# 1 Morphogenesis: Control of Cell Types and Shape

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CONTENTS



## I. Introduction Cell Types and Cell Shapes: a Diverse Array of Form

Fungi generate a variety of cellular morphologies in order to colonize and adapt to new environments. The most commonly utilized cell shapes include spherical, ellipsoidal or cylindrical yeast cells or chains of highly polarised cylindrical cells which form pseudohyphae or hyphae (Fig. 1.1). These common cell shapes encompass a large number of cell types, which may or may not be terminally differentiated, and which differ in their physiologies. Some fungi are capable of growing vegetatively in two or more of these morphologies, and are termed dimorphic. Fungi are also capable of producing a large variety of specialized cell types with unique cellular morphologies during developmental processes such as asexual and sexual reproduction, pathogenesis for host penetration or host association during symbiosis. This chapter will attempt to cover the most common of fungal cell types and highlight the current research into the molecular components which govern cell type and shape establishment through the control of actin polarisation.

## II. Polarity

The establishment and maintenance of polarity is central to the generation of the wide variety of cell morphologies found in organisms. It relies on the ability to mark specific regions of the cell by protein localisation and results in distinct cellular morphologies or function. These marked regions are used for many cellular processes involving the asymmetric distribution of cellular components such as receptors and transporters or in establishing growth polarity by directing growth to specific areas of the cell. Controlling polarisation during growth is required for both the maintenance of cell morphology during vegetative growth and cell division, as well as the alteration of cell morphology which is required for the differentiation of distinct cell types during development (Fig. 1.2; see Chaps. 2–6, this volume, and various chapters in The Mycota, Vol. VIII). The ability of cells to polarise growth is also crucial for rapid morphological responses to the environment. Control of polarisation is directly dependent on the cytoskeleton and its dynamics.

### A. Cytoskeleton

The cytoskeleton is composed of three types of protein filaments – microtubules, microfilaments

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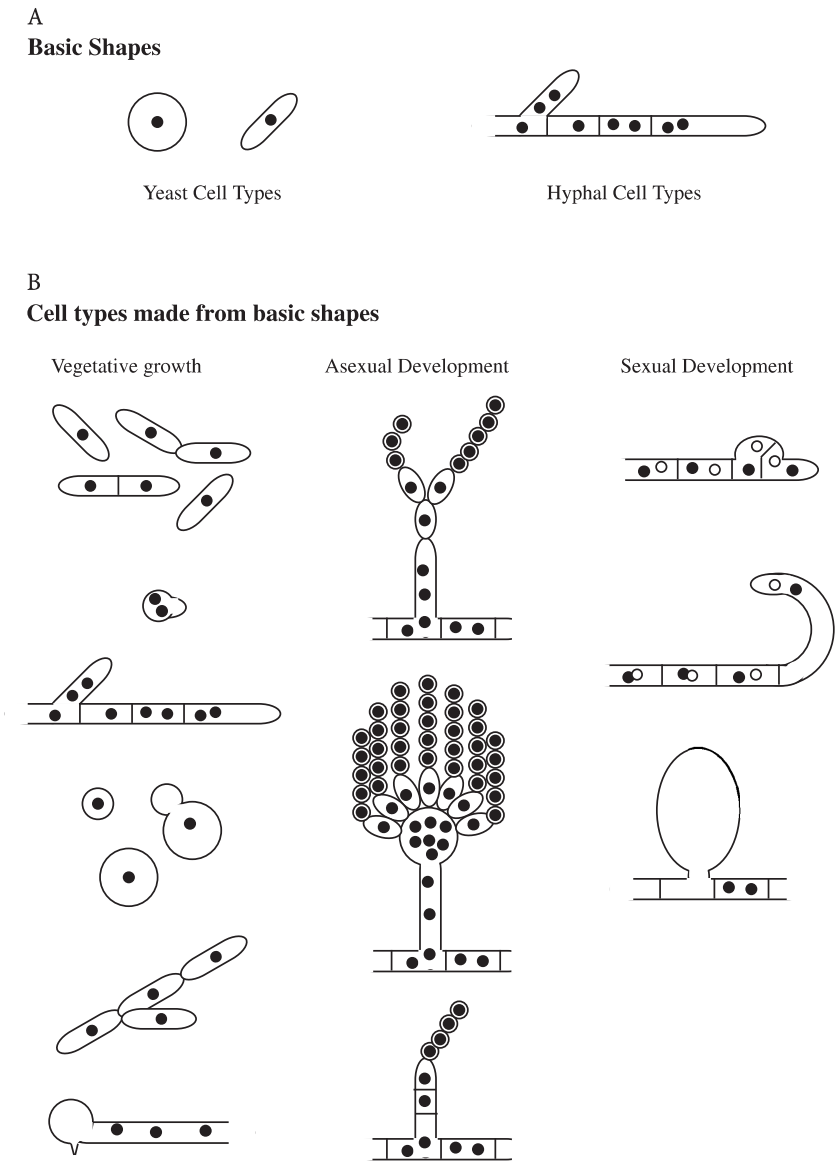


