Life within a community: benefit to yeast long-term survival

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Abstract
Traditionally, living organisms have often been classified into two main categories: unicellular and multicellular. In recent years, however, the boundary between these two groups has become less strict and clear than was previously presumed. Studies on the communities formed by unicellular microorganisms have revealed that various properties and processes so far mainly associated with metazoa are also important for the proper development, survival and behaviour of multicellular microbial populations. In this review, we present various examples of this, using a yeast colony as representative of a structured organized microbial community. Among other things, we will show how the differentiation of yeast cells within a colony can be important for the long-term survival of a community under conditions of nutrient shortage, how colony development and physiology can be influenced by the environment, and how a group of colonies can synchronize their developmental changes. In the last section, we introduce examples of molecular mechanisms that can participate in some aspects of the behaviour of yeast populations.

Introduction
Microorganisms, including yeasts, are routinely investigated as individual cells existing within liquid cultures, usually shaken. This handling prevents cell–cell communication, the formation of gradients of signalling molecules, and the specific orientation of cells within their respective territory, which all spontaneously occur in nature. Studies on liquid exponential cultures of microorganisms have revealed a large amount of information concerning the processes occurring within individual cells during their growth and division. In several cases, these processes are homologous to those occurring within the individual cells of metazoa (plants and/or animals). However, even processes linked to multicellularity (e.g. differentiation), and thus so far only associated with metazoa, have started to be discovered in multicellular microbial communities exhibiting relatively high organization. The multicellular lifestyle of microorganisms (including yeasts) appears to be prevalent under natural conditions, where microorganisms must survive long periods under conditions of environmental stress and limited nutrient supplies. Classical examples are the multicellular fruiting bodies of bacteria (Myxobacteria, Bacillus subtilis) (Branda et al., 2001; Kaiser, 2003) or slime moulds (e.g. Dictostelium discoideum) (Weijer, 2004), bacterial and yeast biofilms (Kierek-Pearson & Karatan, 2005; Mukherjee et al., 2005), and various colonies of different microorganisms (Markx et al., 2004). The cooperation of microorganisms within a multicellular structure leading to its ‘in itself’ development and specific behaviour appears to be, at least in some cases, controlled by mechanisms that are not functional in individual microbial cells growing in shaken liquid cultures. It remains to be elucidated whether some mechanisms specific to the multicellular behaviour of microorganisms have analogues in the multicellular tissues (bodies) of metazoa.

As mentioned above, microorganisms existing in a natural environment only very rarely behave as individuals. On the contrary, they evidently prefer an existence within a community: it significantly potentiates their ability to protect themselves against a harmful environment and offers possibilities for further development that are ruled out in individual cells (Palkova, 2004). For example, the formation of cells carrying out specific tasks by the differentiation of an originally uniform population of microorganisms only makes sense within an organized multicellular community. Such differentiation is usually not advantageous for individual cells, but, on the other hand, helps the
population as a whole to survive. Thus the profit of an individual can be subordinated to that of the community.

In this review, we describe several specific processes that are important for the proper development of yeast communities, examples of the responses of populations (mostly yeast colonies) to a changed environment, as well as various changes in yeast behaviour triggered by yeast–cell interactions with their surroundings.

**Differentiation of yeast existing within multicellular colonies**

The formation of specialized cell types provides various advantages to the yeast community as a whole. These include (1) better protection of a community against a harmful environment, (2) advantages in colonizing territory, (3) provision of nutrients to the most promising cells within a population existing under conditions of long-term nutrient shortage, (4) the formation of cell subpopulations highly resistant to environmental inputs (e.g. spores), and (5) the formation of cells with specialized functions (e.g. those emitting a signal and those accepting a signal). Some examples are given in the following sections.

**Protection of multicellular communities**

In the laboratory, yeasts are usually kept at optimal temperature either in a liquid culture or on wet agar in a covered dish protecting them against drying. In their natural environment, however, yeasts have to cope with changing temperature, humidity (excess of water or drying), the effects of various toxic compounds coming either from the environment (e.g. drugs) or produced by other organisms in their immediate surroundings. Even an individual yeast cell is equipped with systems enabling it to cope with and protect against a perilous environment. These include osmosensing and osmoregulation systems (Hohmann, 2002), systems expelling drugs (e.g. membrane multidrug resistance transporters) (Jungwirth & Kuchler, 2005), and cell-wall integrity mechanisms (Levin, 2005). The range of possibilities is always limited in an individual cell; however, in a yeast community organized into a three-dimensional structure (e.g. a colony, biofilm, stalk), cells can specialize and organize into distinct layers, some of which can have a mostly protective role. Such cell layers are usually located on the surface of the structure and they can be composed of dying cells having abundant cell walls and reduced cellular content. These kinds of cells, forming a skin-like layer, have already been identified on the surface of multicellular yeast stalks (Scherz et al., 2001).

Studies on yeast strains from nature revealed that, like bacteria, yeasts possess the ability to produce an extracellular matrix (ECM). Environmental scanning electron microscopy and additional biochemical analyses revealed that natural Saccharomyces cerevisiae strains create fluffy structured colonies, the cells of which are embedded within an abundant matrix network (Fig. 1a) (Kuthan et al., 2003). This matrix coat may be an analogy of bacterial exopolysaccharides (e.g.

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**Fig. 1.** Extracellular matrix covers cells in yeast colonies. (a) Environmental scanning electron microscopy of wild Saccharomyces cerevisiae BR-F colonies (from Kuthan et al., 2003; Fig. 2). (b) Amorphous (A) and granular (G) material in a colony of Candida albicans. Transmission electron microscopy of agar-embedded colony sections (from Joshi et al., 1975; Fig. 8). (c) Scanning electron microscopy of smooth, pseudohyphal and wrinkled Cryptococcus neoformans colonies (from Fries et al., 1999; Fig. 1).
glycocalyx), which have been identified as important to bacterial biofilm maturation (Dunne, 2002; Jefferson, 2004). A matrix network of fluffy colonies could help the yeast community (from an early developmental stage) to create microcolonies within small chambers connected by channels important for the flow of nutrients and removal of waste products (Fig. 2). Mechanisms of this flow could involve passive capillary movement (Varon & Choder, 2000). In a fluffy colony, the cells located in the central sections are probably less handicapped than those that are tightly attached to each other within the smooth colonies (Fig. 2) formed by laboratory strains (Kuthan et al., 2003). Moreover, the strategy of formation of small microcolonies separated by an abundant ECM, which helps to maintain the distance between individual chambers, enables a fluffy colony to occupy a relatively large territory quickly (Fig. 2) with a relatively small number of cells, and only later to ‘fill’ the ‘chambers’ with new cell generations. In the laboratory, natural strains gradually switch off their ability to produce an ECM and to form fluffy colonies (see later). This indicates that yeast communities can use different strategies of survival under different environmental conditions.

An abundant ECM is also present in the colonies of some Candida species (e.g. Ca. albicans and Ca. tropicalis) (Joshi et al., 1975). It is composed of a homogeneous amorphous material, probably containing highly cross-linked carbohydrates. It appears to surround the intact cells in colonies and to separate them from the irregularly distributed debris of degenerated cells. A large amount of the matrix was found on the colony surface forming a surface coat (Fig. 1b). Degenerated cells are most common in the surface layers. The presence of a polysaccharide capsule is a characteristic feature of another pathogenic yeast, Cryptococcus neoformans. Some of the Cr. neoformans strains also form a variety of colony morphotypes (smooth, wrinkled and pseudohyphal) that differ in the content and composition of their extracelluar capsule (Fig. 1c) (Fries et al., 1999).

