Staying alive
Metabolic adaptations to quiescence

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Quiescence is a state of reversible cell cycle arrest that can grant protection against many environmental insults. In some systems, cellular quiescence is associated with a low metabolic state characterized by a decrease in glucose uptake and glycolysis, reduced translation rates and activation of autophagy as a means to provide nutrients for survival. For cells in multiple different quiescence model systems, including *Saccharomyces cerevisiae*, mammalian lymphocytes and hematopoietic stem cells, the PI3Kinase/TOR signaling pathway helps to integrate information about nutrient availability with cell growth rates. Quiescence signals often inactivate the TOR kinase, resulting in reduced cell growth and biosynthesis. However, quiescence is not always associated with reduced metabolism; it is also possible to achieve a state of cellular quiescence in which glucose uptake, glycolysis and flux through central carbon metabolism are not reduced. In this review, we compare and contrast the metabolic changes that occur with quiescence in different model systems.

Introduction to Quiescence

Ensuring the continuity of life requires that individual cells and entire organisms can survive not only in ideal environments, but also in conditions of scarcity. When conditions are unfavorable for proliferation, many cells have the capacity to enter a non-dividing state, sometimes for years, while retaining their ability to reenter the proliferative cell cycle.[1,2] This state of reversible cell cycle arrest, known as quiescence, is common and can be seen in stem cells, eggs and spores. Some quiescent cells are surprisingly hardy: they can survive long periods of nutrient starvation, cold temperatures and even desiccation. Entry into quiescence is often associated with dramatic changes in metabolism, defined as the uptake of nutrients and the use of these nutrients for the synthesis of macromolecules and energy. Indeed, proliferating and quiescent fibroblasts are expected to have very different metabolic requirements. While proliferating cells must devote much of their metabolic capacity to biosynthesis in order to create the material necessary to form a new cell, quiescent cells are relieved of this steep metabolic requirement. Many but not all quiescent cells respond by downregulating the synthesis of proteins

and activating pathways that sustain them during periods of non-division.

Here we review the metabolic changes during quiescence in multiple model systems: bacteria, yeast, lymphocytes, hematopoietic stem cells, fibroblasts and cancer cells. Quiescent yeast and mammalian cells share a similar set of responses to conditions that are not suitable for proliferation: cell cycle arrest with 2N DNA content,[1,3,4] condensed chromosomes,[5] reduced rRNA synthesis,[6] decreased translation,[7] decreased cell size, activation of autophagy (described more fully below)[8] and increased resistance to a variety of stresses.[2] The signals for quiescence vary for different types of cells. Bacteria and yeast can enter stationary phase, a condition in which they cease cell growth and proliferation, in response to depletion of the glucose in their culture medium or in response to deprivation for a specific nutrient. In mammals, the proliferative state of individual cells is regulated by a host of factors that include not only the presence or absence of nutrients, but also cues from proliferative signaling molecules such as mitogens and situational cues. Quiescent T lymphocytes respond to stimulation of their T-cell receptor with the appropriate antigen by becoming activated, proliferating and secreting cytokines that participate in the immune response. Hematopoietic stem cells (HSCs) are also largely quiescent, but in response to cytokines, they will proliferate to form progeny that form the hematopoietic system. Dermal fibroblasts are typically quiescent, a state in which they secrete the extracellular matrix that forms the external environment. In response to a wound, the fibroblasts are activated, proliferate, migrate and coordinate a wound-healing response. Finally, there may be individual cells within a tumor mass that enter a quiescent or dormant state, possibly in response to a lack of nutrients, detachment from the substratum or hypoxia resulting from the lack of a blood supply. The reemergence of these cells from dormancy may contribute to tumor recurrence.

Cell cycle regulation in response to nutrient deprivation or quiescence cues is often mediated through cyclin-dependent kinase inhibitors. In yeast, the decision to commit to a cell cycle is made in START. Passing through START and entering S phase is regulated by the yeast cyclin-dependent kinase and its associated cyclins.[9,10] When nutrients are limiting, *Saccharomyces cerevisiae (S. cerevisiae)* do not pass through START, but instead exit the mitotic cell cycle in early G_1 and enter stationary phase. The cyclin-dependent kinase inhibitor SIC1 is an important regulator of S-phase entry.[11] In mammalian cells, a complex between cyclin

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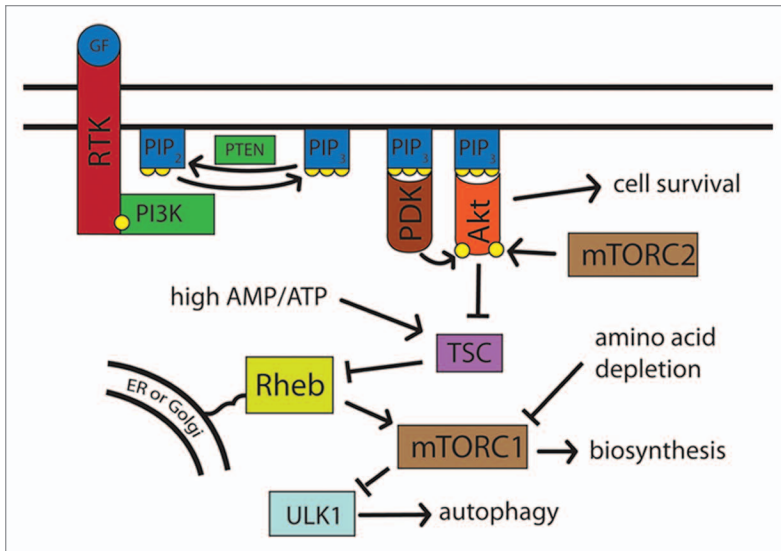
Figure 1. External factors induce cell growth and suppress autophagy via Akt and TOR. Upon stimulation by external factors, such as growth factors, the receptor tyrosine kinase is phosphorylated and recruits PI3K. PI3K phosphorylates PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3, which then recruits PDK and Akt to the cell membrane. Akt, when phosphorylated by mTORC2 and PDK, inhibits TSC, which in turn inhibits Rheb. When activated, Rheb promotes mTORC1 action, which leads to an increase in biosynthesis and suppression of autophagy via ULK1. Thus, nutrient-rich conditions lead to high activity of PI3K, Akt and mTORC1, while nutrient depletion causes reduced PI3K activity and a resulting decrease in mTORC1 activity.

E and cdk2 is central for progression through G_1. Quiescence signals such as the cytokine TGFβ, hormone withdrawal, serum starvation and contact inhibition result in the upregulation of cyclin-dependent kinase inhibitors, including p27[Kip1] (reviewed in refs. 12–16). Cyclin-dependent kinase inhibitors also maintain cells in a quiescent state, as their elimination can result in inappropriate cell cycle entry.[17,18]

In this review, we will first describe the pathways that are important for the regulation of metabolism with quiescence. Then we will describe the metabolic changes associated with quiescence in the model systems listed above. We conclude by comparing and contrasting the metabolic profiles of different quiescent states.

Introduction to the Pathways that Control Metabolic State: TOR

One pathway that is critical for determining the metabolic state of cells is the highly evolutionarily conserved TOR (target of rapamycin) pathway (reviewed in refs. 19 and 20). Extracellular and intracellular information about the availability of nutrients and the cell's energetic state are integrated into the TOR pathway, which then helps to define cell growth rates. When nutrients are plentiful, the TOR regulatory kinase phosphorylates and activates downstream targets that promote cell growth and increase cell size. When nutrients are scarce and in response to other triggers, the TOR pathway becomes inactive. Cells with inactive TOR reduce energy-intensive processes and induce catabolic programs that allow the cell to recover metabolites

and energy from existing macromolecules to ensure their survival.

