

“Sleeping Beauty”: Quiescence in *Saccharomyces cerevisiae*†

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“Beloved, may your sleep be sound
That have found it where you fed”
William Butler Yeats (Lullaby)

INTRODUCTION

All living cells appear to be capable of exiting the normal cell cycle (proliferating state) and entering an alternative (resting) state termed quiescence or G₍₀₎. Quiescent microbes are

thought to represent about 60% of the biomass on Earth and are doubtless the seeds of microbial life in nature. Furthermore, most eukaryotic cells, whether they exist as single-celled or multicellular organisms, spend the majority of their natural lives in a quiescent state (87). Quiescent cells of both prokaryotic and eukaryotic microorganisms can survive for long periods—sometimes years—without added nutrients, a feat of astonishing resilience (167).

Beyond contributing to a more rounded view of the life cycle of cells, understanding quiescence has other potentially significant implications. A deeper understanding of the conserved mechanisms underlying entry into, survival in, and exit from quiescence in eukaryotes may aid the development of novel or supplementary immunosuppressants and anticancer therapies and is also likely to provide significant insights into such di-

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† This paper is dedicated to the memory of Ira Herskowitz and Helmut Ruis.

verse processes as aging (50) and neurodegenerative diseases (139). The discovery of variations on a common theme may allow the development of novel antipathogenic agents. Further, most of the world's microorganisms have yet to be cultured. Among these organisms are likely to be many novel microbes, predominantly in a quiescent state, that can produce medically useful natural products or whose study will provide new insight into evolution, development, and ecology. An understanding of how to stimulate these microbes to exit from quiescence may aid the culturing of such organisms.

Although we ultimately seek to understand aspects of quiescence shared among all eukaryotes, we focus this review on quiescence in the budding yeast *Saccharomyces cerevisiae*. We restrict our focus for several reasons. First, *S. cerevisiae* is one of the best-studied eukaryotes and is tractable to all levels of experimental analysis. Second, because of the conservation of basic cellular processes among eukaryotes, the study of quiescence in yeast is likely to illuminate the equivalent mechanisms and states in many if not all other eukaryotes and possibly prokaryotes as well. Indeed, even mammals possess orthologs of the apparent yeast regulators (see below), such as the TORs, protein kinases A and C, and Snf1p. This is not surprising. The ability of microbes and our microbial ancestors to enter quiescence and thereby maintain viability when starved is likely to have been essential to their survival. A strong selective pressure has doubtless acted to maintain the ability to enter into, survive in, and exit from quiescence over evolutionary time. Third, quiescent yeast and quiescent mammalian cells share a number of salient characteristics such as unreplicated genomes (121); characteristically condensed chromosomes (113), referred to as $G_{(0)}$ chromosomes; increased rates of autophagy; and reduced rates of translation (see below for details). Furthermore, both yeast and mammalian cells respond similarly to rapamycin, an immunosuppressant drug for humans, which inhibits the proliferation of both yeast and mammalian cells and drives each into a state similar to their respective quiescent state (135). Even though entry of cells into and exit of cells from quiescence in metazoan bodies is normally regulated by positional and developmental cues, mammalian cells share with yeast cells the ability to respond to starvation by entering quiescence-like states (91). Finally, with the exploitation of the genome sequence, the new technologies available to study yeast further ensures that this small eukaryote will be central to unlocking the secrets of the quiescent state.

Our knowledge of quiescence in any organism including yeast is fragmented, and the mechanisms that regulate entry into, maintenance of, and exit from quiescence are, at best, poorly understood. Historically, one major factor limiting the study of quiescent cells has been their very modest life-style: classical cell biological, physiological, and biochemical assays detected little or no activity in these cells. Furthermore, the application of genetics to the study of quiescence has been limited (see below for further considerations of this point). As a result, many researchers have long suspected that quiescent cells are difficult to study (historically correct), do not represent a distinct phase of cell (probably inaccurate), do not do anything (inaccurate), or are either uninteresting or dead (very inaccurate). Even now, understanding the mechanisms by which a cell transits between the proliferating and quiescent states and the way in which these states differ presents a for-

midable challenge. As we shall argue, switching between active proliferation and quiescence is likely to involve the wholesale reprogramming of regulatory networks and the remodeling of most if not all intracellular structures and processes.

The "holy grail" for researchers working on quiescence is to define a core quiescence program that prevents cell growth and proliferation, that confers on cells the ability to survive better under adverse conditions, and that allows a rapid transition back to the proliferating state when conditions again become favorable. Here, as a starting point, we present an overview of the current understanding of quiescence in yeast.

Almost 400 papers dealing with some aspect of quiescence in yeast have been published since our last major review of this topic (167, 168). We cannot completely cover this literature, but we provide a list of all the papers that we identified in this area on our website (<http://biology.unm.edu/biology/maggieww/SPreview.htm>). Some literature not covered herein has been discussed in another recent and shorter review of stationary-phase yeast cultures, i.e., those containing quiescent cells (59).

QUIESCENCE IN YEAST

Operational Definition of Quiescence

Quiescent yeast cells are commonly obtained in the laboratory by growing liquid cultures to saturation in rich media, usually for 5 to 7 days at 30°C (Fig. 1). The term "stationary phase" has been used to describe the state of saturated liquid cultures and the state of the constituent cells. We propose a revision of this nomenclature, such that "stationary phase" is used to refer to the state of a saturated culture and the term "quiescence" is used to refer to the state of the constituent cells in such a saturated culture (Fig. 1). It is not known if all cells in a stationary-phase culture are quiescent, but we assume that a substantial proportion are, including the daughter cells that were produced during the final doublings in the post-diauxic phase of culture growth.

We currently define the reference quiescent state in yeast as the state of the cell brought about by growth of a liquid culture of cells to saturation in rich media (yeast-peptone-dextrose [YPD]). Once this and other quiescence-like states of yeast have been more closely examined and compared, this definition of quiescence will doubtless become more refined.

The path by which such a culture of cells reaches saturation is not simple (Fig. 1). Initially, the constituent cells derive their energy from fermentation, the process by which glucose is preferentially metabolized via glycolysis to form nonfermentable carbon compounds, particularly ethanol. During the exponential or logarithmic growth phase, the culture grows rapidly (and the constituent cells proliferate with an average doubling time of approximately 90 min at 30°C) until glucose is exhausted in the medium. At this point, termed the diauxic shift, the culture ceases rapid growth while the constituent cells readjust their metabolism to utilize the nonfermentable carbon sources still present in the medium. After the diauxic shift, the cells in the culture undergo one or two very slow doublings over a period of days before finally ceasing proliferation after the depletion of ethanol and other nonfermentable carbon sources (88). At this point, the culture is in stationary phase and most, if not all, of the constituent cells are quiescent (167).

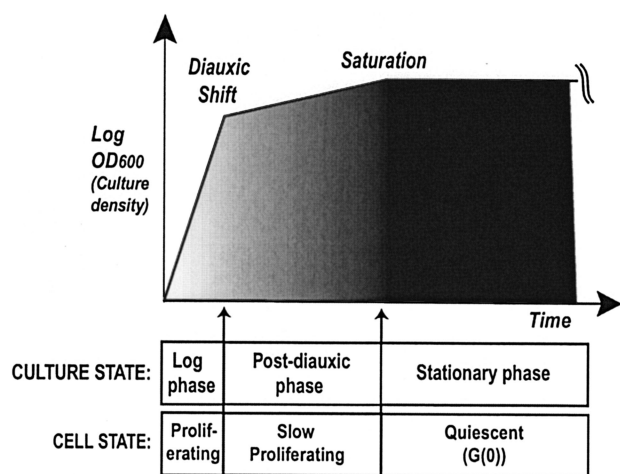


FIG. 1. Relationship between the state of a culture of yeast cells growing to saturation in rich medium (YPD) and the state of the constituent cells. When yeast cells are inoculated into rich medium containing glucose, the cells proliferate rapidly using fermentation and the density of the culture (reflected in optical density at 600 nm [OD₆₀₀]) increases logarithmically with time (log phase). When glucose is consumed in the culture at the diauxic shift (after approximately 1 day), the cells cease rapid cell proliferation and readjust their metabolism from fermentation to respiration to utilize other carbon sources present in the medium. In the resulting post-diauxic shift state of the culture, constituent cells proliferate very slowly. When external carbon sources are exhausted, the culture reaches saturation (at approximately 5 to 7 days postinoculation) and the constituent cells cease proliferation and enter the quiescent state.

All cultures having passed the diauxic shift are often (mistakenly, we believe) classified as being in stationary phase and the constituent cells thus in quiescence. We favor drawing a clear distinction between cells found in the post-diauxic shift state of a culture prior to saturation and quiescent cells found in a saturated culture. This distinction may be more than semantic: post-diauxic shift cells have acquired many, but not all, of the characteristics of quiescent cells and continue to proliferate, albeit very slowly. Entry of cells into quiescence is best viewed as a stepwise process in liquid culture, with various characteristics of the quiescent state acquired either at the diauxic shift or on final cessation of proliferation (see below).

Although our operational definition of quiescence (the state of cells in liquid cultures grown to saturation in rich media) focuses attention on a specific reference state, the definition may be overly restrictive. It is clear that many of the cells in a colony growing on the surface of solid rich media are also in quiescence (108). However, because of the immobility of yeast cells on solid surfaces, distinct microenvironments develop within a colony, allowing multiple subpopulations of cells to coexist. This is in stark contrast to a culture of cells in agitated liquid medium, where every cell experiences the same, homogeneous environment. The response of a colony of cells to starvation is thus likely to be significantly more complicated than, although closely related to, that of cells in liquid cultures.

Our operational definition of quiescence may also be overly restrictive because yeast cells enter somewhat stable nonproliferating states when rapidly starved for nitrogen, sulfur, or phosphate or when transferred directly to water (149). Indeed, spores, formed when diploid cells are starved for nitrogen in

the presence of a poor carbon source, are also in a quiescence-like state: they can remain viable for many years—possibly centuries (99). Because the relationships between these “other” nonproliferating cell states and quiescence are not yet known, we do not discuss these states further here.

It should be noted that not all starvations cause entry into viable nonproliferating states. For example, starvation of inositol auxotrophs for inositol causes rapid cell death (“inositol-less death”), apparently by disrupting the ordered growth of the cell (78). In addition, most laboratory strains lose viability relatively quickly when grown to saturation in synthetic, defined medium (7). It thus appears that entry into quiescence (and quiescence-like states) is a programmed response to specific environmental changes and does not occur simply by default.

