21st Century Guidebook to Fungi, Second Edition of the online version, by David Moore, Geoffrey D. Robson and Anthony P. J. Trinci

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Chapter 5: Fungal cell biology

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Events at the hyphal tip are crucial to the extension of the hypha; so it is vital that we describe the molecular processes taking place in the hyphal tip as far as we can, and this is the main purpose of this chapter.

In this chapter we will give you a complete outline of eukaryotic cell biology with emphasis on how fungal cells work and how the cell biology contributes to mycelial growth. Because they are eukaryotes that are easy to cultivate in the laboratory, several fungi have been adopted as model organisms for experimentation and we will show how yeasts, in particular, have been used in this way since the 19th century. We discuss the essentials of cell structure in some detail, emphasising the molecular biology of the nucleus, nucleolus, nuclear import and export, and mRNA translation and protein sorting. We also briefly cover nuclear genetics and mitotic and meiotic nuclear division. The plasma membrane and signalling pathways, and endomembrane systems, cytoskeletal systems and molecular motors form major topics because directed and rapid transport of materials needed for hyphal tip extension is a crucial and characteristic feature of highly polarised filamentous

growth. Other features of cell biology that are specific to fungi include the fungal cell wall, the cell biology of the hyphal apex, the nature of hyphal fusions and mycelial interconnections, the meaning of cytokinesis in fungi, and septation and the yeast-mycelial dimorphism.

5.1 Mechanisms of mycelial growth

Polarised growth of fungal hyphae is achieved by restricting extension to the hyphal apex. The cell wall at the hyphal tip has viscoelastic properties [see definition box] and yields to the internal turgor pressure within the hypha.

Definition Box

Viscoelastic properties

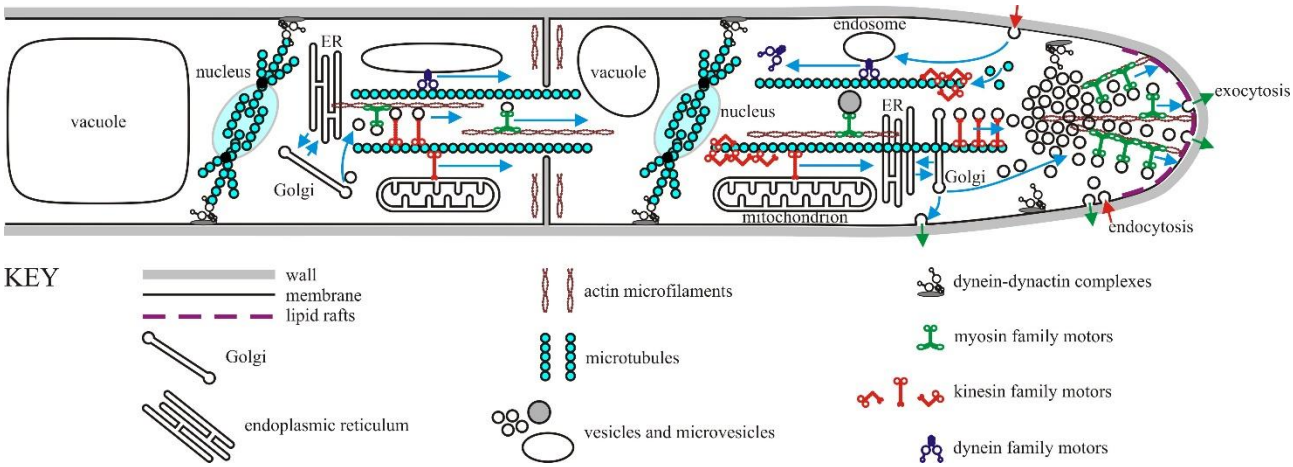
Viscoelastic properties = having some of the characteristics of both a liquid (flowing like a liquid with the consistency of syrup or honey) and a solid (resisting and recovering from stretching, compression or distortion). Elasticity is usually the result of bond stretching along crystallographic planes in an ordered solid; viscoelasticity is the result of the diffusion of atoms or molecules inside an amorphous material.

Further behind the tip the wall is rigidified and resistant to the turgor forces resulting from the osmotic flow of water into the hypha. Turgor pressure generated within the hypha therefore acts as the driving force for hyphal extension.

