Chapter 7
Imaging Living Yeast Cells and Quantifying Their Biophysical Properties by Atomic Force Microscopy

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7.1 Introduction

Yeasts, such as *Saccharomyces cerevisiae,* were used for thousands of years by humans to produce food and beverages, but humans have also cohabited with harmful yeasts such as *Candida albicans*. Both are surrounded by a thick, mechanically strong cell wall that plays several key physiological roles, such as maintaining cell shape and cell integrity, protecting the cell interior from harmful xenobiotics in the environment. The cell wall also harbors several proteins that are implicated in molecular recognition and adhesion (Chaffin 2008). The chemical composition of the yeast cell wall is well known (Lipke and Ovalle 1998). It consists of a microfibrillar network of β-glucans (β-1,3 and β-1,6-glucans) that represent 50–60 % of the cell wall mass, overlaid by highly glycosylated proteins that are decorated by long chains of mannose residues representing 40–50 % of the cell wall mass. Chitin, a linear polysaccharide of β-linked N-acetylglucosamine, is another major component of the yeast cell wall and represents 1–3 % of the cell wall mass. The yeast cell wall is an essential organelle for cell viability because it preserves the cell from osmotic pressure and heat shock, and it serves as a barrier and a filter against harmful molecules. Interestingly, among eukaryotes, this cell wall is unique to fungi, and, as

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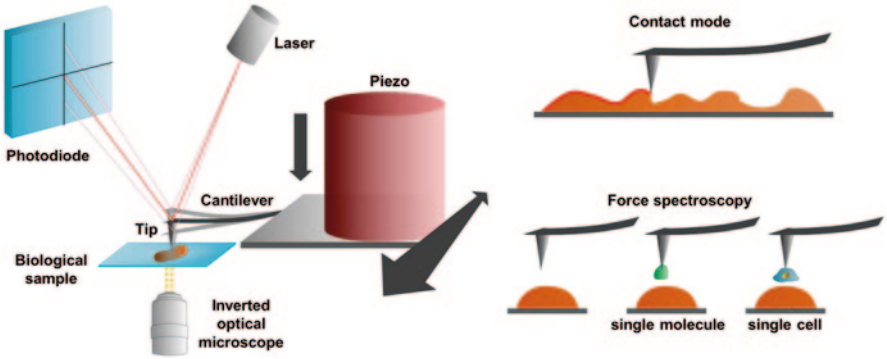

Fig. 7.1 A sharp tip is mounted on a cantilever that can be moved in the *x, y,* and *z* direction by a piezoelectric ceramic. The deflection of the cantilever is monitored on a four-quadrant photodiode as the reflection of a laser beam aligned at the end of the, usually gold-coated, cantilever. The AFM can be used to produce topographical images (i.e., contact and tapping modes with raster scanning) or to measure forces (force spectroscopy mode) between a bare or a functionalized (biomolecule or single-cell) tip and the sample. (Reprinted with permission from Pillet et al. 2014a)

it is essential for yeast viability, it represents an excellent target for antifungal drugs targeted against pathogenic yeasts. In addition, the molecular architecture of the yeast cell wall is not static, but constantly remodeled as a function of growth conditions, morphological development, and in response to cell surface stresses (Orlean 2012). Therefore, atomic force microscopy (AFM), which visualizes and probes the ultrastructure of the cell wall surface, is perfectly suited for the study of its dynamic structure and its molecular modification under different conditions.

Since its invention in 1986 (Binnig and Quate 1986), AFM has been used more and more to explore living cells at the nanoscale. AFM provides the unique opportunity to measure topography, nanomechanical properties, and/or single-molecule interactions, on living cells, with a nanoscale resolution. In the basic contact imaging mode, a sharp tip mounted on a flexible cantilever is scanned by a piezoelectric ceramic over the sample surface (Fig. 7.1). The deflection of the cantilever is continuously monitored through an optical lever (laser and photodiode system) that records vertical and lateral deflection. In the constant force mode, a feedback loop acts on the piezoelectric ceramic to maintain a constant cantilever height, and thus the applied force on the sample is kept constant. In tapping mode, the cantilever is oscillated near its resonant frequency while scanning over the surface. In this mode, the contact between the tip and the sample is defined as a decrease in the resonance amplitude, and the feedback loop adjusts the cantilever height in order to keep the amplitude constant. These two basic imaging modes result in topographic images of the samples recorded line by line while raster scanning the sample with the tip. The AFM, however, is much more than a simple imaging tool as it is also able to detect forces as small as a few piconewtons. In the force spectroscopy (FS) mode (Fig. 7.1), the tip is no longer scanned over the surface, but rather approaches and retracts from the surface. FS results in force *versus* distance curves that can be

analyzed in terms of contact point, nanomechanical properties, and adhesion forces. Moreover, it is also possible to record force curves according to a predefined matrix, resulting in height maps, nanomechanical maps, or adhesion maps acquired point-by-point with a force curve at each point. The latter mode is named force volume and has now evolved toward modes that record force–distance curves at very high speeds (Dufrêne et al. 2013; Chopinet et al. 2013).

