Chapter 6
Microbial Cell Surfaces and Secretion Systems

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Abstract Microbial cell surfaces, surface-exposed organelles, and secreted proteins are important for the interaction with the environment, including adhesion to hosts, protection against host defense mechanisms, nutrient acquisition, and intermicrobial competition. Here, we describe the structures of the cell envelopes of bacteria, fungi, and oomycetes, and the mechanisms they have evolved for the transport of proteins across these envelopes to the cell surface and into the extracellular milieu.

6.1 Basic Structure of Bacterial Cell Envelopes

Based on the Gram-staining method, bacteria are classically divided into two groups, Gram-positives and Gram-negatives, which have different cell envelope architecture. Gram-positives are enveloped by a cytoplasmic membrane (CM) and a thick cell wall. In Gram-negatives, the cell wall is thinner, but an additional membrane is present, the outer membrane (OM), which is located peripheral to the cell wall. The space in between the membranes is called the periplasm and because of the presence of the OM, the CM is also called the inner membrane (Fig. 6.1).

The Cytoplasmic Membrane The CM is a phospholipid bilayer with inserted proteins. The membrane-spanning segments of the integral membrane proteins are α-helical and consist of ~20 amino acids, the vast majority of them containing hydrophobic side chains (Fig. 6.1).

The Cell Wall The rigid cell wall consists of peptidoglycan and provides strength and shape to the bacteria. For example, it protects the bacteria against osmotic

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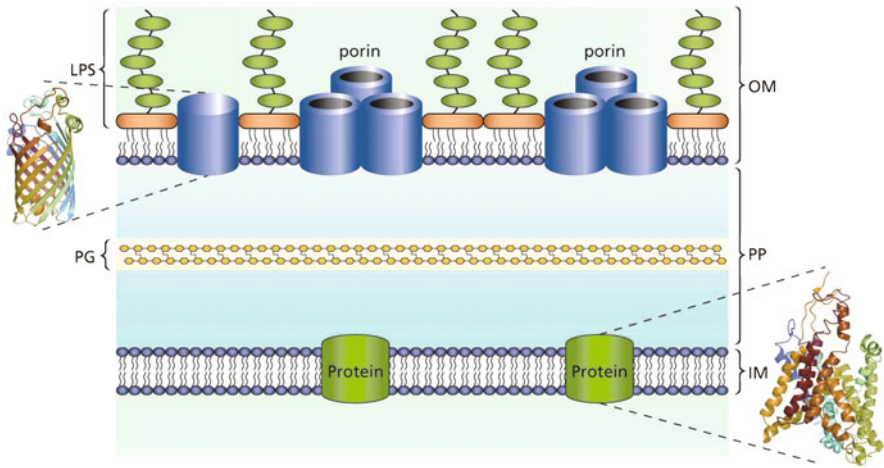
Fig. 6.1 Structure of the Gram-negative bacterial cell envelope. *OM* outer membrane containing LPS in its outer leaflet, *PP* periplasm containing a layer of peptidoglycan (*PG*), *IM* inner membrane. Examples of a typical β-barrel OMP and a typical α-helical inner-membrane protein are shown at the *left* and the *right*, respectively. Reproduced from Tommassen (2010) by permission of the publisher

pressure. Peptidoglycan consists of oligomers of a disaccharide composed of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid, which are covalently interconnected by small peptides, thus creating a network that enwraps the entire bacterial cell (Vollmer and Seligman 2010). In Gram-positive bacteria, the cell wall also contains large amounts of polymers known as (lipo)teichoic acids, which provide a net negative surface charge to the bacteria.

The Outer Membrane The OM protects Gram-negative bacteria from harmful compounds in the environment, including many antibiotics. This asymmetric bilayer contains phospholipids and lipopolysaccharides (LPS) in the inner and outer leaflets, respectively. LPS (Raetz and Whitfield 2002) consists of two or three moieties: lipid A, a core oligosaccharide, and a polysaccharide, the O-antigen, which is absent in several bacterial species. Lipid A is a signaling molecule for the innate immune system. It consists of a phosphorylated glucosamine disaccharide substituted with four 3-OH hydroxylated fatty acids, which can be esterified with secondary fatty acids. Repulsive forces between the negatively charged phosphate groups are compensated by divalent cations. The resulting network, together with the dense packing of the acyl chains, generates a barrier that is barely permeable to hydrophobic substances. Variations in the lipid A structure, affecting the acyl chains or the phosphate groups, are induced by environmental conditions and help bacteria to escape from the host's innate immune responses.

