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Stationary Phase in the Yeast *Saccharomyces cerevisiae*

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INTRODUCTION

Microorganisms, such as the yeast *Saccharomyces cerevisiae*, respond to starvation by ceasing growth and entering a nonproliferating state referred to as stationary phase or G_0. Cells in stationary phase become differentiated in ways that allow maintenance of viability for extended periods without added nutrients but retain the ability to resume growth promptly when appropriate nutrients become available. Stationary-phase cells are also unbudded and contain an unrep-

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licated complement of DNA, characteristic of the G_1 phase of the mitotic cell cycle.

Starvation is one of the most common stresses encountered by living organisms. Indeed, it is estimated that much of the microorganismal biomass in the world exists under nutrient-depleted conditions (123). Thus, entry into starvation-induced stationary phase, which allows long-term maintenance of viability under nutrient-limited conditions, is clearly a process for which there has been and continues to be tremendous selective pressure. The mechanisms by which eucaryotic microorganisms, such as yeast cells, survive prolonged periods of nutrient limitation and resume proliferation remain unclear. The genetic and molecular tractability of *S. cerevisiae* and the universality of the stress of nutrient limitation suggest that a greater understanding of stationary phase in yeast cells may well provide novel insights into nonproliferating states in other eucaryotic cells.

Stationary phase is an identifiable component of the culture cycle of microorganisms that is functionally defined as the time when there is no further net increase in cell number. Several distinct phases of growth are observed prior to entry into stationary phase when yeast cells are grown in glucose-based, rich medium such as YPD (1% yeast extract, 2% peptone, 2% glucose) (Fig. 1). These include exponential phase, diauxic shift, and postdiauxic phase. During exponential phase, yeast cells grow primarily by fermentation. The diauxic shift occurs when glucose becomes exhausted from the medium and cells adapt to respiratory metabolism. During postdiauxic phase, growth resumes at a much lower rate, utilizing energy provided by respiration.

When grown under these conditions, cells enter stationary phase as a result of carbon starvation (124). Although stationary-phase cells are routinely produced in this manner, the conditions under which the cells enter the stationary phase are relatively complex. The preferential catabolism of glucose by fermentation, the modification of metabolism and gene expression by glucose repression, and the subsequent response to depletion of nonfermentable carbon sources make entry into stationary phase by continued growth in YPD a potentially more complicated process than the response to starvation for a single nutrient.

Yeast cells also enter stationary phase when starved for other nutrients, including nitrogen, phosphorus, and sulfur (124). Most studies of the rapid effects of starvation are performed by transferring growing cells to a starvation medium lacking a specific, required nutrient. Under these conditions, the mobilization of endogenous nutrients often allows a limited number of cell divisions, during which stationary-phase characteristics are developed. Cells starved for carbon, nitrogen, sulfur, or phosphorus survive for long periods, accumulate glycogen and trehalose (124), and arrest at a related point with respect to the cell cycle, as determined by *gcs1* mutant analysis (see "Stationary Phase as a Unique State or Stress Response" below) (56), suggesting that stationary phase may be identical in these cells. In this review we refer to the mode of starvation only when the distinction is important.

The timing of entry into stationary phase has also not been clearly defined. Most yeast research is carried out with exponentially growing cells at culture densities less than or equal to 10^7 cells per ml, which is 30- to 50-fold lower than cell concentrations in cultures grown to stationary phase in YPD. Because most research has focused on rapidly growing cells, it is not surprising that the transient growth arrest at the diauxic shift and the extremely slow growth during the postdiauxic phase have been thought to be stationary phase.

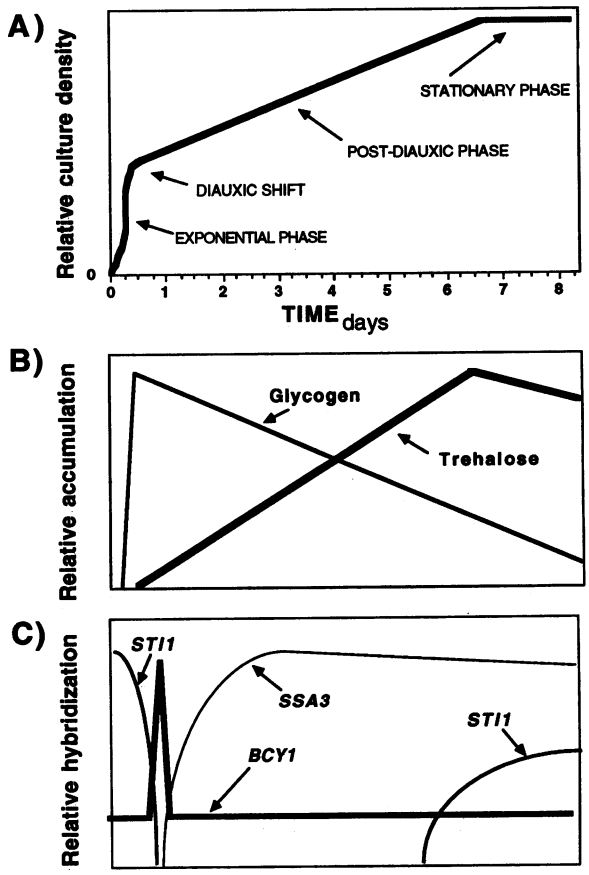

FIG. 1. Relative culture density, storage carbohydrate accumulation, and mRNA abundance as wild-type cells are grown to stationary phase in rich, glucose-based medium (YPD). (A) Diagram of a typical growth curve for which culture density is measured by turbidity at 600 nm or by cell number. For this diagram, the ordinate is linear to emphasize the increase in cell number during the postdiauxic phase. (B) Relative accumulation of storage carbohydrates (72, 124). (C) Relative abundance of three mRNA species based on Northern blot hybridization (147, 219a, 220). The diagram is drawn only to give the relative times at which increases or decreases in mRNA abundance occur and not to suggest the relative amounts of transcript.

In this review we define stationary phase as a physiological state coordinated with a cell cycle arrest. The characteristic of stationary-phase cells that distinguishes them from other cells is their ability to remain viable during prolonged periods of starvation.

In the past 10 years there have been several reviews dealing with carbon metabolism, catabolite repression, storage carbohydrates, and physiological responses to nutrient limitation in *S. cerevisiae* (74, 84, 116, 226). This review does not attempt to cover the material presented in earlier reviews but evaluates our current understanding of entry into and survival during stationary phase.

CHARACTERISTICS OF STATIONARY-PHASE CELLS

Stationary-phase cells are physiologically, biochemically, and morphologically distinct from exponentially growing cells. When observed by phase-contrast microscopy, stationary-phase cells are refractile or phase bright, whereas

exponentially growing cells are not refractile (27). Stationary-phase cells have thick, less porous cell walls (52, 53, 233) and, under some conditions, contain mitochondria that are more rounded and numerous than those observed in exponentially growing cells (126, 194).

As cells approach stationary phase, proteases, including aminopeptidases, accumulate in various subcellular locations (1, 106), electron-dense material (probably polyphosphate) accumulates in the vacuole (126), characteristically folded chromosomes are found in the nucleus (160, 162), and the storage carbohydrates glycogen and trehalose accumulate in the cytoplasm. Lipid vesicles become increasingly abundant in the cytoplasm (126), and triacylglycerol synthesis increases (98, 205). Total phospholipid synthesis decreases, and the relative concentrations of phosphotidylinositol and phosphotidylserine change (95). Cyclic AMP (cAMP) concentrations, which are high during exponential growth and decrease dramatically at the diauxic shift, remain low in stationary-phase cells (72). Stationary-phase cells also exhibit reduced rates of transcription and translation (17) and increases in the resistance to various stresses, such as heat shock (163).

Stationary-phase cells, in contrast to exponentially growing cells, are also able to survive for prolonged periods in either water or spent medium without additional nutrients (124, 219a). Cells have been shown to maintain nearly 100% viability for up to 3 months without added nutrients when grown to stationary phase in rich medium (124).

Regulated Cessation of Cell Proliferation

Stationary-phase cells are almost invariably found as single, unbudded cells containing unreplicated nuclear DNA (165). This indicates that in most starvation situations, enough metabolic activity, including translational capacity (28), remains to allow cells to complete an ongoing round of DNA replication and daughter cell formation.

The unbudded, prereplicative status of a stationary-phase cell is characteristic of a cell that has not yet performed Start, the regulatory step in G_1 of the mitotic cell cycle (168). Start is defined operationally as the cell cycle step blocked by the pheromone response pathway and by conditional *cdc28* mutations (168). Functional tests have shown that, with respect to the mitotic cell cycle, starved cells become blocked at or before Start (165). Conclusive evidence that the starvation-induced arrest point is at or distinct from Start has been hampered by technical difficulties inherent in the functional analysis.

