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OPINION

ARTICLE SERIES: STEM CELLS

Decoding the stem cell quiescence cycle – lessons from yeast for regenerative biology

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ABSTRACT

In the past decade, major advances have occurred in the understanding of mammalian stem cell biology, but roadblocks (including gaps in our fundamental understanding) remain in translating this knowledge to regenerative medicine. Interestingly, a close analysis of the *Saccharomyces cerevisiae* literature leads to an appreciation of how much yeast biology has contributed to the conceptual framework underpinning our understanding of stem cell behavior, to the point where such insights have been internalized into the realm of the known. This Opinion article focuses on one such example, the quiescent adult mammalian stem cell, and examines concepts underlying our understanding of quiescence that can be attributed to studies in yeast. We discuss the metabolic, signaling and gene regulatory events that control entry and exit into quiescence in yeast. These processes and events retain remarkable conservation and conceptual parallels in mammalian systems, and collectively suggest a regulated program beyond the cessation of cell division. We argue that studies in yeast will continue to not only reveal fundamental concepts in quiescence, but also leaven progress in regenerative medicine.

KEY WORDS: Quiescence, Metabolism, Yeast, Adult stem cell, Signaling pathways, Regeneration

Introduction

A distinguishing feature of stem cells is their ability to retain the primal capacity to generate more stem cells whose progeny can have distinct fates, an attribute called (pluri)potency. Uncovering the molecular basis of potency is a key to understanding the distinct biology of stem cells. Current efforts to define 'stemness' focus largely on the regulation of embryonic stem cell (ESC) pluripotency. Adult mammalian tissues also retain resident stem cells, such as the hematopoietic stem cell (HSC), which contributes to their homeostatic maintenance, as well as repair and regeneration. Unlike ESCs, the activity of stem cells within adult tissues is episodic or dependent on damage-induced demand. It is now apparent that understanding how these resident stem cells function will provide fundamental new information on tissue dynamics, as well as hope for regeneration and rejuvenation of damaged or aging tissue. However, the hierarchies and players in control networks for adult stem cells are presently poorly understood. One outstanding question concerns how adult stem cells use quiescence to establish or reinforce the property of self-renewal. Because self-renewal is largely studied by assays that measure proliferation, the paradox of self-renewal programs imposed during non-proliferating conditions has led to much confusion. In this Opinion article, we attempt to deconstruct the quiescent state, highlighting programs characteristic of this particular cell cycle exit that might illuminate the importance of quiescence for adult stem cells.

Unlike the cell division cycle whose complex regulation is well defined, quiescence in mammalian cells is poorly understood, and was earlier considered to result simply from the decline of genetic, signaling and metabolic pathways. However, quiescent adult stem cells must exercise active control in order to fulfill their regenerative role when activated. For example, dormant cells must simultaneously maintain their genomes in a mutation-free state, remember their identity as tissue-specific stem cells, conserve energy and avoid entering alternative programs, such as death and differentiation, as well as retain the ability to respond specifically to activation signals (Fig. 1). Breaking down this complexity into simpler, broadly conserved conceptual frameworks that describe entry and exit into quiescence remains a challenge faced by stem cell biologists.

Importantly, the quiescence program itself appears to be evolutionarily ancient, and has extensively been studied in microorganisms, notably *S. cerevisiae*. A number of studies from yeast have informed our understanding of its regulation, identifying key metabolic, signaling and regulatory events that control entry and exit into quiescence. While the quiescence programs in yeasts (and plants) were once viewed as distinct from mammalian quiescence, increasingly, common features across these systems are emerging. Therefore, can conceptual frameworks on quiescence that have emerged from yeast studies inform and indeed advance the understanding of stem cell biology?

In this Opinion article, we review the evidence linking the pathways controlling quiescence in yeast with those that regulate the function of adult mammalian stem cells. We particularly examine new findings that conceptualize quiescence as a poised state rather than an inert state, and locate them in the context of the molecular history of quiescence, the resting phase of adult stem cells. Specifically, we examine how the framework laid down by yeast biologists has had far-reaching implications for human stem cells and regenerative medicine.

