Acta Biochimica Polonica

Vol. 55 No. 4/2008, 649–662

on-line at: www.actabp.pl

Review

The role of Rsp5 ubiquitin ligase in regulation of diverse processes in yeast cells

Paweł Kaliszewski✉ and Teresa Żołądek

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Received: 04 July, 2008; revised: 08 October, 2008; accepted: 04 November, 2008
available on-line: 28 November, 2008

Rsp5 is a conserved ubiquitin ligase involved in regulation of numerous cellular processes. A growing number of publications describing new functions of the ligase have appeared in recent years. Rsp5 was shown to be involved in the control of intracellular trafficking of proteins *via* endocytosis and multivesicular body sorting. Moreover, nuclear functions of Rsp5 in response to various stresses have been discovered. Rsp5 is also involved in the regulation of unsaturated fatty acid and sterol synthesis and phospholipid composition. Here, an overview of Rsp5 functions with emphasis on its involvement in the regulation of lipid biosynthesis will be presented.

Keywords: Rsp5, ubiquitin ligase, Spt23, Mga2, transcriptional activators, ubiquitination, *Saccharomyces cerevisiae*

UBIQUITINATION

Numerous cellular proteins are modified post-translationally by conjugation of the polypeptide ubiquitin. Among them are cell cycle regulators, transcription activators, signaling proteins, and enzymes involved in metabolic pathways. Therefore, the ubiquitination system regulates a broad array of cellular processes. Aberrations in the system have been implicated in the pathogenesis of major diseases such as cancer, diabetes, and neurodegenerative disorders (reviewed by Weissman, 2001).

Ubiquitination is a process of ubiquitin conjugation to the protein substrate (Hereshko & Ciechanover, 1998). The process is carried out by a multienzyme cascade involving enzymes from different classes. First, ubiquitin is activated by E1 activating enzyme. In yeast cells there is only one E1 enzyme — Uba1, which is essential for growth. The activation is ATP-dependent and occurs with the formation of a thioester bond between a cysteine in the active center of E1 and the C-terminus of ubiquitin. The next step is the transfer of activated ubiquitin to a cysteine residue located in the active center of conjugating enzyme E2. Thirteen E2 enzymes have been identified in yeast. Ubiquitin is then transferred to the acceptor protein either directly from the E2 enzyme or indirectly with an involvement of a ubiquitin ligase, the E3 enzyme. Isopeptide bond is formed between the C-terminal glycine of ubiquitin and a lysine of the substrate protein or from another ubiquitin molecule. The E3 ligases play important roles in recognition and binding specific substrates in a particular moment and compartment of the cell. They are grouped in two classes: protein complexes with a RING-finger catalytic domain, such as APC (anaphase promoting complex) (Jackson *et al.*, 2000), and ligases containing a Hect domain (homologus

✉Corresponding author: Paweł Kaliszewski, Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warszawa, Poland; tel.: (48) 22 592 1304; fax: (48) 22 658 4636; e-mail: pkalisz@ibb.waw.pl

Abbreviations: aa, amino acids; APC, anaphase promoting complex; CTD, C-terminal domain; DAG, diacylglycerol; DMAPP, dimethylallyl diphosphate; E1, ubiquitin activating enzyme; E2, ubiquitin conjugating enzyme; E3, ubiquitin-protein ligase; ER, endoplasmic reticulum; FAR, fatty acid-regulated; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPI, glycosylphosphatidylinositol; GPP, geranyl diphosphate; Hect, homologus to E6-AP carboxy terminus; HMG, 3-hydroxy-3-methylglutarate; HSEs, heat shock elements; IPP, isopentenyl diphosphate; LORE, low-oxygen response elements; MVA, mevalonic acid; MVB, multi vesicular body; Nedd, neural cell-expressed developmentally down-regulated; PA, phosphatidic acid; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIPs, phosphatidylinositides; PL, phospholipid; PS, phosphatidylserine; RNAPII, RNA polymerase II; SE, steryl esters; TAG, triacylglycerol; Ub, ubiquitin; wt, wild-type.

to E6-AP carboxy terminus) (Huibregtse *et al.*, 1995). Five ligases from the Hect class are known in yeast: Rsp5, Ufd4, Tom1, Hul4, and Hul5 (Wang *et al.*, 1999).

Ubiquitin is a 76 amino acid-long polypeptide that is highly evolutionarily conserved. It contains seven lysine residues K6, K11, K27, K29, K33, K48, and K63 (Arnason & Elison, 1994), all of which can be used for conjugation with other ubiquitin molecules. There are three types of ubiquitination: monoubiquitination, the attachment of a single ubiquitin, multiubiquitination — attachment of multiple ubiquitin molecules to a protein, and polyubiquitination, in which ubiquitin is attached to a lysine of another ubiquitin and a long polyubiquitin chain is formed on the protein. Mono- or polyubiquitination of proteins by K63-linked ubiquitins are signals for endocytosis, vacuolar degradation and chromatin remodeling (see a review by Hicke, 2001). Polyubiquitination affects also proteins involved in DNA repair, transcription, cell cycle, and endocytosis of plasma membrane proteins (Weissman, 2001; Lindsten *et al.*, 2002). Polyubiquitination through K48 or K29 of ubiquitin is a signal for 26S proteasomal degradation of short-lived or misfolded proteins (Hochstrasser, 1996; Hershko & Ciechanover, 1998). The functions of the polyubiquitin chain linked by other lysines: K6, K11, K27, and K33 have not been discovered yet.

Rsp5 ubiquitin-protein ligase and its domain structure

The best-studied ubiquitin ligase in yeast, the eukaryotic model organism, is Rsp5 (Huibregtse *et al.*, 1995). It belongs to the Nedd4 family of ubiquitin ligases implicated in diverse cellular functions. Nedd4-like proteins are found in eukaryotes from yeasts to mammals and are defined by a similar domain organization (reviewed by Ingham *et al.*, 2004). In the baker's yeast there is only one protein from the Nedd4 family (Rsp5), but this family has expanded further in higher eukaryotes, for example there are nine paralogous proteins in humans (Fig. 1). The Nedd4 (neural cell-expressed developmentally downregulated) (Kumar *et al.*, 1992) ligase (also referred to as Nedd4-1) is the founding member of the Nedd4 family. Several substrates and binding partners of Nedd4 have been identified and its function in signal transduction, protein trafficking and oncogenesis is documented (Shearwin-Whyatt *et al.*, 2006; Wang *et al.*, 2007). Nedd4 is involved in the regulation of endocytosis of the plasma membrane sodium channel ENaC and implicated in pathogenesis of a hereditary hypertension in humans, the Liddle syndrome (Hamilton & Butt, 2000; Rotin *et al.*, 2000). Besides that Nedd4 is also involved in budding of retroviruses (Segura-Morales *et al.*, 2005). All proteins from the Nedd4 family possess a C2 domain, several WW domains and a catalytic Hect domain. The C2 domain is located at the N-terminus of the protein, multiple WW domains are in the middle, and the Hect domain is at the C-terminus (Harvey & Kumar, 1999).

The C2 domain, approximately 130 amino acids long, is a conserved lipid- and protein-interaction module that is often regulated by calcium (Nalefski & Falke, 1996; Hurley & Misra, 2000). Many C2 domains bind to membranes through electrostatic interactions between basic amino acids and negatively charged lipids (Cho, 2001). It has been shown that mutation of five lysine residues to glutamine within the C2 domain of Rsp5 abolishes its binding to the membranes. The C2 domain of Rsp5 interacts with phosphorylated phosphatidylinositols and is important for localization of Rsp5 to endosomal membranes (Dunn *et al.*, 2004). The C2 domain of Rsp5 is not necessary for the essential function of Rsp5 in standard conditions, but is implicated in Rsp5-dependent sorting of biosynthetic cargo proteins in multivesicular bodies (MVB, late endosomes) (Dunn *et al.*, 2004). Moreover, deletion of the Rsp5 C2 domain impairs internalization of Gap1, a general amino acid permease, without detectably affecting its ubiquitination, suggesting that Rsp5 participates via its C2 domain in endocytosis of ubiquitinated permeases (Springael *et al.*, 1999a).

The WW domains were first described by Sudol (1996) as small modules composed of about 40 amino acids. The WW domains mediate protein–protein interactions and recognize proline-rich sequences called PY motifs. These domains are folded into three-stranded anti-parallel β-sheets forming a hydrophobic pocket (Macias *et al.*, 1996). WW domains are divided into four groups according to their binding specificities (Bedford *et al.*, 2000). Group I bind the PXY, LPXY and PPXY motifs. Group II bind to PPLP, group III recognize the PPR motif and group IV bind short sequences containing phosphoserine or phosphothreonine followed by proline (Lu *et al.*, 1999). Rsp5 contains three WW domains which belong to group I, but not all Rsp5 substrates known contain a PXY, PPXY or LPXY motif (Gupta *et al.*, 2007). These proteins may bind Rsp5 *via* other domains than WW or use adaptor proteins which contain Rsp5-binding motifs. One of these adaptor proteins is Bsd2, which is crucial for Rsp5-dependent ubiqutination of Cps1, a vacuolar carboxypeptidase, and another Tre1, a protein important for ubiqutination and vacuolar degradation of the metal transporter Smf1 (Sullivan *et al.*, 2007).

The catalytic Hect domain of about 350 residues is situated at the C-terminus of Rsp5/Nedd4 proteins. This domain is essential for the ubiquitina-

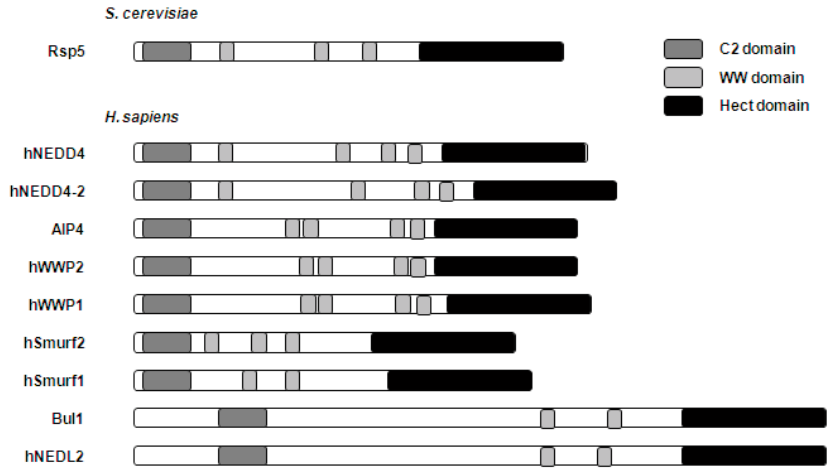

Figure 1. The Nedd4 family of E3 ubiquitin ligases.

tion activity of Rsp5. The conserved cysteine in the active center of the Hect domain forms a thioester bond with ubiquitin upon its transfer from E2 enzymes (Huibregtse *et al.*, 1995). Mutation of this cysteine results in a complete loss of ubiquitination activity of Rsp5 that leads to a dominant negative effect on cell growth.