Activation of the $p34^{CDC28}$ protein kinase, encoded by the *CDC28* gene, occurs as cells traverse Start and is required for performance of Start. $p34^{CDC28}$ protein kinase is activated by association with the G_1-cyclin regulatory proteins (230). Nutrient starvation blocks this activation (135), which may account for the unbudded, G_1 phenotype of stationary-phase cells.

In wild-type cells, G_1-cyclins, which are posttranslationally regulated in a cell-cycle-dependent manner, are degraded as cells leave G_1 and enter S phase and do not reaccumulate until cells have reentered G_1. Nitrogen-starved cells that express a hyperstable G_1-cyclin (*CLN2-1*) (167) arrest as budded cells, indicating that decreased cyclin activity is required for starvation-induced cell cycle arrest (81). This suggests, conversely, that nutrient limitation interferes with the G_1-cyclin activation of $p34^{CDC28}$.

Because of the apparent G_1 arrest of stationary-phase cells, an important question has been whether stationary phase is an integral component or an offshoot of the cell cycle (Fig. 2). The length of time required for stationary-phase cells to resume proliferation suggests that these cells have more to do than simply perform Start to reenter the mitotic cell cycle (102, 105). On the basis of analyses of strains carrying *cdc* mutations that block the mitotic cell cycle, stationary-phase arrest has been hypothesized to occur in G_1 before the *cdc28*-defined cell cycle arrest (101, 102, 165). Evaluation of other mutant strains has provided additional, convincing evidence that stationary phase is genetically distinct from Start and, in fact, is not part of the classically defined mitotic cell cycle (see "Stationary Phase as a Unique State or Stress Response" below) (56, 57).

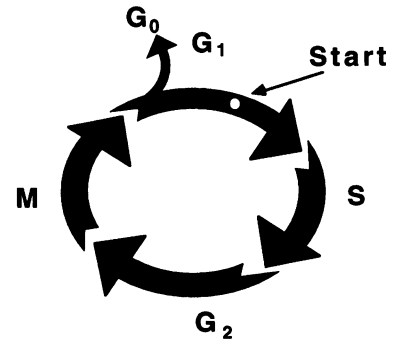

FIG. 2. The yeast cell cycle, showing that starved, stationary-phase cells accumulate at an off-cycle point, as indicated by mutational analyses (56, 57, 103a).

Alteration of Cell Wall Structure

The thickened cell wall that is characteristic of stationary-phase cells is resistant to digestion by the enzymatic activities in zymolyase and glusulase (52). At least part of this resistance to degradative enzymes results from changes in mannoprotein structure. Cell wall mannoproteins, which surround the glucan polysaccharide matrix (8, 30, 31, 56, 182), exhibit altered N-glycosylation (215) and contain increased numbers of disulfide bridges in stationary phase. These modifications increase the resistance of the mannoprotein layer to proteolysis and reduction of disulfide bonds, which is necessary to expose the underlying polysaccharide layer to digestion by zymolyase or glusulase (70, 233).

Accumulation of Storage Carbohydrates

The intracellular concentrations of both glycogen and trehalose vary with the growth status of a cell (Fig. 1) (152, 207) and appear to be a general response to stresses including heat shock and limitation for sources of nitrogen, sulfur, phosphorus, or carbon (10, 124). The patterns of accumulation of these two carbohydrates differ as cell cultures are grown to stationary phase (Fig. 1). Glycogen accumulates prior to the exhaustion of glucose and peaks at the diauxic shift, immediately after glucose exhaustion (124). In contrast, trehalose accumulation begins at the diauxic shift and continues until cells enter stationary phase. Once cells have entered stationary phase, trehalose levels decrease gradually (124).

The differences in the storage and mobilization patterns for glycogen and trehalose suggest that the two carbohydrates may have different functions in the stationary-phase cell. Glycogen is generally considered to be a reserve

carbohydrate that is mobilized to supply glucose during times of nutritional stress. Indeed, the pattern of glycogen metabolism is consistent with this suggested function. Trehalose, however, has been suggested to protect cells from desiccation or other stresses (152, 153, 224) rather than to serve as a reserve carbohydrate. Recent evidence suggests that although there is a direct correlation between trehalose concentrations and thermotolerance in stationary-phase cells (3), long-term viability of stationary-phase cells is not always correlated with intracellular accumulation of trehalose and glycogen (187). On the basis of these results it seems likely that, if a relationship between accumulation of storage carbohydrates and survival during nutrient limitation exists, it is likely to be complex.

Thermotolerance

As cells enter stationary phase, they become constitutively thermotolerant, as assayed by survival at potentially lethal temperatures (50 to 55°C). Thermotolerance is a relatively complex issue, and mutations that cause cells to become constitutively thermotolerant (69) or result in their inability to become thermotolerant (33, 210) have been shown to exert negative effects on survival during stationary phase. The *HSP104* gene product has been shown to contribute to thermotolerance but has only marginal effects on survival of stationary-phase cells (see "Other Types of Mutations" below) (175).

Altered Chromatin Structure and Decreased Transcription

Overall transcription declines dramatically as cells approach stationary phase (37, 220). Most individual mRNA species that are abundant during exponential phase are barely detectable after the diauxic shift (37, 220). Studies of bulk RNA confirm these findings. Stationary-phase cells contain approximately half as much poly(A) RNA as is found in exponentially growing cells (189), which has been estimated to be 5% of the total RNA in exponentially growing cells (133). Characterization of the poly(A) tracts in yeast cells revealed three size classes. The largest size class, (A)90, is threefold less abundant in stationary-phase cells than in exponentially growing cells and accounts for 80% of the decrease in poly(A) RNA concentration in stationary-phase cells (189). The smaller size classes, (A)30 and (A)20, therefore make up at least half of the poly(A) RNA in stationary-phase cells. Surprisingly, 80% of the poly(A) tracts in this small-size-class [(A)20:30] poly(A) RNA are internal, i.e., not at the 3′ end of the transcripts, which suggests that most of these RNAs may not represent classical mRNA (158, 189). These studies suggest that RNA molecules with 3′ poly(A) tails are markedly decreased in abundance (at least threefold) in stationary-phase cells. It is also possible that increases in concentrations of RNases or RNases that copurify with stationary-phase mRNA through phenol-chloroform extraction and oligo(dT)-based mRNA isolation (19a) contribute significantly to the loss of poly(A) RNA.

Yeast nuclear chromatin exhibits changes in overall conformation in stationary-phase cells (160). Nutrient-limited cells develop a slowly sedimenting, or folded, form of chromatin not observed in exponentially growing cells (160). The folded chromosomal conformation is not simply a response to conditions that inhibit cell proliferation, because it is not found in cells arrested by incubation with the mating pheromone α-factor (161) or in cells blocked early in the cell cycle by *cdc* mutations (162).

The higher-order structure of DNA, as reflected in chromatin structure, may influence transcription. The activity of topoisomerase 1, a nuclear enzyme that affects DNA supercoiling, has been shown to be required for the decrease in mRNA abundance after the diauxic shift (37). In *top1* mutant cells, mRNA species that normally undergo a dramatic decrease in abundance after the diauxic shift decrease at much lower rates than in wild-type cells. Moreover, in *top1* mutant cells the incorporation of ^{32}P into mRNA after the diauxic shift is almost fourfold greater than in wild-type cells (37), indicating that decreased RNA polymerase II-mediated transcription in general requires topoisomerase 1 activity. Topoisomerase 1 may be required for folding chromosomes or for repositioning of nucleosomes.

Specific alterations in nucleosome positioning have been found in stationary-phase cells at one locus (156). The *TDH3* gene, encoding the major isozyme of glyceraldehyde-3-phosphate dehydrogenase (129), shows altered nucleosome positioning at the promoter region. The *TDH3* promoter in stationary-phase cells contains two additional nucleosomes, arranged in a phased configuration (156). This alteration is correlated with decreased *TDH3* transcript abundance in starved cells (108). Similar alterations in chromatin conformation at the *TDH3* promoter are seen in mutants lacking the transcriptional regulatory protein Gcr1p (6, 94), which binds DNA (7) and activates *TDH3* expression (40, 94). The lack of Gcr1p results in significantly decreased *TDH3* transcription (94), suggesting that the Gcr1p is necessary to keep the *TDH3* promoter both active and nucleosome free.