The core moiety of LPS is divided in an inner and an outer core. The inner core usually contains L-glycero-d-manno-heptose and 2-keto-3-deoxyoctonate with various substituents and is conserved among different strains of the same species. The outer core is more variable and the O-antigen, which is a polymer of repeating

mono- or oligosaccharides, is highly variable. The O-antigen can form an effective barrier for bacteriophages, bacteriocins, and antibodies, targeting the underlying conserved parts of the OM.

Integral OM proteins (OMPs) structurally deviate from other membrane proteins. The membrane-spanning segments are not α-helices but β-strands, which form a β-barrel (Fig. 6.1). These β-strands are amphipathic with hydrophobic residues facing the lipids and hydrophilic ones directed toward the interior of the barrel. Some of these β-barrels form open channels through which small hydrophilic solutes, including nutrients, such as amino acids and small sugars, can pass by diffusion. Such pore-forming proteins are called porins. Thus, the OM functions as a molecular sieve, allowing the passage of small hydrophilic molecules but holding larger hydrophilic molecules and hydrophobic ones.

Besides integral OMPs, the OM also contains lipoproteins, which are bound to the membrane via an N-terminal lipid moiety. These lipoproteins can be very abundant, e.g. Braun's lipoprotein is, with $\sim10^6$ copies per cell, the most abundant protein in *Escherichia coli*. This lipoprotein is also covalently bound to the peptidoglycan and functions to anchor the OM to the cell wall. Lipoproteins are also found in the CM.

Deviant Cell Envelope Architectures The general architecture of bacterial envelopes as described above was derived from studies on model organisms such as *E. coli* and *Bacillus subtilis*. However, deviations are now well known. For example, although not Gram-negative, Mycobacteria are covered with an OM, however, with a composition entirely different from the Gram-negative OM (Niederweis et al. 2010). It contains a large variety of lipids, including mycolic acids, which are covalently attached to the peptidoglycan via an arabinogalactan polymer. Also, bacteria that lack a cell wall have been described, such as mycoplasmas.

6.2   Additional Layers and Surface Appendices

Peripheral to the cell envelope, bacteria can be covered with additional layers, such as capsules and S-layers, and they can contain organelles, such as flagella, pili and fimbriae, which extend into the extracellular milieu.

Additional Layers Capsules (Bazaka et al. 2011) consist of polysaccharides with a highly variable composition. They have various functions, e.g. in protection against desiccation or against phagocytosis and other defense mechanisms of the host. In addition, they can have a role in the attachment of bacteria to biotic or abiotic surfaces. Bacteria can also produce extracellular polysaccharides (EPS), which do not form a capsule but are released in the environment (Bazaka et al. 2011). EPS can be important components of the extracellular matrix (ECM) of biofilms, which are surface-attached microbial communities embedded in a self-produced ECM (see also Chap. 7). S-layers are paracrystalline arrays of identical protein subunits with a large variety of functions (Fagan and Fairweather 2014). Amongst others, they may function as a molecular sieve, like the OM, or in determining the cell shape, and they may protect against bacteriophages, host defense mechanisms, or osmotic and mechanical stresses.

Flagella Flagella (Van Gerven et al. 2011) are long surface appendages that are used by bacteria to move towards favorable conditions or away from repellents in a process called chemotaxis. A flagellum consists of a long filament composed of multiple copies of a protein called flagellin. The filament is connected via a hook structure to a basal body that anchors the flagellum into the cell envelope and also forms the channel for the export of flagellin from the cytoplasm to the cell surface. The flagellum can rotate like a propeller to move the bacterium. Energy for this process is derived from the proton gradient across the CM. Flagella are also used for initial attachment of bacteria to a substratum during biofilm formation and, like LPS, flagellin is an important signaling molecule for defense mechanisms of animals and plants.