Roles of Rsp5

Rsp5 is a key regulatory protein in the cell, which ubiquitinates numerous proteins and is involved in regulation of a broad array of cellular processes. It is capable of modifying proteins in different cellular compartments, for example on the plasma membrane and in the nucleus.

Intracellular trafficking of proteins

The involvement of Rsp5 in intracellular trafficking of proteins, particularly in endocytosis and MVB (multi vesicular body) sorting, is well studied. Endocytosis is a process by which cells internalize portions of the plasma membrane with proteins and lipids and surrounded molecules from outside the cell. Endocytosis allows cells to remove no longer needed plasma membrane proteins (ion channels, receptors, etc.) but also to supply them with nutrients from the environment. Moreover, it is important for modulation of the cellular responses to external stimuli. Endocytosis starts with invagination of the plasma membrane which buds off and forms an internal vesicle which is later fused with a cellular compartment – early endosome. The early endosome is then converted into MVB. The MVB forms when portions of the late endosome membrane invaginate and pinch off into the lumen, thus forming intralumenal vesicles (Katzmann *et al.*, 2002; Raiborg *et al.*, 2003). The cargo from MVB can be transported either to a vacuole or to the Golgi apparatus. Proteins from endosomes may be also recycled back to the plasma membrane. It has been shown that Rsp5 is important for ubiquitin-mediated endocytosis of several proteins, including the general amino acid permease Gap1 (Springael *et al.*, 1999b), uracil permease Fur4 (Galan *et al.*, 1996, Hein & Andre, 1997), maltose permease Mal61 (Medintz *et al.*, 1998), hexose transporter Hxt6/7, tryptophan permease Tat2 (Beck *et al.*, 1999), zinc transporter Zrt1 (Gitan & Eide, 2000), and the pheromone receptor Ste2 (Dunn & Hicke, 2001). In addition, mutations in the *RSP5* gene cause defects in fluid phase endocytosis as monitored by uptake of the fluorescent dye Lucifer Yellow (Żołądek *et al.*, 1997). The WW domains, but not C2, are important for internalization of Fur4 and Ste2 and for fluid phase endocytosis (Gajewska *et al.*, 2001; Dunn & Hicke, 2001). Plasma membrane transporters and receptors are polydiubiquitinated and the ubiquitin chain is formed *via* lysine K63 (see a rewiev by Hicke, 2001).

The biosynthetic route is the main route for the delivery of resident vacuolar proteins and lipids from their site of synthesis in the ER, *via* Golgi and MVB, to their site of action in the vacuole. Sorting of biosynthetic and endocytic transmembrane proteins into MVB vesicles is controlled by the addition of a single ubiquitin moiety to a cytoplasmic domain of these proteins (Katzmann *et al.*, 2001; Reggiori & Pelham, 2001; Urbanowski & Piper 2001; Morvan *et al.*, 2004). Recent data indicate that Rsp5 is essential for the MVB sorting of the biosynthetic cargo. A mutant lacking the Rsp5 C2 or with mutations in the WW1, 2 or 3 domain was unable to ubiquitinate or properly sort Cps1 into MVB vesicles (Dunn *et al.*, 2004). Other authors showed that Sna3 protein is also diverted from its route in case of Rsp5 deficiency (reviewed by Piper & Katzmann, 2007; Stawiecka-Mirota *et al.*, 2007). Sna3 carries a PPXY motif which mediates its

interaction with Rsp5 WW domains. Mutation of either the Sna3 PPXY motif or the Rsp5 WW3 domain or reduction in the amount of Rsp5 results in mistargeting of Sna3 to multiple mobile vesicles and prevents its sorting to the endosomal pathway. Sna3 is polyubiquitinated on one target lysine, and a mutant Sna3 lacking this lysine displays defective MVB sorting. Sna3 undergoes Rsp5-dependent polyubiquitination with K63-linked ubiquitin chains (Stawiecka-Mirota *et al.,* 2007). Rsp5-dependent ubiquitination is also involved in sorting of the amino acid permease Gap1 at the Golgi apparatus (Helliwell *et al*., 2001).

Rsp5 is also implicated in activation of the plasma membrane H^+-ATPase Pma1 by glucose (de la Fuente *et al.,* 1997), but the mechanism of this regulation is not known. Pma1 is ubiquitinated, which does not affect its stability, but it does affect the stability of a mutant protein Pma1-7 (Pizzirusso & Chang, 2004). Moreover, the G(653)V substitution in the ATP-binding domain of Pma1 suppresses the temperature sensitivity phenotype of *rsp5* mutations (Kamińska *et al.,* 2000).

Nuclear functions

The cell nucleus is delimited by a double membrane also called the nuclear envelope. This double membrane contains nuclear pores which are gates allowing active and selective transport of macromolecules such as proteins and RNAs. Numerous highly regulated processes take place in the nuclear compartment, such as transcription, DNA replication, chromosome segregation, etc. In normal growth condition Rsp5 is localized to multiple cytoplasmic complexes (Gajewska *et al.,* 2001; Katzmann *et al.,* 2004). However, many nuclear functions of Rsp5 have been discovered, which implies that Rsp5 may be a shuttling protein.

Rsp5 affects transcription by regulation of the large subunit of RNA polymerase II (Rpb1 of RNAPII), which is ubiquitinated and targeted for degradation in 26S proteasome in stress conditions. This regulation is mediated by interaction of Rsp5 domains WW2 and WW3 with the C-terminus of Rpb1, CTD (C-terminal domain). CTD is composed of the sequence YSPTSPS repeated 26 times and a core including the PXY motif, which is essential for the interaction with the WW domains. Mutation in the Rsp5 WW2 domain abolishes its interaction with Rpb1 *in vitro* (Wang *et al.,* 1999; Beaudenon *et al.,* 1999). It has been shown that phosphorylation of serine, threonine and tyrosine residues within CTD inhibits its interaction with Rsp5. Dephosphorylation of this domain could be a primary signal targeting Rpb1 to proteasomal degradation (Chang *et al.,* 2000). Def1, an RNAPII degradation factor, is required for the recruiting of Rsp5 to effect RNAPII ubiquitination and subsequent degradation (Reid & Svejstrup, 2004). There are two ubiquitination sites in the yeast Rbp1 and they both play an important role in the elongation step of transcription and the DNA-damage response (Somesh *et al.,* 2007).

Nuclear accumulation of poly(A)$^+$RNA was observed in a temperature sensitive *rsp5-1* mutant strain (mutation in the Hect domain) at the non-permissive temperature (37°C) (Rodriguez *et al.,* 2003). Then, Rsp5-dependent regulation of the nuclear export factor Hpr1 was discovered (Gwizdek *et al.,* 2005). Hpr1 is a member of the THO/TREX (transcription/export) complex which has been implicated in transcription elongation, transcription-dependent recombination, and mRNA export (Zenklusen *et al.,* 2002; Strasser *et al.,* 2002). The THO complex component Hpr1 is ubiquitinated and degraded both *in vitro* and *in vivo* by Rsp5 in conjunction with the E1 and Ubc4p as an E2 (Gwizdek *et al.,* 2005).

Recent data indicate that Rsp5 can affect tRNA localization. Neuman and coworkers (2003) noticed nuclear accumulation of immature tRNA in the *rsp5-3* mutant which contains three mutations of which one lies in the catalytic Hect domain of Rsp5. The *rsp5-3* mutant not only shows strong nuclear accumulation of tRNAs at the restrictive temperature, but also is severely impaired in the nuclear export of mRNAs and 60S pre-ribosomal subunits. Strikingly, the nuclear RNA export defects seen in the *rsp5-3* strain are accompanied by a dramatic inhibition of both rRNA and tRNA processing. Thus, the ubiquitin ligase Rsp5 plays a role in controlling the major nuclear RNA biogenesis/export pathways in yeast. Other authors showed that the *rsp5-19* mutation (P418L substitution in WW3 domain) alters cell sensitivity to antibiotics that affect translation and that *rsp5-19* also increases the fidelity of translation (Kwapisz *et al.,* 2005). Nuclear accumulation of tRNA in this mutant was also observed. Moreover, an additional copy of *TEF2* gene encoding elongation factor eEF1A which delivers tRNAs to the ribosome, suppressed the *rsp5-19* growth defects, translational phenotypes and nuclear accumulation of tRNA. This suggests that nuclear tRNA accumulation may be the primary reason for the altered translational decoding accuracy of *rsp5-19* mutant cells (Kwapisz *et al.,* 2005).

The Rsp5 ligase together with APC (ang. anaphase promoting complex), a ligase from the RING family, are both required for chromatin condensation (Altheim & Schultz, 1999; Harkness *et al.,* 2002). Moreover, it has been shown recently that Rsp5 and Apc5, a subunit of the APC, interact genetically and that Rsp5 acts upstream of Apc5 (Arnason *et al.,* 2005). Those authors also identified an E2 enzyme, Ubc7, implicated in chromatin assembly. Further-

more, they demonstrated that Ubc7 physically and genetically interacts with Rsp5, suggesting that Ubc7 acts as an E2 for Rsp5 at least in this process.