**Fig. 1.1.** Cell types in fungi. **A** The basic shapes of fungal cells associated with the two major growth forms: yeast cells and hyphal cells. **B** Diagrammatic representations of some of the cell types which can be generated using the basic fungal cell shapes. These can be involved in vegetative growth (fission yeast, germinating spores, hyphae, budding yeast, pseudohyphae, appressoria; *top to bottom*), asexual development (biverticilliate conidiophore, vesicular conidiophore, arthroconidiating conidiophore; *top to bottom*) and sexual development (dikaryotic clamp cell, ascogenous hypha, Hülle cell; *top to bottom*)

and intermediate filaments. These filaments provide structure and organisation to the cytoplasm and give shape to the cell. Intermediate filaments, which appear to be higher eukaryote specific and for which there is no firm evidence in fungi, are thought to provide internal mechanical support for cells whereas microtubules are required for transport between intracellular compartments, during formation of the mitotic spindle (division) and during cell motility (beating of cilia and flagella; Glotzer 2005; Palmer et al. 2005). Microfilaments are also necessary for the transport of intracellular components (see below) but differ from microtubular-based movement which often functions over long distances, such as for the transport of vesicles from the endoplasmic reticulum to the expanding growth region of hyphal cells (Steinberg et al. 2000). Details of the specific roles of intermediate filaments and microtubules are beyond the scope of this chapter. Microfilaments are comprised of actin polymers and, when associated with a variety of interacting proteins, comprise the actin cytoskeleton (Schmidt and Hall 1998). The actin cytoskeleton is required for numerous cellular functions, including polarised growth. In mammals and many other organisms, actin is required for cell motility, changes in cell shape, muscle contraction, cytokinesis, cell–substrate