Pili and Fimbriae The names pili and fimbriae are often used interchangeably. These structures are built of subunits called pilins (Van Gerven et al. 2011). An abundant major pilin forms the filament, which usually exposes several minor pilins. Many pili/fimbriae have a role in adhesion, where one of the minor pilins functions as the adhesin that binds, for example, a eukaryotic target cell. The type IV pili form a special class of pili with multiple functions. These pili, which are based in the CM and cross the OM via a large oligomeric protein called secretin, are retractile. Extension and retraction of these pili, both at the expense of ATP, can be used by bacteria such as *Pseudomonas aeruginosa* to move over surfaces, a process called twitching motility. Some bacteria that are naturally transformable use type IV pili to take up DNA from the environment. Type IV pili can also function as nanowires that transfer electrons from the respiratory chain to extracellular electron acceptors. Also sex pili are retractile. They are produced by donor cells in the process of conjugation to establish contact with a recipient cell. Retraction of the pilus then results in the formation of a stable mating pair that allows for the transfer of DNA from donor to recipient. DNA transfer from *Agrobacterium tumefaciens* to plant cells requires similar machinery as in bacterial conjugation (see Chap. 37). A final class of pili is constituted by curli. Curli form amyloid fibers similar to the amyloids that cause neurodegenerative diseases in humans. Curli fibers are involved in adhesion, bacterial aggregation and biofilm formation.

6.3 Protein Export

Translocation Across the CM Proteins destined for export are synthesized as precursors with an N-terminal signal peptide of ∼25 amino-acid residues. Different signal peptides share a similar organization with three domains: an N-domain containing positively charged residues, an H-domain of ∼10–12 hydrophobic residues, and a C-domain containing the motif recognized by the enzyme that cleaves off the signal peptide after export. The signal peptide directs the precursor to the Sec (secretion) machinery, which mediates its transport across the CM (Kudva et al. 2013). The central component of this machinery is a complex of three integral CM proteins, the SecYEG translocon, which forms the protein-conducting channel. It is widely

conserved in nature and corresponds to the Sec61 complex in the endoplasmic reticulum of eukaryotes. Energy for export is provided by the motor protein SecA, which hydrolyzes ATP, and the proton-motive force. The Sec machinery also inserts proteins into the CM. CM proteins are generally not produced with a cleavable signal peptide, but the most N-terminal membrane-spanning α-helix is recognized by the signal-recognition particle (SRP) and targeted via an SRP receptor (FtsY) to the Sec translocon. When such a long hydrophobic α-helix enters the translocon, the substrate is not released in the periplasm, but the translocon opens laterally to insert it into the CM. This is the reason why OMPs have a deviant structure: if OMPs would also consist of hydrophobic α-helices, they would never reach their destination but be inserted by the translocon into the CM. Besides the Sec translocon, YidC protein constitutes an alternate insertase for CM proteins.

The Sec translocon contains a narrow channel and exports delineated proteins. Some proteins need to be exported in a folded conformation, for example because they bind a co-factor in the cytoplasm. These proteins are exported via the Tat system (Kudva et al. 2013). Tat stands for twin-arginine translocation and refers to a characteristic twin-arginine motif in the signal peptides of the substrate proteins. The Tat system consists of three CM proteins, TatA, TatB, and TatC. Energy for transport is provided by the proton gradient.

OMP Assembly The Sec machinery releases OMPs into the periplasm, where they are bound by the chaperones Skp and/or SurA, which prevent their aggregation. Subsequently, they are folded and inserted into the OM by the Bam (β-barrel assembly machinery) complex. The central component of this complex, known as BamA or Omp85, is highly conserved and found in the OM of all Gram-negatives and even in mitochondria and chloroplasts. These eukaryotic cell organelles also contain β-barrel proteins in their OM, probably reflecting their endosymbiont origin. The Bam complex contains a variable number of accessory components, usually lipoproteins, which are less conserved (Tommassen 2010).

6.4 Protein Secretion

In Gram-positive bacteria, exported proteins are bound to the peptidoglycan or released in the environment. In Gram-negatives, secreted proteins have to pass another hurdle, the OM. These bacteria have developed six widely disseminated protein-secretion mechanisms, designated type 1–6 secretion systems (T1-6SS) (Chang et al. 2014). Many of these systems can be present in a single cell, often in multiple copies, each one dedicated to the secretion of a specific protein or set of proteins. For example, *P. aeruginosa* possesses five of the six secretion systems (Fig. 6.2).