The concept of a 'quiescence cycle'

In the early 1990s, well after the underpinning of the cell cycle by oscillatory genetic networks (first described in yeast, clams and starfish and supported by discoveries in tumor viruses) became the dominant model, one phase of the cell cycle remained mysterious. This poorly understood but ubiquitous phase is called quiescence or 'G0'. In fact, mammalian stem cells, much like the majority of microbes on the earth (Lewis and Gattie, 1991), spend much of their lives in this dormant state (Cheung and Rando, 2013), which necessitates a deeper understanding of its particular biology. Although the definition of what constitutes G0 or quiescence continues to evolve, our understanding of how cells enter or exit a G0 phase, and the pathways that regulate these state changes have

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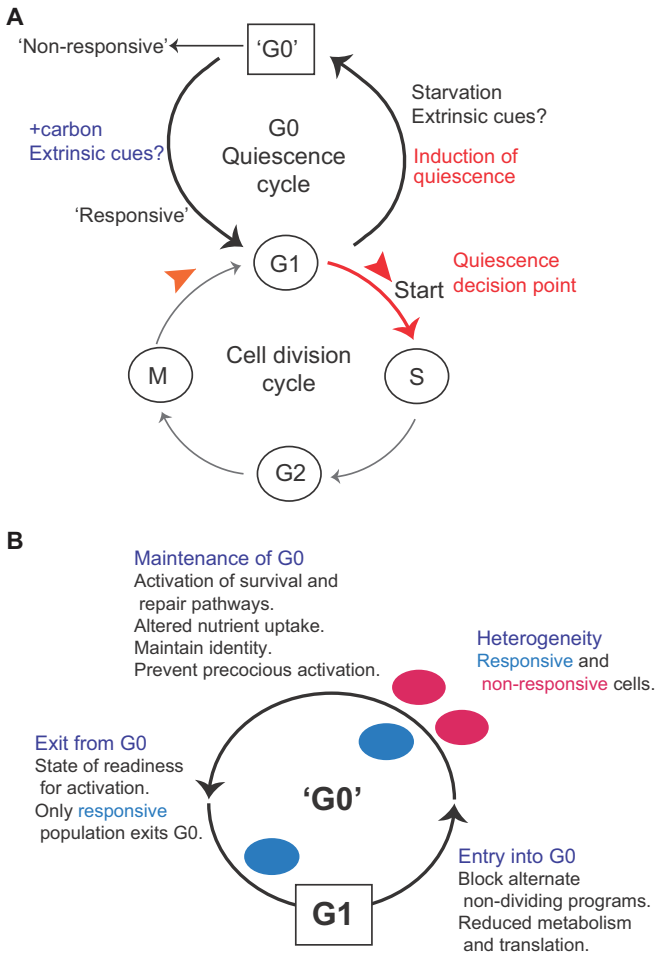

Fig. 1. Reversible withdrawal into a quiescence cycle is an active process. (A) Schematic illustration of the quiescence cycle in context of the general cell cycle. Entry of cells into the quiescence cycle and 'G0' requires nutrient starvation (particularly in yeast cells) and other extrinsic cues, which both in yeast and mammalian cells might all funnel into regulating the activity of TOR, PKA and AMPK to permit induction of a quiescence program. Re-entry into the cell cycle requires nutrients and extrinsic cues. The systemic inputs required for triggering exit from proliferation, or re-entry into the cell cycle from quiescence remain poorly understood. Not all cells within a quiescent population will re-enter the cell cycle upon receiving appropriate cues and some cells appear to be uniquely adapted to re-enter the cell cycle. This program of reversible arrest includes induction of a quiescence program that involves an active suppression of alternative non-dividing fates (see B). Critical determinants of this program remain to be discovered. Nutrient-dependent commitment steps are illustrated in bold with black arrows. In proliferating populations, the decision to enter quiescence was traditionally thought to be in G1 (red arrowhead) when a cell assesses its cellular state in the context of external conditions, but recent evidence suggests that key control mechanisms might already be in place at the end of the preceding cell cycle (orange arrowhead). (B) Quiescence involves the induction of programs beyond mitotic arrest. Signatures of different phases of the quiescence cycle are illustrated. Cells entering G0 from G1 block alternate non-dividing states, such as senescence, death and differentiation. Even within such a population of non-dividing cells, there is heterogeneity in that only some cells are responsive to subsequent cues to be able to exit quiescence. To maintain the G0 state, cells induce survival pathways and nutrient uptake; they also must maintain identity and avoid precocious activation. Upon receiving an appropriate stimulus, which might include nutrient cues, the responsive cells (blue) within this population of cells will exit G0 and re-enter G1. Non-responsive cells are shown in red. Whether the heterogeneity arises from intrinsic variation in activation thresholds of key control mechanisms or temporal asynchrony of the population is not known.

dramatically improved. Remarkably, many of these foundational concepts have come from studies performed in yeasts.