Characteristics of Quiescent Cells

The relationship between the state we term quiescence or G₍₀₎ in yeast and the so-called G₍₀₎ state of nonproliferating, terminally differentiated mammalian cells such as neurons and fibroblasts remains to be established, but it is likely that quiescent yeast will prove to be an important model for understanding the G₍₀₎ state of multicellular eukaryotes, as it has been for so many aspects of the proliferative cell division cycle.

Quiescent yeast cells display numerous specific characteristics that differentiate them from proliferating cells: they do not proliferate; they fail to accumulate mass and volume; they are arrested as unbudded cells (121); the overall transcription rate is three to five times lower than in logarithmic-phase cultures (20); they have a requirement for translation from internal initiation sites (internal ribosome entry site) (108); expression of a subset of genes is severely repressed, e.g., those encoding ribosomal proteins; expression of a subset of genes is strongly induced, e.g., *SNZ1*, *HSP26*, and *UBI4* (168); mRNA degradation is inhibited (73); overall protein synthesis is reduced to approximately 0.3% of the rate found in logarithmically growing cultures (47); chromosomes are condensed [G₍₀₎ chromosomes, (113)]; autophagy (the process of engulfment of the cytoplasm into lipid vesicles for delivery to the vacuole for degradation) is induced (103); cells develop thickened cell walls and are more resistant than are proliferating cells to digestion by zymolyase and to treatment with certain toxic drugs (30); and cells are more thermotolerant and osmotolerant than are their proliferating counterparts (114).

Perhaps surprisingly, quiescent yeast cells are capable of responding to environmental signals in addition to the presence of carbon. Irradiation, heat shock, and treatment with chemicals such as methylmethane sulfonic acid and certain toxins can induce the expression of similar genes in both quiescent and proliferating yeast (68) (M. Werner-Washburne, unpublished data), as can oxidative stress (26).

The above characteristics begin to define a set of landmarks that can be used to identify and characterize cells in quiescence and suggest that the quiescent and proliferating states are distinct. Furthermore, a variety of mutants, including *ubi4*, *ard1*, and some alleles of *bcy1*, are known to selectively or specifically die when starved, supporting the notion that the proliferating and quiescent states are distinct. However, perhaps the most compelling argument that quiescence should be

considered a distinct developmental state comes from the *gcs1Δ* mutant, which proliferates normally in the presence of food, enters quiescence when grown to stationary phase, and maintains viability in quiescence normally but is conditionally defective in exiting from quiescence and returning to active proliferation when nutrients are restored. *gcs1Δ* mutants are cold sensitive only for exiting from quiescence (34, 35).

Cell Quiescence Cycle

The process of entering into quiescence has traditionally been represented as a reversible reaction, with exit from quiescence being simply the reverse of entry. However, we think that this view is too simplistic. Entry into quiescence is triggered when a proliferating cell senses carbon limitation. In contrast, exit is triggered by a different state of the cell (quiescent) sensing the presence of a carbon source. There is no reason to believe that the processes of entry into and exit from quiescence share any common intermediate states of the cell (see "Exiting from quiescence" below).

We therefore propose a revision of this traditional view in which entry into, survival in, and exit from the quiescent state can be regarded as a developmental process that, by analogy to the proliferative cell cycle, can be called the cell quiescence cycle (see Fig. 2). In this view, entry into and exit from quiescence are distinct processes. As with the proliferative cell division cycle, passage around one complete round of the quiescence cycle returns the starting cell, to a first approximation, back to its starting state. In reality, each turn of either cycle changes the state of the starting cell: in the case of the cell cycle, the mother cell becomes one generation older (i.e., has reduced replicative capacity); in the case of the quiescence cycle, the cell also becomes older, again with respect to loss of replicative capacity (2). The cell cycle results in a doubling of cell the number, whereas the quiescence cycle does not.

The cell division cycle and the cell quiescence cycle intersect at the G_1 phase (Fig. 2). In this phase, a cell can enter either the cell division cycle or the quiescence cycle (58, 167). In the presence of ample food supplies (and other conditions permitting), a G_1 cell passes START (58) and enters the proliferative cell cycle. The subsequent removal of nutrients does not generally hamper completion of the ongoing cell cycle, which is driven by internally controlled fluctuations in cyclin-dependent protein kinase activity (104). In the absence of a sufficient carbon source, a G_1 cell fails to pass START and enters the quiescence cycle. Unlike the proliferative cell cycle, the quiescence cycle does not turn under its own steam but cycles with changes in the environment: the lack of nutrients triggers entry into quiescence; the resupply of nutrients triggers exit.

Mutants Defective in the Cell Quiescence Cycle

The isolation of mutants defective in key transitions of the cell quiescence cycle is worthy of some consideration. To date, the most frequently reported class of relevant mutants appears to lose viability when cultured to stationary phase: the mutant cells lose the ability to form colonies when subsequently transferred back to nutrient-rich media. In many cases, this interpretation may be naïve. Three subclasses of "stationary-phase"

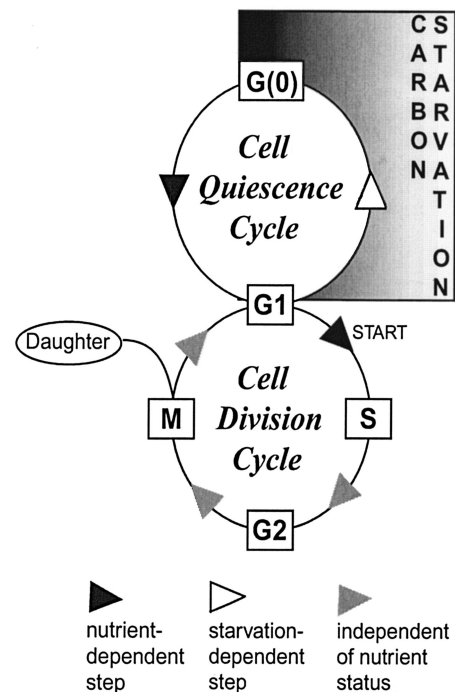


FIG. 2. The cell quiescence cycle and its relationship to the cell division cycle. The cell quiescence cycle is the process by which nutrient limitation (e.g., carbon starvation in our reference case) causes exit from active proliferation (the cell division cycle) and triggers entry into the stable nonproliferating state, quiescence/ $G_{(0)}$. Only after a favorable change in nutrient availability will a turn of the quiescence cycle be completed, since nutrient availability triggers exit from quiescence/ $G_{(0)}$. The cell quiescence cycle and the cell division cycle intersect at the G_1 phase, where a cell has not yet committed to the cell division cycle. In the presence of sufficient nutrients and with no other influences, a G_1 cell will pass START, after which it is committed to completing a turn of the cell division cycle with production of a daughter cell. Slow depletion of an essential nutrient such as carbon will allow the completion of an ongoing cell division cycle but will not allow passage through START. In this case of insufficient nutrient availability, the cell will enter the cell quiescence cycle.

mutants are likely to exist, all of which may be defective in one or more transition of the cell quiescence cycle.

The first subclass includes entry mutants, i.e., those that fail to enter quiescence properly. Such mutants would be expected to die when starved, given the likelihood that successful entry into quiescence is required for cells to remain viable when starved. The second subclass includes maintenance mutants, i.e., those that successfully enter quiescence (acquire all the key characteristics of quiescent cells) but are unable to maintain viability in that state. Two subclasses of these maintenance mutants are likely to exist: those inherently required for viability in the quiescent state itself, and those specifically defective in surviving when starved. The third subclass includes exit mutants, i.e., those that enter quiescence and remain viable normally but are specifically unable to return to the proliferating state when nutrients again become available. The cells of such mutants are viable when starved but are unable to generate CFU on replating.

In only a handful of cases have the above distinctions been entertained. Thus, mutants reported to be lose viability in

stationary-phase cultures may in reality fall into any one of the above classes when properly analyzed. Much of the published literature on “stationary-phase”/quiescent mutants should thus be approached with some caution. A second limitation of the published literature is that many investigators have assumed, incorrectly, that any post-diauxic shift culture is in stationary phase, with its constituent cells in quiescence (see above).

Subclassification of “stationary-phase” mutants is possible. First, many characteristics of quiescent cells are known, and, together, these constitute a reference set of parameters that can be used to determine (or at least estimate) if mutant cells cultured to stationary phase enter quiescence successfully. Second, cell viability can be assayed independently of the ability to subsequently proliferate when refed. It has recently been shown that viability dyes such as methylene blue can be useful to directly determine the viability of starved cells (80). Furthermore, as outlined above, viable quiescent cells can mount transcriptional responses to a variety of environmental stresses and chemical treatments.

It should be borne in mind that a mutant defective in producing a protein may show a terminal defect at a stage of the quiescence cycle distinct from the point at which the protein acts in wild-type cells. For example, it is conceivable that a primary defect in fully entering quiescence may not compromise cell viability but, rather, may prevent successful exit after the stimulation brought about by addition of nutrients. Quiescent yeast cells are poised to respond to nutrients, should they become available, and can do so within seconds of nutrient resupply. It is likely that a critical property of quiescence is the ability to exit from that state as quickly as possible once conditions improve.

ENTRY INTO QUIESCENCE

Significant progress toward understanding the mechanisms regulating entry into quiescence has been made in the last decade. The relevant gene products have been found in a variety of ways: by studying the response of yeast to the immunosuppressant rapamycin; by identifying temperature-sensitive mutants that arrest in a quiescence-like state at nonpermissive temperatures even in the presence of nutrients, e.g., *cdc25* (34); and by studying a subset of mutants fortuitously found to selectively lose viability when cultured to stationary phase. Here, we discuss the signaling pathways thought to regulate entry into quiescence: the TOR and protein kinase A (PKA) pathways, apparent negative regulators of the transition into quiescence; and the protein kinase C (PKC) and Snf1p pathways, apparent positive activators of the transition (Fig. 3). Our understanding of the signaling networks regulating this transition is still fragmented, and other key regulators doubtless remain to be discovered. We therefore cannot yet tell the whole story; instead, we summarize a work in progress.