Two-Step Mechanisms In two-step mechanisms, a periplasmic intermediate is translocated across the OM. The T2SS is dedicated to the secretion of folded proteins. The substrates, usually hydrolytic enzymes or toxins, are either folded in the cytoplasm and exported via the Tat system, or exported via the Sec system and folded in
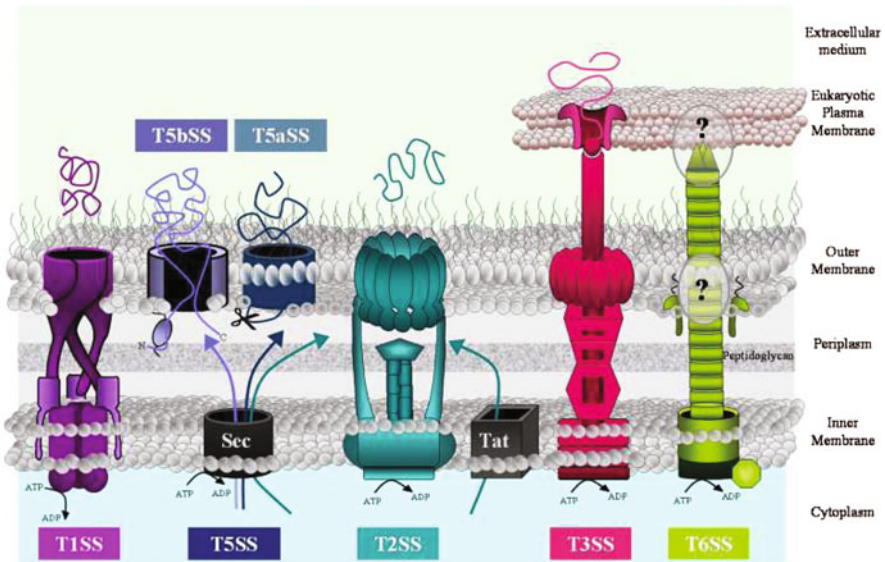
Fig. 6.2 Protein secretions systems in *P. aeruginosa*. Details are described in the text. Reproduced from Bleves et al. (2010) by permission of the publisher

the periplasm (Fig. 6.2). The T2SS consists of 12–16 proteins and resembles the machinery that builds type IV pili. It includes several pilin-like proteins and a secretin in the OM, which forms the protein-conducting channel. The model is that substrates bind the secretin, and a pilus-like structure that grows from the CM provides the mechanical force to push them through the secretin into the milieu.

The T5SS consists of five subtypes (a–e), including four variants of an autotransporter mechanism. Autotransporters consist of a signal peptide for export via the Sec system, a passenger, which is the secreted part, and a translocator domain, which forms a β-barrel that inserts into the OM via the Bam complex. During insertion, the connected passenger is translocated across the OM. Protein folding starts when the first part of the passenger appears at the external side and presumably provides the energy to thread the rest of the protein through the translocation channel. The passenger may stay associated with the translocator and function, for example, as an adhesin, or it may be released into the milieu often via autocatalytic proteolysis. In the fifth T5SS, known as two-partner secretion system or T5bSS (Fig. 6.2), the translocator is not connected to the secreted protein but is a separate protein. The secreted proteins are very large (up to > 6000 residues) β-helical proteins. Many of them contain a small toxic domain at the C terminus that inhibits the growth of related bacteria competing for the same niche (Ruhe et al. 2013).

One-Step Mechanisms The T1SS consists of three proteins, a CM-based ATPase, an OM-based tunnel protein, and a membrane-fusion protein that connects the other two. Together, they form a channel that translocates substrates directly from the

cytoplasm into the external milieu. The substrates are very large proteins, belonging to the RTX (repeat-in-toxin) family, which refers to a glycine/aspartate-rich Ca^{2+}-binding nonapeptide repeat near the C terminus. Many substrates are toxins, but the family also includes adhesins, enzymes, and S-layer proteins.

The T3SS and T4SS translocate substrates directly from the cytoplasm into a eukaryotic target cell (Fig. 6.2), where they interfere with signal transduction and metabolism. The T4SS is very similar to the conjugation apparatus, which translocates DNA with associated proteins into other bacterial or eukaryotic cells. The T3SS contains a basal body resembling the structure that anchors the flagellum in the cell envelope and functions as the translocon for flagellin. In T3SS, the basal body is connected to an extracellular needle or pilus in animal or plant pathogens, respectively. These structures reach through any additional surface layers of the bacteria and the eukaryotic cell wall, if present. The T3SS first inserts a translocon into the eukaryotic membrane that serves to deliver effector proteins into these cells.

The T6SS delivers toxic proteins into competing bacteria or into eukaryotic cells. Several components of the T6SS resemble phage tail proteins, which serve to inject phage DNA into the bacterial cytoplasm. Thus, the T6SS appears to function as an inverted phage driving proteins out of the bacterial cell and delivering them straight into target cells.

