Metabolic status rather than cell cycle signals control quiescence entry and exit

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Quiescence is defined as a temporary arrest of proliferation, yet it likely encompasses various cellular situations. Our knowledge about this widespread cellular state remains limited. In particular, little is known about the molecular determinants that orchestrate quiescence establishment and exit. Here we show that upon carbon source exhaustion, budding yeast can enter quiescence from all cell cycle phases. Moreover, using cellular structures that are candidate markers for quiescence, we found that the first steps of quiescence exit can be triggered independently of cell growth and proliferation by the sole addition of glucose in both Saccharomyces cerevisiae and Schizosaccharomyces pombe. Importantly, glucose needs to be internalized and catabolized all the way down to glycolysis to mobilize quiescent cell specific structures, but, strikingly, ATP replenishment is apparently not the key signal. Altogether, these findings strongly suggest that quiescence entry and exit primarily rely on cellular metabolic status and can be uncoupled from the cell cycle.

Introduction

Most cells spend the majority of their life in quiescence, a state defined as a temporary absence of proliferation. Establishing quiescence and maintaining the capacity to reenter the proliferation cycle are critical for cell survival, and must be tightly orchestrated to avoid pathological proliferation. Remarkably little is known about the molecular bases of the transitions between proliferation and quiescence. For most organisms, it is acknowledged that quiescence entry takes place in the G1 phase, before or at the restriction point, a point after which cells are committed to the cell cycle (Hartwell et al., 1974; Pardee, 1974). The importance of such a point where quiescent cells synchronously arrest is still under debate (Cooper, 2003).

An essential issue is to elucidate whether quiescence results from a passive adaptation to adverse conditions or if it is actively driven by a “program” involving specific genes conserved among eukaryotes. The characterization of quiescent cell transcription profiles in a variety of organisms has demonstrated that specific genes are expressed upon entry into quiescence (Martinez et al., 2004; Wu et al., 2004; Radonjic et al., 2005; Coller et al., 2006; Shimanuki et al., 2007; Sambasivan et al., 2008), these genes being different from those expressed in G1. Importantly, when cells have entered quiescence by different routes, the transcriptional response is partly independent of the input signal (Wu et al., 2004; Coller et al., 2006; Sambasivan et al., 2008). Together, these findings suggest the existence of a cellular “program” that commits cells to quiescence. However, a unified view of the molecular determinants of the quiescent state in eukaryotes is clearly missing.

In unicellular eukaryotes like Saccharomyces cerevisiae, quiescence entry is triggered by nutrient limitation, and is controlled, at least in part, by a complex interplay between various nutrient-sensitive protein kinases such as PKA and Tor (Smets et al., 2010). Quiescence is associated with a variety of features, including a decrease of protein synthesis rate, acquisition of thermo-resistance, and accumulation of storage molecules (Gray et al., 2004). We have shown that yeast starved for carbon...
Figure 1. A yeast stationary phase population contains quiescent cells arrested in all cell cycle stages. [A] No cell growth or division could be observed after 7 d in YPDA. A 7-d-old culture of WT prototroph strain was stained with ConA-FITC, then washed and transferred back into either old or new YPDA medium. Stained, unstained, or hemi-stained cells were counted. [B and C] Actin cytoskeleton organization revealed by phalloidin staining and proteasome localization in WT cells grown for 7 d in YPDA (B) and 30 min after refeeding with YPDA (C). The proteasome localization was followed using Pre6p-GFP (B) or Scl1p-GFP (C). Cell outlines are drawn in white. [D] Colony formation after microseparation of unbudded and budded cells grown for 7 d in YPDA. [E] Budded quiescent cells can be arrested in all cell cycle stages. WT cells expressing Spc97p, a spindle pole body component, fused to GFP were grown for 7 d in YPDA and stained with DAPI (numbers are percentages of budded cells). For each time point, n > 200 cells, two experiments. Errors bars indicate SEM. Bars, 2 µm.
reorganize their actin cytoskeleton into F-actin reservoirs called actin bodies (Sagot et al., 2006). Additionally, we have found that upon carbon exhaustion, yeast relocalize their proteasome from the nucleus into a cytoplasmic structure, named the proteasome storage granule (PSG; Laporte et al., 2008). Recently, using a systematic approach, Narayanaswamy et al. (2009) have shown that many proteins that are localized diffusely in the cytoplasm of dividing yeast relocalize into cytoplasmic foci upon quiescence entry, most of these proteins being metabolic enzymes. Therefore, a broad range of cellular machineries seems to be drastically reorganized upon quiescence entry.

Here, using a combination of criteria, we show that upon carbon source exhaustion, quiescence entry can occur not only in G1, but also in all other cell cycle phases. This suggests that if a “quiescence program” does exist, it has to be independent of the cell cycle. Then, we show that quiescence exit can be triggered independently of cell growth and proliferation by the sole addition of glucose. Finally, we demonstrate that the glucose-induced signal triggering the disassembly of quiescent cells specific structures depends on glucose catabolism through glycolysis. Together, these findings strongly suggest that in yeast, the metabolic status is the critical signal that regulates quiescence establishment and exit.