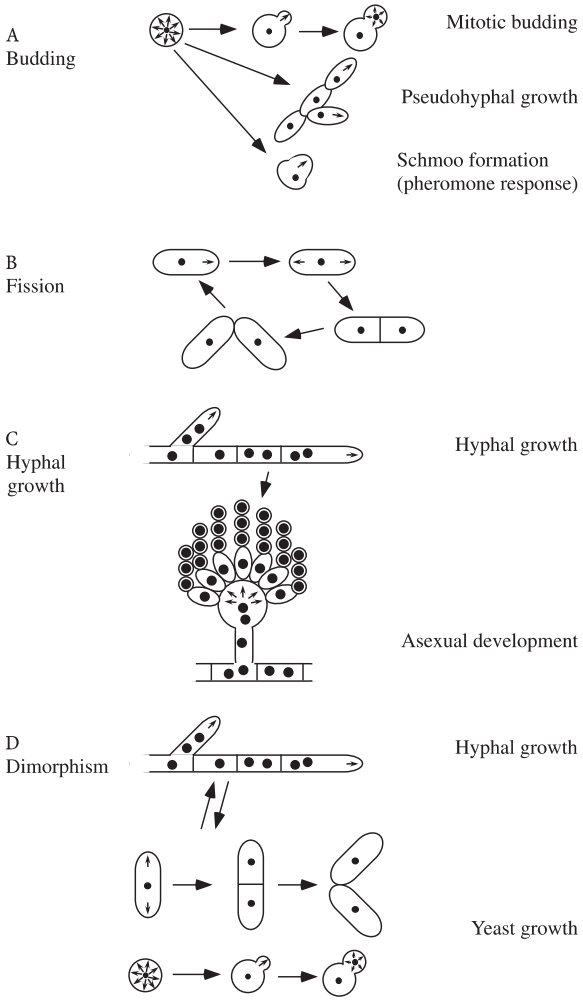


**Fig. 1.2.** Polarisation and fungal cell types. **A** Budding yeast are uninucleate and unicellular, growing by isotropic expansion. The uninucleate cells undergo mitotic budding to reproduce, which requires polarised growth towards the emerging bud. Budding yeasts can also grow as chains of elongated cells termed pseudohyphae. This growth form also requires polarised growth towards the pseudohyphal apex. Upon exposure to pheromone, budding yeasts like *S. cerevisiae* can polarise growth in the direction of the external pheromone source (schmoo formation). **B** Fission yeast are uninucleate and unicellular, growing initially by polarising growth to one end of the cell, followed by polarised growth at both ends. Cell elongation is followed by cell division and cell separation. **C** Mycelial fungi grow as multinucleate, branched hyphae which are divided at regular intervals by septa. Hyphal growth is polarised towards the hyphal and branch apices. Fungi such as *A. nidulans* can also undergo asexual development, a process with similarities to budding in yeast and which requires multiple rounds of initiation of polarised growth. **D** Some fungi can grow both as unicellular yeast (budding or fission) and as multicellular hyphae, and these are termed dimorphic. The switch between these growth states requires regulated changes in the modes of polarised growth. *Arrows* indicate direction of polarisation

interactions, endocytosis and secretion (Schmidt and Hall 1998). Likewise, in *Saccharomyces cerevisiae*, the actin cytoskeleton is required for a range of cellular processes including bud formation, movement of vesicles, localisation of chitin, cytokinesis, endocytosis, organelle movement and shape changes in response to environmental stimuli (Schmidt and Hall 1998).

Cells are reliant on the actin cytoskeleton in order to direct growth in a polarised manner. So, in order to regulate or initiate polarised growth during development, cells must be able to polarise the actin cytoskeleton towards the imminent growth site. However, as the actin cytoskeleton influences a wide variety of other cellular processes, the organisation must be very tightly regulated. This is achieved by regulating actin nucleation and polymerisation, and by regulating the cellular sites where nucleation and polymerisation occur (Schmidt and Hall 1998). Actin exists as either a monomeric form (G-actin) or a polymeric form (F-actin). Actin monomers bind and hydrolyse ATP in order to be incorporated into the polymer. The rate-limiting step in actin polymerisation is nucleation, the assembly of new monomers to form a filament (Schmidt and Hall 1998). This can be enhanced by scaffold proteins at the cell membrane.

## B. Isotropic to Polarised Growth

### 1. The Establishment of Polarised Growth in Budding Yeast

The mechanisms regulating polarised growth establishment and the switch from isotropic to polarised growth in fungal cells has been best characterised in *S. cerevisiae* (reviewed in Johnson 1999). During the mitotic cell cycle, *S. cerevisiae* divides by a process termed budding. This involves the selection of a non-random budding site, the organisation of proteins at this site, and the rearrangement of the actin cytoskeleton (Fig. 1.3). The bud emerges and growth is directed exclusively to the expanding bud. Bud expansion is followed by cytokinesis, septum formation and cell separation.

### 2. Bud Site Selection

During bud initiation in *S. cerevisiae*, the site of bud emergence is selected by the location of cortical markers and the recruitment of the GTP-bound, small Ras-like GTPase, Rsr1p (Bud1p;