6.5 Basic Structure of Cell Envelopes of Fungi and Oomycetes

The Fungal Cell Envelope The fungal cell envelope is the target of microbial control agents. Ergosterol is a main anti-fungal plasma-membrane target, while chitinases, glucanases and proteases attack the cell wall. These enzymes often work synergistically, thereby weakening or even killing the pathogen. The cell envelope has been best studied in *Saccharomyces cerevisiae.* This yeast functions as a model for the plasma membrane and cell walls of plant-pathogenic and plant-beneficial fungi such as *Fusarium* and *Trichoderma*, respectively.

The Fungal Plasma Membrane The plasma membrane of *S. cerevisiae* consists of the phospholipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine as well as inositol sphingolipid and the sterol ergosterol (van Meer et al. 2008). The molar ratio between ergosterol and phospholipids is 0.5. Mechanical stress resistance is acquired by the relative dense packing of sphingolipids and sterols. Plasma membrane proteins are encoded by only ∼4 % of the *S. cerevisiae* genome (i.e. ∼250 proteins). Szopinska et al. (2011) identified > 100 of them and 68 % were integral membrane proteins. About one third were classified as transporters including, for example, seven glucose transporters. The plasma membrane also contains signaling proteins, e.g. of the cell-wall integrity pathway, and proteins involved in cell-wall synthesis, including two chitin synthases, one 1,3-β-D-glucan synthase, and three glucan elongases.

The Fungal Cell Wall   The cell wall stabilizes internal osmotic conditions and turgor pressure and provides physical protection and shape to the cells. It represents a considerable metabolic investment; in S. *cerevisiae*, it accounts for ∼10–25 % of the total cell mass (Klis et al. 2006). Cell walls of fungi generally consist of one or more fibrillar components, one or more matrix components, and may have an outer protein layer. Often, the fibrillar components include both chitin (a 1,4-β-linked GlcNAc polymer) and β-glucans, but occasionally mainly chitin (e.g. *Encephalitozoon cuniculi*) or 1,3-β-glucan (e.g. *Schizosaccharomyces pombe*) is present (Xie and Lipke 2010). Mannoproteins often form the matrix of cell walls, e.g. in *S. cerevisiae*, but also galactomannoproteins and α-glucan are used, e.g. in *S. pombe*. The composition, molecular organization and thickness of the cell wall can vary depending on environmental conditions. This adaption may be functional in an environment where fungi are exposed to antibiotics, lytic enzymes secreted by other microorganisms or to immune systems.

The cell walls of filamentous ascomycetes and even basidiomycetes have been proposed to be very similar to the well-studied cell wall of *S. cerevisiae*, which consists of an inner and outer layer (De Groot et al. 2005). The inner layer consists of 1,3-β-glucan, 1,6-β-glucan and chitin (Fig. 6.3). The moderately branched 1,3-β-glucan is the main polysaccharide. Its side-chains allow only local mutual association by hydrogen bonding. Consequently, a three-dimensional network is formed that is highly elastic and extended under normal osmotic conditions (Klis et al. 2006). Cells of *S. cerevisiae* shrink when exposed to hypertonic conditions. The cell wall also reduces in size then resulting in a surface loss of up to 50 % and a cell-wall porosity of less than 1000 Da, while even medium-sized proteins can pass under normal osmotic conditions. Chitin and 1,6-β-glucan are linked to the inner and outer parts of the 1,3-β-glucan network, respectively. Growing buds have not yet formed chitin; hence this polymer is not essential for cell-wall assembly and function. The 'alkali-sensitive linkage' cell-wall proteins (ASL-CWPs) are linked to the 1,3-β-glucan in the inner layer of the cell wall. Among the ASL-CWPs are the protein with internal repeats (PIR)-CWPs, which interconnect two or even more 1,3-β-glucan chains, thereby strengthening the cell wall (De Groot et al. 2005). Increased presence of PIR-CWPs has been proposed to produce a less elastic cell wall as occurs during the G1 phase of the cell cycle and during cell-wall stress (Klis et al. 2006).

The outer cell-wall layer is formed by mannoproteins, which are heavily glycosylated; each glycochain may contain hundreds of mannose residues. At least 20 different proteins make up this layer. Their composition varies depending on the culture conditions (Klis et al. 2006). The glycosylphosphatidylinositol (GPI)-modified CWPs form the largest group of CWPs within this outer layer. They are covalently linked to 1,6-β-glucan through a truncated form of their original GPI-anchor (De Groot et al. 2005). The genome of *S. cerevisiae* contains 66 GPI-CWP genes (De Groot et al. 2003).