Transcriptional Activation

Not all transcripts decrease in abundance after the diauxic shift or as cells enter stationary phase (Fig. 1). A few transcripts, such as those encoded by *UBI4* (Table 1) (69, 199), *ENO1* (see the following section) (129), *BCY1* (Table 1) (219a), and other as yet uncharacterized mRNAs (37), are present at all times in the growth cycle. Of these, the *ENO1* and *BCY1* transcript abundance is the most constant during the growth to stationary phase, although *BCY1* mRNA does show a transient fivefold increase in accumulation during the diauxic shift (55a). The abundance of *ENO1* mRNA is relatively constant during growth on both glucose and gluconeogenic substrates (129), and *ENO1* mRNA levels during the diauxic shift have not been determined. *UBI4* mRNA is less constant, accumulating severalfold after the diauxic shift and decreasing slightly as cells enter stationary phase (199, 219a).

Other transcripts that are not readily detectable during exponential phase have been found to accumulate to relatively high levels during or after the diauxic shift and as cells enter stationary phase. Among these are transcripts of *CTT1*, encoding catalase (13); *PRB1*, encoding protease B, a vacuolar protease (139); *CYC7*, encoding the minor isoform of the mitochondrial protein cytochrome *c* (159); the heat shock genes *HSP26* (157), *HSP12* (164), *HSP82* (16), and *HSP104* (174); the *HSP70*-related gene *SSA3* (220), which is closely related to genes expressed during exponential growth (220) and is known to be involved in protein translocation (54); *GLC7*, encoding a protein phosphatase (64); *GAC1*, which may encode a protein phosphatase regulatory protein (73); *GPH1*, encoding glycogen phosphorylase (100); *ACH1*, encoding acetyl coenzyme A hydrolase (121); and *UBC5*, encoding a ubiquitin-conjugating protein (183). *GPH1*,

TABLE 1. Genes thought to be involved in entry into and maintenance of the stationary phase

Gene	Synonyms	Gene product	References
ARD1		Subunit of protein amino-terminal acetyltransferase; associates with Nat1p	154, 222, 223
BCY1	*SRA1*	Regulatory subunit of A-kinase	33, 127, 210
CDC25	*CYR2, CTN1*	Guanine nucleotide exchange factor for Ras proteins	25, 107, 170
CDC33		Translation initiation factor eIF-4E, the cap-binding subunit of the translation initiation factor eIF-4F	20, 102, 217
CYR1	*CDC35, IAC1, SRA4*	Catalytic subunit of adenylyl cyclase complex	19, 109, 127
GCD1	*TRA3*	Mutation causing derepression of general control	91, 92, 231
GCD2	*GCD12*	Mutation causing derepression of general control	92
HSP104		Nucleotide-binding protein related to the ClpA/ClpB family from *E. coli* postulated to be involved in protein folding	155, 174, 175
ILS1		Isoleucyl-tRNA synthetase	85, 102, 134
IRA1	*PPD1, GLC1*	Ras GTPase-activating protein (GAP)	26, 200, 201
IRA2	*GLC4, CCS1*	Ras GTPase-activating protein (GAP)	26, 29, 198, 201, 202
KEM1	*XRN1, RAR5, DST2, SEP1*	Protein that may be involved in transduction of nutritional signals; has a number of biochemical activities involving nucleic acid binding	59, 60, 113, 114, 117, 209
NAT1	*AAA1*	Catalytic subunit of protein amino-terminal acetyltransferase; associates with Ard1p	120, 145
OPI3		Methyl transferase involved in phosphatidylcholine biosynthesis	132
PDE1		Low-affinity (high-K_m) cAMP phosphodiesterase	150
PDE2	*SRA5*	High-affinity (low-K_m) cAMP phosphodiesterase	176, 227
PMR1		Ca^{2+} transporter involved in protein secretion	172
RAS1		GTP-binding protein that modulates adenylyl cyclase activity	21, 47, 110, 111, 212
RAS2	*CYR3, CTN5*	GTP-binding protein that modulates adenylyl cyclase activity	47, 110, 111, 142, 212
SLK1		Protein kinase exhibiting synthetic lethality with *spa2*	43
SNC1		Synaptobrevin (VAMP) homolog that suppresses loss of CAP-C function when overexpressed	78
SNF1	*CAT1, CCR1*	Protein kinase required for release of gene expression from glucose repression	34, 39, 61, 146, 232
SNF4	*CAT3*	Subunit of Snf1p protein kinase complex necessary for maximal kinase activity	35, 61, 146
SPA2		Protein localized to the site of bud growth that may be involved in bud site selection	65, 188
SRV2	*supC, CAP*	Adenylyl cyclase-associated protein (CAP) involved in activation of adenylyl cyclase by Ras and in A-kinase independent signal transduction	62, 67, 77
TPK1	*SRA3*	Catalytic subunit of A-kinase	33, 211
TPK2		Catalytic subunit of A-kinase	211
TPK3		Catalytic subunit of A-kinase	211
UBC1		Ubiquitin-conjugating enzyme	185
UBI4		Polyubiquitin, a linear fusion of five ubiquitin sequences	69
WHI2		Protein thought to be involved in signal transduction, possibly of glucose repression	143, 144, 177, 195
YAK1		Protein kinase that may antagonize or act downstream of A-kinase	75, 76
YPT1		GTP-binding protein involved in protein transport through the Golgi complex	4, 5, 179, 181

GLC7, and *GAC1* mRNAs, all required for glycogen synthesis, accumulate prior to glucose exhaustion and the diauxic shift, in concert with intracellular glycogen accumulation (73, 100).

The functional relevance of all of these genes to survival of stationary-phase cells has yet to be determined, although both *UBI4* (69) and *HSP104* (175) have been shown to be essential for long-term survival under nutrient-depleted conditions. Some of the genes expressed as cells enter stationary phase encode proteins required for respiration or metabolism of alternative carbon sources. A large percentage of these genes, however, encode proteins required for proteolysis, and many of the heat shock genes encode proteins postulated to be involved in protein stabilization and transport. These results suggest that protein stability and turnover are likely to be important for survival during stationary phase.

One transcript, encoded by the *STI1* gene, exhibits a novel pattern of expression (147). *STI1* mRNA is detectable by hybridization during exponential phase but decreases below the limits of detection during the postdiauxic phase (147). As cells enter stationary phase, *STI1*-encoded mRNA accumulates again. Pathways regulating *STI1* mRNA accumulation have not been identified.

Sti1p was recently identified as a member of a group of proteins that contain a tetratricopeptide repeat, a 34-amino-acid repeat found in a number of proteins required for mitosis, including Cdc16p and Cdc23p from *S. cerevisiae*, *nuc2* from *Schizosaccharomyces pombe*, BimA from filamentous fungi, and other proteins involved in RNA synthesis and splicing, protein synthesis, mitochondrial protein transport, and *Drosophila* development (80, 122). Recently, a human gene that is 42% identical to *STI1* at the amino acid level was cloned (96). Further characterization of *STI1* is

required to determine whether it has a role in growth regulation or survival during stationary phase.

Understanding the function and regulation of genes induced as cells enter stationary phase is clearly essential for elucidating the processes that control entry into stationary phase. Originally, some genes induced after the diauxic shift, i.e., *SSA3, UBI4*, and *CTT1*, were reported to be repressed by activation of the cAMP-dependent protein kinase. However, recent evidence suggests that regulation of these genes may involve other pathways (see "Mutations Affecting Protein Synthesis" below).

Translation and Posttranslational Regulation

The rate of protein synthesis in stationary-phase cells in minimal medium falls to 10% or less of that observed in exponentially growing cells (17), paralleling the decrease in transcription. Although translation of most cellular proteins decreases, two-dimensional gel electrophoretic studies of radiolabeled proteins isolated from cells starved for particular nutrients or cells harboring conditional mutations in genes such as *CDC35* and *CDC25*, with mutant phenotypes that mimic those of starved cells, suggest that as many as 20 different proteins may be preferentially synthesized during entry into the stationary phase (17, 101, 102). Some of these, such as the Hsp70-related heat shock protein Ssa3p (220), and the regulatory subunit of cAMP-dependent protein kinase, Bcy1p (221), which accumulate in stationary-phase cells, are encoded by mRNAs that are detectable in stationary-phase cells. Rigorous characterization of proteins synthesized in stationary-phase cells should be performed. However, these studies are problematic because of the difficulty in labeling stationary-phase cells and because of our limited knowledge of whether cells arrested by different nutrient limitations or conditional mutations enter identical arrest states.

Although most work has focused on transcriptional regulation as cells approach and enter stationary phase, translational and posttranslational regulation has also been shown to occur. For example, although *RAS2* transcripts are not readily detectable by Northern (RNA) analysis after the diauxic shift, the Ras2 polypeptide is synthesized at nearly the same rate during entry into stationary phase as during exponential growth (21, 22). This suggests that the translatability of *RAS2* mRNA increases after the diauxic shift (22).

