2 Yeast Cell Architecture and Functions

2.1 General Morphology

Cell structure and appearance. Yeast cells exhibit great diversity with respect to cell size, shape, and color. Even individual cells from a pure strain of a single species can display morphological heterogeneity. Additionally, profound alterations in individual **cell morphology** will be induced by changing the physical or chemical conditions at growth. Yeast cell size varies widely – some yeasts may be only 2–3 μm in length, while other species may reach lengths of 20–50 μm. Cell width is less variable at about 1–10 μm. Under a microscope, *Saccharomyces cerevisiae* cells appear as ovoid or ellipsoidal structures, surrounded by a rather thick cell wall (Figure 2.1). Mean values for the large diameter range between 5 and 10 μm, and for the small diameter between 1 and 7 μm. Cell size in brewing strains is usually bigger than that in laboratory strains. Mean cell size of *S. cerevisiae* also increases with age.

With regard to cell shape, many yeast species are ellipsoidal or ovoid. Some, like the *Schizosaccharomyces*, are cylindrical with hemispherical ends. *Candida albicans* and *Yarrowia lipolytica*, for example, are mostly filamentous (with pseudohyphae and septate hyphae). There are also spherical yeasts (like *Debaryomyces* species) or elongated forms (with many yeasts depending on growth conditions).

In principle, the status of *S. cerevisiae* as a eukaryotic cell is reflected by the fact that similar macromolecular constituents are assembled into the structural components of the cell (Table 2.1). There are, however, some compounds that do not occur in mammalian cells or in cells of other higher eukaryotes, such as those building the rigid cell wall or storage compounds in yeast.

For a better understanding of what I will discuss in the following sections, Figure 2.2 presents a micrograph of a dividing yeast cell, indicating some of its major components and organelles. We will deal with the yeast envelope, the cytoplasm, and the cell skeleton, and briefly touch upon the nucleus. The major genetic material distributed throughout the 16 chromosomes residing within the nucleus and other genetic elements, such as the nucleic acids, the retrotransposons, and some extrachromosomal elements, are considered later in Chapter 5. Section 2.5 presents an overview of other yeast cellular structures.

Preparations to view cells. Unstained yeast cells can only be visualized poorly by **light microscopy**. At 1000-fold magnification, it may be possible to see the yeast vacuole and cytosolic inclusion bodies. By using phase-contrast microscopy, together with appropriate staining techniques, several cellular structures become distinguishable. Fluorochromic dyes (*cf.* Table 2.2) can be used with fluorescence microscopy to highlight features within the cells as well as on the cell surface (Pringle *et al.*, 1991).

The range of cellular features visualized is greatly increased, when monospecific antibodies raised against structural proteins are coupled to fluorescent dyes, such as fluorescein isothiocyanate (FITC) or Rhodamine B.

Flow cytometry has several applications in yeast studies (Davey and Kell, 1996). For example, fluorescence-activated cell sorting (FACS) can monitor yeast cell cycle progression, when cell walls are labeled with concanavalin A conjugated to FITC and cell protein with tetramethylrhodamine isothiocyanate (TRITC). These tags enable us to collect quantitative information on the growth properties of individual yeast cells as they progress through their cell cycle.

A very convenient tool to localize and even to follow the movement of particular proteins within yeast cells is the use of the **Green Fluorescent Protein (GFP)** from the jellyfish (*Aequorea victoria*) as a reporter molecule (Prasher *et al.*, 1992), as well as several derivatives of GFP with fluorescence spectra shifted to other wavelengths (Heim *et al.*, 1994; Heim, Cubitt, and Tsien, 1995). Fusions of genes of interest with the fluorescent protein gene (N- or C-terminal) also allow us to follow the expression and destiny of the fusion proteins followed by fluorescence microscopy (Niedenthal *et al.*, 1996; Wach *et al.*, 1997; Hoepfner *et al.*, 2000; see also Chapter 4).

Organelle ultrastructure and macromolecular architecture can only be obtained with the aid of electron microscopy, which in scanning procedures is useful for studying cell topology, while ultrathin sections are essential in transmission electron microscopy to visualize intracellular fine structure (Streiblova, 1988). Atomic force microscopy can be applied to uncoated, unfixed cells for imaging the cell

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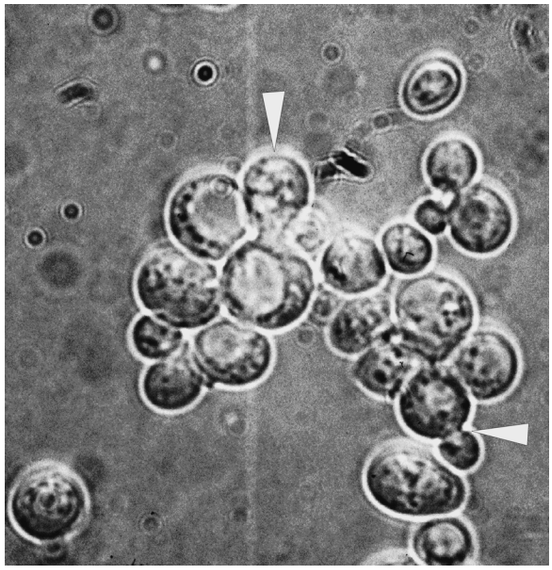

Fig. 2.1 Cells of *S. cerevisiae* under the microscope. The white arrows point to dividing cells.

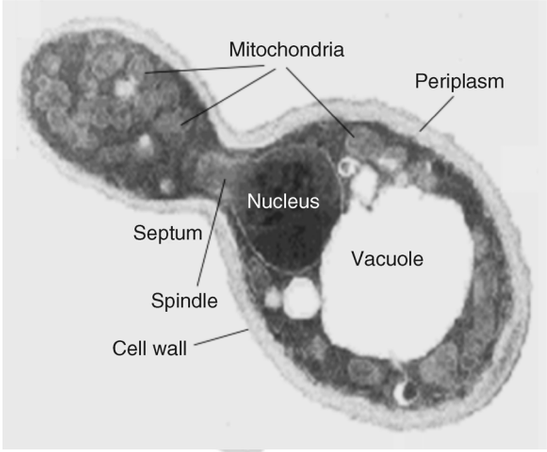

Fig. 2.2 Micrograph of a dividing yeast cell.

Table 2.1 Classes of macromolecules in *S. cerevisiae*.

Class	Category	Major compounds
Proteins	structural	actin, tubulin (cytoskeleton)
		histones (H2A, H2B, H3, H4, H1)
		ribosomal proteins
	hormones	pheromones α and **a**
	functional	enzymes and factors
		transporters
		signaling receptors
		motor proteins (myosins, kinesins, dynein)
Glycoproteins	cell wall components	mannoproteins
	enzymes	many functional enzymes (e.g., invertase)
Polysaccharides	cell wall components	glucan, mannan, chitin
	capsular components	glucan, mannan, chitin
	storage	glycogen, trehalose
Polyphosphates	storage	polyphosphate in vacuole
Lipids	structural	free sterols in membranes
	storage	lipid particles (sterol esters and triglycerides)
	functional	phosphoglyceride derivatives, free fatty acids
Nucleic acids	DNA	genomic DNA (80%), mitochondrial DNA (10–20%)
	RNA	rRNA (80%), mRNA (5% cytosolic, ER, mitochondria), tRNAs, snRNAs, snoRNAs

Table 2.2 Some structure-specific dyes for yeast cells.

Dye	Structures visualized	Comments
Methylene blue	whole cells	nonviable cells stain blue
Aminoacridine	cell walls	indicator of surface potential
F-C ConA	cell walls	binds specifically to mannan
Calcofluor white	bud scars	chitin in scar fluoresces
DAPI	nuclei	DNA fluoresces
DAPI	mitochondria	mitochondria fluoresce pink-white
Neutral red	vacuoles	vacuoles stain red-purple
Iodine	glycogen deposits	glycogen stained red-brown
Rhodamine	mitochondria	

DAPI, 4,6-diamidino-2-phenylindole.

surfaces of different yeast strains or of cells under different growth conditions (De Souza Pereira *et al.*, 1996).

A most convenient method to mark specific cellular structures or compartments is to check for particular marker enzymes that occur in those structures (Table 2.3).

2.2 Cell Envelope

In *S. cerevisiae*, the **cell envelope** occupies about 15% of the total cell volume and plays a major role in controlling the osmotic and permeability properties of the cell. Looking from the inside out, the yeast cytosol is surrounded by the plasma membrane, the periplasmic space, and the cell wall. Structural and functional aspects of the yeast cell envelope have attracted early interest (Phaff, 1963) because – like the cell envelope of fungi in general – it differs from bacterial envelopes and from those of mammalian cells. A peculiarity of yeast is that once the cell has been depleted of its cell wall,

Table 2.3 Marker enzymes for isolated yeast organelles.

Organelle	Compartments	Marker enzyme
Cell wall	periplasm	invertase
	secretory pathway	acid phosphatase
Plasma membrane		vanadate-sensitive ATPase
Cytosol		glucose-6-phosphate dehydrogenase
Nucleus	nucleoplasm	RNA polymerase
	nuclear envelope	transmission electron microscopy
ER	light microsomal fraction	NADPH: cytochrome *c* oxidoreductase
Vacuole	membrane	α-mannosidase
	sap	protease A and B
Golgi apparatus		β-glucan synthase, mannosyltransferase
Mitochondrion	matrix	aconitase, fumarase
	intermembrane space	cytochrome *c* peroxidase
	inner membrane	cytochrome *c* oxidase
	outer membrane	kynurenine hydroxylase
Peroxisome		catalase, isocitrate lyase, flavin oxidase

protoplasts are generated that are able to completely regenerate the wall (Necas, 1971).

2.2.1 Cell Wall

Yeast cell wall. The outer shell is a rigid structure about 100–200 nm thick and constituting about 25% of the total dry mass of the cell (Figure 2.3). The cell wall is composed of only four classes of macromolecules: highly glycosylated glycoproteins ("mannoproteins"), two types of β-glucans, and chitin. The composition of the cell wall is subject to considerable variation according to growth conditions, and the biosynthesis of the single compounds is highly controlled both in space and in time. The literature that has accumulated on these issues has grown so voluminous that reference is given here to only a few review articles (Klis, 1994; Lipke and Ovalle, 1998; Cabib *et al.*, 2001). Details of cell wall synthesis during yeast growth and budding, as well as septum formation (Cid *et al.*, 1995; Cabib *et al.*, 1997; Cabib *et al.*, 2001; Smits, van denEnde, and Klis, 2001), are considered below.

By treatment with lytic enzymes in the presence of osmotic stabilizers, the yeast cell wall can be removed without harming viability or other cellular functions. These "naked" cells are called **spheroplasts**. The cell wall will regenerate and this process has been used to study aspects of cell wall biosynthesis. Spheroplasts are amenable to intergeneric and intrageneric cell fusions; such hybrids are valuable instruments in genetic studies and possess a valuable biotechnological potential. A cell wall protein that contains a putative glycosylphosphatidylinositol (GPI)-attachment site, Pst1p, is secreted by regenerating protoplasts. It is upregulated by activation of the cell integrity pathway, as mediated by Rlm1p, as well as upregulated by cell wall damage via disruption of the *FKS1* gene, representing the catalytic subunit of glucan synthase (*cf.* Chapter 3).