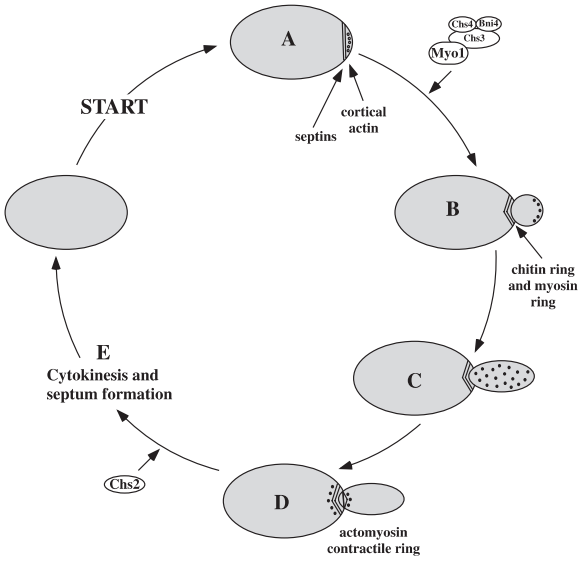


**Fig. 1.3.** Mitotic budding in *S. cerevisiae*. **A** A non-random budding site is selected, and proteins required for polarised growth are localised to this site. **B** Through the action of the type II myosin (Myo1p), chitin synthase (Chs3p and Chs4p) and the bud neck-involved (Bni4p) proteins, the bud emerges and growth is directed specifically to this site via a polarised actin cytoskeleton. **C** The bud begins to grow isotropically, expanding growth in all directions. **D** The actomyosin contractile ring forms in the mother–bud neck region, and a third chitin synthase (Chs2p) is recruited for sepum formation. **E** Cytokinesis occurs, followed by septum formation and cell separation

Bender and Pringle 1989; Fig. 1.4). The Cdc24p guanine exchange factor (GEF) for the small Rho-type GTPase Cdc42p associates with the Rsr1p GTPase and the Bem1p protein located at the incipient bud site. Bound Cdc24p and Bem1p interact with GDP-bound Cdc42p, which is bound to the guanine dissociation inhibitor (GDI) for Cdc42p, Rdi1p (Fig. 1.4). This interaction results in the loss of GDP-Cdc42p-Rdi1p binding, and the GEF Cdc24p catalyses the Cdc42p-bound GDP to GTP exchange (Ziman and Johnson 1994; Zheng et al. 1994, 1995; Richman et al. 2004; Fig. 1.4). These events now establish the site of bud emergence. The mechanisms which select new sites of growth in other fungi are poorly understood. It is clear that bud site selection in the dimorphic, opportunistic human pathogen *Candida albicans* is regulated by some of the same proteins as those identified in *S. cerevisiae* (Table 1.1). The *C. albicans* Rsr1p Ras-like GTPase regulates bud site selection, and the Cdc42p Rho GTPase is required for the establishment of polarity during budding

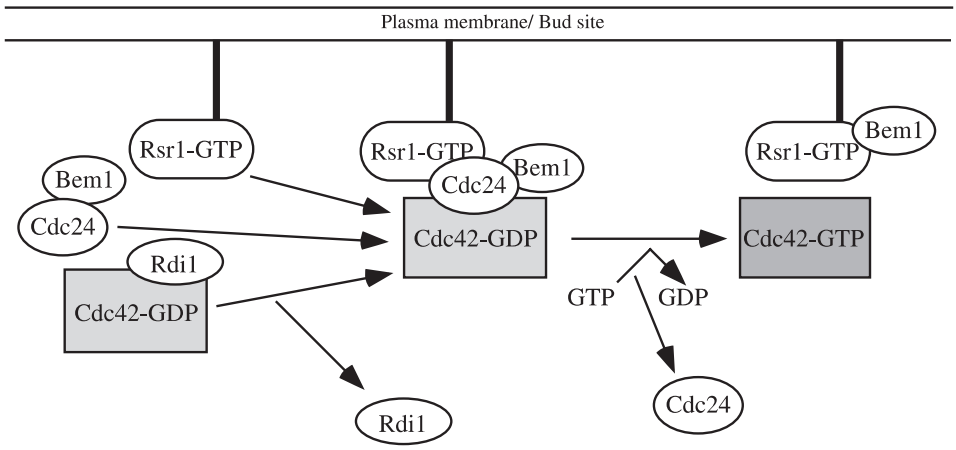


**Fig. 1.4.** Selection of a bud or polarisation site in *S. cerevisiae*. The organisation of protein complexes required for bud site selection and polarised growth during mitotic budding is shown (*left to right*). The process begins with localisation of GTP-Rsr1p to the membrane at the imminent bud site. GTP-bound Rsr1p interacts with Cdc24p, GDP-bound Cdc42p, Rdi1p and Bem1p at the plasma membrane. This interaction results in the loss of GDP-Cdc42p-Rdi1p binding, and the GEF Cdc24p catalyses the Cdc42p-bound GDP to GTP exchange. (Adapted from Johnson 1999)