**Results and discussion**

**Stationary phase cultures are composed of quiescent cells arrested in all cell cycle stages**

Stationary phase haploid yeast cultures have been shown to be heterogeneous based on physical properties (Allen et al., 2006; Aragon et al., 2008). To describe these cultures at the individual cell level, we characterized wild type (WT) yeast cells grown for 7 d in liquid glucose containing rich medium (YPDA), a condition for which carbon is the limiting nutrient (Gray et al., 2004; Smets et al., 2010). After 7 d, the population was composed of both unbudded (90 ± 5%) and budded (10% ± 5) cells. The presence of budded cells in a stationary phase population was previously described for different genetic backgrounds, different growth conditions, and various strain ploidy (Johnston et al., 1977; Sudbery et al., 1980; Allen et al., 2006; Sagot et al., 2006; Sahin et al., 2008). This observation suggested the possible existence of quiescent budded cells, challenging the commonly accepted idea that *S. cerevisiae* cells enter quiescence as unbudded cells in G1.

Given that quiescence is defined as a reversible nonproliferative state, we investigated whether these properties applied to the budded cell subpopulation. First, methylene blue staining revealed that after 7 d, 92 ± 2% of the budded cells and 95 ± 4% of unbudded cells were alive (n > 300 cells, three experiments), which indicates that budded cells have the same propensity to survive when starved for carbon as unbudded cells. Moreover, when cells were pulse-labeled with concanavalin-A–FITC (ConA-FITC; a conjugated lectin that stains the cell wall) and re-inoculated in their 7-d-old medium, no unstained or hemi-stained cells were observed even after an additional 48 h (Fig. 1 A). This experiment demonstrates that after 7 d, cells no longer proliferate. This was further confirmed by the fact that neither unbudded nor budded cells displayed features required for proliferation, namely a polarized actin cytoskeleton or nuclear proteasome. Instead, almost all cells had reorganized these machineries into actin bodies and PSG (Fig. 1 B).

Second, we asked whether these subpopulations were able to reenter the proliferation cycle. Cells were grown for 7 d then re-fed with YPDA. As shown in Fig. 1 C, in both cell types, actin bodies disassembled and the cytoskeleton reorganized into depolarized actin cables and patches. In parallel, proteasome subunits relocated from PSGs into the nucleus (Fig. 1, B and C). Of note, budded cells analyzed 30 min after refeeding were not newly budding cells because upon quiescence exit, new bud emergence required at least 60 min (see Fig. 3 A; Sagot et al., 2006; Sahin et al., 2008). Together, these observations revealed that the stationary phase budded cells subpopulation was resuming the early steps of cell proliferation. In fact, 2 h after refeeding, 30% of these budded cells had repolarized their actin cytoskeleton (Fig. S1, A and B).

Finally, to conclusively establish that the budded cells subpopulation found in stationary phase cultures upon carbon source exhaustion was quiescent, we tested their capacity to exit quiescence and give rise to progeny. Cells from a 7-d-old culture were isolated by micromanipulation, and their ability to form a colony was tested. This experiment showed that 95.1 ± 1.7% of unbudded cells (n = 135) and 65.7 ± 9.2% of budded cells (n = 162) could give rise to a colony (Fig. 1 D), which demonstrates that stationary phase budded cells, although apparently more fragile than the unbudded ones, are indeed bona fide quiescent cells. Importantly, DAPI staining and spindle pole body localization revealed that the quiescent budded cell subpopulation is composed of cells arrested in all cell cycle stages (Fig. 1 E). All together, these observations establish that upon carbon source exhaustion, quiescence entry can occur in all cell cycle phases, which strongly suggests that if a quiescent program exists in *S. cerevisiae*, it is independent of the cell cycle.

**Quiescence establishment can occur independently of cell cycle signals**

In a precursor study, Wei et al. (1993) had shown that upon carbon or nitrogen starvation, yeast cdc mutants were able to acquire heat shock resistance in whatever cell cycle stage they were arrested in, and that these arrested cells were capable of giving rise to a progeny. Although thermo-tolerance is one feature of yeast quiescence, resistance to heat can be induced in proliferating cells by several other routes (Parsell and Lindquist, 1993; Lindquist and Kim, 1996; Piper, 1998; Lu et al., 2009), and this property by itself cannot be used as a method to identify quiescent cells at the individual level. In contrast, actin bodies and PSGs are structures that are specifically assembled upon entry into quiescence after carbon source exhaustion (Fig. S1 C; Sagot et al., 2006; Laporte et al., 2008). In fact, although actin bodies do not form upon nitrogen starvation, probably because of the activation of the autophagy process (Fig. S1 D), both actin bodies and PSGs do form when proliferating cells are transferred to water, a condition where all nutrients are missing (Fig. S1, E and F), which strongly suggests that carbon limitation is a preeminent signal in yeast.
To definitively prove that cells arrested in cell cycle stages other than G1 can enter quiescence, we revisited the experiment of Wei et al. (1993) using actin body formation as a read-out. The cdc13-1 temperature-sensitive mutant is affected for telomere function and, when shifted to 37°C, arrests in G2/M (Hartwell et al., 1974; Burke and Church, 1991). WT and cdc13-1 rho0 cells were grown in YPDA at 25°C to OD600nm 2 and then shifted to 37°C for 14 h. As expected, after the shift, cdc13-1 cells arrested homogeneously as budded cells with a single nucleus (Fig. 2, A and B). Although cell cycle progression was blocked, cdc13-1 cells continued to increase in size and gave rise to large budded cells (Fig. 2 B; Johnston et al., 1977). Before glucose exhaustion, 95.3 ± 0.6% of cdc13-1 rho0 cells displayed a depolarized actin cytoskeleton (Fig. 2 B, top). However, after glucose exhaustion, the cdc13-1 rho0 population was composed of 95.2 ± 1.1% of budded cells, among which 99.3 ± 0.2% displayed actin bodies (Fig. 2 B, bottom). Importantly, these G2/M-arrested cdc13-1 rho0 cells displaying actin bodies were capable of giving rise to progeny (their ability to form colonies was 62.2 ± 2.7% of that of the WT cells). Thus the majority of the G2/M-arrested cdc13-1 cells could reenter the proliferation cycle and therefore meet the criteria of quiescence. To further prove that these cells were in G2/M and not in a pseudo-G1 state, we analyzed the localization of Whi5p, an inhibitor of the G1/S transition that mirrors Cdk activity. Indeed, Whi5p localizes in the nucleus in G1, exits the nucleus upon Cdk activation at the G1/S transition, and reenters the nucleus only in late mitosis (Costanzo et al., 2004). Consistently, in WT unbudded quiescent cells, Whi5p was not required for quiescence establishment, maintenance, or exit.
Finally, using a *cdc24-3* strain, we verified that a sole arrest in G1 is not sufficient to trigger actin body formation (Fig. S2, E and F). This is in good agreement with the fact that in both carbon source exhaustion is sufficient to trigger entry into quiescence even if cells are arrested in G2/M.