As indicated above, extracellular matrix material, i.e. material that is secreted out of the cells and composed of an abundant (poly)saccharide component (but also containing a protein component), appears to be very important for the formation and behaviour of multicellular communities and their interaction with the environment. Such material is usually very sticky and resistant to various treatments, which complicates a detailed analysis of matrix composition. For this reason, little has been discovered about the composition of yeast ECM until now. Baillie & Douglas (2000) analysed the extracellular polymeric material of a matrix of Ca. albicans biofilms and compared it with that obtained from the supernatants of planktonically grown yeast. In both cases they found that the ECM consists of carbohydrate, protein, phosphorus and hexosamine. Biofilm ECM contains less total carbohydrate and protein than planktonic ECM, but, on the other hand, approximately half of its dry weight remained unidentified and may represent one or more unique components (Baillie & Douglas, 2000). The ECM extracted from fluffy colonies (Figs 1a and 2) of a wild S. cerevisiae strain by Kuthan et al. (2003) contained a major specific protein (or protein complex) of mobility >200 kDa. Concanavalin A (ConA)-peroxidase staining revealed its glycosylated nature, and its sensitivity to proteinase K shows its proteinaceous character. However, further characterization of this protein was complicated by the fact that neither glycosidase H, glycosidase O nor N-glycosidase F (PGNase F) were able to remove its glycosidic groups. The predominant structural component of a capsule of Cr. neoformans (Fig. 1c) is the polysaccharide glucuronoxylomannan, composed of (1 → 3)-linked linear
α-δ-mannopyranan with β-δ-xylopyranosyl (Xylp) and β-δ-glucopyranosyluronic acid residues added to the mannose at various positions. The composition of the capsule differs in different colony morphotypes, and appears to be important for Cr. neoformans pathogenicity (Fries et al., 1999).

Morphology of colonies and their surface adhesion

In contrast to the behaviour of laboratory strains of S. cerevisiae, which form mostly smooth colonies, wild S. cerevisiae strains as well as most of the strains of so-called nonconventional yeasts (e.g. Candida, Kluyveromyces, Hansenula etc.) form structured colonies with a pattern that is usually specific to the particular yeast strain and distinct growth conditions. Until now, limited information regarding the rules leading to a particular colony morphotype has been available. Often, the colony morphology is influenced by the presence of the various morphological types of yeast cells (yeast-shaped cells, pseudohyphae and hyphae). Yeast-shaped cells are usually round to ovoid and readily separate from each other. Pseudohyphae resemble ellipsoid yeast-shaped cells that remain attached to one another and usually grow in a branching pattern. True hyphae are long with no obvious constrictions between the cells (Fig. 3a) (Berman & Sudbery, 2002). The colonies of Candida parapsilosis that are composed of yeast-shaped cells are mostly smooth, while the presence of hyphae and pseudohyphae leads to structured colonies (crepe, cocentric, crater, smooth) (Laffey & Butler, 2005) invading the agar substratum. The various structured colony morphotypes of different Ca. albicans mutants (Figs 3c and d) contain different proportions of yeast-shaped cells and filamentous cells in different regions of the colony (Brega et al., 2004; Garcia-Sanchez et al., 2005). Recent analyses on the budding pattern and adhesion properties of S. cerevisiae cells forming colonies of different morphotypes (Vopalenska et al., 2005) revealed that, in contrast to liquid cultures (Chant & Pringle, 1995), strain ploidy does not determine the budding pattern of cells within a colony, which is probably influenced more by the environment.
(surrounding cells, nutrient gradients, etc.). Moreover, a switch from axial or bipolar division to random division (caused by \textit{BUD2} disruption) does not influence the colony morphology. On the other hand, the formation of clusters of incompletely separated yeast cells (Fig. 3e) and the monopolar division pattern appear to play a role in the formation of structured colonies (Vopalenska et al., 2005).

So, these findings indicate that the more structured colony morphotypes correlate with the presence of hyphae/pseudohyphae. However, findings on the changes in cell and colony morphology connected with the long-range ammonia signalling (see later) of \textit{Candida mogii} colonies document that this is not always the case. Here, as the colony enters the ammonia-producing developmental phase, relatively young smooth acidic colony pseudohyphae change to a ruffled spagetti-like structure formed mostly of yeast-shaped oval cells (Fig. 3g) (Palkova & Forstova, 2000). Furthermore, both the smooth and fluffy colonies of \textit{S. cerevisiae} wild strains are formed by yeast-like cells (Kuthan et al., 2003) that, nevertheless, differ in their shape (Fig. 3f). Thus, the presence of hyphae and/or pseudohyphae is not necessarily a prerequisite for the formation of highly structured colonies. One can, however, imagine that oval-shaped monopolarly budding cells can more easily arrange into a structured colony morphology than rounded cells budding randomly or axially.

Another characteristic feature that differs among the various yeast strains is their ability to attach to a solid surface. When looking at the occurrence of microorganisms in nature, it is found that they only very rarely exist as floating cells in a liquid environment. On the contrary, they usually form biofilms or colonies tightly attached to solid surfaces or multicellular pellicles on water surfaces. Several studies indicate that the surface properties of microorganisms are essential for their attachment and adhesion. In \textit{S. cerevisiae}, a well-studied example is flocculation in a liquid environment mediated by surface Flo proteins, which usually results in the efficient sedimentation of yeast cells (Verstrepen et al., 2003). The Flo proteins are often involved also in yeast-cell adhesion to solid surfaces and biofilm formation (Verstrepen & Klis, 2006). Homologues of \textit{S. cerevisiae} Flo proteins, termed adhesins, play a role in \textit{Ca. albicans} virulence and in colonization of the host (Li & Palecek, 2003).

**Cell differentiation and survival of yeast colonies**

In contrast to the fluffy colonies formed by wild \textit{S. cerevisiae}, in the smooth colonies of either standard laboratory strains or domesticated wild strains, yeast cells are tightly attached to each other from the early phases of colony development. Such an arrangement does not allow the efficient new growth of central cells, because they just do not have enough space. When monitoring the fate of individual cells within a giant smooth colony of a \textit{S. cerevisiae} laboratory strain using cells marked with AlexaFluor488 5-tetrafluorophenyl ester, these cells are quickly ‘diluted’ after their inoculation (in 4 days) by new progeny growing at the initial colony inoculation area. Later, the old fluorescent cells are only detectable in the colony centre and their fraction remains almost unchanged until the 28th day of colony growth, while no stained cells are detectable at the newly grown colony margin. This, together with the almost linear radius of expansion of the outer colony margin, suggests that lateral growth is preferred in smooth colonies, and that young cells are largely located in the area of the margin, where they have more chance to reach new areas and obtain nutrients (Vachova & Palkova, 2005). In addition, the regulated yeast cell death (YCD) appears to be essential for the long-term survival of the colony population. The cells located in central regions preferentially die in a manner exhibiting some features of the apoptosis of higher eukaryotes (phosphatidyl serine relocalization, DNA breaks, chromatin fragmentation, etc.). The compounds released from dying cells seem to be important for feeding new cell generations located at the margin. Moreover, newly born cells even appear among the dying cells in the centre. These data imply that regulated cell death is important for the survival and late growth of an ageing yeast colony (Vachova & Palkova, 2005). Thus, like in the fruiting bodies of \textit{Mycobacteria}, where some cells lyse and provide their components to other cells that become spores (Lewis, 2000), in yeast colonies, part of the population sacrifices itself for the benefit of the rest of the population. A brief observation revealed that among ‘young mothers’ and ‘daughters’, quite a small proportion of ‘old mothers’ can be found in central colony areas (L. Vachova & Z. Palkova, unpublished data). This means that the preferential dying of central cells is not because of their replicative ageing, but more because of chronological ageing and the accumulation of stress factors. This site-specific differentiation and localization of dying cells seems to be dependent on ammonia signalling, which triggers metabolic changes important for a decrease in oxidative stress (see later). This consequently locally prevents the dying of new cell generations (Fig. 4) (Vachova & Palkova, 2005).