In this chapter, we describe how AFM can refine our understanding of the yeast cell wall. Firstly, we discuss immobilization methods for yeasts so that they can withstand the tip lateral forces induced during scanning. Immobilization methods must not modify the yeast cell wall. This is an essential problem that needs to be solved properly before any AFM experiments can be pursued. Next, we focus on imaging data to demonstrate interest in live-cell AFM. In particular, we show that AFM is capable of recording cell growth at the single-cell level, as it can work in liquid (culture broth) and at a controlled temperature (microorganism's growth temperature). Then, we examine the measurement of nanomechanical properties of the yeast cell wall and attempt to make a link between the biochemical composition of the cell wall and its nanomechanical properties. We also examine how drugs modify the nanomechanical properties of the yeast cell wall. Finally, we look at single-molecule and single-cell experiments. The prerequisite for such experiments is functionalization of the AFM tip with a molecule of interest or with a cell. We describe these functionalization methods followed by some interesting results of single-molecule or single-cell FS experiments.

7.2 Immobilization of Yeast Cells

AFM is a scanning probe technique and therefore the tip when scanning induces lateral forces on the sample. If the sample (in our case yeast cells) is not properly immobilized, no images and no force curves can be recorded. The first attempt to achieve a firm immobilization of microorganisms, in general, was to fix the cells by air drying (Canetta et al. 2006) or by chemical fixation (Louise Meyer et al. 2010). However, these methods surely induce cell wall modifications. Other strategies were developed to overcome this difficulty; cells were immobilized in gelatin (Gad and Ikai 1995) or trapped into the pores of polycarbonate filters (Touhami et al. 2003a). These techniques have been widely used over the recent years (Francius et al. 2008; Alsteens et al. 2008; Dague et al. 2008b; Gilbert et al. 2007); however, it can lead to tip pollution in the case of gelatin trapping, or it can submit cells to mechanical forces in the case of cells trapped in pores. Also, both techniques are time-consuming because cells are spread all over the sample and can be quite difficult to find. To circumvent these problems, recent developments were dedicated to the fabrication of polydimethylsiloxane (PDMS) stamps structured at the microscale with wells of different sizes adapted to yeast cell diameter (Dague et al. 2011; Formosa et al. 2015a). Then, the cells are assembled into the microwells using the convective/capillary deposition technique, as shown in Fig. 7.2a. This results in arrays of living cells, immobilized

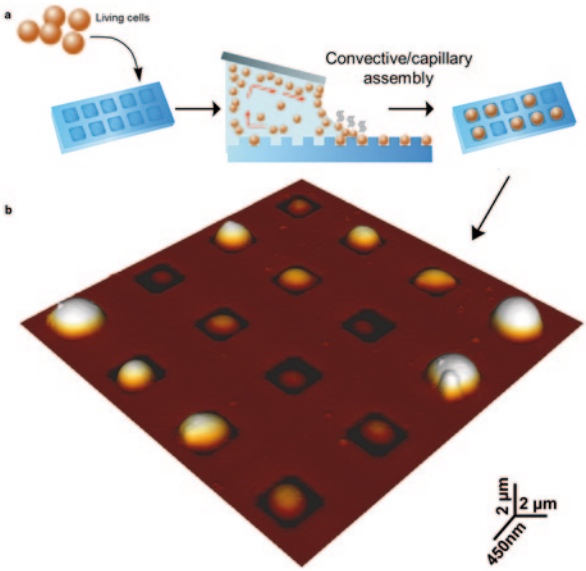

Fig. 7.2 Assembling yeast cells in a polydimethylsiloxane (PDMS) stamp using convective capillary deposition. **a** Principle of convective capillary deposition used to organize the yeast cells in the holes of a PDMS stamp. A meniscus is created between a glass slide and the microstructured stamp. Evaporation at the meniscus creates a convective flux in the liquid that concentrates the cells in the meniscus. The slide is then pulled over the surface. When encountering a hole, the meniscus is caught and a single cell is immobilized in the hole. **b** Atomic force microscopy (AFM) 3D height image of an array of 16 cells organized in a PDMS stamp

in a defined place. Figure 7.2b shows an arrangement of 16 cells organized in 16 holes. This innovative immobilization method makes it possible to analyze many more cells than in the past, which will result in an increase in the statistical meaning of AFM results.

7.3 Imaging Living Yeast Cells by Atomic Force Microscopy

Having overcome the immobilization issue, live, unmodified cells can be imaged at high resolution using AFM. Imaging living microorganisms at high resolution is clearly a challenge. Optical microscopy has been limited for a long time by diffraction (Chaps. 1–4), and its variation now allows sub-diffraction imaging (Jensen and Crossman 2014), while electron microscopy (Chaps. 6, 8) usually requires working in a vacuum and thus is not capable of imaging live cells. In this context, AFM provides the unique opportunity to image yeasts, neither with photons nor electrons, but with a sharp tip in growth media with temperature control and at high resolution (Dupres et al. 2009). The first images of living *S. cerevisiae* cells, immobilized in