Rsp5 functions in response to various stresses

Cells in nature are exposed to various environmental stresses, for example changes in temperature, osmolarity, concentration of nutrients or toxic substances, etc. Stress induces protein denaturation, generates damaged proteins, and leads to growth inhibition or cell death. Two major transcription factors, Hsf1 and Msn2/4, appear to be responsible for stress-induced gene expression (Hashikawa & Sakurai, 2004; Ferguson *et al*., 2005). Hsf1 binds to heat shock elements (HSEs) and Msn2/4 binds to stress response elements (STREs) found in the promoters of many heat-inducible genes encoding stress proteins. The transcription of genes encoding stress proteins: *HSP42* (containing HSE), *DDR2* (containing STRE) and *HSP12* (containing both HSE and STRE) in the *rsp5(A401E)* mutant was significantly lower than that in the wild-type strain when exposed to a temperature up-shift or 9% ethanol (Haitani *et al.,* 2006). Moreover, the amounts of transcription factors Hsf1 and Msn4 were remarkably decreased in the *rsp5(A401E)* mutant in these stress conditions (Haitani *et al.,* 2006) whereas the respective mRNA levels were only slightly lower than those in wild-type cells (Haitani & Takagi, 2008). The mRNAs of *HSF1* and *MSN2/4* were accumulated in the nucleus of *rsp5(A401E)* cells after exposure to temperature up-shift or ethanol, suggesting that Rsp5 is required for the nuclear export of these mRNAs. Those results indicated that, in response to environmental stresses, Rsp5 primarily regulates the expression of *HSF1* and *MSN2/4* at a post-transcriptional level (Haitani & Takagi, 2008).

Regulation of unsaturated fatty acid synthesis

The regulation of enzymes involved in lipid metabolism is an essential process that affects membrane lipid composition and has an impact upon many cell processes, such as cell growth, organelle function and response to stress (Schneiter & Kohlwein, 1997; Carman & Henry, 1999). Therefore, eukaryotes have developed complex mechanisms to regulate lipid biosynthetic pathways. Deregulation of lipid metabolism has been reported in many human diseases, including obesity and atherosclerosis, one of the diseases with the highest morbidity in developed countries (Ntambi, 1999). The ratio of saturated to monounsaturated fatty acids that are incorporated into cell membranes contributes to fluidity of the membrane. In the yeast *Saccharomyces cerevisiae*, this ratio also affects mitochondrial inheritance (Stewart & Yaffe, 1991) and stress responses (Carratu *et al.,* 1996). The enzyme involved in fatty acid desaturation is the D-9 fatty acid desaturase encoded by the essential *OLE1* gene (Stukey *et al.,* 1989). Ole1 protein converts saturated fatty acyl-CoA (palmityl- and stearyl-) to monounsaturated fatty acid species (palmitoleoyl- and oleoyl-) in an oxygen-dependent manner (Stukey *et al*., 1989). The regulation of *OLE1* expression is physiologically very important since unsaturated fatty acids contribute 70–80% of the fatty acyl groups in membrane lipids. The expression of *OLE1* is regulated by nutrient fatty acids and molecular oxygen (Vasconcelles *et al.,* 2001) and other physiological conditions, both at the transcriptional and mRNA stability levels (Gonzalez & Martin, 1996; Choi *et al.,* 1996). Unsaturated fatty acid-dependent repression is mediated by FAR (fatty acid-regulated) elements (Choi *et al.,* 1996) and hypoxic activation is mediated by LORE (low-oxygen response elements) (Vasconcelles *et al.,* 2001).

One essential function of Rsp5 is the regulation of unsaturated fatty acid biosynthesis; the *rsp5Δ* strain is inviable unless the medium is supplemented with oleic acid (Hoppe *et al.,* 2000). Rsp5 controls the activation of two homologous ER-localized transcriptional activators, Spt23 and Mga2 (Hoppe *et al.,* 2000; Shcherbik *et al.,* 2003; 2004), which play a role in the up-regulation of expression of *OLE1* gene (Zhang *et al.,* 1999). They are both functionally redundant since neither of the two genes, *SPT23* and *MGA2,* is essential for viability, whereas the double *spt23Δ mga2Δ* mutation is lethal (Zhang *et al.,* 1999). This lethality is suppressed by the presence of oleic acid in the growth medium. Spt23 and Mga2 are produced as p120 precursors which are anchored as homodimers in the membrane of the ER *via* their C-terminal transmembrane domains (Hoppe *et al.,* 2000; Shcherbik *et al.,* 2003). When unsaturated fatty acids become limiting, the Spt23 and Mga2 precursors are ubiquitinated, and one of the dimer subunits is processed into a mature p90 form. Subsequently, with the assistance of the chaperone complex Cdc49/Ufd1/Npl4 and the ubiquitin-proteasome pathway, p90 is released from the membrane-bound p120 subunit and transported into the nucleus where it functions as a transcriptional activator of *OLE1* (Shcherbik *et al.,* 2003). Mga2 is also essential for the hypoxic induction of *OLE1* expression and is a component of the LORE-bound complex (Nakagawa *et al.,* 2002). The WW2 or WW3 domain of Rsp5 binds Spt23 and Mga2 *via* the LPKY motif and ubiquitination takes place enabling release of these processed proteins from the ER (Shcherbik *et al.,* 2003; Bhattacharya *et al.,* 2008). In the *rsp5-19* temperature sensitive mutant saturated fatty acid accumulation contributes to cell lethality at elevated temperatures (Kaliszewski *et al.,* 2006).

A recent study has revealed several classes of genes, including those for ribosomal proteins, mating-type and lipid metabolism genes, with which activated Spt23 and Mga2 associate (Auld *et al.*, 2006). Most of the lipid metabolism genes also bound proteasome components suggesting that the ubiquitin-proteasome pathway might have a role in regulation of these genes downstream of Spt23 and Mga2 activation. The list of lipid metabolism genes bound by Spt23 and Mga2 includes the *OLE1* gene, other fatty acid biosynthesis genes (*SUR4*, *FAS1*, *ELO1*, *FAA4*, *ACC1*, *FAA3*, *OAR1*) and genes encoding enzymes of the mevalonate pathway (see below). This suggests that Rsp5 is not only involved in the regulation of unsaturated fatty acid content, but due to Spt23 and Mga2 regulation it is important for the maintenance of lipid homeostasis.

Effects on phospholipid and triacylglycerol synthesis

Phospholipids are the main components of cellular membranes. They play crucial roles in cell growth and metabolism. Phospholipids are important for membrane-associated functions such as enzyme catalysis, receptor-mediated signaling, and solute transport (Dowhan, 1997; Dowhan *et al.*, 2004). In addition, phospholipids are precursors for the synthesis of large molecules such as glycosylphosphatidylinositol membrane anchors (Fankhauser *et al.*, 1993) and sphingolipids (Lester & Dickson, 1993). They act as molecular chaperones (Bogdanov *et al.*, 1996; 1999), participate in protein modification for membrane association (Ichimura *et al.*, 2000), and are precursors of second messengers (Exton *et al.*, 1994). The main phospholipids of *S. cerevisiae* membranes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) (reviwed by Carman & Henry, 1989). The most common fatty acids found in the phospholipids include the saturated palmitic and stearic acids, and monounsaturated palmitoleic and oleic acids (Rattray *et al.*, 1975). PS, PE, and PC are synthesized from phosphatidic acid (PA) *via* the CDP-DAG (CDP-diacylglycerol) pathway (Fig. 2). The CDP-DAG liponucleotide is synthesized from PA and CTP by the Cds1 CDP-DAG synthase (Carter & Kennedy, 1966; Shen & Dowhan, 1996). CDP-DAG is then converted to PS by the Cho1 PS synthase (Nikawa *et al.*, 1987; Kiyono *et al.*, 1987) and it is decarboxylated to PE by the Psd1 (Clancey *et al.*, 1993) and Psd2 (Trotter & Voelker, 1995) PS decarboxylase enzymes. PE is then converted to PC by a three-step methylation reaction catalyzed by Cho2 and Opi3 (Bremer & Greenberg, 1960).

PE and PC can also be synthesized from ethanolamine and choline *via* the Kennedy pathway (reviwed by Carman & Han, 2007) (Fig. 2). In PI synthesis the Pis1 PI synthase (Nikawa & Yamashita, 1984) utilizes CDP-DAG and inositol as substrates (Paulus & Kennedy, 1960). The inositol used in this reaction is synthesized from glucose-6-phosphate by the Ino1 inositol-3-phosphate synthase (Klig & Henry, 1984; Dean-Johnson & Henry, 1989) and the Inm1 inositol-3-phosphate phosphatase (Murray & Greenberg, 2000).

Rsp5 regulates the synthesis of unsaturated fatty acids (see previous paragraph) which are built into phospholipids and therefore it also affects the phospholipids' composition. It has been shown that *rsp5-3* cells grown at the restrictive temperature exhibit significantly reduced levels of di-unsaturated PE species (Neumann *et al.*, 2003). Moreover, accu-

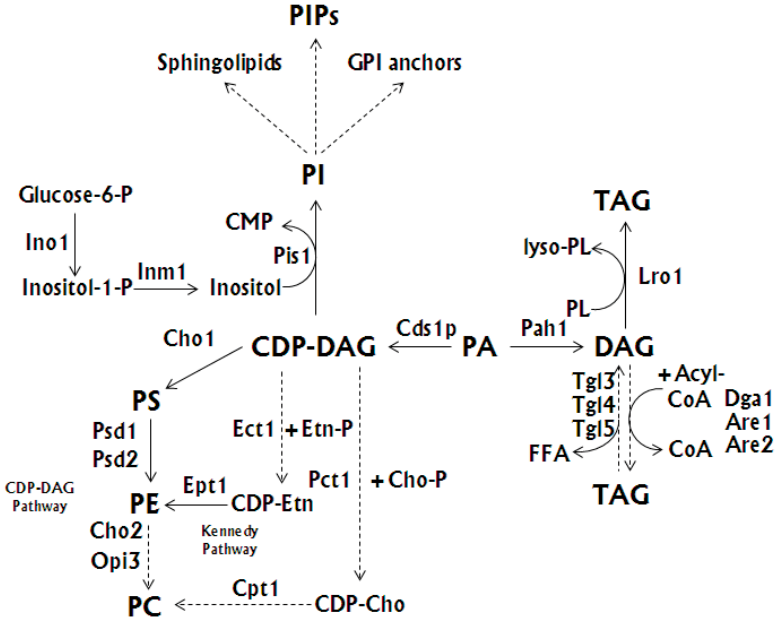

Figure 2. Biosynthesis of phospholipids and triacylglycerol.
Description in the text.

mulation of saturated fatty acids in particular phospholipids can be one of the reasons of the decreased viability of the *rsp5-19* mutant cells at the restrictive temperature. Interestingly, this growth defect can be suppressed by overexpression of the *PIS1* gene encoding an enzyme involved in PI synthesis (Fig. 2) (Kaliszewski *et al.,* 2006). The *PIS1* gene appeared to be a nonspecific suppressor since it suppressed also growth defects of other *rsp5* mutants at the restrictive temperature, suggesting that the suppression mechanism was not connected with a particular *rsp5* mutation. It was demonstrated that enhanced phosphatidylinositol synthesis was important for the suppression because expression of *PIS1* was higher in the *rsp5-19* mutant than in the wild-type, whereas the introduction of *PIS1* on a multicopy plasmid resulted in a further increase of the Pis1 level in both backgrounds and the catalytic activity of Pis1 was essential for the suppression (Kaliszewski *et al.,* 2006). Moreover, the synthesis and utilization of inositol (a substrate of Pis1) was increased, since the expression of *INO1* (inositol synthase, see Fig. 2) was elevated in the *rsp5-19* mutant, and inositol added to the medium improved growth of *rsp5* mutants at the restrictive temperature. Finally, it was shown that overexpression of *PIS1* did not correct the cellular unsaturated fatty acid content in *rsp5-19;* however, the *rsp5-19* mutation induced saturated fatty acid accumulation in PE, a phenomenon that could be fully suppressed by overexpression of *PIS1* due to rerouting of saturated acyl chains towards PI (Kaliszewski *et al.,* 2006). This suggests that the primary reason of *rsp5* mutant lethality at the restrictive temperature can be accumulation of saturated fatty acids in PE, the phospholipid which normally is the most unsaturated one (Ferreira *et al.,* 2004).