TOR Pathway

The immunosuppressant drug rapamycin inhibits proliferation of both yeast and mammalian cells. Rapamycin-treated yeast cells appear to enter a quiescence-like state (3). Treated haploids arrest as small, unbudded cells with 1N DNA content and undergo many of the gene expression changes characteristic of quiescent cells including repression of the ribosomal

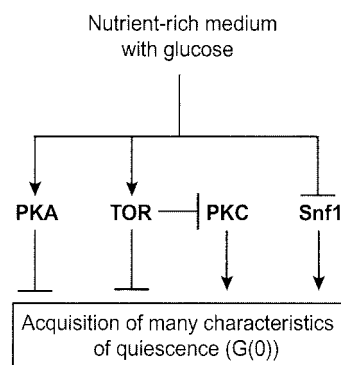


FIG. 3. Summary of the known signaling pathways thought to control aspects of entry into quiescence. The TOR and PKA pathways are active in the presence of nutrients and act to repress aspects of quiescence. When cells are starved of carbon, both pathways are downregulated. Inactivation of the TORs causes activation (albeit transiently) of PKC, leading to some characteristics of quiescence such as a remodeled cell wall. The Snf1 pathway is inhibited by the presence of fermentable carbon sources such as glucose. When such sources are depleted, Snf1 is activated and contributes to the switch from fermentative to respiratory metabolism that is essential for entry into quiescence.

protein genes and induction of *UBI4* and *HSP26* (57). Rapamycin-treated yeast also synthesize proteins at 50 to 60% of the level of logarithmically growing cultures, display reduced activity of RNA Polymerase I (PolI) and PolIII and high levels of autophagy (103), and accumulate the storage carbohydrates glycogen and trehalose.

The cytosolic target of rapamycin is FKBP12, an immunophilin (135). This binary rapamycin-FKBP12 complex binds to and inhibits the partially redundant proteins Tor1p and Tor2p when complexed with two other essential proteins, Lst8p and Kog1p, in the so-called TORC1 complex (89). The TOR proteins (for “target of rapamycin”) are phosphatidylinositol kinase-related protein kinases (69). Loss of both Tor1p and Tor2p largely phenocopies rapamycin treatment (3). Based on these and other observations, Hall and coworkers have proposed that the TOR proteins function to repress a quiescence program when nutrients are abundant. They envisage that nitrogen or carbon starvation would lead to inactivation of the TOR pathway, liberation of the quiescence program, and consequent entry into quiescence (25, 36, 94, 119, 131, 135).

Some progress has been made in recent years in identifying the downstream functions of Tor proteins, although direct *in vivo* targets of the kinases are still not known. One important downstream component is Tap42p, which binds to and regulates the catalytic subunits of PP2A protein phosphatases such as Sit4p, Pph21p, and Pph22p (32, 69). The TORs promote association of Tap42p with PP2A catalytic subunits when nutrients are plentiful. Both rapamycin treatment and transit through the diauxic shift cause dissociation of Tap42p from its phosphatase partners (32, 36, 66). Thus, the diauxic shift and rapamycin act to downregulate TOR function.

Using microarray profiling, a global picture of the gene expression changes caused by rapamycin treatment (and thus, by implication, inhibition of the common function of Tor1p and Tor2p) has emerged (see, e.g., reference 138). Inhibition of

TOR function causes activation of Gln3p and Gat1p transcription factors via Tap42p, resulting in induced expression of nitrogen discrimination pathway (NDP) and carbon discrimination pathway (CDP) genes, which are normally induced by a shift from good to poor nitrogen or carbon nutrient sources, respectively (22). Inhibition of TOR function also causes activation of Mks1p and consequent induction of Rtg1p- and Rtg3p-regulated genes, particularly those encoding some of the Krebs cycle enzymes (147), and activation of Hap2/3/4/5p with consequent induction of genes encoding other Krebs cycle enzymes.

Inactivation of the TORs also causes gene expression changes independently of Tap42p, such as decreased expression of the ribosomal protein genes and coordinated genes encoding components of the translational apparatus (120) and activation of the transcription factors Msn2p and Msn4p, by promoting their dissociation from 14-3-3 protein anchors in the cytoplasm. These redundant transcription factors drive the expression of the stress response element-containing genes, which are also induced by multiple other environmental stress such as heat shocks and hyperosmotic shocks (4, 49).

Is inhibition of the TOR pathway important for the formation of quiescent cells triggered by starvation? Probably. The phenotype of rapamycin-treated cells and the inactivation of the pathway on carbon starvation suggests that inhibition of the TOR pathway is important, if not critical, for entry into quiescence. In addition, mutants defective in a number of downstream targets of the TORs, e.g., in autophagy or in the protein kinase C pathway (see below), die on starvation, supporting a key role for the TORs (77, 80, 154). Unfortunately, no constitutively activated alleles of *TOR1* and *TOR2* exist, precluding a definitive test of this hypothesis.

Inactivation of the TORs may not be sufficient for the formation of truly quiescent cells since rapamycin-treated cells do not appear identical to quiescent cells. First, rapamycin inhibits translation by only 50 to 60% (i.e., up to half) of the rate measured for untreated, logarithmically growing cultures whereas quiescent cells display 0.3% (i.e., 1/333) of that translation rate (3, 47, 82). Second, rapamycin-treated cells appear to continue to accumulate mass and volume, unlike truly quiescent cells (3). Finally, rapamycin treatment induces both NDP and CDP gene expression but carbon limitation induces only CDP (57).

Whatever the importance of the TORs in defining the quiescent state, it is clear that an understanding of their regulation should inform any model of the elusive nutrient detection systems that regulate the entry into quiescence. The TORs are inactivated to some extent at the diauxic shift, by transfer from good- to poor-quality carbon or nitrogen sources or by starvation for carbon or nitrogen. The TORs are thus responding to the absence of high-quality nutrient sources as opposed to the presence of low-quality ones. Many potential regulators of mTOR (mammalian TOR) have been proposed, including the possibility that it is a direct sensor of cytoplasmic ATP by virtue of an unusually high K_m for ATP (29). How mTOR is regulated by nutrients and by growth factors is still hotly disputed at the time of writing, and the identity of the mechanisms that regulate the yeast TORs is also unknown.

Protein Kinase C Pathway

The yeast PKC, encoded by *PKC1*, responds to cell surface stresses and changes in the actin cytoskeleton during vegetative proliferation (53, 75, 86). Pkc1p in part regulates a mitogen-activated protein (MAP) kinase cascade involving the MAP kinase Mpk1p (23, 65, 83, 84). Mutants lacking *BCK1*, encoding the MAP kinase kinase kinase that acts in this cascade, were reported to die rapidly on nitrogen limitation (23, 24). Based on this observation, it was proposed that the Pkc1p-MAP kinase pathway may be a nutrient sensor.

It was recently reported that the Pkc1p-MAP kinase pathway is required for viability on carbon or nitrogen starvation or growth of a culture to stationary phase (80). However, the pathway is unlikely to be a nutrient sensor. Rather, it acts downstream of and is transiently activated by TOR inactivation (80). Mpk1p is also activated transiently at the diauxic shift (i.e., concomitant with TOR inactivation) and *mpk1Δ* mutants begin to lose viability at the same point (154). Activation of the Pkc1p pathway by TOR inhibition occurs by a novel mechanism independent of the Hcs77p and Mid2p sensors required for detecting cell surface stresses during vegetative proliferation (53, 67, 79, 124, 160).

The Pkc1p pathway acts, in part, to promote the acquisition of one key characteristic of quiescent cells on starvation: a reinforced and remodeled cell surface wall (80). First, mutants defective in the Pkc1p pathway lyse when starved, and this lysis is coincident with cell death. Second, starvation or rapamycin treatment rapidly causes increased resistance to the cell wall-digesting enzyme zymolyase, and this acquisition of zymolyase resistance is dependent on Pkc1p. This failure of Pkc1p pathway mutants to acquire resistance to zymolyase occurs before cell death, indicating that the pathway is a bona fide positive regulator of entry into quiescence, which probably acts downstream of TOR inactivation.

Curiously, rapamycin treatment alone, even in rich media, is sufficient to kill mutants defective in the Pkc1p pathway (80). Thus, mutants lacking components of the Pkc1 pathway die under all the conditions tested that drive cells into quiescent or quiescence-like states and that inhibit the TORs. We infer that the Pkc1p pathway is inherently required for the formation of viable quiescent cells and not simply for the formation of quiescent cells that can survive starvation.

Protein Kinase A Pathway

The cyclic AMP (cAMP)-dependent protein kinase (PKA) pathway is conserved in all eukaryotic cells and, although the structure of the pathway is not identical in all cells, this pathway is invariably involved in regulating cell growth and development (37, 162). When cAMP concentrations are low, PKA is inactive and exists as a tetramer composed of two catalytic subunits and two regulatory subunits (81, 148). There are three forms of the catalytic subunit encoded by the three partially redundant genes, *TPK1*, *TPK2*, and *TPK3* (152). The regulatory subunit is encoded by *BCY1* (17, 151). When cAMP concentrations are high, the nucleotide binds to the inhibitory Bcy1p subunits, causing dissociation from and activation of the catalytic subunits (148). Tpk1p, Tpk2p, and Tpk3p appear to have different functions. For example, cells lacking Tpk2p

grow better than wild-type cells on nonfermentable carbon sources, while Tpk1p is actually required for growth on nonfermentable carbon sources (129), possibly because these cells arrest prematurely on such nonfermentable carbon sources.

The PKA pathway acts, in general, as an inhibitor of entry into quiescence. Mutants lacking adenylate cyclase activity are unable to proliferate and arrest in a state superficially similar to quiescence (11, 15, 155, 158). Constitutive activation of PKA, e.g., by deletion of *BCY1*, causes cell death at the diauxic shift (16, 17, 166), indicating that proper downregulation of the PKA pathway is necessary for successful transit to the post-diauxic phase (96, 140, 157). Altered Bcy1p protein, in which the serine 145 residue had been changed to alanine, has a 10-fold-higher affinity than does the wild-type protein for the catalytic subunits. Cultures of cells carrying this allele transit the diauxic shift and enter stationary phase at a lower cell density than do wild-type cells (169). In addition, cells harboring different alleles of *bcy1* with mutations in the C terminus die at different times during the post-diauxic and stationary phases when cultured to saturation (110). Localization of Bcy1p and the holoenzyme is dynamic during entry into quiescence (54), switching from nuclear localization in exponentially growing cells to cytoplasmic localization as the cells approach and enter the quiescent state.

If inactivation of the PKA pathway is critical for entry into quiescence, then activation of the pathway should be important for successful exit from quiescence when nutrients are again available. This seems to be the case. Quiescent mutant cells containing low constitutive activity of the PKA pathway (i.e., harboring Tpk-wimpy alleles) display a long delay in reentering the cell cycle on addition of glucose-based rich medium (70).