In mammalian cells, mTOR (mammalian target of rapamycin), in association with its cooperating proteins, phosphorylates targets that promote protein synthesis and cell growth.[21] In mammals, there are two TOR proteins, mTORC1, which is sensitive to rapamycin, and mTORC2, which is not.[19,22] mTORC1 plays an important role in promoting translation and two important targets of its kinase activity are 4E-BP1 and S6 kinase.[23] Phosphorylation of 4E-BP1 releases its inhibition of eIF-4E,[24] which can, in turn, recruit the translation initiation complex to 5' caps and increase cap-dependent translation rates.[25,26] mTOR also phosphorylates and activates S6 kinase. S6K phosphorylates S6 protein of the 40S ribosomal subunit and other targets and activates translation.[27-29] mTORC2, in contrast, phosphorylates and activates Akt (also called protein kinase B),[19,30-32] which inhibits apoptosis and promotes survival. mTORC2 also targets proteins important for cytoskeletal organization.[19,22,33] Excessive TOR activity results in increased cell growth,[34,35] while TOR inhibition results in reduced cell growth and smaller cell size.[34,35] The TOR proteins are conserved in yeast, and they perform a similar functional role through distinct targets as described further below.[33,36]

Introduction to Pathways that Regulate Metabolism: PI3K

The phosphatidylinositol-3-kinase (PI3K) pathway is the pathway through which extracellular insulin and growth factors affect mTOR activity and thereby modulate the cell's metabolic state.[37,38] PI3K phosphorylates the lipid phosphatidylinositol-4,5-bisphosphate to generate phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3], which creates lipid docking sites on the cytoplasmic face of the plasma membrane (**Fig. 1**). This activity is opposed by the dephosphorylating action of tumor suppressor PTEN phosphatase.[39] The pro-survival kinase Akt can bind to PtdIns(3,4,5)P_3 on the cytosolic face of the lipid membrane, become activated and phosphorylate downstream targets. Among the targets of Akt is the tuberous sclerosis complex (TSC), which it inhibits.[40-42] TSC acts as a GTPase-activating protein and thereby inhibits the activity of the Rheb GTPase.[43-45] Rheb, in turn, activates TOR.[43,45-49] When nutrients are plentiful, Akt is active; TSC is inactive, and Rheb-GTPase activates mTOR. When nutrients are scarce, Akt is inactive; the TSC complex is active; Rheb is inhibited, and mTORC1 is inactivated.[50,51] Mutations in the TSC complex result in constitutive activation of mTOR and tuberous sclerosis disease,[40,41,52-55] an autosomal dominant disorder characterized by benign tumors affecting virtually all organ systems of the body.[56]

mTOR activity is also responsive to intracellular energy levels, which are sensed by the Lkb1 kinase. A high ratio of AMP to ATP, indicative of an energy-depleted state, leads to activation

of the tumor suppressor Lkb1. Lkb1 then activates AMPK and 12 other related kinases.[57-60] AMPK restores cellular ATP levels through phosphorylation of key regulatory proteins involved in protein synthesis, fatty acid and glucose metabolism and glucose transport,[61] resulting in a reduction in energy-intensive cellular activity. AMPK also phosphorylates TSC, which results in inactivation of mTOR and a decrease in biosynthesis when the cell is depleted of energy equivalents.[44,62,63] Thus, when extracellular conditions indicate that nutrients are rich and ATP is plentiful, mTOR transitions the cell into a growth mode. If the cell is in an energy-poor state or nutrients are unavailable, mTOR inactivation results in decreased translational activity, and, instead, there is a shift to an alternative metabolic program in which the cell reclaims energy and metabolites.

There is also a close connection between TOR activation and the cell cycle. Treatment with the TOR inhibitor rapamycin causes yeast to arrest in G_0/G_1 in a quiescence-like state.[64] The PI3K-TOR pathway is also implicated in control of quiescence exit and entry in other model systems, for instance, *Drosophila melanogaster* neuroblasts, progenitor cells that give rise to the central nervous system. Drosophila neuroblasts enter a quiescent state from which they exit in response to a nutritional checkpoint that requires amino acids. Cell cycle reentry of neuroblasts from quiescence was found to be dependent on PI3K and TOR signaling.[65]

Introduction to Pathways that Regulate Metabolism: Autophagy

Cells that are in nutrient-poor environments or are not actively growing tend to coordinately regulate two distinct processes: on the one hand, they reduce the rate at which macromolecules including proteins are synthesized, and on the other hand, they activate catabolic processes that allow them to recapture energy by breaking down macromolecules. One approach to achieving an increase in catabolism is the activation of autophagy (**Fig. 2**). Autophagy, or macroautophagy more specifically, is an evolutionarily conserved mechanism through which cytoplasmic proteins and organelles are engulfed into autophagosomes and degraded in lysosomes. The biological functions of autophagy include the reclamation of metabolites and ATP, the elimination of damaged proteins and organelles, the elimination of pathogens, tumor suppression and antigen presentation.[66] Defects in autophagy have been implicated in liver disease, neurodegeneration, Crohn's disease and metabolic syndrome.[67]

The TOR complex can act as an intermediary between the presence of glucose, insulin and amino acids and the catabolic process of autophagy. Amino acid depletion, for instance, results in reduced TOR activity[68-70] and an induction of autophagy, which releases free amino acids from lysosomes for the rebuilding of proteins. mTOR actively represses autophagy, and thus, when cells are in a nutrient-rich, energy-replete state, only basal autophagy is activated. In mammals, TOR controls autophagy levels in part by phosphorylating and inhibiting the autophagy-specific kinase ULK1.[71-73] When TOR is inactivated, ULK1 is released from inhibitory phosphorylation events. ULK1 in
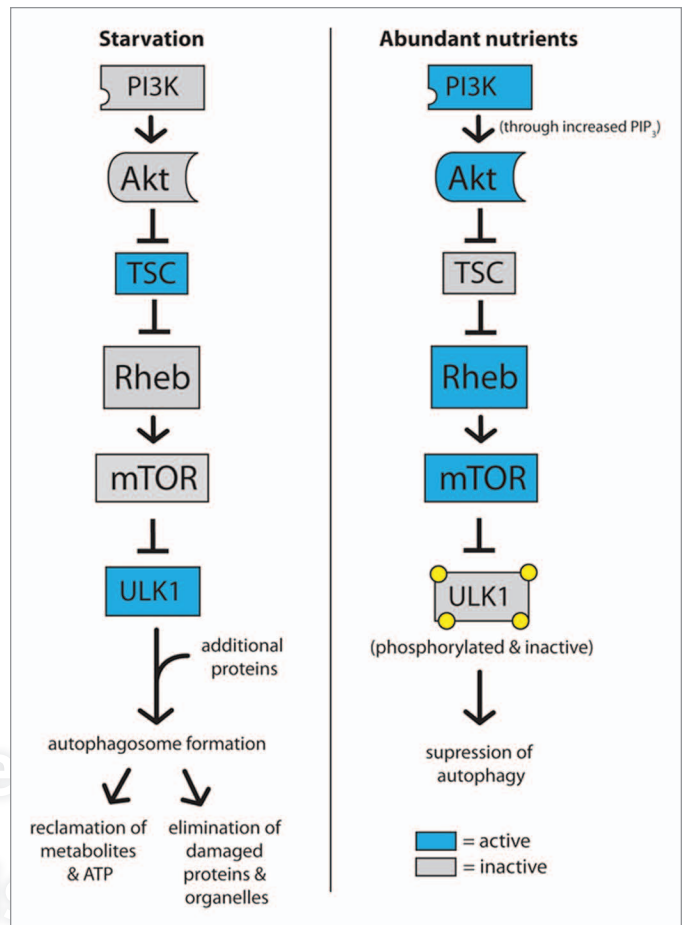
Figure 2. Autophagy is activated by low mTOR signaling and activation of ULK1. In starvation conditions, high TSC activity represses mTOR, which allows ULK1 to become active. After recruiting additional proteins to form a complex, ULK1 promotes autophagosome formation and autophagy. In contrast, high mTOR activity in high-nutrient conditions phorphorylates ULK1 and suppresses the induction of autophagy.

conjunction with other coordinating proteins can then form complexes that initiate the formation of autophagosomes.[74] Thus, under conditions of starvation signaled by low insulin, glucose or amino acids, autophagy can provide nutrients to an energetically depleted or starved cell.