The transcript encoded by *ENO1*, one of two genes that encode the enzyme enolase of the glycolytic pathway (93), may also exhibit a similar change in translatability. The Eno1 protein (also termed hsp48 for its heat inducibility) (103) is present at higher levels in stationary-phase cells than in proliferating cells (101, 128). Nevertheless, *ENO1* transcript abundance is similar for cells growing on glucose or on nonfermentable carbon sources (128) and is present at detectable levels in stationary-phase cells (104). The increased amount of Eno1p in stationary-phase cells could result from increased translatability of *ENO1* mRNA, although changes in Eno1p stability cannot be ruled out.

Similar observations of increased protein concentration in stationary phase with little variation in mRNA levels have been made for the Bcy1 protein (221). The contributions to the increased Bcy1p accumulation from changes in *BCY1* mRNA translatability and from changes in protein stability have not been determined. The increased Bcy1p accumulation is associated with posttranslational modification of Bcy1p (221), which may influence protein stability (see below).

Posttranslational regulation has also been shown to affect activation of the vacuolar protease B, encoded by *PRB1*, during entry into stationary phase. *PRB1* mRNA accumulates during or immediately after the diauxic shift (140). However, the newly synthesized protease is not activated at that time. Protease B activation, which requires glycosylation and several proteolytic processing events, including carboxy-terminal cleavage (140, 141), is not observed until several hours after *PRB1* mRNA levels increase (139, 140). Now that antibodies to Prb1p are available, it will be of interest to determine whether the timing and kinetics of synthesis of the Prb1 precursor protein correspond to mRNA accumulation or occur later, when active protease is detectable.

Posttranslational Modification

Modifications of Bcy1p occur as cells are grown to stationary phase (221). During exponential phase, one 50-kDa, anti-Bcy1-reactive band is observed by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. During the diauxic shift, at least two additional bands of 52 and 55 kDa are observed and the relative abundance of Bcy1p increases three- to fourfold. After 1 week of incubation of postdiauxic-phase cells, two additional bands are observed, with apparent molecular masses of 59 and 61 kDa. At this time, Bcy1p increases in abundance at least eightfold relative to its level in exponentially growing cells (221). The Yak1p protein kinase, which works antagonistically to cAMP-dependent protein kinase (A-kinase) (75), is necessary for the accumulation of the 59- and 61-kDa Bcy1p isoforms in starved cells (221).

Although the nature and functional implications of Bcy1p modifications are not known, they occur at times when the Bcy1p regulatory subunit is increasingly important to cellular function. Studies with a mutant *BCY1* allele that encodes a more tightly binding regulatory subunit suggest that increasing inhibition of A-kinase activity is required at the diauxic shift, during the diauxic phase, and when cells enter stationary phase, which are all times when dramatic changes in the accumulation of modified Bcy1p isoforms are observed (221). It is possible that the modifications alter the affinity of the regulatory subunit for the catalytic subunit, the localization of the regulatory subunit, or the interactions of the regulatory subunit with other cellular proteins.

STATIONARY PHASE AS A UNIQUE STATE OR STRESS RESPONSE

Until recently it was unclear whether stationary phase was colinear with or an offshoot of the cell cycle (Fig. 2). The isolation and characterization of cells with a novel mutant phenotype, exhibited by *gcs1* and *gcs1 sed1* mutant cells, have now shown that the starvation-induced stationary phase is indeed an off-cycle developmental state, with requirements to resume cell proliferation that are distinct from the requirements to maintain progress through the mitotic cell cycle (57, 103a). By this mutational criterion, therefore, stationary-phase cells are in a unique state and are not arrested at a point on the mitotic cell cycle (Fig. 2).

The original *gcs1 sed1* mutant was isolated serendipitously as a mutant that was cold sensitive only for proliferation from stationary phase (57). Subsequent work has shown that loss-of-function *gcs1* mutant alleles are sufficient to produce this novel mutant phenotype and that a *sed1* mutation is not necessary (103a). Stationary-phase *gcs1* or *gcs1 sed1* cells,

when transferred to fresh medium at the restrictive temperature, are unable to resume cell proliferation (57, 103a). In contrast, exponentially growing *gcs1* or *gcs1 sed1* mutant cells continue to proliferate when transferred to fresh medium at the restrictive temperature. It is important to note that stationary-phase *gcs1* cells do respond to nutrient addition with increased metabolic activity, mobilization of storage carbohydrates, and loss of thermotolerance. Thus, *gcs1* cells undergo physiological changes associated with the resumption of proliferation, even though they are unable to resume proliferation. An order-of-function test has shown that, in some genetic backgrounds, the block to resumption of proliferation prevents mutant cells from reentering the mitotic cell cycle and that cells become blocked before the Start regulatory step (57, 103a).

Although stationary phase has been shown genetically to be a distinct state, the biochemical, morphological, and physiological changes associated with it are clearly not unique. Because some of these changes also occur in response to stresses other than starvation, it is possible that the response to starvation is part of a more general stress response. For example, both heat-shocked exponentially growing cells and stationary-phase cells accumulate trehalose and become thermotolerant (152, 153). Nevertheless, studies of the *gcs1 sed1* mutant indicate that exponentially growing cells that experience a heat shock do not enter stationary phase (56). These results suggest that trehalose accumulation and thermotolerance induction alone are not sufficiently diagnostic of stationary-phase cells.

Morphological changes that result in refractile, phase-light cells, resistant to cell wall-degrading enzymes, are another characteristic of stationary-phase cultures. These types of morphological changes have also been observed in cells growing in glucose-limited, chemostat cultures (27). The presence of the same types of cells both in slowly growing cultures and in stationary-phase cultures suggests either that phase brightness is a characteristic of slowly growing but not necessarily stationary-phase cells or that during suboptimal growth some cells may transiently enter stationary phase. Similarly, it has been hypothesized that the small percentage of constitutively thermotolerant cells in exponentially growing cultures are in stationary phase and, furthermore, that a certain percentage of cells are in stationary phase during the entire culture cycle (163). More rigorous characterization of the putative "stationary-phase" cells in growing cultures must be done to determine whether some cells in a population do respond prematurely to starvation signals.

The ability to survive prolonged periods without added nutrients may be another characteristic of stationary-phase cells. However, this property has not yet been correlated with the genetic test for stationary phase provide by the *gcs1* mutation and has generally been a neglected criterion for stationary phase.

MUTATIONS AFFECTING ENTRY INTO AND MAINTENANCE OF STATIONARY PHASE

Despite the lack of a systematic search for mutants exhibiting defects in entry into stationary phase, a number of mutations affecting this process have been identified. Certain conditional cell cycle mutations, such as *cdc25* and *cdc33* (see below), cause cells to adopt some stationary-phase characteristics even when nutrients are not limiting (165). Other mutations appear to impede the ability to enter stationary phase or to maintain viability during stationary phase. In many cases it is difficult to distinguish between the inability to enter stationary phase, the inability to perform functions necessary for survival during stationary phase, and the inability to resume proliferation from stationary phase. The following sections describe genes involved in these processes.

Mutations Affecting Signal Transduction

Signal transduction pathways provide an essential framework in every living organism for coordination of cellular responses to external stimuli. These pathways are especially important in microorganisms, which must be able to respond in a coordinated manner to changes in their environment, including nutrient depletion. Although a variety of stimuli are known to activate signal transduction pathways in different organisms, the pathways themselves have been remarkably well conserved in eucaryotes.

Two of the best-studied signal transduction pathways in *S. cerevisiae*, the Ras-regulated cAMP-dependent protein kinase (A-kinase) activation pathway (Fig. 3) and the Snf1p protein kinase pathway involved in catabolite derepression, are involved in responding to nutrient depletion. Both of these pathways have been reviewed recently (23, 24, 74, 226). This review does not attempt to cover the material presented in these reviews but emphasizes the role played by A-kinase and Snf1p protein kinase during entry into stationary phase.

Phenotypes of Ras/A-kinase mutants. Phenotypes of strains carrying mutations in the Ras/cAMP pathway can be divided into two categories depending on whether the mutations result in the activation or inactivation of A-kinase (23, 24, 79, 203). Mutants in which A-kinase is inactivated are constitutively thermotolerant, hyperaccumulate glycogen, and, in the case of temperature-sensitive mutants shifted to the nonpermissive temperature, arrest as unbudded cells (25, 109, 212). Mutants in which A-kinase is activated cannot accumulate glycogen, are extremely sensitive to carbon and nitrogen starvation, cannot become thermotolerant, and do not arrest as unbudded cells when deprived of nutrients (33, 110, 210). Diploids in which A-kinase is inactivated sporulate on rich medium, and those in which A-kinase is activated are unable to sporulate (25, 33, 109, 110, 210, 212).