**Changes in lifestyle of yeast populations evoked by environment**

Yeasts, like other microorganisms, possess the ability to adapt efficiently to changed environmental conditions. Various pathways involved in such adaptation have been identified. They include osmoadaptation, and heat-shock and cold-shock responses (Hohmann, 2002; Riezman,
In general, the first reaction of yeast to a stress usually involves the activation of so-called ‘environmental stress response’ (ESR) genes (Gasch et al., 2000), including the genes encoding proteins that help cells to survive immediate stress. Examples are the reactive oxygen species (ROS) defence enzymes catalase and superoxide dismutase, heat-shock proteins, and others. The consequent fate of the cell then usually depends on its ability to adapt to a particular stress and to survive its longer-term attack. This usually requires a different set of genes from those of the ESR group, and the final fate of the cell depends on the efficiency of its adaptation and on the level of a particular stress factor.

Ammonia-induced adaptation of yeast colonies

The ammonia-induced adaptation of _S. cerevisiae_ colonies can serve as an example of a yeast community reaction to a changed environment. During their long-term growth on solid media, yeast colonies switch from the acidic growth phase to the alkali phase of ammonia signal production when their growth is transiently inhibited (Palkova et al., 1997). The switch is connected with extensive gene expression changes (Palkova et al., 2002), which include the activation of genes encoding proteins important to the biogenesis of peroxisomes and fatty acid β-oxidation,

Fig. 4. Model of ammonia-triggered differentiation and specific localization of YCD in _Saccharomyces cerevisiae_ colonies. In first-acidic-phase colonies, ROS and other harmful products are produced by cells throughout the whole colonies and induce YCD. To escape damage, wild-type (wt) cells start to emit (outgoing violet arrows) and accept (incoming arrows) ammonia signal, which triggers metabolic changes that consequently allow cells to lower their ROS production. Healthy cells located mainly at the colony border (where the concentration of ROS is low) can thus escape YCD. Consequently, at the colony border, there are mainly slowly growing and dividing healthy cells (green) in later developmental phases (second acidic phase), while in the colony centre, dying cells (red) predominate. Compounds released (red arrows) from these cells in late stages of YCD sustain border cell growth and reproduction. Cells in sok2 colonies are not able to produce ammonia and change their metabolism, and cell dying proceeds quickly to the final stages, even in a high proportion of sok2 cells located in ‘younger’ colony areas. Far left, giant wild-type colonies in the first acidic, alkali and second acidic phases; the blue arrow indicates the position of the colony considered in the model.
amino acid metabolism, enzymes of mitochondrial glyoxylate bypass and various transporters (e.g. putative ammonium exporters Ato1p, Ato2p and Ato3p). This indicates that the yeast colony population changes its metabolism to a more economical one that enables it to exploit intracellular and extracellular reserves. A parallel gradual decrease in the expression of genes of oxidative phosphorylation indicates that mitochondrial respiration may be reduced (for a more detailed description of metabolic changes, see the recent review by Palkova & Vachova, 2003).

The metabolic alterations occurring in colonies during their transition to the ammonia-producing phase (Palkova et al., 2002) partially resemble those induced in yeast by mitochondrial dysfunction, that is, those related to the so-called retrograde response (Epstein et al., 2001). Here, respiratory-deficient yeast cells respond to the loss of mitochondrial oxidative phosphorylation by reconfiguring metabolism to increase supplies of acetyl-CoA from peroxisomal activities (Epstein et al., 2001). Some of the genes induced in colonies (e.g. CIT3 and ICL2) (Palkova et al., 2002) encode enzymes of methylcitrate cycle that was shown to be involved in the assimilation of propionate via propionyl-CoA, which is generated from the oxidation of odd-chain fatty acids or amino acids (Luttik et al., 2000). The induction of methylcitrate genes was also induced by a defect in some of the mitochondrial enzymes of the citrate cycle (McCannmon et al., 2003). In addition, one of the ATO genes, encoding putative ammonium exporters, was found to be a retrograde responsive gene by Guaragnella & Butow (2003). These authors proposed that ATO3 is induced in respiratory-deficient yeast cells to eliminate the excess ammonia that arises because of a possible defect in ammonia assimilation in such cells. Thus, in colonies, the observed decline of ‘mitochondrial’ oxidative phosphorylation, starting as early as during the early phases of the transition to ammonia production (Palkova et al., 2002), may participate in the induction of the later metabolic changes.

Changes in the expression of metabolic genes and the parallel decrease in the expression of ESR genes as well as in the activities of some of the stress-defence enzymes imply that the ammonia-producing period of colony development is connected with the activation of an adaptive metabolism enabling the cells of colonies to overcome the stress that was previously escalating in the acidic phase (Palkova et al., 2002). The changes related to ammonia action persist and some of them are even enhanced in later periods of colony development, that is, in the second acidic phase.

Colonies formed by a strain defective in the Sok2p transcription factor that can neither produce sufficient amounts of ammonia nor accept an ammonia signal are not able to switch on the genes of adaptive metabolism and they exhibit defects in long-term survival (Vachova et al., 2004). Interestingly, despite the fact that sok2 colonies appear to increase the activities of some of the stress-defence enzymes, cell dying is spread throughout the whole sok2 colony, and so not even newly born outer cells are spared (Vachova & Palkova, 2005). This is in agreement with the prediction of the role of ammonia in the regulation of cell dying in particular colony areas and indicates that, at least in some developmental phases, a metabolic adaptation can be more important for the long-term survival of a colony population than the activation of stress-defence mechanisms. Such adaptation could result in a decrease in stress-generating systems (e.g. oxidative phosphorylation in mitochondria generating most of the cellular ROS), and thus a decrease in the necessity to cope with a stress. In other words, it implies that the prevention of a stress may be more advantageous than the defence against an already-generated stress (Fig. 4).