(Fig. S2, A to D). Together, these experiments demonstrate that
yeast and mammals, cell cycle arrest in G1 is not sufficient to induce a quiescence-specific gene expression profile (Coller et al., 2006; Aragon et al., 2008). Therefore, although quiescence entry and G1 arrest are generally concomitant, an arrest in G1 is neither necessary nor sufficient for quiescence establishment. Protein synthesis is crucial for reaching the critical cell size required for the passage through “start.” In fact, in S. cerevisiae, limited protein synthesis extends G1 length but has little effect on other cell cycle stages (Unger and Hartwell, 1976; Hartwell and Unger, 1977; Johnston et al., 1977; Slater et al., 1977; Popolo et al., 1982). Thus, the fact that quiescence entry occurs preferentially in G1 could simply be the passive result of a translation rate slowdown.

Glucose can trigger quiescence exit

Quiescence establishment is a slow phenomenon; therefore, the identification of signaling cascades involved in this process is difficult. In contrast, both actin body and PSG mobilization occur within seconds after nutrient addition (Sagot et al., 2006; Laporte et al., 2008; Sahin et al., 2008). In this context, we made the striking observation that the sole addition of glucose was leading to the mobilization of these structures. When cells grown for 7 d were transferred into a 2% glucose solution, both actin bodies and PSGs disassembled just as rapidly as upon transfer to YPDA (Fig. 3, A and B). This is reminiscent of a pioneer work by Granot and Snyder (1991, 1993) showing that these cells were no longer quiescent (Granot and Snyder, 1991, 1993).

Interestingly, we found that glucose is also sufficient to trigger quiescent cells’ specific structure mobilization in Schizosaccharomyces pombe. When S. pombe cells were starved for carbon, they arrested in G2 with a 2N DNA content (Costello et al., 1986) and assembled PSGs (Fig. 3 C; Laporte et al., 2008). As shown in Fig. 3 C, in S. pombe, just as in S. cerevisiae, glucose could promote PSGs mobilization. Importantly, in both yeast species, cell growth was only observed when quiescence exit was triggered by the addition of YPDA (Table I), and in S. cerevisiae, no new bud emergence was observed in the presence of glucose alone. This indicated that the sole addition of glucose was sufficient to induce the early steps of quiescence exit but did not suffice to trigger cell growth and proliferation. Consistently, we have previously demonstrated that both actin bodies and PSGs can be mobilized upon nutrient replenishment even in the absence of de novo protein synthesis (Sagot et al., 2006; Laporte et al., 2008), a condition preventing cell growth and synthesis of cell cycle regulators. Collectively, these results strongly suggest that the first steps of quiescence exit can be triggered independently of cell growth and proliferation by the addition of glucose.

### Quiescence exit requires glucose catabolism but appears to be independent of ATP replenishment

We then asked whether glucose-induced mobilization of quiescent cell structures was caused by the activation of nutrient-sensing cascades or whether it was a consequence of glucose metabolism. First, we showed that glucose-induced actin body mobilization could occur in the absence of de novo protein synthesis (Fig. S3 A). Then, we demonstrated that actin bodies could be mobilized upon glucose addition even if the Ras or the Tor pathways were inactivated (Fig. S3, B–E). This is in good agreement with the fact that all known glucose sensors were dispensable for glucose-induced actin body mobilization (Fig. 4 B, Δsensors strain). In contrast, yeast strains impaired for glucose uptake (Fig. 4 B, Δtransporters strain; Wieczorke et al., 1999) or glucose phosphorylation (Fig. 4 B, Δhexokinase strain) were totally unable to disassemble actin bodies. Finally, various mutants that block individual glycolysis steps were tested, and none of them could remobilize actin bodies when fed with glucose (Fig. 4 B). This demonstrates that glucose has to be catabolized through the glycolytic pathway at least into pyruvate to trigger actin body mobilization. This was further supported by the fact that no actin body mobilization was observed with nonmetabolizable glucose analogues or when glucose was added together with iodo-acetate, a drug that inhibits glycolysis (Fig. S3 F).

To get an overview of the metabolic consequences of glucose addition onto quiescent cells, we monitored the intracellular concentration of several metabolites. As shown in Fig. 4 C, glucose addition onto WT quiescent cells caused a significant increase in 5′-phosphoribosyl-5-amino-4-imidazole carboxamide (AICAR), SAICAR (succinyl-AICAR), inosine, GTP, CTP, and UDP, and a drop of AMP. Using mutants or drugs, we showed that none of these variations were required for glucose-induced actin body mobilization (Fig. S3, F and H). Further, as expected, glucose addition also caused a drastic ATP concentration increase in WT cells (Fig. 4, C and D). In contrast, in pgi1Δ cells, ATP concentration did not increase 15 min after transfer into either a glucose- or a fructose-containing solution (Fig. 4 D).