Components of the Ras/A-kinase pathway. *S. cerevisiae* carries two *RAS* genes that encode low-molecular-weight, guanine-nucleotide-binding proteins (G-proteins) (212) (Fig. 3). The *RAS* genes make up an essential gene family (111). The only known downstream effector of the yeast Ras proteins is adenylyl cyclase, which synthesizes cAMP (212). In *S. cerevisiae*, as in most eucaryotic cells, increased intracellular cAMP concentrations activate A-kinase (206).

Like other G-proteins, the activity of the Ras proteins is controlled by the guanine nucleotide that is bound, such that Ras proteins are inactive when bound to GDP and active when bound to GTP (9). The intrinsic GTPase activity of Ras proteins (18) is enhanced by the *IRA* gene products (198, 200–202), which down-regulate Ras activity. The *CDC25* gene product is a guanine nucleotide exchange factor (107) that acts, in opposition to Ira, to activate Ras (25, 170). The balance of Ira and Cdc25p activities controls the guanine nucleotides bound to Ras proteins and, as a consequence, the activity of the Ras proteins (Fig. 3).

Adenylyl cyclase, encoded by the *CYR1* gene, catalyzes the synthesis of cAMP (Fig. 3) (109). Temperature-sensitive alleles of *CYR1* have been identified as *cdc35*, which causes G_1 arrest (19). Adenylyl cyclase is membrane associated (66, 86, 138), in part through interaction with Ira1p (137), and

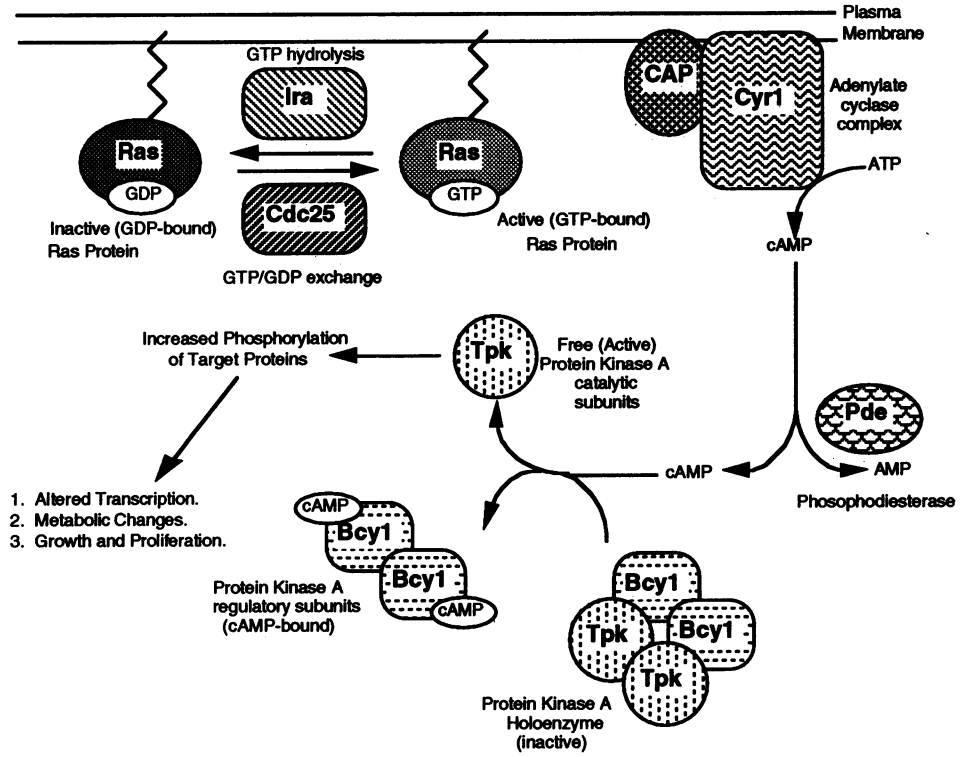

FIG. 3. Ras-activated pathway for regulation of cAMP-dependent protein kinase. Proteins are named for the genes that encode them. Cyr1p is shown as a membrane protein, although Cyr1p (adenylate cyclase) has been shown to be a peripheral membrane protein whose membrane association is regulated during the cell cycle (138). cAMP binds to the regulatory subunit, Bcy1p, which is part of the tetrameric holoenzyme, causing the release of Bcy1p and active catalytic subunits. In stationary phase, when cAMP concentrations are low, catalytic subunits are sequestered in the holoenzyme, inactivating protein kinase activity.

contains potential regulatory domains (42, 63, 87, 111, 125, 214).

The hydrolysis of cAMP is catalyzed by two cAMP phosphodiesterases, a high-affinity (low-K_m) phosphodiesterase encoded by the *PDE2* gene (176, 227) and a low-affinity (high-K_m) phosphodiesterase encoded by the *PDE1* gene (150).

When cAMP concentrations are low, the apparent target of Ras signaling, A-kinase, exists in its inactive form as a holoenzyme complex (206). The A-kinase holoenzyme is composed of two regulatory (R) subunits, encoded by the *BCY1* gene (Fig. 3) (33, 210), and two catalytic (C) subunits, encoded by three related genes, *TPK1*, *TPK2*, and *TPK3* (33, 211). When the intracellular cAMP concentration is high, Bcy1p binds cAMP and dissociates from the complex, releasing active C subunits. Although the exact relationship among the three *TPK* genes is unclear, it is known that any two *TPK* genes are dispensable and that at least one functional *TPK* gene is necessary for viability (211).

Activated *RAS* alleles such as $RAS2^{val19}$, which encodes a protein with impaired GTPase activity; loss-of-function mutations in the *IRA* genes, such as the *ccs1-1* allele of *IRA2*; and *bcy1* mutants all cause increased sensitivity to carbon and nitrogen starvation (58, 200, 202, 212). Strains carrying these mutations exhibit the same phenotypes as do cells with unregulated A-kinase activity. Similarly, *ras2* mutant cells exhibit phenotypes typical of strains with down-regulated A-kinase, with the exception that *ras2* mutants cannot grow on nonfermentable carbon sources (204). The probable basis for this phenotype is that the Ras1 protein is not synthesized when strains are grown on nonfermentable carbon sources and that *ras1 ras2* mutants are inviable (22). Temperature-sensitive mutants such as *cdc25* and $ras1\ ras2^{ts}$ exhibit G_1 arrest in a manner similar to strains carrying $cdc35/cyr1^{ts}$ alleles (22, 23).

cAMP-independent regulation of entry into stationary phase. Despite the apparent importance of regulated A-kinase activity in the proper response to nutrient signaling, recent results suggest that entry into stationary phase is probably regulated by something other than A-kinase (32). tpk^w ("wimpy") alleles, thought to encode catalytic subunits with attenuated kinase activity, have been identified that suppress *bcy1* mutant phenotypes and can restore relatively normal responses to starvation in *bcy1* cells. The responses to nutrient deprivation by *bcy1* tpk^w mutant cells are also independent of the products of *CYR1*, *RAS1*, *RAS2*, and *CDC25* genes (32). Accumulation of glycogen and development of thermotolerance occurs normally in *bcy1* tpk^w strains, indicating that regulation of these processes during entry into stationary phase requires low levels of A-kinase activity but not cAMP-dependent regulation of A-kinase activity. It can be concluded from these results that down-regulation of A-kinase activity is necessary but probably not sufficient for regulation of entry into stationary phase.

Interactions with other pathways. In addition to the evidence for cAMP-independent, Ras-independent regulation of entry into stationary phase, there is some evidence that the *RAS* signaling pathway can also function in a cAMP-

independent manner. Unfortunately, cAMP-dependent pathway activity is likely to mask these signals, even in *bcy1 tpk*^{w} mutants, making them difficult to detect.

CAP. The CAP protein, a 70-kDa protein physically associated with the adenylyl cyclase complex, provides the first evidence for a cAMP-independent pathway associated with Ras (67). The *SRV2* gene, which encodes the CAP protein, was identified by suppression of the heat-shock sensitivity caused by the activated *RAS2*^{val19} allele (62). CAP is a bifunctional protein for which the N-terminal portion has been shown to enhance Ras-mediated stimulation of adenylyl cyclase activity (77). Ras activation of adenylyl cyclase is not completely dependent on CAP, however, since cells lacking CAP are viable (109). Deletions of the CAP carboxyl-terminal domain result in several phenotypes, including sensitivity to nitrogen starvation, the inability to grow on rich medium (77), morphological defects including the formation of extremely rounded and large cells (67, 77), cytoskeletal abnormalities (218), and a random budding pattern (218). Mutations that suppress *cyr1* mutations do not suppress the *cap* carboxyl-terminal mutations, indicating that CAP-mediated responses to nitrogen starvation are independent of adenylate cyclase activation (77).