**Domestication of wild S. cerevisiae strains**

The existence of a variety of strategies of yeast populations for coping with a modified environmental impact is documented by the efficient domestication of wild S. cerevisiae under laboratory conditions (Fig. 2). The cultivation of fluffy colonies of wild S. cerevisiae strains on rich agar media leads to an increase in the number of cells that in subsequent generations form smooth colonies resembling those formed by laboratory strains (Kuthan et al., 2003). The frequency of this fluffy-to-smooth switch is relatively high (c. 4% according to the medium composition), which suggests that it is more probably caused by stable regulatory change (i.e. by an epigenetic event/s) than by a mutation. Smooth colonies differ from fluffy ones in several respects, including the absence of an extracellular matrix, a more compact colony structure, and a different cell shape within the colonies (ovoid cells in smooth colonies vs. elongated cells in fluffy colonies) (Kuthan et al., 2003). Genome-wide expression studies revealed differences in the expression of a large set of genes in fluffy vs. smooth colonies. Among others, these include the genes for various glucosidases (maltsases and glucanases), the cell wall proteins (e.g. TIP1, SPS100, CHS1 etc.), water channels (AQY1), and a large group of transposon and subtelomeric genes (Kuthan et al., 2003). These findings suggest that a yeast population effectively adapts its behaviour to the environment, trying to decide what is more important in particular circumstances, either to keep mechanisms enabling more efficient protection (e.g. to produce an extracellular matrix) or to save the energy consumed for such protection. For example, under relatively invariable laboratory conditions, there is no reason to waste energy on the formation of an abundant extracellular matrix that is mainly important in a hostile natural environment.
Yeast dimorphic transition reflects environmental changes

Efficient adaptation of multicellular communities to changed environment can include an aspect of differentiation and thus a different fate for different individuals within a particular community. A typical example is the switch of *S. cerevisiae* from yeast-like growth to pseudohyphal growth under conditions of nitrogen (or carbon) starvation (Gimeno et al., 1992). This requires complex cell reprogramming in a way that enables more efficient cell expansion from a colony to a free space. Recent studies have revealed that the genes located near telomeres (e.g. those encoding flocculin lines) are involved in this change (Halme et al., 2004). Their subtelomeric location is connected with the so-called ‘position effect’, i.e. chromatin regulation, enabling the alteration of gene expression from the ON state to OFF and vice versa. This consequently results in a mixed cell population composed of yeast-like cells and pseudohypal cells. One can speculate that this situation is advantageous as it enables the population to react quickly to actual environmental conditions that could change from those where yeast-like cells are preferred to those where pseudohyphae are preferred. The actual reaction of a mixed population can be much quicker than the reaction of a homogeneous one that must extensively change its gene expression during the switch. The fact that a number of surface proteins change during a yeast-to-pseudohyphal switch suggests that cell–cell contact and short-distance communication play a role in the behaviour of such a community.

Furthermore, many fungal pathogens of humans (and plants), including *Ca. albicans*, are dimorphic; that is, they are capable of reversible transitions between yeast-shaped cells and filamentous forms (hyphae or pseudohyphae) depending on the environmental conditions. The dimorphic switch of *Ca. albicans* is controlled by at least five positive (MAP-kinase pathway, cAMP pathway, Cph2p pathway, Rim101p pH response pathway, Czf1p matrix pathway) and two negative (Tup1p–Nrg1p–Rpg1p pathway, Rbf1p pathway) regulatory pathways enabling the yeast to respond properly to the environment (Berman & Sudbery, 2002). In some of these situations, dimorphic transitions correlate with pathogenic yeast infectivity.

White-to-opaque transition of *Ca. albicans*

The dimorphic transition is one example of ‘phenotypic switching’, that is, a global change in cell and population morphology and physiology. Various ‘phenotypic switching’ systems have been identified in the yeasts of the *Candida* genus. They usually lead to efficient cell and colony morphological changes and they are very often influenced by the environment. It has been shown that the majority of strains of the clinical pathogen *Ca. albicans* undergo reversible high-frequency switching, leading to a number of different phenotypes exhibiting diverse adhesive properties and invasive growth, and thus also differing in their virulence (Soll, 2004).

The white-to-opaque transition (Slutsky et al., 1987) probably represents the most studied phenotypic switching process. Here, cells switch spontaneously and reversibly between the ‘white phase’, characterized by hemispherical white colonies consisting of cells with a shape, size, and budding pattern similar to those of the cells of common laboratory strains, and the ‘opaque phase’, in which large flat and grey colonies are composed of bean-shaped cells three times the volume and twice the mass of white cells. This switching can also occur during colony development, as white colonies with opaque sectors, and vice versa, can emerge (Slutsky et al., 1987). The white-to-opaque switching system is expressed in <10% of *Ca. albicans* isolates (white-to-opaque switchers). The white-to-opaque transition occurs with a frequency of $10^{-2}$–$10^{-3}$ per cell generation, while the opposite opaque-to-white switch occurs with a frequency of $10^{-3}$–$10^{-4}$ (Slutsky et al., 1987; Ian et al., 2002; Lockhart et al., 2002) and increases substantially when the cells are shifted from 25 to 37°C or 42°C (Slutsky et al., 1987; Lockhart et al., 2002). However, in some other experiments (e.g. Rikkerink et al., 1988), the frequencies differ significantly, which indicates that the efficiency of switching is dependent on the actual conditions (media, temperature, humidity, etc.).

Interestingly, recent findings link together white-to-opaque switching, cell mating ability, and strain virulence (Fig. 5) (Magee & Magee, 2004; Soll, 2004). It was found that the white-to-opaque switching is tightly connected with the mating process that is controlled by the *MTL* locus eMagee & Magee, 2004). Only white strains homozygous at the *MTL* locus (a/a and α/α or a/- and α/-) can undergo white-to-opaque switching, thus forming opaque homozygotes, which can consequently mate and form white phenotype heterozygotes. On the other hand, heterozygosity at the *MTL* locus of white a/α strains represses the white-to-opaque switch as well as the genes involved in the mating process. Moreover, the *MTL* locus regulates (by an as yet unknown mechanism) the virulence of *Ca. albicans*, providing a competitive advantage to heterozygous a/α cells over homozygotes, at least in systemic infections (Lockhart et al., 2005). These findings can explain the relatively small proportion of white-to-opaque switchers observed in clinical *Ca. albicans* isolates. Contrary to the case in systemic infections, opaque-phase cells appear to be more proficient in colonizing mouse skin than white cells. This can be explained by the fact that the switch of homozygous opaque cells to the white phenotype is quite efficiently induced at 37°C, the physiological temperature in the blood stream (Lachke et al., 2003). On the other hand, skin represents an...
environment of lower than physiological temperature, thus stabilizing the opaque phenotype. Moreover, the mating of opaque cells is more efficient when the cells are immobilized on the skin than when they are in the blood stream. Interestingly, there are some indications that some unique features of the skin surface (other than reduced temperature) facilitate cell fusion during mating (Lachke et al., 2003).

White and opaque cells also differ in several other virulence characteristics. White and opaque cells exhibit different expressions of the drug-resistance genes CDR3 and CDR4. They also differ in adhesivity, in their sensitivity to white blood cells and oxidants, and in the efficiency of their bud–hypha transition (for a review, see Soll, 2004). White cells release a compound that is recognized by polymorphonuclear leucocytes (PMNs) as a chemo-attractant, thus stimulating the defence systems of the host organism against systemic Ca. albicans infections. In contrast, opaque cells do not attract PMNs (Geiger et al., 2004), and thus evade the innate immune response and express a large set of adherence factors. This is an ideal situation for establishing commensalism, the predominant relationship between Ca. albicans and its host (Magee & Magee, 2004). All these data imply that phenotypic switching can play an important role during the natural coexistence of a yeast with its host organism. On the yeast side this includes adaptation to different host-body environments, leading to better survival of the microorganism; on the host side, to the development of protective mechanisms preventing efficient expansion of the microorganism within the body. Thus, detailed knowledge of the molecular mechanisms involved in the phenotypic switching of different yeasts could be essential for finding new ways of protection against pathogen infection.