During starvation, autophagy can provide a nutrient source and thereby promote survival.[67,75,76] Autophagy is required immediately after oocyte fertilization to feed the developing embryo before it can access maternal nutrients.[77] Autophagy is required again during the period of neonatal starvation after separation from their mother.[78-80] Autophagy-deficient mice contain lower amino acid levels in their tissues and plasma during this period. Autophagy invoked during the extensive remodeling that occurs during insect metamorphosis may provide amino acids to be used for the synthesis of proteins needed for the insect's new form.[81] From this perspective, autophagy is not just a mechanism for degradation of proteins and other macromolecules, but also an opportunity for renewal: the cell can erase its memory of its previous path and commit to a new cellular or even organismic lifestyle.[82]

Quiescence in *E. coli*

Starved bacteria can enter "stationary phase," a state in which there is no increase in cell number.[83] Starvation triggers higher levels of the alternative sigma factor RpoS, which controls expression of up to 10% of *Escherichia coli (E. coli)* genes.[84,85] RpoS is responsive to low levels of carbon, nitrogen, phosphorus or amino acids.[86] RpoS prepares cells for survival by activating genes that protect against starvation, osmotic stress[87] and temperature stress.[88] During stationary phase, successive rounds of mutants with improved fitness appear within the population and come to dominate the culture.[83,89] At the same time, less fit cells die and provide nutrients to the survivors. The result is an equilibrium that leads to a stable, viable population.

Starved bacteria can be considered to enter a hypometabolism state, in that they slow their growth rate dramatically.[90] Stationary phase *E. coli* reduce protein synthesis by about 20%. Levels of rRNA and tRNA decline compared with cells in exponential growth. In stationary-phase bacteria, ribosomes become dimerized, which could represent a way to store them.[83,91] Upon readdition of nutrients, the ribosomes are released from their dimerized state within two minutes.[91] Thus, decreased translation is associated with stationary phase in bacteria.

In stationary-phase bacteria, the activity of transport systems and the metabolism of carbohydrates, amino acids and phospholipids also decreases.[92] In bacteria, metabolism shifts during stationary phase, and the nondividing state is characterized by an increase in glycolysis enzymes and lower production of TCA enzymes.[93] Bacteria with mutations in ArcA, which is an important regulator of the shift to glycolytic enzymes, do not survive during starvation,[94] indicating that the shift toward glycolysis away from the TCA cycle is important for the survival of stationary-phase bacteria. The shift away from TCA cycle use may be necessary to protect the bacteria from reactive oxygen species produced as a byproduct of oxidative phosphorylation. Overproduction of superoxide can rescue the survival of starved ArcA-deficient *E. coli*.[94] *E. coli* strains lacking TCA cycle-related genes, specifically a subunit of succinate dehydrogenase, exhibited longer stationary-phase survival characterized by a reduced production of superoxide.

Protein catabolism is also critical for stationary phase, nutrient-deprived bacteria. Stationary-phase bacteria activate dwarfing, a form of self-digestion that results from degradation of endogenous cellular material, especially the cytoplasmic and outer mebranes.[93] In bacteria, protein turnover increases 5-fold in starved *E. coli,* as many of the proteins synthesized in the early stages of starvation are proteases and peptidases.[95] Mutations that reduce peptidase activity drastically reduce survival in stationary phase.[96] Cells that lack specific proteases exhibit an accelerated rate of death during extended stationary phase, which further supports the importance of protein degradation and amino acid reclamation, for survival in reduced nutrient conditions.[97]

Quiescence in *S. cerevisiae*

S. cerevisiae can also achieve a quiescent state when they are grown to stationary phase, which is typically accomplished by allowing the cultures to grow in rich medium until they have exhausted the glucose present, usually for 5 to 7 d.[1] During this period, the yeast undergo a change in metabolism termed diauxic shift: they transition from fermenting plentiful glucose via glycolysis into ethanol to metabolizing the accumulated ethanol through the citric acid cycle and respiration. As the glucose is depleted, the cells undergo one or two slow doublings and then cease proliferation and enter stationary phase.[1] *S. cerevisiae* can also enter stationary phase in response to limitation for a specific nutrient (e.g., nitrogen, sulfur or phosphate).[98] Yeast that enter stationary phase through diauxic shift form two distinct populations, the heavier of which exhibits more of the characteristics of quiescent cells.[1] The heavier, quiescent population is unbudded, which indicates cell cycle arrest, and these heavier cells synchronously reenter the cell cycle when provided with nutrients.[99] The heavier cells also exhibit higher long-term viability and reproductive capacity, have greater thermotolerance, and exhibit decreased aging and oxidative stress.[99]

Stationary-phase cultures of yeast enter a state of decreased metabolism and biosynthesis, in which quiescent cells do not increase in mass or volume.[1] The overall transcription rate in stationary phase cultures is three to five times lower than in logarithmic-phase cultures.[100] Genes encoding ribosomal proteins are expressed at lower levels,[6] and the translation rate is reduced to approximately 0.3% of the rate in logarithmically growing cultures.[7] A hallmark of stationary phase in yeast, as in bacteria, is the ability to survive for prolonged periods without added nutrients.[1] This may reflect not only the slower metabolic rate of the quiescent yeast, but also the fact that the non-quiescent cells provide nutrients for the quiescent population as they die.[99]

Another possible mechanism for ensuring the long-term survival of stationary phase yeast without added nutrients is the accumulation and use of storage carbohydrates.[101-103] During diauxic growth and upon starvation for nitrogen, sulfur or phosphorus,[101] yeast accumulate glycogen, a glucose polymer, and trehalose, a disaccharide of two glucose molecules. The fraction of dry weight represented by glycogen can be as high as 8%, while the fraction represented by trehalose can be as high as 20%.[101,104] Glycogen is visible by electron microscopy in the denser, quiescent cells within a stationary phase population, and the accumulation of storage carbohydrates is likely responsible for the characteristic increased density of the quiescent cells in stationary phase batch cultures.[99,105] During long-term stationary phase, glycogen and then trehalose stores are depleted from cultures.[101,105] However, the importance of these two storage carbohydrates to survival during stationary phase is not resolved, as the absolute amounts of the storage carbohydrates do not directly correlate with viability.[106] On the other hand, cells grown in a medium that does not allow them to accumulate trehalose or glycogen exhibit reduced survivability and lifespan.[105] When stationary phase yeast are provided with fresh, rich medium, trehalose stores are depleted rapidly, and activity of the trehalase enzyme increases.[105] Cells lacking trehalose exit quiescence more slowly, suggesting that trehalose may be the carbohydrate of choice upon cell cycle reentry from quiescence in *S. cerevisiae*. Since trehalose is a disaccharide of two glucose molecules, cleavage of one glycosidic trehalose

bond rapidly provides two glucose molecules, whereas cleavage of a single glycosidic bond in a glycogen molecule provides only one glucose molecule. Trehalose may be a carbohydrate of choice for cell cycle reentry, because it can supply glucose rapidly.

Trehalose levels accumulate not only upon entry into stationary phase, but also in response to stresses such as heat shock, cold shock,[107] and especially, desiccation.[108] Mutant yeast unable to synthesize trehalose die more rapidly than wild-type yeast upon freezing.[109] Trehalose may also facilitate desiccation and protect cells during freezing, because it is less likely to form crystals and can, instead, take-on a gel-like consistency.[108,110] Trehalose may also stabilize lipid membranes upon desiccation, possibly by replacing water and hydrogen bonding with polar groups on membrane lipids and proteins.[108,111] Finally, trehalose has been reported to function as a chemical chaperone and thereby facilitate protein folding.[112] Thus, the accumulation of storage carbohydrates may represent an important mechanism for *S. cerevisiae* to survive during quiescence and reentry by providing carbohydrates to be metabolized for energy and by protecting yeast from stress during quiescence.

