage of protoplasm within a multinucleate sporangium
* ∗ Several mechanisms
    * ● Large number of cleavage vesicles migrate around nuclei, then fuse to form the membrane of the spores
    * ● Central vacuole forms “arms” that fuse with the membrane of the sporangium to delimit the individual spores
* ∗ Flagellar apparatus in motile spores (e.g., *Phytophthora*)
    * ● Separate flagellar vesicle is separate, but fuses with the spore membrane after enclosing the nucleus presenting itself on the outside
    * ● Significant process in that there appear to be different chemoreceptors for the flagellar apparatus (in its membrane) and the spore
* ∗ Entire process of zoospore development and release is environmentally sensitive to nutrients, temperature, antibiotics, etc.

◆ Conidia
* ∗ Formed in various manners, but always external to the hypha or conidiophore
* ∗ Two basic types of conidial development:
    * ● Blastic - swelling or budding of hyphae
    * ● Thallic - fragmentation of hyphae

∗ Regulation of conidiation
- Traditionally difficult to study due to fact that cell growth is not synchronous across a colony
- Solved via the culture of *Aspergillus niger* using a chemostat
- In *A. niger*, three different nutritionally-related phases were uncovered
  - ✦ Initiation of conidiophore (switch from vegetative to sporulation) - nitrogen-limited, carbon-rich media
  - ✦ Development of conidiophore - requires nitrogen and citrate (or similar Krebs cycle intermediate)
  - ✦ Phialide formation - nitrogen and glucose required
- Whole process occurs on agar medium in a 1-2 mm zone located a few mm behind the leading edge of a hyphal colony
- Presumably, in an asynchronous agar culture, physiological changes bringing about conidia formation is co-ordinated
- Genetics of sporulation studied in *A. nidulans* leading to the discovery of three gene groups:
  - ✦ Switch from somatic growth to sporulation
  - ✦ Regulation of sporulation development
  - ✦ Secondary aspects (e.g., spore color)
- Some fungi require light to trigger sporulation
  - ✦ Near-UV light - 1 hour exposure can induce system
  - ✦ Blue light - represses sporulation (e.g., *Botrytis cinerea*)

∗ Role of hydrophobins
- Hydrophobins are secreted proteins that are unique to fungi
- Soluble in water, except at water/air interface where they form a film that surrounds a hyphae extending outwards, making it hydrophobic in nature and leading to different interactions among the hyphae/performing various functions

Sexual Development

◆ Sexual reproduction involves three fundamental processes:

∗ Plasmogamy - fusion of haploid cells

∗ Karyogamy - fusion of haploid nuclei

∗ Meiosis - reduction division

- Two fundamental points of sexual reproduction
  - Nature of sexuality
  - Serves as a survival mechanism
  - Nature of sexuality
    - Homothallic vs. heterothallic
    - Governed by mating type genes (compatibility)
    - Arrangement of mating types
      - Bipolar compatibility - governed by a single gene locus where one of a non-allelic pair of genes (idiomorph) exists
      - Tetrpolar compatibility - two mating type gene pairs of multiple idiomorphs
  - Survival mechanism
    - Dormancy
    - Mating type switching
  - Mating type and hormonal control
    - Chytridiomycota
      - *Allomyces* is a homothallic fungus that produces separate male and female gametangia that release motile gametes
      - Females release a pheromone, serinin, that attracts the male gametes
      - Male gametes move along a concentration gradient
      - Sirenin and carotenoid color produced in male gametangia are produced from the same precursor, indicating mating type gene controls development of the sex organs
    - Oomycota
      - Homothallic or heterothallic, but in most cases produces a colony with both male and female sex organs (antheridia and oogonia)
      - Mating type genes control capatibility
      - Hormonal control in *Achlya*
        - Female produces antheridiol causing the male to increase production of cellulase which induces hyphal branching to increase
        - Once triggered by antheridiol, males release oogoniols that induce oogonia development
        - Eventually, male branches (antherida) fuse with oogonia

- Zygomycota
    - Homothallic or heterothallic
    - Two mating type genes that govern conversion of β-carotene to a prohormone
    - Prohormone is eventually converted by mating-type specific gene to trisporic acid
    - Trisporic acid volatilizes and causes hyphae of opposite mating type to grow towards one another and fuse to form a zygospore
- Ascomycota
    - Typically two mating types **a** cells and α cells
    - Best characterized system is that of *Saccharomyces*
    - Mating is controlled by the *MAT* gene locus of flanked by two other loci, *MATa* and *MAT*α A copy of one loci is made and inserted into *MAT* gene locus - this is now the mating type of the cell
    - This copy can switch out after each new bud cell is produced
    - *MATa* and *MAT*α are responsible for producing:
        - Peptide hormones a-factor and α-factor
        - Hormone receptors
        - Cell surface agglutinins
    - α cells constitutively release α-factor that is recognized by a receptor on **a** cells
    - **a** cells cease growth and arrest at G_1 phase of the cell cycle, then release a-factor
    - Different mating types then form outgrowths (“schmoo” cells) with strain specific agglutinins on their surfaces
    - Agglutinins cause cells to bind to one another, which then leads to fusion (plasmogamy), followed by karyogamy (diploid formation)
    - Subsequent induction of meiosis produces four ascospores
- Basidiomycota
    - Most are heterothallic having one or two mating type loci (typically termed A and B) with mulitiple idiomorphs at each locus (e.g., A_1, A_2, A_3, etc.)
    - Successful matings occur with different idiomorphs at each locus (e.g., A_1, B_1 x A_2, B_2)
    - Different pairings of idiomorphs have allowed a dissection of the functions of the mating-type genes
        - A locus - controls pairing and synchronous division of nuclei and initiation of clamp formation
        - B locus - controls septal dissolution, fusion of clamp branches, and increased glucanase activity