The cell wall of *S. cerevisiae* also contains proteins that are not covalently linked to β-glucans. Collectively, the covalently linked and the non-covalently linked CWPs have a wide range of functions. Both classes are involved in cell-wall synthesis and remodeling. The GPI-CWPs also have a structural role by making the cell wall
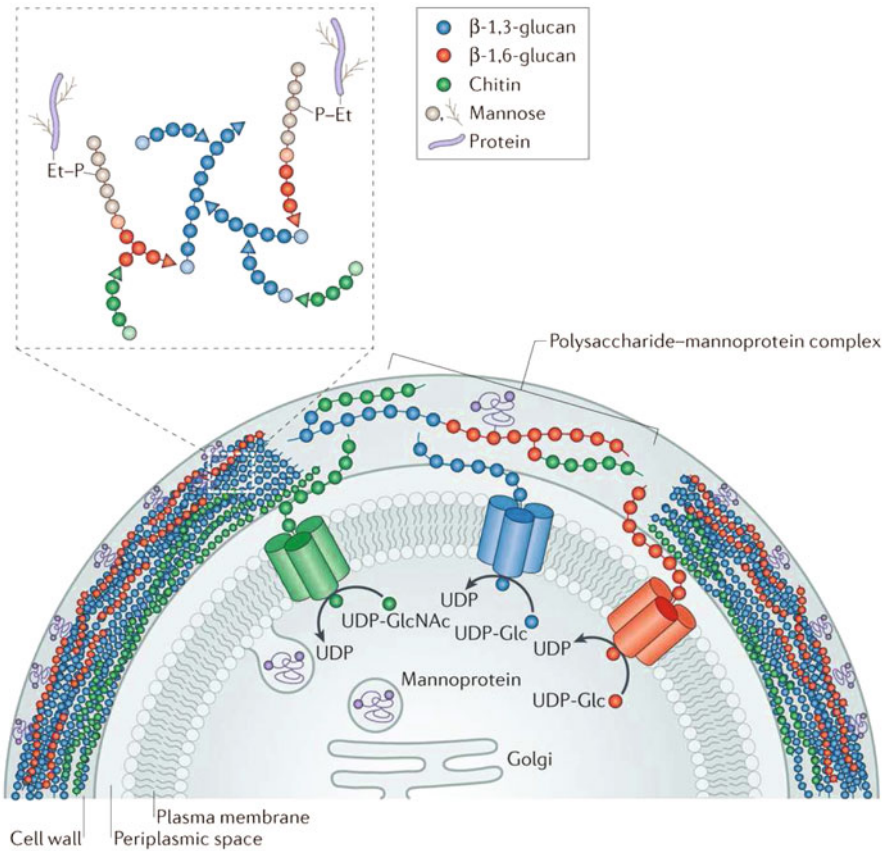
Fig. 6.3 Representation of the cell wall of *S. cerevisae*. Mannoproteins are delivered to the cell wall via secretory vesicles that fuse with the plasma membrane. The polysaccharides β-1,3-glucan and chitin are synthesized by synthases in the plasma membrane. The mechanism by which β-1,6-glucan is formed is not known. The cell wall polysaccharides and mannoproteins form a complex in the cell wall. The ASL-CWPs and proteins that are not covalently linked are not shown. *Et* ethanolamine, *Glc* Glucose, *P* phosphate. Reproduced from Cabib and Arroyo (2013) by permission of the publisher

less porous (Klis et al. 2006). Reduced porosity has been proposed to retain high-molecular-weight soluble proteins in the cell-wall matrix and may also protect the cell against lytic enzymes of competing microbes or plants or animals. In the latter case, it thus contributes to virulence. CWPs can also be involved in virulence by acquiring iron in the host or by inactivation of oxygen radicals released by the immune system (De Groot et al. 2005). Adhesins also contribute to the infection process. Adhesion of *S. cerevisiae* to foreign surfaces depends on the GPI-CWP Flo11 (Bojsen et al. 2012). This protein, which can also mediate mutual binding of yeast cells, is characterized by an A, B, and C domain. The A domain mediates cell-surface or cell-cell adherence, while the C domain contains the GPI anchor. Flo1, Flo5, Flo9 and Flo10 show high

mutual homology and similarity to Flo11. These proteins also have A, B, and C domains. The A domain is a β-barrel involved in mutual binding of yeast cells. Binding to a cell surface or to each other is often accompanied by the formation of an ECM, which creates a micro-environment that may prevent dehydration or access of antibiotics to the cells. In the case of *S. cerevisiae,* glucose and mannose polysaccharides and proteins constitute the ECM (Beauvais et al. 2009).