In their visionary review, Werner-Washburne and colleagues proposed that, despite the distinct biology of unicellular and multicellular organisms, understanding the quiescent state would improve our understanding of tumor biology as well as degenerative disease (Gray et al., 2004). How might this concept be viable, given the perceived disparity between yeast biology and human disease? If viewed from the perspective of the quiescent adult stem cell, cancer and degeneration represent opposite ends of a spectrum: cancer can result from a failure of cells to enter a resting state following activation, leading to excess proliferation, whereas degenerative disease can result from a failure to exit the resting state in response to injury, leading to loss of tissue over time. This idea (of the centrality of the quiescent state) has in fact been built upon extensive studies, in particular from yeast, that described nutrient deprivations that lead to cells entering stationary, quiescent phases, as well as pinpointing the signaling pathways that are crucial for entering into or exiting from this stationary phase (Granot and Snyder, 1993; Gray et al., 2004; Werner-Washburne et al., 1993). Integrating studies from molecular and mutant analysis, this body of work suggests that cells can leave the conventional cell cycle, and enter an alternate cycle termed the 'quiescence cycle', where entry, maintenance and exit are controlled by specific genetic and signaling networks that are distinct from networks regulating the conventional cell cycle (Gray et al., 2004). The emerging conceptual framework envisaged that the quiescence cycle intersected with the cell division cycle at the G1 phase (Gray et al., 2004). Functionally, the quiescence cycle could be interpreted to reflect a switching between responsive ('dozing') and non-responsive ('sleeping') states: cells were considered to cycle between these two states, neither of which results in two daughter cells; instead each state represents a period with variable potential for producing daughters (Fig. 1). In this model, if external conditions were conducive, these responsive cells could be activated, entering G1 and completing the process of cell division (Fig. 1). Alternately, these responsive cells could return to quiescence without passing through a cell division cycle, suggesting that this transition is reversible. This model has been crucial for our current understanding of quiescence, because all previous models have suggested that any transition away from dormancy meant an obligatory return to the cell division cycle, even if later checkpoints stalled proliferation. Thus, quiescence has come to be viewed not as a singular state, but a continuum of cyclically related states.

Distinct states of quiescence exist

This yeast-derived model of a quiescence cycle remains remarkably prescient today across different eukaryotic cells, even as studies have continued to expand and refine it. Several yeast studies, in which the properties of cells have been investigated in intact yeast colonies as well as in well-mixed liquid cultures, are bringing greater clarity and substance to this model of a quiescence cycle. The first systematic observations of quiescence in yeast came from cells that had been grown in liquid cultures to saturation, or cells subjected to systematic carbon, nitrogen, phosphorus or sulfur starvation (De Virgilio, 2012). Here, as nutrients deplete, cells reach non-dividing 'stationary phases' and enter into quiescence after having undergone a series of adaptations (Lillie and Pringle, 1980; Werner-Washburne et al., 1993). This maximizes their long-term survival or 'chronological lifespan' (Kaeberlein et al., 2007; Piper, 2006). Importantly, it appears that yeast establishes a core

Journal of Cell Science (2015) 128, 4467-4474 doi:10.1242/jcs.177758

quiescence program regardless of the type of nutrient starvation and exhibits the ability to survive long periods of starvation to re-enter proliferative states upon refeeding (Klosinska et al., 2011; Lillie and Pringle, 1980; Werner-Washburne et al., 1993). Interestingly, although all cells in such a population face the same nutrient deprivations and enter non-dividing phases, only a sub-population of cells retain the ability to survive such periods of starvation, and re-enter the cell cycle upon stimulus, suggesting that there are distinct G0 or quiescent states within the population (Fig. 1) (Kaeberlein et al., 2007; Lillie and Pringle, 1980; Piper, 2006). In such heterogeneously quiescent yeast populations, the reasons that some cells do not survive extended starvation are not fully unknown, although there is a steady accumulation of dead cells in late stationary phase cultures. However, studies from the Werner-Washburne laboratory were among the first to provide interesting clues with regard to what distinguishes the cells that are able to survive starvation and re-enter the cell cycle. In these studies, distinct subpopulations of stationary cells were isolated, with each population having a different cell density (Allen et al., 2006; Aragon et al., 2008). Cells with a higher density showed an increased ability to survive and synchronously re-enter the mitotic cycle (Allen et al., 2006). This study was amongst the first to indicate that 'stationary phase' cells were not all alike, but had distinct properties that are likely derived from metabolic differences regulating their ability to re-enter the cell cycle. In support of this notion, earlier work had shown that switching from glycolytic to respiratory metabolism increased the potential of a yeast cell to exit quiescence (MacLean et al., 2001). These studies therefore brought clarity to the decades-old observations that cells entering quiescence due to nutrient limitation utilized glycolytic metabolism during initial rapid proliferation, before shifting to respiratory metabolism as they move towards quiescence (De Virgilio, 2012; Lillie and Pringle, 1980). More recent studies suggest that the unique ability of some yeast cells within a population to re-enter the mitotic cycle appears to be made possible by a combination of their increased ability to store specific forms of carbon for use as a future energy source (Shi et al., 2010), the transcriptional repression of specific growth and cell-cycle-related genes (Miles et al., 2013), and post-transcriptional regulation of mRNAs (Li et al., 2013). One interesting feature of quiescence in yeast is that different starvation conditions induce distinct quiescence states, suggesting that there are different ways to enter quiescence (Daignan-Fornier and Sagot, 2011; Klosinska et al., 2011). However, the transcriptional response to these different inputs remains similar for all of these starvations (Klosinska et al., 2011). Furthermore, although there are different metabolic responses under these different starvation conditions, these cells share many common features, including increases in storage carbohydrates and the respiratory cycle metabolites (Klosinska et al., 2011). Collectively, there is emerging evidence that specific metabolic transitions, such as shifts from glycolytic to respiratory metabolism, might be a prerequisite for cells to successfully transition into reversible quiescence.