Analyses of the global expression profiles of white and opaque cells have revealed differences in various genes, including those involved in cell-surface composition and adhesion, stress response, signalling, mating type, and virulence (Lan et al., 2002). Interestingly, approximately one-third of the differently expressed genes are related to metabolic pathways. Opaque cells activate the expression of the genes of oxidative metabolism, including fatty acid β-oxidation, the citrate cycle and glyoxylate bypass. These metabolic pathways, if activated, can help opaque cells to colonize mouse skin (Kvaal et al., 1999), a habitat lacking free sugar but rich in lipids (Lan et al., 2002). In contrast, white cells activate expression of the genes of fermentative metabolism, which is useful for white cells causing systemic infections (Lockhart et al., 2005). Other differences concern the expression of amino acid metabolic genes and transporter genes. For example, the metabolic genes CDG1 (cysteine dioxygenase), CHA1 and CHA2 (serine/threonine dehydratases) are preferentially expressed in opaque cells, but the amino acid permease genes CAN3, GAP1, and AGP2, in

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**Fig. 5.** Conditions for white-to-opaque switching of Candida albicans. Conditions and frequencies of white-to-opaque and opaque-to-white switching are indicated by arrows. Only homozygotes in the MTL locus can switch to opaque-phase cells, and only opaque-phase cells of opposite mating types can mate. Photos: (a) white and opaque colony morphology on agar containing phloxine B, which stains opaque-phase colonies red, while white-phase colonies remain white; and (b) white and opaque cell morphology visualized by scanning electron microscopy (from Lockhart et al., 2002; part of Figs 1 and 2).
white cells. Furthermore, the expression of high-affinity phosphate and sulphate transporter genes (PHO84, PHO89, and SUL2) is induced in opaque cells, whereas another set of phosphate transporter genes (PHO87 and PHO98) is more highly expressed in white cells. The different equipping of cells by these membrane proteins could be important for the proper uptake of essential nutrients, but can also mediate cell communication and the sensing of different environments (Lan et al., 2002).

Changes in behaviour of pathogenic yeasts after interaction with the host

The interaction and coexistence of various yeasts with metazoas and the related changes occurring both in the microbial population and in the host are of great interest, particularly because of changes in yeast behaviour from harmless commensalism to dangerous parasitism. Such a transition is an active process, which is regulated by the interplay between the host and the yeast community. It seems that the host alone (the fitness of its immune system) determines the balance between commensalism and pathogenicity (Hube, 2004). In most healthy people, Ca. albicans is a harmless commensal, but it can cause severe infections in immunocompromised patients. Owing to its high adaptability to different host niches, which involves the activation of appropriate sets of genes in response to complex environmental signals, this yeast can colonize or infect almost all sites in the body. One example is the differential activation of individual members of the gene family encoding secreted aspartic proteinases (Saps), which are implicated in Ca. albicans virulence. Different SAP isogenes are activated during systemic disease as compared with a mucosal infection, and the progress of the infection influences individual SAP gene activation. Some members of the SAP gene family are induced immediately after yeast contact with the host; others are expressed only after dissemination into deep organs (Staib et al., 2000).

A link between morphogenetic variations and the virulence of pathogenic dimorphic fungi has been presumed for a long time (Gow et al., 2002; Romani et al., 2003). The yeast-shaped cells of various Candida species form filamentous multicellular pseudohyphae and hyphae in tissues, and their conversion to hyphae is in at least some cases essential for a tissue invasion (Berman & Sudbery, 2002; Gow et al., 2002), indicating the importance of yeast dimorphic transition. Tissue penetration can be accomplished by growth of the hyphae, generating significant pressure of the hyphal tip on the tissue. Those hyphae expanding from the margin of a well-anchored filamentous yeast population can more easily mechanically penetrate into solid surfaces (Gow et al., 2002). In addition to mechanical pressure, chemical compounds can help the yeast in tissue penetration. It was shown that the tip of hyphae is also the site of secretion of enzymes degrading proteins, lipids and other cellular components. These can liquefy the substrate in front of a penetrating cell (Hube & Naglik, 2001), thus facilitating yeast infiltration into solid substrates and tissues (Gow et al., 2002). Moreover, hyphal cells produce cell-wall proteins that facilitate yeast adhesion, which can be the first important step of tissue invasion, thus helping the yeast to avoid phagocytosis by neutrophils or macrophages (Berman & Sudbery, 2002). An example is Ca. albicans Als1p adhesin, a structural and functional homologue of S. cerevisiae Flo11p, which is required for the filamentation of Ca. albicans in vitro (Fu et al., 2002). The importance of hyphae formation for yeast virulence is also supported by the fact that mutant Ca. albicans strains incapable of hyphal formation are, in general, avirulent in mouse models of disseminated or mucosal candidiasis (Fu et al., 2002). However, decreased infectivity has also been observed without changes in the ability to form hyphae (Van Dijck et al., 2002; Romani et al., 2003).

After entering the bloodstream during systemic infections, Ca. albicans undergoes dimorphic transition and the hyphae start to produce hypha-associated factors that have the potential to protect hyphae against neutrophils. Moreover, Ca. albicans cells that are phagocytosed by macrophages form hyphae (producing specific proteinases), which consequently penetrate the membrane and kill the macrophages (Borg-von Zepelin et al., 1998). Hyphal cells are able to induce phagocytosis by endothelial cells, which can help the yeast to escape from the bloodstream. The higher level of superoxide dismutases in hyphae may counteract the oxidative burst of phagocytic cells. The strong adhesivity of hyphal cells together with their improved invasive properties (see above) can help yeast adhesion to endothelial cells and penetration into the deeper tissue of blood vessels. Although Ca. albicans usually switches to hyphal growth after exposure to blood plasma, hyphal morphology itself is not sufficient for pathogenesis, being only part of the complex metabolic change associated with the transition that gives the yeast the capability to counteract host-cell responses and defences (Hube, 2004).

Besides the importance of the transition to hyphae for yeast infectivity, other studies suggest that yeast-shaped cells can also initiate a host invasion (Gow et al., 2002). It has been shown that strains that are unable to grow as yeast-shaped cells are less virulent than dimorphic strains (Laprade et al., 2002; Romani et al., 2003). Yeast-shaped cells can be important for the dissemination of the pathogen through the blood stream (Berman & Sudbery, 2002). Candida albicans, Candida glabrata and S. cerevisiae yeast-shaped cells appear to be able to traverse the gut wall in infant mice (Pope & Cole, 1982). The production of a proteinase by Ca. albicans yeast-shaped cells leads to the
formation of pits in the surface of mouse skin ex vivo (Ray & Payne, 1988; Gow et al., 2002). So, these data indicate that both yeast-shaped cells and hyphae can have properties well suited to tissue invasion and evasion of the immune system (Gow et al., 2002).

