REVIEW ARTICLE

The essence of yeast quiescence

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Received 28 February 2011; accepted 19 May 2011. Final version published online 14 July 2011.

DOI:10.1111/j.1574-6976.2011.00287.x

Editor: Martin Kupiec

Keywords
quiescence; nutrient-signaling pathways; target of rapamycin complex 1 (TORC1); protein kinase A (PKA).

Abstract

Like all microorganisms, yeast cells spend most of their natural lifetime in a reversible, quiescent state that is primarily induced by limitation for essential nutrients. Substantial progress has been made in defining the features of quiescent cells and the nutrient-signaling pathways that shape these features. A view that emerges from the wealth of new data is that yeast cells dynamically configure the quiescent state in response to nutritional challenges by using a set of key nutrient-signaling pathways, which (1) regulate pathway-specific effectors, (2) converge on a few regulatory nodes that bundle multiple inputs to communicate unified, graded responses, and (3) mutually modulate their competences to transmit signals. Here, I present an overview of our current understanding of the architecture of these pathways, focusing on how the corresponding core signaling protein kinases (i.e. PKA, TORC1, Snf1, and Pho85) are wired to ensure an adequate response to nutrient starvation, which enables cells to tide over decades, if not centuries, of famine.

Introduction

Around 1800, a sailing barge carrying a consignment of bottled champagne and beer, possibly sent by France's King Louis XVI to the Russian Imperial Court, sunk in the Baltic Sea. This marked the beginning of a 200-year-long period during which the 'sleeping beauty', a member of the *Saccharomyces cerevisiae* family, rested quiescently in a beer bottle in a dark and gloomy spot of the seabed. In 2010, a diving expedition brought this 'beauty' back to daylight, where, after gentle awakening, she may unveil some of the most treasured secrets of 18th-century beer brewing (Neuhaus, 2010).

As this modern fairy tale exemplifies, quiescent yeast cells, which, by (a controversial) analogy to terminally differentiated mammalian cells, are often referred to as G_0 cells, can survive for very long time periods under certain environmental conditions. Like all microorganisms, yeasts spend most of their natural lifetime in a reversible, quiescent/G_0 state that is primarily induced by limitation for essential nutrients. Accordingly, when starved for carbon, nitrogen, phosphate, or sulfur, *S. cerevisiae* cells cease growing, arrest cell division in the G_1 phase of the cell cycle, and acquire a distinct array of physiological, biochemical, and morphological traits that collectively confer on cells both the ability to survive extended periods of starvation and to transit back to the proliferating state upon refeeding (Lillie & Pringle, 1980). While some aspects of the quiescence program are clearly nutrient specific (Gasch *et al.*, 2000; Carroll & O'Shea, 2002), it is generally assumed that yeast cells establish a core quiescence program regardless of which nutrient is limiting.

Our current knowledge on quiescent yeast cells is predominantly based on analyses of cells harvested from liquid cultures grown to saturation (i.e. stationary phase) in rich glucose-containing media. Under such conditions, cells enter into quiescence following progression through distinct adaptive phases, which critically affect the cells' life span and their ability to withstand environmental stresses (Werner-Washburne *et al.*, 1993; Herman, 2002). The earliest of these phases begins when nearly half of the initial glucose has been consumed and is characterized by the onset of glycogen synthesis (Lillie & Pringle, 1980). Subsequent phases, which are also critical for the development of stress resistance, include specific transcriptional changes and the synthesis of trehalose before and following glucose exhaustion, respectively (Lillie & Pringle, 1980; Mager & De Kruijff, 1995; Ruis & Schüller, 1995; Boy-Marcotte *et al.*, 1998; Thevelein & de Winde, 1999; Estruch, 2000). In the diauxic shift phase (following glucose depletion), cells transiently reduce their growth rate to readjust their metabolism for the subsequent postdiauxic phase of slow, respiratory growth

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on nonfermentable carbon sources, such as ethanol and acetate. The cellular responses initiated at the diauxic transition include the transcriptional induction of genes whose products are involved in respiration, fatty acid metabolism, and glyoxylate cycle reactions, and, likely as a consequence of the on-setting respiratory activity, of genes encoding antioxidant defenses that allow scavenging and/or the destruction of reactive oxygen species (ROS) (Jamieson, 1998; Costa & Moradas-Ferreira, 2001). The final characteristics of quiescent cells reflect their integrated responses and adaptations triggered by progression through distinct, sequential physiological phases (Werner-Washburne *et al.*, 1993, 1996; Braun *et al.*, 1996; Padilla *et al.*, 1998).

It is worth noting that stationary-phase cultures (defined as $>$ 7 days old) exhibit a complex, heterogeneous community structure, composed of a large fraction of quiescent, long-lived (almost exclusively daughter and young mother) cells and a nonquiescent fraction of cells, which rapidly lose their ability to reproduce and gradually accumulate ROS, exhibit genomic instability, and become senescent or apoptotic (Allen *et al.*, 2006; Aragon *et al.*, 2008; Davidson *et al.*, 2011). This diverse array of physiologically different cell populations with both different reproductive histories and distinct survival rates [and hence different chronological life spans (CLS)] contributes to the temporal plasticity of the mortality rate (generally determined as the relative loss of CFUs) within an aging stationary-phase culture (Minois *et al.*, 2009). Notably, both the heterogeneity within stationary-phase cultures and the fact that some of the reproductively incompetent, living cells remain unaccounted for by CFU measurements (Minois *et al.*, 2009) were hitherto largely overlooked in various CLS studies. Nevertheless, genetic and physiological studies of aging factors that affect CLS in yeast, a potentially valuable model for aging in postmitotic mammalian cells (Fabrizio & Longo, 2003; Kaeberlein, 2010), have identified distinct properties of quiescent cells that collectively define the essence of the quiescence program in yeast.

The quiescence program of stationary-phase cells

Cell cycle

Starvation for various nutrients such as carbon, ammonia, sulfate, phosphate, or biotin causes prototrophic yeast strains to arrest at START A within the G_1 phase of the cell cycle that, as mapped by classical reciprocal shift experiments, just precedes START B [defined operationally as the pheromone-repressible cell cycle event mediated by the cyclin-dependent protein kinase (CDK) $p34^{CDC28}$] (Hartwell, 1974; Pringle & Hartwell, 1981; Iida & Yahara, 1984; Sherlock & Rosamond, 1993). These findings have led to the commonly accepted conclusion that essential nutrients impinge on the cell's decision during late G_1 to commit to the initiation and completion of a new cell cycle, even when suddenly starved for nutrients. Interestingly, auxotrophic mutants that are starved for essential compounds (e.g. leucine, uracil, inositol, or fatty acids) are impaired for proper $G_{1/0}$ arrest and, likely as a consequence, exhibit a rather short life span (Henry, 1973; Hartwell *et al.*, 1974; Keith *et al.*, 1977; Saldanha *et al.*, 2004; Boer *et al.*, 2008). Cell cycle arrest at START A and entry into quiescence therefore appear to be tightly programmed responses to starvation for a distinct set of essential nutrients and are not just simple consequences of growth arrest. Whether cells have access to the quiescent state via G_1 arrest only at START A is of conceptual importance as it may indicate the existence of a distinct restriction point in G_1 that is similar to the one in mammalian cells (Pardee, 1989). This remains a matter of debate. Accordingly, while cells are able to induce specific responses to nutrient starvation (e.g. acquire an increased level of stress resistance) at any point in the cell cycle (Wei *et al.*, 1993; Laporte *et al.*, 2011), it is not known whether impeding G_1 arrest (e.g. by expressing hyperstable G_1 cyclins; Hadwiger *et al.*, 1989) may compromise the proper setup of the quiescence program. Furthermore, the ArfGAP Gcs1 has been claimed to be specifically required for cells to pass START B when exiting from quiescence at 15 °C, even though it is apparently dispensable under these conditions for both the initial physiological responses of quiescent cells to the readdition of nutrients and cell proliferation in general (Drebot *et al.*, 1987; Ireland *et al.*, 1994). This claim may support the existence of a nutrient-controlled restriction point in G_1. However, more recent studies have shown that Gcs1 performs an essential function in proliferating cells by facilitating post-Golgi transport redundantly with Age2 (Poon *et al.*, 2001). It is therefore possible that the observed defect of *gcs1*Δ cells in START B passage may simply reflect a synthetic effect uncovered by the loss of Gcs1 combined with nutrient starvation-induced reduction in Age2 function. In conclusion, whether stationary-phase cells arrest at a unique off-cycle point in G_1 remains a challenging issue to be addressed in future studies.

Metabolism

Glycogen

Limitation for nitrogen, sulfur, phosphate, and carbon sources triggers the cells to accumulate the reserve carbohydrate glycogen within both the cytoplasm and, as a result of on-setting macroautophagy, the vacuolar compartment (Lillie & Pringle, 1980; Wang *et al.*, 2001; Wilson *et al.*, 2002). In batch cultures, glycogen synthesis begins before glucose exhaustion and peaks at the beginning of the diauxic

shift phase. Glycogen stores are then partially utilized to fuel the metabolic adaptations to respirative growth and the synthesis of the nonreducing disaccharide trehalose (François & Parrou, 2001). During the subsequent growth phase on glucose-derived fermentation products such as ethanol, glycogen stores are refilled to ultimately serve as an energy depot during extended periods of starvation. The synthesis of glycogen requires the glycogenins Glg1/2, a pair of functionally redundant self-glucosylating initiator proteins that provide initial oligosaccharide primers, the glycogen synthases Gsy1/2 that use UDP-glucose (UDPG) to catalyze the successive addition of α-1,4-linked glucose residues to the nonreducing ends of these primers and/or glycogen molecules, and the branching enzyme Glc3, which introduces α-1,6-glucosidic bonds to form the highly branched form of mature glycogen (see Wilson *et al.*, 2010, for a recent review). Mobilization of cytoplasmic or vacuolar glycogen pools is catalyzed by the combined action of the glycogen debranching enzyme Gdb1 and the glycogen phosphorylase Gph1 or the vacuolar glucoamylase Sga1, respectively (Teste *et al.*, 2000; Wang *et al.*, 2001). Glycogen levels are fine-tuned in response to external nutrients mainly by the transcriptional control of glycogen anabolic (*GLG1/2*, *GSY1/2*, and *GLC3*) and catabolic (*GDB1*, *GPH1*, and *SGA1*) genes and by post-translational control of their corresponding gene products. The latter includes allosteric control of enzyme activities [e.g. activation and inactivation of Gsy2 and Gph1, respectively, by glucose-6-phosphate (Glu-6P)], and phosphorylation/dephosphorylation events that modulate for instance the activities of Gsy2 and Gph1 (for reviews, see François & Parrou, 2001; Wilson *et al.*, 2010).

Trehalose

Various environmental stresses including desiccation, heat shock, or starvation for nitrogen, sulfur, phosphate, or carbon induce cells to accumulate high levels (up to 0.5 M) of the nonreducing disaccharide trehalose (Lillie & Pringle, 1980; De Virgilio *et al.*, 1990, 1994; Crowe *et al.*, 1992; Hottiger *et al.*, 1994). Because of its particular biophysical properties, trehalose is thought to contribute to the stress tolerance of cells by preserving membranes in a liquid crystalline phase during desiccation or freezing and by stabilizing proteins and suppressing the aggregation of denatured proteins during heat shock (Singer & Lindquist, 1998; Crowe, 2007; Jain & Roy, 2009). During the diauxic shift and the subsequent growth phase on ethanol, yeast cells accumulate trehalose, which is then degraded steadily as starvation proceeds (> 7 days), notably at a higher pace once glycogen stores are depleted (Lillie & Pringle, 1980). Thus, in addition to its general protective role, trehalose may also contribute to energy homeostasis in quiescent cells. The enzymes that catalyze the two key reactions of trehalose biosynthesis, Tps1 [which transfers the glucosyl residue of UDPG to Glu-6P to yield trehalose-6-phosphate (Tre-6P)] and Tps2 (which hydrolyzes Tre-6P to trehalose and phosphate), are part of a protein complex that also harbors the regulatory Tsl1 and Tps3 proteins (Bell *et al.*, 1992, 1998; De Virgilio *et al.*, 1993; Vuorio *et al.*, 1993; Reinders *et al.*, 1997). While trehalose synthesis is partially regulated at the transcriptional level (i.e. transcription of all four genes *TPS1*, *TPS2*, *TPS3*, and *TSL1* is activated before or during the diauxic shift; DeRisi *et al.*, 1997), both the allosteric activation and inactivation of Tps1 by fructose-6-phosphate and phosphate, respectively, and the metabolic supply of substrates (i.e. UDPG and Glu-6P) appear to be major determinants of net trehalose synthesis (Vandercammen *et al.*, 1989; Londesborough & Vuorio, 1993). Although Tps1, Tps2, Tps3, and Tsl1 are all phosphorylated proteins *in vivo* (Albuquerque *et al.*, 2008), it is not known whether their functions are regulated by phosphorylation. Upon refeeding of stationary-phase cells with carbohydrates, trehalose is rapidly mobilized by hydrolysis, which may at least in part serve to fuel cell cycle progression upon return to growth (Shi *et al.*, 2010). Key for this event is the cytoplasmic, neutral trehalase Nth1 that is thought to be activated following refeeding by one or several phosphorylation events (Thevelein, 1984). The identity of the functionally critical residues within Nth1 remains a matter of debate because unequivocal evidence regarding the nature of the implicated protein kinases, which likely include the protein kinase A (PKA) and/or Sch9, is still lacking (Uno *et al.*, 1983; Thevelein, 1984; Zähringer *et al.*, 1998; Wera *et al.*, 1999; Roosen *et al.*, 2005; Panni *et al.*, 2008). Both the Nth1-homolog Nth2 and the acidic, vacuolar trehalase Ath1 apparently play a minor role, if any, in trehalose mobilization upon exit from quiescence (Jules *et al.*, 2004, 2008; Parrou *et al.*, 2005).