Distinct pathways to quiescence have also been described in mammalian cells. Early studies in cultured fibroblasts (Benecke et al., 1978; Dean et al., 1986) showed that mitogen deprivation, high cell density, amino acid deprivation and anchorage deprivation all impose quiescence; this was later broadly supported by molecular profiling (Coller et al., 2006) and metabolic analysis (Lemons et al., 2010), although each of these distinct arrested states has not yet been analyzed to the same depth. Entry into reversible arrest that is represented by the quiescence program also inhibits alternate non-dividing states, such as apoptosis, senescence and differentiation (Milasincic et al., 1996; Sachidanandan et al., 2002; Sousa-Victor et al., 2014). The suppression of tissue-specific programs in quiescent, lineage-determined cells, such as myoblasts, is reversed by re-entry into the cell cycle (Kitzmann et al., 1998; Sachidanandan et al., 2002; Sebastian et al., 2009), indicating a complex coupling between differentiation potential and proliferation.

Metabolic states, heterogeneity and quiescence

Several striking findings on quiescence come from studies of robust oscillations in oxygen consumption that are observed in nutrient-limited yeast cultures (Parulekar et al., 1986; Satroutdinov et al., 1992; Tu et al., 2005). During these yeast metabolic cycles, over half the yeast genome is periodically expressed, with a striking logic of gene expression. Genes encoding proteins with common functions show highly correlated expression patterns, and cellular and metabolic processes are orchestrated in three distinct phases; a growth phase where cells consume oxygen, following by their synchronous division, and a subsequent phase of extended survival, during which genes associated with starvation, the stationary phase and quiescence are induced (Brauer et al., 2008; Tu et al., 2005). However, only a sub-population of the non-dividing cells exit this survival phase and commit once again to cell growth, followed by cell division (Laxman et al., 2010; Tu et al., 2005). This is reminiscent of the observations from stationary phase yeast cells where only a sub-population of cells exit quiescence and re-enter proliferation (De Virgilio, 2012). Studies of cells undergoing these metabolic cycles suggest that this exit from quiescence into proliferation is dependent on the availability of the central carbon metabolite acetyl-CoA, which can drive the acetylation of histones at specific loci that encode for growth regulatory genes, thereby promoting their activation and enabling exit from the quiescent-like state (Cai et al., 2011; Shi and Tu, 2013). These studies suggest that exit from quiescence follows a central logic, wherein metabolic activation that results in a rapid accumulation of acetyl-CoA enables cells to exit quiescence. Quiescent cells with sufficient stores of carbon, which are primed to convert these carbon forms into acetyl-CoA upon stimulus (such as the denser sub-population of cells described above), might therefore be best poised to survive starvation and exit quiescence. A testable hypothesis could be to ask whether such a simple model is also conserved in quiescent adult mammalian stem cells.

Similar studies of mature yeast colonies, where groups of cells of different ages survive in distinct niches, have begun to provide important insights into the differences in outcomes of these 'older', non-dividing cells (Váchová et al., 2012). Even within a mature isogenic colony, different cells show distinct metabolic states, indicating that they produce, utilize and share different metabolic precursors (Čáp et al., 2012). The older, non-dividing cells within the population maintain different properties and functions, or altruistically die to support the growth and survival of younger cells (Palková et al., 2014; Váchová and Palková, 2011). Taken together, these studies show that the ability of a cell to exit quiescence and re-enter mitosis upon appropriate stimuli is determined by factors such as metabolic, transcriptional and post-transcriptional events, which are distinct between sub-populations within stationary phase cells and conditioned by different environmental factors. Of particular note, these studies have revealed the existence of cells with distinct identities and abilities to re-enter the cell cycle even within a population of cells that is typically considered quiescent, and

Journal of Cell Science (2015) 128, 4467-4474 doi:10.1242/jcs.177758

particularly, common metabolic principles that enable cells to exit quiescence.