Yeast cell aggregation. A phenomenon of particular importance in brewing is flocculation. It is based on asexual cellular aggregation when cells adhere, reversibly, to one another, which leads to the formation of macroscopic flocs sedimenting out of suspension. Traditionally, brewing yeast strains are distinguished as highly flocculent bottom yeasts (used for lager or Pilsner fermentations) or weakly flocculent top yeasts (used for ale fermentations or, in Germany, to prepare "top-fermented" beers). Although flocculation is far from being completely understood, it appears that the phenomenon is due to specific cell wall lectins in yeast (so-called flocculins) – surface glycoproteins capable of directly binding mannoproteins of adjacent cells. Yeast flocculation is genetically determined by the presence of different *FLO* genes. One such protein is Flo1p, a lectin-like cell-surface protein that aggregates cells into "flocs" by binding to mannose sugar chains on the surfaces of other cells. Both the

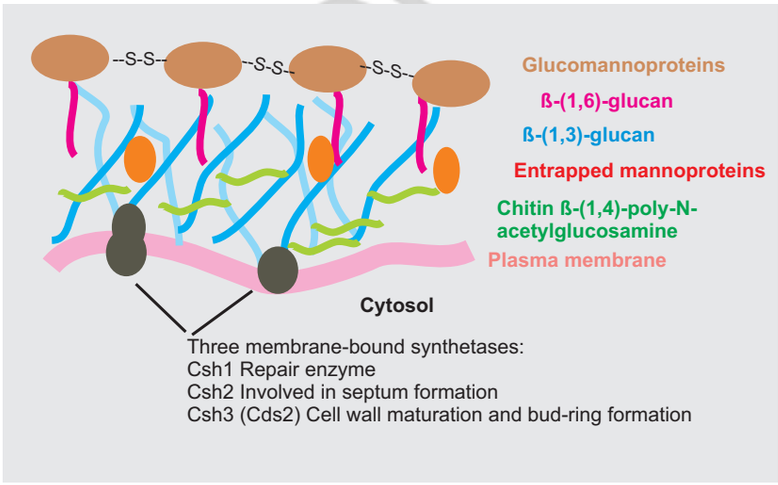

Fig. 2.3 Schematic representation of the yeast cell wall.

phenotypic characterization of *FLO5* strains and the sequence similarity between Flo1p and Flo5p suggest that Flo5p is also a mannose-binding lectin-like cell surface protein.

As the yeast cell wall is involved in sexual **agglutination**, some attention has been given to this particular aspect (Lipke and Kurjan, 1992). **a-** and α-cells can be distinguished by their agglutinin proteins. The anchorage subunit of **a**-agglutinin, Aga1p, is a highly *O*-glycosylated protein with an N-terminal secretion signal and a C-terminal signal for the addition of a GPI anchor (*cf.* Section 3.4.3.2). Linked to the anchoring subunit by two disulfide bonds is the adhesion subunit Aga2p. The α-agglutinin of α-cells is Sag1p. It binds to Aga1p during agglutination; its N-terminus is homologous to members of the immunoglobulin superfamily, containing binding sites for **a**-agglutinin, while the C-terminus is highly glycosylated and harbors GPI anchor sites.

The cell wall as a target for the defeat of mycoses. Similarly, several peculiarities of fungal cell wall synthesis such as the occurrence of ergosterol have led to the development of strategies for their inhibition as a means to defeat severe **mycoses** (Gozalbo *et al.*, 1993). A more recent brief account is given in an article by Levin (2005) describing cell wall integrity regulation in *S. cerevisiae*, which is considered a good model for the development of safe and effective antifungal agents. At present, effective antifungal therapy is very limited and dominated by the azole class of ergosterol biosynthesis inhibitors. Members of this class of antifungals are cytostatic rather than cytotoxic and therefore require long therapeutic regimens. The antifungal drugs can be applied to the major opportunistic human pathogens (*Candida* species, *Aspergillus fumigatus*, and *Cryptococcus neoformans*) causing systemic infections among immunocompromised patients. As this population has grown over the past three decades due to HIV infection, cancer chemotherapy, and organ transplants, and the number of life-threatening systemic fungal infections has increased accordingly, there is a need to develop safe, cytotoxic antifungal drugs (*cf.* Chapter 14).

2.2.2 Plasma Membrane

Like other biological membranes, the surface **plasma membrane** of yeast can be described as a lipid bilayer, which harbors proteins serving as cytoskeletal anchors, and enzymes for cell wall synthesis, signal transduction, and transport. The *S. cerevisiae* plasma membrane is about 7.5 nm thick, with occasional invaginations protruding into the cytoplasm. The lipid components comprise mainly phospholipids (phosphatidylcholine, phosphatidylethanolamine, and minor proportions of phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol) as well as sterols (principally ergosterol and zymosterol). Like the cell wall, the plasma membrane changes both structurally and functionally depending on the conditions of growth.

The primary functions of the yeast plasma membrane are:

i) Physical protection of the cell.
ii) Control of osmotic stability.
iii) Control of cell wall biosynthesis.
iv) Anchor for cytoskeletal compounds.
v) Selective permeability barrier controlling compounds that enter or that leave the cell. Of prime importance in active transport of solutes is the activity of the plasma membrane proton-pumping ATPase (see Section 5.6.1).
vi) Transport-related functions in endocytosis and exocytosis.
vii) Location of the components of signal transduction pathways.
viii) Sites of cell–cell recognition and cell–cell adhesion (Van der Rest *et al.*, 1995).

A comprehensive coverage of the lipids and the yeast plasma membrane, as well as on the biogenesis of the cell wall, can be found in a book by Dickinson and Schweitzer (2004).

The **periplasmic space** (Arnold, 1991) is a thin (35–45 Å), cell wall-associated region external to the plasma membrane. It comprises mainly secreted proteins that are unable to permeate the cell wall, such as invertase and phosphatase, which catabolize substrates that do not cross the plasma membrane. The unique properties of invertase have inspired its commercial preparation for the confectionary industry. The signal sequences of invertase (*SUC2*) and phosphatase (*PHO5*) have been used in recombinant DNA technology to generate heterologous proteins that can be secreted (Hadfield *et al.*, 1993). Most frequently used for secretion of heterologous proteins is the prepro-α-factor (*MFα1*) (Brake, 1989) (*cf.* Section 4.2.2.3).

2.3 Cytoplasm and Cytoskeleton

2.3.1 Yeast Cytoplasm

Like in all other cellular organisms, the **yeast cytoplasm** is the site for many cellular activities and the space for intracellular traffic. In yeast, it is an aqueous, slightly acidic (pH 5.2) colloidal fluid that contains low- and intermediate-molecular-weight weight compounds, such as proteins, glycogen, and other soluble macromolecules. Larger macromolecular entities like ribosomes, proteasomes, or lipid particles are suspended in the cytoplasm. The cytosolic (nonorganellar) enzymes include the glycolytic enzymes, the fatty acid synthase complex, and the components and enzymes for protein biosynthesis. Many functions essential for cellular integrity are localized to the cytoplasm (e.g., the components that form and control the cytoskeletal scaffold).

2.3.2 Yeast Cytoskeleton

The **cytoskeleton** of yeast cells, most intensely and successfully studied from early on by D. Botstein's and J. Pringle's groups, comprises **microtubules** and **microfilaments** (Botstein, 1986; Schatz *et al.*, 1986; Huffacker, Hoyt, and Botstein, 1987). These are dynamic structures that perform mechanical work in the cell through assembly and disassembly of individual protein subunits. Yeast microtubules and microfilaments are involved in several aspects of yeast physiology, including mitosis and meiosis, organelle motility, and septation. It is noteworthy that the skeleton in yeast cells exhibits a marked asymmetry, which becomes evident in the way it divides during vegetative growth (*cf.* Section 7.1.1).

2.3.2.1 Microtubules

Microtubules are conserved cytoskeletal elements. They are formed by polymerization of polymerization-competent α- and β-tubulin heterodimers (Figure 2.4). Yeast cells are unusual among other eukaryotes in that they possess very few cytoplasmic microtubules, thus explaining that most aspects of cell polarity largely reside in the actin skeleton (Pruyne and Bretscher, 2000; Schott, Huffaker, and Bretscher, 2002).

Yeast has two **α-tubulins**, Tub1p and Tub3p, and one **β-tubulin**, Tub2p. During biogenesis, the tubulins are protected by a specific chaperonin ring complex, CCT, which contains several subunits, Cct2p–Cct8p. (Note that the CCT complex is also needed in actin assembly.) Competence means that α-tubulin and β-tubulin need be properly folded, a reaction that requires specific **cofactors** for the folding of α- and β-tubulin (Alf1p/cofactor B for α-tubulin; Cin1p/cofactor D and Cin2p/cofactor C for β-tubulin). Homologs of these cofactors have been found in numerous organisms. An effector in this heterodimer formation is Pac2p (cofactor E) that binds to α-tubulin. One of the players in tubulin formation is Cin4p, a small GTPase in the ADP ribosylation factor (ARF) subfamily (*cf.* Section 6.1.2); it genetically interacts with several of the yeast tubulin cofactors, such as Pac2p, Cin1p, and Cin2p (the GTPase-activating protein (GAP) for Cin4p). As it appears (from analogy with the human homolog, Arl2), Cin4p is involved in regulating the yeast activity of the postchaperonin tubulin folding pathway, in part by decreasing the affinity of Cin1p/cofactor D for native tubulin. Yeast *CIN4* was isolated in a genetic screen for mutants displaying supersensitivity to benomyl, a microtubule-depolymerizing drug; it was independently isolated in a genetic screen for elevated chromosome loss. Δ*cin4* mutants are cold-sensitive, show synthetic phenotypes in combination with tubulin mutants, and have defects in nuclear migration and nuclear fusion. Rbl2p, the homolog of mammalian cofactor A, participates in the morphogenesis of tubulin in that it protects the cell from excess of free β-tubulin, which would be lethal as it leads to disassembly of tubulin.

Tub4p, the **γ-tubulin**, is a conserved component of microtubule organizing centers (MTOCs) and is essential for microtubule nucleation in the spindle pole bodies (SPBs). Tub4p localizes to both nuclear (inner plaque) and cytoplasmic (outer plaque) faces of the SPB, and is essential for nucleating microtubules from both faces (see Section 7.1.1).

2.3.2.2 Actin Structures

Actin-based transport. Unlike animal cells, which rely primarily on microtubule-based transport to establish and maintain cell polarity, yeast cells utilize actin-based transport along cables to direct polarized cell growth and to segregate organelles prior to cell division. In budding, **actin cable** assembly is initiated from the bud, leading to reorientation

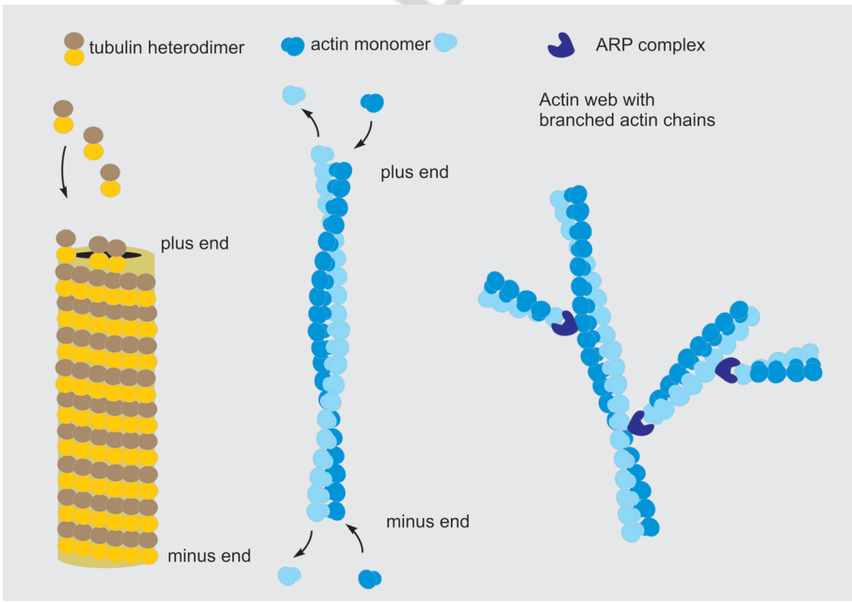

Fig. 2.4 Yeast microtubules and actin filaments (not to scale). Note that the actin monomers are differently colored only for better visualization.

of actin cables, and thus targeting of growth and secretion to the future bud tip (*cf*. Section 7.1). Polarized growth towards the bud tip (or cap) continues through a medium-budded stage, and depends on actin cables emanating from the bud tip and neck. These cables serve as polarized tracks for type V myosin-dependent delivery of cargos needed to build the daughter cell.