Cell wall

The macromolecular composition, molecular organization, and thickness of yeast cell walls vary considerably depending on environmental conditions and are tightly controlled in space and time. The backbone of the cell wall consists mainly of β-glucans (formed by β-1,3- and β-1,6-β-bonds), with a minor amount (about 3%) of chitin that is attached to it via β-1,4-bonds. Highly *N*- or *O*-glycosylated mannoproteins, which are either noncovalently or covalently bound to the β-glucan backbone, form an outer layer that shields the glucan polysaccharide matrix from β-glucanase-containing enzyme preparations such as zymolyase and glusulase (for a review, see Lesage & Bussey, 2006). Stationary-phase cells express high levels of mannoproteins such as Sed1 and exhibit specific changes in *N*-glycosylation and disulfide bridge formation within the mannoprotein layer, both of which

contribute significantly to the effectiveness of this layer's protective function and render cells highly resistant to different lytic enzyme mixtures (Zlotnik *et al*., 1984; Valentin *et al*., 1987; de Nobel *et al*., 1990; Shimoi *et al*., 1998). Stationary-phase cells also have characteristically thick cell walls, which partially result from the increased expression of the cell wall-synthesizing enzyme β-1,3-glucan synthase Gsc2 and the localized synthesis of its substrate UDPG during the post-diauxic growth phase (Lesage & Bussey, 2006). The latter process is controlled by the activity of Per-Arnt-Sim (PAS) kinases (particularly Psk1) that directly phosphorylate and regulate the enrichment of the UDPG pyrophosphorylase Ugp1 at the plasma membrane (Grose *et al*., 2007, 2009).

Polyphosphate (polyP)

As yeast cultures approach stationary phase, the uptake of phosphate likely exceeds its metabolic demand. As a result, excess phosphate accumulates mainly in the vacuole in the form of polyP, a linear-chain phosphate polymer that buffers the intracellular phosphate concentration in yeast (Kornberg *et al*., 1999; Thomas & O'Shea, 2005). In the absence of both the endopolyphosphatase Ppn1 and the exopolyphosphatase Ppx1, cells rapidly lose viability in stationary phase (Sethuraman *et al*., 2001), suggesting that polyP degradation represents an important aspect of phosphate homeostasis in quiescent cells.

Triglycerides (TGs) and steryl esters (SEs)

Storage and degradation of TGs and SEs are nutrient-regulated processes that play important roles in homeostasis of cellular energy and membrane biosynthesis. During the diauxic shift, yeast cells build up large amounts of TG and SE depots in specific subcellular organelles termed lipid droplets (LDs). Following nutrient depletion (in stationary phase), these fat depots are then slowly degraded by the release and subsequent β-oxidation of fatty acids, which yield metabolic energy for long-term survival in the absence of external nutrients (Hiltunen *et al*., 2003). In contrast, upon refeeding with carbohydrates, stationary-phase cells rapidly degrade their entire fat depots and resume growth (Kurat *et al*., 2006). The fatty acids that are released from TGs and SEs under these conditions are not metabolized via peroxisomes, but serve as precursors for the rapid reinitiation of membrane lipid synthesis (Trotter, 2001; van Roermund *et al*., 2003; Gray *et al*., 2004). Yeast LDs are thought to form by budding from the endoplasmic reticulum, which harbors the key enzymes required for TG and SE synthesis, such as the conserved Dga1 (acyl-CoA:diacylglycerol acyltransferase), Lro1 (phospholipid:diacylglycerol acyltransferase), and Are1/2 (acyl-CoA:cholesterol acyltranferase) proteins (Dahlqvist *et al*., 2000; Oelkers *et al*., 2000, 2002; Zweytick *et al*., 2000; Sandager *et al*., 2002; Sorger & Daum, 2002). The simultaneous loss of all four proteins renders yeast cells virtually incapable of TG/SE synthesis and reduces their ability to survive under long-term starvation conditions (Sandager *et al*., 2002). Mobilization of neutral lipids is catalyzed by TG lipases (i.e. Tgl3, Tgl4, and Tgl5) and SE hydrolases (i.e. Tgl1, Yeh1, and Yeh2), which – with the exception of Yeh2 – all localize to LDs (Athenstaedt & Daum, 2003, 2005; Jandrositz *et al*., 2005; Köffel *et al*., 2005; Köffel & Schneiter, 2006; Kurat *et al*., 2006). While the simultaneous loss of all TG and SE lipases has not yet been examined, studies of double *tgl3*Δ *tgl4*Δ mutant cells indicate that mobilization of neutral lipids from LDs is required for the rapid resumption of growth following refeeding of stationary-phase cells with carbohydrates (Kurat *et al*., 2009). Given both the dynamic regulation of LD appearance and disappearance and the reported colocalization of TG synthesis (i.e. Dga1) and TG/SE degradation enzymes on LDs, it appears likely that some of these enzymes are regulated via transcriptional, translational, or post-translational mechanisms in response to nutrient availability.

In stationary-phase cells, the acyl-CoA forming fatty acid activator Faa4, which synthesizes the cosubstrate for the acylation of diacylglycerol through Dga1, is localized exclusively to LDs (Natter *et al*., 2005; Kurat *et al*., 2006). Faa4 may therefore be metabolically coupled to TG storage or may serve to channel free fatty acids released from the breakdown of TGs (or SEs) towards activation and further metabolic utilization when cells are starved for longer periods. In this context, it is interesting to note that the loss of Faa4 causes a strong synthetic defect in stationary-phase survival when combined with impaired activity of the myristoyl-CoA:protein *N*-myristoyltransferase Nmt1, which requires the cosubstrate myristoyl-CoA provided by Faa4 (or Faa1) (Ashrafi *et al*., 1998). Thus, proper *N*-myristoylation of a set of proteins, which may include Arf1/2, Sip2, Van1, Ptc2, Ego1/Meh1, Moh1, and Vps20, is critical for stationary-phase survival (Ashrafi *et al*., 1998).

Respiration and redox balance

Mitochondrial respiration results in the generation of a variety of ROS within cells that can damage cellular constituents such as DNA, lipids, and proteins. Proliferating yeast cells can sense and respond to oxidizing agents by inducing a specific series of antioxidant mechanisms including the synthesis of glutathione and the production of enzymes [e.g. superoxide dismutases (Sod1/2), catalases (Ctt1 and Cta1), glutathione peroxidases (Gpx1/2), glutathione reductase (Glr1), glutaredoxins (Grx1/2), thioredoxins (Trx1/2), and a thioredoxin reductase (Trr1)], which detoxify oxidants or repair the damage caused by them (Jamieson, 1998). Quiescent cells retain some capacity to

respond to oxidative stress (Cyrne *et al.*, 2003) and exhibit an intrinsically high level of resistance towards oxidants, which may result from their adaptive response to mitochondrial respiratory metabolism-derived ROS production (including the synthesis of glutathione and the induction of Sod1/2, Ctt1/Cta1, Gpx1, Glr1, and Grx1/2; Costa & Moradas-Ferreira, 2001; Greetham *et al.*, 2010). In line with this interpretation, respiratory-deficient, stationary-phase yeast cells are hypersensitive to oxidants (Jamieson, 1992). Thus, oxidative stress may be a major factor that limits survival in stationary phase. Accordingly, enhanced expression of the cytosolic copper, zinc-superoxide dismutase (Cu,Zn-SOD) Sod1, and the mitochondrial manganese-superoxide dismutase (Mn-SOD) Sod2 during adaptation to efficient respiratory metabolism (for instance during the diauxic shift phase) is critical for maximal stationary-phase survival (or CLS) (Longo *et al.*, 1996; Flattery-O'Brien *et al.*, 1997; Harris *et al.*, 2003, 2005; Fabrizio *et al.*, 2004; Weinberger *et al.*, 2010). Despite the apparent negative effects of mitochondrial respiration-derived ROS, efficient respiration *per se* appears to play a positive role in life span extension in certain mutant backgrounds (Bonawitz *et al.*, 2007; Lavoie & Whiteway, 2008; Aerts *et al.*, 2009) and may be critical for the survival of quiescent cells, possibly by maintaining the redox balance and/or $NAD^+/NADP^+$ pools (Martinez *et al.*, 2004; Aragon *et al.*, 2008; Davidson *et al.*, 2011). Finally, carbon or nitrogen starvation, independent of ROS production, induces protein glutathionylation, a reversible post-translational modification that protects cysteine residues from irreversible oxidation. Because efficient exit from quiescence requires thioredoxin Trx1/2-mediated protein deglutathionylation, some of the corresponding modifications may have protein-regulatory functions (Greetham *et al.*, 2010).

Transcription

Transcriptional reprogramming during the diauxic shift, postdiauxic shift (PDS), and stationary phases involves at least one quarter of the yeast genome and is controlled by various signaling pathways (DeRisi *et al.*, 1997; Gasch *et al.*, 2000; Radonjic *et al.*, 2005). Many of the corresponding transcriptional changes are brought about by the control of promoter-specific activator proteins that recruit the RNA polymerase (RNA Pol) II in a holoenzyme form consisting of general transcription factors (GTFs), coactivators such as the Mediator, and chromatin-modifying complexes. In contrast, promoter-specific repressor proteins inhibit transcription by interfering with activator binding, preventing recruitment of the transcription apparatus by activator proteins, and modifying chromatin structure (Lee & Young, 2000). In addition to these rather specific regulatory mechanisms, transcriptional control in response to nutrient starvation is also exerted at a more general level and implicates GTFs and auxiliary proteins of RNA Pol I, II, and III (Lempiäinen & Shore, 2009). For instance, the general shutdown of transcription by RNA Pol II has been attributed partially to changes in DNA topology (Choder, 1991) or a drastic reduction in the levels of GTFs, including the TATA box-binding protein, $TAF_{II}145$, and several additional TFIID subunits (Walker *et al.*, 1997). Global transcription during the postdiauxic growth phase and survival in stationary phase also requires Rpb4, which increases its association with RNA Pol II as cells enter quiescence (Choder, 1993; Choder & Young, 1993). Similarly, the conserved carboxy-terminal domain (CTD) of the largest Pol II subunit, which comprises tandem (YSPTSPS) heptad repeats, is implicated in global transcription during the transition into stationary phase by serving as a dynamic landing pad for proteins that interact with the transcription elongation complex, carry out cotranscriptional pre-mRNA processing, and modify histones (Carlson, 1997; Phatnani & Greenleaf, 2006). Specifically, phosphorylation of Ser^2 within the CTD heptapeptide sequence increases during the diauxic shift and impairment of this phosphorylation (e.g. in cells harboring a mutation in the Ser^2-targeting Ctk1 kinase) or CTD truncation causes extensive defects in gene expression when cells enter stationary phase (Howard *et al.*, 2002; Ostapenko & Solomon, 2005). In addition, a four-protein regulatory module of the Mediator, composed of Med12 (Srb8) and Med13 (Srb9) plus the cyclin-dependent kinase Cdk8 (Srb10) and its cyclin partner CycC (Srb11) (Borggrefe *et al.*, 2002), functions as a negative regulator of a substantial fraction of genes that are repressed when cells grow on rich media and are induced as cells experience nutrient deprivation (Holstege *et al.*, 1998; van de Peppel *et al.*, 2005). While induction of this set of genes likely results from the depletion of Cdk8 (Srb10) and CycC (Srb11) when cells enter the diauxic shift, unscheduled transcriptional activation in cells carrying mutations in this particular Mediator regulatory module results in poor stationary-phase viability (Cooper *et al.*, 1997; Holstege *et al.*, 1998; Chang *et al.*, 2001). Interestingly, in quiescent cells, Mediator may serve as a platform for sequestering Pol II upstream of specific inactive genes that are rapidly induced when cells exit quiescence (Radonjic *et al.*, 2005). Lastly, the general downregulation of transcription in quiescent cells appears to allow the dynamically exchanging linker histone H1 (Hho1) to bind DNA. This process is essential for chromatin compaction in quiescent cells and may contribute to the genome integrity in these cells (Piñon, 1978; Schäfer *et al.*, 2008).