The TOR pathway is also a key component of the low metabolic rate in stationary-phase yeast.[64] Treatment with rapamycin, a TOR inhibitor, induces yeast to enter a quiescence-like, G_1-arrested, unbudded state similar to that achieved after diauxic shift.[64,113,114] Quiescent yeast contain reduced levels of transcripts for G_1 cyclins, including the sensor cyclin CLN3.[64] Overexpression of CLN3 in rapamycin-treated cells results in an inability to properly enter quiescence and a loss of rapamycin-induced G_1 arrest.[64] Rapamycin treatment also results in reduced expression of ribosomal protein genes[115] and reduced rates of protein synthesis.[64] Rapamycin-treated yeast accumulate storage carbohydrates[1,64] and activate autophagy.[116] TOR also regulates multiple genes known to change upon entry into quiescence.[64] Thus, TOR inhibition results in a state with similarities to stationary phase: catabolic processes such as autophagy are induced, while anabolic processes such as transcription, translation and ribosome biogenesis are reduced.[117,118] For these reasons, TOR has been considered by some to be a master regulator of quiescence in yeast.[20,119] However, there may be other contributory pathways to stationary phase as well, such as protein kinase A and C.[1] TOR pathway inhibition by itself does not recapitulate the dramatic decrease in protein synthesis rates of stationary phase cells,[7,64,120] and *S. cerevisiae* treated with rapamycin continue to accumulate mass and volume.[1,64]

TOR has wide-ranging cellular effects in yeast (reviewed in ref. 117). As described above, in mammals, two important targets of TOR are 4E-BP1 and p70 S6K. In *S. cerevisiae,* like in mammalian cells,[121] TOR may prevent a protein, in this case, EAP1, from inhibiting eIF-4E, thus resulting in eIF-4E activation and increased cap-dependent translation.[122] TOR activation in yeast also stimulates protein synthesis by increasing the abundance of ribosomes.[123,124] TOR target Sch9 (reviewed in ref. 125) fulfills a role similar to S6 kinase in mammals.[117] Carbon starvation or rapamycin treatment results in dephosphorylation and inactivation of Sch9 (reviewed in ref. 126), whereas activation of Sch9 leads to increased transcription of ribosomal biogenesis and ribosomal protein genes through phosphorylation-sensitive transcriptional repressors.[125,127-129] Also, TOR itself can shuttle back and forth between the cytoplasm and the nucleus.[130] TOR enters the nucleus in a nutrient- and rapamycin-sensitive way, binds promoters of rRNA genes and promotes their expression. Decreased TOR activity upon entry into quiescence in yeast likely contributes to the reduced translation rate, although, as described above, it may not be the only contributing factor.[7]

In *S. cerevisiae*, TOR also regulates cell cycle activity, in part through its regulation of two phosphatases, protein phosphatase type 2A (PP2A) and Sit4, a type 2A-related protein phosphatase.[131,132] PP2A is involved in the activation of Cdc28, which promotes cell cycle entry.[133] Sit4 is important for the initiation of the cell cycle at START, bud formation, the initiation of DNA synthesis and spindle pole body duplication.[134,135] Strains with a temperature-sensitive allele of SIT4 arrest in the G_1 phase of the cell cycle and resemble the phenotype of rapamycin-treated cells, indicating a possible mechanism by which TOR inactivation may contribute to the cell cycle arrest in stationary phase.[133,135]

TOR activation also results in exclusion of specific transcription factors from the nucleus,[119] including the GLN3 transcription factor, which transcribes genes that allow the cell to use poor nitrogen sources.[131,132,136-139] When TOR is active, Gln3 is phosphorylated and localized to the cytoplasm. If TOR is inhibited by rapamycin, Gln3 becomes dephosphorylated, accumulates in the nucleus and activates the transcription of permeases that facilitate import of nitrogen metabolites and genes that allow yeast to metabolize poorer nitrogen sources. Decreased TOR activity results in the relocalization of high-affinity amino acid permeases to vacuoles.[136,137] TOR can also regulate the activity of the Gcn4 transcription factor, which activates genes that regulate the biosynthesis of amino acids.[140] Rapamycin treatment of *S. cerevisiae* also induces the expression of genes involved in autophagy.[115]

Stationary-phase yeast reduce their glycolytic rate, but they continue to rely on mitochondrial respiration. They are, therefore, expected to produce ROS, which can damage cellular macromolecules. Active TOR prevents nuclear localization of the stress response transcription factors MSN2/4 (reviewed in ref. 136). These transcriptional regulators accumulate in starved cells, and their targets, such as superoxide dismutase 1 and superoxide dismutase 2 (SOD1 and SOD2),[141,142] aid in managing the stress induced by nutrient deprivation. SOD1 or SOD2 deletion results in more rapid loss of viability compared with wild-type cells when stationary cells are aerated,[143] while SOD1/SOD2 double mutant cells die even more quickly. In the absence of respiration, the SOD mutant yeast retained viability. The results indicate that respiration contributes to the oxidative burden of yeast, and that SODs are important for protecting stationary phase yeast from the reactive species formed. Thus, in *S. cerevisiae*, TOR activity is a major regulator of gene expression; it regulates the expression of ribosomal biogenesis proteins and ribosomal proteins, genes that regulate the biosynthesis of amino acids, genes that facilitate amino acid utilization, genes that allow for use of poor nitrogen sources, genes that defend against oxidative damage and genes that regulate autophagy.

Activation of autophagy is important for the survival of yeast in nutrient-deficient environments, as the amino acids reclaimed by autophagy are recycled and utilized for the translation of proteins needed to adapt to starvation.[82] Autophagy-deficient yeast die rapidly upon starvation for glucose or nitrogen.[144,145] In these yeast, the total intracellular amino acid pool is diminished, and, as a consequence, bulk protein synthesis is substantially reduced.[145] Proteins that continue to be produced in wild-type starved yeast, such as vacuolar/lysosomal enzymes, respiratory chain proteins, antioxidant enzymes and proteins in pathways of amino acid biosynthesis, are not synthesized.[145] Lacking the proteins needed for the respiratory chain, autophagy-deficient yeast lose respiratory function and accumulate higher levels of ROS when starved.[146] Autophagy deficiency also affects carbohydrate metabolism, as autophagy-deficient yeast are able to synthesize glycogen when approaching stationary phase, but are unable to maintain their glycogen stores. Modulation of autophagy in stationary phase is achieved in part through TOR, which negatively regulates autophagy through the activity of yeast autophagy regulatory proteins.[147,148]

Quiescence in Lymphocytes

In contrast to bacteria and yeast, mammalian cells rarely encounter nutrient depletion in physiological conditions, because they are bathed in glucose, amino acids and other nutrients provided by nearby capillaries. In mammalian cells, the presence or absence of growth factors and situational cues are commonly used as triggers for quiescence. Lymphocytes, a central component of the adaptive immune response, coordinate the body's reaction to antigens. Activation of T lymphocytes requires co-stimulation through the T-cell receptor (TCR) and a co-stimulatory receptor CD28. Upon ligation of the T-cell receptor, the cytoplasmic tail of CD28 is phosphorylated, and it then serves as an adaptor for the recruitment and activation of PI3K.[149-151] Lymphocytes can also be activated by mitogenic lectins and some lymphocytes, including the hematopoietic cell line FL5.12, are dependent upon the cytokine interleukin-3. Upon activation, a quiescent lymphocyte grows to approximately double its resting size, rapidly proliferates and secretes large amounts of cytokines.[152] This transition is associated with a major shift in the cell's metabolic profile.[153-156]