How does nutrient availability regulate the PKA pathway? The immediate upstream regulators of cAMP synthesis are known. The partially redundant G proteins Ras1 and Ras2 are activated by signals from the environment, e.g., nutrient availability. Cdc25p, an exchange factor (130), activates Ras1p and Ras2p by promoting the replacement of bound GDP to GTP. The activated (i.e., GTP-bound) forms of these small G proteins directly bind to and activate adenylate cyclase (Cdc35/Cyr1p) (153), leading to an increase in the level of intracellular cAMP (for a review, see reference 150).

One G-protein-coupled receptor system (Gpr1p-Gpa2p) appears to act upstream of PKA as a sensor of external glucose (46) and is important for glucose activation of cAMP synthesis (150). However, mutants lacking Gpr1p or Gpa2p are viable and proliferate normally in glucose-containing media, indicating that this sensing system plays a minor or specialized role in the regulation of the PKA pathway. The key regulators of the pathway during entry into quiescence remain elusive. Although many observations point to glucose and other carbon sources as being sensed by the pathway, it has recently been reported that starvation for nutrients other than carbon can also result in decreased PKA activity (149).

What are the downstream targets of the PKA pathway? The pathway inhibits the transcription factors Msn2p and Msn4p, which are also targets of the TOR pathway (see above) (4, 142). Rim15p, a protein kinase previously shown to stimulate meiotic gene expression, acts downstream of and is negatively regulated by PKA (127). Additionally, Gis1p, a putative zinc finger protein, acts downstream of Rim15p and mediates tran-

scriptional activation via the post-diauxic shift element found upstream of many genes whose expression increases at the diauxic shift (111). It has recently been suggested that the PKA pathway also regulates the Ccr4p-Not complex, which appears to regulate gene expression both positively and negatively via the general transcription factor TFIID (85). This Ccr4p-Not complex may mediate the repression of Msn2p and Msn4p by the PKA pathway. The PKA pathway may also alter chromatin structure (184). Finally, the PKA pathway has recently been shown to be a direct activator of pyruvate kinase (Cdc19p) (126) and Cox6p (176) in proliferating cells, suggesting a possible role for the pathway in regulating carbohydrate metabolism and mitochondrial function at the diauxic shift. The specific role for PKA in the post-diauxic and quiescent phases is not known, although it may in part regulate the Rye proteins, several of which are Ssn/Srb subunits of PolIII and are required for survival in stationary phase (reviewed in reference 59).

The Msn2p and Msn4p transcription factors are negatively regulated by both the TOR and PKA pathways and are activated at the diauxic shift. Curiously, in strains lacking both Msn2p and Msn4p, PKA activity is dispensable for vegetative proliferation (142). Thus, inactivation of the Ras-cAMP pathway in rich media leads to arrest in a quiescence-like state, in part because of activation of Msn2p and Msn4p. Importantly, loss of Msn2p and Msn4p function also confers modest resistance to rapamycin (4). Thus, Msn2p and Msn4p are necessary, at least in part, for arrest in a quiescence-like state triggered by PKA or TOR inactivation in the presence of nutrients. However, activation of Msn2p and Msn4p is not necessary for successful entry into quiescence triggered by growth of cultures to stationary phase: *msn2 msn4* double mutants have been reported to maintain viability for protracted periods, although not as long as do wild-type cells, when starved (95).

Snf1p Pathway

The *SNF1* gene encodes the yeast homologue of AMP-activated protein kinase (AMPK) (56). AMPK is activated by a variety of stresses to mammalian cells that change the ATP/AMP ratio, and the activation occurs by direct allosteric changes (56). Yeast Snf1p is also activated when the in vivo ATP/AMP ratio drops, but the activation is thought to be indirect since the purified kinase is refractive to these nucleotides (174). In the presence of glucose, Snf1p is inactive, resulting in the preferential use of glucose as the carbon source (174, 175). When glucose levels drop, Snf1p is rapidly activated (within 5 mins) and derepresses the expression of genes required for the use of alternative carbon sources and metabolic pathways that generate ATP (174).

Mutants lacking *SNF1* cannot utilize alternative carbon sources such as ethanol and glycerol (56), and they die when the cultures are grown to high density (actually soon after the diauxic shift). This and other evidence (see below) suggests that adaptation to the use of poor carbon sources and the ability to respire are necessary for proper entry into a stable quiescent state.

An Emerging Signaling Network

There is accumulating evidence that Snf1p function converges with both the PKA and TOR pathways (and thereby the

Pkc1 pathway) in modulating various outputs, including Gln3p and Msn2p/Msn4p (see above) (6, 97). Snf1p activation also induces peroxisomal genes, such as *POT1* (102), which is negatively controlled by PKA (63). In addition to the above interactions with Snf1p, the PKA and Pkc1p pathways appear to intersect based on studies of Rpi1p, an upstream antagonist of RAS that also regulates cell wall integrity (143) and the WSC genes (160). Interactions between the PKA and TOR pathways are also known to exist through their effects on Msn2p and Msn4p (39, 159). Thus, the signaling pathways thought to regulate entry into quiescence appear to form an interacting network that acts at the diauxic shift in response to the change in carbon quality. The architecture of this putative network is still poorly defined and may be constant through the life cycle of yeast or may itself dynamically change as the cells transit into quiescence.

The link between the TOR and PKA pathways is becoming clearer. Mutants lacking both Gln3p and Gat2p, known effectors of the Tip41/Tip42 branch of the TOR pathway, are only moderately resistant to rapamycin. It has been reported that high- or low-level constitutive activation of the PKA pathway confers robust rapamycin resistance on such mutants but not on wild-type cells (134). Further, even in wild-type cells, such misregulation of the PKA pathway prevents the acquisition of most, if not all, of the characteristics attributed to regulation of the Tip41/Tip42-independent branch of the TOR pathway, including repression of the ribosomal protein genes. Finally, it has been shown that rapamycin treatment alone causes nuclear localization of Bcy1, thereby mimicking cAMP depletion and nitrogen limitation. It thus appears that the Ras-cAMP/PKA pathway may act, at least in part, downstream of the TORs and in the Tip41/Tip42-independent branch.

Another recent paper (112) suggests that both pathways control the activity of the Rim15p protein kinase (128). Rim15p regulates the expression of genes containing post-diauxic shift elements in their promoter at or soon after the diauxic shift. Importantly, *rim15Δ* mutants appear to lose viability to some extent when cultured to stationary phase (to 10% CFU in 30 days), fail to acquire some key characteristics of quiescence (128), and suppress the growth defects of strains lacking the PKA pathway activity (as do *msn2Δ msn4Δ* mutants [see above]).

It now appears that the TOR and PKA pathways have at least one common target, Rim15p, and regulate multiple common outputs. It is less clear how this regulation takes place. It could be that the PKA pathway acts downstream of the TOR kinases, as suggested by Schmelzle et al. (134). Alternatively, both pathways may act in parallel, as argued by Pedruzzi et al. (112). This issue awaits resolution.

Working Model for the Regulation of Entry into Quiescence

Although cells acquire many of the characteristics of quiescence at the diauxic shift, it is clear that in rich, glucose-based medium, entry into quiescence proper occurs when carbon is finally depleted, concomitant with permanent proliferation arrest. It thus appears, to a first approximation, that growth of a culture to stationary phase causes at least two distinct changes in cell state: (i) rapid proliferating (fermenting) to slow proliferating (respiring), concomitant with the diauxic shift of the

culture, and (ii) slow proliferating (respiring) to quiescent, concomitant with saturation of the culture. The first change (at the diauxic shift) reprograms cells for respiration, which may be a necessary precursor for the second change: entry into the nonproliferating quiescent state (see Fig. 4). Each transition in liquid medium is triggered by a distinct environmental change: the first by the lack of a good carbon source, and the second by the lack of any carbon source (Fig. 4).

The recent work by Gasch et al. (49) has supported this two-transition model of entry into quiescence after growth of cultures to stationary phase. One of the experiments in this work involved monitoring the genome-wide gene expression changes (by microarray expression profiling) on growth of a culture for 5 days in rich medium (i.e., to late post-diauxic shift/early quiescence). Two results are clear. First, the changes in gene expression that occur at the diauxic shift persist for at least 5 days in culture. Second, numerous additional changes in gene expression happen selectively or exclusively after 4 or 5 days, i.e., as the cells approach full quiescence (e.g., induction of the *YDR504w* and *SNZ1* genes). These late changes in gene expression are not triggered when cells are starved for nitrogen. These changes may thus be specific for entry into quiescence proper triggered by carbon starvation.

The putative signaling network involving the TOR, PKA, PKC, and Snf1p pathways appears to act predominantly at the first transition, concomitant with the diauxic shift (Fig. 4). Other, yet to be implicated, pathways may also act here. We know nothing about the regulators and mediators of the second step, final entry into quiescence. Because mutants harboring different loss-of-function alleles of *BCY1*, the gene encoding the inhibitor of PKA, appear to lose viability at different points when cultured to saturation, ranging from the diauxic shift (reminiscent of a null mutant) to stationary phase (110), it is possible that stepwise regulation of some or all of the same signaling network that acts at the diauxic shift also contributes to the final entry into quiescence.

MAINTENANCE OF VIABILITY IN QUIESCENCE

The processes of entry into and survival in quiescence are intimately linked. One characteristic of successful entry into quiescence by wild-type cells must be the acquisition of the ability to survive in that state. However, success comes in degrees. A distinction between the processes of entry into and maintenance in quiescence is possible and useful. Entry can be viewed as the process by which the key measurable characteristics of quiescence (yet to be defined) are attained (e.g., involving signaling pathways and mediators of change of state [see above]); maintenance encompasses the processes by which the characteristics of quiescence acquired on entry contribute to long-term viability of that state. Most mutants that lose cell viability when cultured to stationary phase can in practice be subclassified as being entry defective or maintenance defective (see above). In this section, we focus on a few selected processes that are thought to be important specifically for maintenance of viability in quiescence.