Translation

During transition into the quiescent state, the coordinated downregulation of ribosomal protein (RP) and translation factor gene expression and the inhibition of translation

initiation contribute to the dramatic (~300-fold) reduction in protein synthesis rates (Boucherie, 1985; Fuge *et al.*, 1994; Ju & Warner, 1994; DeRisi *et al.*, 1997). The remaining translational capacity is both sufficient to translate a number of mRNAs – including *HSP26* mRNAs or mRNAs of the *SNO* and *SNZ* families, which are involved in the synthesis of pyridoxine/vitamin B_6 that may become limiting during prolonged starvation (Dickson & Brown, 1998; Padilla *et al.*, 1998; Bean *et al.*, 2001; Radonjic *et al.*, 2005) – and necessary for maintaining the viability of cells in stationary phase (Paz & Choder, 2001).

Some of the molecular pathways that couple nutrient availability to translation initiation in yeast converge on Ser^{51} of the α-subunit of the eukaryotic translation initiation factor 2 (eIF2α). eIF2α delivers methionyl-$tRNA^{Met}$ in a ternary complex (TC) with GTP to the 40S ribosomal subunit. Phosphorylation of eIF2α-Ser^{51} inhibits TC formation and consequently all subsequent steps in the translation initiation pathway (Hinnebusch, 2005). The levels of eIF2α-Ser^{51} phosphorylation are tightly controlled by the eIF2α-kinase Gcn2 and eIF2α-phosphatases (eIF2α-PPs) that include the type I protein phosphatase (PP1) Glc7 and the type 2A protein phosphatase (PP2A)-related Sit4 (Wek *et al.*, 1992; Cherkasova *et al.*, 2010). Gcn2 is activated by (1) uncharged tRNAs that accumulate during amino acid starvation and that bind to its carboxy-terminal, histidyl-tRNA synthetase-related domain, (2) Sit4-mediated dephosphorylation of its negative regulatory Ser^{577} residue, and (3) autophosphorylation of Thr^{882} within its activation loop, which relies to some extent on the activity of Snf1, an ortholog of mammalian AMP-activated kinase that is responsible for the activation of glucose-repressed genes at low glucose levels (Cherkasova & Hinnebusch, 2003; Hinnebusch, 2005; Cherkasova *et al.*, 2010). The regulatory mechanisms that impinge on eIF2α-PPs are less well understood, but include Snf1-mediated (direct or indirect) inhibition of Glc7 and Sit4 when cells are grown on galactose (Cherkasova *et al.*, 2010). Notably, Sit4 can be found in distinct complexes containing Tap42 and either Rrd1 or Rrd2, which are regulated by the target of rapamycin complex 1 (TORC1) (Di Como & Arndt, 1996; Jiang & Broach, 1999; Zheng & Jiang, 2005). Thus, several major nutrient-signaling kinases including Gcn2, Snf1, and TORC1 contribute to the fine-tuning of translation initiation by regulating the levels of eIF2α phosphorylation. Nevertheless, yeast cells harboring a nonphosphorylatable $eIF2\alpha^{S51A}$ allele are still able to inhibit translation initiation in response to glucose withdrawal. This suggests the existence of additional translation initiation control mechanisms, which may target the formation of 48S preinitiation complexes (Hoyle *et al.*, 2007). Intriguingly, Snf1 also appears to play a role in this latter process (Ashe *et al.*, 2000).

While the decrease in TC levels following nutrient starvation reduces protein synthesis globally, the 5′- and 3′-untranslated regions (UTRs) of mRNAs also direct individual control of mRNA translation. For instance, a specialized reinitiation mechanism involving four short upstream ORFs (uORFs) in the 5′-UTR of the *GCN4* mRNA serves to repress *GCN4* translation under nonstarvation conditions and to derepress it in response to eIF2α phosphorylation in amino acid-starved cells (Hinnebusch, 2005). In contrast, the 5′-UTR of the *CLN3* mRNA, which codes for the CDK $p34^{CDC28}$-activatory G_1 cyclin Cln3, contains a short uORF that renders its translation, and consequently passage of cells through START, specifically sensitive to the inhibition of translation initiation (Polymenis & Schmidt, 1997). Another functionally important structural aspect of mRNAs is the length of their 5′-UTR, which is critical both for loading sufficient 40S subunits and for the scanning ribosome to gain initiation competence (Kozak, 1991). Accordingly, mRNAs with very short 5′-UTRs (e.g. *SSA3*) are poorly translated when ribosome assembly becomes less efficient as TC levels decline in cells entering stationary phase (Paz *et al.*, 1999b). Alternatively, some mRNAs have been proposed to escape cap-dependent translation particularly under starvation conditions by directing ribosomes towards an internal AUG via an internal ribosome entry sequence (Paz *et al.*, 1999a; Gilbert *et al.*, 2007). Finally, it is becoming increasingly clear that the 3′-UTRs of mRNAs also play important roles in post-transcriptional gene expression by regulating translational efficiency and/or mRNA stability. The Puf proteins, for instance, each of which has its own set of functionally related target transcripts to coordinately regulate certain cellular processes, recognize UG-rich sequences within 3′-UTRs and direct the accelerated decay of their target mRNAs by recruiting the Ccr4–Pop2–Not1-5 deadenylase complex (Gerber *et al.*, 2004; Goldstrohm *et al.*, 2006). For illustration, Puf4 specifically destabilizes transcripts encoding RPs and ribosome biogenesis factors in response to nutrient starvation when cells enter stationary phase, thereby contributing to the general downregulation of protein synthesis under these conditions (Foat *et al.*, 2005).

Another consequence of glucose withdrawal is that, following the inhibition of translation, mRNAs broadly dissociate from specific translation factors, associate with translational repressors, and accumulate as repressed messenger ribonucleoprotein complexes within cytoplasmic granules, also termed processing bodies (P-bodies or PBs) (Sheth & Parker, 2003; Brengues *et al.*, 2005). These mRNAs are then either degraded, repressed and stored, or diverted back to translation following a passage through stress granule-like eIF4E-, eIF4G-, and Pab1-containing bodies (EGPBs) (Hoyle *et al.*, 2007; Parker & Sheth, 2007; Buchan *et al.*, 2008). PBs contain a conserved core of proteins consisting of the mRNA decapping machinery, including

the decapping enzymes Dcp1/2, the activators of decapping (i.e. Dhh1, Pat1, Scd6, Edc3, and the heptameric Lsm1-7 complex), and the 5′–3′-exonuclease Xrn1 (Eulalio *et al.*, 2007; Parker & Sheth, 2007). PBs also contain the conserved Ccr4–Pop2–Not1-5 complex that initiates deadenylation of the 3′-poly(A) tail of mRNAs, which, besides allowing 3′ to 5′ degradation of mRNAs by the exosome complex, primarily induces Dcp1/2-mediated removal of the 5′ end cap structure, followed by 5′ to 3′ transcript degradation (Anderson & Kedersha, 2006; Parker & Sheth, 2007). Maintenance of normal 5′ to 3′ mRNA decay rates further requires Dcs1, which catalyzes the cleavage of m^7GDP generated by Dcp1/2-mediated decapping (and of 5′ end m^7G-oligoribonucleotide fragments generated by the 3′ to 5′ exonucleolytic decay), a process that is important for the survival of cells in stationary phase possibly because uncleaved m^7GDP may compete with capped mRNAs for eIF4F binding and thereby inhibit translation initiation (Malys *et al.*, 2004; Liu & Kiledjian, 2005; Malys & McCarthy, 2006). Finally, the core of conserved PB components, also termed the 5′–3′ mRNA decay machinery, functions in both translation repression and mRNA degradation and competes with the assembly of translational factors (Eulalio *et al.*, 2007; Parker & Sheth, 2007). How nutrient limitation impinges on and regulates this competition remains elusive.

Autophagy and protein degradation

Macroautophagy (referred to as autophagy for the rest of this review) is a vacuolar degradative pathway for bulk proteins and damaged and/or unnecessary organelles (He & Klionsky, 2009). Autophagy is most potently stimulated by nitrogen starvation and, to a somewhat lesser extent, by starvation for other essential nutrients including carbon (Takeshige *et al.*, 1992). Autophagy begins with the formation of double-membrane vesicles, termed autophagosomes, which sequester cytoplasmic material and ultimately fuse with the vacuole. The inner vesicle (autophagic body) that is released into the vacuolar lumen is then degraded by a series of vacuolar hydrolases such as the lipase Atg15 and the stationary-phase-induced proteinases A (Pep4) and B (Prb1) (Van Den Hazel *et al.*, 1996; Teter *et al.*, 2001). Following efflux from the vacuole, the corresponding degradation products can then be metabolically recycled, a process that contributes significantly to the survival of cells during starvation (Tsukada & Ohsumi, 1993; Yang *et al.*, 2006; He & Klionsky, 2009; Gresham *et al.*, 2011). Interestingly, while 40S and 60S ribosomal subunits are engulfed and delivered to the vacuole via nonselective autophagy when cells are starved for nutrients, their degradation also relies on a second, specific Ubp3/Bre5 ubiquitin protease-requiring ribophagy pathway, which also contributes to cell survival during starvation (Kraft *et al.*, 2008).

Ubiquitin-dependent protein degradation probably does not contribute significantly to bulk proteolysis in cells entering stationary phase. However, it appears that proper regulation of this process is critical for the maintenance of viability in quiescent cells. Accordingly, loss of (1) Ubi4, the polyubiquitin precursor comprised of five head-to-tail ubiquitin repeats, (2) the ubiquitin-conjugating enzymes Ubc5 and Ubc1, (3) the E3 ubiquitin ligase Rsp5, or (4) the deubiquitinating enzyme Doa4 all reduce the viability of cells as they approach stationary phase (Finley *et al.*, 1987; Seufert & Jentsch, 1990; Swaminathan *et al.*, 1999; Cardona *et al.*, 2009). Proteasome-dependent proteolysis is generally enhanced during early, but then reduced in late stationary-phase cells. This reduction is likely due to the disassembly of 26S holoenzymes into their 20S core particle (CP) and 19S regulatory particle components and/or the massive relocalization of proteasome subunits from the nucleus to cytoplasmic proteasome storage granules that serve as proteasome reservoirs for cells exiting quiescence (Finley *et al.*, 1987; Fujimuro *et al.*, 1998; Bajorek *et al.*, 2003; Laporte *et al.*, 2008). Uncontrolled, accelerated proteasome activity causes a precipitous decline in cell viability in 10-day-old stationary-phase cultures (Bajorek *et al.*, 2003). Conversely, significant remodeling of the 20S CP composition in cells approaching stationary phase may also be important to ensure a basal level of proteasome-mediated protein degradation to help eliminate oxidatively damaged proteins (Chen *et al.*, 2004). In line with this idea, loss or overproduction of a specific 20S CP maturation factor (i.e. Ump1) decreases or enhances, respectively, the cell's capacity to survive in stationary phase (Chen *et al.*, 2006).

While quiescent cells historically have attracted much less attention than proliferating cells, our appreciation of their properties and life style, as illustrated above, has grown tremendously during the last couple of years. The currently available depiction of the quiescent state therefore provides a sufficiently elaborated basis for studies addressing the challenging question of how nutrient-signaling pathways are wired to warrant optimal setup of the quiescence program in response to specific environmental challenges.

Signaling networks regulating quiescence

Both PKA and TORC1 are positive key regulators of cell growth that critically participate in the cell's decision whether or not to enter into quiescence. For instance, cells with uncontrolled, elevated PKA activity typically fail to acquire many (if not most) physiological characteristics of the quiescence program as they approach stationary phase. Conversely, PKA deficiency, similar to TORC1 inhibition, causes growth arrest and locks cells in a G_0-like state (Tatchell, 1986; Thevelein & de Winde, 1999; Gray *et al.*, 2004; De Virgilio & Loewith, 2006b; Wullschleger

et al., 2006; and references therein). An additional signaling network with the Snf1 protein kinase at its core is dispensable for growth on glucose, but – unlike PKA and TORC1 – positively regulates the transition into quiescence (Gray *et al.*, 2004). Lastly, recent evidence suggests that the Pho85-signaling pathway significantly modulates the setup of the quiescence program. The structure of these signaling networks and their corresponding cellular targets will be discussed in the following paragraphs.

The PKA-signaling network

The heterotetrameric PKA complex is composed of a combination of two out of three closely related Tpk1, Tpk2, and Tpk3 catalytic subunits and two regulatory Bcy1 subunits, which restrict the activity of the catalytic subunits by acting as pseudosubstrates. Binding of cyclic AMP (cAMP) to Bcy1 subunits alleviates their inhibitory activity and releases the catalytic subunits, each of which phosphorylates distinct, but partially overlapping sets of target proteins (Robertson & Fink, 1998; Ptacek *et al.*, 2005).

What regulates PKA?