Types of actin filaments. **Actin** is an ATP-binding protein that exists both in monomeric (G-actin) and filamentous (F-actin) forms. Actin is encoded in yeast by the single gene *ACT1* (Ng and Abelson, 1980). Actin filaments are assembled by the reversible polymerization of monomers and have an intrinsic polarity; the fast-growing end is called the barbed end and the slow-growing end is called the pointed end (Figure 2.4). Yeast cells contain three types of filamentous actin structures: (i) **actin cables**, (ii) an actin-myosin **contractile ring** (Bi *et al*., 1998), and (iii) actin **cortical patches**, all of which are subjected to extensive reorganization throughout the cell cycle. Actin cables serve as tracks for polarized secretion, organelle and mRNA transport, and mitotic spindle alignment. The actin–myosin contractile ring forms transiently at the mother–daughter neck and is important for cytokinesis. Cortical patches are branched actin filaments involved in endocytosis and membrane growth and polarity. Genetic screens and biochemical purifications have been fruitful in identifying numerous factors that regulate actin cytoskeleton dynamics, organization, and function (review: Moseley and Goode, 2006).

Assembly of actin filaments. The *S. cerevisiae* genome encodes two genes, *BNI1* and *BNR1*, that are members of the formin family assembling linear **actin cables** in the bud and bud neck, respectively. **Formins** constitute a well-conserved family of proteins that promote the assembly of actin filaments, which are necessary in remodeling of the actin cytoskeleton during such processes as budding, mating, cytokinesis, or endocytosis (and in higher cells, cell adhesion and migration). The formin proteins are characterized by the presence of two highly conserved FH (formin homology) domains: the FH1 domain, containing polyproline motifs that mediate binding to **profilin** (actin- and phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$)-binding protein, Pfy1p), which in turn binds actin monomers, and the FH2 domain, which nucleates actin assembly. The FH2 domains of Bni1p and Bnr1p are distinct from those of the metazoan groups, containing a yeast-specific insert that is not found in other organisms. In addition to FH1 and FH2 domains, formins contain a regulatory Rho-binding domain (RBD) and a Dia-autoregulatory domain (DAD).

A model for **formin-mediated actin assembly** has suggested the following sequence of events. Activated Rho protein binds to the formin RBD domain and releases the formin from a conformation in which it is autoinhibited (due to an interaction between its N- and C-termini) to adopt a conformation that exposes the FH1 and FH2 domains. The FH1 domain then interacts with profilin-bound actin monomers, handing them over to the FH2 domain, a dimeric structure that may interact with two actin monomers to stabilize a dimeric actin form, prior to polymerization, whereby actin cables are formed. The FH2 domain remains associated with the growing end of the filament to protect it from interaction with capping proteins (a FH2 function termed "processive capping").

Consistent with this model, Bni1p has been identified as a downstream target of Rho1p, which regulates reorganization of the actin cytoskeleton, and hence the process of bud formation (*cf*. Section 7.1.1). Additionally, Bni1p activation is regulated by the small GTPases Rho3p and Rho4p, which affect the inhibitory interaction between the RBD and the DAD domains in the formin, while the Rho protein Cdc42p is needed for proper cable assembly during initiation of bud growth. Bni1p autoinhibition (as mentioned before) can also be aborted by phosphorylation of Bni1p affected by Prk1p kinase. Support for the model also comes from crystal structure studies of the Bni1p FH2 domain complexed with actin.

Actin filament assembly. Long actin filament **bundles** are formed by Crn1p (coronin) (Rybakin and Clemen, 2005), which binds actin filaments (F-actin) and cross-links them. Crn1p also regulates the actin filament nucleation and the formation of branched actin filaments as found in **cortical patches**. Crn1p is composed of five N-terminal WD repeats, forming a β-propeller structure, a microtubule binding domain, and a C-terminal α-helical coiled-coil structure, whereby the β-propeller and coiled-coil domains are required for recruitment of Crn1p to cortical patches.

The highly conserved actin **nucleation center** required for the motility and integrity of actin patches, involved in endocytosis and membrane growth, is the **Arp2/3 complex**. In yeast, the complex consists of seven proteins, two of which (Arp2p and Arp3p) are actin-related, while five components (Arc15p, Arc18p, Arc19p, Arc35p, and Arc40p) are non-actin-related proteins (Winter *et al*., 1997; Evangelista *et al*., 2002). The Arp2/3 complex nucleates the formation of branched actin filaments by binding to the side of an existing (mother) filament and nucleating the formation of a new (daughter) actin filament at a 70° angle (Figure 2.4). Arp2p and Arp3p serve as the first two subunits of the daughter filament, likely mimicking actin monomers due to their structural similarity to actin. However, the Arp2/3 complex does not play a role in the formation of actin cables (unbranched actin structures). To achieve optimal actin nucleation activity, the Arp2/3 complex is assisted by an assembly protein, such as Las17p (also Bee1p, of the SCAR/WASP family), myosin I, Abp1p (Olazabal and Machesky, 2001), or Pan1p.

Las17p/Bee1p as an activator of the Arp2/3 protein complex is the only *S. cerevisiae* homolog of the human Wiskott–Aldrich syndrome protein (WASP), which itself is a member of the larger WASP/SCAR/WAVE protein family. Las17p was identified biochemically as an essential nucleation factor in the reconstitution of cortical actin patches. Las17p localizes with the Arp2/3 complex to actin patches; disruption of *LAS17* leads to the loss of actin patches and a block in endocytosis. In the physical interaction between Las17p and the

Arp2/3 complex, the C-terminal WA (WH2 (WASP homology 2) and A (acidic)) domain of Las17p are required as are the two subunits of the Arp2/3 complex, Arc15p and Arc19p. The WA domain is sufficient for Arp2/3 complex binding and activation; it shares sequence similarity with an acidic domain in myosin type I (Myo3p and Myo5p in *S. cerevisiae*), which also interacts with the Arp2/3 complex. Genetic and biochemical studies have identified numerous proteins that physically interact with Las17p. The WH1 domain of Las17p binds strongly to verprolin (Vrp1p/End5p (Thanabalu and Munn, 2001)), the yeast homolog of human WIP (WASP-interacting protein), which is involved in Las17p localization. The proline-rich region of Las17p binds to SH3 domain-containing proteins, including Sla1p (an actin patch protein with a role in endocytosis) and many others that may regulate the activity of Las17p.

Two other proteins involved in formation and stabilization of actin bundles in cables and patches are Sac6p (fimbrin) and Scp1p (calponin/transgelin), which work together. The stabilization of actin filaments in patches also strictly depends on capping of the "barbed" ends by small capping proteins, Cap1p and Cap2p.

Actin filament disassembly. Debranching of the actin filaments in cortical patches by the Arp2/3 complex is induced by Gmf2p/Aim7p, which also inhibits further actin nucleation (Gandhi *et al.*, 2010). The protein has similarity to yeast Cof1p (cofilin) and to the human glia maturation factor (GMF). Cofilin, Cof1p, promotes actin filament depolarization in a pH-dependent manner. It binds both actin monomers and filaments; its main task is to sever filaments (Moon *et al.*, 1993; Theriot, 1997). Cofilin is regulated by phosphorylation at Ser4; homologs are ubiquitous and essential in eukaryotes. Aip1p promotes filament disassembly by enhancing cofilin severing and protecting severed filaments by capping.

Scd5p is an essential protein that colocalizes with **cortical actin** and as an adapter protein functionally links cortical actin organization with **endocytosis**. Scd5p and the clathrin heavy and light chains (Chc1p and Clc1p, respectively) physically associate with Sla2p (Wesp *et al.*, 1997), a transmembrane actin-binding protein involved in membrane cytoskeleton assembly and cell polarization, which is also a homolog of the mammalian huntingtin-interacting protein HIP1 and the related HIP1R. Both Scd5p and clathrin are required for Sla2p localization at the cell cortex. Scd5p activity appears to be regulated by phosphorylation/dephosphorylation. Phosphorylation of Scd5p by protein kinase Prk1p results in its negative regulation, whereas dephosphorylation by the Glc7p type 1 protein phosphatase relieves this inhibition. Mutations in *GLC7* that abolish Glc7p interactions with Scd5p result in defects in endocytosis and actin organization. Loss of function *scd5* mutants suffer from defects in receptor-mediated endocytosis and normal actin organization. They exhibit larger and depolarized cortical actin patches and a prevalence of G-actin bars.

2.3.2.3 Motor Proteins

Myosins, kinesins, and dynein are **three classes of motor proteins** that are highly conserved throughout evolution; several members of these proteins occur in yeast (Figure 2.5). Remarkably, myosins and kinesins are proteins that are able to bind to polarized cytoskeletal filaments and use the energy

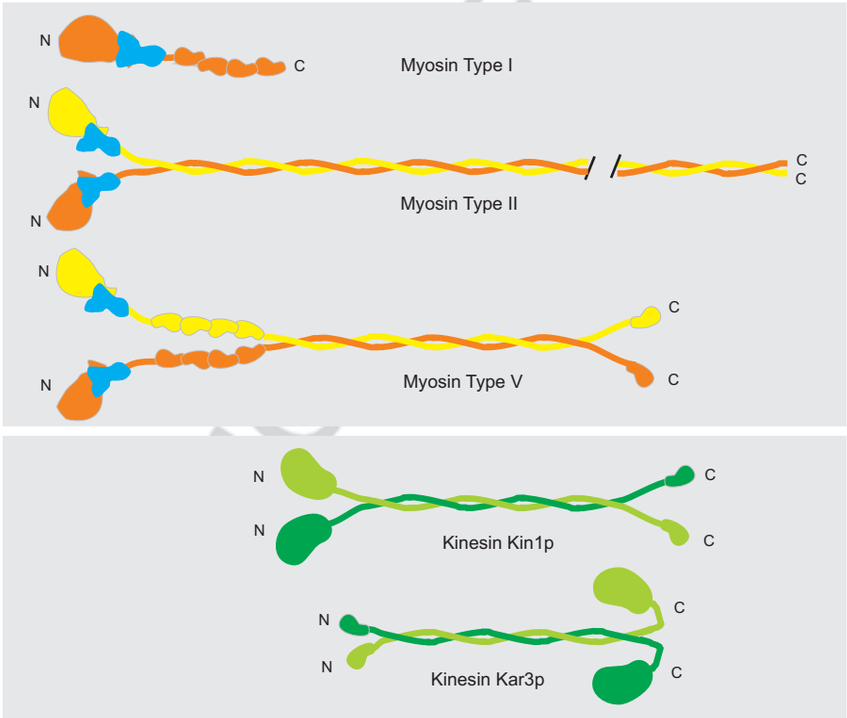

Fig. 2.5 Motor proteins in yeast. The chains in myosin and kinesin are identical; distinction by color is only for better perception. Kin1p is a "plus"-end motor; Kar3p is a "minus"-end motor.

derived from repeated cycles of ATP hydrolysis to move along them. By unidirectional movement, these molecules can carry cargo from one point to a distant location within the cell; other motor proteins may cause filaments to slide against each other, so that the generated force drives processes like nuclear migration and cell division (Hoyt, Hyman, and Bähler, 1997; Moore and Cooper, 2010).