Triacylglycerols (TAG) serve as a storage of energy and of fatty acids required for the synthesis of membrane lipids in cells. TAG cannot integrate into a phospholipid bilayer membrane, so they are deposited in lipid particles. TAG synthesis in yeast is mainly catalyzed by two enzymes: Dga1, DAG-acyltransferase which catalyzes acyl-CoA-dependent acylation of DAG, and Lro1, which is a phospholipid: DAG acyltransferase (Oelkers *et al.,* 2002). Lro1p converts DAG to TAG in an acyl-CoA-independent reaction and uses glycerophospholipids, preferentially PC and PE, as the acyl source (Dahlqvis *et al.,* 2000; Oelkers *et al.,* 2000). These two enzymes play different roles in the cell. Lro1 is mainly responsible for TAG synthesis during logarithmic phase of growth, whereas Dga1 is more active in the stationary phase of growth. Another difference between them is their subcellular distribution. Dga1 is located in the ER and lipid particles, the storage compartment for neutral lipids, whereas Lro1 seems to be located only in the ER (Sorger & Daum, 2002; Sorger & Daum, 2003). Lro1 and Dga1 are not the only TAG-synthesizing enzymes in yeast cells because when both *LRO1* and *DGA1* genes are disrupted the cells retain approximately 5% of the DAG esterification activity as compared to wild type (Sorger & Daum, 2002; Oelkers *et al.,* 2002). For that activity Are1 and Are2 sterol acyl transferases are responsible (see Fig. 3) which mainly use activated fatty acids to synthesize steryl esters (SE), another form of lipids stored in lipid particles (Sandager *et al.,* 2002; Sorger *et al.,* 2004). TAG in yeast cells mainly contain unsaturated fatty acids and they cannot accommodate too much saturated ones (Ferreira *et al.,* 2004). In agreement with this finding it has been shown that TAG amount is decreased in the *rsp5-19* mutant which shows elevated levels of saturated fatty acids (Kaliszewski *et al.,* 2006; 2008). Overproduction of Spt23 or Mga2 devoid of transmembrane domain and constitutively active (see previous paragraph) enhanced TAG synthesis in the wild type and the *rsp5-19* mutant and led to an accumulation of unsaturated fatty acids stored within TAG. Those results indicate that Rsp5 *via* Spt23 and Mga2 affects not only the unsaturation ratio but also the TAG level. The overproduction of Spt23 or Mga2 was also accompanied by the appearance of large lipid particles in the wild type and *rsp5-19* strains, probably as a result of enhanced TAG synthesis (Kaliszewski *et al.,* 2008).

Regulation of mevalonate pathway

The mevalonate (MVA) pathway supplies the cell with sterols and isoprenoid precursors which are used to produce dolichols, prenylated proteins, ubiquinone and heme. The first step in the pathway is the synthesis of acetoacetyl-CoA by the Erg10 enzyme — acetoacetyl-CoA thiolase (see a review by Kornblatt & Rudney, 1971; Daum *et al.,* 1998). Then acetoacetyl-CoA is converted to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in a multistep reaction catalyzed by six enzymes: Erg13 — hydroxymethylglutaryl-CoA (HMGCoA) synthase, Hmg1/Hmg2 HMGCoA reductases, Erg12 MVA kinase, Erg8 phosphomevalonate kinase, Erg19 MVA pyrophosphate decarboxylase, and Idi1 isopentenyl diphosphate isomerase (see a review by Daum *et al.,* 1998) (Fig. 3). The branch point enzyme of the isoprenoid pathway is farnesyl diphosphate synthase (Erg20) which catalyses the sequential condensation of DMAPP with IPP to form geranyl diphosphate (GPP) and further farnesyl diphosphate (FPP) (Song & Poulter, 1994). DMAPP used by Erg20 is also a substrate for Mod5, a tRNA isopentenyltransferase (Dihanich *et al.,* 1987), an enzyme which is dually localized in the cytoplasm and mitochondria. It has been shown that *rsp5* mutant cells exhibit a

decreased mitochondrial pool of Mod5 as compared to wild type (Żołądek *et al.*, 1995).

The main product of the MVA pathway is ergosterol in yeast and cholesterol in humans. Ergosterol is an important component of the plasma and organellar membranes which affects their fluidity, permeability and other features. Physiological characterization of various *erg* mutants in yeast has revealed roles for sterols in endocytosis (Munn *et al.*, 1999; Heese-Peck *et al.*, 2002), lipid raft formation (Bagnat *et al.*, 2000; Umebayashi & Nakano, A. 2003), cation and amino acid uptake (Welihinda *et al.*, 1994; Umebayashi & Nakano, 2003), cell cycle regulation (Rodriguez & Parks, 1983), vacuole fusion (Kato & Wickner, 2001), and mitochondrial respiration (Parks & Casey, 1995). Ergosterol is synthesized from FPP through a cascade of enzymatic reactions (Fig. 3). In the first step squalene synthase Erg9 condenses two FPP molecules to form squalene (Jennings *et al.*, 1991). Then the first sterol molecule of the pathway is synthesized by action of two enzymes, the Erg1 squalene epoxidase (monooxygenase) which converts squalene to 2,3-oxidosqualene (Jahnke & Klein, 1983), and 2,3-oxidosqualene cyclase Erg7 which converts it to lanosterol (Corey *et al.*, 1994; Shi *et al.*, 1994). Lanosterol conversion to zymosterol and further to ergosterol is catalyzed by ten other enzymes (Erg 11, Erg24, Erg25, Erg26, Erg27, Erg6, Erg2, Erg3, Erg5, Erg4) (Fig. 3; see a review by Daum *et al.*, 1998). Ergosterol exists in free and esterified forms (SE). SE are synthesized by two enzymes Are1 and Are2 which form SE from sterols and activated fatty acids (Yu *et al.*, 1996; Yang *et al.*, 1996). Esterified sterols are strongly hydrophobic and form the core of lipid particles (reviewed by Czabany *et al.*, 2007).

Sterol depletion in mammalian cells causes activation of the transcription factors known as sterol regulatory element (SRE)-binding proteins (SREBPs)

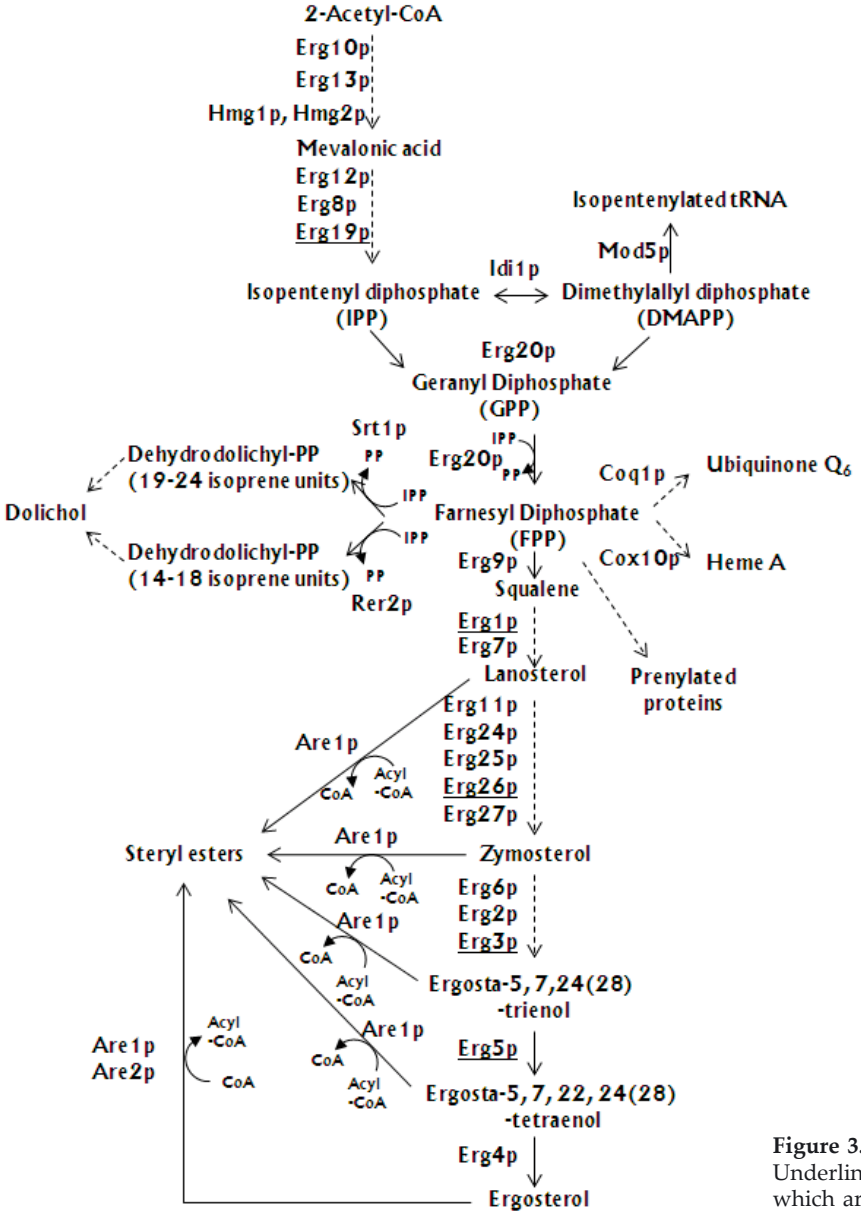

Figure 3. The mevalonate pathway.
Underlined enzymes are encoded by genes which are bound by Spt23 and Mga2.