Genes Required for Maintaining Viability

Given the caveat that mutants designated as being required for "survival" in quiescence/stationary phase can be defective

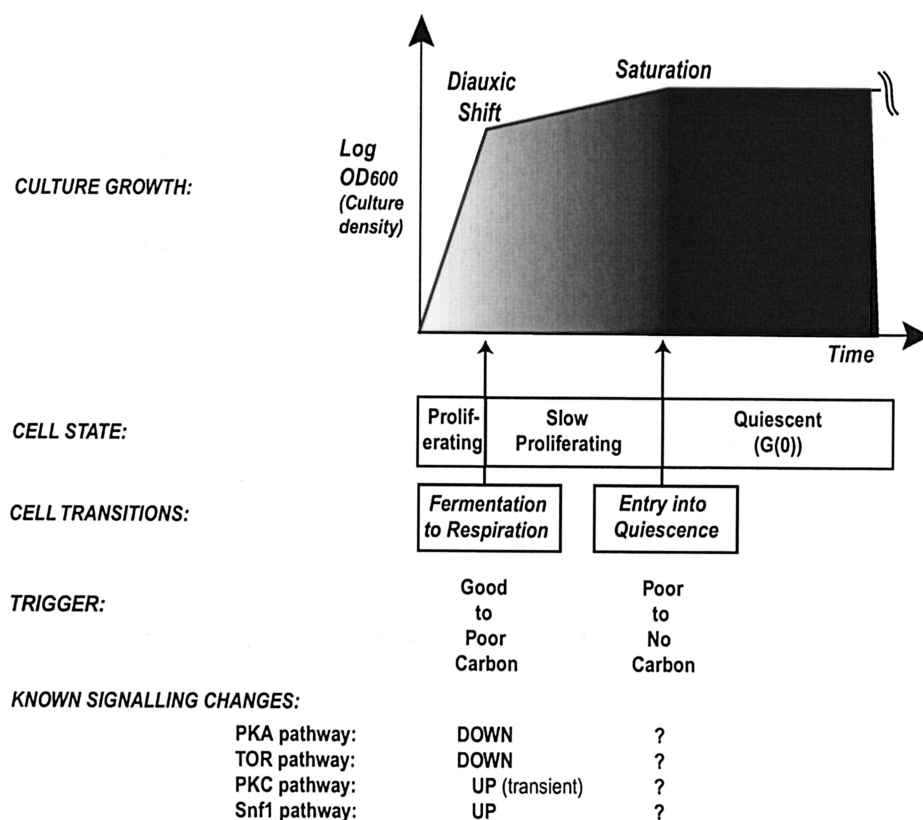


FIG. 4. Cell transitions that occur when a culture is grown to saturation cell density. As shown in Fig. 1, as a culture of cells is grown to stationary phase, two distinct and temporally separated transitions occur with concomitant transitions of the constituent cells. At the diauxic shift transition of the culture, cells switch from fermentation to respiration and from rapid proliferation to slow proliferation. The trigger for this transition is thought to be exhaustion of a fermentable carbon source. At this transition, the PKA and TOR pathways are downregulated and the PKC and Snf1 pathways are activated, the former only transiently. At saturation of the culture, cells switch from a slowly proliferating and respiring state to a quiescent state/ G_0 that is also thought to be respiring. The trigger for this transition is thought to be depletion of nonfermentable carbon sources in the medium, i.e., carbon starvation. Mediators of this latter transition are not known, nor has a role for the PKA, TOR, PKA, or Snf1 pathways been established.

in entry into, maintenance in, or exit from quiescence, many such mutants are likely to be required for maintenance of viability in quiescence. An increasing number of such mutants are being identified, and the collection as a whole gives a low-resolution view of the cellular processes that are more critical for the survival of quiescent cells than for the survival of proliferating cells. Mutants known prior to 1993 have been extensively reviewed already (167). A deficiency in any of a wide range of cellular functions can cause viability loss in quiescence; these include oxidative stress responses, e.g., *sod2* mutants (45, 90); accumulation of polyphosphate in the vacuole, e.g., *ppn1* mutants (136); ubiquitination, e.g., *doa4* mutants (145); and those lacking specific myristolated proteins, e.g., *arf1*, *arf2*, *cdc40/prp17* and *las17* mutants (1). Most recently, genes required for survival in quiescence at 37°C have been identified among genes coordinately repressed on exiting quiescence (171), predominant among which are genes encoding proteins involved in growth regulation, oxidative phosphorylation, and other processes involved in mitochondrial function (M. J. Martinez, A. B. Archuletta, A. I. Rodriguez, A. D. A. Aragon, S. Roy, C. P. Allen, P. D. Wentzell, and M. Werner-Washburne, submitted for publication).

“Essential” Genes That Are Not Required for Viability

Another equally interesting set of genes appear to exist: those that are essential for the proliferative state but are not required for viability in quiescence. One such example is the gene encoding the translation factor eIF4E (109). It is thought that translation initiation in quiescent cells does not involve recognition of the mRNA cap. Thus, some functions are more important to a proliferating cell than they are to a quiescent cell. Although other obvious possibilities could easily be postulated, e.g., proteins required for cell division cycle progression should be dispensable during quiescence, we that expect other, more informative cases will be discovered.

Control of Gene Expression

Correct regulation of gene expression is a key process in the cell quiescence cycle. A change in the expression of any given gene can result from altered activity of a particular transcription factor (see multiple examples elsewhere in this review) but can also be affected by changes in general transcription factors. The latter may also be significant for the cell quiescence cycle.

For example, the general transcription factor TFIID comprises the TATA box-binding protein and a set of highly conserved associated factors (TAFIIs). TAFII145, the core subunit of the yeast TAFII complex, is dispensable for normal transcription of most yeast genes but is specifically required for progression through the G₁/S transition of the cell division cycle. Walker et al. have shown that the levels of TAFII145, several other TAFIIs, and TATA box-binding protein are drastically reduced in quiescent cells relative to their levels in proliferating cells (161). Another example is the PolIII subunit Rpb4p. Yeast cells lacking Rpb4p proliferate normally at moderate temperatures (18 to 22°C) but not at temperatures outside this range. When subjected to a heat shock, proliferating cells lacking *RPB4* rapidly lose PolIII transcriptional activity and subsequently die. When cultured to stationary phase at a permissive temperature (i.e., permissive for proliferating cells), *rpb4Δ* mutants also exhibit a substantial decline in mRNA synthesis relative to wild-type cells and die. Moreover, in wild-type cells, the portion of PolIII complexes that contain Rrb4p increases substantially as the cells enter quiescence (21). There is evidence that PolIII complexes need to be covalently modified in some way in order to recruit Rpb4p, and, again, the portion of modified complexes increases as cells enter quiescence, becoming the predominant form (132).

Translation

Protein synthesis consumes a huge amount of the energy in an exponentially growing yeast cell. rRNA transcription represents ~60% of the total transcription, and ribosomal protein synthesis represents ~15% of total translation. It is not surprising, therefore, that the first coordinated downregulation of genes that seems to occur during the transition into the quiescent state is the coordinated, global shutdown of the transcription of genes coding for the proteins in both subunits of the ribosome. How this coordinated shutdown is accomplished is not known. Despite this shutdown of ribosomal protein biosynthesis, quiescent cells maintain excess translational capacity (31) and protein synthesis continues, albeit at very reduced rates (some 0.3% of the rate in proliferating cells) (47).

A few proteins have so far been identified that are selectively synthesized after entry into quiescence (47). One such protein, designated Snz1p, is induced later than all other known proteins, and its relative rate of synthesis increases with time in quiescence. *SNZ1* expression also increases in response to starvation for other specific nutrients, such as tryptophan, adenine, or uracil (106). Increased Snz1p levels may be a hallmark of a general core quiescence program, one that is shared by the responses to different starvation regimens and one that is likely to be highly conserved, as is Snz1p itself (12).

It transpires that Snz1p is required for pyridoxine (vitamin B₆) biosynthesis (38, 105). Why, then, is Snz1p (and presumably Snz1p activity) induced in quiescence? There are two likely possibilities. Vitamin B₆ derivatives may be important cofactors for metabolism in quiescent cells, possibly in aminotransferase reactions. Alternatively, these vitamins may function as antioxidant compounds, providing a defense against endogenously generated reactive oxygen species, especially singlet oxygen (see below).

Curiously, there is no direct correlation between steady-state

mRNA accumulation and protein synthesis for another protein, Ssa3p, that is synthesized preferentially in quiescent cells. It thus appears that the synthesis of at least some important proteins in the quiescent state is regulated by mechanisms other than mere control of steady-state mRNA abundance.

Protein Turnover and Covalent Modification of N Termini

Cells in stationary-phase yeast cultures do not increase in mass with time. Since protein synthesis is known to continue in these quiescent cells (albeit at a low rate), the obvious conclusion is that protein synthesis and degradation must be tightly coupled in the quiescent state. However, little is known about the regulation of protein turnover in quiescent yeast cells.

Evidence from mutants clearly implicates ubiquitin-dependent protein degradation as a process essential for the maintenance of viability in quiescent cells. *ubi4* mutants, which lack polyubiquitin (a natural gene fusion of five ubiquitin sequences), are unable to maintain viability when cultured to stationary phase or after starvation for nitrogen or carbon (43, 44). *ubc5* and *ubc1* mutants, which lack the corresponding ubiquitin-conjugating enzymes, also display reduced viability in quiescence (137). In the *doa4Δ* mutant, which is defective in the recycling ubiquitin from ubiquitinated substrates, ubiquitin is strongly depleted from cells under certain conditions, most notably as the cultures approach stationary phase (145). Ubiquitin depletion precedes a striking loss of cell viability in saturated cultures of *doa4Δ* cells. This loss of viability of *doa4Δ* cells is rescued by provision of additional intracellular ubiquitin. Presumably, ubiquitin becomes depleted in the mutant because it is degraded much more rapidly than in wild-type cells. Aberrant ubiquitin degradation in the *doa4Δ* mutant can be partially suppressed by mutation of the proteasome or by inactivation of vacuolar proteolysis or endocytosis. This latter observation connects protein homeostasis to protein trafficking.

Indirect evidence for the importance of specific targets for regulated turnover comes from experiments with mutations that affect N-terminal acetylation. Loss of function of either of the two subunits of the *N*-acetyltransferase encoded by the *NAT* and *ARD1* genes causes a failure of yeast to survive carbon starvation (107, 172). The proteomes of both wild-type and *nat1* mutant cells in proliferating cultures have been examined (48). Although only a small subset of the 6,000 yeast proteins were identified in this analysis, at least 56 proteins appear to be acetylated by Nat1p under normal proliferative conditions. Intriguingly, these modified proteins included Yst1p and Yst2p, structural proteins of the ribosome; Asc1p, a protein known to interact with the translational machinery; Ebf1p, a GDP-GTP exchange factor for the translational protein EF-1; Bmh1p and Bmh2p, proteins known to affect the PKA and TOR pathways; and Tif1p, translation initiation factor eIF4A. Others included the ubiquitin-activating enzyme Uba1p and several peroxisomal proteins. Thus, a number of proteins involved in functions already implicated in entry into or maintenance of the quiescent state are targets of N acetylation.