2.3.2.3.1 **Myosins** Myosins are rod-like, extended structures (around 2 nm wide and greater than 150 nm long) normally consisting of two heavy and four light chains, whereby the heavy chains wrap around each other to form a coiled-coil of two α-helices (called the tail), while the light chains are part of motor domains at the N-terminus (called the head); between the head and tail are so-called IQ domains. Of the 14 different types of myosins in the myosin superfamily, *S. cere*visiae has members of type I, type II, and type V (Brown, 1997). Type I members are characterized by the occurrence of only one head per molecule, whereas type II members carry two heads, and type V members have two extended head regions.

Type II myosins. The only type II myosin in yeast is Myo1p; it fulfills a specialized function as part of the ring-shaped **actomyosin** complex that (early in the cell cycle) localizes to the presumptive bud site and remains at the mother–bud neck until cytokinesis is completed (VerPlank and Li, 2005). Formation, but not maintenance, of this contractile ring requires the intact septin collar at the bud neck (*cf.* Section 7.2). Late in anaphase, F-actin Act1p and the IQGAP-related protein, Iqg1p (Epp and Chant, 1997), also accumulate in the neck ring, whereby incorporation of F-actin depends on Myo1p, and Iqg1p determines the localization of axial markers Bud4p and Cdc12p. At the end of anaphase, the actinomyosin ring begins to contract. Myo1p is regulated by two light chains, an essential light chain (ELC), Mlc1p, and a regulatory light chain (RLC), Mlc2p, which displays significant sequence homology to calmodulin or myosin light chain related proteins. Like other light chains, Mlc2p contains an EF hand and a phosphorylatable serine residue, both close to the N-terminus. Mlc1p interacts with one of the two motifs (IQ1), which, however, does not play a major role in regulating Myo1p; instead, this interaction regulates actin ring formation and targeted secretion through further interactions with Myo1p, Iqg1p, and Myo2p. Mlc2p interacts with the IQ2 motif and most likely plays a role in the disassembly of the Myo1p ring. The human counterpart to Myo1p, MYH11, may give rise to leukemia or familial aortic aneurysm.

Type V myosin subfamily. Myo2p and **Myo4p** belong to the type V myosin subfamily. Myo2p promotes polarized growth by orienting the mitotic spindle and by taking over the vectorial transport of organelles along actin cables to sites such as the growing bud during vegetative growth, the bud neck during cytokinesis, and the shmoo tip during mating. Even organelles, including secretory vesicles, vacuoles, peroxisomes, and late Golgi elements, are transported into the growing bud (Johnston, Prendergast, and Singer, 1991). These tasks afford cargo-specific myosin receptors making contact between the cargo and the myosin tail. For example, there are specific receptors on vacuoles (Vac8p–Vac17p) or on peroxisomes (Inp2p). Sec4p, a vesicle-bound Rab protein, associates with Myo2p, and along with Sec2p and Smy1p, is critical for vesicle transport (Figure 2.6). Myo2p participates in spindle orientation by actively transporting Kar9p/Bim1p-decorated microtubule ends into the bud. Myo2p together with the Rab protein Ypt11p are required for distribution and retention of newly inherited mitochondria in the bud (Ito *et al.*, 2002). Myo4p has the main function of moving mRNAs within the cell (Haarer *et al.*, 1994).

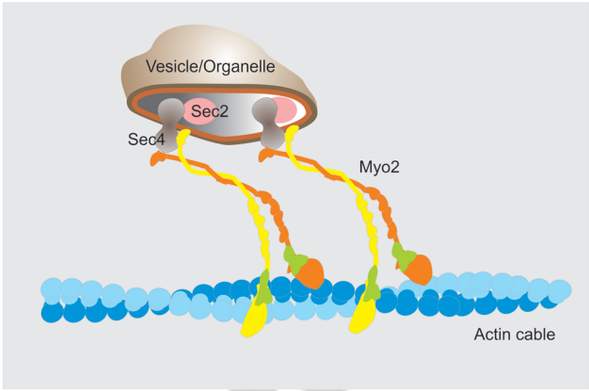

Fig. 2.6 Vesicle (and organelle) transport in yeast.

Type V myosins have a particular domain architecture and distinct modes of regulation. Myo2p and Myo4p, in addition to the N-terminal actin-binding motor domain, have a globular C-terminal domain at the tail of the coiled-coil dimerization domain. Adjacent to the motor domain, there is a neck region that contains six IQ motifs that can bind calmodulin (Cmd1p). Through this interaction, calmodulin participates in polarized growth of yeast cells and inheritance of the vacuole by daughter cells. Calmodulin may also interact with the heavy chain of Myo4p. Through interactions with both the unconventional type I myosin (Myo5p) and Arc35p, a component of the Arp2/3 complex, calmodulin is also involved in receptor-mediated endocytosis.

Type V myosins are typically regulated by interactions with light chains. Mlc1p physically interacts with and regulates Myo2p. The binding of the Myo2p tail by the kinesin-like protein Smy1p promotes the polarized localization of Myo2p. The light chain(s) that regulate Myo4p are yet to be defined, but a novel motor-binding protein, She4p, may modulate Myo4p activity. While Myo2p predominantly moves organellar compounds, Myo4p moves mRNAs and acts as part of the mRNA localization machinery (see below).

Type I myosins. There are two yeast type I myosins represented by **Myo3p** and **Myo5p** that localize to **actin cortical patches**. Physical interaction between Myo5p and calmodulin (Cmd1p) has been detected, and was found to be required

for endocytosis. Myo5p also interacts physically with verprolin (Vrp1p), a proline-rich protein. Deletion of the gene *VRP1* causes delocalization of Myo5p-containing patches.

Tropomyosin. In addition to the myosins, yeast harbors two isoforms of tropomyosin. Tmp1p is the major isoform that binds to and stabilizes actin cables and filaments, which direct polarized cell growth and the distribution of several organelles. The protein is acetylated by the NatB complex; the acetylated form will bind actin more efficiently. Tmp2p, the minor isoform, largely has functions overlapping with those of Tmp1p.

2.3.2.3.2 **Kinesins** Both **kinesins and kinesin-related proteins** are motor proteins remarkably similar to type V myosins. They generally function in **mitotic spindle** assembly and organization (see also Section 7.2.2.2), although each one takes over specialized functions. Cin8p, a kinesin motor protein, has an additional role in chromosome segregation. Functionally redundant with Cin8p is the kinesin-related motor protein Kip1(Cin9p), which, however, has an additional role in partitioning the 2 μm plasmid. The kinesin-related motor protein Kip2p stabilizes microtubules by targeting Bik1p, a microtubule-associated protein and component of the interface between microtubules and kinetochore (Berlin, Styles, and Fink, 1990; Moore and Cooper, 2010), to the plus end; Kip2p levels are controlled during the cell cycle. Kip3p is a further kinesin-related protein involved in spindle positioning. Cik1p is a kinesin-associated protein that stably and specifically targets the karyogamy protein Kar3p, a minus-end-directed microtubule motor that functions in mitosis and meiosis, localizes to the SPB, and is required for nuclear fusion during mating. Smy1p, a protein whose N-terminal domain is related to the motor domain of kinesins and that interacts with Myo2p, has already been mentioned; it may be required for exocytosis.

2.3.2.3.3 **Dynein** Cytoplasmic **dynein**, Dyn1p (Pac6p), is the largest motor protein in yeast and a "minus"-end motor of microtubules. Dyn1p is active in the movement of the mitotic spindle that must move into the narrow neck between the mother cell and the bud in order to segregate duplicated chromosomes accurately. The process begins with the **dynactin complex**, directing spindle orientation and nuclear migration. This complex is composed of the actin-related protein Arp1p, together with Jnm1p (Pac3p) and Nip100p (Pac13p).

The **movement of the spindle** occurs in two main steps as part of nuclear migration into the neck region. (i) The nucleus moves to a position adjacent to the neck, a process involving cytoplasmic microtubules, the motor protein Kip3p, and Kar9p, a karyogamy protein required for correct positioning of the mitotic spindle and for orienting cytoplasmic microtubules; Kar9p localizes to the shmoo tip in mating cells and to the tip of the growing bud. (ii) The mitotic spindle is moved into the neck, which requires cytoplasmic microtubules from the SPB sliding along the bud cortex, and pulling the nucleus and the elongating spindle. Sliding depends on the heavy chain of cytoplasmic dynein (Dyn1p), the dynactin complex, and the regulators Num1p (Pac12p) and Ndl1p. In the second step, Pac1p functions in aiding the recruitment of dynein to the "plus" ends of microtubules. In this function, Pac1p is regulated by Ndl1p, a homolog of nuclear distribution factor NudE that interacts with Pac1p (Li, Lee, and Cooper, 2005). Cortical Num1p brings together the dynein intermediate chain Pac11p and the cytoplasmic microtubules (Farkasovsky and Kuntzel, 2001). Finally, Bim1p, a microtubule-binding protein, also known as Yeb1p (EB1, microtubule plus-end binding) together with Kar9p serves as the cortical microtubule capture site. In case the spindle is oriented abnormally, Bim1p will delay the exit from mitosis (Schwartz, Richards, and Botstein, 1997; Miller, Cheng, and Rose, 2000; Moore, Stuchell-Brereton, and Cooper, 2009).

2.3.2.4 Other Cytoskeletal Factors

2.3.2.4.1 **Proteins Interacting with the Cytoskeleton** Other proteins that have been implicated in actin cytoskeleton reorganization and establishment of cell polarity are the proteins Boi1p and its functionally redundant homolog Boi2p. Both Boi1p and Boi2p contain SH3, pleckstrin homology (PH), and proline-rich domains. Several structure–function and genetic analysis experiments have tried to determine which domains are important for interactions with other proteins involved in the above processes. These studies showed that the Boi proteins interact physically and/or genetically with Bem1p, another SH3 domain protein, as well as three Rho-type GTPases – Cdc42p, Rho3p and the Rho3-related Rho4p (*cf.* Section 7.1.1).

Stt4p, the **phosphatidylinositol-4-kinase** involved in sphingolipid biosynthesis and in regulation of the intracellular transport of aminophospholipid phosphatidylserine from the endoplasmic reticulum (ER) to the Golgi, is required for actin cytoskeleton organization as well. Stt4p binds to the plasma membrane via the protein Sfk1p, thus promoting cell wall synthesis, actin cytoskeleton organization, and the Rho1/Pkc1-mediated mitogen-activated protein (MAP) kinase cascade (*cf.* Section 10.2). *STT4* is an essential gene in some backgrounds, but not in others. Δ*stt4* mutants lack most of the phosphatidylinositol-4-kinase activity that is detected in the wild-type and are arrested in the G_2/M phase of the cell cycle. Inactivation of Stt4p results in mislocalization of the Rho-GTPase guanine nucleotide exchange factor (GEF) Rom2p and also in the rapid attenuation of translation initiation.

2.3.2.4.2 **Transport of Organellar Components** Of importance for the proper transfer of organellar components to the bud or, on the contrary, to restrict certain compounds to be accumulated in the bud is a specific **mRNA localization machinery** that becomes active during budding. In particular, mating-type switching should occur only in mother cells,

meaning that HO transcription in daughter cells has to be prevented (*cf.* Chapter 7). This effect is brought about by Ash1p, a protein specifically localized to daughter nuclei late in the cell cycle, where it is poised to inhibit HO transcription in the following G_1 phase. This asymmetric localization is achieved by the delivery of *ASH1* mRNA to daughter cells by the products of the *SHE* genes. She2p and Loc1p bind to *ASH1* mRNA in mother and daughter nuclei, and mediate export to the cytoplasm. She3p then associates with the ribonucleoprotein particle (mRNP) and acts as an adapter for its association with myosin Myo4p (also called She1p). Myo4p transports the mRNP complex along actin cables to the bud tip. During telophase, *ASH1* mRNA becomes anchored to the bud tip by Bni1p and/or Hek2p and/or Bud6p. Translation of *ASH1* mRNA is delayed as long as the message is in transit.