(reviewed in Edwards *et al.,* 2000). When sterols are abundant the SREBPs are inactive and tethered to the endoplasmic reticulum membrane by their transmembrane domains. When sterol level drops, regulated proteolysis releases the transcrpitional activation domain of SREBPs allowing its nuclear transport. SREBPs activate transcription of genes involved in sterol and fatty acid synthesis. The human gene encoding FPP synthase contains a SRE sequence (Sato *et al.,* 1996). However, less is known about this regulatory mechanism in yeast. Many genes of the mevalonate pathway are transcriptionaly regulated in response to *erg* mutations, inhibitors of MVA pathway, and anaerobiosis, as determined by genome-wide expression profile analyses (Dimster-Denk *et al.,* 1999; Bammert & Fostel, 2000; Kwast *et al.,* 2002; Agarwal *et al.,* 2003). On the other hand, *ERG20* is constitutively expressed after inhibition of the downstream part of the ergosterol synthesis pathway by azoles (Bammert & Fostel, 2000; Agarwal *et al.,* 2003) and in anaerobiosis (Kwast *et al.,* 2002), and only 2–3-fold upregulated by lovastatin, an inhibitor of HMG-CoA reductase in the upstream part of the pathway (Dimster-Denk *et al.,* 1999). An about two-fold increase of *ERG20* expression was also observed in an RNA polymerase III regulatory mutant that shows enhanced tRNA synthesis (Kamińska *et al.,* 2002). Many *ERG* genes (*ERG1*, *ERG2*, *ERG3*, *ERG7*, *ERG25*, *ERG26*, and *ERG27*) are activated by the Upc2 and Ecm22 transcription factors which bind yeast SRE (Vik & Rine, 2001), and are repressed by Mot3 and Rox1 (Kwast *et al.,* 2002; Henry *et al.,* 2002). One of the enzymes of the isoprenoid pathway, HMG-CoA reductase (Hmg2), is physiologically regulated by ubiquitination and degradation in proteasomes (Gardner *et al.,* 2001). It was observed that in the *rsp5-19* mutant the level of sterols was lower compared to wild type and the steady state level of *ERG20* transcript was diminished, but this latter effect appeared to be Spt23-independent (Kamińska *et al.,* 2005). It was demonstrated that the *rsp5-19* strain had a decreased level of ergosterol and its intermediates downstream from lanosterol in the pathway (Kaliszewski *et al.,* 2008), which implies that Rsp5 may affect the level of FPP.

It has been shown that activated Spt23 and Mga2 bind to genes involved in the ergosterol biosynthetic pathway: *ERG1*, *ERG3*, *ERG5,* and *ERG26* (Auld *et al.,* 2006). Moreover, Mga2 binds to the *ERG19* gene encoding an enzyme which acts upstream of Erg20 in the ergosterol pathway (see Fig. 3). Recently it was observed that overproduction of Spt23 and Mga2 transcriptional activators increased the level of sterols in the wild type and, to a lower extent, in the *rsp5-19* mutant strain (Kaliszewski *et al.,* 2008), which led to the conclusion that Rsp5 regulates sterol synthesis *via* activation of Spt23 and Mga2 and *via* other post-activation step(s) (Kaliszewski *et al.,* 2008).

Ubiquinone, another product of the MVA pathway, is present in all cells and membranes and in addition to being a component of the mitochondrial respiratory chain it has other functions as well: it participates in extra-mitochondrial electron transport, is the only lipid-soluble antioxidant, a regulator of the physicochemical properties of membranes, etc. (see a review by Turunen *et al.,* 2004). Ubiquinone is composed of a benzoquinone moiety and an isoprenoid side chain. The number (n) of isoprene units in the polyprenyl tail (Qn) is species specific, in humans it is 10 and in *S. cerevisiae* 6. In the yeast the isoprenoid chain is formed by Coq1, a *trans*-prenyltransferase (Ashby & Edwards, 1990) which catalyzes the condensation of FPP with three IPPs, all in the *trans* configuration. The isoprenoid chain is then transfered to the benzoquinone precursor 4-hydroxybenzoic acid by Coq2 (Ashby *et al.*, 1992). The final steps of ubiquinone synthesis are subsequent ring modifications by the Coq3, Coq5, Coq6, and Coq7/Clk-1 enzymes (see a review by Turunen *et al.,* 2004).

Dolichol is a long-chain polyprenol with a saturated α-isoprene unit, and its phosphorylated form (dolichyl phosphate, Dol-P) participate in the synthesis of N- or O-glycosidically linked oligosaccharide chains of glycoproteins and in the formation of glycosylphosphatidylinositol (GPI) membrane anchors (Herscovics & Orlean, 1993; reviewed by Grabinska & Palamarczyk, 2002). Dolichol synthesis is catalyzed by a *cis*-prenyltransferase enzyme which catalyses successive condensations of IPP with FPP in the *cis* configuration to form long-chain polyprenyl diphosphate which is further converted to dolichol by dephosphorylation and saturation of the α-isoprene unit (Chojnacki & Dallner, 1988; Sagami *et al.,* 1993; 1996). There are two *cis*-prenyltransferases, Rer2 and Srt1, in yeast (Sato *et al.,* 2001). The polyprenol product of Srt1 is longer (19–24 isoprene units) than that of Rer2 (14–18 isoprene units) (Sato *et al.,* 2001). The expression of these two *cis*-prenyltransferases is differently regulated during the yeast life cycle. The cellular level of Srt1 is maximal in the late-logarithmic and stationary phases, while the level of Rer2 is the highest in the early logarithmic phase (Sato *et al.,* 2001).

It was shown that in the *rsp5-19* mutant strain, in addition to a decreased sterol level, the level of dolichols and ubiquinone was also decreased (Kaliszewski *et al.*, 2008). This suggests that the synthesis of FPP (the common substrate for these products, see Fig. 3) could be diminished by the *rsp5-19* mutation. Moreover, the Spt23 and Mga2 transcriptional activators appeared to play a role in the regulation of dolichol synthesis. The level of dolichols

was decreased in *rsp5-19* and wild type strain overproducing Spt23 or Mga2, which could be an effect of enhanced utilization of FPP in sterol synthesis (Kaliszewski *et al.*, 2008). Moreover, the Spt23 and Mga2 factors affected the synthesis of long chain dolichols, products of the Srt1 *cis*-prenyl transferase (Kaliszewski *et al*., 2008). Spt23 or Mga2 overproduction resulted in lowering of the pool of long-chain dolichols from 30% to 3–5% of total polyprenols. Similarly, the *rsp5-19* strain transformed with plasmids encoding Spt23 or Mga2 exhibited no Srt1 activity (Kaliszewski *et al.*, 2008). These results indicate that Rsp5 together with Spt23 and Mga2 have broad physiological effects on lipid homeostasis.

CONCLUSIONS

Ubiqutination is an extensively studied process in yeast and mammalian cells. It has been shown that deregulation of this pathway is implicated in pathogenesis of many diseases, including neurodegenerative diseases and cancer. Therefore, discovering new functions of highly homologous ligases, such as Rsp5 in yeast, provides useful information which can be easily utilized in the deciphering of similar processes in higher eukaryotes. Moreover, the knowledge of lipid synthesis regulation can be used to construct yeast strains with high lipid content which can be useful in biotechnology.

Acknowledgements

The laboratory work was supported by grant 0137/P01/2006/31 from the Ministry of Science and Higher Education (Poland to T.Ż. and P.K.) and by a Collaborative Experimental Scholarship for Central and Eastern Europe from FEBS to P.K.

REFERENCES

Agarwal AK, Rogers PD, Baerson SR, Jacob MR, Barker KS, Cleary JD, Walker LA, Nagle DG, Clark AM (2003) Genome-wide expression profiling of the response to polyene, pyrimidine, azole, and echinocandin antifungal agents in *Saccharomyces cerevisiae*. *J Biol Chem* **278**: 34998–35015.

Altheim BA, Schultz MC (1999) Histone modification governs the cell cycle regulation of a replication-independent chromatin assembly pathway in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **96**: 1345–1350.

Arnason T, Ellison MJ (1994) Stress resistance in *Saccharomyces cerevisiae* is strongly correlated with assembly of a novel type of multiubiquitin chain. *Mol Cell Biol* **14**: 7876–7883.

Arnason TG, Pisclevich MG, Dash MD, Davies GF, Harkness TA (2005) Novel interaction between Apc5p and Rsp5p in an intracellular signaling pathway in *Saccharomyces cerevisiae. Eukaryot Cell* **4**: 134–146.

Ashby MN, Edwards PA (1990) Elucidation of the deficiency in two yeast coenzyme Q mutants. Characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase. *J Biol Chem* **265**: 13157–13164.

Ashby MN, Kutsunai SY, Ackerman S, Tzagoloff A, Edwards PA (1992) COQ2 is a candidate for the structural gene encoding *para*-hydroxybenzoate:polyprenyltransferase. *J Biol Chem* **267**: 4128–4136.

Auld KL, Brown CR, Casolari JM, Komili S, Silver PA (2006) Genomic association of the proteasome demonstrates overlapping gene regulatory activity with transcription factor substrates. *Mol Cell* **21**: 861–871.

Bagnat M, Keranen S, Shevchenko A, Simons K (2000) Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. *Proc Natl Acad Sci USA* **97**: 3254–3259.

Bammert GF, Fostel JM (2000) Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. *Antimicrob Agents Chemother* **44**: 1255–1265.

Beaudenon SL, Huacani MR, Wang G, McDonnell DP, Huibregtse JM (1999) Rsp5 ubiquitin-protein ligase mediates DNA damage-induced degradation of the large subunit of RNA polymerase II in *Saccharomyces cerevisiae*. *Mol Cell Biol* **19**: 6972–6979.

Beck T, Schmidt A, Hall MN (1999) Starvation induces vacuolar targeting and degradation of the tryptophan permease in yeast. *J Cell Biol* **146**: 1227–1238.

Bedford MT, Sarbassova D, Xu J, Leder P, Yaffe MB (2000) A novel pro-Arg motif recognized by WW domains. *J Biol Chem* **275**: 10359–10369.