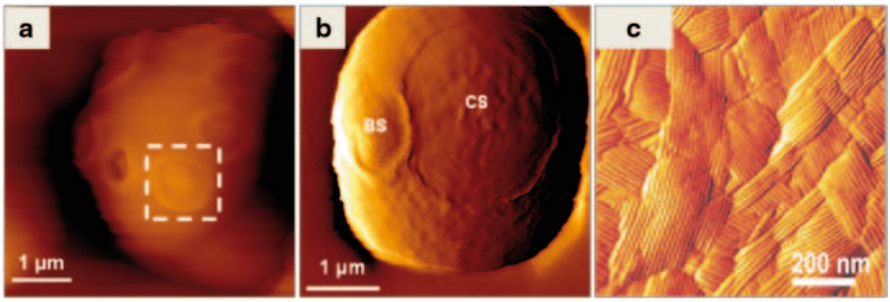

Fig. 7.3 Atomic force microscopy (AFM) high-resolution imaging of *Saccharomyces cerevisiae* with a ring made of eight bud scars *(BSs)* surrounding the cell. The *dotted line square* is centered on a *BS* (**a**), the circular structure *(CS)* induced by heat shock on *S. cerevisiae,* next to a *BS* (**b**), and the rodlet layer made of hydrophobins of *Aspergillus fumigatus* (**c**)

gelatin, showed bud and birth scars (Kasas and Ikai 1995). Figure 7.3a presents an example of a mother cell of *S. cerevisiae* with multiple bud scars (BSs), one of which is surrounded by a dashed square (Pillet et al. 2014b). Such features at the surface of yeast cells have been described in multiple studies (Touhami et al. 2003a; Dague et al. 2010; Alsteens et al. 2008; Adya et al. 2006) by different authors. In Fig. 7.3b, a BS can be seen next to a different and larger structure, named by the authors of this study a circular structure (CS, Pillet et al. 2014b), that appears on the *S. cerevisiae* surface after an hour of heat shock at 42 °C. This circular ring reaches 3 µm in diameter and is initiated on a BS. The authors showed that the formation of this structure required a functional budding process, as no CSs were observed on yeasts in which genes involved in the budding process were deleted.

At the surface of fungal spores, such as spores of *Aspergillus fumigatus,* proteins named hydrophobins are auto-organized and confer hydrophobicity to the conidia (Fig. 7.3c). These proteins create a rodlet layer (Beever and Dempsey 1978; Aimanianda et al. 2009) that can be imaged by AFM at high resolution (Dufrêne et al. 1999; Dague et al. 2008a; Zykwinska et al. 2014; Ma et al. 2005). Hydrophobins are amyloid proteins that are assembled into fibrils spaced from each other with 10 nm. This rodlet layer is also described on spores of bacteria such as *Bacillus atropheus* (Plomp et al. 2007a) or *Clostridium novyi* (Plomp et al. 2007b). The disruption of this layer during spore germination has been followed in real time (Dague et al. 2008c) which demonstrated that AFM was able to image dynamic processes such as germination on living cells. After 1 h of germination, the rodlet layer is slightly disrupted. After 2 h, the spore surface is heterogeneous; some regions still have an altered rodlet layer, whereas other regions present an amorphous surface. Finally, after 3 h, the whole surface of the spore is amorphous. Unfortunately, it was impossible to image the emission of a germ tube, probably because the immobilization of the conidia in the pore of a polycarbonate filter made that impossible. However, measures directly performed on small areas of hyphae were possible. The Dahms' lab demonstrates, using this method, that galactofuranose is a key constituent for the organization of the *Aspergillus nidulans* hyphal wall (Paul et al. 2011).

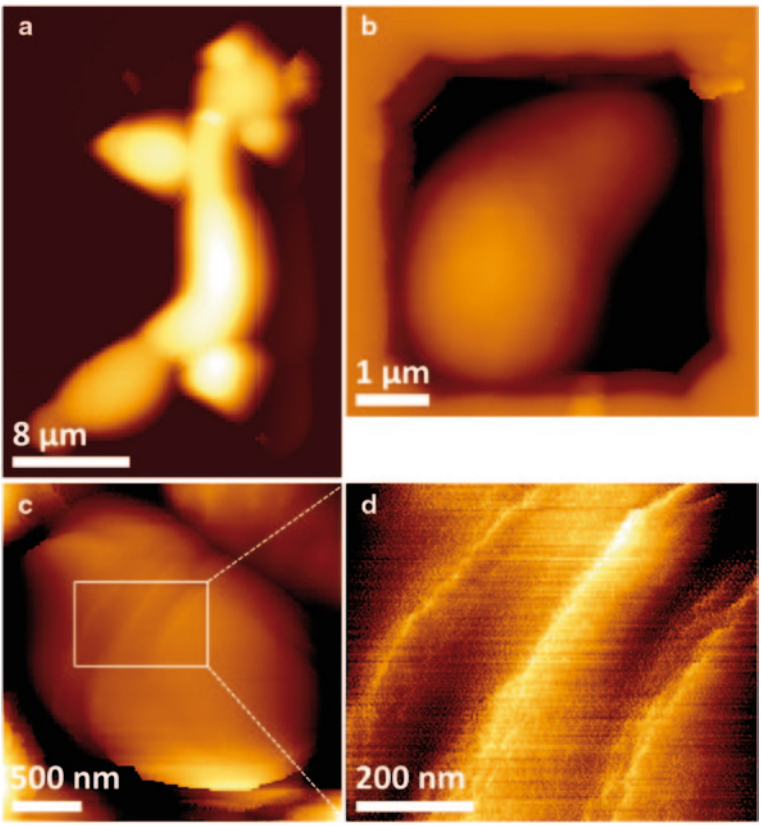

Fig. 7.4 Imaging morphological changes in yeast cells. High-resolution imaging of **a** a *Candida albicans* hyphae immobilized in polydimethylsiloxane (PDMS), **b** of a mating projection of *Saccharomyces cerevisiae* immobilized in a PDMS stamp, and **c** of a *Saccharomyces cerevisiae* cell exposed to caspofungin at 4 × MIC. The *white square* on **c** is imaged at higher resolution in **d**. *MIC* minimal inhibitory concentration