Hyphal extension at the apex requires synthesis and insertion of **new wall material** and **new membranes** in a way that does not weaken the tip. This highly organised process is supported by the **continuous flow of vesicles** generated within the cytoplasm behind the tip and is co-ordinated with the growth and replication of all the other cytoplasmic organelles and their **migration towards the extending apex**. In this Chapter we will be describing all these individual processes, and in Chapter 6 this mosaic of processes will be assembled into what we hope will be a full picture of hyphal tip growth. What *is* important at this point is to recognise how much of the detailed cell biology of filamentous fungi is adapted, devoted and committed to forward thrusting of the hyphal apex; that is, **hyphal extension growth**. Making this the supreme characteristic of filamentous fungi that sets them off from the other crown eukaryotes, animals and plants.

The following drawing is a cartoon representation of an overall molecular model of hyphal growth. The key feature of hyphal apical growth is rapid movement towards the apex of all the materials needed to create new wall, new membranes and new cytoplasmic components. Most of these materials are exported in vesicles by the endoplasmic reticulum (ER) and Golgi organelles, the vesicles being delivered to the apical vesicle cluster (called the Spitzenkörper) along microtubules powered by motor proteins of the kinesin and dynein families. The Spitzenkörper organises the final distribution of microvesicles along actin microfilaments to the plasma membrane at the extending tip. Vesicle fusion with the membrane is enabled by t-SNARE and v-SNARE proteins. Sterol-rich 'lipid rafts' at the hyphal tip could provide domains for apical proteins like signalling and binding complexes and might facilitate endocytosis. Endocytosis at the hyphal tip is dependent upon actin patches where myosin-1 polymerises actin into filaments that take the endocytotic vesicles away from the membrane. The extreme apex of hyphal tips undergoes extensive ***exo***cytosis, which is mainly devoted to synthesis of wall polymers outside the membrane and wall construction and maturation. ***Endo***cytosis features in the flanking regions of the hyphal tip, and this both recycles membrane components (originally delivered as exocytotic vesicles) and imports nutrients; both of which are transported to the endomembrane system for sorting and appropriate use. This figure also shows that subterminal hyphal cells (potentially many) contribute to the apical migration of resources and mitochondria are all transported towards the apex in streams of vesicles and trains of rapidly moving vacuoles, and this transport extends through hyphal septa. Also note that the

position of nuclear division spindles is probably specified by interaction between astral microtubules and membrane-bound dynein-dynactin complexes, and septal positioning is associated with rings of actin microfilaments. Remember: this ***is*** a cartoon, no attempt is made to portray relative scale or relative timing (some structures, like division spindles) are more transient than others (like the Spitzenkörper). Also, everything happens, ***quickly***; in the text we show that 38,000 vesicles have to fuse with the apical membrane each minute (that's over 600 every second) to support extension of each hyphal tip of *Neurospora crassa* when it is growing at its maximum rate. Chapters 5 and 6 provide a complete explanation of the details in this summary diagram, and you will meet this diagram again in Section 6.7.



5.2 The fungus as a model eukaryote

The cell we are describing is the generalised cell of a eukaryote. In most textbooks when this is attempted it is usually the animal cell that takes centre stage (e.g. the classic cell biology text, Alberts *et al*., 2014); plant cells might be described occasionally, when there's a need to deal with photosynthesis, and yeasts may get a mention as the source of some of the molecular detail. There's nothing wrong with that (although animal cells do not have the cell wall that's so important to the other eukaryotic kingdoms, this feature being lost in the distant past by the single-celled opisthokonts that gave rise to Kingdom Animalia), but it does downplay the enormous contribution that fungi have made to development of our knowledge of **eukaryotic cell biology**. As well, perhaps, as downplaying the enormous contribution that the fungal life style has made to the evolution of eukaryotic cell biology.

Although several unicellular eukaryotes have been used as models in cell and molecular biology (Simon & Plattner, 2014), the fact is that most of what we know about the biology of the cell of higher organisms derives from work with ***yeasts***. Most biologists would recognise the contribution made by yeast research to molecular biology in the 1990s. The first complete **sequence** analysis of any **eukaryote chromosome** was that of the entire DNA sequence of *Saccharomyces cerevisiae* chromosome III, published in 1992 by a large international team led by **Steve Oliver**.

This was followed in 1996 by the sequencing of the whole of the genome of *S. cerevisiae*, which was the **first eukaryote genome** to be sequenced (the 13-year human genome project, which got all the headlines, was completed in 2003).

Some biologists will know that the Nobel Prize for Physiology or Medicine in 2001 was awarded to three scientists '...for their discoveries of key regulators of the cell cycle', and two of them worked with **yeasts** (**Leland Hartwell** worked with *Saccharomyces cerevisiae*, and **Paul Nurse** with