Lipidation of the N terminus of some proteins, e.g., by N-myristoylation, is also important for the maintenance of viability in quiescence. *S. cerevisiae* contains four known acyl co-

enzyme A (acyl-CoA) synthetases, Faa1p to Faa4p (for “fatty acid activation proteins”). Acyl-CoA metabolism regulates protein N myristoylation, a reaction catalyzed by the essential enzyme, myristoyl-CoA:protein N-myristoyltransferase (Nmt1p). The combination of a partial-loss-of-function mutation in *NMT1* and a null mutation in *FAA4* results in a progressive millionfold reduction in CFU in quiescence that is associated with a deficiency in protein N myristoylation (1). This apparent viability defect first appears during logarithmic growth of cultures, worsens through the post-diauxic phase, and becomes extreme in stationary phase. Curiously, Nmt1p activity is normally present in cells cultured to log and diauxic/post-diauxic phases but is absent from cells at stationary phase. It thus appears that N-myristoylated proteins present in quiescent cells, and the requirement for them, are “inherited” from prior proliferating states.

Many known and putative N-myristoylated proteins have been identified in yeast (1). Of the 64 genes identified that encode such proteins, removal of any 1 of the following 9 causes a severe loss of CFU in quiescence: *ARF1*, *ARF2*, *SIP2*, *VANI*, *PTC2*, YBL049W (homology to *SNF7*), YJR114W, YKR007W, and VPS20. Thus, protein N myristoylation (during prior proliferating states) and a number of individual targets of this modification appear to be required for viability in quiescence.

Autophagy

For turnover of cellular components, eukaryotic cells are equipped with several other degradation systems, one of which is the process of autophagy. Autophagy is a membrane transport pathway leading from the cytoplasm to the vacuole in yeast (or to the lysosomes in mammalian cells) for degradation and recycling. In addition to nonspecific bulk cytosol, selective cargoes such as peroxisomes are sorted for autophagic transport under specific physiological conditions. In a nutrient-rich growth environment, many of the autophagic components are recruited to execute a specific biosynthetic trafficking process, the cytoplasm-to-vacuole targeting (Cvt) pathway, that transports the resident hydrolases aminopeptidase I and α -mannosidase to the vacuole. Recent studies have identified pathway-specific components that are necessary to divert a protein kinase and a lipid kinase complex to regulate the conversion between the Cvt pathway and autophagy (62).

During the autophagic process, a single-membrane structure, the so-called isolation membrane, surrounds portions of the cytoplasm and organelles. Fusion of the tips of the isolation membrane to each other forms a double-membrane spherical autophagosome with a diameter of about 1 μ m. The autophagosome then fuses with lysosomes, and the sequestered contents, along with the inner membranes, are degraded by lysosomal hydrolases (100).

In most cells under most conditions, autophagy is usually suppressed to a very low basal level. Some conditions, including starvation (yeast) and hormonal stimulation (mammalian cells), can trigger dramatic enhancement of autophagy. Autophagy at the basal rate most probably contributes to the turnover of cellular components at steady state, whereas starvation-induced autophagy is thought to aid in maintaining an amino

acid pool for gluconeogenesis and for the synthesis of proteins essential to survival under starvation conditions.

Autophagy-deficient yeast mutants die rapidly on starvation (156). Autophagy in yeast has traditionally been stimulated in rich medium by starving for nitrogen, and the relationship of this state to the quiescent state attained on starvation for carbon is unclear. However, increased autophagic activity has been observed in wild-type cells in cultures entering stationary phase, and this induction was impaired in a *snf1* strain (164). Snf1p is a putative regulator of entry into quiescence (see above) and is required for glucose derepression.

Glycogen storage is also defective in autophagy mutants: mutants defective for autophagy are able to synthesize glycogen when approaching the stationary phase but are unable to maintain their glycogen stores, because subsequent synthesis is impaired and degradation by phosphorylase, Gph1p, is enhanced. Deletion of *GPH1* partially reverses the loss of glycogen accumulation in autophagy mutants. Loss of the vacuolar glucosidase, *SGAI*, also protects glycogen stores but does so only very late in stationary phase, suggesting that Gph1p and Sga1p may degrade distinct pools of glycogen (164). Defective glycogen storage in *snf1* cells may be due to both defective synthesis on entry into stationary phase and impaired maintenance of glycogen levels caused by the lack of autophagy, suggesting an important role for this process in the ability of cells to survive carbon starvation.

Autophagy may actually help connect transcription and translation in starved cells. The yeast eIF2 α kinase, Gcn2 (which is required for translation of the transcription factor Gcn4p), and the transcription factor Gcn4, which is regulated by Gcn2, are required for autophagy induced by starvation (146). This induction process for autophagy is likely to be functionally conserved since the mammalian eIF2 α kinase, PKR, is able to restore starvation-induced autophagy in yeast cells lacking the *GCN2* gene (146).

Interestingly, murine embryonic fibroblasts lacking the mammalian eIF2 α kinase or with a nonphosphorylatable mutant form of eIF2 α (due to a Ser-51 mutation) are defective in autophagy that can be triggered by herpes simplex virus infection. Furthermore, PKR and eIF2 α Ser-51-dependent autophagy is antagonized by the herpes simplex virus neurovirulence protein, ICP34.5. Thus, autophagy is a novel evolutionarily conserved function of the eIF2 α kinase pathway that is both required for viral virulence and targeted by viral virulence gene products (146).

Many of the known mediators of entry into quiescence can modulate autophagy. As noted above, Snf1p is required for increased autophagy as cultures are grown to saturation (164). In addition, inhibition of the TOR proteins by rapamycin in rich media is sufficient to induce autophagy, an induction that is prevented by hyperactivation of the PKA pathway (134). Thus, TOR and PKA activities act to repress autophagy in the vegetative state, and inactivation of these same pathways during entry into quiescence probably derepresses autophagy. The mechanism by which the TOR proteins modulate autophagic activity is partly understood. The protein kinase activity of Apg1p, the autophagy-regulating kinase, is enhanced by starvation or rapamycin treatment (76). In addition, Apg13p, which binds to and activates Apg1p, is hyperphosphorylated in a TOR-dependent manner, reducing its affinity to Apg1p.

Apg1p-Apg13p association is required for starvation-induced autophagy but not for the Cvt pathway (74, 76). *YPT1*, a small GTPase important for vesicular transport, which is a process known to be essential for exit from quiescence (see below), has also been implicated in autophagy via the effects of its GTPase-activating proteins (Ypt1p-GAPs). Ypt1p-GAP deletion strains exhibit various morphological alterations resembling constitutive activation of autophagy (27).

Metabolism

Yeast cells in quiescence have increased amounts of storage carbohydrates (glycogen and trehalose), whose levels decrease slowly with time in quiescence. Are these carbohydrates metabolized for fuel? Probably not. The long-term viability of cells in stationary-phase cultures does not always correlate with trehalose or glycogen accumulation (141). The primary function of trehalose may be to protect proteins in quiescent cells from denaturation and damage by oxygen radicals (5).

There is no answer at present to the obvious question: what are quiescent cells using as an energy source? The highest energy output per weight of material in cellular metabolism comes from the β -oxidation of fatty acids. It seems likely, therefore, that cells in stationary-phase cultures derive their energy from the slow metabolism of lipids, but no direct evidence for this has been published.

There is indirect evidence to suggest that lipid metabolism is important in quiescent cells. Loss-of-function mutations in *OPI3*, the gene coding for the enzyme that catalyzes the final methylation reaction in phosphatidylcholine biosynthesis, cause cells to lose viability when cultured to stationary phase (98). It is known that as cells enter the quiescent state, triacylglycerol synthesis increases (61), even though total phospholipid biosynthesis decreases (60). These results suggest that oxidation of triacylglycerols may be an energy reserve for quiescent cells.

Since β -oxidation of fatty acids occurs in the peroxisomes of eukaryotic cells, it seems reasonable to assume that there is a role for the peroxisome in survival of quiescence, but no such studies on this organelle have been published. Our preliminary observations (J. L. Collins and G. A. Petsko, unpublished data) indicate that peroxisomal fatty acid metabolism is not important for the maintenance of viability in quiescence. Most other fatty acid metabolism occurs in mitochondria. Are these organelles important to quiescent cells? Little work has been done to answer this question, but there are already good reasons to think that mitochondrial oxidative metabolism may be the chief source of energy for quiescent cells. Glyoxalate pathway genes are upregulated in cell cultures on entry into stationary phase (49). More directly, petite mutants and mutants harboring other mitochondrial loss-of-function defects die rapidly when starved for carbon in rich media (J. L. Collins, G. A. Petsko and D. Ringe, unpublished data; Martinez et al., submitted).

Redox Homeostasis

Unlike proliferative cells, quiescent yeast cells cannot dilute out damage to proteins and DNA by rapid synthesis of new macromolecules and cell division. Hence, quiescent cells are

potentially more vulnerable to internal and external stresses than are proliferating cells. It is reasonable to assume that quiescent cells have active, maybe even specialized, protection mechanisms to counter any accumulating damage. Here, we focus on one such stress, oxidative damage.

Mitochondrial respiration appears to be a major source of energy for quiescent cells. Unfortunately, respiration produces large amounts of reactive oxygen species, whose toxic effects must be countered if viability is to be maintained. We think that proper redox homeostasis is of great importance to quiescent cell viability. Multiple findings support this view. For example, the expression of genes encoding antioxidant enzymes, Mn superoxide dismutase (MnSOD), Cu,Zn superoxide dismutase (Cu,ZnSOD), and glutathione reductase, is induced when quiescent cells are exposed to menadione, an oxidizing agent (26). Thus, quiescent cells retain a capacity to detect and respond to oxidative damage.

It is clear that the response to oxidative stress is important. Longo et al. (92) studied yeast mutants lacking CuZnSOD and MnSOD (*sod1* and *sod2*, respectively) and determined their long-term viability (by measuring CFU) in stationary-phase cultures in minimal medium. Such cells would be in a state related to, but not identical to, our reference quiescent state. In well-aerated cultures, the lack of either SOD resulted in dramatic loss of viability over the first few weeks in culture. However, the double mutant died more quickly still, i.e., within a few days. Reduction of respiration via a second mutation dramatically increased short-term survival. These results strongly suggest that ongoing mitochondrial respiration is itself a major stress to starved yeast cells.

Aging versus Maintenance of Viability

The measurable, time-dependent loss of CFU in stationary-phase yeast cultures in synthetic media has been proposed as a model for cellular aging, "chronological" ageing (41). Stationary-phase cultures in rich media are much more resilient to apparent loss of viability than are saturated cultures in synthetic media for reasons that are not yet understood. Nevertheless, the processes in cells grown to saturation in synthetic medium should, in large part, inform us of the evolution of our reference quiescent state, and vice versa.