As in bacteria, some surface-exposed fungal proteins can form amyloid-like structures (Gebbink et al. 2005). Amyloids are filamentous protein structures of ∼10 nm wide and 0.1–10 μm long that share a structural motif, the cross-beta structure, and have been associated with neurodegenerative diseases. One of the best studied amyloid-forming microbial proteins is the hydrophobin SC3 of *Schizophyllum commune.* The water-soluble form of this protein affects the polysaccharide cell-wall composition. When confronted with the interface between the cell wall and the air or a hydrophobic surface, such as that of a plant, the structure of SC3 changes. It self-assembles into an amphipathic two-dimensional mosaic film of parallel amyloid fibrils. In this conformation, SC3 has different functions. It allows fungi to escape the aqueous substrate to grow into the air, it confers hydrophobicity to aerial hyphae and mediates attachment of the fungus to a hydrophobic support (Wösten 2001). Adhesins in the yeast cell wall (see above) have also been proposed to adopt the amyloid structure. This would explain the paradox that the adhesins often show weak binding to ligands, yet mediate remarkably strong adherence. Experimental evidence indicates that the strength of adhesion results partly from amyloid-like clustering of hundreds of adhesin molecules to form an array of ordered binding sites (Lipke et al. 2012).

The Cell Wall of Oomycetes Oomycetes are more related to brown algae and diatoms than to fungi and include some of the most devastating plant and animal pathogens. Their cell envelopes represent an excellent target to control disease but little is known about their composition. The cell wall is classically described to consist of 1,3-β- and 1,6-β-glucans and 4–20 % of cellulose (Aronson et al. 1967). A recent detailed cell wall analysis of 10 species from two oomycete orders revealed high heterogeneity (Mélida et al. 2013). Three different cell wall types were distinguished primarily based on GlcNAc content. Types I, II and III contain 0 %, up to 5 %, and > 5 % of this sugar, respectively. Each type is also characterized by additional compositional features. For example, the type I cell walls of *Phytophtora* spp. contain glucuronic acid and mannose and have a cellulose content of 32–35 %. *Saprolegnia* has a type II cell wall. Its GlcNAc residues are contained in chitin. A unique feature of this type is the 1,3,4-linked glucosyl residues, which are indicative of cross-links between cellulose and 1,3-β-glucans. *Aphanomyces euteiches* has a type III cell wall and contains nearly 10 % GlcNAc including 1,6-linked polymers.

6.6 Protein Export in Fungi

Most fungal proteins are transported to the cell wall or beyond via the ER (Conesa et al. 2001). *S. cerevisiae* translocates proteins over the ER membrane via SRP-dependent and -independent pathways, in which translocation occurs co-

or post-translationally, respectively. Proteins with a less hydrophobic signal peptide are targeted through the SRP-independent route, which involves the ER chaperone BiP, whereas proteins with a more hydrophobic signal can take both routes. Both pathways make use of the same translocon, the Sec61 complex. Also in other fungi, both pathways are probably operational. In the ER lumen, proteins are modified and folded and then transported to the Golgi, where they are further processed, e.g. by proteolytic modification and/or by modifications of their glycan-chains. Subsequently, the proteins are packed in vesicles which fuse with the plasma membrane to release the proteins into the cell wall. Vesicle fusion does not occur uniformly along the plasma membrane but mainly at the growing bud of the daughter cell and later re-localizes at the mother-bud neck just prior to cytokinesis. This implies that proteins are mainly incorporated in the cell wall or released into the medium when the cell is formed and not once cell division has occurred (Sietsma and Wessels 2006). In filamentous fungi, which form hyphae that extend at their tips, vesicles also fuse at the growth site. Once extruded at the hyphal tip, proteins migrate together with the newly synthesized cell-wall polymers to the outer part of the wall. This migration is driven by the turgor pressure in the hyphae and the apposition of newly synthesized polymers at the inner part of the wall. At the outside, the proteins diffuse into the medium (Sietsma and de Vries 2006). The newly synthesized cell-wall polymers are initially not cross-linked, resulting in a deformable cell wall at the tip. Once deposited, the polymers start to crosslink, and the cell wall becomes more and more rigid and less porous in subapical direction. This implies that proteins that are secreted more subapically will be captured in the cell wall. Yeast buds grow over their whole surface and do not have an "ever" extending tip that creates a continuous flow of proteins to the medium. Therefore, protein release in this case depends more on pores in the cell wall.