AFM also allowed the direct imaging of *C. albicans* hyphal growth, as shown in Fig. 7.4a (Formosa et al., personal communication), which follows from studies in which both fixed and live/growing hyphae were imaged by AFM (Ma et al. 2005, 2006). In Fig. 7.4a, the ramifications of the fungal hyphae are clearly observed. Hyphal forms of *C. albicans* are being used by the fungus to invade host tissues; the possibility to study them by AFM is therefore relevant for clinical microbiologists. It opens the doors to fundamental questions on the formation of these hyphae; for example, mutated strains for surface proteins could present modified growth processes, which could be monitored by AFM. Also, their interactions with host cells can be probed using AFM; for example, a recent study by El-Kirat-Chatel et al. focused on the interaction of *C. albicans* hyphae with macrophages and showed the ability of the fungus to grow hyphae directly into the macrophagic cells that resist

them (El-Kirat-Chatel and Dufrêne 2012). Another phenomenon that can be imaged, thanks this time to the immobilization method in PDMS stamps, is the emission of mating projection by *S. cerevisiae* exposed to the α-factor (Fig. 7.4b). The α-factor, a yeast sexual hormone, triggers the formation of characteristic mating projections, also named "shmoos," by haploid yeast strains of a mating type (Merlini et al. 2013). This is an important process that facilitates contact between two partner cells so that they can fuse to form a diploid zygote. The study of yeast mating has many implications (e.g., in understanding analogous fundamental biological processes in higher eukaryotes), and its study by AFM could give new insights into the mechanisms underlying this process. For example, different genes could be implicated in this process using mutated strains in the presence of α-factor. However, many other types of experiments, including experiments with functionalized AFM tips recognizing specific proteins at the tip of shmoos or on the cell surface, can also be envisioned, knowing the great possibilities of such a technology.

Finally, AFM also allows to image changes in surface morphology induced by drugs such as antifungals. Figure 7.4c, d shows an *S. cerevisiae* cell treated with a high dose (4 × minimal inhibitory concentration) of caspofungin, an antifungal drug used for fungal infections (Denning 2003; Formosa et al. 2013). Upon treatment, the cell is no longer spherical but is elongated, resembling *Schizosaccharomyces pombe* cells. The antifungal-treated cell also presents a surprising feature on its surface (Fig. 7.3d), with rings up to 15 nm high, indicating altered cell division likely associated with impairment of cytokinesis.

Taken together, these examples show the importance of AFM, which offers the possibility to work on live yeast cells in liquid conditions. Indeed, AFM can be used to image yeast cell morphologies at the nanoscale, such as hyphae or shmoos, the ultrastructures naturally present at their surface (bud and birth scars), or those induced by stress (e.g., a protease; Ahimou et al. 2003) or the circular structure induced by thermal stress and rings induced by an antifungal treatment.

7.4 Probing the Nanomechanical Properties of Yeast Cells

To probe the nanomechanical properties of living yeasts, such as elasticity, spring constant, or turgor pressure, AFM is used in the FS mode. The nanomechanical property most often used to describe the cell wall of yeasts is elasticity; therefore, we focus on this parameter in this chapter. The elasticity can be deduced from the approach force–distance curves obtained in the FS mode. Force–distance curves can be converted into indentation curves, which are then fitted through the Hertz model, to extract the Young modulus value, meaning the elasticity, expressed in pascals (Hertz 1881). The elasticity of the cell wall reflects its composition but also its molecular organization. In 2003, Touhami and coworkers (Touhami et al. 2003b) mapped the nanoscale elasticity of *S. cerevisiae*. Specifically, they compared the nanomechanical properties of a BS with the rest of the cell wall. They mapped a higher resistance on the BS than on the rest of the cell and correlated this result with

the increased amount of chitin in the BS. A few years later, this result was reproduced on *S. cerevisiae* but not for another yeast species, *Saccharomyces carlbergensis* (Alsteens et al. 2008). This result demonstrated that the correlation between nanomechanical data and the cell wall composition is not always straightforward.