### Table I. Glucose addition does not trigger cell growth.

| Species       | Before transfer | H2O     | YPDA  | 2% glucose |
|---------------|-----------------|---------|-------|------------|
| S. cerevisiae | 34.2 ± 1.9 µm³  | 30.5 ± 1.9 µm³ | 51.8 ± 2.0 µm³ | 32.9 ± 1.9 µm³ |
| S. pombe      | 8.76 ± 1.33 µm  | 8.8 ± 1.2 µm  | 13.2 ± 2.3 µm  | 9.52 ± 1.49 µm  |

*Cells were grown for 7 d in YPDA and then transferred into the indicated medium. The cell length was measured 3 h after transfer (mean length ± SEM, two experiments).

*Cells were grown for 4 d in YPDA then transferred into the indicated medium. The cell volume was measured 2 h after transfer (mean volume, n = 50,000 cells ± SEM, two experiments).
Figure 4. **Actin body mobilization requires glucose catabolism.** (A) Schematic representation of the glycolysis pathway. Genes encoding each activity in *S. cerevisiae* are indicated in green. G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F1,6BP, fructose 1,6 bisphosphate; DHAP, dihydroxy acetone phosphate; G3P, glycerolaldehyde 3 phosphate; 1,3BPG, 1,3 bis phosphoglycerate; 3-PG, 3 phosphoglycerate; 2-PG, 2 phosphoglycerate; PEP, phosphoenolpyruvate. (B) WT, snf3Δ, rgt2Δ, gpr1Δ (sensors) and hxk1Δ, hxk2Δ, and glk1Δ (hexokinases) strains were grown in galactose-rich medium. WT and a strain that cannot uptake glucose (transporters strain; Wieczorke et al., 1999) were grown in maltose-rich medium. WT and pgil1 mutant were grown in fructose-rich medium. WT, pfk1Δ, pfk2Δ double mutant, and pgk1Δ mutants were grown in lactate-rich medium. After 7 d, cells were transferred into glucose (2%) and the actin cytoskeleton remodeling was monitored by phalloidin staining. (C) UV chromatograms from WT cells grown for 7 d in YPDA (black line), then transferred for 15 min into 2% glucose (red line) or into water (green line). Blue insets show enlarged views of the of AICAR and SAICAR peaks, the black inset shows amperometric detection of G6P and F1,6BP. (D and E) WT and pgil1 strains were grown for 7 d in rich medium containing 2% fructose, then transferred into either a 2% glucose or fructose solution. (D) ATP, G6P, and F1,6BP concentration measured before (7 d) and 15 min after transfer. (E) Actin cytoskeleton organization revealed by phalloidin staining. For each time point, n > 200 cells, at least two experiments. Error bars indicate SEM.
Yet, upon fructose, but not glucose, addition, this mutant mobilized its actin bodies just as efficiently as the WT strain (Fig. 4 E). This surprising result clearly establishes that an ATP concentration increase is not required for actin body mobilization. In addition, we found that when quiescent cells were transferred into water, the ATP concentration transiently increased, whereas no actin body disassembly was observed (Fig. 3 A and Fig. 4 C). We therefore conclude that ATP replenishment is neither sufficient nor necessary for actin body mobilization. At the present time, we do not know which metabolites resulting from glucose catabolism are responsible for this critical signaling step; yet our study adds to the emerging idea that metabolism is not just for fueling cells, but that small molecules are directly involved in the regulation of a myriad of cellular processes (McKnight, 2010), including proliferation/quiescence transitions.

Materials and methods

Yeast strains and growth conditions

All the S. cerevisiae strains used in this study are isogenic to BY4741, BY4742, or BY4743, available from Euroscoruf unless specified. Yeast strains carrying GFP fusions were obtained fromInvitrogen. The S. pombe strain expressing Pad1-GFP from the endogenous locus was from J.P. Javerzat (University of Bordeaux, Centre National de la Recherche Scientifique, Institut de Biochimie et Genetique Cellulaires, UMR 5095, Bordeaux, France; Wilkinson et al., 1998). Thermo-sensitive calc13-1 rho2 and calc24-3 strains were from C. Boone (The Donnelly Centre for Cellular and Molecular Research, University of Toronto, Toronto, Ontario, Canada). The strain referred as tottransporters is leu2-3,112 ura3-52 trp1-289 his3-1 MAL2-8C hxt14Δ::loxP htx23::loxP htx30::loxP htx37A::loxP htx8::loxP htx92A::loxP htx10Δ::loxP htx11::loxP htx12A::loxP htx13::loxP htx14::loxP htx15::loxP htx7D::loxP htx17::gag12A::loxP htx17::gag12A::loxP agr1::loxP mph23::loxP mph33::loxP snf3::loxP rpi23::loxP, and is from E. Boles (Institute of Molecular Biosciences, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany; Wieczorke et al., 1999). The strain expressing Tpk1 (M164G), Tpk2 (M147G), Tpk3 (M165G), and Sch9 (T492G) was a gift from J. Broach [Princeton University, Princeton, NJ; Zaman et al., 2009]. The YPD medium contained 1% yeast extract, 2% peptone, 2% glucose, and 0.04 g/liter adenine. Unless specified, yeast cells were grown in liquid medium at 30°C in flasks with 220 rpm shaking. For refueling experiments, cells were centrifuged and washed twice with water, then inoculated into the indicated medium at a final OD$_{600}$ of 0.6–1 and incubated at 30°C unless specified otherwise.