Another type of aging in yeast is replicative aging, defined as the loss of potential to undergo subsequent rounds of cell division cycle in rich media. A newly born daughter cell can undergo only a finite number of subsequent cell divisions before becoming senescent: mutants with a longer replicative life span can undergo more rounds of division from birth to senescence in the continuous presence of ample food. It is not clear how the two aging processes, chronological and replicative, are related: some mutation appear to have opposite effects on them, lengthening one while shortening the other, whereas other mutants affect one process only. However, there are a few genes whose deletion appears to affect both ageing mechanisms in the same way, suggesting that there is some commonality between the two mechanisms. For example, deletion of *SCH9*, a gene encoding a protein kinase that is a possible yeast homologue to the human antiapoptotic kinase Akt/PKB, dramatically extends the chronological life span of yeast (42) in a *SOD2*-dependent manner (40).

There is also evidence from yeast studies that longevity in eukaryotes may be negatively regulated by the PKA pathway, which is implicated in entry into quiescence. Mutations that decrease the activity of the Ras-Cyr1p-PKA pathway extend the longevity of yeast cells and increase stress resistance by activating transcription factors Msn2 and Msn4 and the mitochondrial superoxide dismutase Sod2p. Specifically, deletion of *RAS2*, one of the two Ras genes in yeast, doubles the chronological life span (overexpression of *RAS2* also increases the life span, suggesting that the dependence of life span on Ras2p activity is denoted by a bell-shaped curve); transposon mutation of *CYR1*, the adenylate cyclase that is a downstream target of Ras2p, has a similar effect (40). We presume that extension of life span, as opposed to its shortening, cannot be due to a failure to enter quiescence proper, so these observations suggest a role for the Ras-Cyr1p-PKA pathway in maintenance as well as entry and also suggest a possible connection between these stages of the quiescence cycle.

It is known that cells lose replicative capacity with time spent in quiescence proper, demonstrating a direct relationship between replicative ageing and evolution of quiescence. It must be noted, however, that the very concept of yeast as a model for aging in other organisms has been questioned (50). The specific objections raised do not invalidate the relationships between replicative ageing, chronological ageing, and maintenance of viability in quiescence in yeast; the study of one should, in part at least, inform the others.

EXITING FROM QUIESCENCE

Resuspending quiescent cells in media containing all necessary nutrients (including a carbon source) stimulates exit from quiescence and completion of the quiescence cycle. Stimulated (refed) quiescent cells lose thermotolerance, become sensitive to cell wall-degrading enzymes, and display increased rates of RNA and protein synthesis. Internal carbohydrate stores such as glycogen and trehalose are also mobilized. Ultimately, stimulated cells resume cell growth and begin proliferation, i.e., enter the proliferative cell cycle (167).

Sensing Nutrients

How does a quiescent cell sense the presence of nutrients? It is possible that the cell has the ability to simultaneously sense the presence of all essential nutrients such that it initiates exit from quiescence only in complete medium. Alternatively, and more economically, a quiescent cell may be poised to detect only one or a few key nutrients that would indicate, with sufficiently low risk, that the environment has become permissive again for active proliferation.

Quiescent yeast cells appear to be “risk takers” and primarily use the presence of an external carbon source as the key indicator of a favorable change in nutritional fortune. Cells allowed to enter quiescence in glucose-based rich medium are able to maintain viability for long periods even when subsequently transferred to distilled water (51, 52). Nevertheless, the simple addition of a carbon source such as glucose to quiescent cells in water causes the loss of many characteristics of quiescence (52).

This striking finding indicates that the presence of a carbon

source alone is sufficient to initiate, at least in part, the process of exiting from quiescence. In contrast, quiescent cells in water are refractile to the addition of a nitrogen source alone, supporting the high predictive value placed by these cells on carbon availability. This predictive gamble can be risky: the addition of glucose to quiescent cells in water ultimately results in cell death, presumably due to the lack of other essential nutrients. The presence of a carbon source alone appears, therefore, to irreversibly commit quiescent cells to attempting to exit from quiescence.

Transcriptional Changes

An analysis of genome-wide gene expression changes that occur during exit from quiescence has been performed using slide-based microarrays and at 5-min intervals after stimulation (Martinez et al., submitted). A correlation map of this time course revealed that the greatest changes in gene expression occur within the first 10 to 15 min of stimulation by addition of nutrients. For example, at least 127 genes, including *SNZ1*, are rapidly and coordinately repressed. In addition, the “ribosomal” gene set encoding ribosomal proteins and related translation factors (and a potential target of the PKA and TOR pathways [see “Entry into quiescence” above]) is coordinately induced within the first 10 min. Over the extended time course, expression of approximately one-third of all genes is altered by a factor of 2 or greater. Distinct temporal patterns of expression are observed, indicating that exiting from quiescence is an ordered set of sequential events.

It has long been thought that stimulated cells exit from quiescence into the G_1 phase of the cell cycle. Ultimately this may be the case, and some genes characteristically expressed in G_1 , such as *SWI4*, are rapidly induced upon refeeding. However, exiting cells appear to transit through a unique set of states that do not appear to be similar to any other known state of yeast. The expression profiles of exiting cells appear to be distinct from that of cells in the G_1 phase of the cell cycle or cells at any intermediate stage during entry into quiescence (M. J. Martinez and M. Werner-Washburne, unpublished data). This result requires further study to distinguish the physiological from the cell cycle responses during the exit process and to ensure that the cells being studied are synchronous. Nevertheless, this surprising finding suggests that cells exiting from quiescence take a unique path back to the proliferating state, a path that is not simply the reverse of that taken during entry into quiescence.

The rapid transcriptional response of a quiescent cell to the addition of nutrients leads to additional questions. What is the source of nucleoside triphosphates for this synthesis, and where are they stored? It is possible that early transcription utilizes internal stockpiles retained during quiescence. How is a quiescent cell poised to make such an enormous and coordinated response? Such a rapid response doubtless requires activation or remodeling of signal transduction pathways, chromatin structure, transcription factors, and RNA polymerases. We do not know the answers to these questions.

In another study, Brejning et al. (13, 14) examined global changes in gene expression after resuspending cells that were post-diauxic (but not yet quiescent) in synthetic defined medium containing all essential nutrients. They focused on the lag

phase, i.e., the time between stimulation by addition of nutrient and the actual resumption of rapid cell proliferation. Approximately 240 genes were induced, and 122 genes were repressed at least fivefold during the lag phase. Again, the expression profiles indicate that lag-phase cells display expression patterns that are distinct from those of actively proliferating or post-diauxic cells.

Proteome Changes

No systematic analysis of changes in the proteome during exit from quiescence has been reported to date. However, the proteome has been monitored by two-dimensional gel electrophoresis during the lag phase on resuspension of post-diauxic shift cells in fresh synthetic complete medium (13). The overall rate of protein synthesis increased dramatically during the lag phase, with a concomitant increase in the number of proteins detectable on a single two-dimensional gel, from approximately 500 in early lag phase to 1,500 in late lag phase. The increased abundance of a protein correlated well with an increased amount of the corresponding transcript. Thus, there is little evidence to date for wholesale posttranslational control of protein abundance during the lag phase. These results may be relevant to the process of exiting from quiescence.

Exit Mutants

The study of mutants defective in exiting from quiescence should complement studies of mutants defective in entry. An analogy can be drawn to the response of haploid cells to the presence of mating pheromone. The addition of mating pheromone to haploid yeast cells causes a change in cell state from the proliferative state to the nonproliferative shmoo. Subsequent removal of pheromone reverses this change in state. Mutants lacking activators of the mating-signal transduction pathway, such as components of a MAP kinase cascade, fail to respond to pheromone treatment, i.e., cannot enter the shmoo state (144). In contrast, mutants lacking inhibitors of the mating pathway, e.g., protein phosphatases that inactivate the MAP kinase cascade, are defective in resuming proliferation after pheromone removal, i.e., cannot exit from the shmoo state (18, 19). Similarly, mutants unable to exit from quiescence should be defective in genes encoding distinct and opposing regulators and mediators to those identified from genetic analysis of entry.

The process of exit from quiescence has received little attention from geneticists to date, although, as outlined above, many such mutants may have been inappropriately classified. Only one mutant, *gcs1*, has been confirmed to be specifically defective in exiting from the quiescent state (34, 35, 59, 167).

Gcs1 and Vesicular Traffic

Mutants lacking the *GCSI* gene are conditionally (at low temperature, e.g., 15°C) unable to successfully exit from quiescence on stimulation by fresh nutrients (34, 35, 59, 64, 167). Because null alleles of *GCSI* display this phenotype, the conditionality is due to a conditional requirement for Gcs1p activity. The defect is specific for exit from quiescence, since *gcs1* mutants do not display any problems in other phases of the life

cycle, although diploid cells lacking Gcs1p function are impaired for sporulation (34, 35, 71, 72) (G. C. Johnston and R. A. Singer, unpublished data). Quiescent *gcs1* mutants are viable (see below) and can successfully return to active cell proliferation at temperatures higher than 15°C. The special requirement for Gcs1p during exit from quiescence at low temperatures is imposed relatively early in the process of entering into quiescence, since *gcs1* cells become conditionally unable to resume proliferation soon after the diauxic shift (35, 64, 71, 72). As indicated above, this degree of starvation is also sufficient to cause cells to acquire many of the hallmarks of quiescence (for reviews, see references 72 and 167).

At 15°C, the resupply of nutrients to starved *gcs1* cells stimulates cell growth (i.e., mass and volume increase), RNA and protein synthesis, degradation of storage carbohydrates, and gene expression changes characteristic of exiting cells (35). Indeed, resupply of nutrients causes the appearance of mRNA transcripts from the *GCSI* gene itself at a time when other transcripts also become detectable (64). The kinetics of these responses is similar to that seen for wild-type cells exiting from quiescence under the same conditions. However, *gcs1* mutant cells subsequently fail to reenter the mitotic cell cycle and pass the START checkpoint (34, 35, 64). This behavior suggests that the *gcs1* mutation does not affect the ability of a quiescent cell to sense and initially respond to the presence of nutrients but, rather, impairs some later process required to fully achieve the proliferative state.