2.4 Yeast Nucleus

2.4.1 Overview

The **nuclear structure** in yeasts is a nearly round organelle of about 1.5 μm diameter located in the center of the cell or slightly excentrically. The nucleoplasm is surrounded by a double membrane bilayer (inner and outer nuclear membrane), thus separating the nucleoplasm from the cytoplasm. **Nuclear pore complexes** (NPCs) of about 50–100 nm in diameter form the natural channels for exchange of components between the nucleus and cytosol, whereby export and import pathways can be distinguished (see Section 8.2). The outer nuclear membrane is largely contiguous with the membrane of the **ER**. Unlike most eukaryotic cells, the yeast nuclear membrane is not resolved during mitosis, while is breaks down in higher eukaryotic cells. This latter fact necessitates the resynthesis of the complete nuclear structure, including nuclear pores, for example, in animal cells. Nonetheless, biogenesis of nuclear pores has also been studied in yeast, as *de novo* synthesis has to occur also in this organism (D'Angelo and Hetzer, 2008).

On its outside, the nucleus carries a **SPB** that serves as an anchor for continuous and discontinuous microtubules across the nucleus as well as for cytosolic microtubules (Figure 2.7). During mitosis, the SPB will be duplicated and this apparatus effects the movement of the duplicated chromosomes into mother and daughter cell before cell separation. Details are presented in Section 7.1.

The **nucleolus** is a dense region within the nucleus that disappears during mitosis and reappears in interphase (Thiry and Lafontaine, 2005). The nucleolus locates the rRNA genes, and is the site for the synthesis and processing of rRNA. It is also involved in the assembly of the ribosomal subunits and in pre-mRNA processing (see Section 2.4.2.3).

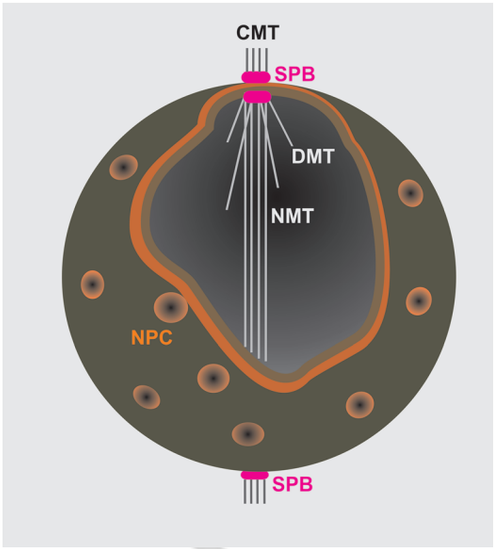

Fig. 2.7 Yeast nucleus. NPC, nuclear pore; SPB, spindle pole body; CMT, cytosolic microtubules; NMT, nuclear microtubules; DMT, discontinuous microtubules.

2.4.2 Nuclear Pore

2.4.2.1 Historical Developments

Nuclear export and import. Rather early, it became clear that not only cellular components synthesized in the nucleus have to be exported to the cytosol, but that also a vivid shuttling of various components has to occur. Nuclear pores – cellular superstructures 30 times the size of a ribosome – were defined as the gates for all traffic between the nucleus and the cytoplasm (reviews: Görlich and Mattaj, 1996; Görlich, 1997; Nigg, 1997; Englmeier, Olivo, and Mattaj, 1999; Hoelz and Blobel, 2004; Becskei and Mattaj, 2005). The NPC consists of two integral membrane proteins and a large set (greater than 30) of so-called nucleoporins recruited from the cytoplasm. Together these are assembled at points of fusion between the inner and outer nuclear membranes (Strambio-de-Castillia, Blobel, and Rout, 1999).

Actually, the detection of the nuclear envelope and nuclear pores dates back to the late 1950s (Watson, 1954). From an article by Aaronson and Blobel (Aaronson and Blobel, 1974, and the literature cited therein), one can infer that at that time the main technique for describing the nuclear envelope was electron microscopy, applied to isolates from a number of vertebrates. Aaronson and Blobel then set out to characterize the single components by biochemical methods, choosing rat liver nuclei for their first experiments. This means that yeast entered this field much later. In fact, biochemical characterization of components of the nuclear envelope in yeast started in the early 1990s (Wente, Rout, and Blobel, 1992; Rout and Blobel, 1993; Aitchison, Blobel, and Rout, 1995; Strambio-de-+Castillia, Blobel, and Rout, 1995). In these years, yeast

factors implicated in nuclear import and export were also characterized (Corbett *et al.*, 1995; Koepp and Silver, 1996). By the end of the 1990s, a rather comprehensive description of the components of the yeast nuclear pore (which is somewhat simpler than that of metazoans) was available (Rout *et al.*, 2000). Likewise, the majority of the yeast and vertebrate components, as well as interesting aspects of nuclear traffic, were described (Ryan and Wente, 2000; Vasu and Forbes, 2001).

2.4.2.2 Current View of the Nuclear Pore

The nuclear pore as a gated channel. Meanwhile, more detailed facets of both NPC structure and assembly have been obtained. Figure 2.8 presents a recent schematic model of the NPC; both yeast and vertebrate pores have a central core, the major scaffold of the pore. This scaffold is 960 Å in diameter by 350–380 Å in height in yeast. The central transporter in yeast is 350–360 Å in diameter by 300 Å in height. Eight filaments of around 500 Å extend into the cytoplasm. On the nuclear side of the pore, eight long filaments (950 Å in yeast) connect at their distal end to a small ring. This structure is termed the nuclear pore basket. Pore-associated filaments extend from the basket of the pore into the nucleus, and contain the proteins Mlp1/2p in yeast and Tpr in vertebrates.

Previous sequence analysis by many groups had revealed that one-third of the yeast nucleoporins contain phenylalanine–glycine (FG) repeats, in some cases FXFG or GLFG repeats (but collectively referred to as FG repeats). Different FG nucleoporins are major sites of interaction for specific transport factors (extensively reviewed in Ryan and Wente, 2000).

In recent years, more details on the structural and functional aspects of the yeast NPC have become apparent (Lim and Fahrenkrog, 2006; Peters, 2006; Alber *et al.*, 2007; Cook *et al.*, 2007). In particular, the yeast FG proteins have been intensively studied not only by conventional methods, such as electron microscopy and biochemical strategies, but also by more advanced applications, such as X-ray crystallography and atomic force microscopy (Frey, Richter, and Görlich, 2006; Hsia *et al.*, 2007; Lim *et al.*, 2006; Lim *et al.*, 2007a; Lim *et al.*, 2007b; Patel *et al.*, 2007).

As schematized in Figure 2.9, the symmetric core of the NPC appears to adopt the shape of a set of concentric cylinders. A peripheral cylinder coating the pore membrane contains subcomplexes, the structures of which have been solved experimentally (Hsia *et al.*, 2007). The core contains an elongated heptamer (the Nup84 complex) that harbors the Sec13–Nup145C complex in its middle section as well as the complexes Seh1–Nup85 and Nup133–Nup84, plus Nup120. A hetero-octamer of Sec13–Nup145C forms a slightly curved but rigid rod, whose dimensions are compatible with the suggestion that it extends over the full height of the proposed membrane-adjacent cylinder. Nup145Cp is mainly structured from α-helices, while Sec13p consists of six blades of a β-propeller domain, which interacts with Nup145Cp that contributes the seventh blade to the β-propeller domain. At a first sight, the occurrence of Sec13p in a NPC came as a surprise, since Sec13p was known to occur as a membrane-bending activity in

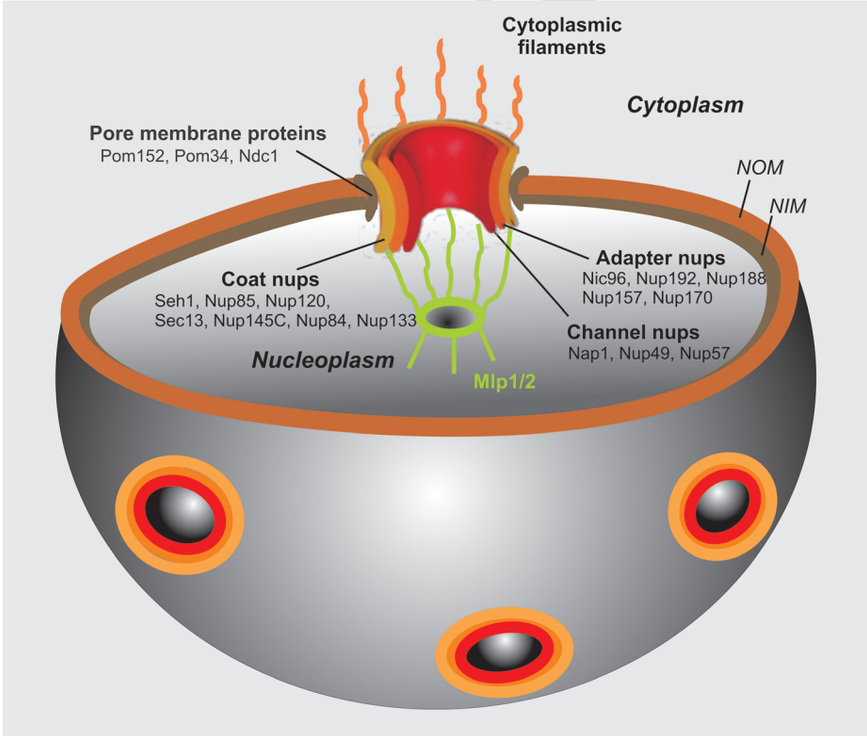

Fig. 2.8 Schematic view of the NPC. NOM, nuclear outer membrane; NIM, nuclear inner membrane.

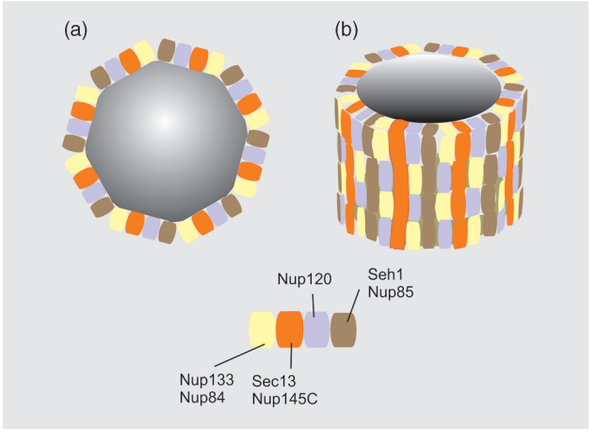

Fig. 2.9 Model of the outer core complex of the nuclear pore.

COPII-coated vesicles. Presently, a debate has arisen about whether Sec13p might fulfill a similar function during pore biogenesis (see below). In accord with the dimensions and symmetry of the NPC core, Hsia *et al.* (2007) proposed a model (Figure 2.9) in which the entire cylinder is composed of four antiparallel rings, each ring consisting of eight heptamers horizontally arranged in a head-to-tail fashion. This model also suggested that the hetero-octamer would vertically traverse and connect the four stacked cylinders.