**Table. 1.1.** Major protein families required for polarity establishment in fungi

| Protein type | Protein | Organism | Role | Reference |
|---|---|---|---|---|
| Guanine exchange factor (GEF) | Cdc24p | *S. cerevisiae* | Catalyses GDP to GTP exchange of Cdc42p | Adams et al. (1990) |
| | Cdc24p | *U. maydis* | Cell separation during budding | Weinzierl et al. (2002) |
| | Cdc24p | *A. gossypii* | Establishment of actin polymerisation and polarised hyphal growth | Wendland and Philippsen (2001) |
| | Cdc24p | *C. albicans* | Hyphal germ tube emergence | Bassilana et al. (2003) |
| Guanine dissociation inhibitor (GDI) | Rdi1p | *S. cerevisiae* | Prevents Cdc42p-GDP associating with the membrane | Johnson (1999) |
| Bud initiation | Bem1p | *S. cerevisiae* | Associates with Cdc24p and Rsr1p during the activation of Cdc42p | Peterson et al. (1994) |
| GTPase activating protein (GAP) | Bem2p | *S. cerevisiae* | Activates Rho1p and Cdc42p during bud initiation | Marquitz et al. (2002) |
| | Bem2p | *A. gossypii* | Polarised hyphal growth and actin polarisation | Wendland and Philippsen (2000) |
| Ras-like GTPase | Rsr1p/Bud1p | *S. cerevisiae* | Bud site selection | Bender and Pringle (1989) |
| | Rsr1p | *C. albicans* | Bud site selection | Yaar et al. (1997) |
| | Rsr1p | *A. gossypii* | Hyphal growth guidance and morphology. Required for the localisation of the polarisome component Spa2 | Bauer et al. (2004) |
| Rho GTPase | Cdc42p | *S. cerevisiae* | Regulates budding frequency. Organises proteins required for polarised growth at bud site. Activates PAK kinases, resulting in the formation of septin, chitin and myosin ring at the presumptive bud site. Localises actin. Assembles cytokinesis proteins and actin complex in mother–bud region. Leads to activation of MAPK cascade during pseudohyphal growth | Hartwell (1974); reviewed in Johnson (1999) |
| | Cdc42p | *C. albicans* | Required for bud formation and polarised growth of hyphae | Ushinsky et al. (2002) |
| | Cdc42p | *A. gossypii* | Establishment of actin polymerisation and polarised hyphal growth | Wendland and Philippsen (2001) |
| | CflA | *P. marneffei* | Initiation of germination (establishment of polarised hyphal growth), actin polarisation during hyphal growth (maintenance of polarised growth) and the polarised growth of yeast cells | Boyce et al. (2001) |
| | CflB | *P. marneffei* | Actin-dependent polarisation of hyphae (maintenance of polarised hyphal growth) and conidiophores | Boyce et al. (2003) |
| | Rac1 | *C. trifolii* | Required for polarised hyphal growth | Chen and Dickman (2004) |
| | Rho1p | *S. cerevisiae* | Establishment of cell polarity. Regulates protein kinase C (Pkc1p) and cell wall-synthesizing enzyme 1,3-beta-glucan synthase (Fks1p and Gsc2p) | Drgonova et al. (1996) |
| | Rho2p | *S. cerevisiae* | Establishment of cell polarity and microtubule assembly | Madaule et al. (1987) |
| | Rho3p | *S. cerevisiae* | Establishment of cell polarity | Matsui and Toh-E (1992) |
| | Rho4p | *S. cerevisiae* | Establishment of cell polarity | Matsui and Toh-E (1992) |
| | Rho5p | *S. cerevisiae* | Involved in protein kinase C-dependent signal pathway which controls cell integrity | Schmitz et al. (2002) |
| | Rho1p | *A. gossypii* | Maintenance of polarised hyphal growth | Wendland and Philippsen (2001) |

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