The intracellular cAMP level is balanced by Cdc35 adenylate cyclase-mediated synthesis and Pde1/2 phosphodiesterase-mediated breakdown of cAMP. Two parallel molecular pathways that likely couple intracellular and extracellular nutrient signals, respectively, to PKA regulation converge on adenylate cyclase (Fig. 1). Firstly, the partially redundant GTP-binding proteins Ras1 and Ras2 directly activate adenylate cyclase when present in their GTP-bound state. The GTP-loading status of Ras proteins is regulated by both a pair of GTPase-activating proteins (GAPs), Ira1 and Ira2, which stimulate the intrinsic GTPase activity of Ras proteins, and by the guanine nucleotide exchange factors (GEF) Cdc25 and Sdc25 (for reviews, see Thevelein & de Winde, 1999; Schneper *et al.*, 2004). While Ras proteins are required to maintain basal cAMP/PKA levels, glucose addition to starved cells strongly increases the relative amount of Ras-GTP and consequently the intracellular cAMP concentrations. This increase, however, is only transient because activated PKA inhibits cAMP synthesis and activates cAMP hydrolysis (via Pde1/2) as part of a regulatory feedback loop (Tanaka *et al.*, 1989, 1990; Gross *et al.*, 1992; Ma *et al.*, 1999; Colombo *et al.*, 2004; Jian *et al.*, 2009; Hu *et al.*, 2010). In batch cultures, basal cAMP levels are rather high when cells are growing exponentially, but decline sharply as cells reach the diauxic shift phase (Russell *et al.*, 1993). Although the molecular mechanisms by which glucose affects Ras-GTP levels remain largely unknown, they appear to be dependent on intracellular phosphorylation of glucose and proper regulation of both Cdc25 and Ira proteins (Colombo *et al.*, 1998, 2004; Gross *et al.*, 1999; Rolland *et al.*, 2001; Paiardi

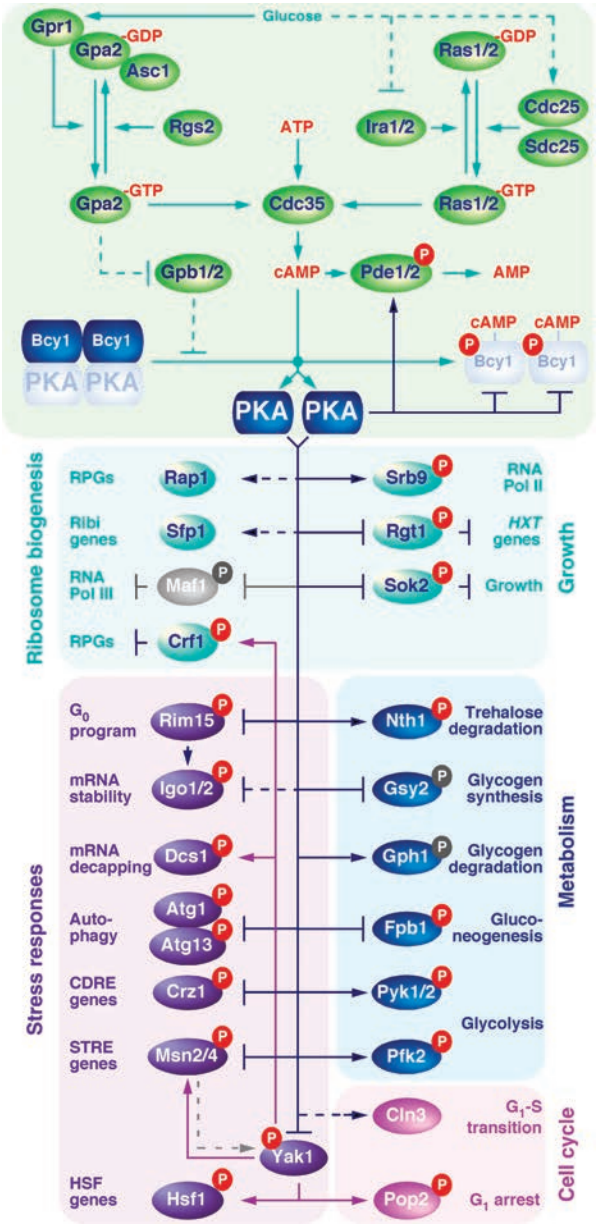

Fig. 1. Diagram of the *Saccharomyces cerevisiae* PKA-signaling network. PKA regulates growth by promoting ribosome biogenesis via controlling the expression of ribosomal protein genes (RPGs), rDNA genes, and ribosome biogenesis (Ribi) genes, and by inhibiting transcription factors that function in growth repression. PKA further inhibits stress responses, regulates G_1–S progression, and controls key metabolic events in response to glucose availability. Upstream of PKA, the small G-proteins Ras1/2 and Gpa2 mediate glucose signaling through the activation of adenylate cyclase Cdc35. Solid arrows and bars refer to direct interactions; dashed arrows and bars refer to indirect and/or potential interactions. Red circles containing the letter P denote phosphorylated amino acid residues; the corresponding gray circles denote potentially phosphorylated amino acid residues. CDRE, calcineurin-dependent response element; HSF, heat shock factor; *HXT*, hexose transporter; STRE, stress-responsive element. See text for further details.

et al., 2007). Secondly, adenylate cyclase integrates extracellular (likely glucose and sucrose) nutrient signals via a G-protein-coupled receptor (GPCR) system that consists of the receptor Gpr1, the Gα protein Gpa2 with its GAP Rgs2 (for a review, see Santangelo, 2006), and the Gβ-subunit Asc1 (Zeller *et al.*, 2007). This GPCR system, probably in conjunction with Ras proteins that may properly position and/or prime adenylate cyclase at the plasma membrane (Colombo *et al.*, 2004), is also important for the transient glucose activation of cAMP synthesis (Thevelein & de Winde, 1999). However, unlike the Cdc25-Ras-Cdc35 branch, the GPCR module is not required for growth and its absence does not drive cells into quiescence (at START A) when grown on rich media (Iida & Yahara, 1984; Toda *et al.*, 1985; Plesset *et al.*, 1987; Sherlock & Rosamond, 1993). Thus, the Gpr1-Gpa2 branch plays a minor auxiliary role in controlling entry or exit from quiescence (Colombo *et al.*, 1998; Harashima & Heitman, 2002; Wang *et al.*, 2004).

In addition to its regulation by cAMP, PKA may be subject to several less well-established control mechanisms. For instance, as part of an autoactivation process, PKA phosphorylates Bcy1 at Ser^{145}, thereby destabilizing Bcy1 via an unknown mechanism (Kuret *et al.*, 1988; Werner-Washburne *et al.*, 1991; Budhwar *et al.*, 2010). In addition, Bcy1 dynamically relocalizes from the nucleus to the cytoplasm as cells approach stationary phase (Griffioen *et al.*, 2000), suggesting that PKA activity is subject to both temporal and spatial control. Moreover, recent evidence indicates that the kelch repeat proteins Gpb1/2, rather than functioning as Gβ-subunit mimics for Gpa2 as initially suggested (Harashima & Heitman, 2002), and in addition to their controversial role in controlling the stability of Ira proteins (Harashima & Heitman, 2005; Phan *et al.*, 2010), may reinforce stable Bcy1–Tpk interactions downstream of Gpa2 (Peeters *et al.*, 2006, 2007; Budhwar *et al.*, 2010). Although the simultaneous loss of Gpb1/2 appears to preclude cells from accessing a proper quiescent state in stationary phase (Harashima & Heitman, 2002), it is not known whether (or how) nutrients regulate Gpb1/2. Furthermore, it has also been proposed that autophosphorylated Mck1 binds to and directly inhibits, but does not phosphorylate, PKA catalytic subunits (Rayner *et al.*, 2002). Lastly, nutrient permeases such as the general amino acid permease Gap1, the ammonium permease Mep2, and the phosphate carrier Pho84 (for a review, see Rubio-Texeira *et al.*, 2010), as well as the vacuolar ATPase (Dechant *et al.*, 2010) have all been implicated in PKA activation, but their precise role in entry and/or exit from quiescence remains to be elucidated.

What does PKA regulate?

PKA regulates growth in part by promoting ribosome biogenesis, via control of the expression of RP genes, rDNA genes, and ribosome biogenesis (Ribi) genes, which encode rRNA processing, ribosome assembly, and translation factors (Jorgensen *et al.*, 2004; Chen & Powers, 2006). PKA further inhibits stress responses, some of which are incompatible with growth, and regulates key metabolic events as cells approach and/or enter the diauxic shift phase (Fig. 1).

Ribosome biogenesis

PKA controls growth by favoring the expression of the translation machinery via a number of yet poorly defined processes. For instance, PKA activates Rap1 (Klein & Struhl, 1994; Neuman-Silberberg *et al.*, 1995), which, together with the high-mobility group protein Hmo1 (Wade *et al.*, 2004; Hall *et al.*, 2006), recruits the nutrient-controlled Fhl1–Ifh1 complex exclusively to RP gene promoters to activate the expression of the corresponding genes (Martin *et al.*, 2004; Schawalder *et al.*, 2004; Wade *et al.*, 2004; Rudra *et al.*, 2005; Kasahara *et al.*, 2007). PKA further prevents Yak1-mediated activation of the transcriptional corepressor Crf1, which, following its phosphorylation by Yak1, replaces (in some strains) the coactivator Ifh1 of the fork head transcription factor Fhl1 to repress RP gene expression (Martin *et al.*, 2004; Zhao *et al.*, 2006). PKA also favors nuclear localization of the transcription factor Sfp1, which positively influences RP and Ribi gene expression (Jorgensen *et al.*, 2004; Marion *et al.*, 2004; Budovskaya *et al.*, 2005; Cipollina *et al.*, 2008a, b; Lempiäinen & Shore, 2009), and may (Moir *et al.*, 2006) or may not (Huber *et al.*, 2009) phosphorylate and thereby inhibit the RNA Pol III repressor Maf1 to ensure 5S rDNA and tRNA transcription. In addition to regulating ribosome biogenesis, PKA also controls growth in part by (1) regulating the elongation step of RNA Pol II-mediated transcription (Howard *et al.*, 2003), (2) controlling directly the Mediator subunit Srb9 (Chang *et al.*, 2004), (3) inhibiting the transcriptional repressor activity of Sok2 (Ward *et al.*, 1995; Shenhar & Kassir, 2001), (4) altering the function of Rgt1 and relieving its repressive effects on the expression of hexose transporter genes (Özcan & Johnston, 1999; Kim & Johnston, 2006), and (5) specifically regulating the translation of Cln3 (presumably via control of translation initiation), thereby coupling growth cues with cell cycle decisions (Hall *et al.*, 1998) (Fig. 1).

Stress responses

In addition to stimulating growth, PKA suppresses several stress responses by different means. For instance, PKA inhibits the dual-specificity tyrosine phosphorylation-regulated protein kinase Yak1, which was originally isolated as a growth antagonist as its loss renders cells largely independent of PKA activity (Garrett & Broach, 1989). PKA sequesters Yak1 in the cytoplasm by phosphorylating it at

Ser^{295} (and two additional minor sites) (Garrett *et al.*, 1991; Zappacosta *et al.*, 2002; Budovskaya *et al.*, 2005; Lee *et al.*, 2011; Malcher *et al.*, 2011). Downregulation of PKA as cells enter the diauxic shift phase enables Yak1 to gain access to some of its targets in the nucleus. These include (1) Bcy1, which is phosphorylated and subsequently partitioned into the cytoplasm in a Yak1-dependent manner (Werner-Washburne *et al.*, 1991; Griffioen *et al.*, 2001), (2) Pop2 of the Ccr4–Pop2–Not1-5 complex, whose phosphorylation by Yak1 is required for proper G_1 arrest as cells approach stationary phase (Moriya *et al.*, 2001), (3) the 'decapping' scavenger Dcs1 (Malys *et al.*, 2004), (4) Crf1, which acts as a corepressor of RP gene expression (Martin *et al.*, 2004; Zhao *et al.*, 2006), (5) the heat shock transcription factor Hsf1, which binds more strongly to DNA following Yak1-mediated phosphorylation (Lee *et al.*, 2008), and (6) the Zn^{2+}-finger transcription factor Msn2 (Lee *et al.*, 2008), which, together with its partially redundant paralog Msn4, drives the expression of about 200 stress response element-containing genes in response to multiple environmental stress conditions including glucose limitation at the diauxic shift (Boy-Marcotte *et al.*, 1998; Moskvina *et al.*, 1998; Garreau *et al.*, 2000; Gasch *et al.*, 2000; Cameroni *et al.*, 2004; for reviews, see also Ruis & Schüller, 1995; Estruch, 2000; Smets *et al.*, 2010) (Fig. 1). Yak1-dependent phosphorylation activates Msn2, yet the underlying mechanism remains elusive.