Bhattacharya S, Żołądek T, Haines DS (2008) WW domains 2 and 3 of Rsp5p play overlapping roles in binding to the LPKY motif of Spt23p and Mga2p. *Int J Biochem Cell Biol* **40**: 147–157.

Bogdanov M, Sun J, Kaback HR, Dowhan W (1996) A phospholipid acts as a chaperone in assembly of a membrane transport protein. *J Biol Chem* **271**: 11615–11618.

Bogdanov M, Umeda M, Dowhan W (1999) Phospholipid-assisted refolding of an integral membrane protein. Minimum structural features for phosphatidylethanolamine to act as a molecular chaperone. *J Biol Chem* **274**: 12339–12345.

Bremer J, Greenberg DM (1960) Biosynthesis of choline *in vitro*. *Biochim Biophys Acta* **37**: 173–175.

Carman GM, Henry SA (1989) Phospholipid biosynthesis in yeast. *Annu Rev Biochem* **58**: 635–669.

Carman GM, Henry SA (1999) Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. *Prog Lipid Res* **38**: 361–399.

Carman GM, Han GS (2007) Regulation of phospholipid synthesis in *Saccharomyces cerevisiae* by zinc depletion. *Biochim Biophys Acta* **1771**: 322–330.

Carratu L, Franceschelli S, Pardini CL, Kobayashi GS, Horvath I, Vigh L, Maresca B (1996) Membrane lipid perturbation modifies the set point of the temperature of heat shock response in yeast. *Proc Natl Acad Sci USA* **93**: 3870–3875.

Carter JR, Kennedy EP (1966) Enzymatic synthesis of cytidine diphosphate diglyceride. *J Lipid Res* **7**: 678–683.

Chang A, Cheang S, Espanel X, Sudol M (2000) Rsp5 WW domains interact directly with the carboxyl-terminal domain of RNA polymerase II. *J Biol Chem* **275**: 20562–20571.

Chavez S, Beilharz T, Rondon AG, Erdjument-Bromage H, Tempst P, Svejstrup JQ, Lithgow T, Aguilera A (2000)

A protein complex containing Tho2 Hpr1 Mft1 and a novel protein Thp2 connects transcription elongation with mitotic recombination in *Saccharomyces cerevisiae*. *EMBO J* **19**: 5824–5834.

Cho W (2001) Membrane targeting by C1 and C2 domains. *J Biol Chem* **276**: 32407–32410.

Choi JY, Stukey J, Hwang SY, Martin CE (1996) Regulatory elements that control transcription activation and unsaturated fatty acid-mediated repression of the *Saccharomyces cerevisiae* OLE1 gene. *J Biol Chem* **271**: 3581–3589.

Chojnacki T, Dallner G (1988) The biological role of dolichol. *Biochem J* **251**: 1–9.

Clancey CJ, Chang SC, Dowhan W (1993) Cloning of a gene (PSD1) encoding phosphatidylserine decarboxylase from *Saccharomyces cerevisiae* by complementation of an *Escherichia coli* mutant. *J Biol Chem* **268**: 24580–24590.

Corey EJ, Matsuda SP, Bartel B (1994) Molecular cloning characterization and overexpression of ERG7 the *Saccharomyces cerevisiae* gene encoding lanosterol synthase. *Proc Natl Acad Sci USA* **91**: 2211–2215.

Czabany T, Athenstaedt K, Daum G (2007) Synthesis storage and degradation of neutral lipids in yeast. *Biochim Biophys Acta* **1771**: 299–309.

Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H, Stymne S (2000) Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. *Proc Natl Acad Sci USA* **97**: 6487–6492.

Daum G, Lees ND, Bard M, Dickson R (1998) Biochemistry cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*. *Yeast* **14**: 1471–1510.

de la Fuente N, Maldonado AM, Portillo F (1997) Yeast gene YOR137c is involved in the activation of the yeast plasma membrane H+-ATPase by glucose. *FEBS Lett* **420**: 17–19.

Dean-Johnson M, Henry SA (1989) Biosynthesis of inositol in yeast. Primary structure of myo-inositol-1-phosphate synthase (EC 5.5.1.4) and functional analysis of its structural gene the INO1 locus. *J Biol Chem* **264**: 1274–1283.

Dihanich ME, Najarian D, Clark R, Gillman EC, Martin NC, Hopper AK (1987) Isolation and characterization of MOD5 a gene required for isopentenylation of cytoplasmic and mitochondrial tRNAs of *Saccharomyces cerevisiae*. *Mol Cell Biol* **7**: 177–184.

Dimster-Denk D, Rine J, Phillips J, Scherer S, Cundiff P, DeBord K, Gilliland D, Hickman S, Jarvis A, Tong L, Ashby M (1999) Comprehensive evaluation of isoprenoid biosynthesis regulation in *Saccharomyces cerevisiae* utilizing the Genome Reporter Matrix. *J Lipid Res* **40**: 850–860.

Dowhan W (1997) Molecular basis for membrane phospholipid diversity: why are there so many lipids? *Annu Rev Biochem* **66**: 199–232.

Dowhan W, Mileykovskaya E, Bogdanov M (2004) Diversity and versatility of lipid-protein interactions revealed by molecular genetic approaches. *Biochim Biophys Acta* **1666**: 19–39.

Dunn R, Hicke L (2001) Multiple roles for Rsp5p-dependent ubiquitination at the internalization step of endocytosis. *J Biol Chem* **276**: 25974–25981.

Dunn R, Klos DA, Adler AS, Hicke L (2004) The C2 domain of the Rsp5 ubiquitin ligase binds membrane phosphoinositides and directs ubiquitination of endosomal cargo. *J Cell Biol* **165**: 135–144.

Edwards PA, Tabor D, Kast HR, Venkateswaran A (2000) Regulation of gene expression by SREBP and SCAP. *Biochim Biophys Acta* **1529**: 103–113.

Exton JH (1994) Messenger molecules derived from membrane lipids. *Curr Opin Cell Biol* **6**: 226–229.

Fankhauser C, Homans SW, Thomas-Oates JE, McConville MJ, Desponds C, Conzelmann A, Ferguson MA (1993) Structures of glycosylphosphatidylinositol membrane anchors from *Saccharomyces cerevisiae*. *J Biol Chem* **268**: 26365–26374.

Ferguson SB, Anderson ES, Harshaw RB, Thate T, Craig NL, Nelson HC (2005) Protein kinase A regulates constitutive expression of small heat-shock genes in an Msn2/4p-independent and Hsf1p-dependent manner in *Saccharomyces cerevisiae*. *Genetics* **169**: 1203–1214.

Ferreira T, Regnacq M, Alimardani P, Moreau-Vauzelle C, Berges T (2004) Lipid dynamics in yeast under haem-induced unsaturated fatty acid and/or sterol depletion. *Biochem J* **378**: 899–908.

Fisk HA, Yaffe MP (1999) A role for ubiquitination in mitochondrial inheritance in *Saccharomyces cerevisiae*. *J Cell Biol* **145**: 1199–1208.

Gajewska B, Kaminska J, Jesionowska A, Martin NC, Hopper AK, Żołądek T (2001) WW domains of Rsp5p define different functions: determination of roles in fluid phase and uracil permease endocytosis in *Saccharomyces cerevisiae*. *Genetics* **157**: 91–101.

Galan JM, Moreau V, Andre B, Volland C, Haguenauer-Tsapis R (1996) Ubiquitination mediated by the Npi1p/Rsp5p ubiquitin-protein ligase is required for endocytosis of the yeast uracil permease. *J Biol Chem* **271**: 10946–10952.

Gardner RG, Shearer AG, Hampton RY (2001) *In vivo* action of the HRD ubiquitin ligase complex: mechanisms of endoplasmic reticulum quality control and sterol regulation. *Mol Cell Biol* **21**: 4276–4291.

Gitan RS, Eide DJ (2000) Zinc-regulated ubiquitin conjugation signals endocytosis of the yeast ZRT1 zinc transporter. *Biochem J* **346** Pt 2: 329–336.

Gonzalez CI, Martin CE (1996) Fatty acid-responsive control of mRNA stability. Unsaturated fatty acid-induced degradation of the *Saccharomyces* OLE1 transcript. *J Biol Chem* **271**: 25801–25809.

Grabinska K, Palamarczyk G (2002) Dolichol biosynthesis in the yeast *Saccharomyces cerevisiae*: an insight into the regulatory role of farnesyl diphosphate synthase. *FEMS Yeast Res* **2**: 259–265.

Gupta R, Kus B, Fladd C, Wasmuth J, Tonikian R, Sidhu S, Krogan NJ, Parkinson J, Rotin D (2007) Ubiquitination screen using protein microarrays for comprehensive identification of Rsp5 substrates in yeast. *Mol Syst Biol* **3**: 116.

Gwizdek C, Hobeika M, Kus B, Ossareh-Nazari B, Dargemont C, Rodriguez MS (2005) The mRNA nuclear export factor Hpr1 is regulated by Rsp5-mediated ubiquitylation. *J Biol Chem* **280**: 13401–13405.

Haitani Y, Takagi H (2008) Rsp5 is required for the nuclear export of mRNA of HSF1 and MSN2/4 under stress conditions in *Saccharomyces cerevisiae*. *Genes Cells* **13**: 105–116.

Haitani Y, Shimoi H, Takagi H (2006) Rsp5 regulates expression of stress proteins via post-translational modification of Hsf1 and Msn4 in *Saccharomyces cerevisiae*. *FEBS Lett* **580**: 3433–3438.

Hamilton KL, Butt AG (2000) The molecular basis of renal tubular transport disorders. *Comp Biochem Physiol A Mol Integr Physiol* **126**: 305–321.

Harkness TA, Davies GF, Ramaswamy V, Arnason TG (2002) The ubiquitin-dependent targeting pathway in

Saccharomyces cerevisiae plays a critical role in multiple chromatin assembly regulatory steps. *Genetics* **162**: 615–632.

Harvey KF, Kumar S (1999) Nedd4-like proteins: an emerging family of ubiquitin-protein ligases implicated in diverse cellular functions. *Trends Cell Biol* **9**: 166–169.

Hashikawa N, Sakurai H (2004) Phosphorylation of the yeast heat shock transcription factor is implicated in gene-specific activation dependent on the architecture of the heat shock element. *Mol Cell Biol* **24**: 3648–3659.