T-cell activation triggers a dramatic increase in glucose transport and metabolism through glycolysis. Quiescent lymphocytes derive most of their ATP from oxidative phosphorylation.[152,157,158] In contrast, activated lymphocytes depend heavily on glucose to serve as a carbon source and to provide ATP needed for biosynthesis and secretion. For rat T lymphocytes stimulated with lectins and interleukins, activation results in a 53-fold increase in glucose metabolism.[159-163] The activity of individual glycolytic enzymes increased 12- to 30-fold upon stimulation. Increasing the dose of IL-3 administered to FL5.12 cells results in an 8-fold increase in glycolytic flux.[164]

Activated T cells are so committed to glycolysis, in fact, that they continue the process anaerobically even when oxygen is available; that is, they continue to convert glucose to pyruvate and secrete it as lactate rather than use oxidative phosphorylation

for energy despite the presence of oxygen that could be used as an electron acceptor.[159] In activated thymocytes, 90% of the glucose was converted into lactate, whereas resting cells metabolized only 56% to lactate.[159] Anaerobic glycolysis is also performed by cancer cells, a process called the Warburg effect.[165,166] In fact, the lymphocytes increase glycolysis over and above what they need to maintain their ATP/ADP levels, which has been interpreted as an accumulation of ATP in anticipation of future metabolic demands.[164] Glutamine utilization also increases in activated rat T lymphocytes and was 8-fold higher in proliferating than quiescent thymocytes.[159]

Increased glycolytic flux in activated T lymphocytes is achieved by increasing the amount of glucose transporters on the cell surface. In adipose tissue, and cells of the muscle and liver, the glucose transporter Glut4 is rapidly translocated from intracellular storage vesicles to the cell surface in response to insulin.[167-169] Lymphocytes do not express Glut4, but rather rely on the ubiquitously expressed Glut1 as their primary source of intracellular glucose.[154] In quiescent lymphocytes, Glut1 mRNA and protein levels decline, and the levels of Glut1 transporters on the cell surface decline as cells endocytose and degrade the transporters responsible for glucose uptake[153,154,170,171] as well as transporters for amino acids, low-density lipoprotein and other nutrients.[172] TCR-CD28 costimulation or IL3 addition promotes glucose uptake in part by promoting localization and retention of Glut1 on the cell surface.[171,173-175] IL3 receptor engagement attenuates Glut1 internalization to promote maximal surface levels.[172,173] PI3K and Akt affect endocytosis, recycling and trafficking of the Glut1 (and Glut4) transporters to maximize cell surface expression. Glut1 overexpression in T cells results in increased cell size, increased glucose uptake in peripheral T cells and enhanced expression of activation markers. Akt is important for the regulation of surface transporters not only for glucose, but also for amino acids, low-density lipoprotein and iron.[153] Constitutively active Akt results in the maintenance of these transporters on the cell surface in the absence of growth factors and increased cell size.[153] Rapamycin treatment of Akt-expressing cells diminishes their size, indicating that Akt signaling is mediated through mTOR. Thus, T-cell activation through TCR engagement or growth factors controls cellular growth and survival in part by regulating access to extracellular nutrients through the Akt and mTOR pathways.

When lymphocytes are deprived of growth factors, glucose uptake is limited, and the cells activate an alternative pathway in which glucose uptake and metabolism are reduced. This pathway leads to cell death, which makes it difficult to define the quiescent state of lymphocytes.[154,164] To address this problem, cells deficient for both of the key initiator apoptotic proteins Bax and Bak were created.[176,177] Cells from Bax^{-/-}Bak^{-/-} animals do not undergo apoptosis in response to serum deprivation or growth factor withdrawal, and thus, the metabolic changes that occur in quiescent cells can be investigated over a longer time frame.[177,178] Following growth factor withdrawal, Bax^{-/-}Bak^{-/-} cells undergo progressive atrophy, shrink in size approximately 50% over three months of self-cannibalization and ultimately die. In the absence of growth factors, these cells do not express glucose transporters

on their cell surface, and therefore, they fail to take up nutrients from their environment. To compensate for the lack of externally-provided nutrients, they activate autophagy to maintain their ATP levels. If autophagy is inhibited, there is rapid cell death. Thus, while the cells slowly die of nutrient deprivation in the absence of growth factors, they die much more rapidly when autophagy is inhibited, indicating that autophagy is important for protecting them from starvation. If the cells are provided with a cell-permeable form of pyruvate, thus providing a substrate for the TCA cycle, cell death is suppressed. When growth factors are added back to growth factor-deprived cells, their ability to take up and metabolize glucose is restored, and the cells recover their original size and proliferative potential. Therefore, growth factors promote cell survival by maintaining the ability of cells to take up sufficient nutrients to maintain ATP production and to support synthesis of needed macromolecules.

In accord with the prediction that autophagy plays a protective role for quiescent lymphocytes in vivo, primary, quiescent mouse and human T lymphocytes contain autophagosomes.[179-181] To investigate further, the immune system of adult mice was reconstituted with autophagy-deficient hematopoietic precursors from mice lacking Atg5.[179] Autophagy-deficient mice were unable to reconstitute the lymphoid compartment. Atg5^{-/-} CD8^{+} T lymphocytes exhibited increased apoptotic cell death compared with wild-type controls, thus the ability to induce autophagy is important for the survival of CD8^{+} T cells. Atg5^{-/-} CD4^{+} and CD8^{+} T cells also failed to undergo efficient proliferation after TCR stimulation, indicating that autophagy is important for the survival and proliferation of T lymphocytes.

Quiescence in Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are the source of all of the short-lived cells within the blood that are needed over the course of our lifetimes.[182] To repopulate all of the necessary lineages of cells within the blood, millions of mature cells must be generated daily from progenitors that proliferate extensively. The HSCs are operationally defined as the cells capable of recreating the entire blood system of a recipient. HSCs are found in a specific niche or microenvironment within the bone marrow[183-185] and are in a mostly quiescent state.[186,187] In response to intrinsic or extrinsic cues about the need for mature blood cells, HSCs can be stimulated to regenerate another HSC or, in an asymmetric cell division, to generate a progenitor cell that can then repopulate differentiated hematopoietic cell lineages.[182]

If HSCs inappropriately commit to proliferation and differentiation rather than maintain quiescence, there can be a depletion of the HSC pool, which will ultimately result in an organism that lacks the HSCs needed for blood cell replenishment.[186] In support of this model, the proliferative rate of HSCs among mouse strains is strongly anti-correlated with the strain's maximum lifespan, suggesting a link between HSC depletion and aging.[188,189] In animals with homozygous deletion of the cyclin-dependent kinase inhibitor p21[Cip1], there is increased proliferation of stem cells, followed by a loss of stem cells and increased susceptibility to stress-induced exhaustion of the stem cell pool.[190]

In addition to cyclin-dependent kinase inhibitors, the PI3K-mTOR pathway is also an important regulator of quiescence in HSCs as evidenced by the results when regulatory subunits of PI3K—PTEN, Akt and TSC—were modulated.[191-195] HSCs from PI3K-deficient embryos are less able to respond to growth factors and less able to repopulate hematopoietic lineages compared with wild-type controls,[191] demonstrating the importance of PI3K signaling for cell cycle entry. Conversely, conditional deletion of PTEN, a phosphatase that counters PI3K phosphorylation events, results in short-term HSC expansion and long-term decline. PTEN loss resulted in excessive HSC proliferation, leading to leukemias, followed by stem cell depletion. PTEN-deficient HSCs could not repopulate the blood system of irradiated mice. HSC function could be restored by treatment with rapamycin, indicating that TOR likely mediated the effects of PTEN deletion.[192,193] Similar to the phenotype of PTEN-deleted HSCs, constitutive activation of Akt using myristoylated Akt resulted in a hyperproliferative state and subsequent HSC depletion.[194] Finally, deletion of TSC also resulted in increased TOR activity, HSC cycling and depletion, defective repopulating potential and defects in hematopoietic lineage development.[195-197] The effects of TSC deletion on repopulating potential and hematopoietic lineage development were mediated through TOR signaling.[195] Thus, multiple different types of modulation of the PI3K signaling pathway from PI3K to TOR suggest that the PI3K-TOR axis is central for the control of the commitment of HSCs to proliferation from quiescence.