PKA also phosphorylates Msn2 directly at critical residues within a nuclear localization signal (NLS) domain and presumably within a nuclear export signal (NES) domain to inhibit its nuclear import and possibly favor its nuclear export, respectively (Görner *et al.*, 1998, 2002; Garreau *et al.*, 2000). Moreover, because the expression of Yak1 strongly depends on Msn2/4, this PKA-controlled mechanism serves to downregulate Yak1 and may explain why loss of Msn2/4, like loss of Yak1, renders cells largely independent of PKA activity (Garrett & Broach, 1989; Smith *et al.*, 1998). Furthermore, the protein kinase Rim15 appears to play an equally important role in mediating growth inhibition in the absence of PKA as Msn2/4 and Yak1. Rim15 represents a distinct member of the PAS protein kinase family that broadly and positively controls the proper setup of the quiescence program and its kinase activity is directly inhibited by PKA-mediated phosphorylation (Reinders *et al.*, 1998). The molecular elements linking Rim15 to its distal readouts, including the expression of specific nutrient-regulated and oxidative stress genes, trehalose and glycogen accumulation, proper cell cycle arrest (likely at START A), stationary-phase survival, and induction of autophagy, are only partially characterized, but also involve Msn2/4 and the closely related transcription factor Gis1, which drives the expression of PDS element-controlled genes (Pedruzzi *et al.*, 2000; Fabrizio *et al.*, 2001; Cameroni *et al.*, 2004; Roosen *et al.*, 2005; Yorimitsu *et al.*, 2007; Wei *et al.*, 2008; Zhang *et al.*, 2009; Weinberger *et al.*, 2010). Rim15 may coordinate the transcription of Msn2/4- and Gis1-dependent genes (Lenssen *et al.*, 2002; Lenssen *et al.*, 2005) with post-transcriptional mRNA protection by phosphorylating the paralogous Igo1 and Igo2 proteins (Talarek *et al.*, 2010). This event stimulates Igo proteins to associate with the mRNA decapping activator Dhh1 and shelters specific mRNAs, which are newly expressed as cells approach stationary phase, from degradation via the 5′–3′ mRNA decay pathway, thereby ensuring their translation during the initiation of the quiescence program (Luo *et al.*, 2011).

PKA further inhibits stress responses by phosphorylating and thereby inhibiting the nuclear import of the Zn^{2+}-finger transcription factor Crz1, which is necessary for the expression of calcineurin-dependent response element-containing genes whose products (e.g. the β-1,3-glucan synthase Gsc2) promote adaptation to stress (Mazur *et al.*, 1995; Yoshimoto *et al.*, 2002; Kafadar & Cyert, 2004). Lastly, PKA inhibits autophagy by phosphorylating the protein kinase Atg1 as well as its regulator Atg13. This prevents the recruitment of the Atg1–Atg13 complex to the preautophagosomal structure, the nucleation site from which autophagy pathway intermediates are formed (Budovskaya *et al.*, 2004, 2005; Stephan *et al.*, 2009).

Metabolism

Some of the physiological changes that occur as cells approach and/or enter the diauxic shift phase are also subject to post-transcriptional control by PKA. Accordingly, PKA antagonizes both the metabolic transition from glycolysis to gluconeogenesis and the induction of trehalose and glycogen synthesis by different means, including (1) the stimulation of the glycolytic 6-phosphofructo-2-kinase Pfk2 and pyruvate kinases Pyk1/2 (Cytryńska *et al.*, 2001; Vaseghi *et al.*, 2001; Portela *et al.*, 2002, 2006; Rayner *et al.*, 2002; Dihazi *et al.*, 2003; Galello *et al.*, 2010), (2) the inhibition of the gluconeogenic fructose 1,6-bisphosphatase Fpb1 (Gancedo *et al.*, 1983; Rittenhouse *et al.*, 1987), (3) the activation of the neutral trehalase Nth1 (Ortiz *et al.*, 1983; Uno *et al.*, 1983; Wera *et al.*, 1999; Panni *et al.*, 2008), (4) the activation of the glycogen phosphorylase Gph1 (Wingender-Drissen & Becker, 1983; Lin *et al.*, 1996), and (5) the inhibition of the glycogen synthase Gsy2 (Hardy & Roach, 1993) (Fig. 1). Particularly for Gph1 and Gsy2 (and to some extent for Nth1), it is still a matter of debate as to whether these proteins are directly or indirectly controlled by PKA.

The TORC1-signaling network

The highly conserved TOR proteins are central components of another key signaling pathway that controls the growth of

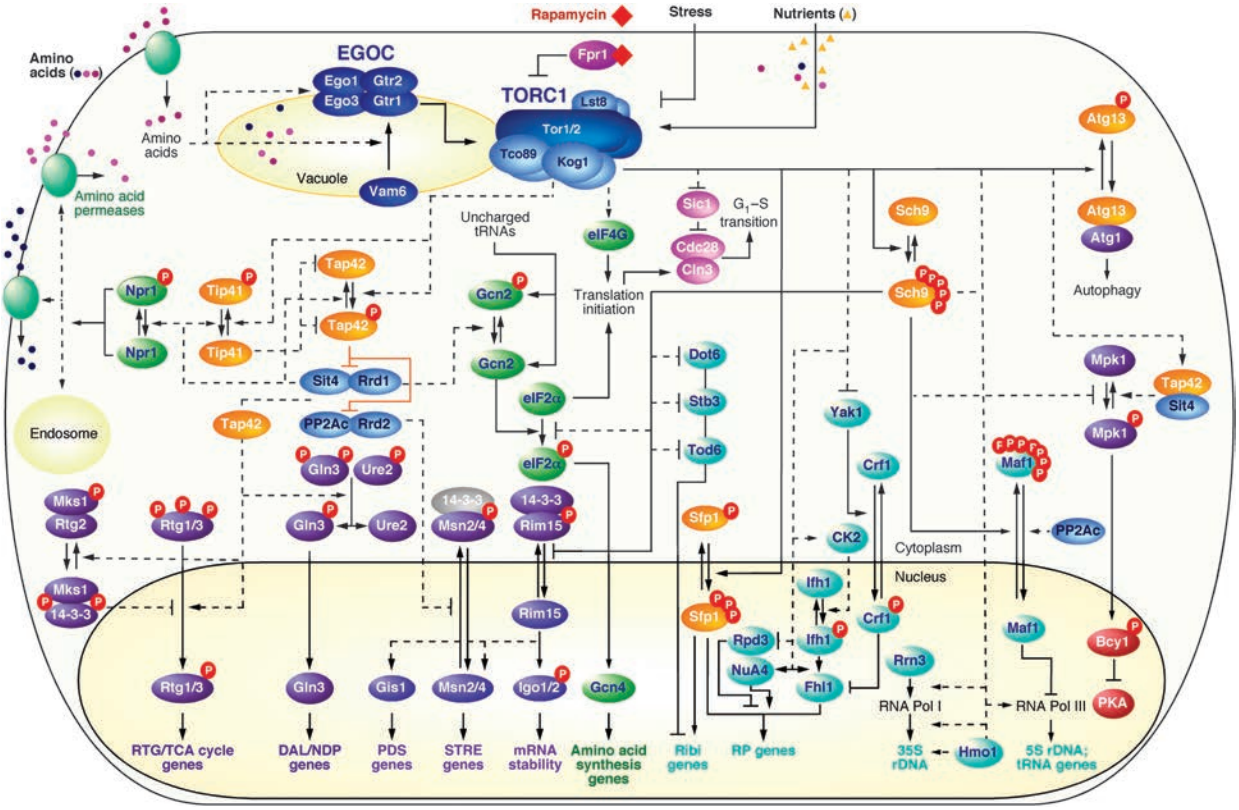

Fig. 2. Diagram of the *Saccharomyces cerevisiae* TORC1-signaling network. TORC1 (including Tor1 and/or Tor2 and its subunits Kog1, Tco89, and Lst8) is pictured as a dimer. TORC1 promotes cell growth by stimulating anabolic processes such as translation initiation and permease activity (green proteins), and by stimulating expression of the translation machinery (turquoise proteins). TORC1 propagates signals mainly via the protein kinase Sch9 and the catalytic subunits of the type 2A protein phosphatases (PP2Ac) Pph21 and Pph22 or the related Sit4 protein phosphatase when associated with Tap42 and Rrd2 or Rrd1, respectively. Proximal TORC1 effectors include Sch9, Tip41, Tap42, Sfp1, and Atg13 (orange). TORC1 inhibits catabolic processes such as autophagy and blocks transcriptional stress responses mediated by Rtg1/3, Gln3, Gis1, Msn2/4, Rim15, Igo1/2, and Mpk1 (violet proteins). Upstream of TORC1, cytoplasmic and/or intravacuolar amino acids may influence the activity of Vam6, which regulates the nucleotide-binding status of the small GTPase Gtr1. As part of the EGO complex (EGOC; dark blue), Gtr1-GTP binds to and somehow activates TORC1. Rapamycin specifically inhibits TORC1 when in complex with the peptidyl-prolyl isomerase Fpr1. Arrows and bars denote positive and negative interactions, respectively. Solid arrows and bars refer to direct interactions; dashed arrows and bars refer to indirect and/or potential interactions. Red circles containing the letter P denote phosphorylated amino acid residues. STRE, stress-responsive element; PDS, postdiauxic shift; DAL, degradation of urea and allantoin; NDP, nitrogen discrimination pathway; RTG, retrograde regulation; TCA, tricarboxylic acid cycle. See text for further details.

proliferating yeast in response to nutrients (Fig. 2). *Saccharomyces cerevisiae* cells express two TOR homologs, Tor1 and Tor2, both of which – when associated with Lst8, Kog1, and Tco89 in TORC1 – are targets of the therapeutically important, immune-suppressive macrolide rapamycin in complex with the peptidyl-prolyl isomerase Fpr1 [also known as FK506-binding protein 12 (FKBP12) in mammals] (Loewith *et al.*, 2002; Jacinto & Hall, 2003). Binding of the rapamycin–FKBP12 complex to TORC1, a mode of action that is conserved from yeasts to humans (Hara *et al.*, 2002; Kim *et al.*, 2002; De Virgilio & Loewith, 2006a), inhibits the activity of the TOR kinases and elicits a number of responses that mimic nutrient starvation, including a decrease in protein synthesis and ribosome biogenesis, specific changes in gene transcription, sorting and turnover of nutrient permeases, induction of autophagy, G_1 cell cycle arrest, and entry into quiescence (for reviews, see Rohde *et al.*, 2001; Jacinto & Hall, 2003; De Virgilio & Loewith, 2006b).

What regulates TORC1?

Transfer from preferred to poor-quality carbon or nitrogen sources, starvation for carbon or nitrogen, or exposure to noxious stress elicit responses in yeast analogous to those observed following rapamycin treatment (for a review, see De Virgilio & Loewith, 2006b). It is therefore assumed that TORC1 is regulated by the abundance and/or the quality of the available carbon and nitrogen sources, as well as by the presence or absence of different forms of stresses. In line with this assumption, starvation of cells for carbon or

nitrogen, induction of oxidative or osmotic stress, and caffeine treatment result in TORC1 inhibition, as measured by its proficiency to phosphorylate the bona fide substrate Sch9 (Urban *et al.*, 2007; Wanke *et al.*, 2008). Caffeine directly inhibits the TORC1 kinase (Kuranda *et al.*, 2006; Reinke *et al.*, 2006; Wanke *et al.*, 2008), but it is not known how other stress signals impinge on TORC1. Moreover, intracellular metabolites such as amino acids may play a particular role in regulating TORC1 activity. For instance, the treatment of cells with the translation elongation inhibitor cycloheximide strongly activates TORC1, possibly by increasing the intracellular pool of free amino acids (Beugnet *et al.*, 2003; Urban *et al.*, 2007; Binda *et al.*, 2009). Based on the observation that glutamine starvation phenocopies the effects of rapamycin-mediated TORC1 inactivation inasmuch as it causes nuclear localization and activation of the transcription factors Gln3 and Rtg1/3, the amino acid glutamine has been proposed to act upstream of TORC1 (Crespo *et al.*, 2002; Butow & Avadhani, 2004). However, because other TORC1 readouts (such as the subcellular distribution of Msn2) remain unaffected by glutamine starvation, TORC1 may also respond to additional nutrients (and elicit to some extent nutrient-specific responses).

The EGO (exit from rapamycin-induced growth arrest) protein complex (EGOC) (Dubouloz *et al.*, 2005), which consists of Ego1, Ego3, Gtr1, and Gtr2, has recently been proposed to function as a critical hub that directly relays an amino acid signal to TORC1 (Binda *et al.*, 2009) (Fig. 2). EGOC is evolutionarily conserved (Kogan *et al.*, 2010) and colocalizes with TORC1 mainly at the limiting membrane of the vacuole (Reinke *et al.*, 2004; Araki *et al.*, 2005; Gao & Kaiser, 2006; Urban *et al.*, 2007; Sturgill *et al.*, 2008; Berchtold & Walther, 2009; Binda *et al.*, 2009). More importantly, its subunit Gtr1, which is homologous to mammalian Rag GTPases (Binda *et al.*, 2010; and references therein), directly interacts with and activates TORC1 in an amino acid-sensitive and nucleotide-dependent manner (Binda *et al.*, 2009). Accordingly, expression of a constitutively active (GTP bound) $Gtr1^{GTP}$ interacts with TORC1 and renders TORC1 partially resistant to leucine deprivation, while expression of a growth-inhibitory $Gtr1^{GDP}$ causes constitutively low TORC1 activity. Complementary studies in *Drosophila* and mammalian cells have also reported that the conserved Rag GTPases act as upstream regulators of TORC1 and play important roles in coupling amino acid-derived signals to TORC1 (Kim *et al.*, 2008; Sancak *et al.*, 2008). The mechanisms by which amino acids impinge on EGOC are still unknown, but may involve the Vam6 GEF, a conserved vacuolar membrane protein that binds to and regulates the nucleotide-binding status of Gtr1 (Binda *et al.*, 2009). Interestingly, a genome-wide screen for TORC1 regulators further identified Npr2 and Npr3 (Neklesa & Davis, 2009), which, possibly as part of the conserved, vacuolar membrane-localized SEA complex (Dokudovskaya *et al.*, 2011), also mediate amino acid signals to TORC1.