Indeed, different components of the yeast cell wall are interconnected to form macromolecular complexes (Orlean 2012; Free 2013), which can be modified upon stress or if genes involved in cell wall synthesis are missing. A recent study showing the differences that can take place in the nanomechanical properties of yeast cells focused on yeast mutants of *S. cerevisiae* defective in cell wall architecture (Dague et al. 2010). In this work, the authors showed that native wild-type cells of *S. cerevisiae* had a global cell wall elasticity of 1.6 MPa, whereas its isogenic mutants defective in enzymes involved in cell wall cross-linking and assembly (*gas1, chr1chr2* mutants), or with a reduction of their chitin content (*chs3* mutant), had a reduced Young's modulus compared to wild-type cells. However, mutants with reduced β-glucan (*fks1*), mannan *(mnn9)* content, or defective in the regulation of the cell wall biosynthesis *(knr4)* presented higher Young's modulus values compared to wild-type cells. Thus, these results show that the nanomechanical properties of yeast cells are dependent not only on the composition of the cell wall but also on the intrinsic molecular organization of the cell wall, as shown by Paul et al. (2011).

The relationship between elasticity of the yeast cell wall and its composition/molecular organization has also been observed in a different context—thermal stress (Pillet et al. 2014b). In this study, the authors showed that thermal stress induced an increase in the chitin content of the cell wall, which was accompanied by an increase in the Young's modulus values. When yeast cells are submitted to a parietal stress, one of the first defense mechanisms is the overproduction of chitin (Ram et al. 1998). Chitin, being a rigid polymer, increases the elasticity of the cell wall when overproduced. Finally, previous studies on the effects of caspofungin on the yeast cell wall of *S. cerevisiae* and *C. albicans* also showed modification of the viscoelastic properties of cells upon treatment with this antifungal (Formosa et al. 2013; El-Kirat-Chatel et al. 2013). Figure 7.5 presents nanoindentation measurements performed on cells of *C. albicans* in native conditions, or treated by two different doses of caspofungin (0.5 and 4 × MIC). Figure 7.5a, b, c depicts elasticity maps of the whole cells immobilized in PDMS stamps; Fig. 7.5d, e, f shows elasticity maps recorded on small areas of 1 μm^2 on top of the corresponding cells; and, finally, Fig. 7.5g, h, i shows the distributions of the Young's moduli obtained for each pixel on the local elasticity maps for which the darker red the pixel, the higher the Young's modulus. We can see clearly from this figure that treatment with caspofungin results in an increase in the Young's moduli. Remarkably, the higher the caspofungin dose, the higher the Young's moduli, which was correlated with higher chitin content in the cell wall of caspofungin-treated yeasts. This work shows the correlation between elasticity of the yeast cell wall and its composition.

These results show how AFM can be used as a force machine to probe the cell wall of yeast cells in their native state and when submitted to genetic stress (mutants) or external stresses (heat shock and caspofungin treatment). Altogether, these nanomechanical data give new insights into the yeast cell wall organization and remodeling in response to different types of stresses.

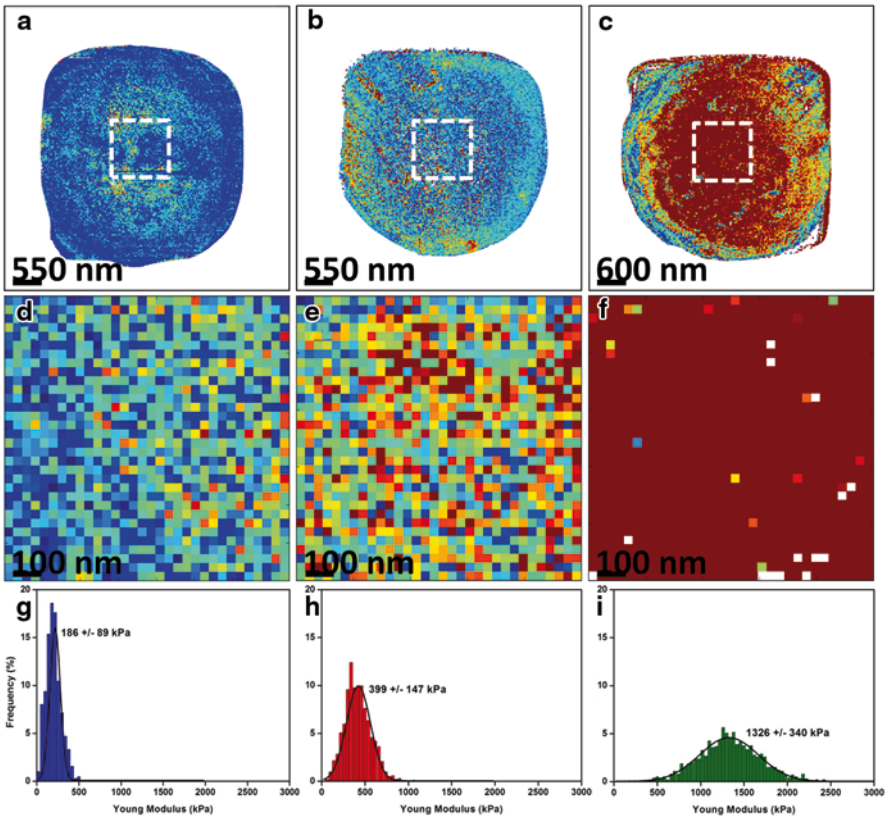

Fig. 7.5 Mapping of *C. albicans* cell surface elasticity. **a**–**c** Elasticity maps (z range = 0.5 MPa) of a native cell (**a**), of a cell treated with caspofungin at 0.5 × MIC (0.047 μg/ml) (**b**), and of a cell treated with caspofungin at 4 × MIC (0.376 μg/ml) (**c**). **d**, **e**, and **f** Local elasticity maps (z range = 0.5 MPa) recorded on a 1-μm area *(white dashed squares)* on the top of the cells in panels **a**–**c**, respectively. **g**, **h**, and **i** Distributions of Young's moduli ($n = 1024$) corresponding to the local elasticity maps in panels **d**–**f**, respectively. (Reprinted with permission from Formosa et al. 2013)