In contrast, how such heterogeneity arises remains poorly understood in quiescent adult mammalian stem cells. However, given the growing literature in yeast, it is plausible to assume that this heterogeneity involves differential responses to extrinsic signals that amplify any subtle intrinsic differences, as has recently been demonstrated in cultured mammalian cells (Spencer et al., 2013). Here, using a sensor to report on levels of activity of a key signal-dependent cell cycle control protein (Cdk2), the Meyer laboratory traced cell lineages through successive rounds of cell division in culture, which revealed that ∼25% of cells in an asynchronous population did not express the levels of Cdk2 activity required to progress through G1, and thus they entered G0. The bifurcation point appears to be located during a 'restriction window' at the end of the previous cell cycle and not at the G1 restriction point that has been classically determined to control entry into S phase. Thus, intrinsic variations in regulatory protein expression and/or activity might underlie or enhance functional heterogeneity (Yao, 2014). Indeed, stem cells in tissues display functional heterogeneity *in vivo* (Collins et al., 2007; Sherwood et al., 2004). Although stem cell heterogeneity has long been associated with a variable proliferative potential (Guenechea et al., 2001; Roeder and Loeffler, 2002), molecular correlates have been elusive. Recent studies by the Goodell laboratory in bone marrow revealed that intrinsic differences in a continuum of HSC subtypes might be amplified by their variable responsiveness to the extrinsic signal TGFβ, which is well known to target quiescence pathways (Challen et al., 2010; Copley et al., 2012). Therefore, it is plausible to assume that the concepts of differential responses of cells to extrinsic cues that have been identified based on yeast studies, particularly those that depend on understanding the underlying metabolic state of the responding cell, might also apply to quiescent adult mammalian stem cells.

Signaling pathways in quiescence – from yeast to stem cells

Yeast studies have been pivotal in defining the signaling pathways that regulate entry into and exit from quiescence. Three pathways, the cAMP–protein-kinase-A (PKA) pathway, the AMP-activated protein kinase (AMPK) pathway (or Snf1 pathway in yeast), and the target of rapamycin complex 1 (TORC1) pathway, have been found to be crucial for entry into G0 in response to nutrient starvation or desiccation (Fabrizio et al., 2001; Longo et al., 2012; Martin and Hall, 2005; Thevelein and de Winde, 1999; Welch et al., 2013). The PKA and TORC1 pathways are growth-promoting pathways activated by glucose and amino acids, and it is now apparent that these pathways can determine whether or not a cell will enter into quiescence; for example, yeast cells with excessive PKA or TORC1 activity do not enter quiescence. Conversely, PKA-deficient cells, or TORC1 inhibition results in growth-arrested cells in a G0 state (De Virgilio, 2012; Gray et al., 2004; Thevelein and de Winde, 1999). In contrast, the AMPK (Snf1) pathway is activated upon glucose starvation (Ghillebert et al., 2011) and acts as a positive regulator of entry into quiescence (Gray et al., 2004; Smets et al., 2010; Zaman et al., 2008). In particular, these studies have revealed a central role for TORC1 and components of the TOR signaling pathway that enable cells to integrate environmental, nutrient and metabolic cues in order to control entry into and exit from quiescence (Fig. 2) (De Virgilio, 2012; Longo et al., 2012; Smets et al., 2010), wherein the downregulation of TORC1 is required for yeast cells to enter quiescence, and its activation appears to be crucial for re-entry into the growth phase (De Virgilio, 2012; Zaman et al., 2008).

Collectively, these yeast studies have described a range of concepts central to our current understanding of quiescence, from the existence of distinct sub-populations within quiescent cells, to the molecular and signaling pathways that regulate quiescence. These have subsequently been extended to mammalian systems, with functions that are remarkably well conserved, as discussed below (Fig. 2).