What does TORC1 regulate?

TORC1 propagates signals mainly via two key effector branches (Huber *et al.*, 2009), which include (1) the presumed mammalian S6 kinase (S6K) ortholog Sch9 (Powers, 2007), whose activity depends on TORC1-mediated phosphorylation of five to six C-terminal serine and threonine residues (Urban *et al.*, 2007), and (2) the PP2A catalytic subunits (PP2Ac) Pph21/22 or the related Sit4 protein phosphatase when associated with Tap42 and the peptidyl-prolyl *cis*/*trans*-isomerases Rrd2 or Rrd1, respectively (Di Como & Arndt, 1996; Jiang & Broach, 1999; Zheng & Jiang, 2005) (Fig. 2). TORC1 is thought to stabilize Tap42–PP2Ac–Rrd2 and Tap42–Sit4–Rrd1 complexes under nutrient-rich conditions, either by directly phosphorylating Tap42 (Jiang & Broach, 1999) or by preventing Tap42 dissociation via the phosphoprotein Tip41 (Jacinto *et al.*, 2001). Thus, TORC1 inactivation results in dephosphorylation of and increased association between Tap42 and Tip41, and consequently, the release of the PP2Ac–Rrd2 and Sit4–Rrd1 dimers. These released dimers then presumably become active and/or have altered substrate specificities (Düvel *et al.*, 2003; Düvel & Broach, 2004; Van Hoof *et al.*, 2005; Zheng & Jiang, 2005; Yan *et al.*, 2006). In line with genome-wide transcription analyses, which suggest that TORC1 is downregulated as cells transit through the diauxic shift (Hardwick *et al.*, 1999), PP2Ac–Rrd2 and Sit4–Rrd1 dimers are also released from Tap42 when cells approach stationary phase (Di Como & Arndt, 1996). TORC1 signals, mainly via its proximal effectors Sch9 and the phosphatase · Rrd1/2 modules, to distal readouts to positively regulate ribosome biogenesis and translation and to inhibit stress responses that are incompatible with growth and typically induced in quiescent cells.

Ribosome biogenesis

TORC1 controls growth by favoring the expression and assembly of the translational machinery, which requires the coordinated regulation of RNA Pol I-, II-, and III-mediated transcription of 35S rDNA repeats, RP/Ribi genes, and tRNA genes, respectively (Zaragoza *et al.*, 1998; Cardenas *et al.*, 1999; Hardwick *et al.*, 1999; Powers & Walter, 1999; Jorgensen & Tyers, 2004; Lempiäinen & Shore, 2009). To this end, TORC1 regulates the function of several transcription factors by different means (Fig. 2). These include (1) stabilization of the initiation-competent Rrn3–RNA Pol I complex and Sch9-controlled recruitment of RNA Pol I to rDNA loci, which may also require direct binding of TORC1

to rDNA promoters (Claypool *et al.*, 2004; Li *et al.*, 2006; Huber *et al.*, 2009; Singh & Tyers, 2009), (2) stabilization of Hmo1 at 35S rDNA loci to endorse RNA Pol I-mediated transcription (Berger *et al.*, 2007), (3) promotion of Ifh1–Fhl1 complex formation to favor RNA Pol II-dependent RP gene expression [possibly in part via casein kinase 2 (CK2)-mediated phosphorylation of Ifh1] (Martin *et al.*, 2004; Schawalder *et al.*, 2004; Wade *et al.*, 2004; Rudra *et al.*, 2005, 2007), (4) reciprocal recruitment of the NuA4 histone acetyltransferases and Rpd3 histone deacetylases to RP gene promoters when TORC1 is active and inactive, respectively (Reid *et al.*, 2000; Rohde & Cardenas, 2003; Humphrey *et al.*, 2004), (5) promotion, apparently as a result of direct TORC1-mediated phosphorylation (Lempiäinen *et al.*, 2009), of Sfp1 nuclear localization and consequently activation of Ribi and – following extraction of Ifh1–Flh1-bound RP gene promoters from repressive domains within the nucleolus – RP gene expression (Jorgensen *et al.*, 2004; Marion *et al.*, 2004), (6) inhibition, likely in part via Sch9 (Huber *et al.*, 2009), of Stb3 and Dot6/Tod6, which repress Ribi gene transcription, presumably by recruiting histone deacetylase complexes to rRNA-processing elements (RRPEs) and RNA Pol A and C (PAC) motifs, respectively (Kasten & Stillman, 1997; Humphrey *et al.*, 2004; Liko *et al.*, 2007; Badis *et al.*, 2008; Freckleton *et al.*, 2009; Lippman & Broach, 2009; Zhu *et al.*, 2009; Liko *et al.*, 2010), (7) stimulation of RNA Pol III-dependent 5S rRNA and tRNA expression as a result of direct or indirect (via Sch9) TORC1-mediated inhibition of the conserved RNA Pol III repressor Maf1 (Oficjalska-Pham *et al.*, 2006; Roberts *et al.*, 2006; Huber *et al.*, 2009; Wei & Zheng, 2009; Wei *et al.*, 2009b), and (8) promotion of ribosome assembly by preventing entrapment of the 40S ribosome synthesis factors Dim2 and Rrp12 within the nucleolus (Vanrobays *et al.*, 2008).

Translation

TORC1 positively controls growth at the level of translation initiation by inhibiting Sit4-mediated dephosphorylation of the negative regulatory p-Ser^{577} residue within the eIF2α kinase Gcn2 and a parallel Sch9-mediated mechanism that antagonizes eIF2α phosphorylation (Cherkasova & Hinnebusch, 2003; Urban *et al.*, 2007) (Fig. 2), as well as by still poorly understood mechanisms that implicate the adaptor protein eIF4G and the eIF4E-binding protein Eap1 (Barbet *et al.*, 1996; Berset *et al.*, 1998; Danaie *et al.*, 1999; Cosentino *et al.*, 2000; Kuruvilla *et al.*, 2001). By activating translation initiation, TORC1 impinges indirectly on cell cycle decisions, because, as noted above, *CLN3* mRNA translation and consequently passage of cells through START is specifically sensitive to the inhibition of translation initiation (Barbet *et al.*, 1996). TORC1 also regulates the decision to pass START by destabilizing the CDK inhibitor Sic1 via a mechanism that is still under study and that appears to involve Cdc34-dependent ubiquitination (Verma *et al.*, 1997; Zinzalla *et al.*, 2007).

Stress responses

In addition to stimulating growth, TORC1 plays an equally important role in suppressing a number of (nutrient) stress responses (Fig. 2). Firstly, TORC1 inhibits the transcription of nitrogen-catabolite repression-sensitive genes by favoring cytoplasmic anchorage of the GATA transcription factors Gln3 (via its association with Ure2) and Gat1 (presumably via another yet unidentified anchor protein) (Beck & Hall, 1999; Cardenas *et al.*, 1999; Hardwick *et al.*, 1999; Bertram *et al.*, 2000; Shamji *et al.*, 2000; Carvalho *et al.*, 2001; Carvalho & Zheng, 2003; Georis *et al.*, 2011). Cytoplasmic retention of Gln3/Gat1 appears to be partially controlled by Tap42–phosphatases (Tap42–PPases). Recent evidence indicates that the regulation of Gln3/Gat1 function is complex, varies among different yeast strains, and involves TORC1-independent nutrient-sensing mechanisms (Georis *et al.*, 2009; Tate *et al.*, 2009, 2010). Secondly, TORC1 antagonizes nuclear accumulation of and consequently transcription mediated by the heterodimeric Rtg1–Rtg3 transcription factor complex, a central element of the mitochondria-to-nucleus signaling (or retrograde response) pathway that activates genes whose products (including mitochondrial and peroxisomal enzymes) are required for glutamate and glutamine homeostasis (for a review, see Liu & Butow, 2006). TORC1 exerts this control by favoring, presumably via the regulation of Tap42–PPases (Düvel *et al.*, 2003), the association of Rtg1–Rtg3 with a cytoplasmic Mks1- and 14-3-3 protein Bmh1/2-containing complex and by precluding the disruption of this complex by Rtg2 (Liao & Butow, 1993; Komeili *et al.*, 2000; Sekito *et al.*, 2000, 2002; Dilova *et al.*, 2002, 2004; Tate *et al.*, 2002; Liu *et al.*, 2003). Thirdly, TORC1 promotes cytoplasmic accumulation of Msn2, which may (Beck & Hall, 1999) or may not (Santhanam *et al.*, 2004) require Bmh1/2, via the Tap42–PPase branch that likely impinges in parallel to PKA on the NES of Msn2 (Görner *et al.*, 2002; Düvel *et al.*, 2003). Fourthly, TORC1 acts through Sch9 and possibly a PPase to anchor Rim15 via Bmh1/2 in the cytoplasm (Reinders *et al.*, 1998; Pedruzzi *et al.*, 2003; Wanke *et al.*, 2005). Fifthly, TORC1 inhibits autophagy by directly phosphorylating Atg13, thereby preventing the assembly of the Atg1–Atg13 complex, and possibly by an additional mechanism that implicates Tap42–PPases (Funakoshi *et al.*, 1997; Kamada *et al.*, 2000, 2010; Yorimitsu *et al.*, 2009). Lastly, TORC1 regulates the sorting of diverse nutrient permeases to and from the plasma membrane via the Tap42–PPase target Npr1 (Vandenbol *et al.*, 1990; Schmidt *et al.*, 1998; Beck *et al.*, 1999; De

Craene *et al.*, 2001). How Npr1 regulates these sorting events is currently not well understood, but recent evidence suggests that Npr1 targets arrestins (e.g. Aly2) to control intracellular trafficking of permeases (such as the general amino acid permease Gap1) (O'Donnell *et al.*, 2010).

Induction of stress responses is an important determinant for the survival of yeast cells during stationary phase. In this context, well-designed genome-wide analyses of chronological longevity factors (Powers *et al.*, 2006; Burtner *et al.*, 2011) have significantly contributed to the current view that partial inhibition of TORC1 or loss of Sch9 increases stationary-phase survival (or CLS) in a Rim15- and Msn2/4-dependent manner (Wanke *et al.*, 2008; Wei *et al.*, 2008, 2009a; Talarek *et al.*, 2010).

The cell wall integrity (CWI) pathway

The CWI pathway is comprised of a family of cell surface sensors (belonging to the CWI and stress response component WSC family of proteins) that are coupled via the Rom1/2 GEFs to the small GTPase Rho1, which activates a set of effectors including the β-1,3-glucan synthase and protein kinase C (Pkc1; reviewed in Levin, 2005). Pkc1 has multiple effectors, the best characterized being a mitogen-activated protein kinase (MAPK) cascade, composed of the MAPKKK Bck1, the redundant MAPKKs Mkk1/2, and the MAPK Slt2/Mpk1, which regulates (in part by activating the transcription factor Rml1 and by inactivating Sir3-mediated subtelomeric gene silencing; Ai *et al.*, 2002; Levin, 2005) the expression of cell wall biosynthetic enzymes implicated in remodeling the cell wall during normal growth and in response to stress. Loss of Pkc1, Bck1, or Mpk1 causes zymolyase sensitivity and drastically reduces cell viability following carbon or nitrogen starvation, suggesting that CWI pathway-controlled cell wall remodeling is an important aspect of the quiescence program (Krause & Gray, 2002; Torres *et al.*, 2002). Intriguingly, both cells entering stationary phase and cells treated with rapamycin exhibit enhanced phosphorylation of Mpk1 at sites required for its activation (Ai *et al.*, 2002; Krause & Gray, 2002; Torres *et al.*, 2002). Consequently, TORC1 may, possibly via Sch9 and/or Tap42–Sit4 (Fig. 2), impinge upon the CWI pathway, but whether this occurs at the level of the WSC family members, Rom2, or the Pkc1–Bck1–Mpk1 cascade is currently unknown (Ai *et al.*, 2002; Torres *et al.*, 2002; Reinke *et al.*, 2004; Araki *et al.*, 2005; Kuranda *et al.*, 2006; Soulard *et al.*, 2010).

The Snf1-signaling network

The Snf1 protein kinase, like its mammalian ortholog the AMP-activated protein kinase (AMPK), functions within a heterotrimeric complex, which, in yeast, is composed of the Snf1 (α) catalytic subunit, one of three β-subunit isoforms (Gal83, Sip1, or Sip2), and the Snf4 (γ) subunit (reviewed in Hardie *et al.*, 1998; Sanz, 2003; Hedbacker & Carlson, 2008). This heterotrimeric complex is a central controller of energy homeostasis that is primarily required for the adaptation of cells to glucose limitation and for growth both on less preferred fermentable carbon sources (e.g. sucrose, galactose, or maltose) and on nonfermentable carbon sources (e.g. ethanol and glycerol). Accordingly, Snf1 plays a particularly prominent role when cells enter the diauxic shift phase in part by controlling the expression of a large set of genes that are involved in the metabolism of alternative carbon sources, in gluconeogenesis, and in respiration. Consequently, in the absence of Snf1, cells fail to properly acquire many of the key traits of quiescent cells and rapidly lose viability as they approach stationary phase (Thompson-Jaeger *et al.*, 1991), underlining the importance of the metabolic reprogramming at the diauxic shift in priming the cells for proper entry into quiescence at later stages when nutrients become exhausted (Gray *et al.*, 2004; Martinez *et al.*, 2004).

