Stationary phase in yeast

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Eukaryotic cell proliferation is controlled by specific growth factors and the availability of essential nutrients. If either of these signals is lacking, cells may enter into a specialized nondividing resting state, known as stationary phase or G_0. The entry into such resting states is typically accompanied by a dramatic decrease in the overall growth rate and an increased resistance to a variety of environmental stresses. Since most cells spend most of their life in these quiescent states, it is important that we develop a full understanding of the biology of the stationary phase/G_0 cell. This knowledge would provide important insights into the control of two of the most fundamental aspects of eukaryotic cell biology: cell proliferation and long-term cell survival. This review will discuss some recent advances in our understanding of the stationary phase of growth in the budding yeast, *Saccharomyces cerevisiae*.

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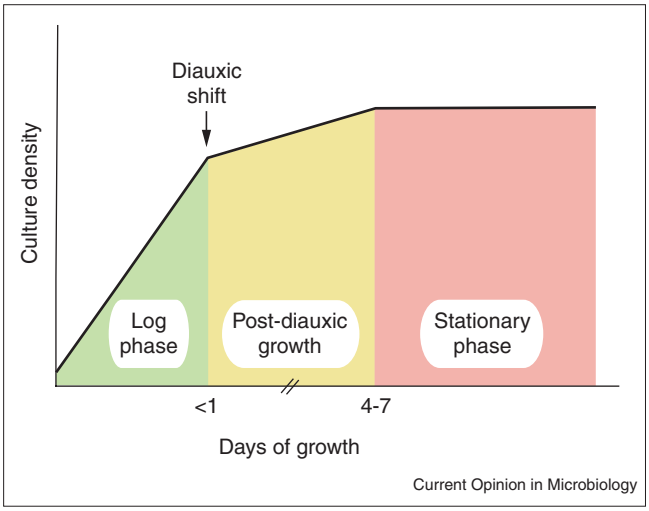
Abbreviations
CTD carboxy-terminal domain
PKA cAMP-dependent protein kinase

Introduction

When starved of an essential nutrient, cells of *Saccharomyces cerevisiae* cease mitotic division and arrest within the G_1 phase of the mitotic cell cycle. The arrested cells subsequently acquire a variety of characteristics that collectively define the stationary phase of growth [1,2]. These changes include a dramatic reduction in the overall rate of growth, an accumulation of the storage carbohydrate, glycogen, an increased resistance to a variety of environmental stresses, including heat shock, a thickening of the cell wall, and an increased ability to survive extended periods of starvation. A similar set of changes occurs when cells are starved of either a nitrogen, phosphate or carbon source [1,3]. However, it is not yet clear if the final resting state is identical in each of these instances. In particular, it has been suggested that a true stationary phase might only be reached following carbon-source deprivation [2,4] (Figure 1). In any case, the above differences between G_1 and stationary phase suggest that this resting state might be a distinct, out-of-cycle phase of growth.

Although stationary phase is a critical aspect of yeast cell biology, research in this area has lagged far behind that on the mitotic cell cycle. There have been few systematic genetic studies of stationary phase and we still do not have many useful molecular markers for this growth phase. As a result, some of the most basic questions regarding this resting state remain unanswered. This review will examine some of the reasons for this rather 'stationary' pace of progress and will suggest experiments aimed at stimulating new interest in this research area. In particular, the potential utility of genomic strategies for stationary phase research will be discussed.