One possible explanation for the deleterious effects of increased TOR activity on stem cell survival is that the transition from quiescence to proliferation, and the associated increase in TOR activity, results in a higher metabolic state and an increase in ROS. HSCs are usually present in an anaerobic niche within the low-oxygen portion of the bone marrow, far from circulating blood and the oxygen it carries.[198,199] Low oxygen tension in the stem cell niche may be important for maintaining HSCs in an undifferentiated state.[200] Metabolic activation of HSCs may occur when they migrate to a more oxygen-rich portion of their microenvironment, as the increase in oxygen levels may promote their differentiation.[201] Indeed, HSCs maintain tight regulation of mitochondrial homeostasis, and this control is important for their integrity.[202-205] Normal HSCs have few mitochondria, and increased mitochondrial biogenesis results in defective HSC maintenance.[197,206] HSCs can be identified based on their low levels of staining with the vital dye Rhodamine-123, an indicator of their low mitochondrial activity.[206,207] HSCs with low Rhodamine-123 are enriched for cells in the G_0/G_1 phase of the cell cycle,[208] indicating that quiescence in HSCs is closely associated with decreased mitochondrial activity. In another study using a dye for intracellular ROS, HSCs were separated into ROS[low] and ROS[high] fractions.[201] The ROS^{low} cells were enriched for cells in G_0, expressed higher levels of cyclin-dependent kinase inhibitors and better retained their long-term self-renewal ability.

A role for TOR in maintaining ROS levels is supported by the observation that an antioxidant or rapamycin could restore the self-renewal ability of the ROS[high] HSCs. Mice with overactive TOR as a result of Tsc1 inactivation also contained higher

ROS, as well as increased mitochondrial mass and mitochondrial DNA copy number.[196,197,209] Antioxidant treatment also significantly rescued the ability of the Tsc1^{-/-} HSCs to reconstitute blood. Rapamycin treatment and siRNAs against mTOR binding partners causes decreased mitochondrial polarization, oxygen consumption and ATP production.[210] In aged HSCs, TOR inhibition increases HSC lifespan and restored the self-renewal capacity of the stem cells.[196] TOR may thus play a role in regulating the balance between mitochondrial and non-mitochondrial sources of ATP.

The importance of low ROS levels in HSCs is further supported by experiments with the FoxO family of transcription factors. In quiescent murine HSCs, Akt is not activated, and members of the FoxO family of transcription factors, which are important for suppressing ROS levels,[211] are localized to the nucleus. Upon cytokine stimulation of HSCs, Akt becomes activated, and FoxO proteins are exported from the nucleus to the cytoplasm.[212] HSCs with conditional deletion of FoxO proteins exhibit lineage expansion, lymphoid developmental abnormalities and a decrease in the HSC population.[213] Bone marrow from FoxO-deficient mice is deficient in repopulating the blood system of mice. In vivo antioxidant treatment reversed the FoxO-deficient phenotype.

Lkb1, a kinase that senses ATP depletion as described above, was recently shown to play an important role in addition to and distinct from the role played by TOR in the regulation of HSC quiescence.[202-204] Conditional deletion of Lkb1 resulted in an increase in the number of HSCs followed by depletion of these cells.[202-204] In mice in which Lkb1 was conditionally deleted, HSCs escaped from quiescence and proliferated.[202,204] Lkb1-deficient HSCs were unable to generate sufficient progeny to repopulate blood cells in irradiated mice and did not form colonies in culture.[202,203] While rapamycin can rescue the phenotypes of Tsc1-knockout mice, rapamycin did not rescue Lkb1 deletion defects[202-204] and, consequently, mTOR activation is not a major mediator of HSC depletion after Lkb1 deletion.[204] Treatment with metformin, an AMPK activator, also did not rescue the transient expansion and subsequent depletion of HSCs in Lkb1 mice,[204] indicating that Lkb1 alters metabolism through AMPK-independent mechanisms in HSCs. In addition, Lkb1-deficient bone marrow cells exhibited alterations in lipid and nucleotide metabolism and depletion of cellular ATP.[203] These findings indicate a broad role for Lkb1 in energy metabolism in HSCs and that Lkb1 may serve as another rheostat, in addition to TOR, that also sets the appropriate balance between anabolic and catabolic activities in HSCs.[202-204]

More recently, it has also become clear that autophagy is critical for HSC maintenance. HSCs in which there was conditional deletion of an essential autophagy protein exhibited a loss of normal function, and the mice died within weeks.[214] Autophagy-deficient HSCs could not reconstitute the hematopoietic system of irradiated mice. Autophagy-deficient HSCs also accumulated aberrant mitochondria and contained increased levels of mitochondrial superoxide and DNA damage, indicating a role for autophagy in regulating mitochondrial quantity and quality in HSCs. Mice with autophagy-deficient HSCs exhibited myeloid proliferation, indicating that autophagy may protect against leukemogenesis. Thus, quiescent HSCs rely on autophagy for maintenance of viability, quiescence and mitochondrial quality.

Quiescence in Fibroblasts

Fibroblasts are the cells that carry most of the responsibility for secreting extracellular matrix proteins, such as collagen fibers, that create the basement membrane and provide structural support for connective tissue. Dermal fibroblasts are usually quiescent and are stimulated to proliferate in response to wounds. When skin is wounded, the fibroblasts proliferate and coordinate the wound-healing response, including secreting collagen and other extracellular matrix proteins,[215] recruiting endothelial cells to promote angiogenesis in the area[216,217] and secreting other molecules that facilitate the wound-healing response.[217] If fibroblasts fail to quiesce at the end of a wound-healing event, the result can be excessive scarring or fibrotic disease.[218,219]

Cultured primary human fibroblasts can be induced into quiescence by multiple methods, including contact inhibition and serum starvation. Fibroblasts induced into quiescence by either of these methods accumulate in G_0/G_1 cell cycle phases with about 80% efficiency. Quiescent fibroblasts express higher levels of the cyclin-dependent kinase inhibitor p27[Kip1] (CDKN1B).[220-222] Microarray analysis of cultured human fibroblasts has shown that the quiescent state of the fibroblasts is associated with widespread changes in gene expression.[16,217,223]

An analysis of the metabolic profiles of primary human fibroblasts induced into quiescence by contact inhibition revealed that, surprisingly and in contrast to most other quiescence model systems, contact-inhibited fibroblasts maintain comparable metabolic rates to proliferating fibroblasts despite not actively proliferating.[220] Glucose uptake and lactate secretion rates were only 2-fold lower in contact-inhibited compared with proliferating fibroblasts. An analysis of flux through metabolic pathways revealed comparable flux through glycolysis in proliferating compared with contact-inhibited fibroblasts. Thus, cells can be quiescent, in that they are not actively dividing to make two cells, without entering a state of hypometabolism characterized by low glucose uptake.

Quiescent fibroblasts did differ from proliferating fibroblasts with regard to the mechanism through which the products of glycolysis entered the TCA cycle.[220] Metabolic intermediates can enter the TCA cycle from pyruvate via the pyruvate dehydrogenase-catalyzed transfer of an acetyl group from acetyl-CoA to oxaloacetate. Alternatively, pyruvate can be converted to oxaloacetate by pyruvate carboxylase, which allows for anaplerosis or the replenishment of TCA cycle intermediates extracted for catabolism. Quiescent fibroblasts, especially fibroblasts made quiescent by a combination of contact inhibition and serum starvation, exhibited a decline in pyruvate dehydrogenase-catalyzed TCA cycle entry. There was an increase in pyruvate carboxylase-catalyzed TCA cycle entry with quiescence, indicating increased anaplerotic filling of TCA intermediates.