**Viable but nonculturable microorganisms**

Despite the findings described above, our present knowledge of the cell variants existing within different yeast populations is poor. Interesting implications come from studies on so-called viable but nonculturable (VBNC) microorganisms. These have been described in several situations, where microorganisms that reach starvation or meet other stressful conditions enter a state that is characterized by their inability to regrow without ‘resuscitation’. During conversion to the VBNC stage, the cells change metabolism (Porter et al., 1995; Oliver, 2005) and protein composition (Heim et al., 2002), synthesize some new starvation and stress proteins (Morton & Oliver, 1994), maintain their ATP level, and usually reduce their cellular size. Before new regrowth, VBNC dormant cells require resuscitation either by a changed environment (e.g. temperature) or by some factors produced by other cells. For example, after long-term starvation (several months), *Micrococcus luteus* bacteria convert to dormant cells that are able to survive for long time-periods but have lost the ability to grow on agar plates. This ability is restored by a picomolar concentration of the specific resuscitation factor Rpf, which is the peptide that is produced by a growing *M. luteus* culture. A special example of VBNC could be spores of bacilli, which can remain dormant for long periods of the time. Once formed, spores need for their better outgrowth special conditions, for example the addition of amino acids (referred as germinants) and sugars or ions (termed cogerminants) as well as the presence and cooperation of specific receptors (GerA, GerB and GerK) (Atluri et al., 2006).

Microorganisms that are grown and studied in laboratories represent only a negligible percentage of the large variety of microorganisms in nature that appear to represent one-half of all cellular carbon. Ninety-nine percent of these microorganisms that exist in different environments are unknown and cannot be grown on standard media in laboratories (Whitman et al., 1998). At least some of these microorganisms in nature can survive long periods of starvation and other stresses as dormant cells, and only leave dormancy and start to grow again after receiving a specific resuscitation signal, either from the environment or provided by other (micro)organism(s), indicating a possible favourable change in the surroundings. The VBNC state has been identified in more than 60 bacterial species so far, while only a few resuscitation conditions have been found. Recently, the first evidence of the ability of yeast to enter the VBNC state was identified by Divol & Lonvaud-Funel (2005). These authors showed that the various species of yeast (*S. cerevisiae, Candida stellata, Rhodotorula mucilaginosa*) etc. that are present in *Botrytis*-affected wine enter the VBNC state after the addition of SO$_2$, which is used for wine stabilization. After the removal of the stress of free SO$_2$ and dormant yeast resuscitation in a liquid culture, the cells are again able to form colonies on standard plates (Divol & Lonvaud-Funel, 2005).

These data suggest that the ability to enter the VBNC state could be a general property of microorganisms. It could occur under conditions in which a cell transition to the ‘normal’ stationary-like stage, from which the cells can easily regrow when they receive nutrients (with no need for specific resuscitation), is not sufficient for long-term cell survival. Thus, one can imagine that, within an ageing multicellular population, some of the cells occur in the VBNC dormancy state (probably the yeast form most highly resistant to the environment). A mixed-cell population, in which cells differ not only in their ability to withstand different stresses but also in their ability to initiate division quickly when conditions become more favourable, has more chances to weather periods of various hostile conditions (Fig. 6).

**Synchronized responses of microorganisms and multicellular communities**

Coordinated behaviour is another important property of metazoa that can also be found in populations of microorganisms. One of the most intriguing examples is a coordinated movement of *Myxobacteria* called rippling, in which groups of bacteria organize in a space and start to periodically move back and forth in defined time-intervals (Shimkets & Kaiser, 1982; Stevens & Sogaard-Andersen, 2005). Despite the fact that little is known about the reasons why bacteria start to ripple, the observed link between rippling and moving of population to aggregation centres during fruiting body formation as well as the connection between rippling and *Myxobacteria* attack on other bacteria serving as nutrients (e.g. *Micrococcus luteus*) suggest that coordination increases the effectiveness of *Myxobacteria* action. Another example of coordination is connected with the various quorum-sensing systems of bacteria, in which the accumulation of a signalling molecule due to an increase in the density of a bacterial population leads to a response by the whole population (e.g. Waters & Bassler, 2005). It has recently been shown that the yeast *Ca. albicans* also displays several cell-density-dependent phenomena based on quorum-sensing molecules (QSM). Farnesol (Hornby et al., 2001) and farnesoic acid (Oh et al., 2001) were identified as autoregulatory extracellular QSM, which are continuously...
produced during the growth of a *Ca. albicans* population (Hornby *et al.*, 2001). These QSM block the yeast-to-hypha morphological transition. *Candida albicans* is also able to produce another autoregulatory QSM, tyrosol. Tyrosol abolishes the delay of growth after overnight culture dilution and stimulates filamentation under conditions permissive for germ tube formation (Chen *et al.*, 2004). QSM also seem to play a role in *Ca. albicans* biofilm development. The preincubation of *Ca. albicans* cells with a high farnesol concentration almost completely inhibits the formation of biofilms (Ramage *et al.*, 2002). Moreover, farnesol blocks the mycelial development of yeast cells newly produced in a biofilm; these cells may detach and colonize a new substrate. The role of tyrosol in biofilm development has not yet been investigated (Ramage *et al.*, 2002).

Ultradian respiratory oscillations in populations of continuous yeast cultures are another example of synchronized behaviour at the cellular level. These oscillations occur with a period of \( \approx 40 \text{ min} \), and can be monitored as oscillations of residual \( \text{O}_2 \) concentration. The yeast population significantly changes the gene expression during the oscillation cycle. One group of genes is expressed during the phase of active respiration (i.e. in the oxidative phase of the cycle), while the expression of another large group of genes is observed during the ‘reductive’ phase (Lloyd & Murray, 2005).

In addition to the synchronized behaviour of cells within a population, the synchronized reaction of whole individual populations has also been described (Palkova & Forstova, 2000). During ammonia signalling, all individual yeast colonies occurring at respective territory accept the ammonia signal released by the colony that first reaches the transition state, and all of them switch on their own ammonia production and related metabolic changes in a coordinated manner (and independently of their original growth phase). This means that even a very young colony in its early acidic phase can be prematurely induced to its own alkali \( \text{NH}_3 \)-producing developmental phase. In this way, a whole group of colonies of different ages synchronize their ammonia production and subsequent development. Taking the model of ammonia emission functioning as an alarm signal announcing an incoming shortage of nutrients to the other colonies (Palkova & Forstova, 2000), the premature induction of all colonies allows them to adapt in time and increases their chances of surviving the incoming unfavourable conditions. A volatile molecule diffusing through the air allows a more effective induction of the surrounding colonies in nature, where various barriers can block a signal through a liquid layer. Moreover, a gaseous signal can act over longer distances.