Figure 1



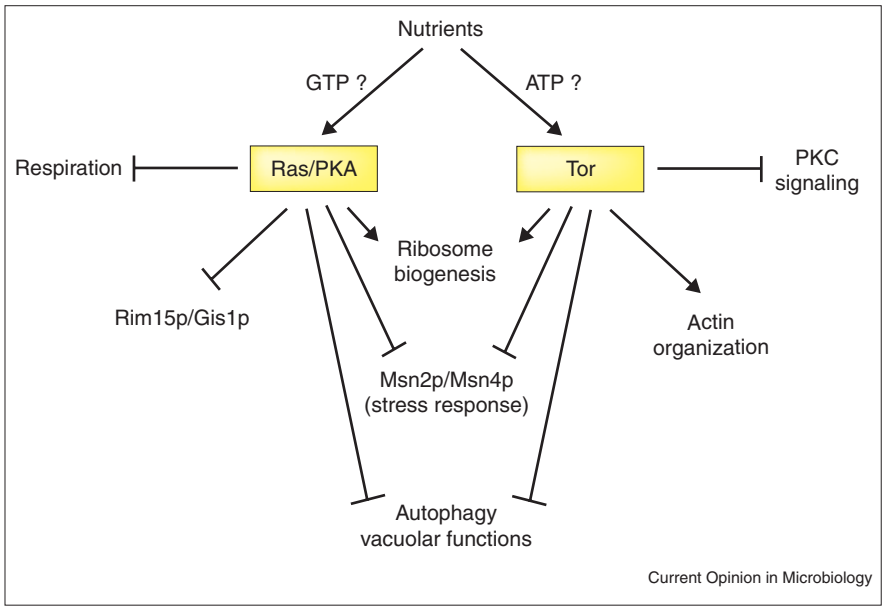
Growth phases exhibited by *S. cerevisiae* cultures grown on glucose-based media. The best characterized growth arrest in *S. cerevisiae* is that which occurs following growth on glucose-containing media. During the initial logarithmic phase of growth, this budding yeast grows by fermentation of the available glucose. When glucose becomes limiting, the cells transiently arrest growth and switch to a respiratory mode of energy production. This period of transition is known as the 'diauxic shift'. During the subsequent post-diauxic growth period, the cells grow rather slowly and utilize the ethanol that was produced during the previous period of fermentation. When this ethanol is finally exhausted, the cells enter into the true stationary phase, the growth period when the cell number is no longer increasing. In traditional rich media, such as yeast extract/peptone/dextrose (YEPD), cells may not reach the stationary phase until seven or more days of growth. This is an important observation, as many studies of 'stationary phase' are in fact performed with cultures that are in the post-diauxic phase of growth and caution should be applied to the interpretations of any such experiments. The final characteristics of stationary phase cells are likely to be the result of changes occurring in both the post-diauxic and stationary phases of growth.

Signaling pathways regulating stationary phase biology

The entry into stationary phase is regulated by the Ras and Tor signal transduction pathways, both of which are critical modulators of cell growth [5,6•]. The *S. cerevisiae* Ras proteins, Ras1p and Ras2p, are small GTP-binding proteins that activate the cAMP-dependent protein kinase PKA [5]. The Tor proteins, Tor1p and Tor2p, are themselves

Figure 2

Targets of the Ras/PKA and Tor signaling pathways in *S. cerevisiae*. Some of the targets of these two signaling pathways are shown. For a more complete list of potential targets, the reader is directed to recent reviews of these signaling pathways [6•,8,53]. Recent work indicates that the Ras/PKA pathway might be regulated by cytoplasmic GTP levels and that the mammalian Tor proteins might act as ATP sensors [54–56].



serine/threonine-specific protein kinases [6•,7]. Both of these signaling pathways positively regulate a variety of processes, such as protein translation, that are essential for cell growth, while at the same time inhibit other activities that are refractory to growth and proliferation [6•,7,8] (Figure 2). This latter category includes processes involved in protein degradation and organellar turnover (e.g. autophagy) and in the response to cellular stresses. Together, these data suggest that the Ras and Tor pathways are central components of a growth checkpoint mechanism in *S. cerevisiae* [6]. This checkpoint would serve to ensure that the balance between synthetic and degradative processes is properly coordinated with the available nutrient supply. Several recently identified targets of the Ras and Tor pathways that may be important for stationary phase biology are discussed below.

Ras/PKA pathway targets

Recent studies have identified the Rim15p protein kinase as a PKA substrate required for stationary phase entry [9]. Mutants lacking Rim15p are viable but fail to assume the characteristics of stationary phase upon nutrient deprivation. These effects on stationary phase appear to be mediated, at least in part, by the Gis1p transcription factor [10•]. It is not yet known if Gis1p is a substrate of Rim15p or if the control by this protein kinase is more indirect. Interestingly, *gis1* mutants are only modestly defective for glycogen accumulation and G_1 arrest but exhibit a significant defect in stationary phase viability [10•]. Thus, an analysis of the transcription defects in *gis1* mutants might identify genes important specifically for the long-term survival of stationary phase cultures.

Recent studies have suggested an interesting link between stationary phase entry and the carboxy-terminal domain (CTD) of Rpb1p, the largest subunit of RNA polymerase II [11•,12]. The Rpb1p CTD is a highly conserved, repetitive structure that is an important site of regulation for multiple steps during the production of a mature mRNA molecule [13,14]. Howard *et al.* [11•] found that truncations of the Rpb1p CTD prevented entry into a normal stationary phase and were lethal in combination with mutations that elevated the levels of Ras/PKA activity. In these studies [11•,12], it was suggested that the Ras/PKA pathway might coordinate gene expression with nutrient availability by regulating the function of proteins associated with the Rpb1p CTD. Unfortunately, the Ras/PKA target responsible for these effects has not yet been identified. One interesting possibility is that Ras activity could be affecting the phosphorylation state of the CTD in specific growth conditions. In this regard, the phosphorylation level at a specific residue in the CTD repeats has been found to increase during the diauxic shift [15]. It will be interesting to test whether this increased phosphorylation is regulated by Ras activity and is important for stationary phase entry.