TCA cycle metabolism also shifted with contact inhibition-induced quiescence. When contact-inhibited fibroblasts were

fed isotopically labeled glucose, the glucose was readily incorporated throughout the TCA cycle. In proliferating fibroblasts, there was a substantial decrease in the transmission of labeled carbons from citrate to α-ketoglutarate.[220] Instead, the citrate was transported to the cytoplasm, from which it could be used for fatty acid synthesis or to modify histones. Proliferating fibroblasts, rather than rely on glucose for TCA cycle anaplerosis, shifted to a dependence upon the amino acid glutamine, which can enter the TCA cycle at α-ketoglutarate. The reliance of proliferating cells on exogenous glutamine for TCA cycle anaplerosis is consistent with the increased requirement of proliferating cultured cells for exogenous glutamine to support their growth in culture.[224-226]

These experiments would suggest that primary dermal fibroblasts induced into quiescence by contact inhibition differ from most other cell types in that they maintain a comparable metabolic rate during quiescence compared with proliferating cells. In most cells, biosynthetic activity is downregulated upon quiescence because non-proliferating cells do not need to synthesize the components required to create new cells. One possible explanation for the maintenance of higher metabolic rates during quiescence in contact-inhibited dermal fibroblasts is that they do have an important biosynthetic function during quiescence, which is the secretion of extracellular matrix proteins. Indeed, contact-inhibited dermal fibroblasts increased the secretion of multiple extracellular matrix proteins as compared with proliferating fibroblasts.[220] A redirection of metabolic potential away from biosynthesis of intracellular components and toward synthesis of extracellular matrix proteins represents one possible explanation for the observation that quiescent, contact-inhibited fibroblasts do not enter a hypometabolic state.

Other studies have investigated the importance of TOR in fibroblasts induced into quiescence by serum withdrawal. When fibroblasts from the lung fibroblast cell line WI-38 are serum-starved, TOR activity decreases.[227] In contrast, TOR levels remained high in fibroblasts treated with doxorubicin to induce senescence, irreversible arrest that results from the end of a cell's replicative lifespan or stress.[227] Treatment with rapamycin reduced doxorubicin-induced senescence, suggesting that a reduction in mTOR activity can protect WI-38 fibroblasts from senescence. Thus, the TOR pathway may be an important determinant of whether a cell responds to anti-proliferative signals by entering a reversible quiescent state or an irreversible senescent state. To further these findings, the authors also used a model system of quiescence or senescence induced by activation of the tumor suppressor p53.[228-230] p53-mediated cell cycle arrest by treatment with nutlin-3a, a small molecule that activates p53 (reviewed in refs. 231 and 232), resulted in quiescence if TOR was inhibited and senescence if it was not.[233,234] In WI-38 lung fibroblasts, nutlin-3a suppressed senescence and inhibited mTOR. In mouse embryo fibroblasts, which are much more prone to senescence than human fibroblasts, p53 activation with nutlin did not reduce mTOR activity and led to senescence. Rapamycin rescued the cells from senescence and allowed them to enter a state more similar to quiescence. Thus, the status of the mTOR pathway may be an important contributor to the

decision between senescence and quiescence in fibroblasts: cell cycle arrested coupled with inappropriately high mTOR activity was found to be associated with senescence.

In fibroblasts, like yeast, TOR regulation can also affect the transition between proliferation and quiescence. Fibroblasts in which TOR is overexpressed as a result of antisense inhibition of the TSC protein do not undergo G_0 arrest upon serum withdrawal.[235] Inhibition of TSC also activated quiescent fibroblasts to reenter the cell cycle through upregulation of cyclin D1 expression and increased activity of G_1 CDKs,[235] and possibly destabilization and relocalization of p27[Kip1] (reviewed in ref. 236). Further, loss of TSC2 induced soft agar growth of the fibroblasts, a phenotype reminiscent of tumor growth.

Mouse embryo fibroblasts (MEFs) and immortalized mouse fibroblasts, NIH3T3 cells, have also been used to monitor the relationship between autophagy and quiescence. MEFs made quiescent by contact inhibition or serum withdrawal activated autophagy.[8] However, depletion of an essential autophagy protein in MEFs had no measurable effect on the ability of the cells to enter quiescence. Thus, in contrast to cells like lymphocytes that die rapidly upon autophagy inhibition when they are not stimulated with growth factors,[178] fibroblasts can achieve a quiescent state without functioning autophagy.[8] Thus, consistent with some of the experiments on metabolism in quiescent fibroblasts,[220] quiescent fibroblasts may not rely as heavily on nutrients or ATP equivalents reclaimed via autophagy in the quiescent state as other types of quiescent cells, although longer timepoints would be needed to make further conclusions.

Quiescence in Dormant Cancer Cells

There may also be cells within a tumor that enter a quiescent state characterized by hypometabolism and increased survivability. One argument for the existence of such cells is that tumors can recur many years after removal of the primary tumor. Breast cancers often recur years after a mastectomy was performed, even if there was no evidence of clinical disease at the time of surgery.[237] During this time between tumors, there may be minimal residual disease that results from solitary disseminated tumor cells.[238,239] The presence of these cells is associated with higher risk of recurrence.[237,240-244] The long intervals between primary tumors and their recurrences suggest that tumor cells may have developed mechanisms that allow for their long-term survival in a dormant state. Indeed, disseminated cancer cells may face nutrient starvation and may readjust their metabolic programs to allow them to use alternative energy sources during their dormant period. Some recent studies have explored the role of metabolic pathways in model systems designed to understand the properties of quiescent cells within tumors.

One model system for studying tumor dormancy is based on a highly tumorigenic and metastatic human squamous carcinoma cell line (T-Hep3) that, when passaged in culture for > 40 generations, reprograms to a reversibly dormant phenotype. The dormant cells undergo a G_0/G_1 arrest in culture and fail to form tumors in vivo.[245-247] Aguirre-Ghiso and colleagues discovered that there is an important role for the Rheb-mTOR pathway in

the dormant cells in this model.[248] The Rheb-mTOR pathway is regulated by the ATF6 survival factor. In response to endoplasmic reticulum stress, the cytosolic domain of ATF6 translocates to the nucleus and activates transcription of unfolded protein response genes. ATF6 levels were higher in the dormant compared with the tumorigenic matched cell lines. ATF6 was found to regulate Rheb and thereby activate mTOR signaling in the dormant cells, and the dormant cells were much more resistant to rapamycin treatment than their tumorigenic counterparts. Knockdown of Rheb decreased the survival of the dormant cells in vivo, suggesting that TOR signaling serves a role as a survival factor in the dormant tumor cells. Thus, in this model system of tumor dormancy, in contrast to the other quiescent states discussed, TOR activity is upregulated in quiescence.

Another approach to studying the signaling of dormant tumor cells was taken by Ramaswamy and colleagues.[249] They observed significant heterogeneity in cell cycle progression in the MCF7 cell line, a highly proliferative, aneuploid, ER+/HER2- human breast cancer cell line. Some cells remained in interphase for much longer than other cells before dividing. Based on previous studies indicating that slowly cycling hematopoietic, neural and breast adult stem cells and cancer stem cells produce low levels of ROS, they isolated the ROS^{low} subpopulation of the MCF7 cells. ROS^{low} MCF7 cells were predominantly in the G_0/G_1 phase of the cell cycle compared with actively cycling ROS^{high} cells. These G_0-like cells were characterized by low levels of proteins associated with proliferation, low levels of certain histone modifications and high levels of Hes1, which had been identified as a molecule important for the ability of quiescent fibroblasts to resist senescence and differentiation.[250] ROS^{low} cells were found not only in MCF7 cells but also cancer cell lines MDA-MB-231 and HCT116.