Although several studies in cultured mammalian cells have now expanded our understanding of these three pivotal pathways, their importance in stem cell quiescence *in vivo* is less well known. In the past 15 years, several mouse models have been developed to study stem cells in their native location and at different stages of development and physiological alteration (Beauchamp et al., 2000; Blanpain et al., 2004). Improved access to mammalian stem cells has meant that it became possible to pose more difficult questions addressing the crosstalk between distinct populations of stem cells. Muscle stem cells are an attractive stem cell model because there are excellent molecular markers for the different stages of muscle stem cell specification and participation in regeneration. Importantly, unlike HSCs, which are preferred models in stem cell biology (Becker et al., 1963; Mendelson and Frenette, 2014; Nakamura-Ishizu et al., 2014), muscle stem cells are easily imaged within their niche in skeletal muscle (Wang et al., 2014), because they are sandwiched between the sheets of the myofiber plasma membrane and the ensheathing basement membrane (Abou-Khalil et al., 2009; Conboy and Rando, 2002; Kuang et al., 2008; Seale et al., 2000; Rocheteau et al., 2012).

So how have studies in yeast foreshadowed studies in stem cells? Recent discoveries by the Rando group have demonstrated that mammalian TOR (mTOR) signaling regulates the quiescent state in muscle stem cells (Rodgers et al., 2014). This finding not only pinpoints the conserved mTOR pathway as a crucial determinant of quiescence and stem cell function, but also reveals a previously unappreciated long-range or systemic signaling between damaged tissue and stem cells of more than one type in distant tissues. Their data also further suggest the existence of a new level of quiescence (G0), conceptualized as a 'half-awake' or 'dozing' state, or G(Alert) (discussed in detail below). Early studies of the migration of muscle stem cells between muscle fibers provided evidence for a signaling between damaged and undamaged areas of the same muscle (Hughes and Blau, 1990; Morgan et al., 1993), but there was no evidence for any other long-range signals. Indeed, contralateral muscles of the same animal have long been considered an adequate experimental 'control' for the damaged site, as they provide the exact same physiological context, minus the damage. However, the important observation by Rodgers et al. that stem cells isolated from contralateral muscle, that is, responsive cells, have a slightly different volume than those isolated from uninjured muscle tissue now changes this view and suggests that responsive 'dozing' and non-responsive 'sleeping' quiescent stem cells can be distinguished from each other and, importantly, can also be separated. This is in accordance with earlier yeast studies that identified distinct populations of yeast cells in G0. These experiments therefore not only described a rapid and sustained activation of G0 muscle stem cells into a G(Alert) state, but also revealed that the TORC1 pathway involved in induction of this state has this conserved function across species. Furthermore, the authors also showed that G(Alert) cells can return to G0 without passing through a replicative cycle, further supporting the notion that an alternate quiescence cycle exists in mammalian cells, similar to what has been observed in yeast. Although the observations in mouse muscle stem cells revolutionize our understanding of the impact of circulatory signals (which are not