What regulates Snf1?

In mammalian cells, energy stress results in increased levels of AMP, which allosterically activates AMPK and protects it from dephosphorylation of a critical p-Thr within the activation loop of the catalytic α-subunit (Sanders *et al.*, 2007). In yeast, the role of AMP in the activation of Snf1 is uncertain (Mitchelhill *et al.*, 1994; Woods *et al.*, 1994; Wilson *et al.*, 1996; Momcilovic *et al.*, 2008), and the molecular details of how nutrients impinge on Snf1 activation remain poorly understood. Nonetheless, glucose depletion is known to activate Snf1 by alleviating (via Snf4) its intramolecular autoinhibition and by promoting phosphorylation of Thr^{210} within its protein kinase activation loop by any of three Snf1 kinases (Sak1, Tos3, or Elm1) (Jiang & Carlson, 1996; Hong *et al.*, 2003; Nath *et al.*, 2003; Sutherland *et al.*, 2003; Momcilovic *et al.*, 2008; Liu *et al.*, 2011). However, the Snf1 kinases are not regulated by glucose and nutrient control of Thr^{210} phosphorylation appears to be mainly exerted via Reg1, which, in conjunction with Snf1, controls the access of the PP1 Glc7 to the Thr^{210} residue within Snf1 (Tu & Carlson, 1995; Ludin *et al.*, 1998; McCartney & Schmidt, 2001; Rubenstein *et al.*, 2008; Tabba *et al.*, 2010). Glucose also regulates Snf1 complexes at the level of substrate accessibility by controlling the subcellular localization of the β-subunits Gal83 and Sip1, which relocate from the cytoplasm to the nucleus (Gal83) or to the vacuolar membrane (Sip1) upon glucose depletion (Vincent *et al.*, 2001; Hedbacker & Carlson, 2006). Interestingly, PKA appears to inhibit Sip1 vacuolar localization (Hedbacker *et al.*, 2004), but the significance of this regulatory step is unknown. In summary, glucose modulates both the

phosphorylation of Snf1 to control its activity and its subcellular localization to control its access to specific substrates, but the underlying mechanism(s) remains elusive.

What does Snf1 regulate?

Snf1 regulates the transcription of approximately 400 genes (Young *et al.*, 2003), either by inhibiting transcriptional repressors (e.g. Mig1), stimulating transcriptional activators (e.g. Adr1, Cat8, and Sip4), or controlling the transcriptional machinery directly. Snf1 also plays a role in various other processes including chromatin modification, translation, autophagy, and control of metabolic enzyme activities as briefly summarized below.

Transcriptional activators, repressors, and RNA Pol II holoenzyme

As a central regulator of the adaptive transcriptional program that serves the cells to cope with reduced glucose availability, Snf1 exerts its control by various means. Firstly, Snf1 induces many glucose-repressed genes by phosphorylation of the transcriptional repressor Mig1, which alters the Mig1–Ssn6–Tup1 repressor–corepressor interaction and promotes Mig1 nuclear export. This alleviates repression of certain high-affinity hexose carrier genes and repression of genes that are required for the metabolism of alternative carbon sources (Treitel & Carlson, 1995; Tzamarias & Struhl, 1995; Östling *et al.*, 1996; Özcan & Johnston, 1996; Treitel *et al.*, 1998; DeVit & Johnston, 1999; Smith *et al.*, 1999; Papamichos-Chronakis *et al.*, 2004). Secondly, Snf1 plays a dual role in the activation of gluconeogenic genes by the carbon source-responsive element-binding transcription factors Cat8 and Sip4. Accordingly, Snf1-mediated inactivation of Mig1 allows biosynthesis of Cat8. Phosphorylation (directly or indirectly mediated by Snf1) converts Cat8 into a transcriptional activator, which subsequently stimulates the expression of Sip4 (Hedges *et al.*, 1995; Lesage *et al.*, 1996; Rahner *et al.*, 1996; Randez-Gil *et al.*, 1997). Cat8 and Sip4, which is likely activated through a Gal83-mediated interaction with and phosphorylation by Snf1, contribute to the transcriptional activation of gluconeogenic genes, with Cat8 being the more important activator (Lesage *et al.*, 1996; Vincent & Carlson, 1998). Thirdly, Snf1 is required for promoter binding, coactivator recruitment, and (indirect) control of the Ser^{230} phosphorylation level of the transcription factor Adr1, which activates the expression of genes involved in the catabolism of nonfermentable carbon sources and β-oxidation of fatty acids (Young *et al.*, 2002, 2003; Tachibana *et al.*, 2005; Biddick *et al.*, 2008; Ratnakumar *et al.*, 2009). Fourthly, Snf1 phosphorylates the Hsf1 transcription factor to promote its binding to and subsequent transcription from specific promoters of stress-inducible genes in response to glucose starvation (Tamai *et al.*, 1994; Hahn & Thiele, 2004). Notably, Hsf1 may, in some cases, cooperate with Mns2/4 to induce transcription of stress genes (Amorós & Estruch, 2001; Grably *et al.*, 2002). Fifthly, Snf1 phosphorylates Msn2 to inhibit its nuclear accumulation as part of an adaptation process to long-term carbon starvation (Mayordomo *et al.*, 2002; De Wever *et al.*, 2005). Sixthly, Snf1 favors (possibly by direct phosphorylation) nuclear accumulation of Gln3 in response to glucose starvation (Bertram *et al.*, 2002). Seventhly, Snf1 (directly or indirectly) phosphorylates Rgt1 to promote its binding to and repress transcription from the *HXK2* promoter under low-glucose conditions (Palomino *et al.*, 2006). This regulation may be relevant because hexokinase 2 (Hxk2) plays a role in antagonizing Snf1 function, possibly through direct binding to and preventing inactivation of Mig1 by Snf1-mediated phosphorylation (Sanz *et al.*, 2000; Ahuatzi *et al.*, 2004, 2007). Lastly, Snf1 may, besides impinging on transcriptional activators and repressors, also directly control the function of the RNA Pol II holoenzyme, but the corresponding mechanism(s) remains unknown (Kuchin *et al.*, 2000; Shirra *et al.*, 2005; Tachibana *et al.*, 2007).

Chromatin modification

Upon glucose depletion, Snf1 phosphorylates at certain promoters Ser^{10} within histone H3 (Lo *et al.*, 2001), which may (Lo *et al.*, 2001, 2005) or may not (Geng & Laurent, 2004; Liu *et al.*, 2005; Shirra *et al.*, 2005) be relevant for activation of the corresponding genes. In some cases, Snf1 influences the recruitment of the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex to specific promoters by processes that depend on either Snf1-mediated H3 Ser^{10} phosphorylation, Snf1-dependent relief of Ssn6–Tup1-mediated repression, or a more direct role of Snf1 in SAGA complex regulation, as it physically interacts with and likely phosphorylates a number of residues within the catalytic domain of the histone acetyltransferase Gcn5 (Lo *et al.*, 2001; Liu *et al.*, 2005, 2010; van Oevelen *et al.*, 2006).

Metabolism and translation

Snf1 controls cellular energy homeostasis by regulating carbohydrate and fatty acid metabolism at a post-transcriptional level. For instance, Snf1 favors the induction of glycogen synthesis upon glucose limitation in part because it antagonizes Pcl8/10–Pho85 cyclin–CDK-mediated phosphorylation and inhibition of glycogen synthase Gsy2 (Thompson-Jaeger *et al.*, 1991; Hardy *et al.*, 1994; Huang *et al.*, 1996; Wilson *et al.*, 1999). Snf1 appears to positively act (via poorly understood mechanisms) on autophagy, thereby contributing to the partial sequestration of glycogen within the vacuole where glycogen is protected from

degradation during the early stages of stationary phase as long as the activity of the vacuolar glucoamylase Sga1 remains low (Wang *et al.*, 2001). During growth on non-fermentable carbon sources, Snf1 is required for the activation of the PAS kinase Psk1 (and hence for Ugp1 phosphorylation) (Grose *et al.*, 2007, 2009), which likely favors cell wall biosynthesis at the expense of glycogen synthesis when cells grow in the postdiauxic shift phase. Activated Snf1 kinase represses anabolic processes, such as the biosynthesis of fatty acids, likely by direct phosphorylation and inactivation of acetyl-CoA carboxylase (Acc1), which is the key regulatory step in the biosynthesis of fatty acids (Mitchelhill *et al.*, 1994; Woods *et al.*, 1994). As noted above, Snf1 is further thought to inhibit translation initiation by at least two different mechanisms. On the one hand, Snf1 may be involved in preventing, by still unknown means, the formation of 48S preinitiation complex formation when cells are deprived of glucose (Ashe *et al.*, 2000; Hoyle *et al.*, 2007). On the other hand, Snf1 favors eIF2α phosphorylation by promoting the autophosphorylation of Thr^{882} within the activation loop of the eIF2α kinase Gcn2 and inhibiting (directly or indirectly) the proposed eIF2α-PPs Glc7 and Sit4 under defined nutrient conditions (Cherkasova *et al.*, 2010). Finally, because Gcn2 has been found to be required for specific aspects of nitrogen-starvation-induced autophagy (Tallóczy *et al.*, 2002; Ecker *et al.*, 2010), it may be informative to address the question of whether Snf1 modulates autophagy via Gcn2.

The Pho85-signaling network

As stated above, entry into quiescence can be triggered by phosphate starvation (Lillie & Pringle, 1980), albeit the corresponding regulatory mechanisms are largely unknown. The key nutrient-signaling kinase that orchestrates the phosphate starvation response in yeast is the CDK Pho85, which associates with a family of 10 cyclins, each of which can potentially direct Pho85 to different target substrates (Carroll & O'Shea, 2002). The best-studied partner of Pho85 is the cyclin Pho80. Accordingly, in the presence of sufficient phosphate, the Pho80–Pho85 cyclin–CDK complex inhibits the phosphate starvation response by controlling the localization and activity of the transcription factor Pho4, which activates the transcription of genes involved in both phosphate scavenging and metabolism (Carroll & O'Shea, 2002). Pho85 also negatively controls the expression of an additional set of genes (including glycogen and trehalose synthesis, oxidoreductive stress, and protein-folding genes) that are typically induced under glucose-limiting conditions before entry into quiescence (DeRisi *et al.*, 1997; Timblin & Bergman, 1997; Ogawa *et al.*, 2000; Carroll *et al.*, 2001; Nishizawa *et al.*, 2004; Swinnen *et al.*, 2005). Some of these effects may be explained by Pho80–Pho85 cyclin–CDK complex-mediated phosphorylation and consequently enhanced nuclear exclusion of Rim15 and Crz1 (Wanke *et al.*, 2005; Sopko *et al.*, 2006). Moreover, as mentioned above, Pcl8/10–Pho85 cyclin–CDK also controls glycogen synthesis by inhibiting Gsy2 (Huang *et al.*, 1996). Thus, proper execution of the quiescence program includes, to some extent, integration of Pho85-mediated signals.

Network integration

A major challenge in the field is to understand how the different signals transmitted by the TORC1, PKA, Snf1, and Pho85 pathways are integrated to ensure the induction of a quiescence program that allows the cells to survive starvation for any of the key nutrients. Relevant to this discussion is the view, emerging from recent systems biology studies, that quiescent states are likely built on a common core program, but may also be individually structured in response to the nature of the encountered nutrient stress (Gasch *et al.*, 2000; Wu *et al.*, 2004; Gutteridge *et al.*, 2010; Klosinska *et al.*, 2011). Conceptually, this may be achieved by shaping signaling pathways that, in addition to regulating pathway-specific readouts, converge on a set of key effectors and mutually modulate responsiveness to and/or transmission of signals. Recent evidence suggests that yeast cells in fact utilize both of these strategies to dynamically configure the quiescent state according to the environmental challenges encountered.