7.5 Single-Cell and Molecule Force Spectroscopy

Another property that can be probed by AFM is adhesion. Indeed, specific molecular interactions are the basis of various biochemical and biological processes. In order to gain significant data on these interactions, AFM tips can be functionalized with molecules that will interact specifically with target molecules at the surface of the cells. These experiments, performed with functionalized AFM tips, are called single-molecule force spectroscopy (SMFS) experiments. Of the various strategies to functionalize AFM tips with biomolecules, some consist of nonspecific adsorption of proteins (e.g., bovine serum albumin; BSA) to the silicon nitride surface

of AFM tips (Florin et al. 1994), or the chemical fixation of biomolecules by sulfur–gold bonds to gold-coated AFM tips. This last strategy has been successfully used to functionalize AFM tips with methyl groups, CH_3, to probe the hydrophobic characteristics of the rodlet layer of *A. fumigatus* (Alsteens et al. 2007; Dague et al. 2007). In this study, the authors showed that hydrophobic tips enable quantification of surface hydrophobicity on live cell surfaces, and how this hydrophobicity relates to a function such as surface adhesion or drug interaction.

It is also possible to covalently link a molecule containing amino groups directly to the silicon nitride AFM tip. Toward this end, AFM tips must be first amino-functionalized either by esterification with ethanolamine (Hinterdorfer et al. 1996) or silanization with aminopropyl-triethoxysilane (APTES; Ros et al. 1998). Then, the amino-functionalized tip must be bridged to the biomolecule of interest, achieved through the use of hetero-bifunctionalized polyethylene glycol (PEG; Wildling et al. 2011; Ebner et al. 2008; Kamruzzahan et al. 2006) or an aldehyde–phosphorus dendrimer, as we previously described (Jauvert et al. 2012). The second strategy has been used to map the polysaccharides at the surface of living yeast cells, with AFM tips functionalized with concanavalin A, a protein that interacts specifically with carbohydrates (Gad et al. 1997). In this study, the adhesive forces were calculated from the AFM retract portion of force curves by measuring the piezoelectric retraction force required to break the interaction between the lectin and the recognized carbohydrate. Such measurements allowed the authors to conclude that mannans were uniformly distributed on the cell wall surface.

This functionalization strategy has also been used to map the surface properties of the pathogenic yeast *C. albicans*. This pathogenic yeast species has emerged as a major public health problem in the past two decades. This opportunistic pathogen causes a wide range of infections from surface to mucosal and bloodstream infections (Gow and Hube 2012). In order to colonize and subsequently disseminate in the bloodstream, *C. albicans* first needs to adhere to different biotic substrates. This first stage of infection (Naglik et al. 2011) is mediated by adhesins that are found on the surface of the yeast cell wall. Many of these adhesins are mannoproteins; among them, one identified as having a major role in host cell attachment is the agglutinin-like sequences (Als) family (Hoyer 2001). The Als were initially reported as having homology to the proteins responsible for autoagglutination in the baker's yeast *S. cerevisiae*. Eight *ALS* genes have been identified, and all are primarily involved in host–pathogen interactions (Hoyer et al. 2008). In a recent study by Beaussart et al., the authors used SMFS experiments to map the localization of Als3 on the surface of *C. albicans* hyphae (Beaussart et al. 2012) using an anti-Als3 antibody-functionalized tip to probe different parts of the germinating tube, that is, on the germinating yeast (Fig. 7.6a, b, c) and on the germ tube (Fig. 7.6d, e, f). The Als3 protein distribution was very different with the number and length of unfolding events higher on the germ tube, accompanied by longer unfoldings, indicating that the adhesin Als3 is much more exposed on the germ tube than on the germinating yeast. These results are consistent with the specific expression of the *ALS3* gene during the yeast–hyphae transition (Liu and Filler 2011).