Journal of Cell Science (2015) 128, 4467-4474 doi:10.1242/jcs.177758

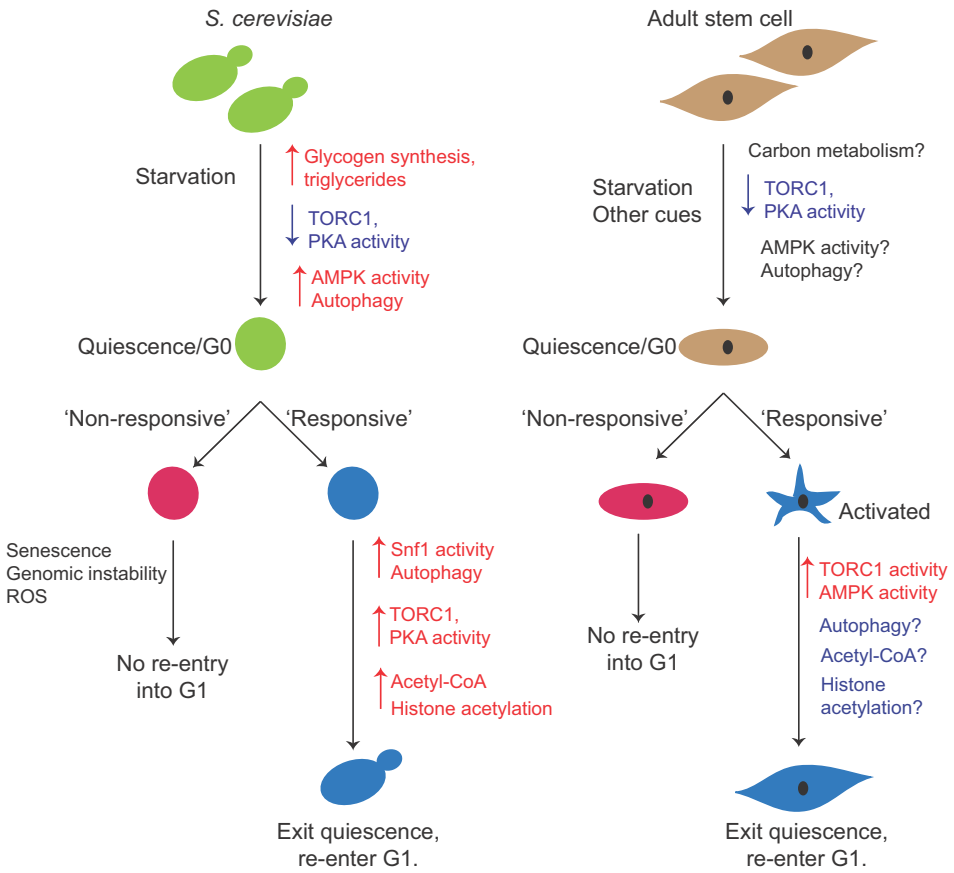

Fig. 2. Conserved mechanisms might govern the entry into and exit from quiescence in stem cells. Studies from yeast have elucidated the metabolic events and signaling pathways that control entry into quiescence during starvation (shown on the left). In particular, starvation strongly inhibits the TORC1 and PKA pathways, with an initial activation of the AMPK (Snf1) pathway. Exit from quiescence requires TORC1 activation, and an increase in the key metabolite acetyl-CoA, which regulates the activation of growth-promoting genes through specific acetylation events. Many parallels have been observed in adult mammalian stem cells (shown on the right). The population of yeast cells in G0 is heterogeneous, and only responsive cells (indicated in blue), which typically appear to have higher stores of carbon that can be converted into acetyl-CoA, exit quiescence upon stimulus (see also Fig. 1). Exit from quiescence in yeast correlates with increased TOR and PKA activity, and histone acetylation, which controls the transcription of growth-related genes. Several parallels appear to be conserved in mammalian adult stem cells, such as in the myoblast cell depicted on the right. However, unlike in yeast cells, the changes in carbon metabolism upon entry into quiescence and the role of the conserved energy-sensing kinase AMPK have not been well studied in mammalian cells. However, all three pathways identified in yeast have also been implicated in quiescence control in mammalian cells. Mammalian cell populations that enter quiescence are also heterogeneous and only responsive cells (in blue) exit quiescence upon sensing appropriate cues. Although recent studies have shown a crucial role for the mTORC1 pathway in exiting quiescence, other aspects observed in yeast cells (such as acetyl-CoA-dependent histone acetylation and gene activation) have not yet been investigated in mammalian cells. Indeed, several aspects of both metabolic events and signaling responses in stem cells remain unclear; however, owing to the high degree of conservation of these processes across different eukaryotes, it is likely that many of the pathways observed in yeast have similar roles in stem cells.

a feature of yeast cultures) on stem cell quiescence, notably, these findings converge on principles of the control of G0 by TOR that have emerged from earlier observations made in yeast cells.

Stages of awakening of quiescent cells

Several years ago, the activation of quiescent cells in stages had been described, leading to the proposal that a sequential induction of distinct pathways was required for dormant cells to enter a proliferative cycle. This 'competence-progression model' was built on the observation that cultured fibroblasts that had been arrested by high cell density or serum-starvation could be activated to re-enter the cell cycle by the sequential administration of combinations of growth factors (Pledger et al., 1978). Importantly, the factors that were required to 'activate' quiescent cells and move them into G1 were different from those required for full progression through the cell cycle into S phase. Platelet-derived growth factor (PDGF), termed by the authors a 'competence factor', was required for the G0–G1 transition, whereas epidermal growth factor (EGF) and insulin were needed for progression through G1 to DNA synthesis. The authors proposed that PDGF mediated the induction of a set of genes – the immediate early genes, including many transcription factors – which was required to activate the next step in the cascade of G1 progression, but that on their own these immediate early genes could not initiate a full mitogenic response (Pledger et al., 1978). Since its first description, the 'competence-progression model' has been updated and a number of signaling pathways have been found to be associated with cell cycle control and cancer. However, in view of the recent findings by Rodgers et al., where, temporally, the earliest stem-cell-activating signal induces the activation of TOR, the competence phase in this early model can be considered equivalent to the activated or G(Alert) state of muscle stem cells or the 'responsive' stage of the yeast quiescence cycle. Viewed in the light of the quiescence cycle model, competence might be equated with the responsive sub-population,

which in the absence of a combination of supporting mitogenic, nutrient or metabolic cues, will revert to their previous non-responsive state.