The *SNC1* gene, which is a high-copy-number suppressor of loss of CAP carboxyl-terminal function, encodes a yeast homolog of an integral membrane protein, called synaptobrevin (VAMP), found in synaptic vesicles (78). Suppression of the loss of CAP carboxyl-terminal function by overexpression of *SNC1* is dependent on the presence of activated alleles of *RAS2*, confirming the involvement of Ras proteins in the CAP carboxyl-terminal-dependent pathway.

YAK1. *YAK1*, encoding a nonessential protein kinase, was identified as a suppressor of the growth arrest of *ras1 ras2*^{ts} mutants at a restrictive temperature (75). Loss of *YAK1* activity also suppresses the lethality caused by the deletion of all three *TPK* genes, although this suppression is not complete, since cells lacking both functional *TPK* and *YAK1* genes still hyperaccumulate glycogen, one of the phenotypes of strains carrying *TPK* deletions (75). Yak1p is required for the stationary-phase modifications and accumulation of Bcy1p (221) and the stationary-phase induction of the *CTT1* gene (76). Overexpression of Yak1p kinase results in enhanced expression of *CTT1* during exponential phase and inhibits the growth of *ras1 ras2*^{ts} cells and *bcy1 tpk*^{w} cells (76). These and other findings suggest that Yak1p protein kinase is an inhibitor of proliferation and an antagonist of the A-kinase pathway (76).

SNF1. The Snf1p protein kinase has been implicated in the phenomenon of catabolite derepression. Mutations in the *SNF1* (*CCR1*/*CAT1*) gene were first identified because they prevent expression of the *SUC2* invertase gene and generally prevent catabolite derepression (39, 61, 146). Snf1p has been shown to interact with the Snf4 protein, and mutations in *SNF4* cause similar but less severe phenotypes than do mutations in *SNF1* (35, 146). More recent genetic studies demonstrate a functional interaction between the Snf1p protein kinase and components of the Ras/cAMP pathway.

Loss of Snf1p function prevents many of the normal responses to nutrient depletion, including glycogen accumulation, acquisition of thermotolerance, and maintenance of viability (208). Mutations affecting adenylyl cyclase or Ras2p, alterations that diminish A-kinase activity (208), or overexpression of Pde2p, a phosphodiesterase that reduces intracellular cAMP concentrations (99), all attenuate the *snf1* mutant phenotype. Interactions between the Ras/A-kinase pathway and Snf1p are also evident in the virtual inviability of *snf1 bcy1* double-mutant cells under normal growth conditions, and the unusually slow growth of *snf1 RAS2*^{val19} double-mutant cells (208). These observations all suggest that Snf1p protein kinase and A-kinase have antagonistic effects and that the balance between the activities of Snf1p protein kinase and A-kinase is important for adaptations to changing nutrient conditions (208).

It is clear that Snf1p interacts at some level with the Ras/A-kinase signal transduction pathway. However, cells lacking Snf1p and functional A-kinase catalytic subunits (*tpk1 tpk2 tpk3 yak1* cells) are unable to accumulate glycogen or grow on acetate-based rich medium (208). Because the phenotype of these cells is more like that of *snf1* cells than of *tpk1 tpk2 tpk3 yak1* cells, this result suggests that Snf1p can also act independently of the RAS-activated, cAMP-dependent signaling pathway (208).

Mutations Affecting Protein Synthesis

***ILS1* and general control.** The temperature-sensitive *ils1-1* mutation in the gene encoding isoleucyl-tRNA synthetase (85, 134, 136) blocks cell proliferation and brings about certain stationary-phase characteristics, including maintenance of viability (102, 148). These properties of *ils1-1* mutant cells are acquired through the action of general control (92), a global regulatory system that coordinates the expression of amino acid biosynthetic genes in response to amino acid deprivation. General control has been suggested to respond, through the Gcn2p protein kinase, to an accumulation of uncharged tRNAs (55, 219). The viability of *ils1-1* mutant cells is compromised by *gcn1*, *gcn2*, and *gcn3* mutations, which impair general control (148), suggesting that general control is involved in *ils1-1*-mediated cell arrest. However, general control is neither activated (149) nor necessary for viability (148) upon starvation for nitrogen or other central nutrients. This may indicate that specialized regulatory systems such as general control come into play only for certain types of nutrient deprivation.

General control and normal responses to nutrient deprivation may be affected by mutations of a novel type, termed *crl* (cycloheximide resistant lethal) (130, 131). The complex *crl* phenotype suggests that mutants are unable to derepress amino acid biosynthetic pathways and resembles that of *gcn* mutants in the impaired G_1 arrest upon amino acid starvation but not upon nitrogen starvation. Other aspects of the *crl* phenotype, including temperature sensitivity, arrest as unbudded cells, and glycogen hyperaccumulation upon exhaustion of the carbon source, have not been reported for *gcn* mutants. The functions of *CRL* gene products are not yet known, but they may affect ribosome function (130). It is not known whether these mutations affect viability during stationary phase. However, a suppressor of *crl3* lies in the *PRS2* gene, encoding an essential subunit of the proteosome (118), the large multicatalytic proteinase complex that functions in ubiquitin-mediated protein degradation (184). The implications of this finding for *CRL* gene product activity and for stationary phase are not yet clear. Mutations in the ubiquitin pathway are discussed below.

***GCD* and *CDC33*: global translation.** Mutations in *GCD* genes constitutively derepress general control (92) through increased efficiency of translation of the *GCN4* mRNA, which encodes a transcription activator. The *gcd* mutant phenotype has also been observed in strains carrying mutations in genes required for translation initiation (225). Mutations in these genes often confer temperature sensitivity that is independent of *GCN4* (83). The temperature-sensitive *gcd1*

and *gcd2* mutations decrease the rate of amino acid incorporation (91, 92, 231) and block cells in G_1 (82, 231). In this arrested state, *gcd1* mutants retain their viability for extended periods (91), suggesting that they arrest in stationary phase.

The *CDC33* gene encodes eukaryotic initiation factor 4E (eIF-4E) (20), the small cap-binding subunit of translation factor eIF-4F (169). The temperature-sensitive *cdc33-1* mutation causes cells to acquire stationary-phase characteristics (101, 102, 217). The role of Cdc33p in translation initiation suggests that *cdc33* mutations may bring about arrest in stationary phase through inhibition of the synthesis of certain key proteins. Cells containing *cdc33-1* and other temperature-sensitive *cdc33* alleles (2) do in fact show allele-specific effects on newly synthesized proteins and transcript-specific alterations in the efficiency of translation (9a).

Mutations Affecting Protein N-Terminal Acetylation

Mutations in two genes affecting protein N-terminal acetylation, *ARD1* and *NAT1*, eliminate the response to starvation signals. *ard1* and *nat1* haploids cease proliferation when starved without the acquisition of stationary-phase characteristics (120, 145, 223), whereas diploids harboring these mutations cannot undergo sporulation (145, 223).

The *ARD1* and *NAT1* genes are also required for repression of the cryptic mating-type information at the *HML* locus, resulting in a mating defect for *MAT***a** haploids (145, 222). This mating defect is distinct from the stationary-phase phenotype, since the mating defect is suppressed by deleting or placing *MAT***a** information in the *HML* locus, whereas the responses to nutrient depletion are unchanged in this background (222).

The *NAT1* gene is thought to encode the catalytic subunit of a protein acetyltransferase, since a polypeptide corresponding in size to Nat1p copurifies with protein acetyltransferase activity (119) and the *NAT1*-encoded polypeptide shows similarities to the bacterial *cat*-encoded protein, chloramphenicol acetyltransferase (145). Nat1p and Ard1p have also been shown to associate in a complex with protein acetyltransferase activity (154).

The basis for the stationary-phase phenotypes of these mutants is not known. It is possible that N-acetylation of a regulatory protein is required during starvation-induced arrest (145).

Mutations Affecting Protein Turnover

The control of protein degradation plays an important role in the regulation of cellular function, including the regulation of proliferation and the ability to survive starvation (69). One of the best-studied proteolytic systems involves ubiquitin, a highly conserved 76-residue protein (151) that is conjugated to other proteins as a signal for degradation. The ubiquitin system has recently been reviewed (68, 166).

The *UBI4* gene encodes polyubiquitin, which is a natural gene fusion of five ubiquitin sequences (69). *UBI4* is a heat shock gene that is expressed at all times in the culture cycle. However, *UBI4* is expressed at higher levels after the diauxic shift and as cells approach stationary phase, whereas the other three ubiquitin genes are expressed only during exponential phase (69, 199).

ubi4 mutants are unable to maintain viability during stationary phase or on starvation for nitrogen or carbon (69, 199). On starvation, *ubi4* mutants do not arrest as unbudded cells (199) and are constitutively thermotolerant (69). The latter findings suggest that the *UBI4* gene is required for cessation of proliferation and that either growth arrest or subsequent proteolytic events are essential for maintenance of stationary phase (69).