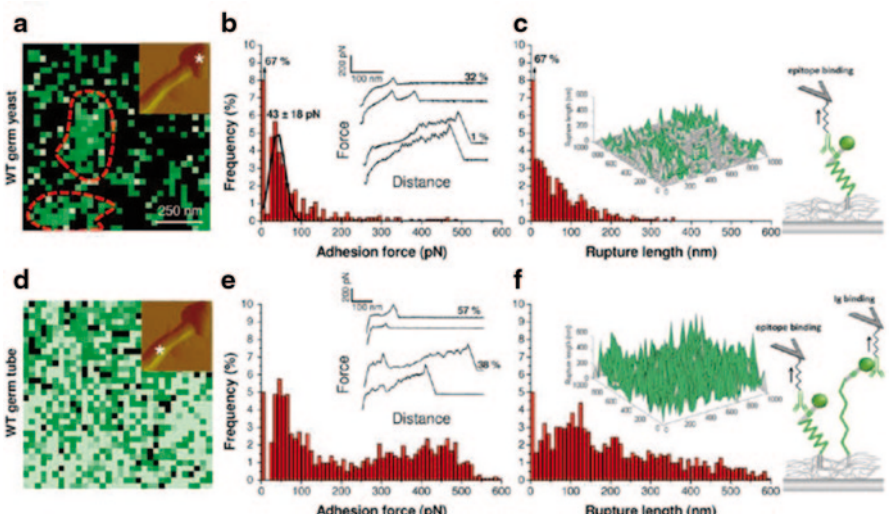

Fig. 7.6 Cellular morphogenesis leads to a major increase in the distribution and extension of Als3 proteins. **a**, **d** Adhesion force maps (1 μm^2, z range = 300 pN) recorded in buffer on the yeast (**a**) and germ tube (**d**) of a germinating cell using an anti-Als3 tip. *Insets:* deflection images in which the *asterisk* indicates where the force maps were recorded. The *dashed lines* in **a** emphasize Als3 clusters. **b**, **e** Corresponding adhesion force histograms ($n = 1024$) together with representative force curves. **c**, **f** Histograms of rupture distances ($n = 1024$) and 3D-reconstructed polymer maps (false colors, adhesion forces in *green*). The data are representative of several independent experiments using different tip preparations and cell cultures. (Reprinted with permission from Beaussart et al. 2012)

Another characteristic of the Als proteins is the presence of an amyloid-forming sequence, which enables their aggregation, under certain conditions, into amyloids at the surface of *C. albicans* cells. A recent study by Alsteens et al. showed that the formation and propagation of Als5 protein nanodomains of the Als5 protein at the surface of *S. cerevisiae* yeast cells overexpressing Als5 was force induced (Alsteens et al. 2010). AFM tips functionalized with an anti-Als5 antibody showed that a localized delivery of piconewton forces by the AFM tip could initiate the formation and propagation of Als5 nanodomains over the cell (Fig. 7.7a, b). Thus, this process may be involved in cellular adhesion, in response to mechanical stimuli—important processes for the early stages of *C. albicans* infection.

The clustering of adhesins at the yeast surface has also been described in an industrial nonpathogenic strain of *S. cerevisiae* (Schiavone et al. 2015) (Fig. 7.7c, d). A recent study by Schiavone et al. used a transcriptomic analysis to identify the protein responsible for the adhesive nanodomains. Their results showed that the strain producing adhesive nanodomains also overexpressed the Flo11 protein, an adhesin similar to the Als protein family that also contains an amyloid sequence. Finally, the use of Quantitative Imaging™ (an advanced force-volume-based mode) made it possible to map, with unprecedented resolution, adhesive nanodomains at the *C. albicans* cell surface (Fig. 7.7e, f; Formosa et al. 2015b). In this work by Formosa