Components that contribute to the architecture of the nuclear pore are listed in Table 2.4, comparing them to the constituents found in vertebrate NPCs (Fernandez-Martinez and Rout, 2009). This listing slightly differs from the picture presented in Figure 2.8; it clearly indicates that most of the FG proteins are located to the "channel" of the NPC and only a few are found at the inner ring.

FG molecules are unfolded and highly flexible, and they can form opposing "**sliding helices**." Patel *et al.* (2007) found that phenylalanine-mediated inter-repeat interactions indeed cross-link G-repeat domains into elastic and reversible hydrogels and that such hydrogel formation is required for viability in yeast. The laboratory of U. Aebi (Lim *et al.*, 2007a; Lim *et al.*, 2007b) constructed an experimental device to study the collective behavior of surface-tethered FG proteins at the nanoscale. These measurements indicated that such FG molecules induce an exponentially decaying long-range steric repulsive force. This observation suggests that the molecules are thermally mobile in an extended polymer brush-like conformation. Therefore, FG-repeat domains may simultaneously function as an entropic barrier and a selective trap of NPCs, explaining why nucleocytoplasmic transport is specific not only in terms of cargo recognition, but also in terms of directionality (e.g., with nuclear proteins imported into the nucleus and RNAs exported out of it). The data support a **two-gate model** of nuclear pore architecture, with the central diffusion gate formed by a meshwork of cohesive FG nucleoporin filaments and a peripheral gate formed by repulsive FG nucleoporin filaments.

Table 2.4 Components (nucleoporins) and subcomplexes of the nuclear pore.

Subcomplexes	Yeast	FG repeats	Vertebrates
Transmembrane	Pom152		Gp210
	Pom34		
	Ndc1		Ndc1
			Pom121
Outer ring	Nup133		Nup133
	Nup120		Nup160
	Nup145C		Nup96
	Nup85		Nup75
	Nup84		Nup107
	Seh1		Seh1
	Sec13		Sec13
			Nup43
			Nup37
			Aladin
Inner ring	Nup192		Nup205
	Nup188		Nup188
	Nup170		Nup155
	Nup157	GLFG	
Linker nucleoporins	Nup59	FG-6	Nup35
	Nup53	FG-4	
	Nic96		Nup93
	Nup82		Nup88
"Channel" FG proteins	Nup159	FG	Nup214
	Nup145N	GLFG	Nup98
	Nup116	GLFG	
	Nup100	GLFG	
	Nsp1	FG	Nup62
	Nup57	FG	Nup54
	Nup49		Nup58/45
	Nup42	FG	NLP1
	Nup1	FG	
	Nup2	FG	
	Nup60		
	Cdc31		
	Gle1		
	Gle2		

Nup, nucleoporin; POM, integral membrane protein; FG, phenylalanine–glycine repeat, GLFG, glycine–leucine–phenylalanine–glycine repeat.

Biogenesis of the nuclear pore. Meanwhile, biogenesis of novel NPCs is quite well understood (Fernandez-Martinez and Rout, 2009): NPCs have their own lives – they are generated, exist for a while, age, are dissolved into subcomplexes, and can eventually be reassembled. Genetic dissection of NPC biogenesis in yeast has contributed many clues towards the mechanism of NPC assembly. In screens for mutants defective in NPC formation, mutants corresponding to Ran, Ran-GEF, Ran-GAP, Ran transport cofactor Ntf2p, and importin Kap95 were identified (Ryan, Zhou, and Wente, 2007). Further, elegant *in vivo* approaches by tagging nucleoporins with Dendra (Makio *et al.*, 2009; Onischenko *et al.*, 2009) provided evidence that at least two pools of nucleoporins contribute to forming functional NPC intermediates, and can easily be included into the following scheme. First, transmembrane nucleoporins and components that form the inner ring (Nup170/Nup157 complex) in mature NPCs

congregate on both sides of the nuclear envelope (D'Angelo *et al.*, 2006), probably starting the process of bending the outer and inner membranes. Perhaps to accomplish this, Nup170p homologs make use of a membrane-binding amphipathic α-helix; this complex fuses to form a prepore. In a second step, the outer ring Nup84 complex builds up a scaffold to coat the whole pore membrane; nucleoporins Nup53p and Nup59p might directly interact with the Nup170p complex to stabilize this prepore structure. The scaffold finally recruits the residual linker nucleoporins and the FG nucleoporins to complete a mature NPC.

It appears that other candidates for assembly factors of novel NPCs include the ER protein Apq12p (Scarcelli, Hodge, and Cole, 2007), and members of the reticulons (RTNs) and Yop1p protein families (Dawson *et al.*, 2009). RTNs and Yop1p (DP1 in vertebrates) proteins are of particular interest, as they can bend membranes and also have functions in tubular ER maintenance.

2.4.2.3 Yeast Nucleolus

As in all other eukaryotes, the nucleolus in yeast is a separate compartment within the nucleus, forming a crescent-shaped region abutting the nuclear envelope (Shaw and Doonan, 2005). This differs from nucleoli in higher organisms, where they appear as more or less spherical bodies. In all cases, the nucleolus is the specialized subnuclear compartment for ribosome synthesis, centered around the nucleolar organizing regions (NORs) – landmarks within the genome that encode the repeated rRNA genes (Boisvert *et al.*, 2007).

The genes for the rRNAs attached in tandem copies, are transcribed by RNA polymerase I (*cf.* Chapter 9) with the exception of the 5S RNA gene. The rRNA precursor molecules are processed in the nucleolus by specific trimming enzymes and modified at roughly greater than 200 nucleotide positions – either by the action of specific methylases or pseudouridine synthases. Likewise, a large number of assembly steps of the rRNAs with ribosomal proteins occur in this compartment (*cf.* Chapter 5). Accordingly, a plethora of proteins must be involved in these procedures. Proteome analyses in human nucleoli have identified more than 700 proteins acting in this compartment. However, some of these components (such as the small nucleolar RNAs (snoRNAs)) seem to be involved in processes other than ribosome biogenesis (e.g., in mRNA splicing).

During mitosis the nuclear envelope, NPCs, and nucleolus must also be segregated. Yeast cells achieve this in a "closed" form of mitosis (i.e., in yeast these nuclear structures remain intact), while in higher organisms mitosis occurs in more or less "open" forms in which these nuclear structures are disassembled (DeSouza and Osmani, 2009). Although not all problems have been solved about how chromosome segregation is achieved (*cf.* Chapter 7), it has been established that breakdown and separation of the nucleolus in yeast occurs late in mitosis; it persists as an intact region until anaphase. A peculiarity of rDNA-containing chromosomes is their direct association with condensin and thus high compaction of rDNA chromatin in the nucleolus. This condensation is promoted by Cdc14p in the FEAR pathway of mitotic exit, but independent from the MEN pathway (Freeman, Aragon-Alcaide, and Strunnikov, 2000) (*cf.* Chapter 7).

Table 2.5 Genome sizes of some yeasts.

Species	Ploidy	Chromosome number	Genome size (Mb)
Saccharomyces cerevisiae	*n*	16	12.1
Saccharomyces paradoxus	2*n*	16	12.2
Saccharomyces bayanus	2*n*	16	10.2
Saccharomyces exiguus	2*n*	14–16	
Saccharomyces servazii	2*n*	9–13	
Candida glabrata	*n*	13	14.7
Saccharomyces castellii		9	11.4
Kluyveromyces waltii		8	10.7
Kluyveromyces marxianus		10	
Saccharomyces kluyveri	2*n*	8	11.3
Eremothecium gossypii	*n*	7	8.7
Kluyveromyces lactis	*n*	6	10.7
Debaryomyces hansenii	*n*	7	12.2
Yarrowia lipolytica	*n*	6	20.5

See also Chapters NaN, 16, and Appendix B.

2.4.3 Yeast Chromosomes

The nucleoplasm harbors the nuclear **chromosomes** packed into chromatin structure. In contrast to higher eukaryotic cells, yeast nucleosomes occupy a length of around 145 bp of DNA. While the genome sizes of (Hemiascomycetous) yeasts are relatively constant and generally range from 10 to 15 Mb, the number and sizes of the single chromosomes vary between species (Table 2.5).

Yeast genomes have been analyzed by karyotyping – the separation and size determination of the single chromosomes by pulsed-field gel electrophoresis (PFGE; Figure 2.10) (Carle and Olson, 1985).

Genetic elements of the nuclear chromosomes and the extrachromosomal genetic elements are considered in detail in Chapter 5.

2.5 Organellar Compartments

Various compartments surrounded by individual membranes are located within the yeast cytoplasm, which play key roles in the manufacturing and trafficking of proteins (Figure 2.11). Transport of proteins between cellular compartments is bound to different forms of transport vesicles and is found in all eukaryotic cells, but yeast has

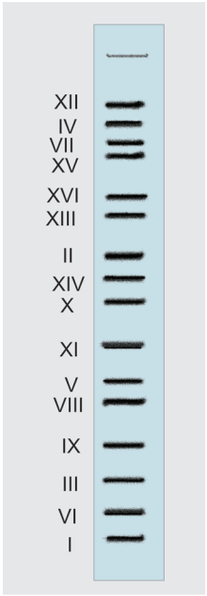

Fig. 2.10 PFGE of *S. cerevisiae* chromosomes.

served as a fundamental model of understanding such processes.

2.5.1 ER and the Golgi Apparatus

The **ER** is a key organelle for all processes controlling the stability, modification, and transport of proteins; it is organized into an extended system of branching tubules surrounded by a lipid double-layer membrane, which is intimately connected to the nuclear membrane. The ER cisternal space may make up to 10% of the cell's volume. The ER is the cellular site for the production of all transmembrane proteins and lipids of most of the cell's organelles: the ER itself, the Golgi apparatus, lysosomes (vacuoles in yeast), endosomes, secretory vesicles, and the plasma membrane. Likewise, proteins designed for secretion are manufactured in this compartment. Proteins synthesized on (poly)ribosomes are translocated through the ER membrane from the cytosol into the lumen of the ER. In the ER, chaperone-assisted protein folding takes place along with part of protein glycosylation. Correct folding is a prerequisite for successful "export" of proteins from the ER. In the ER, proteins are packed into vesicles that bud from the ER membrane and are transferred to the Golgi apparatus, where they fuse to the Golgi membrane.

The **Golgi apparatus** (or Golgi complex) consists of a series of parallel stacks of membranous compartments. The ER-proximal part of the Golgi is called the "early" or *cis*-Golgi network (CGN), followed by the internal cisternae, while the ER-distal part is called the "late" or *trans*-Golgi network (TGN). This nomenclature refers to the fact that the Golgi establishes an ordered sequence of processing of proteins that enter the network on its *cis* face and leave on its *trans* face; processing and sorting events include synthesis and processing of complex oligosaccharide chains of *N*-glycosylated proteins, phosphorylation of oligosaccharides destined for the vacuole, proteoglycan synthesis (i.e., *O*-linked glycosylation of proteins), modification of lipids, sulfation of tyrosine, and so on. The transport of cargo between the different Golgi compartments is accomplished by Golgi vesicles.