Heese-Peck A, Pichler H, Zanolari B, Watanabe R, Daum G, Riezman H (2002) Multiple functions of sterols in yeast endocytosis. *Mol Biol Cell* **13**: 2664–2680.

Hein C, André B (1997) A C-terminal di-leucine motif and nearby sequences are required for NH^{4+}-induced inactivation and degradation of the general amino acid permease Gap1p of *Saccharomyces cerevisiae*. *Mol Microbiol* **24**: 607–616.

Helliwell SB, Losko S, Kaiser CA (2001) Components of a ubiquitin ligase complex specify polyubiquitination and intracellular trafficking of the general amino acid permease. *J Cell Biol* **153**: 649–662.

Henry KW, Nickels JT, Edlind TD (2002) *ROX1* and *ERG* regulation in *Saccharomyces cerevisiae*: implications for antifungal susceptibility. *Eukaryot Cell* **1**: 1041–1044.

Herscovics A, Orlean P (1993) Glycoprotein biosynthesis in yeast. *FASEB J* **7**: 540–550.

Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* **67**: 425–479.

Hicke L (2001) Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol* **2**: 195–201.

Hochstrasser M (1996) Ubiquitin-dependent protein degradation. *Annu Rev Genet* **30**: 405–439.

Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD, Jentsch S (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell* **102**: 577–586.

Huang L, Kinnucan E, Wang G, Beaudenon S, Howley PM, Huibregtse JM, Pavletich NP (1999) Structure of an E6AP–UbcH7 complex: insights into ubiquitination by the E2–E3 enzyme cascade. *Science* **286**: 1321–1326.

Huibregtse JM, Scheffner M, Beaudenon S, Howley PM (1995) A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc Natl Acad Sci USA* **92**: 5249.

Hurley JH, Misra S (2000) Signaling and subcellular targeting by membrane-binding domains. *Annu Rev Biophys Biomol Struct* **29**: 49–79.

Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, Noda T, Ohsumi Y (2000) A ubiquitin-like system mediates protein lipidation. *Nature* **408**: 488–492.

Ingham RJ, Gish G, Pawson T (2004) The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. *Oncogene* **23**: 1972–1984.

Jackson PK, Eldridge AG, Freed E, Furstenthal L, Hsu JY, Kaiser BK, Reimann JD (2000) The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases. *Trends Cell Biol* **10**: 429–439.

Jahnke L, Klein HP (1983) Oxygen requirements for formation and activity of the squalene epoxidase in *Saccharomyces cerevisiae*. *J Bacteriol* **155**: 488–492.

Jennings SM, Tsay YH, Fisch TM, Robinson GW (1991) Molecular cloning and characterization of the yeast gene for squalene synthetase. *Proc Natl Acad Sci USA* **88**: 6038–6042.

Kaliszewski P, Ferreira T, Gajewska B, Szkopinska A, Berges T, Żołądek T (2006) Enhanced levels of Pis1p (phosphatidylinositol synthase) improve the growth of *Saccharomyces cerevisiae* cells deficient in Rsp5 ubiquitin ligase. *Biochem J* **395**: 173–181.

Kaliszewski P, Szkopinska A, Ferreira T, Swiezewska E, Berges T, Żołądek T (2008) Rsp5p ubiquitin ligase and the transcriptional activators Spt23p and Mga2p are involved in co-regulation of biosynthesis of end products of the mevalonate pathway and triacylglycerol in yeast *Saccharomyces cerevisiae. Biochim Biophys Acta* **1781**: 627–634.

Kaminska J, Tobiasz A, Gniewosz M, Żołądek T (2000) The growth of mdp1/rsp5 mutants of *Saccharomyces cerevisiae* is affected by mutations in the ATP-binding domain of the plasma membrane H+-ATPase. *Gene* **242**: 133–140.

Kaminska J, Grabinska K, Kwapisz M, Sikora J, Smagowicz WJ, Palamarczyk G, Żołądek T, Boguta M (2002) The isoprenoid biosynthetic pathway in *Saccharomyces cerevisiae* is affected in a maf1-1 mutant with altered tRNA synthesis. *FEMS Yeast Res* **2**: 31–37.

Kaminska J, Kwapisz M, Grabinska K, Orlowski J, Boguta M, Palamarczyk G, Żołądek T (2005) Rsp5 ubiquitin ligase affects isoprenoid pathway and cell wall organization in *S. cerevisiae*. *Acta Biochim Polon* **52**: 207–220.

Kato M, Wickner W (2001) Ergosterol is required for the Sec18/ATP-dependent priming step of homotypic vacuole fusion. *EMBO J* **20**: 4035–4040.

Katzmann DJ, Babst M, Emr SD (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex ESCRT-I. *Cell* **106**: 145–155.

Katzmann DJ, Odorizzi G, Emr SD (2002) Receptor down-regulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol* **3**: 893–905.

Katzmann DJ, Sarkar S, Chu T, Audhya A, Emr SD (2004) Multivesicular body sorting: ubiquitin ligase Rsp5 is required for the modification and sorting of carboxypeptidase S. *Mol Biol Cell* **15**: 468–480.

Kiyono K, Miura K, Kushima Y, Hikiji T, Fukushima M, Shibuya I, Ohta A (1987) Primary structure and product characterization of the *Saccharomyces cerevisiae* CHO1 gene that encodes phosphatidylserine synthase. *J Biochem* **102**: 1089–1100.

Klig LS, Henry SA (1984) Isolation of the yeast INO1 gene: located on an autonomously replicating plasmid the gene is fully regulated. *Proc Natl Acad Sci USA* **81**: 3816–3820.

Kornblatt JA, Rudney H (1971) Two forms of acetoacetyl coenzyme A thiolase in yeast. I Separation and properties. *J Biol Chem* **246**: 4417–4423.

Kumar S, Tomooka Y, Noda M (1992) Identification of a set of genes with developmentally down-regulated expression in the mouse brain. *Biochem Biophys Res Commun* **185**: 1155–1161.

Kwapisz M, Cholbinski P, Hopper AK, Rousset JP, Żołądek T (2005) Rsp5 ubiquitin ligase modulates translation accuracy in yeast *Saccharomyces cerevisiae*. *RNA* **11**: 1710–1718.

Kwast KE, Lai L-C, Menda N, James DT 3rd, Aref S, Burke PV (2002) Genomic analyses of anaerobically induced genes in *Saccharomyces cerevisiae*: functional roles of Rox1 and other factors in mediating the anoxic response. *J Bacteriol* **184**: 250–265.

Lester RL, Dickson RC (1993) Sphingolipids with inositolphosphate-containing head groups. *Adv Lipid Res* **26**: 253–274.

Lindsten K, de Vrij FM, Verhoef LG, Fischer DF, van Leeuwen FW, Hol EM, Masucci MG, Dantuma NP (2002) Mutant ubiquitin found in neurodegenerative disorders