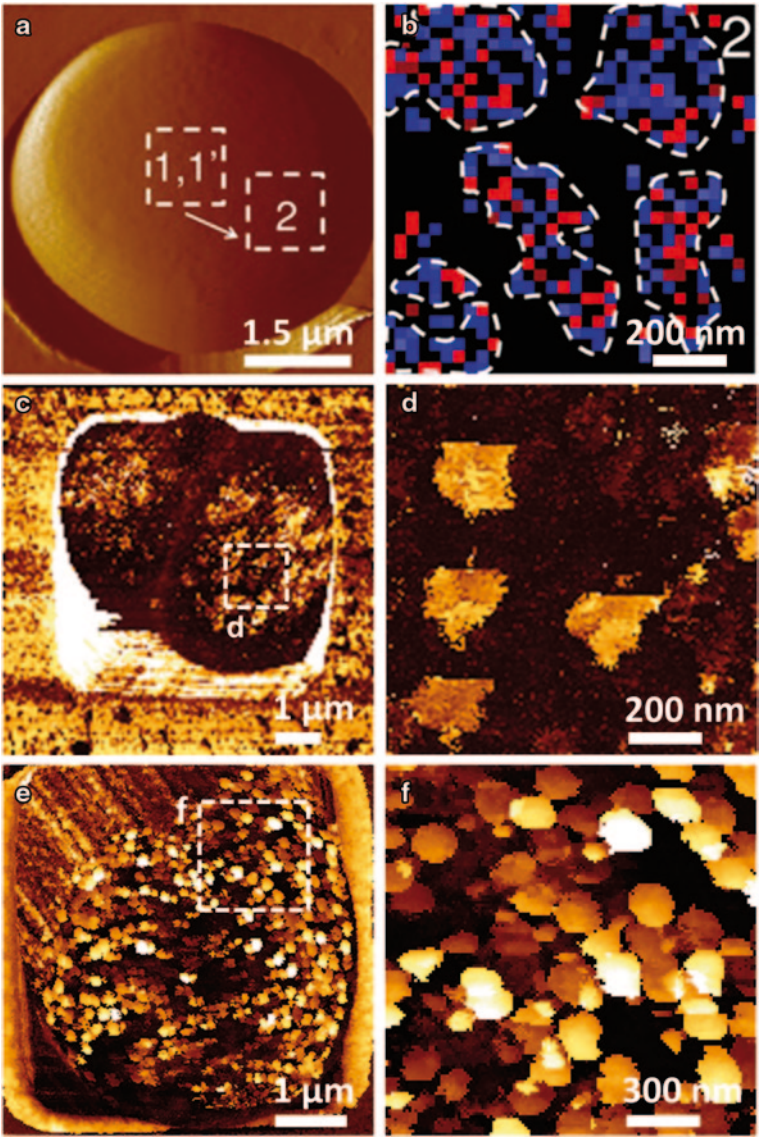

Fig. 7.7 Adhesive nanodomains at the yeast surface. **a** Atomic force microscopy (AFM) height image of *S. cerevisiae* trapped in a pore of a polycarbonate membrane. **b** Adhesion map, recorded in the *dotted square 2,* after force inducing the clustering in area *1,1',* showing the aggregation in nanodomains of the Als5 protein. *Blue* and *red pixels* correspond to forces smaller and larger than 150 pN, respectively. **c** AFM adhesion map of an *S. cerevisiae* cell immobilized in a microstructured polydimethylsiloxane (PDMS) stamp. **d** Adhesion map corresponding to the *dotted square d*. The adhesion forces range from 0 to 2 nN. **e** Adhesion map recorded on a *C. albicans* cell immobilized in a microstructured PDMS stamp. **f** Adhesion map corresponding to the *dotted square f*. Adhesion forces range from 0 to 2 nN

et al., nanodomain size, stiffness, and adhesive properties were characterized, with nanodomains divided into two classes. Some are stiff and behave like hydrophobic structures (high adhesion force), whereas others are softer and unfold in a manner similar to proteins. The first class of nanodomains was interpreted as being amyloid plaques and the second class as protein aggregates. Altogether, the formation of adhesive nanodomains at the yeast cell surface seems to be a general phenomenon; further work is required to understand what triggers their formation, if and how they are used by cells to adhere to or infect hosts.

With these last examples, we show that FS in conjunction with functionalized AFM tips is a powerful tool to better understand the molecular adhesive properties of living pathogenic yeast cells. These data will eventually contribute to preventing *C. albicans* infections, as well as in the identification of new potential targets for antifungal drugs.

Since it is possible to functionalize an AFM tip with a molecule, it is also possible to functionalize an AFM tip with a living cell. This type of experiment is called single-cell force spectroscopy (SCFS) experiment. The principle is the same as for SMFS, but this time a living cell is immobilized onto the cantilever becoming the AFM tip, which is engaged to interact with the surface or with another cell (Bowen et al. 2000, 2001). This strategy is reemerging, and there are only few papers in which it is used for the study of living yeast cells (Bowen et al. 2000, 2001; Stewart et al. 2013). However, Dufrêne's group has recently published a study that aims to measure the adhesive forces between a yeast cell immobilized on a cantilever and a hyphae of the same yeast species *C. albicans* (Alsteens et al. 2013). They show that the *C. albicans* adhesin, Als3, on the hypal surface was responsible for their adhesion with yeast cells. In the context of biofilm formation, these new data offer the first new insight into the interactions between two morphotypes of the same yeast species. The same team also used SCFS to quantify adhesion between cells of *C. albicans* and another type of pathogenic microorganism, *Staphylococcus epidermidis* (Beaussart et al. 2013). Indeed, these two types of microorganisms are often colocalized in human infections (Peleg et al. 2010). The results of this study showed that the fungal molecules involved in the interactions with *S. epidermidis* were adhesins, once again, as well as 0-mannosylations. It is interesting to note that, using the same cell probe strategy, the interactions between vaginal staphylococci and lactococci were probed (Younes et al. 2012). This work, pioneering in the field of microbiology, opens doors to new questions about fungal adhesion, an important process involved in the first stages of human yeast infections. Such studies could lead, for example, to the identification of anti-adhesion drugs that would prevent coinfections by *C. albicans* and *S. epidermidis*. The lack of statistical data has been recently addressed, and promising perspectives are evolving from the work of Potthoff (Potthoff et al. 2012), which aims to serially quantify adhesion forces using yeast probes.

7.6 Conclusions

AFM has emerged as a significant technology in the life sciences over the past 20 years. The number of publications using AFM is exponentially growing (Pillet et al. 2014a), even if the number of studies dedicated to living cells only slowly increases. The latter issue may relate to the difficulty of immobilizing live cells and avoiding damage to the cell membrane during immobilization. For yeast cells, physical trapping in pores of polycarbonate membranes or in holes of microstructured PDMS stamps is appropriate. As an imaging technology, AFM helps refine our understanding of structures exposed on the fungal cell wall, such as BSs and rodlet layers. Interestingly, AFM is also able to track morphological modifications during cell growth. More than an imaging technology, AFM is also a force machine able to probe the cell nanomechanical properties or adhesion. The data collected from FS experiments have broadened our perception of the fungal cell wall. The well-known biochemical composition of the cell wall covers up the extremely complex and dynamic interplay of its organization and architecture. Therefore, much more work is required for a fully comprehensive description of fungal cell walls and the application of that knowledge to relevant issues in the life sciences.

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