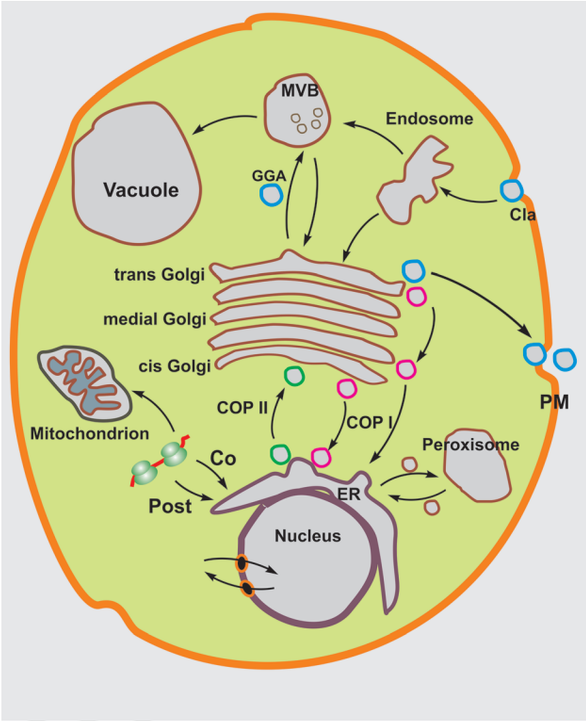

Fig. 2.11 Pathways and vesicle types in intracellular traffic. Colored vesicles: green, COPII; red, COPI; blue, clathrin. MVB, multi-vesicular body; Cla, Clathrin; PM, plasma membrane. Other abbreviations are explained in the text.

Retrograde transport of proteins (retrieval from the Golgi back to the ER) also takes place and is of high importance to sort out misfolded or wrongly modified proteins; retrograde transport can occur from all subcompartments of the Golgi. A special "quality control" system of the ER (ER-associated degradation (ERAD)) prevents misfolded or improperly assembled proteins to be secreted from the cell.

2.5.2 Transport Vesicles

Depending on the final target site of the cargo components, distinct vesicles and pathways are involved in intracellular transport. For example, different vesicles mediate the transport of components designated for the plasma membrane (as well as other membranes of the cell) and those for secreted proteins (see Section 8.1).

Transport vesicles are generally generated from different organellar membranes by **budding**. Depending on the cargo

Table 2.6 Components of coated vesicles in yeast.

Vesicle	Subcomponent	Gene product	Description
COPII		Sec12	GEF; ER membrane protein; Sar1p activator
		Sar1	small GTP-binding protein; Sar1p-GTP recruits Sec23–Sec24 complex
		Sec23	GAP for Sar1p
		Sec24	involved in cargo selection and formation of prebudding complex
		Sfb2	
		Sfb3	
		Sec13	forming outer layer (scaffold) of COPII coat; Sec13p has membrane bending activity
		Sec31	
		Sec16	stabilizes prebudding vesicles
COPI	coatomer α	Cop1	COPI vesicle coatomer complex
	coatomer β	Sec26	involved in ER–Golgi protein trafficking and maintenance of normal ER morphology
	coatomer β′	Sec27	involved in ER–Golgi and Golgi–ER transport; contains WD40 domains that mediate cargo selective interactions
	coatomer γ	Sec21	involved in ER–Golgi transport of selective cargo
	coatomer δ	Ret2	involved in retrograde transport between Golgi and ER
	coatomer ε	Sec28	regulates retrograde Golgi–ER protein traffic; stabilizes Cop1p and the coatomer complex
	coatomer ζ	Ret3	involved in retrograde transport between Golgi and ER
		Arf1	small GTP-binding protein
		Dsl1	peripheral membrane protein needed for Golgi–ER retrograde traffic; forms a complex with Sec39p and Tip20p that interacts with ER SNAREs Sec20p and Use1p; component of the ER target site that interacts with coatomer
Clathrin	triskelion	Chc1	clathrin heavy chain, triskelion structural component
		Clc1	clathrin light chain, triskelion structural component; regulates clathrin function
	AP-1	Apl2	β-adaptin, large subunit of the clathrin-associated protein (AP-1) complex; binds clathrin
		Apl4	γ-adaptin, large subunit of the clathrin-associated protein (AP-1) complex; binds clathrin
		Aps1	small subunit of the clathrin-associated adapter complex AP-1
		Laa1	AP-1 accessory protein; colocalizes with clathrin to the late Golgi apparatus; involved in TGN–endosome transport; physically interacts with AP-1
		Apm1	Mu1-like medium subunit of the clathrin-associated protein complex (AP-1); binds clathrin
	AP-2	Apl1	β-adaptin, large subunit of the clathrin-associated protein (AP-2) complex; binds clathrin
		Apl3	α-adaptin, large subunit of the clathrin associated protein complex (AP-2)
		Aps2	small subunit of the clathrin-associated adapter complex AP-2; involved in protein sorting at the plasma membrane
		Apm4	Mu2-like subunit of the clathrin associated protein complex (AP-2)
	AP-3	Apl5	δ-adaptin-like subunit of the clathrin associated protein complex (AP-3); functions in transport of alkaline phosphatase to the vacuole
		Aps3	small subunit of the clathrin-associated adapter complex AP-3, involved in vacuolar protein sorting
		Apm3p	Mu3-like subunit of the clathrin-associated protein complex (AP-3); functions in transport of alkaline phosphatase to the vacuole
		Apm2	protein of unknown function, homologous to the medium chain of mammalian clathrin-associated protein complex
		Gga1	Golgi-localized protein with homology to γ-adaptin, regulates Arf1p and Arf2p in a GTP-dependent manner to facilitate traffic through the late Golgi
		Gga2	protein that regulates Arf1p and Arf2p in a GTP-dependent manner to facilitate traffic through the late Golgi; binds InsP(4), which plays a role in TGN localization
		Swa2	clathrin-binding protein required for uncoating of clathrin-coated vesicles

and its destination, the vesicles – in addition to their lipid bilayer envelope, are endowed with a characteristic coat. In yeast, three types of coated vesicles can be distinguished (Table 2.6).

(i) **COPII-coated vesicles** are employed for the anterograde (forward) transport of cargo molecules from the ER to the Golgi, a function that is meanwhile well understood also in a mechanistic sense; the yeast system has contributed integral information (Hughes and Stephens, 2008). COPII-coated vesicles are assembled at the ER membrane from three components: the small GTP-binding protein (Sar1p), the Sec23/24p complex, and the Sec13/31p complex; these are sufficient to build a vesicle *in vitro*. Packaging of the types of transported molecules is not random, but a selective process (Bickford, Mossessova, and Goldberg, 2004; Lee and Miller, 2007; Sato and Nakano, 2007; Fromme, Orci, and Schekman, 2008). Each outward movement has to be counterbalanced by a retrieval step whereby membrane and selected proteins are returned to their original compartment of origin; components of the complex may undergo several rounds of export

from the ER. COPII recruitment is initiated by the activation of the small GTPase Sar1p (Nakano *et al*. 1989) and by its ER-localized GEF Sec12p. Upon exchange of GDP for GTP, Sar1p exposes an N-terminal amphipathic tail, which is tightly inserted into the lipid bilayer. This recruits a heterodimeric complex Sec23/24p required for cargo binding, and together with cargo and Sar1p the prebudding complex is formed. Addition of Sec13/31p, consisting of two Sec13p and two Sec31p subunits, acting as a scaffold for the outer layer, permits minimal cage formation. Finally, Sec16p, predominantly cytosolic and thought to cycle on and off the ER, completes the complex. In addition, the Rab-interacting protein Yip1p is also implicated in COPII vesicle formation.

In order to exit the ER, proteins must be properly folded and assembled into their multimeric protein complexes. Misfolded or aggregated proteins are recognized by a **quality control** mechanism for proteins leaving the ER. More recently, a second model for quality control of exported proteins has been suggested involving the chaperone complex 14-3-3 that can detect any misfolding that occurs along the route (Yuan *et al*., 2003).

The prebudding COPII complex is stabilized via a combination of GTPase, GEF, and GAP activities, whereby Sec12p, a transmembrane protein that acts primarily as a GEF for Sar1p, takes the role of maintaining COPII coat assembly. Further complexity occurs upon recruitment of Sec13/31 to the membrane and this outer layer further stimulates the GAP activity of Sec23/24p by an order of magnitude (Figure 2.12).

(ii) **COPI-coated vesicles** consist of coatomer, a multimeric protein complex, and the small GTP-binding protein Arf1p (an ARF). COPI-coated vesicles mark the retrieval pathway, which begins in the *cis*-Golgi and continues to the late Golgi (Beck *et al*., 2009).

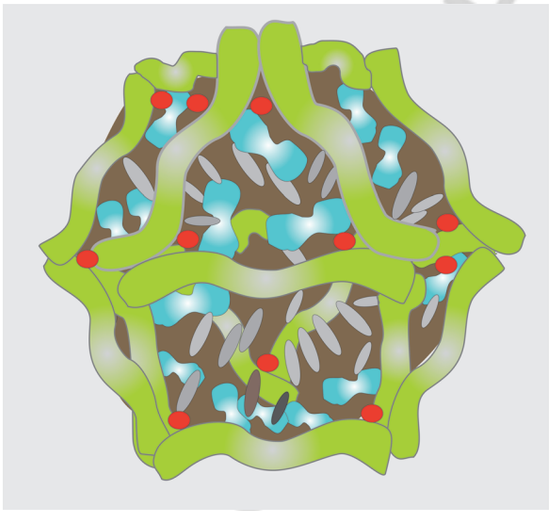

Fig. 2.12 Model of a COPII vesicle cage in cuboctahedron geometry. The outer scaffold (Sec13/Sec31) is shown in green; red, Sar1; blue, Sec23/Sec24; gray, cargo.

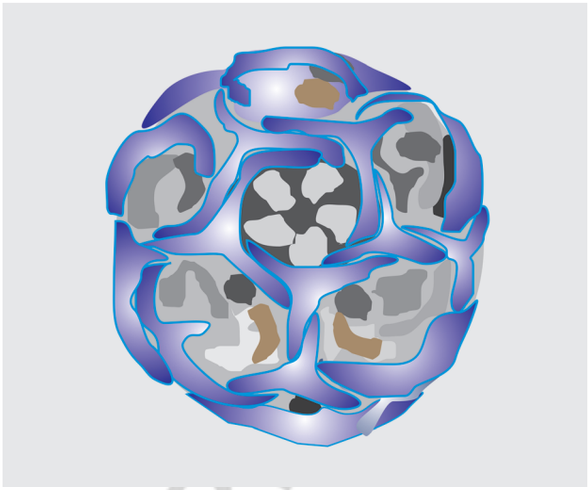

Fig. 2.13 Clathrin; triskelion scaffold shown in blue.

(iii) **Clathrin-coated vesicles** direct transport steps in the late secretory pathway, budding from several membranes, such as the plasma membrane (for endocytotic transport), the TGN (for transport to the vacuole), or secretory vesicles that are retrieved to the Golgi network. Clathrin-coated vesicles are built from clathrin, an adapter protein complex (AP-1; AP-2; AP-3, being adapted to particular transport functions), and the small GTP-binding protein Arf1p (an ARF). A special class of late Golgi clathrins employs the recently discovered GGA proteins (Zhdankina *et al*., 2001; Demmel *et al*., 2008b), which exhibit homology to γ-adaptin, and regulate Arf1p and Arf2p in a GTP-dependent mode. Release of the clathrin-coated vesicles is assisted by the action of dynamin, a GTPase, which together with other soluble cytosolic proteins cuts off the budding vesicles from the extruding lipid bilayer. Shortly after release, the clathrin coats are rapidly removed from the vesicles. See Figure 2.13.

Mechanisms that are responsible for the generation and directionality as well as the uptake of the vesicles into their target compartments are discussed in more detail in Section 8.1.

2.5.3 Vacuolar System

2.5.3.1.1 **Yeast Vacuole** The vacuole is a lysosome-like compartment, and is a key organelle involved in intracellular protein trafficking and nonspecific intracellular proteolysis (Schekman, 1985). Vacuoles may not always be clearly distinct and independent organelles (like mitochondria), but form an integral component of the ER–Golgi–vesicle route. Vacuoles arise by a regulated process from growth, multiplication, and separation of pre-existing entities rather than by *de novo* synthesis (Weisman, Bacallao, and Wickner, 1987). They are dynamic structures that may exist in cells as a single large compartment or as several smaller compartments,

called "prevacuolar compartments" (PVCs), "prevacuolar endosomes" (PVEs), or "late endosomes" (LEs). They are bound by a single membrane, which has a phospholipid, unsaturated fatty acid, and sterol content different from the plasma membrane. Phosphatidylinositol phosphates (e.g., phosphatidylinositol-4-phosphate (PI(4)P)) (Audhya, Foti, and Emr, 2000) are essential for the maintenance of vacuolar morphology. In yeast, the vacuole(s) usually occupy up to 30% of the total cellular volume.