Cell staining

For regrowth experiments, a 7-d-old culture was centrifuged, and the resulting cell pellets were resuspended in fresh liquid 200M. Cells were then incubated for 1 h at 25°C, then washed twice with old filtered YPDA, and finally transferred into the indicated medium containing 70% glycerol and 5 mg/liter paraphenylenediamine [PPD, Invitrogen]. Cells were then washed twice, resuspended in a mounting solution containing 2% Difco agarose and 1 mg/ml actin phallidin (MW=440 000), and were incubated at 37°C unless specified otherwise.

Microscopy

Images are maximal projection of z-stacks performed using a 0.2 µm step using SlideBook.

Metabolomics

Cellular extracts were prepared by an ethanol extraction method adapted from Loret et al. (2007). In brief, cells were harvested by rapid filtration on a 0.2 µm cellulose filter, immediately dropped into 5 ml ethanol/10 mM Hepes, pH 7.2 (4/1 vol/vol), and incubated at 80°C for 3 min. Samples were evaporated using a rotovapor apparatus. The residue was resuspended in 500 µl of water and insoluble particles were eliminated by centrifugation. Separation of metabolites was performed on a CarboPac PA1 column, linked to an IC53000 chromatography workstation (Dionex), by using a NaOH/Na acetate gradient. Sugar phosphate and nucleotide derivatives were, respectively, detected by amperometry (IntAmp, carbohydrates standard-quad potential ED50; Dionex) and UV diode array (Ultimate 3000; Dionex) detectors.

Miscellaneous

Yeast strains were micro-manipulated using a Singer MSM system (Singer Instrument Co. Ltd.). Glucose concentration was measured using the z-glucose/-fructose UV test kit (Roche). Cell volume was measured using a Multi-sizer 4 (Beckman Coulter).

Online supplemental material

Fig. S1 shows actin cytoskeleton and proteasome reorganization upon various nutrient exhaustions and upon cell refueling. Fig. S2 shows that quiescence establishment does not require Whi5 and is not triggered by a sole arrest in G1. Fig. S3 shows that quiescence exit requires glucose catabolism but appears to be independent of Ras or Tor pathways and of nucleotide replenishment. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201009028/DC1.

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References

Allen, C., S. Büttner, A.D. Aragon, J.A. Thomas, O. Meirelles, J.E. Jacot, D. Benn, S.W. Ruby, M. Veenhuizen, F. Madeo, and M. Werner-Washburne. 2006. Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. J. Cell Biol. 174:89–100. doi:10.1083/jcb.200604072

Aragon, A.D., A.L. Rodriguez, O. Meirelles, S. Roy, G.S. Davidson, P.H. Tapia, C. Allen, R. Joe, D. Benn, and M. Werner-Washburne. 2008. Characterization of differentiated quiescent and nonquiescent cells in yeast stationary-phase cultures. Mol. Biol. Cell. 19:1271–1280. doi:10.1091/mbc.E07-07-0666

Burke, D.J., and D. Church. 1991. Protein synthesis requirements for nuclear division, cytokinesis, and cell separation in Saccharomyces cerevisiae. Mol. Cell. Biol. 11:3691–3698.

Coller, H.A., L. Sang, and J.M. Roberts. 2006. A new description of cellular quiescence. PLoS Biol. 4:e83. doi:10.1371/journal.pbio.0040083

Cooper, S. 2003. Reappraisal of serum starvation, the restriction point, G1 and GI phase arrest points. FASEB J. 17:333–340. doi:10.1096/fasebj.02-0352rev

Costanzo, M., J.L. Nishikawa, X. Tang, J.S. Millman, O. Schub, K. Breitkreuz, C. Allen, C., S. Büttner, A.D. Aragon, J.A. Thomas, O. Meirelles, J.E. Jaetao, D. Benn, I. Rupes, B. Andrews, and M. Tyers. 2004. CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. PLoS Biol. 2:e83. doi:10.1371/journal.pbio.0040083

Costello, G., L. Rodgers, and D. Breach. 1986. Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. Curr. Genet. 11:119–125. doi:10.1007/BF00378203

Granot, D., and M. Snyder. 1991. Glucose induces cAMP-independent growth-related changes in stationary-phase cells of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA. 88:5724–5728. doi:10.1073/pnas.88.13.5724
Granot, D., and M. Snyder. 1993. Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. Yeast 9:465–479. doi:10.1002/yea.300090503

Gray, J.V., G.A.Petsko, G.C. Johnston, D. Ringe, R.A. Singer, and M. Werner-Washburne. 2004. “Sleeping beauty”: quiescence in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 68:187–206. doi:10.1128/MMBR.68.2.187-206.2004

Hartwell, L.H., and M.W. Unger. 1977. Unequal division in Saccharomyces cerevisiae and its implications for the control of cell division. J. Cell Biol. 75:422–435. doi:10.1083/jcb.75.2.422

Hartwell, L.H., J. Culotti, J.R. Pringle, and B.J. Reid. 1974. Genetic control of the cell division cycle in yeast. Science 183:46–51. doi:10.1126/science.183.4120.46

Johnston, G.C., J.R. Pringle, and L.H. Hartwell. 1977. Coordination of growth with cell division in the yeast Saccharomyces cerevisiae. Exp. Cell Res. 105:79–98. doi:10.1016/0014-4827(77)90115-4