Molecules and regulations involved in the development of multicellular yeast populations

During recent years, more and more information regarding the behaviour of populations of microorganisms, cell–cell interactions and signalling has appeared. Initial studies mostly focused on bacteria (studies on the social behaviour of myxobacteriae, quorum sensing in bacteria, etc.); however, the importance of the behaviour of pathogenic yeasts and their interaction with a mammalian host in medical care also significantly extended research and knowledge in the field of yeast population studies. Despite relatively extensive...
new findings on yeast population phenotypes and their changes evoked by the environment, however, there is still limited information concerning the molecular mechanisms regulating the differentiation of individuals in the populations, their coordinated behaviour, and particularly those mechanisms that are relevant to the behaviour of microorganisms in nature. In contrast to traditional studies in liquid cultures, studies on populations require new approaches taking yeast (or other microorganism) multicellularity into account. For example, taking cells away from the structure must be done with caution, as it could lead to immediate changes in some cellular processes and to modification of cell behaviour. Monitoring the behaviour of communities of defined mutants is one of the prevalent approaches that can provide important information. However, the pleiotropic effect of some central regulators that can differ in the distinct phases of population development could make final interpretation difficult. In some cases, present knowledge looks at the behaviour and prominent changes in the population as a whole, rather than in the individual subpopulations of the differentiated structures. For example, the adaptation changes in ageing yeast colonies evoked by ammonia were detected with respect to entire colonies (Palkova et al., 2002); that is, they represent either a moderate change in the majority of the colony population or a strong change in part of the population. More detailed studies on the differentiated subpopulations are required to distinguish these two possibilities. In expression studies on whole colonies, only a few transcription factors and kinases were identified as significantly changing during colony ageing (Palkova et al., 2002). Contrary to changes in the expression of metabolic genes that are usually quite noticeable, and could even be registered when they occur in a colony subpopulation, moderate changes in the expression of a regulatory gene in a cell subpopulation are probably masked by the background and therefore can hardly be detected.

Despite these problems, there have been several discoveries concerning the regulatory mechanisms involved in the behaviour of multicellular yeast communities: these are summarized below.

**Role of surface proteins in cell–cell interaction**

As indicated earlier, the surface properties of yeast cells are very important for the formation and behaviour of multicellular communities and their interaction with the environment. Studies on colonies of wild *S. cerevisiae* and of some *Candida* spp. have revealed important roles for extracellular matrix material, despite the fact that information regarding its composition is limited (see above). On the other hand, more information is available regarding the possible roles of different yeast surface proteins in cell–cell attachment and cell interaction with solid surfaces. About 100 *S. cerevisiae* proteins are predicted to belong to a group of extracellular cell-wall proteins containing the glycosylphosphatidylinositol (GPI) anchor (YPD, www.proteome.com), which indicates that yeasts are relatively well equipped with surface proteins that can participate in their interaction, communication and surface attachment. Probably the most studied group of GPI-anchored cell-wall proteins participating in cell–cell interaction are the Flo proteins, which are related to the adherens of pathogenic fungi (Teunissen & Steensma, 1995; Caro et al., 1997; Lo & Dranginis, 1998). The surface Flo proteins are usually classified into two groups, differing in their adhesion properties. The first group includes classical lectines flocculins (encoded by the genes *FLO1*, *FLO5*, *FLO9* and *FLO10*) causing cell–cell adhesion that is Ca$^{2+}$-dependent and therefore disrupted in a citrate buffer. This type of adhesion is also inhibited by the presence of saccharides such a mannose (Stratford et al., 1988). Information concerning the properties of Flo11p, representative of the second group, is rather inconsistent. It has been shown by Lo & Dranginis (1996) that flocculation of the strain producing normal levels of Flo11p is Ca$^{2+}$-dependent and disrupted by a citrate buffer. The same authors recently reported that Flo11p-dependent flocculation is inhibited by mannose (but not by glucose, maltose or sucrose) and occurs only at higher cell densities (more than 10$^7$ cells per mL) at acidic pH (Bayly et al., 2005). In contrast, Guo et al. (2000) reported that aggregation of the $\Sigma 1278$ strain overexpressing the *FLO11* gene is Ca$^{2+}$-independent and is not inhibited by the presence of mannose (Guo et al., 2000). The Flo11p, besides its involvement in yeast-cell adhesion to various solid surfaces (e.g. to an agar or plastic surface, Reynolds & Fink, 2001), seems to be required for invasive cell growth and for pseudohyphal growth (Lambrechts et al., 1996), for the organization of an air–liquid interfacial biofilm during the growth of a Sardinian wine strain of *S. cerevisiae* (Zara et al., 2005) and for biofilm formation (Verstrepen & Klis, 2006). It can also participate in the formation of the fluffy structure of wild *S. cerevisiae* colonies (Kuthan et al., 2003).

The Flo11p functional homologue Cph3p (Eap1p) was recently identified in *Ca. albicans*. This protein is involved in the adherence of *Ca. albicans* to mammalian cells, and, when produced in *S. cerevisiae*, it restores invasive growth of the haploid *flo8* and *flo11* strains as well as filamentous growth of the diploid *flo8/flo8* and *flo11/flo11* strains (Li & Palecek, 2003).

**Role of APSES regulators in yeast morphogenesis and metabolism**

Studies investigating ammonia signalling (Palkova et al., 2002; Vachova et al., 2004) indicated an important role for
the S. cerevisiae Sok2p transcription factor in yeast colony development. However, several other distinct roles of Sok2p have been identified, indicating a pleiotropic function for this transcription regulator. Among others, Sok2p functions as a negative regulator of sporulation (Shenhar & Kassir, 2001) and of the switch to pseudohyphal growth in diploid cells under conditions of nitrogen limitation (Pan & Heitman, 2000). Sok2p negatively regulates the expression of the ASH1 and SWI5 genes, which encode the positive regulators of the genes required for pseudohyphal growth (e.g. FLO11) (Pan & Heitman, 2000). The Sok2p homologue Phd1p, which stimulates filamentous growth in S. cerevisiae, is also negatively regulated by Sok2p (Gancedo, 2001).

In Ca. albicans, two homologues of Sok2p were identified. These proteins, Efg1p and Efhh1p, together with S. cerevisiae Sok2p and Phd1p, belong to the family of APSES proteins (proteins containing the APSES domain, Pfam PF02292, www.sanger.ac.uk/Software/Pfam/), a conserved class of transcriptional regulators participating in the control of morphogenetic processes in Ascomycetes. In contrast to Sok2p, which negatively influences the pseudohyphal transition of S. cerevisiae, Efg1p plays a central role in three activating pathways (the MAP-kinase pathway, cAMP pathway and Cph2p pathway), regulating the morphogenetic switch of Ca. albicans from yeast-shaped cells to pseudohyphae and hyphae, and it participates in opaque-to-white switching (Doedt et al., 2004). Deletion of the EFG1 gene blocks Ca. albicans filamentation under most conditions (Berman & Sudbery, 2002). Efhh1p enhances the functionality of Efg1p, presumably by the activation of EFG1 expression (Doedt et al., 2004). Efg1p also participates in Ca. albicans adhesion to host tissues, as it regulates the transcription of the EAP1 gene encoding a Ca. albicans cell-wall protein important for yeast attachment to kidney epithelial cells (Li & Palecek, 2003).

Interestingly, genome-wide transcriptional profiling revealed that the Ca. albicans APSES proteins Efg1p and Efhh1p not only regulate genes linked to filamentation and morphogenetic changes, but also strongly influence the expression of metabolic genes, including glycolytic genes and genes of oxidative metabolism. In doing this, Efg1p appears to function as a repressor, whereas Efhh1p functions as an activator of gene expression (Doedt et al., 2004). These findings indicate a dual role of Ca. albicans APSES proteins in the regulation of yeast morphogenesis and metabolism. Findings on the role of Sok2p in the switching of S. cerevisiae colonies to the ammonia-producing developmental period that is accompanied by extensive metabolic alterations (Vachova et al., 2004) and on its role in pseudohyphal differentiation (Pan & Heitman, 2000) also suggest a dual function of Sok2p in S. cerevisiae.