Studies of *Drosophila* stem cell niches support the notion that sub-populations of cells exhibit distinct abilities to exit quiescence based on their different metabolic states and cues from neighboring or distant cells (Shim et al., 2012). Placing these studies in the context of models established in yeast might allow researchers to tease out the roles of processes that depend on metabolites or nutrients from those that require mitogenic cues (Fig. 2). Although distinct growth factors might affect multiple cells and enable their long-distance communication, it is possible that only quiescent cells in a suitable metabolic state might be capable of responding to these cues.

Circulating factors and stem cell dormancy – the hope of rejuvenation

In adult mammals, are there secreted factors that impose quiescence on stem cells, or are crucial for sustained dormancy in the absence of mitogenic signals? Studies by the Weissman, Rando and Wagers laboratories (Conboy et al., 2005) have pioneered a new understanding of systemic influences on adult stem cells by revitalizing the use of parabiosis (Carrel, 1913); in this approach, the circulatory systems of two mice of differing physiology (age, genetic background etc.) are surgically connected over long periods in order to assess the effect of systemically circulating factors. An important finding from this work is that defective regeneration in aged mice can be attributed to alterations in circulating factors and not a defective intrinsic function (Conboy et al., 2005). Indeed, in a heterochronic parabiotic mouse pairing, the deleterious signals originate from the old partner and condition the response of the young partner (Brack et al., 2007). Furthermore, rejuvenating signals pass from the young partner to restore the declining regenerative response of the old partner (Katsimpardi et al., 2014; Sinha et al., 2014). The importance of these findings for the eventual therapeutic deployment of stem cells cannot be emphasized enough, as transplantation of purified populations of stem cells have repeatedly fallen short of their expected ability to regenerate damaged tissues. Indeed, the transplantation of purified tissue stem cells faces many challenges, including an incomplete understanding of the regulatory pathways that govern the survival of injected stem cells and their incorporation into preformed tissue architectures. The ability to modulate the systemic environment using pharmacological agents with a desired consequence for the behavior of endogenous stem cells is a more tractable therapeutic approach. Identifying these systemic or secreted cues, as well as the underlying features of a cell that make them responsive to these cues is therefore central to this problem. Although there will undoubtedly be conditional or contextual responses, an understanding of common underlying metabolic and signaling features as identified from models such as yeast, might inform on what makes a cell responsive to enter or exit quiescence.

Therapeutic opportunities – identifying candidates to control stem cell function using yeast

There are two directions that could propel a deeper understanding of stem cell quiescence towards possible clinical applications. The first approach is to mobilize endogenous stem cells by systemic treatments that activate quiescent cells or induce stem cells to return to their fully quiescent G0 state to preserve them for future activation. Stem cell exhaustion has been proposed to underlie degenerative disorders such as muscular dystrophy (Sacco et al., 2010); here, ongoing tissue loss and inflammation keeps stem cells activated. A transient induction of stem cell quiescence during the degenerative phase might therefore prevent the depletion of the stem cell compartment and mitigate the effects of this disease. Given the high degree of conservation in quiescence pathways, using small molecules that can be easily identified in yeast based on their ability to affect quiescence could help to manipulate mammalian stem cell fate and be of pharmacologic value in modulating the response to damage. A second opportunity lies in gaining a better understanding of the difference between responsive and non-responsive cells in mammalian models, making use of the concepts and molecules described for yeast. Here, the heterogeneity in quiescent populations might provide the opportunity to elucidate and dissect the metabolic and signaling pathways that permit a graded or distributed response to a given signal. In particular, developing better molecular descriptions of quiescence at the level of single cells would be useful. Together, these approaches might pave the way for the application of findings originally made in yeast to be applied to stem cell therapeutics.

Perspectives – yeast biology and regenerative medicine

We suggest that the lessons learned from yeast biology might help stem cell researchers and clinicians to apply the overarching principles that define how and when cells can enter into or exit quiescence. Yeast is an ideal species to address unresolved questions, particularly the crucial metabolic and molecular differences between responsive cells that can re-enter the cell cycle upon stimulation and other cells in G0. Yeast are also ideal to elucidate the signaling pathways that trigger the required activation of TORC1 in different cells within a population, and perform screens to identify molecules that alter the ability of a cell to enter or exit quiescence. In particular, yeast studies could help to distinguish between growth-factor-dependent mechanisms from pathways that depend on metabolic and nutrient cues to regulate exit from quiescence. As illustrated in Fig. 2, although there are obvious parallels between yeast and mammalian systems, important gaps remain in our understanding of quiescent mammalian cells. Just as studies on yeast, marine invertebrates, frogs, viruses and fibroblasts uncovered the stunningly conserved machinery at the core of the cell cycle and ignited our current understanding of cancer, we propose that a close mapping of yeast metabolic pathways that control quiescence will have profound implications for regenerative medicine.

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Competing interests

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