Laporte, D., B. Salin, B. Daignan-Fornier, and I. Sagot. 2008. Reversible cytoplasmic localization of the proteasome in quiescent yeast cells. J. Cell Biol. 181:737–745. doi:10.1083/jcb.200711154

Lindquist, S., and G. Kim. 1996. Heat-shock protein 104 expression is sufficient for thermotolerance in yeast. Proc. Natl. Acad. Sci. USA 93:5301–5306. doi:10.1073/pnas.93.11.5301

Lorent, M.O., L. Pedersen, and J. François. 2007. Revised procedures for yeast metabolites extraction: application to a glucose pulse to carbon-limited yeasts, which reveals a transient activation of the purine salvage pathway. Yeast 24:47–60. doi:10.1002/yea.1435

Lu, C., M.J. Brauer, and D. Botstein. 2009. Slow growth induces heat-shock resistance in normal and respiratory-deficient yeast. Mol. Biol. Cell 20:891–903. doi:10.1091/mbc.E08-08-0582

Martinez, M.J., S. Roy, A.B. Archuleta, P.D. Wentzell, S.S. Anna-Arruola, A.L. Rodriguez, A.D. Aragon, G.A. Quijones, C. Allen, and M. Werner-Washburne. 2004. Genomic analysis of stationary-phase and exit in Saccharomyces cerevisiae: gene expression and identification of novel essential genes. Mol. Biol. Cell. 15:5295–5305. doi:10.1091/mbc.E03-11-0856

McKnight, S.L. 2010. On getting there from here. Science. 330:1338–1339. doi:10.1126/science.1199080

Narayanawamy, R., M. Levy, M. Tsechansky, G.M. Stovall, J.D. O’Connell, J. Mirrielees, A.D. Ellington, and E.M. Marcotte. 2009. Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. Proc. Natl. Acad. Sci. USA. 106:10147–10152. doi:10.1073/pnas.0812771106

Pardue, A.B. 1974. A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. USA. 71:1286–1290. doi:10.1073/pnas.71.4.1286

Parsell, D.A., and S. Lindquist. 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27:437–496. doi:10.1146/annurev.ge.27.120193.002253

Piper, P. 1998. Differential role of Hps and trehalose in stress tolerance. Trends Microbiol. 6:43–44. doi:10.1016/S0966-842X(97)01190-6

Popolo, L., M. Vanoni, and L. Alberghina. 1982. Control of the yeast cell cycle by protein synthesis. Exp. Cell Res. 142:69–78. doi:10.1016/0014-5793(82)90410-4

Radonic, M., J.C. Andraud, P. Lijntzaad, P. Kemmeren, T.T. Kockelkorn, D. van Leenen, N.L. van Berkm, and F.C. Holstege. 2005. Genome-wide analyses reveal RNA polymerase II located upstream of genes poised for rapid response upon S. cerevisiae stationary phase exit. Mol. Cell. 18:171–183. doi:10.1016/j.molcel.2005.03.010

Sagot, I., S.K. Klee, and D. Pellman. 2002. Yeast formins regulate cell polarity by controlling the assembly of actin cables. Nat. Cell Biol. 4:42–50.

Sagot, I., B. Pinson, B. Salin, and B. Daignan-Fornier. 2006. Actin bodies in yeast quiescent cells: an immediately available actin reserve? Mol. Biol. Cell. 17:4645–4655. doi:10.1091/mbc.E06-04-00282

Sahin, A., B. Daignan-Fornier, and I. Sagot. 2008. Polarized growth in the absence of F-actin in Saccharomyces cerevisiae exiting quiescence. PLoS ONE 3:e2556. doi:10.1371/journal.pone.0002556

Sambusivan, R., G.K. Pavlath, and J. Dhawan. 2008. A gene-trap strategy identifies quiescence-induc ed genes in synchronized myoblasts. J. Biosci. 33:27–44. doi:10.1007/s12038-008-0019-6

Shimanuki, M., S.Y. Chung, Y. Chikashige, Y. Kawasaki, L. Uehara, C. Tsutsunami, M. Hatamaka, Y. Hirooka, K. Nagao, and M. Yanagida. 2007. Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in Schizosaccharomyces pombe. Genes Cells. 12:677–692. doi:10.1111/j.1365-2443.2007.01079.x

Slater, M.L., S.O. Sharrow, and J.J. Gart. 1977. Cell cycle of Saccharomyces cerevisiae in populations growing at different rates. Proc. Natl. Acad. Sci. USA. 74:3850–3854. doi:10.1073/pnas.74.9.3850

Snedberg, P., R. Ghillebert, P. De SNijder, M. Bindu, E. Swinnen, C. De Virgilio, and J. Windericks. 2010. Life in the midst of scarcity: adaptations to nutrient availability in Saccharomyces cerevisiae. Curr. Genet. 56:1–32. doi:10.1007/s00294-009-0287-1

Sudbery, P.E., A.R. Goodey, and B.L. Carter. 1980. Genes which control cell proliferation in the yeast Saccharomyces cerevisiae. Nature. 288:401–404. doi:10.1038/288401a0

Unger, M.W., and L.H. Hartwell. 1976. Control of cell division in Saccharomyces cerevisiae by methionyl-tRNA. Proc. Natl. Acad. Sci. USA. 73:1664–1668. doi:10.1073/pnas.73.5.1664

Wei, W., P. Nurse, and D. Brock. 1993. Yeast cells can enter a quiescent state through G1, S, G2, or M phase of the cell cycle. Cancer Res. 53:1867–1870.