Convergence of pathways on key nodes

A recurrent theme that emerges from the above outline is that the PKA, TORC1, Snf1, and Pho85 pathways impinge, in various combinations, on common target proteins that often serve as regulatory nodes, which in turn critically determine the proper establishment of the quiescence program. The increasing list of such regulatory focal points includes two classes of proteins that are oppositely regulated, i.e. proteins that are required for the proper setup of the quiescence program (e.g. Rim15, Msn2, Atg1–Atg13, Gln3, Hsf1, Crz1, and Gsy2) and proteins that are indispensable for robust growth (e.g. eIF2α and Sfp1). Some of these critical nodes and their control by nutrient-signaling pathways shall be briefly recalled here (Fig. 3a). For instance, TORC1 and the Pho80–Pho85 cyclin–CDK promote cytoplasmic sequestration, while PKA inhibits the kinase activity of Rim15 (Fig. 3b) (Reinders *et al.*, 1998; Pedruzzi *et al.*, 2003; Wanke *et al.*, 2005). In a similar vein, TORC1 and PKA independently antagonize nuclear accumulation of Msn2 to prevent the induction of stress-responsive genes (Görner *et al.*, 1998, 2002; Santhanam *et al.*, 2004; De Wever *et al.*, 2005), target the Atg1–Atg13 complex to inhibit autophagy (Stephan *et al.*, 2009), and promote nuclear localization of the transcription factor Sfp1 to favor the transcription of

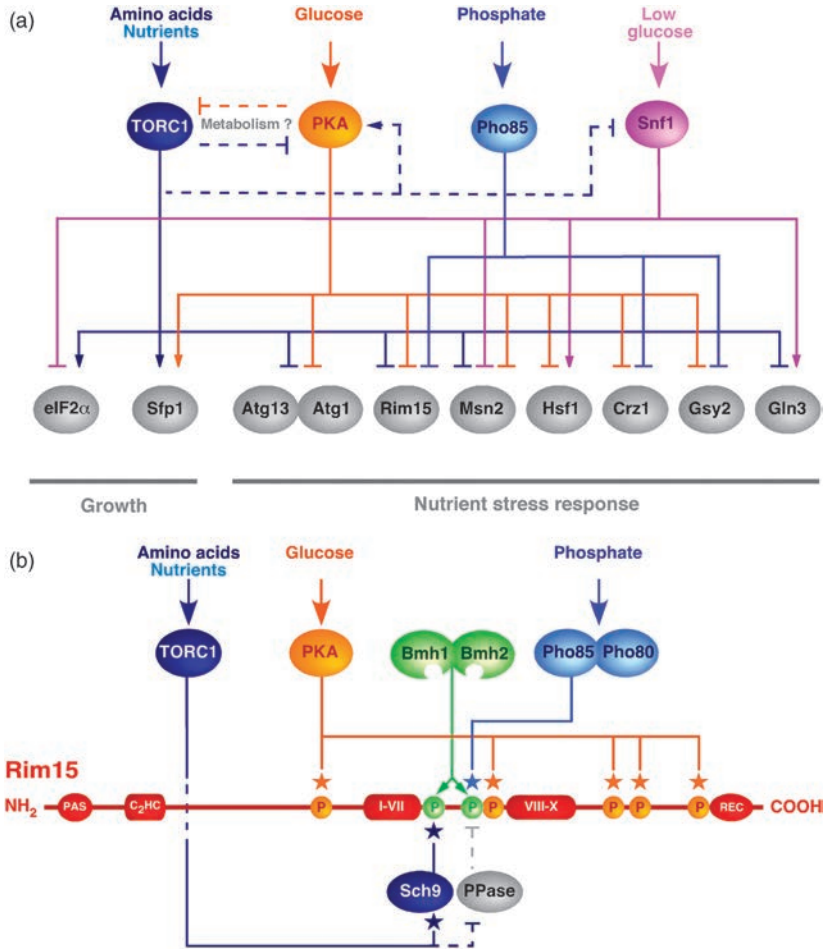

Fig. 3. Convergence of pathways on key nodes. (a) The TORC1, PKA, Pho85, and Snf1 pathways impinge, in various combinations, on common target proteins that serve as regulatory nodes, which critically determine the proper establishment of the quiescence program. Arrows and bars denote positive and negative interactions, respectively, which can either be direct or indirect. Dashed arrows and bars refer to potential cross-talk mechanisms between TORC1, PKA, and/or Snf1. See text for further details. (b) Nutrient signal integration by Rim15. The schematic diagram illustrates the domain architecture of Rim15, which is drawn approximately to scale. Rim15 belongs to a small group of conserved fungal proteins, which exhibit the same domain organization. These include the N-terminal PAS and C_2HC-type zinc finger domains, the central protein kinase domain, and a C-terminal receiver domain. Notably, Rim15 is a distant member of the conserved nuclear Dbf2-related and large tumor suppressor serine/threonine kinase subclass of the protein kinase A, G, and C class of kinases, which share the unique feature of harboring an insert of at least 30 amino acids between the protein kinase subdomains VII and VIII (Tamaskovic *et al.*, 2003). Rim15 function is regulated by at least four nutrient-regulated protein kinases. Accordingly, cytoplasmic Rim15, anchored through its binding to the 14-3-3 proteins Bmh1/2, is maintained inactive through PKA-mediated phosphorylation of at least five of its amino acid residues (i.e. Ser^{709}, Ser^{1094}, Ser^{1416}, Ser^{1463}, and Ser^{1661}; orange circles containing the letter P; Reinders *et al.*, 1998). Moreover, phosphorylation of Thr^{1075} and Ser^{1061} (green circles containing the letter P) engages Rim15 in binding the two monomeric subunits within a single 14-3-3 protein dimer in the cytoplasm. Ser^{1061} is directly phosphorylated by the TORC1 target Sch9 and Thr^{1075} phosphorylation is independently regulated by the Pho80-Pho85 cyclin-CDK (by direct phosphorylation) and by TORC1 (presumably via inhibition of a protein phosphatase; PPase) (Pedruzzi *et al.*, 2003; Wanke *et al.*, 2005, 2008). Solid arrows and bars refer to direct interactions; dashed bars refer to indirect and/or potential interactions. Stars refer to direct phosphorylation events mediated by TORC1, PKA, Pho85, or Sch9. See text for further details.

Ribi/RP genes (Jorgensen *et al.*, 2004; Marion *et al.*, 2004; Oficjalska-Pham *et al.*, 2006; Roberts *et al.*, 2006; Huber *et al.*, 2009; Wei & Zheng, 2009; Wei *et al.*, 2009b). In line with these observations, TORC1 and PKA have also been suggested, on the basis of transcriptional profile studies, to provide separate inputs to control various (e.g. Ribi/RP) gene clusters (Zurita-Martinez & Cardenas, 2005; Chen & Powers, 2006; Lippman & Broach, 2009). Other examples include Gln3 and eIF2α, both of which are independently and oppositely regulated by TORC1 and Snf1 (Beck & Hall, 1999; Bertram *et al.*, 2002; Cherkasova & Hinnebusch, 2003; Cherkasova *et al.*, 2010). PKA, Snf1, and Pho85 all appear to converge on Gsy2, although the molecular details of the individual regulatory steps remain to be elucidated

(Thompson-Jaeger *et al.*, 1991; Hardy & Roach, 1993; Hardy *et al.*, 1994; Huang *et al.*, 1996; Wilson *et al.*, 1999). Lastly, PKA and Pho85 favor the nuclear exclusion of Crz1 (Kafadar & Cyert, 2004; Sopko *et al.*, 2006). In summary, a wealth of data supports the idea that key nutrient-signaling pathways regulate both pathway specific as well as common effectors that communicate unified, but differentiated responses.

Mutual control of signaling pathways

Whether and how the various nutrient-signaling pathways cross-talk to each other is currently very poorly studied, although recent data are beginning to shed light on this important aspect of the quiescence program. For instance, PKA and TORC1 pathways have been suggested to antagonize each other within a certain physiological range, thereby buffering relatively minor environmental changes to ensure rather constant growth rates (Ramachandran & Herman, 2011). In support of this model, PKA downregulation was found to rescue the temperature-sensitive growth defect of a *las24-1*/*kog1*^{ts} strain, indicating that PKA negatively regulates TORC1 function (Araki *et al.*, 2005). The molecular details of the antagonism between PKA and TORC1 are currently unknown, but it is possible that downregulation of either pathway causes a short-term overflow of critical nutrient signals that spill over into neighboring nutrient-signaling pathways. In support of this assumption, metabolic profile analyses have shown that glutamate tends to accumulate during carbon starvation (i.e. when PKA activity is expected to be low), while various glycolytic and tricarboxylic acid cycle intermediates accumulate during nitrogen starvation (i.e. when TORC1 activity is expected to be low) (Brauer *et al.*, 2006; Boer *et al.*, 2010). Based on these considerations, a profound appreciation of the cross-talk between different nutrient-signaling pathways will require integrative analyses of the changes in metabolic fluxes that are triggered by the modulation of individual nutrient-signaling pathways.

Recent data further suggest the existence of direct control mechanisms between nutrient-signaling pathways (Fig. 3a). Firstly, TORC1 prevents, via an unknown mechanism, phosphorylation (at Thr^{210}) and thus activation of Snf1 (Orlova *et al.*, 2006). Secondly, TORC1 also impedes, by a largely unknown mechanism, the nuclear accumulation of both PKA (i.e. Tpk1) and Yak1 (Schmelzle *et al.*, 2004). Because Bcy1 resides predominantly in the nucleus, TORC1 inactivation might consequently favor the engagement of Tpk1 subunits into the formation of inactive Tpk1-Bcy1 holoenzymes within the nucleus (Griffioen *et al.*, 2000; Martin *et al.*, 2004; Schmelzle *et al.*, 2004). Thirdly, in line with several genetic studies suggesting that TORC1 negatively regulates the CWI pathway and that the CWI pathway antagonizes PKA (Verna *et al.*, 1997; Park *et al.*, 2005; Kuranda *et al.*, 2006), TORC1 was recently found to prevent (indirectly via a circuit that implicates Sch9) Mpk1 activation and consequently Mpk1-mediated phosphorylation of Bcy1, which is thought to inhibit PKA towards specific substrates (Soulard *et al.*, 2010). A model that unifies the latter observations is that TORC1, via its effects on Tpk1 localization and Bcy1 phosphorylation, controls the spatial distribution of PKA activity. Accordingly, TORC1 inactivation may convert the nucleus into a low PKA environment that should, nonetheless, retain cAMP responsiveness (Griffioen *et al.*, 2000, 2001; Soulard *et al.*, 2010). Such a scenario also provides an elegant explanation for why Rim15, which is anchored in the cytoplasm due to TORC1 function and maintained inactive by PKA-mediated phosphorylation (Reinders *et al.*, 1998; Pedruzzi *et al.*, 2003; Fig. 3b), can be activated by TORC1 inactivation, i.e., once released from its cytoplasmic anchors and transferred into the nucleus, Rim15 may encounter a low PKA environment and hence be released from PKA inhibition (Pedruzzi *et al.*, 2003; Wanke *et al.*, 2005). Conversely, it remains unknown why inactivation of only PKA (which does not cause nuclear accumulation of Rim15) also suffices to induce Rim15-dependent aspects of the quiescence program. Among conceivable models to be tested in the future are the possibilities that critical Rim15 target proteins (e.g. Igo1/2) may be activated in the cytoplasm and subsequently imported into the nucleus to carry out their functions, or that a small nuclear fraction of the pool of (GFP)-Rim15 molecules, which may escape detection by conventional fluorescence microscopy, is sufficient to ascertain a significant response upon PKA inactivation. All of the recent evidence, taken together, suggests that the PKA, TORC1, and Snf1 pathways perform their functions within a complex wired network to adequately shape the cellular response to nutrient starvation.

Concluding remarks and future issues

Substantial progress has been made in defining the physiological state of quiescent cells and the nutrient-signaling pathways that shape this state, particularly when cells are grown in liquid cultures to saturation on a rich medium. An emerging view is that cells, rather than relying on a binary 'on-off' decision, dynamically configure the quiescent program according to the various environmental challenges by using a set of different key nutrient-signaling pathways that, in addition to regulating pathway-specific effectors, converge on a set of integrative nodes (e.g. Rim15, Msn2, and Atg1–Atg13) and mutually modulate their competence to transmit signals. This model implies that, even though quiescent cells share a distinguished set of common traits, all quiescent programs, whether they are induced by nitrogen, phosphate, sulfur, or carbon starvation, are not the

same. While most recent studies indeed support this idea, the tremendous progress in the development of analytical tools such as transcript, metabolic, and proteomic profiling, is likely to shed more light on the presumed diversity of quiescent states. In this context, it is useful to emphasize that stationary-phase cultures exhibit a complex, heterogeneous community structure and that available studies on stationary-phase cells generally represent data on the average behavior of a cell within a population. Thus, it is possible that even within a stationary-phase culture, individual cells may differ with respect to their interpretation of and response to the environmental signals. The existence of heterogeneity at this level (be it of genetic, epigenetic, or physiological nature) is at present speculative, but may be conceptually important for the overall fitness of the population.

Despite the wealth of existing data on quiescence, there are still a number of important gaps in our understanding on how cells decide and subsequently proceed to enter into quiescence. Among the most pertinent questions are the following: Do quiescent cells arrest at a unique off-cycle point in G_1? How do nutrient-signaling pathways impinge on the cell cycle machinery? What is the precise nature of the nutrient cues that control TORC1? How is glucose or its absence sensed by the Ras/PKA or Snf1 pathway, respectively? How are the different nutrient-signaling pathways wired to each other to coordinate, to some extent, a unified developmental program? And last, but not least, what are the essential attributes of quiescent cells that ensure survival over a 200-year-long period in the dark and gloomy seabed of the Baltic Sea? Together with the numerous additional questions elaborated throughout this review, these questions illustrate that the 'sleeping beauty' is not yet ready to unveil the most treasured secrets of the essence of quiescence.

Acknowledgements

I thank Drs Debbie Ang, Costa Georgopoulos, Kelly Hughes, Robbie Loewith, Marie-Pierre Péli-Gulli, and Nicolas Talarek for critically reading the manuscript. C.D.V. is supported by the Swiss National Science Foundation and the Canton of Fribourg.

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