Further investigation showed that there were asymmetric cancer cell divisions that resulted in one proliferative and one G_0-like cell. The proliferative cells contained higher levels of Akt signaling and nuclear FOXO1 compared with the daughters. Treatment with a small molecule Akt inhibitor resulted in an increase in the G_0-like cells, which suggested that low Akt signaling may be an important regulator of the commitment to the G_0-like state. While G_0-like cancer cells were rare in pretreatment biopsies from five individuals, they were enriched in the biopsies from the same patients after neoadjuvant chemotherapy. The authors concluded that cancer cells flux between symmetric and asymmetric division depending on the state of Akt.

Other approaches to define a cell type that contributes to tumor dormancy have focused on the reported subset of cancer cells called "cancer stem cells," which are defined by their ability to survive and maintain their undifferentiated state.[251] Autophagy might be important for the survival of the disseminated cancer stem cell-like cells in nutrient and oxygen-deprived environments. Inhibiting autophagy with shRNAs that target autophagy genes in the stem cell-like compartment of a breast epithelial cancer cell line reduced the fraction of cells with the undifferentiated cancer stem cell-like phenotype.[252] The results suggest that autophagy is important for the maintenance of a cancer stem cell compartment.

From another perspective, all of the cells within a tumor would be expected to experience signals similar to those that trigger quiescence. Detachment from the substratum, low levels of nutrients, and hypoxia would all be expected to be characteristics of the tumor microenvironment. Thus, the PI3K/TOR pathway might be anticipated to be an important pathway in cancer, as mutations that activate the PI3K/TOR pathway could promote cancer cell survival in the absence of nutrients.[253] Breast cancers, for instance, often contain activating mutations in PI3K, inactivating mutations in PTEN or activating mutations in Akt. These mutations confer growth-factor and anchorage-independent growth.[253] Preventing tumor cells from activating the PI3K/TOR pathway could disable them and over 20 drugs that target the PI3K/TOR pathway are in clinical development.[253,254]

Similarly, autophagy is critical for the tumorigenic process for the bulk tumor as well.[67,255] Autophagy within a tumor is enhanced in regions furthest from blood vessels.[255] Suppressing autophagy reduces the growth of tumors with ras mutations,[256,257] myc-induced lymphomas and polyoma middle T-induced mammary tumors.[258,259] Autophagy may also be induced as an adaptive response to chemotherapy.[260,261] Indeed, autophagy could be a pharmacological target for anti-cancer agents.[261,262] Autophagy inhibition can be used in combination with other drugs, for instance, to enhance the effectiveness of growth factor inhibitors used to treat cancer.[263] An advantage of targeting autophagy in tumors is that autophagy inhibitors that affect lysosomal pH have already been shown to be safe and effective in people based on their use as antimalarials and as treatment for autoimmune disease.[264]

Summary: Comparing and Contrasting Quiescent States

Cells in many different species exhibit a common response to extracellular and intracellular signals that indicate that conditions are not suitable for proliferation. As described above, yeast and mammalian cells arrest their cell cycles prior to DNA synthesis. In the nematode, *C. elegans*, nutrient limitation and crowding results in the activation of an alternative developmental program called dauer.[265] During dauer, *C. elegans* remain immature and stockpile reserve lipids for survival during times of limitation.[266] Other examples of cells in quiescent states include neuroblasts in Drosophila and reproductive cells, such as oocytes, seeds and bacterial spores. Changes in central carbon metabolism vary in the different species. In bacteria, entry into stationary phase is associated with decreased TCA use.[94] Yeast, in contrast, can enter stationary phase from glucose deprivation and consequently shift from a reliance on glucose for energy to metabolizing the ethanol through respiration.[1] On the other hand, *S. cerevisiae* that can switch from TCA cycle/respiration to glycolysis during stationary phase exhibit lifespan extension.[267] Thus, decreased TCA cycle use may be advantageous during stationary phase for yeast as well as bacteria. Lymphocytes consume less glucose in a quiescent state compared with actively proliferating lymphocytes, which reflects a decrease in the expression of glucose transporters and in their localization to the plasma membrane.[268] In

lymphocytes, the quiescent state is associated with more oxidative phosphorylation and the activated state is associated with a shift toward increased glycolysis. HSCs are very sensitive to ROS levels.[200] They perform very little mitochondrially localized oxidative phosphorylation in the quiescent state compared with their activated progenitors.[206] Primitive oocytes share this property with HSCs, as they also have low levels of mitochondria or mitochondrial activity.[269,270] Contact-inhibited fibroblasts display yet another pattern with comparable glycolytic and TCA rates in both the proliferating and quiescent state.[220] The most deeply quiescent fibroblasts investigated, those that were quiescent from contact inhibition and serum starvation, did decrease flux from glycolysis to the TCA cycle through pyruvate dehydrogenase, and thus fibroblasts may share some properties with the cells that minimize TCA usage when they are in deeper quiescent states.

In yeast, storage carbohydrates may be used as an energy storage system during quiescence, while trehalose in particular is used as a nutrient source upon cell cycle reentry in response to nutrient readdition. Trehalose is likely important for stationary phase in species other than yeast as well, possibly for its role in protecting against desiccation,[108] as it is present in stationary phase *E. coli*,[271] and many species of plants, insects and fungi, including plant seeds and fungal spores,[272] but not mammals. In humans, buffering against starvation occurs at an organism level. The hepatocytes of the liver, muscles and red blood cells store glycogen and deploy it when glucose levels are low, while adipocytes store large lipid droplets that can also be mobilized in times of starvation. Lymphocytes, for instance, do not have large internal glycogen or lipid stores, and are highly dependent on glucose import to meet their metabolic needs.[273]

The TOR signaling pathway is frequently regulated with quiescence and can allow the cell to respond to extracellular nutritional conditions by modulating the rate of protein synthesis and growth.[19] In yeast, lymphocytes and HSCs, downregulation of the TOR pathway occurs with quiescence. In yeast, inhibiting TOR is sufficient to recapitulate a quiescence-like phenotype.[64,113,114] In HSCs, inappropriate activation of the TOR pathway results in excessive cell cycle commitment and ultimately stem cell exhaustion.[196,197,209] TOR signaling in HSCs results in elevated ROS

levels that are damaging to HSCs in the quiescent state.[196,197,209] In fibroblasts, TOR activation during quiescence is associated with a commitment to a senescent rather than quiescent state.[227] In contrast to the other quiescent states considered, in dormant tumor cells, TOR was activated in the dormant state and contributed to cancer cell survival.[248]

Autophagy and protein turnover are also critically important aspects of the quiescent state for many quiescence model systems. As described above, yeast, lymphocytes, HSCs and cancer cells all rely on autophagy for survival in their quiescent states. Inactivation of autophagy proteins in *C. elegans* disrupts normal dauer formation.[266] Fruit flies and spores also require autophagy to adapt to organismal nutrient starvation.[144,274,275] Thus, in many instances of nutrient deprivation, cells rely on autophagy for survival.

A better understanding of the mechanisms used by quiescent cells to maintain their viability during quiescence and in low nutrient environments may result in tools that allow us to manipulate the quiescent state. This could potentially have ramifications for a wide range of fields. Many of the earth's environments are low in nutrients and cannot sustain the growth of microbes.[83] An understanding of these natural environments and the stationary phase state of the microorganisms in them could suggest improved antifungals or antibiotics.[276,277] Anticancer therapies that target dormant cancer cells could represent a potent and new approach to preventing tumor recurrence. A mechanism to manipulate the quiescent state of lymphocytes could suggest new approaches for immunization strategies and new immunosuppressants. Finally, the phenotypes of aging may reflect the transition of quiescent cells to senescence or irreversible cell cycle arrest.[278] Aging may also result from inappropriate activation and secondary depletion of stem cells. The ability to prevent this process could suggest strategies to slow the aging process. The ability to enter a hypometabolic state provides cells with a powerful mechanism for surviving during times when proliferation isn't favored and nutrients are limiting. This capacity makes species resilient and makes it possible for life to flourish in a much wider array of conditions, locations and times than if cells did not have this remarkable capacity.

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