is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. *J Cell Biol* **157**: 417–427.
Lu PJ, Zhou XZ, Shen M, Lu KP (1999) Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science* **283**: 1325–1328.
Lu JY, Lin YY, Qian J, Tao SC, Zhu J, Pickart C, Zhu H (2008) Functional dissection of a HECT ubiquitin E3 ligase. *Mol Cell Proteomics* **7**: 35–45.
Macias MJ, Hyvonen M, Baraldi E, Schultz J, Sudol M, Saraste M, Oschkinat H (1996) Structure of the WW domain of a kinase-associated protein complexed with a proline-rich peptide. *Nature* **382**: 646–649.
Medintz I, Jiang H, Michels CA (1998) The role of ubiquitin conjugation in glucose-induced proteolysis of *Saccharomyces* maltose permease. *J Biol Chem* **273**: 34454–34462.
Morvan J, Froissard M, Haguenauer-Tsapis R, Urban-Grimal D (2004) The ubiquitin ligase Rsp5p is required for modification and sorting of membrane proteins into multivesicular bodies. *Traffic* **5**: 383–392.
Munn AL, Heese-Peck A, Stevenson BJ, Pichler H, Riezman H (1999) Specific sterols required for the internalization step of endocytosis in yeast. *Mol Biol Cell* **10**: 3943–3957.
Murray M, Greenberg ML (2000) Expression of yeast INM1 encoding inositol monophosphatase is regulated by inositol carbon source and growth stage and is decreased by lithium and valproate. *Mol Microbiol* **36**: 651–661.
Nakagawa Y, Sakumoto N, Kaneko Y, Harashima S (2002) Mga2p is a putative sensor for low temperature and oxygen to induce OLE1 transcription in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* **291**: 707–713.
Nalefski EA, Falke JJ (1996) The C2 domain calcium-binding motif: structural and functional diversity. *Protein Sci* **5**: 2375–2390.
Neumann S, Petfalski E, Brugger B, Grosshans H, Wieland F, Tollervey D, Hurt E (2003) Formation and nuclear export of tRNA rRNA and mRNA is regulated by the ubiquitin ligase Rsp5p. *EMBO Rep* **4**: 1156–1162.
Nikawa J, Yamashita S (1984) Molecular cloning of the gene encoding CDPdiacylglycerol–inositol 3-phosphatidyl transferase in *Saccharomyces cerevisiae*. *Eur J Biochem* **143**: 251–256.
Nikawa J, Tsukagoshi Y, Kodaki T, Yamashita S (1987) Nucleotide sequence and characterization of the yeast PSS gene encoding phosphatidylserine synthase. *Eur J Biochem* **167**: 7–12.
Ntambi JM (1999) Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J Lipid Res* **40**: 1549–1558.
Oelkers P, Tinkelenberg A, Erdeniz N, Cromley D, Billheimer JT, Sturley SL (2000) A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. *J Biol Chem* **275**: 15609–15612.
Oelkers P, Cromley D, Padamsee M, Billheimer JT, Sturley SL (2002) The DGA1 gene determines a second triglyceride synthetic pathway in yeast. *J Biol Chem* **277**: 8877–8881.
Parks LW, Casey WM (1995) Physiological implications of sterol biosynthesis in yeast. *Annu Rev Microbiol* **49**: 95–116.
Paulus H, Kennedy EP (1960) The enzymatic synthesis of inositol monophosphatide. *J Biol Chem* **235**: 1303–1311.
Piper RC, Katzmann DJ (2007) Biogenesis and function of multivesicular bodies. *Annu Rev Cell Dev Biol* **23**: 519–547.
Pizzirusso M, Chang A (2004) Ubiquitin-mediated targeting of a mutant plasma membrane ATPase Pma1-7 to the endosomal/vacuolar system in yeast. *Mol Biol Cell* **15**: 2401–2409.
Raiborg C, Rusten TE, Stenmark H (2003) Protein sorting into multivesicular endosomes. *Curr Opin Cell Biol* **15**: 446–455.
Rattray JB, Schibeci A, Kidby DK (1975) Lipids of yeasts. *Bacteriol Rev* **39**: 197–231.
Reggiori F, Pelham HR (2001) Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J* **20**: 5176–5186.
Reid J, Svejstrup JQ (2004) DNA damage-induced Def1–RNA polymerase II interaction and Def1 requirement for polymerase ubiquitylation *in vitro*. *J Biol Chem* **279**: 29875–29878.
Rodriguez RJ, Parks LW (1983) Structural and physiological features of sterols necessary to satisfy bulk membrane and sparking requirements in yeast sterol auxotrophs. *Arch Biochem Biophys* **225**: 861–871.
Rodriguez MS, Gwizdek C, Haguenauer-Tsapis R, Dargemont C (2003) The HECT ubiquitin ligase Rsp5p is required for proper nuclear export of mRNA in *Saccharomyces cerevisiae*. *Traffic* **4**: 566–575.
Rotin D, Staub O, Haguenauer-Tsapis R (2000) Ubiquitination and endocytosis of plasma membrane proteins: role of Nedd4/Rsp5p family of ubiquitin-protein ligases. *J Membr Biol* **176**: 1–17.
Sagami H, Igarashi Y, Tateyama S, Ogura K, Roos J, Lennarz WJ (1996) Enzymatic formation of dehydrodolichal and dolichal, new products related to yeast dolichol biosynthesis. *J Biol Chem* **271**: 9560–9566.
Sagami H, Korenaga T, Ogura K (1993) Geranylgeranyl diphosphate synthase catalyzing the single condensation between isopentenyl diphosphate and farnesyl diphosphate. *J Biochem* **114**: 118–121.
Sandager L, Gustavsson MH, Stahl U, Dahlqvist A, Wiberg E, Banas A, Lenman M, Ronne H, Stymne S (2002) Storage lipid synthesis is non-essential in yeast. *J Biol Chem* **277**: 6478–6482.
Sato R, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M (1996) Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. *J Biol Chem* **271**: 26461–26464.
Sato M, Fujisaki S, Sato K, Nishimura Y, Nakano A (2001) Yeast *Saccharomyces cerevisiae* has two *cis*-prenyltransferases with different properties and localizations. Implication for their distinct physiological roles in dolichol synthesis. *Genes Cells* **6**: 495–506.
Schneiter R, Kohlwein SD (1997) Organelle structure function and inheritance in yeast: a role for fatty acid synthesis? *Cell* **88**: 431–434.
Segura-Morales C, Pescia C, Chatellard-Causse C, Sadoul R, Bertrand E, Basyuk E (2005) Tsg101 and Alix interact with murine leukemia virus Gag and cooperate with Nedd4 ubiquitin ligases during budding. *J Biol Chem* **280**: 27004–27012.
Shcherbik N, Żołądek T, Nickels JT, Haines DS (2003) Rsp5p is required for ER bound Mga2p120 polyubiquitination and release of the processed/tethered transactivator Mga2p90. *Curr Biol* **13**: 1227–1233.
Shcherbik N, Kee Y, Lyon N, Huibregtse JM, Haines DS (2004) A single PXY motif located within the carboxyl terminus of Spt23p and Mga2p mediates a physical and functional interaction with ubiquitin ligase Rsp5p. *J Biol Chem* **279**: 53892–53898.
Shearwin-Whyatt L, Dalton HE, Foot N, Kumar S (2006) Regulation of functional diversity within the Nedd4 family by accessory and adaptor proteins. *BioEssays* **28**: 617–628.
Shen H, Dowhan W (1996) Reduction of CDP-diacylglycerol synthase activity results in the excretion of inosi-

tol by *Saccharomyces cerevisiae*. *J Biol Chem* **271**: 29043–29048.

Shi Z, Buntel CJ, Griffin JH (1994) Isolation and characterization of the gene encoding 23-oxidosqualene-lanosterol cyclase from *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **91**: 7370–7374.

Somesh BP, Sigurdsson S, Saeki H, Erdjument-Bromage H, Tempst P, Svejstrup JQ (2007) Communication between distant sites in RNA polymerase II through ubiquitylation factors and the polymerase CTD. *Cell* **129**: 57–68.

Song L, Poulter CD (1994) Yeast farnesyl-diphosphate synthase: site-directed mutagenesis of residues in highly conserved prenyltransferase domains I and II. *Proc Natl Acad Sci USA* **91**: 3044–3048.

Sorger D, Daum G (2002). Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast *Saccharomyces cerevisiae*. *J Bacteriol* **184**: 519–524.

Sorger D, Daum G (2003) Triacylglycerol biosynthesis in yeast. *Appl Microbiol Biotechnol* **61**: 289–299.

Sorger D, Athenstaedt K, Hrastnik C, Daum G (2004) A yeast strain lacking lipid particles bears a defect in ergosterol formation. *J Biol Chem* **279**: 31190–31196.

Springael JY, De Craene JO, Andre B (1999a) The yeast Npi1/Rsp5 ubiquitin ligase lacking its N-terminal C2 domain is competent for ubiquitination but not for subsequent endocytosis of the gap1 permease. *Biochem Biophys Res Commun* **257**: 561–566.

Springael JY, Galan JM, Haguenauer-Tsapis R, Andre B (1999b) NH^{4+}-induced down-regulation of the *Saccharomyces cerevisiae* Gap1p permease involves its ubiquitination with lysine-63-linked chains. *J Cell Sci* **112**: 1375–1383.

Stawiecka-Mirota M, Pokrzywa W, Morvan J, Żołądek T, Haguenauer-Tsapis R, Urban-Grimal D, Morsomme P (2007) Targeting of Sna3p to the endosomal pathway depends on its interaction with Rsp5p and multivesicular body sorting on its ubiquitylation. *Traffic* **8**: 1280–1296.

Stewart LC, Yaffe MP (1991) A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J Cell Biol* **115**: 1249–1257.

Strasser K, Masuda S, Mason P, Pfannstiel J, Oppizzi M, Rodriguez-Navarro S, Rondon AG, Aguilera A, Struhl K, Reed R, Hurt E (2002) TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* **417**: 304–308.

Stukey JE, McDonough VM, Martin CE (1989) Isolation and characterization of OLE1 a gene affecting fatty acid desaturation from *Saccharomyces cerevisiae*. *J Biol Chem* **264**: 16537–16544.

Sudol M (1996) Structure and function of the WW domain. *Prog Biophys Mol Biol* **65**: 113–132.

Sullivan JA, Lewis MJ, Nikko E, Pelham HR (2007) Multiple interactions drive adaptor-mediated recruitment of the ubiquitin ligase rsp5 to membrane proteins *in vivo* and *in vitro*. *Mol Biol Cell* **18**: 2429–2440.

Trotter PJ, Voelker DR (1995) Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (PSD2) in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* **270**: 6062–6070.

Turunen M, Olsson J, Dallner G (2004) Metabolism and function of coenzyme Q. *Biochim Biophys Acta* **1660**: 171–199.

Umebayashi K, Nakano A (2003) Ergosterol is required for targeting of tryptophan permease to the yeast plasma membrane. *J Cell Biol* **161**: 1117–1131.

Urbanowski JL, Piper RC (2001) Ubiquitin sorts proteins into the intralumenal degradative compartment of the late-endosome/vacuole. *Traffic* **2**: 622–630.

Vasconcelles MJ, Jiang Y, McDaid K, Gilooly L, Wretzel S, Porter DL, Martin CE, Goldberg MA (2001) Identification and characterization of a low oxygen response element involved in the hypoxic induction of a family of *Saccharomyces cerevisiae* genes. Implications for the conservation of oxygen sensing in eukaryotes. *J Biol Chem* **276**: 14374–14384.

Vik A, Rine J (2001) Upc2p and Ecm22p dual regulators of sterol biosynthesis in *Saccharomyces cerevisiae*. *Mol Cell Biol* **21**: 6395–6405.

Wang G, Yang J, Huibregtse JM (1999) Functional domains of the Rsp5 ubiquitin-protein ligase. *Mol Cell Biol* **19**: 342–352.

Wang X, Trotman LC, Koppie T, Alimonti A, Chen Z, Gao Z, Wang J, Erdjument-Bromage H, Tempst P, Cordon-Cardo C, Pandolfi PP, Jiang X (2007) NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN. *Cell* **128**: 129–139.

Weissman AM (2001) Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol* **2**: 169–178.

Welihinda AA, Beavis AD, Trumbly RJ (1994) Mutations in LIS1 (ERG6) gene confer increased sodium and lithium uptake in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1193**: 107–117.

Yang H, Bard M, Bruner DA, Gleeson A, Deckelbaum RJ, Aljinovic G, Pohl TM, Rothstein R, Sturley SL (1996) Sterol esterification in yeast: a two-gene process. *Science* **272**: 1353–1356.

Yu C, Kennedy NJ, Chang CC, Rothblatt JA (1996) Molecular cloning and characterization of two isoforms of *Saccharomyces cerevisiae* acyl-CoA : sterol acyltransferase. *J Biol Chem* **271**: 24157–24163.

Zenklusen D, Vinciguerra P, Wyss JC, Stutz F (2002) Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p. *Mol Cell Biol* **22**: 8241–8253.

Zhang S, Skalsky Y, Garfinkel DJ (1999) *MGA2* or *SPT23* is required for transcription of the Δ9 fatty acid desaturase gene, *OLE1* and nuclear membrane integrity in *Saccharomyces cerevisiae*. *Genetics* **151**: 473–483.

Żołądek T, Vaduva G, Hunter LA, Boguta M, Go BD, Martin NC, Hopper AK (1995) Mutations altering the mitochondrial-cytoplasmic distribution of Mod5p implicate the actin cytoskeleton and mRNA 3′ ends and/or protein synthesis in mitochondrial delivery. *Mol Cell Biol* **15**: 6884–6894.

Żołądek T, Tobiasz A, Vaduva G, Boguta M, Martin NC, Hopper AK (1997) MDP1 a *Saccharomyces cerevisiae* gene involved in mitochondrial/cytoplasmic protein distribution is identical to the ubiquitin-protein ligase gene RSP5. *Genetics* **145**: 595–603.