References

Aronson JM, Barbara A, Cooper BA et al (1967) Glucans of oomycete cell walls. Science 155:332–335

Bazaka K, Crawford RJ, Nazarenko EL et al (2011) Bacterial extracellular polysaccharides. Adv Exp Med Biol 715:213–226

Beauvais A, Loussert C, Prevost MC et al (2009) Characterization of a biofilm-like extracellular matrix in FLO1-expressing *Saccharomyces cerevisiae* cells. FEMS Yeast Res 9:411–419

Bleves S, Viarre V, Salacha R et al (2010) Protein secretion systems in *Pseudomonas aeruginosa*: A wealth of pathogenic weapons. Int J Med Microbiol 300:534–543

Bojsen RK, Andersen KS, Regenberg B (2012) *Saccharomyces cerevisiae*–a model to uncover molecular mechanisms for yeast biofilm biology. FEMS Immunol Med Microbiol 65:169–182

Cabib E, Arroyo J (2013) How carbohydrates sculpt cells: chemical control of morphogenesis in the yeast cell wall. Nat Rev Microbiol 11:648–655

Chang JH, Desveaux D, Creason AL (2014) The ABCs and 123s of bacterial secretion systems in plant pathogenesis. Annu Rev Phytopathol. doi:10.1146/annurev-phyto-011014-015624

Conesa A, Punt PJ, van Luijk N et al (2001) The secretion pathway in filamentous fungi: a biotechnological view. Fungal Genet Biol 33:155–171

De Groot PW, Hellingwerf KJ, Klis FM (2003) Genome-wide identification of fungal GPI proteins. Yeast 20:781–796

De Groot PWJ, Ram A, Klis F (2005) Features and functions of covalently linked proteins in fungal cell walls. Fungal Genet Biol 42:657–675

Fagan RP, Fairweather NF (2014) Biogenesis and functions of bacterial S-layers. Nat Rev Microbiol 12:211–222

Gebbink MF, Claessen D, Bouma B et al (2005) Amyloids–a functional coat for microorganisms. Nat Rev Microbiol 3:333–341

Klis FM, Boorsma A, De Groot PW (2006) Cell wall construction in *Saccharomyces cerevisiae*. Yeast 23:185–202

Kudva R, Denks K, Kuhn P et al (2013) Protein translocation across the inner membrane of Gram-negative bacteria: the Sec and Tat dependent protein transport pathways. Res Microbiol 164:505–534

Lipke PN, Garcia MC, Alsteens D et al (2012) Strengthening relationships: amyloids create adhesion nanodomains in yeasts. Trends Microbiol 20:59–65

Mélida H, Sandoval-Sierra JV, Diéguez-Uribeondo J et al (2013) Analyses of extracellular carbohydrates in oomycetes unveil the existence of three different cell wall types. Eukaryot Cell 12:194–203

Niederweis M, Danilchanka O, Huff J et al (2010) Mycobacterial outer membranes: in search of proteins. Trends Microbiol 18:109–116

Raetz CRH, Whitfield C (2002) Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635–700

Ruhe ZC, Low DA, Hayes CS (2013) Bacterial contact-dependent growth inhibition. Trends Microbiol 21:230–237

Sietsma JH, Wessels JGH (2006) Apical wall biogenesis. In: Kues U, Fisher R (eds) The mycota, part 1: growth, differentiation and sexuality, 2nd edn. Springer, Berlin, pp 53–73

Szopinska A, Degand H, Hochstenbach JF et al (2011) Rapid response of the yeast plasma membrane proteome to salt stress. Mol Cell Proteomics 10:M111.009589

Tommassen J (2010) Assembly of outer-membrane proteins in bacteria and mitochondria. Microbiology 156:2587–2596

Van Gerven N, Waksman G, Remaut H (2011) Pili and flagella: biology, structure, and biotechnological applications. Prog Mol Biol Transl Sci 103:21–72

van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9:112–124

Vollmer W, Seligman SJ (2010) Architecture of peptidoglycan: more data and more models. Trends Microbiol 18:59–66

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646

Xie X, Lipke PN (2010) On the evolution of fungal and yeast cell walls. Yeast 27:479–488