The vacuole is a "drain." The vacuole is the compartment that receives proteins from different routes: (i) proteins sorted away from the secretory pathway at the Golgi apparatus, (ii) proteins derived from the plasma membrane, (iii) proteins imported by endocytic traffic, and (iv) products from autophagy, which represents a "destructive" pathway to liberate the cell from old organelles or organellar remnants (*cf.* Section 8.1.3.5.1).

In the first stages of endocytosis, plasma membrane invaginations are formed that pinch off to generate vesicles that finally deliver their load to the **endosomes**. In most cases, the endocytosed proteins are directed – via several forms of multivesicular bodies (MVBs) – to the vacuole for degradation. However, recently it became clear that also retrieval pathways (to the Golgi) for endocytosed proteins do exist in yeast. Details of these processes are presented in Section 8.1.

2.5.3.1.2 **Vacuolar Degradation** The degradative processes are catalyzed by the activities of the more than 40 different intravacuolar hydrolases: endopeptidases, aminopeptidases, and carboxypeptidases (Achstetter *et al.*, 1984; Jones, 1984; Jones, 1991; Vida *et al.*, 1991; Knop *et al.*, 1993), and nucleases, glycosidases, lipases, phospholipases, and phosphatases. Delivery of these enzymes to the vacuole is mediated by a portion of the secretory pathway (Rothman *et al.*, 1989; Fratti *et al.*, 2004) and there is a selective uptake of substrates to be degraded (Chiang and Schekman, 1991; Chiang, Schekman, and Hamamoto, 1996). Apart from their role in degradative processes, vacuoles are involved in several other physiological functions, such as being storage compartments for basic amino acids, polyphosphates, and certain metal cations (K^+, Mg^{2+}, and Ca^{2+}). They also participate in osmoregulation and the homeostatic regulation of cytosolic ion concentration and pH. pH is controlled by the vacuolar plasma membrane ATPase (see Section 8.3); while the cytosolic pH is about 7.2, the vacuolar pH is adapted to 5.0 – the optimum for the hydrolytic enzymes.

2.5.4 Endocytosis and Exocytosis

Endocytosis has to fulfill two tasks: (i) internalize and degrade components that might be hazardous to the cell, and (ii) recycle membrane components for repeated use (retrieval of receptors) or downregulate the activity of particular membrane receptors, both of which are of major importance to keep cellular integrity. In many cases, selected extracellular macromolecules are endocytosed by binding to specific membrane receptors. One example in yeast is the receptor protein for the **a**- or α-mating pheromones.

Two methods are employed in preparing the cargo to be imported; further details are discussed in Section 8.1.3.6.

Exocytosed material is packaged into clathrin-coated vesicles in the late Golgi network. There exists a constitutive secretory pathway for proteoglycans and glycoproteins that will form constituents of the plasma membrane. Regulated pathways are designed for the export of transmembrane proteins, such as receptors or transporters.

One prominent example of exocytosed material are **lipid rafts**, which form in the membrane of the *trans*-Golgi by self-aggregation into microaggregates and thus can transport particular combinations of membrane constituents to the cell surface. Lipid rafts may comprise proteins with extended transmembrane domains, glycolipids, and GPI-anchored proteins (*cf.* Section 3.4.3.2).

2.5.5 Mitochondria

For a long time, yeast **mitochondria** have been employed by many researchers as *the* model system in which mitochondrial structure, function, and biogenesis have been studied. Yeast mitochondria not only resemble these organelles found in higher eukaryotes, but are of outstanding importance for the understanding of fermentation processes. Yeast mitochondria are easy to isolate as respiratory-competent organelles and the genetics of yeast mitochondria has been studied in great detail.

2.5.5.1 Mitochondrial Structure

Yeast mitochondria, like their mammalian counterparts, are surrounded by two types of lipid bilayers, an outer membrane (MOM) and an inner membrane (MIM), the two of which embody an intermembrane space (IMS). The inner of the mitochondrion is called the "mitochondrial matrix." The outer membrane is sort of a shelter that also contains enzymes involved in lipid metabolism. The inner membrane contains (i) cytochromes for the respiratory chain, (ii) the ATP synthase coupled to the respiratory chain, and (iii) a number of transport proteins for the exchange of low-molecular-weight components. The matrix is the site for the citric acid cycle (tricarboxylic acid (TCA) cycle) and contains the mitochondrial DNA, together with the protein synthesizing machinery including mitochondrial ribosomes. One of the most important features of the setup comprising **all** compartments of the mitochondria are the systems for the internalization and processing of proteins that are manufactured on cytosolic ribosomes and imported into the mitochondria (Figure 2.14). Only a few proteins are synthesized by the use of the mitochondrial machinery, whereas the vast majority of the mitochondrial proteins (greater than 800) have to be

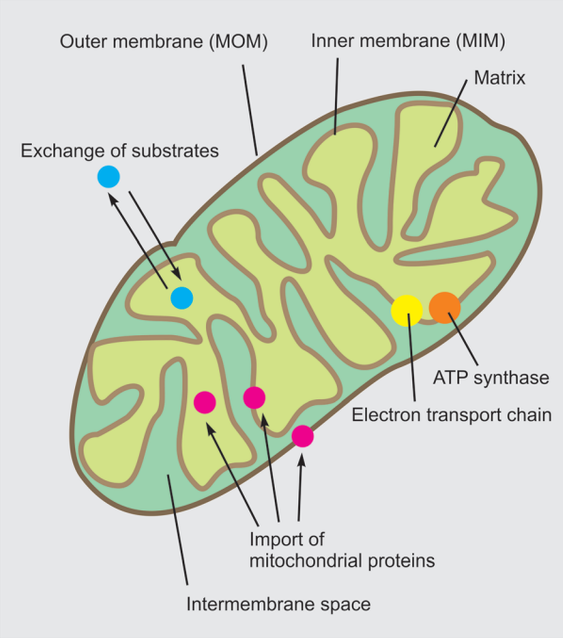

Fig. 2.14 Schematic view of a yeast mitochondrion.

imported. The specialized roles of yeast mitochondria are presented in Section 11.1.

Mitochondria will adopt **different morphologies**, depending on the conditions under which *S. cerevisiae* cells are grown. Under aerobic conditions, with sufficient oxygen supply and availability of carbon sources, mitochondria are primarily occupied with respiration and synthesis of ATP; there are few but large (and efficient) organelles. Under anaerobic conditions, they seem to be redundant in a respiratory sense, because of the absence of the final electron receptor – oxygen. Nonetheless, mitochondria do fulfill other functions that have to be maintained under anaerobic conditions, which underlines their physiological importance. For example, the synthesis and desaturation of fatty acids as well as the biosynthesis of ergosterol (involving mitochondrial cytochromes) have to be continued. Adaptation to stresses (by free oxygen radicals) has to be maintained. Enzymes for the synthesis of amino acids, nucleobases, porphyrin, or pteridins have to be provided and mobilization of glycogen has to be enabled.

2.5.6 Peroxisomes

Peroxisomes, also called microbodies, are organelles enwrapped by a single double-layer lipid membrane (Lazarow and Fujiki, 1985). Characteristic for these organelles is the occurrence of, at minimum, one hydrogen peroxide-producing oxidase and a detoxifying catalase, an enzyme that is capable of metabolizing hydrogen peroxide. The number and morphology of peroxisomes in yeast cells is largely determined by the growth conditions. They are endowed with several metabolic functions that are of outstanding importance for cell viability. In addition to the oxidase/catalase system, yeast peroxisomes harbor a complete system for β-oxidation of fatty acids. In yeast, fatty acids are oxidized exclusively by peroxisomes, while in animal cells only long-chain fatty acids are metabolized.

Peroxisomes do not possess any intrinsic genetic material nor translation machinery, so that their complete proteinaceous equipment needs to be provided by nuclear-encoded entities. The import of peroxisomal proteins into the peroxisomal matrix is directed by specific target sequences to these proteins; targets are recognized by specialized receptors and the cargo internalized by a specific "importomer" (Grizalski *et al.*, 2009; Meinecke *et al.*, 2010). After delivery of their cargo, the components of the "importomer" are recycled to the cytosol. Details of these processes are discussed in Section 11.2.

Summary

- This chapter presents an overview of how a cell of *S. cerevisiae* is built from elementary structures, each of which has been designed to fulfill particular functions in order to guarantee successful propagation of all individuals in a given population, and at the same time respond to the conditions imposed by its (natural) environment. Except for a rigid cell wall, we will encounter all of the organellar structures of a yeast cell in other single-cell as well as in multicellular eukaryotic organisms. Based on this fact and given the uncomplicated handling (both in terms of genetic manipulation and easy preparation of subcellular entities), yeast became the preferred model system in many basic research areas of molecular biology.
- Part of this chapter is devoted to the description of yeast cell morphology and of how subcellular structures can be recognized. Later, in Chapter 4, methods for the isolation of organelles and cellular components will be presented. The yeast cell envelope and, in particular, the rigid cell wall are of utmost importance for a safe life. The cell wall protects against mechanical injury and unwanted ingress of material. On the other hand, the cell wall is not an inflexible cage, but has to be adapted to the changing shape of the cell during growth and propagation in a controlled fashion. The plasma membrane serves many structural and functional obligations – it harbors protein anchors for the cell cytoskeleton, enzymes of cell

wall synthesis, receptors for communication with the environment, including osmotic control, and selective transporters for compounds that enter or that leave the cell. We describe in some detail the components (i.e., microtubules and microfilaments) that build up a dynamic cytoskeletal scaffold. The cytoskeleton is mainly designed for two functions in yeast physiology: (i) transport of cargo (from simple molecules through complex structures to whole organelles) across the cell cytoplasm, and (ii) participation in mitosis and meiosis, determining cell polarity during budding or mating as well as septation before cell separation. Motor proteins such as myosins, kinesins, or dynein provide the energy necessary for motility.

- The cell nucleus is the central organelle that handles the duplication of the chromosomes during cell division and the expression of the majority of genetic information. Thereby, the nuclear pores act as instruments controlling the exchange of macromolecules between the nucleus and the cytosol, or sequestration of particular types of factors within the nucleus. These features are paralleled in yeast and mammals, as intense work in both systems has demonstrated. Here, we rather concentrate on morphological aspects and will discuss some functional aspects later in Chapter 8.

- Cellular organelles taking care of protein processing, their cellular distribution, and programmed degradation are the ER, connected to the nuclear membrane, the Golgi apparatus, and the yeast vacuole (which is similar to lysosomes), including the various vesicular bodies that mediate interorganellar transport. Most of the proteins are fabricated by cytoplasmic ribosomes and imported into the ER, which then controls their correct folding. Handed over to the Golgi, the proteins are subjected to various modifications, thus endowing each of them with attributes that facilitate their targeting to the final destination within the cell or label them for exocytosis. Proteins imported into the cell or retrieved from its surface are channeled into this system as well. The intracellular trafficking was one of the earliest issues studied in yeast and opened up ways to look into similar aspects in higher organisms.

- Finally, this chapter briefly outlines the structural and functional characteristics of the mitochondria and the peroxisomes, both of which are organelles also indispensable in higher eukaryotes.

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