Wieczorke, R., S. Krampe, T. Weierstall, K. Freidel, C.P. Hollenberg, and E. Boles. 1999. Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in Saccharomyces cerevisiae. FEBS Lett. 464:123–128. doi:10.1016/S0014-5793(99)01698-1

Wilkinson, C.R., M. Wallace, M. Morphe, P. Perry, R. Allshire, I.P. Inverzat, J.R. McIntosh, and C. Gordon. 1998. Localization of the 26S proteasome during mitosis and meiosis in fission yeast. EMBO J. 17:6465–6476. doi:10.1093/embj/17.22.6465

Wu, J., N. Zhang, A. Hayes, K. Panoutsopoulou, and S.G. Oliver. 2004. Global analysis of nutrient control of gene expression in Saccharomyces cerevisiae during growth and starvation. Proc. Natl. Acad. Sci. USA. 101:3148–3153. doi:10.1073/pnas.0308321100

Zaman, S., S.I. Lippman, L. Schneper, N. Slonim, and J.R. Bouch. 2009. Glucose regulates transcription in yeast through a network of signaling pathways. Mol. Syst. Biol. 5:245.
Figure S1.  Actin cytoskeleton and proteasome reorganization upon various nutrient exhaustions and upon cell refeeding. (A and B) Reorganization of the actin cytoskeleton upon quiescence exit. (A) Actin cytoskeleton organization in each cell type before and 2 h after transfer to YPDA. (B) Examples of budded cells stained with ConA-FITC and displaying a polarized actin cytoskeleton 2 h after refeeding. Bar, 2 µm. (C) Percentage of cells displaying actin bodies and PSG upon exhaustion of various carbon sources. WT cells were grown in YPA medium containing 2% of the indicated carbon source. (D) Actin cytoskeleton organization upon starvation for other nutrients than carbon. A medium containing 20 times less yeast extract and peptone, 0.5 mg/ml and 1 mg/ml, respectively, than the regular YP medium was prepared (YP/20 medium). WT prototroph cells were inoculated at OD_{600nm} 0.25 in YP/20 medium containing glucose (100g/l). OD_{600nm} (black squares) and glucose concentration (white diamonds) were measured. The actin cytoskeleton organization was analyzed by phalloidin staining. (E and F) Actin bodies and PSG formation upon starvation for all nutrients. (E) WT cells were grown in YPDA to OD_{600nm} 1. Cells were then pelleted, washed twice with water, and transferred into water (final OD_{600nm} was 2). The actin cytoskeleton organization was analyzed in a function of time after transfer into water. (F) WT cells expressing Rpn11-GFP, a proteasome subunit, were grown in YPDA to OD_{600nm} 0.6. Cells were then pelleted, washed twice with water, and transferred into water (final OD_{600nm} was 1). The proteasome localization was scored in function of time after transfer into water. For each time point, n > 200 cells, at least two experiments. Error bars indicate SEM.)
Figure S2. Quiescence establishment does not require Whi5 and is not triggered by a sole arrest in G1. (A–D) Whi5p is not essential for quiescence establishment. (A) Actin cytoskeleton organization in WT and whi5Δ cells after 7 d of growth in YPDA. Bar, 2 µm. (B) Whi5Δ cells form colonies after passage through stationary phase. After 7 d of growth in YPDA, 200 cells were spread on YPDA plates. After 3 d at 30°C, colonies were counted (results are the mean of two independent experiments; error bars indicate SEM; a Student’s t test indicated a probability of up to 0.05 for no difference between the two strains). (C) Percentage of budded cells upon quiescence exit of a WT strain and a whi5Δ strain. After 7 d of growth, cells were transferred into new YPDA (n > 200 cells were counted for each point; two experiments; error bars indicate SEM). (D) WT and whi5Δ cell proliferation upon quiescence monitored by OD_{600}. (E and F) An arrest in G1 does not trigger actin body formation until glucose is exhausted. (E) Diagram representing the experimental protocol: WT and cdc24-3 cells were grown in YPDA at 25°C. At OD_{600}=4, cells were transferred to 37°C. The budding index for the WT (gray diamonds) and the cdc24-3 (black squares) strains are shown (n > 200 cells for each time point; two experiments; error bars indicate SEM). (F) Actin cytoskeleton organization in WT and cdc24-3 before and after glucose exhaustion, respectively, 5 h and 27 h after shift to 37°C. Bars, 2 µm.
Figure S3. Quiescence exit requires glucose catabolism but appears to be independent of Ras or Tor pathways and nucleotide replenishment. (A) De novo protein synthesis is not required for glucose-induced actin body mobilization. WT cells were grown for 7 d in YPDA, then washed and resuspended into a glucose solution (2%) to the final OD$_{600nm}$ of 1. For cycloheximide (CHX) treatment, cells were preincubated with 100 µg/ml cycloheximide by addition of the drug directly into the growth medium. After 1 h of pretreatment, cells were washed and resuspended into a glucose solution (2%) containing 100 µg/ml CHX (final OD$_{600nm}$ = 1). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored. n > 200 cells were counted for each point, two experiments. Error bars indicate SEM. (B) Rapamycin treatment does not prevent actin body mobilization. WT cells were grown for 7 d in YPDA, then washed and resuspended into a glucose solution (2%). For rapamycin treatment, cells were preincubated with 2 µg/ml rapamycin by addition of the drug directly into the growth medium. After 1 h of pretreatment, cells were washed and resuspended into a glucose solution (2%) containing 200 ng/ml rapamycin (final OD$_{600nm}$ = 1). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored. n > 200 cells were counted for each point, two experiments. Error bars indicate SEM. (C) Strains deleted for genes involved in Ras or Tor pathways are capable of mobilizing actin bodies upon glucose addition. Strains deleted for the indicated genes were grown for 7 d in YPDA, then washed and resuspended into a glucose solution (2%). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored (n > 200). (D) Strains expressing a thermo-sensitive allele of genes involved in Ras or Tor pathways are capable of mobilizing actin bodies upon glucose addition. Strains expressing a thermo-sensitive allele of the indicated gene were grown for 7 d in YPDA at 25°C. The stationary phase cultures were then either kept at 25°C or transferred at 37°C for 3 h. Cells were then washed with prewarmed water and resuspended into a glucose solution (2%) at 25°C or 37°C. Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored (n > 200). (E) Tpk1, Tpk2, Tpk3, and Sch9 kinases are not required for glucose-induced actin body mobilization. To inactivate both PKA and Sch9, we used a strain expressing mutants that are sensitive to the ATP analogue C3-1-naphthyl-methyl PP1 (NMPP1). A strain expressing the NMPP1-sensitive proteins Tpk1 [M164G], Tpk2 [M147G], Tpk3 [M165G], and Sch9 [T492G; a gift from J. Broach; Zaman et al., 2009], and the corresponding WT strain were grown for 7 d in YPDA. For NMPP1 treatment, cells were preincubated for 1 h with 1 µM of the drug, then washed and resuspended into a glucose solution (2%) containing 1 µM NMPP1 (Merck). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored (n > 200). (F) Nonmetabolizable glucose analogues or iodoacetate do not allow actin body mobilization. WT cells were grown for 7 d in YPDA, then washed and resuspended into a glucose-containing solution (final concentration was 2%). For iodoacetate treatment, cells were preincubated with 10 mM iodoacetate by addition of the drug directly into the growth medium. Before 20 min of pretreatment, cells were washed and resuspended into a glucose solution containing 10 mM iodoacetate (final OD$_{600nm}$ was 1). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored. n > 200 cells counted for each point, two experiments. Error bars indicate SEM. (G) Glucose-induced actin body mobilization does not require accumulation of inosine, AICAR, or SAICAR, nor AMP depletion. A ade1 ade7 ade17 his1 strain that cannot synthesize AICAR or SAICAR (Pinson et al., 2009) can mobilize actin bodies upon glucose addition. Similarly, a strain that cannot synthesize inosine from IMP (isin1Δ; Itch et al., 2003) or a strain that cannot transform AMP into IMP (amf1Δ; Saint-Marc et al., 2009) are both capable of mobilizing actin bodies upon glucose addition. Cells were grown for 7 d in YPDA, then washed and resuspended (final OD$_{600nm}$ was 1) into a glucose solution (2%). Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored (n > 200). (H) Glucose-induced actin body mobilization does not require accumulation of GTP or UTP. Mycophenolic acid (MPA) inhibits inosine monophosphate dehydrogenases and prevents GMP (and consequently GDP and GTP) synthesis from IMP (Saint-Marc et al., 2009),
whereas 6-aza-uridine (6-AzaU) inhibits the synthesis of both UTP and GTP (Exinger and Lacroute, 1992). However, these two drugs have no effect on glucose-induced actin body mobilization. WT cells were grown for 7 d in YPDA. Cells were then preincubated for 1 h with 100 µg/ml of MPA or 60 µg/ml of 6-AzaU, or no drug as a control, then washed and resuspended into a glucose solution (2%) with, respectively, 100 µg/ml of MPA or 60 µg/ml of 6-AzaU, or no drug. Before or 15 min after transfer to glucose, cells were fixed and stained with phalloidin. Cells with depolarized actin cytoskeleton (blue) or actin bodies (red) were scored (n > 200). (I) Actin body formation and mobilization upon exhaustion and refeeding with various carbon sources. WT cells were grown for 7 d in YPA containing 2% of various carbon sources. Cells were then washed and resuspended into YPA medium containing 2% the indicated carbon source. Before, 15 min, and 2 h after refeeding, cells were fixed and stained with phalloidin, and the percentages of cells displaying a depolarized actin cytoskeleton or actin bodies were scored (n > 200 cells).