The *UBC1* gene, which encodes a ubiquitin-conjugating enzyme, is also induced during growth to stationary phase, and *ubc1* mutants are slow to resume growth from stationary phase (185). Ubc1p is a member of a subfamily of ubiquitin-conjugating enzymes that includes the products of the *UBC4* and *UBC5* genes (185). *ubc1 ubc4* double mutants grow more slowly than either of the single-mutant strains, and *ubc1 ubc4* spores fail to germinate, further underscoring the importance of ubiqutin-conjugating activity early in reproliferation from an arrested state (185).

Although these results suggest that protein turnover is important for survival during stationary phase, specific proteolytic targets involved in cell survival have not been identified. Ubiquitin has been hypothesized to be involved in processes in addition to proteolysis (68). Thus, the identification of the targets of ubiquitination would greatly aid in evaluating the role of ubiquitin-mediated proteolysis in the process of entry into and survival during stationary phase.

Mutations Affecting Protein Secretion

YPT1. Two proteins required for secretion are also necessary for the maintenance of viability in stationary phase. One of these, the *YPT1* gene product, is a small GTP-binding protein (179) that is found associated with the Golgi apparatus (181). Ypt1p is necessary for transport from the endoplasmic reticulum through the Golgi complex both in vivo (181) and in vitro (4, 5). On nitrogen starvation, but not carbon starvation, the essential Ypt1p protein (178, 180) is necessary for maintenance of viability following normal accumulation of unbudded cells (180). The loss of Ypt1p function is suppressed by extra gene copies encoding synaptobrevin-like membrane proteins, the *SLY2* and *SLY12* (*BET1*) genes (46). Interestingly, CAP carboxyl-terminal deletion mutants (see above) are also suppressed by increased dosage of a synaptobrevin-like protein, *SNC1* (78).

PMR1. Viability in stationary phase is also compromised by disruption of the *PMR1* gene, which encodes one of at least two proteins that resemble transmembrane Ca^{2+} ion pumps (172). Like *YPT1*, *PMR1* also affects transit from the endoplasmic reticulum to the Golgi complex, which may reflect calcium homeostasis. The slow growth of *pmr1* mutants is suppressed by increasing external calcium levels (178); analogous partial suppression by increased calcium levels is also seen for *ypt1* mutant cells. In contrast, the viability loss of *pmr1* mutants is actually exacerbated rather that alleviated by increased external calcium concentrations. *PMR1* gene disruption suppresses the cold sensitivity of a *ypt1* mutation, a finding that has prompted the suggestion that *pmr1* mutations lead to the bypass of a segment of the secretory pathway (172).

Mutations Affecting Membrane Biosynthesis

OPI3. The *OPI3* gene functions in the final methylation reactions during phosphatidylcholine biosynthesis (132). Mutations in *OPI3* impair the growth of mutant cells in media unsupplemented with choline or immediate precursors; however, more significantly, they cause cells to lose viability when the growth medium has been exhausted (132). This loss of viability is attributed to abnormal membrane lipid composition in *opi3* mutant cells. The reasons for the

enhanced sensitivity of *opi3* mutants during stationary phase is not clear, but results clearly suggest that membrane structure is important for survival during stationary phase.

It should be pointed out that the loss of viability by *opi3* mutant cells in stationary phase differs fundamentally from the viability loss suffered by cells defective in phosphoinositol biosynthesis. *ino1* mutants are auxotrophic for inositol, and mutant cells lose viability without inositol (45). Under these conditions, however, *ino1* mutant cells die while undergoing active growth and cell division and do not enter stationary phase (88). In fact, stationary-phase *ino1* cells, or mutant cells starved for a variety of nutrients, are spared from inositol-less death (81, 89, 90).

Mutations Affecting Cell Polarity

SPA2. In proliferating yeast cells, new cell surface growth of budded cells is highly polarized and is confined to the tip of the enlarging bud (65). The Spa2 protein is localized to the growing bud tip, and *spa2* mutant cells exhibit altered patterns of bud formation (188). This mutant phenotype suggests that Spa2p is involved in the bud site selection. The Spa2p may also influence overall growth responses, because *spa2* mutant cells also exhibit aberrant phenotypes in stationary phase. After exhausting the growth medium (probably by carbon source depletion), *spa2* mutant cells remain budded to a greater extent than do wild-type cells and are about twofold less thermotolerant than are wild-type controls (188). These findings suggest that *spa2* cells are marginally defective for entry into stationary phase.

SLK1. A more severe defect for entry into stationary phase is conferred by loss of Slk1p protein kinase activity. The *SLK1* (synthetic lethal kinase) gene was identified by a mutation that makes mutant cells dependent on *SPA2* for growth (43). Strains lacking *SLK1* function have a pleiotropic phenotype, including temperature sensitivity and morphological defects, but, more importantly here, fail to respond properly to nutrient deprivation (43). *slk1* mutants are partially rescued by overexpression of the *SSD1/SRK1* gene, which encodes a protein involved in protein phosphatase activity (197). Enhanced protein dephosphorylation mediated by increased *SSD1* activity may therefore allow mutant cells without Slk1p protein kinase to survive starvation. If so, the protein kinase counteracted by increased *SSD1* activity cannot be the Slk1 protein kinase but may be the A-kinase. Extra *SSD1* gene copies also suppress some of the aberrant responses to nutrient depletion in strains carrying mutations in *pde2* (phosphodiesterase), *bcy1* (A-kinase regulatory subunit), and *ins1* (a gene product that appears to be involved in cell cycle regulation at the level of DNA replication) (228). The starvation defects of *slk1* mutant cells, coupled with the suppression of these defects and those caused by increased A-kinase activity, by a few extra copies of *SSD1* suggests that Slk1p and the A-kinase have opposing functions for nutrient signaling (43). Thus, Slk1p protein kinase and A-kinase could well constitute yet another pair of protein kinases with antagonistic effects during times of nutrient deprivation.

Other Types of Mutations

HSP104. *HSP104* was first identified as a yeast heat shock gene required for complete induction of thermotolerance at 37°C (174). The Hsp104 protein, a member of the ClpA/ClpB family from *Escherichia coli*, contains two nucleotide-binding regions that are essential for thermotolerance functions, which are postulated to involve protein folding (155). Stationary-phase cells carrying a null mutation in *HSP104* suffer increased mortality on heat shock and are marginally impaired in the retention of viability in stationary phase, which, on long-term incubation, leads to significant differences between mutant and wild-type populations (175).

WHI2. Cells harboring mutations in the *WHI2* gene produce daughter cells that are smaller than normal and mother cells that bud at decreasingly small cell sizes (195). The *whi2* mutant phenotype is evident only after the diauxic shift, requires aerobic growth conditions and a gluconeogenic carbon source, and is thought to result from excess cell division which cannot be supported by the available carbon source (144). In the postdiauxic phase, *whi2* cells do not arrest proliferation in concerted fashion, become thermotolerant, resistant to lytic enzymes, or accumulate storage carbohydrates (143, 177). These results suggest that the *WHI2* gene acts as a component of a nutritional signal transduction pathway.

WHI2 mRNA is not detectable after the diauxic shift but is easily detected during the exponential phase (143). However, the decrease in *WHI2* mRNA abundance does not seem to be due to glucose depletion at the diauxic shift, since experiments performed in the presence of glucosamine, a gratuitous inducer of glucose repression, demonstrated that *WHI2* mRNA accumulation correlates with the rate of proliferation rather than the level of glucose repression (144). *whi2* mutants also show an increased resistance to glucosamine, suggesting that *WHI2* functions in glucose repression. Consistent with this hypothesis, *whi2* mutants exhibit increased respiration during growth on glucose, and cells overexpressing *WHI2* exhibit decreased respiration and are unable to grow on glycerol (144).

KEM1. Mutations in the *KEM1* gene were initially identified because they exacerbate the defect in nuclear fusion caused by *kar1* mutations (113). From this finding it was suggested that the Kem1 protein may have a role in the relay of nutritional information to the spindle pole body, the structure that is affected by *kar1* mutations (171). The Kem1 protein is necessary for sporulation at elevated temperatures (60, 113, 209) and for good spore viability (209), consistent with a role for Kem1p in nutrient signaling. It is not clear whether, in *kem1* mutants, sporulation itself is affected or whether the cells are simply unable to survive in sporulation medium.

Additional mutant phenotypes show that Kem1 protein is involved in other processes not obviously related to nutrient signaling (112). The *KEM1* gene, also cloned as *RAR5*, *DST2*, *SEP1*, and *XRN1* (60, 114, 117, 209), also affects the mitotic transmission of plasmids (114), and encodes a 5′-to-3′ exonuclease (192) with RNase H activity (193) that can catalyze a DNA strand transfer reaction (59, 115). These observations suggest many possible physiological roles for the gene product (112).