The role of Gcs1p in the late quiescence cycle is suggested by the nature of the Gcs1p protein itself (64). Gcs1p is a GTPase-activating protein (GAP) that stimulates GTP hydrolysis by the Arf small GTP-binding proteins (118). Arf proteins are known to regulate various stages of vesicular transport in proliferating cells (reviewed in references 9 and 33), suggesting that the remodeling of intracellular vesicular transport may be critical for the transition from the quiescent to the proliferating state.

GCSI expression is not restricted to, and is not uniquely affected by, exit from quiescence. Thus, Gcs1p probably plays a role both in the vegetative state and in exiting from the quiescent state. These roles may be the same or distinct.

What is the role of Gcs1p in proliferating cells? The yeast genome encodes several proteins that are related in structure and function to Gcs1 (115, 116). One of these, Age2p, is another ArfGAP whose *in vivo* function in proliferating cells overlaps that of Gcs1p: although *gcs1* or *age1* single mutants cells proliferate normally, the *gcs1 age1* double mutant is inviable (117, 182). This overlapping and essential function of the two proteins in proliferating cells appears to be to enable transport of vesicles from the *trans*-Golgi network (117). The double mutant lacking both proteins displays a severe impairment in endosomal vesicle traffic.

What is the role of Gcs1p during exit from quiescence? *gcs1* mutants undergoing the transition from quiescence back to cell proliferation at a low (restrictive) temperature also display a severe endosomal impairment (163), similar to that shown by vegetative cells lacking both Gcs1p and Age1p (117). Although it has not yet been demonstrated conclusively, this vesicle trafficking defect may account for the failure of *gcs1* mutants to successfully exit from quiescence. In support of this possibility, replacing the *in vivo* wild-type allele of *GCSI* with a mutated version encoding a GAP-dead form of the protein (178) mim-

ics the exit defect of *gcs1* null mutants (C. L. Adams, S. Lewis, R. A. Singer, and G. C. Johnston, unpublished data).

If Gcs1p performs the same function in cells exiting from quiescence as it does in proliferating cells, why do *gcs1* single mutants display a specific defect in transiting from the quiescent to the proliferating state at low temperature? There are three likely possibilities. First, a cell exiting from quiescence may require an unusual amount of endosomal trafficking to successfully and physically transform itself into a proliferation-competent state, especially at low temperature. However, reduction of the overall ArfGAP activity of a cell by deletion of the *AGE2* gene alone does not affect its ability to exit from quiescence at any temperature, making this possibility unlikely. Second, it may be that Age2p function (or some component of an Age2p-mediated pathway) is impaired during exit from quiescence, particularly at low temperatures. In this scenario, a stimulated *gcs1* mutant cell would effectively lack both Age2p (or Age2p-related) and Gcs1p functions at low temperature, only the latter because of mutation. Finally, Gcs1 may perform another function that may directly or indirectly affect endosomal traffic and that is not shared with Age2p. Such a function could be unique to, or uniquely required for, exit from quiescence at low temperatures. A possible function has recently come to light. A subset of mammalian ArfGAP proteins, the centaurins (125), regulate both vesicle transport and rearrangements of the actin cytoskeleton at the plasma membrane and endosomal compartments. Gcs1p appears to be the yeast homologue of centaurin- α (8). Interestingly, *gcs1* mutants display abnormal actin cytoskeletal organization specifically when exiting from quiescence at 15°C (Johnston and Singer, unpublished). Thus, a defect in actin organization may underlie, at least in part, the defect in resumption of cell proliferation from quiescence (28) exhibited by *gcs1* mutants.

CONCLUSIONS AND PERSPECTIVES

Quiescence is poorly understood for any organism. Even for yeast, progress has been limited. There are many possible reasons for this, as discussed above, including the apparent modest life-style of quiescent cells and misconceptions about their viability. However, additional factors appear to have clouded the field.

Herein, we focus attention on only one quiescent state of the cell, i.e., that achieved by growth of liquid cultures to saturation in rich medium. We currently call this state "quiescence" and limit the use of the term to this case alone. This is an operational definition only. It allows us relate the findings of different researchers who have worked on cells derived by the same operation. In reality, it is likely that yeast cells can enter any one of a series of related and stable quiescence-like states depending on the environmental trigger. For example, nonproliferating states can be triggered by starvation for carbon, nitrogen, or phosphate in rich or synthetic defined media, by sporulation of diploids, and even by transfer of proliferating cells to distilled water. We do not know if the states entered as a result of all these treatments are largely similar (i.e., if there is a core quiescence program) or not. Because of this uncertainty, data acquired for any one state may or may not be relevant to any of the others.

For rapid progress to be possible in the study of quiescent

states, it is critical that each state be studied separately. This has not been the case to date. For example, as argued above, cells in cultures that have just arrested at or passed the diauxic shift are not in the same state as cells in saturated cultures, yet the two cell states have often been confused. Cells from stationary-phase cultures (i.e., cells starved for carbon) have been studied more intensively than have other nonproliferating cells and as such are the most understandable.

However, focusing attention on one state for study does not necessarily help with gleaning information and understanding from cells that do not appear to do much. Thankfully, recent advances in experimental methodologies and increasing availability of reagents derived from the exploitation of the genome sequence are coming to the rescue.

A first step toward fully understanding the biology of quiescent cells is to characterize the quiescent state itself and the way in which it differs from the proliferating state. For example, genome-wide gene expression profiling (see, e.g., reference 177) can be and is being done to uncover the differences between the gene expression patterns of quiescent and proliferating cells (49, 171). Furthermore, large-scale analysis of the proteome is increasingly viable, including assays of the protein concentration, localization, covalent modification, and complex formation (see, e.g., references 165 and 183).

Thus, in addition to the known measurable phenotypes of quiescent cells, such new technologies will add countless others. The definition of the quiescent state would allow a comparison with other nonproliferating states, permitting the relationships between all of them to be finally estimated with good confidence. Furthermore, knowledge of the unique properties of quiescent cells will contribute to our understanding of the processes by which these cells remain so stubbornly viable.

Studying the dynamic processes of entry into and exit from quiescence in similar detail should be particularly informative. Which signaling pathways regulate each stage during entry and exit? This question is potentially answerable. Furthermore, it should not be assumed that maintaining viability in quiescence is not itself dynamic. It is likely that the state of the cell evolves with time in quiescence, as internal energy reserves become depleted and as the cell copes with ongoing environmental assault, e.g., oxidative stress. We know that this dynamic evolution of the quiescent state is likely to occur, because a related phenomenon in yeast is well known, i.e., chronological aging (see above).

Understanding how a cell physically transits back and forth between the proliferating and quiescent states is a more formidable challenge, since such transitions appear to involve the wholesale remodeling of many (if not most) cell processes and structures. Even a partial list of the known and coordinated changes associated with these transitions includes changes in many signal transduction pathways; chromatin structure; transcription rate and pattern; mRNA stability; translation rate, pattern, and mechanism; protein stability; covalent modification of protein; vesicular traffic; mitochondrial structure; and cell wall structure. Beneath this complexity lies a big reward: understanding the mechanisms by which the many cellular processes that underlie active growth, and that are mostly studied in isolation in proliferating cells, are coordinately regulated.

Analytical methods alone will be very useful, but the com-

bination of genetics and these technologies promises to generate the main revolution in our understanding of quiescence. As outlined above, the subclassification of mutants that lose colony-forming potential after growth to stationary phase means that mutants defective in individual aspects of entry into, maintenance in, and exit from quiescence can be identified. The availability of genome-wide deletion collections and the ability to screen such collections robotically means that large numbers of cell quiescence cycle (*cqc*) mutants will soon be identified.

One of the motivations for studying quiescence in yeast is the hope that it might prove to be a model for the behavior of quiescent mammalian cells (93). A number of cells in more complex eukaryotes exit the mitotic cell cycle permanently on terminal differentiation. The most important in terms of human health and disease are the neurons (181). Humans are prey to a host of neurodegenerative diseases, both sporadic and inherited. Most of these diseases seem to result from the accumulation in neurons of aggregates of misfolded proteins (139), although the exact mechanism of neurotoxicity is not established. Increased oxidative stress has been implicated in the etiology of many of these diseases: for example, oxidatively damaged proteins are present in the aggregates found in neurons of patients with Alzheimer's disease and Parkinson's disease (133), and loss-of-function mutations in DJ-1, a protein implicated in the response of the cell to oxidative stress, cause early-onset Parkinson's disease (10, 173, 180). In the familial forms of many neurodegenerative diseases, the mutated proteins are expressed in many different cell types (DJ-1, for example, is ubiquitous), yet the predominant phenotype is the death of specific classes of neurons.

What makes neurons particularly vulnerable to protein damage and/or the loss of proteins that protect against such damage? It is tempting to conclude that at least part of the explanation is that these are quiescent cells (albeit ones that are highly active in certain specific metabolic processes) and therefore cannot dilute out or repair the damage as efficiently as proliferating cells can (139). Understanding the factors responsible for the survival—and death—of quiescent yeast cells, which have homologues for many of the genes encoding proteins such as DJ-1 and SOD, associated with human disease, may lead to a better understanding of the vulnerability of neurons to degeneration (122) and, it is hoped, also give clues to how such degeneration may be prevented.

Recently, evidence has also emerged that degenerating neurons in several different human diseases display markers indicating that they have attempted to exit from $G_{(0)}$ /quiescence and reenter the cell cycle, including expression of cyclin-dependent protein kinases and their regulators (55). Reexpression or deregulation of the genes involved in exit from quiescence may thus be an important step in neurodegeneration. A better understanding of this stage of the cell quiescence cycle may present new opportunities for therapy (101, 123, 179).

In conclusion, we hope that it has become clear that the cell quiescence cycle is as important a process to life on this planet as is the mitotic cell division cycle. By the time of our next review, we hope that the cell quiescence cycle is as active and productive a topic of study as is the cell division cycle today.

ACKNOWLEDGMENTS

We thank the members of the Gray, Werner-Washburne, Petsko/Ringe, and Johnston/Singer laboratories for helpful discussions. One of us (G.A.P.) is especially grateful to Jeremy Thorner, who first brought the phenomenon of stationary-phase survival to his attention and who encouraged his budding interest (pun intended).

Work in the Gray laboratory has been supported by funds from The Royal Society, the Wellcome Trust, the IBLS Research Committee, and the Biotechnology and Biological Sciences Research Council. The Petsko and Ringe laboratories thank the Ellison Medical Foundation for support. Research in the Johnston/Singer laboratory has been supported by funds from the Canadian Cancer Society through grants from the National Cancer Institute of Canada and grants from the Canadian Institutes for Health Research. Research in the Werner-Washburne laboratory has been supported by funds from the National Institutes of Health.

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