References

Exinger, F., and F. Lacroute. 1992. 6-Azauracil inhibition of GTP biosynthesis in Saccharomyces cerevisiae. *Curr. Genet.* 22:9–11. doi:10.1007/BF00351735

Itoh, R., C. Saint-Marc, S. Chaignepain, R. Katahira, J.M. Schmitter, and B. Daignan-Fornier. 2003. The yeast ISN1 (YOR155c) gene encodes a new type of IMP-specific 5′-nucleotidase. *BMC Biochem.* 4:4. doi:10.1186/1471-2091-4-4

Pinson, B., S. Vaur, I. Sagot, F. Coulpier, S. Lemoine, and B. Daignan-Fornier. 2009. Metabolic intermediates selectively stimulate transcription factor interaction and modulate phosphate and purine pathways. *Genes Dev.* 23:1399–1407. doi:10.1101/gad.521809

Saint-Marc, C., B. Pinson, F. Coulpier, L. Jourdren, O. Lisova, and B. Daignan-Fornier. 2009. Phenotypic consequences of purine nucleotide imbalance in Saccharomyces cerevisiae. *Genetics*. 183:529–538. doi:10.1534/genetics.109.105858

Zaman, S., S.I. Lippman, L. Schneper, N. Slonim, and J.R. Broach. 2009. Glucose regulates transcription in yeast through a network of signaling pathways. *Mol. Syst. Biol.* 5:245.