Does chromatin participate in changes of yeast lifestyle?

There are several indications that changes in chromatin and related regulatory processes are involved in phenotypic switching and other processes during which the yeast alters its lifestyle as a response to the environment. There are several indications of important roles for, among others, histone modification enzymes (acytelylases/deacetylases) in influencing silencing and thus the expression state of distinct areas of chromosomes.

It has been shown that two Ca. albicans histone deacetylases, Hda1p and Rpd3p, play roles in the phenotypic switching of Ca. albicans colonies, probably regulating the expression of phase-specific genes. The deletion of either of the HDA1 or RPD3 genes affects white-to-opaque switching (Srikantha et al., 2001). Similarly, the inhibition of Hda1p histone deacetylase by the specific inhibitor trichostatin-A results in an increase in white-to-opaque switching (Klar et al., 2001). In S. cerevisiae, Hda1p deacetylates large continuous subtelomeric regions of the yeast genome (Robyt et al., 2002). It leads to stabilization of the chromatin structure and to the silencing of genes located in this area. One of the genes, the expression of which appears to be regulated by Hda1p, is the FLO11 gene that is important for invasive and pseudohyphal growth, biofilm formation, and other processes. In contrast to the S. cerevisiae Σ1278 parental strain that forms a mixture of filamentous FLO11-expressing cells and yeast-shaped cells (in which FLO11 is repressed), the FLO11 gene is desilenced in the hda1 mutant, owing to the absence of histone deacetylation, and hda1 cells are uniformly filamentous (Halme et al., 2004). Similarly, the expression of another epigenetically regulated adhesine gene, FLO10, is silenced by the distinct histone deacetylas Hst1p and Hst2p.

There are several other examples (mostly fragmentary) of the possible involvement of chromatin alterations and acetylase/deacetylase function in colony morphology switching. The transcription regulator Tup1p participates in the white-to-opaque switching of Ca. albicans (Zhao et al., 2002). The homologous Tup1p repressor of S. cerevisiae binds to underacetylated histone tails and it requires histone deacetylases, including Rpd3p, a homologue of Rpd3p histone deacetylase of Ca. albicans (Davie et al., 2003), for its repressive function. In silencing and in high-frequency Ca. albicans colony morphology switching, the putative histone deacetylase Sir2p (Sir3p according toYPD, www.proteome.com) of Ca. albicans (homologous to the Sir2p and Hst4p histone deacetylases of S. cerevisiae) is also involved. Deletion of SIR2 results in a dramatic rise in variant colony morphologies and it also leads to high-frequency karyotypic changes (Perez-Martin et al., 1999). Observed changes in the expression of genes encoding
proteins involved in chromatin modification (SAS3, HPA2) indicate that chromatin rearrangement may also play a role in the domestication of wild-type S. cerevisiae strains (Kuthan et al., 2003).

All the data mentioned above suggest that chromatin-mediated regulation may play a crucial role in the differentiation of a yeast community and in its response to a changed environment. As in the case of mating-type switching, in which derepression of the new mating cassette leads to pleiotropic changes in yeast-cell behaviour, the acetylation/deacetylation of particular chromatin areas that may contain principal regulatory genes could also cause global changes in population behaviour.

Open questions

A classical paradigm of microbiology, invented in the nineteenth century by the famous microbiologist Robert Koch, defines bacteria as ‘primitive individual organisms exploiting a limited ability to respond to changing environmental conditions’ (Shapiro, 1997). Since that time immense progress has been made towards accepting the multicellularity of microorganisms, which are able to form organized differentiated structures in a regulated way. However, even now, this insight into microorganisms is still not generally accepted, as various papers and discussions at conferences clearly document.

In this review, we have attempted to introduce various aspects of the behaviour of multicellular yeast populations, but also to show that our knowledge is still somewhat limited. However, as a result of escalating progress in this field, crucial new findings can be expected during the coming years not only in the direction of fundamental knowledge, but, consequently, also in its medical and ecological applications.

What are the main differences in the anticipated findings on yeast populations compared with those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, differences in the anticipated findings on yeast populations compared with those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking, multicellular communities of microorganisms are far from those of metazoa. On the other hand, historically speaking,
crucial discoveries concerning various basic cellular processes (e.g. cell-cycle regulation) were made on simple eukaryotes, yeast. Moreover, there are already several examples of signalling compounds and proteins involved in the regulatory machinery important for the ageing and survival of yeast communities that have an analogy in mammals. Examples are ammonia or as a signalling molecule in yeast colonies (Palkova et al., 1997) and in mammalian long-living neurones (Marcaggi & Coles, 2001), Sir2p histone deacetylase of yeast and its mammalian homologue SirT1 (Vaziri et al., 2001), and some others. Moreover, there are several yeast proteins whose functions appear to be important in the development of yeast populations that have mammalian homologues of unknown function. Thus, the future will show whether at least some of the molecular mechanisms that appear to be important for the development of multicellular microorganisms have counterparts in higher eukaryotes.

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Photos in Fig. 1a, Fig. 2 and Fig. 3f were reprinted from Kuthan et al. (2004). Domestication of wild *Saccharomyces cerevisiae* is accompanied by changes in gene expression and colony morphology. *Mol Microbiol* **47**: 745–754 with permission from Blackwell Publishing. Photos in Fig. 1b were reprinted from Joshi et al. (1975). Intercellular matrix in colonies of *Candida*. *J Bacteriol* **123**: 1139–1143, in Fig. 1c from Fries et al. (1999). Phenotypic switching in *Cryptococcus neoformans* results in changes in cellular morphology and glucuronoxylomannan structure. *Infect Immun* **67**: 6076–6083, and in Fig. 3c from Brega et al. (2004). *Candida albicans* Csy1p is a nutrient sensor important for activation of amino acid uptake and hyphal morphogenesis. *Eukaryotic Cell* **3**: 135–143, with permission from the authors and the American Society for Microbiology. Photos in Fig. 3a were reprinted from Sudbery P, Gow N & Berman J (2004). The distinct morphogenetic states of *Candida albicans*. *Trends Microbiol* **12**: 317–324 and in Fig. 3e from Vopalenska I, Hulкова M, Janderova B & Palkova Z. (2005). The morphology of *Saccharomyces cerevisiae* colonies is affected by cell adhesion and the budding pattern. *Res Microbiol* **156**: 921–931, both with permission from Elsevier. Photos in Fig. 3b were reprinted from Laffey SF & Butler G (2005). Phenotype switching affects biofilm formation by *Candida parapsilosis*. *Microbiology* **151**: 1073–1081, with permission from the Society for General Microbiology. Photos in Fig. 3d were reprinted from Molecular Biology of the Cell (Mol. Biol. Cell 2005 671–692; published online before print as 10.1091/mbc.E05-01-0071) with permission from The American Society for Cell Biology.

**References**


Srikantha T, Tsai L, Daniels K, Klar AJ & Soll DR (2001) The histone deacetylase genes HDA1 and RPD3 play distinct roles...
in regulation of high-frequency phenotypic switching in 