RVS161. Mutants lacking the *RVS161* gene are sensitive to nitrogen, carbon, and sulfur starvation (44). These mutants are also sensitive to high salt conditions and are unable to grow on nonfermentable carbon sources but are essentially wild type with respect to glycogen accumulation and heat shock sensitivity. Mutant cells, starved or incubated with amino acid analogs or salts, exhibit dramatic, dimorphic morphologies, with large cells two to three times larger than small cells and a high percentage of multibudded cells. *RSV161* encodes a novel 30-kDa protein that appears by DNA hybridization analysis to be a member of a gene family (44).

REGULATION OF STATIONARY-PHASE GENE EXPRESSION

Because the process of entry into stationary phase itself is poorly defined, it is not surprising that the regulation of stationary-phase gene expression is also not well defined. To begin to identify pathways that regulate gene expression during entry into stationary phase, we need to consider what is known about regulation of gene expression during the postdiauxic phase.

Several pathways appear to regulate glucose derepression, which occurs at the diauxic shift. The best studied of these is regulated by the *SNF1*-encoded protein kinase. *SNF1* is allelic to *CCR1* and *CAT1* (38, 61). Strains carrying mutations in *snf1* cannot derepress *SUC2* expression after glucose exhaustion (146). Other genes regulated by Snf1p include *GAL1* and *ADH2* (see below). Regulation of glucose derepression by Snf1p has been reviewed recently (74, 213, 226). Although a number of *snf1* mutations and suppressor (*SSN*) mutations have been isolated, a clear picture of the mechanism of glucose repression and catabolite derepression in *S. cerevisiae* has yet to be developed.

It is likely that one difficulty in establishing a coherent signal transduction pathway for catabolite derepression is due to the complexity of carbon source regulation. For example, the *GAL1* promoter has at least two upstream activating sequence (UAS) elements, named UASg and URSg, involved in catabolite repression but regulated somewhat independently (71). UASg requires *GAL4* for derepression; URSg is *GAL4* independent. URSg requires the *URR1, GAL82*, and *HXK2* gene products for repression; UASg does not. Despite these differences, there is some overlap in the requirements of these UASs. Both elements require *SNF1* for *GAL1* derepression and *GAL83*, *REG1*, *GRR1*, and *SSN6* for repression (71). On the basis of these results, it is understandable why a signal transduction pathway for glucose repression was not identified by classical genetic analysis and that a clear picture of glucose regulation will require molecular analysis of other glucose-regulated promoters.

ADH2

ADH2, which is one of two genes encoding alcohol dehydrogenase, is induced after the diauxic shift and, like *GAL1*, exhibits a complex pattern of regulation of gene expression (226). *ADH2* is known to be positively regulated by Snf1p and negatively regulated by A-kinase, independently of Snf1p activation (48–51). A-kinase inhibition, which accounts for only a small portion of glucose-mediated repression, occurs via the Adr1p transcriptional activator (36, 49). In addition to negative regulation by A-kinase, *ADH2* is also negatively regulated by the transcription factors encoded by *ADR4*, *CRE1*, and *CRE2* (226). Sch9p, a kinase which, when overexpressed, can compensate for the lack of A-kinase activity, acts as a positive regulator of *ADH2* expression (49).

Interestingly, the transcriptional activator Adr1p, which is phosphorylated by A-kinase, is also regulated at the level of translation. Adr1 protein concentrations correlate directly with *ADH2* expression, and within 1 h of glucose depletion, Adr1p synthesis increases 10- to 16-fold (216). This increase in Adr1p occurs concurrently with an increase in *ADH2* expression. In addition to its involvement in *ADH2* expression, Adr1p is required for expression of genes required for peroxisomal function (186) and for factors essential for growth on nonfermentable carbon sources (12).

These results indicate that *ADH2* is not unique among glucose-regulated genes in its complexity of regulation. However, because so much is known about *ADH2* regulation, it may provide a good model for understanding regulation of glucose-repressed genes and especially for understanding the interactions between Snf1p-regulated gene expression and A-kinase-regulated gene expression.

The complexity of promoters of glucose-regulated genes suggests that the ability to respond to changing carbon sources, especially from fermentable to nonfermentable carbon sources, has exerted strong selective pressure over time. Because of the importance of these metabolic switches, it is not too surprising that there are multiple layers of control in this process. Understanding the coordination of these controls is a significant but difficult goal for biologists studying yeast cells.

Several genes induced after the diauxic shift are also induced in adenylyl cyclase mutants when exogenous cAMP is limiting. These genes are not induced in *bcy1* strains, lacking the regulatory subunit of A-kinase (11, 13, 14). Originally these results were interpreted to mean that decreasing cAMP concentrations, which are known to occur at the diauxic shift (72), mediate alterations in gene expression, presumably though effects on altered A-kinase activity. With the availability of *bcy1 tpk1^{w}* mutant strains, lacking two of the three A-kinase catalytic subunits and harboring an attenuated or "wimpy" form of the third A-kinase catalytic subunit (32), evidence has accumulated that the down-regulation of A-kinase activity is necessary but not sufficient for this expression. In *bcy1 tpk1^{w}* mutant strains, mRNAs accumulate for both the *HSP70*-related *SSA3* and *CTT1*, encoding the cytoplasmic catalase gene, suggesting that gene products other than A-kinase can regulate this expression (11, 219a).

SSA3

Characterization of the promoter of the *SSA3* gene led to the identification of a UAS, named the PDS (postdiauxic shift) element, which regulates accumulation of *SSA3* transcripts after the diauxic shift (14, 15). The PDS element is also found in *CTT1* and *UBI4* promoters (11, 199). The gene products directly involved in the induction of *SSA3* expression have not been identified.

HSP26

Mutational analysis has shown that the *HSP26* promoter, like that of the *SSA3* gene, is regulated at the transcriptional level by repression as well as by activation (196). The sequences responsible for increased expression during the stationary phase were shown to be redundant in the *HSP26* promoter and could not be separated experimentally from the sequence elements responsible for increased expression on heat shock (196).

UBI and *UBC* Genes

A family of genes encoding ubiquitin-conjugating enzymes directs a posttranslational modification of proteins by covalently attaching the small protein ubiquitin to the targeted protein. Two members of one subfamily of ubiquitin-conjugating enzymes, *UBC5* and *UBC1*, are induced in stationary-phase cells (183, 185).

Ubiquitin itself is encoded by four genes, but only the *UBI4* gene, encoding a polyubiquitin protein, is induced in

the stationary phase (69, 151). Further work has shown that *UBI4* is induced by reduced cAMP concentrations, most probably through the action of A-kinase (199).

CTT1

S. cerevisiae contains two different catalase activities, the peroxisomal catalase A encoded by the *CTA1* gene and the cytoplasmic catalase encoded by *CTT1* (41, 173, 190, 191). Both catalase genes encode the apoproteins of hemeprotein catalases that are regulated by glucose, heme, and oxygen (97). The *CTT1* transcript accumulates in response to nitrogen, sulfur, or phosphorus starvation (13) and on heat shock (11).

Transcriptional control of the *CTT1* gene involves synergistic interactions between heme, cAMP, and heat shock. Heme and, in an indirect manner, oxygen control of the *CTT1* gene has been shown to be mediated by the Hap1 protein through an upstream element showing a surprisingly low degree of similarity to other Hap1p-binding sequences (191, 229). A putative PDS element similar to that identified in the *SSA3* promoter (14) has been identified in an upstream region that is necessary for proper response to nitrogen starvation (11). Like the *SSA3* gene, the PDS element upstream of *CTT1* is flanked by a sequence having similarity to canonical heat shock elements, which may account for the heat shock induction of *CTT1* (11).

CTT1 mRNA also shows a significant increase in accumulation during starvation in a *bcy1 tpk*^{w} strain lacking the regulatory subunit of the protein kinase A, indicating that *CTT1* mRNA accumulation is at least partially cAMP independent (11). Overexpression of the *YAK1* gene has been shown to cause a 50-fold increase in postdiauxic *CTT1* mRNA accumulation (76).

CONCLUSIONS

The process of entry into stationary phase is a complex, poorly understood, and inherently interesting part of the yeast life cycle. The *gcs1* mutant phenotype provides genetic evidence that stationary phase is a unique developmental state and a genetic assay for attainment of stationary phase (57).

Although it is clear that stationary phase is a unique state, no single change that is uniquely characteristic of stationary phase has been identified. We propose that the ability to maintain viability without added nutrients is likely to be the most rigorous physiological definition of entry into stationary phase.

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