Tor pathway targets

The protein kinase C homologue Pkc1p is part of a signaling pathway that regulates yeast cell integrity by controlling cell wall biosynthesis and the actin cytoskeleton [16]. Interestingly, this Pkc1p pathway was recently found to be both required for stationary phase survival and inhibited by the activity of the Tor pathway [17,18]. In addition, inactivation of the Tor pathway was shown to result in cell wall alterations that were dependent upon Pkc1p activity [17]. Since the yeast cell wall is known to be significantly remodeled upon stationary phase entry [1,19], it is tempting to speculate that the Pkc1p pathway plays a role in mediating these changes.

Tor activity has also been shown to inhibit autophagy — a membrane trafficking pathway that is induced by starvation and is essential for normal stationary phase survival [20,21]. The autophagy pathway carries bulk protein, organelles and other cytoplasmic components to the vacuole for degradation [22]. In a recent study [23••], the Tor pathway was found to inhibit the activity of Apg1p, a protein kinase essential for the earliest steps of the autophagy process. In particular, Tor activity reduced the affinity of Apg1p for its regulatory subunit, Apg13p, possibly by direct phosphorylation of Apg13p [23••]. Autophagy also appears to be inhibited by the Ras/PKA pathway, but the target of this inhibition is not yet known [20].

Coordinating stationary phase entry

A key question that remains concerns the manner in which the yeast cell coordinates the control by the Ras and Tor signaling pathways. The inactivation of either of these pathways results in a constitutive stationary phase-like arrest [5,6•]. This happens even in rich growth media, where the other pathway might be expected to remain active and to continue signaling for growth. One explanation for these results is that these two pathways might be coordinately controlled in some manner that has not yet been identified. One possibility is that processes essential for cell growth, such as protein translation, might simply require input from both the Ras and Tor pathways. Alternatively, there could be some form of communication or crosstalk between these two signaling pathways. In this way, the inactivation of one pathway could generate a signal that would result in the shutdown of the other. With current technology, it should be possible to discern whether such crosstalk does indeed take place.

Mutants defective for stationary phase survival

A recent study has shown that proteins in the Srb complex of the RNA polymerase II holoenzyme are required for the entry into a normal stationary phase [24••]. Mutations that inactivate this complex disrupt the normal patterns of gene expression that occur upon nutrient deprivation [24••,25]. These observations led to the suggestion that these Srb proteins might be targets of signaling pathways responsible for coordinating yeast cell growth with nutrient availability [24••]. This prediction appears to be correct, as this complex has recently been identified as a direct target of the Ras/PKA signaling pathway (YW Chang and PK Herman, unpublished data).

The rate of protein synthesis drops ~300-fold upon entry into stationary phase [2]; however, a recent study found that this low level of protein synthesis is essential for stationary phase survival [26•]. Surprisingly, the translation initiation factor eIF4E, or the cap-binding protein, does not appear to be required for this essential translation. Instead, the authors suggest that the survival of stationary phase cells might be dependent upon a low level of protein translation that can occur in a cap-independent manner, perhaps by initiation at internal ribosome entry sites [26•,27].

The *TRX1* and *TRX2* genes encode cytoplasmic thioredoxins that are important for the response to oxidative stress [28,29]. Mutants lacking both Trx1p and Trx2p are more sensitive to oxidative stress during stationary phase and exhibit a dramatic decrease in stationary phase viability [29]. These observations are consistent with previous reports indicating that oxidative damage contributes to the cell death that occurs in stationary phase cultures [30]. Finally, stationary phase survival defects have also been observed in mutants defective for inorganic polyphosphate metabolism, the Rpi1p transcriptional regulator and the regulation of the G_α protein, Gpa2p [31–33].

Stationary phase as a model for the study of aging?

S. cerevisiae cells can undergo two different types of aging. The first is 'replicative aging', and is measured by the finite number of divisions that a particular cell has undergone [34,35]. The second, 'chronological aging', refers to the total lifespan of a given cell and is the sum of the replicative lifespan and the time spent in a quiescent state [36]. Recent studies have shown that stationary phase figures prominently in both of these aging processes [36,37••,38,39]. In particular, since stationary phase can be much longer than the total replicative lifespan, several studies have simply used stationary phase survival as a measure of the chronological aging in yeast cultures [36,37••,38]. Interestingly, this work has suggested that proteins important for the regulation of longevity in metazoans also play a critical role in determining the chronological lifespan of yeast [30,37••]. Therefore, the study of yeast stationary phase could provide important insights into the mechanisms underlying aging in other organisms, including humans [36,38].

Stationary phase residence was also found to influence the replicative lifespan of yeast cells [39]. Cells that had been passaged through stationary phase were found to exhibit a significantly shorter replicative lifespan than those cells that had never been starved. The authors suggested that this stationary phase incubation contributed to the accumulation of an 'aging factor' that subsequently led to a reduction in the normal number of divisions these cells could carry out [39]. Once the identity of this factor is known, it will be interesting to see if it is also an important determinant of the chronological lifespan.

Is stationary phase a distinct, out-of-cycle growth phase?

A central question that remains unresolved concerns the very nature of the *S. cerevisiae* stationary phase. Is this resting state truly an unique phase of growth, distinct from all major phases of the mitotic cycle? An alternative hypothesis is that stationary phase represents an extended G_1 phase, where the cells are exhibiting an especially slow rate of growth. This alternative was raised in response to observations indicating that several stationary phase characteristics were also exhibited by slow-growing, but mitotically active,

yeast cultures [40]. The authors of this study suggested that the degree of stress resistance might be inversely related to growth rate and that stationary phase might represent the furthest point on this continuum. It is important to stress that this is not simply an issue of semantics, as the existence of a unique resting state would provide the cell with a separate point at which to control cell proliferation [41].

To establish that stationary phase is indeed a distinct phase of growth, it will be necessary to satisfy one of the two following conditions. The first would be to identify genes that are specifically required for the transitions between stationary phase and the cell cycle. Ideally, these genes would be dispensable for mitotic cell division. The second condition would involve the identification of a biochemical activity that is specific to the stationary phase of growth. To date, there has been some progress made towards satisfying the first requirement with studies of the *GCS1* gene. Cells containing mutations in *GCS1* were conditionally defective for the exit from stationary phase but exhibited no significant defect in mitotic growth [42]. Although the *gcs1* mutation has been used extensively to characterize the genetic requirements for stationary phase exit [1,43], subsequent work has indicated that the protein Gcs1p has a mitotic function, and thus the search for a specific function in the $G_1 \rightarrow$ stationary phase transition continues [44,45].

Less progress has been made with the second condition, and there are presently no specific molecular markers for the stationary phase of growth. Several early reports had indicated that the *SNZ* gene family might be expressed specifically in stationary phase cultures [46,47]. However, subsequent analyses of *SNZ* orthologues in other fungi have indicated that this gene-family is involved in the biosynthesis of pyroxidine, otherwise known as vitamin B6 [48,49]. Thus, the stationary phase induction of these genes might simply be due to the fact that pyroxidine becomes limiting at this time. Although this story is not yet complete, it does serve as a cautionary tale for these types of expression studies. The identification of any stationary phase-specific expression pattern should be corroborated with other functional information, such as the demonstration of the necessity of this gene for stationary phase survival. Recent success with such an analysis is discussed below. Finally, it should be noted that a stationary phase marker need not involve new gene expression and could instead be a re-localization of a protein or a new post-translational modification [50,51].

Genomic approaches to the study of stationary phase

The recent advent of functional genomics has provided tools that should facilitate future research on stationary phase biology. For example, two recent studies have used these technologies to directly examine the possibility that stationary phase is a distinct phase of growth. In the first study, a whole-genome expression analysis with microarrays identified 45 genes with a stationary phase-specific expression pattern (M Werner-Washburne, personal communication). Importantly, 14 of these genes have also been shown to be essential for stationary phase viability. The second study aimed to identify genes important for stationary phase survival (SC Howard and PK Herman, unpublished data). The study makes use of a deletion strain set that contains ~4700 yeast mutants, each deleted for a particular nonessential gene [52]. This collection should be very useful for stationary phase research, because it has been effectively pre-screened for mutants that do not have a significant effect on mitotic growth. Further characterization of the genes identified in these studies should shed important insights on the regulation of stationary phase biology and provide us with useful markers for the stationary phase of growth. The availability of more facile markers will hopefully encourage other researchers to begin to examine additional aspects of this quiescent state.

Conclusions

Although I have focused on the budding yeast *S. cerevisiae* in this review, the issues discussed are relevant to resting states in many, if not all, organisms. In most cases, it is still not clear whether a given resting state is a distinct phase of growth, and on the whole we do not have many useful markers for a quiescent state. However, this situation is likely to change significantly in the near future. The development of new technologies has poised the field for rapid progress in addressing some of the outstanding questions concerning growth control. For example, the experiments described above should identify both genes that are required for stationary phase survival and those expressed specifically in resting cells. The subsequent characterization of these genes should provide us with important insights into not just stationary phase, but also into a variety of biological processes. These are likely to include insights into both the expected, such as general growth control, and the unexpected, such as human aging. The key point is that there is a wealth of biology awaiting discovery in the nondividing cell and that we need to start focusing our scientific resources on these resting states.

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