Transcriptional Regulation in Yeast during Diauxic Shift and Stationary Phase

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Abstract

The preferred source of carbon and energy for yeast cells is glucose. When yeast cells are grown in liquid cultures, they metabolize glucose predominantly by glycolysis, releasing ethanol in the medium. When glucose becomes limiting, the cells enter diauxic shift characterized by decreased growth rate and by switching metabolism from glycolysis to aerobic utilization of ethanol. When ethanol is depleted from the medium, cells enter quiescent or stationary phase G_0 . Cells in diauxic shift and stationary phase are stressed by the lack of nutrients and by accumulation of toxic metabolites, primarily from the oxidative metabolism, and are differentiated in ways that allow them to maintain viability for extended periods of time. The transition of yeast cells from exponential phase to quiescence is regulated by protein kinase A, TOR, Snf1p, and Rim15p pathways that signal changes in availability of nutrients, converge on transcriptional factors Msn2p, Msn4p, and Gis1p, and elicit extensive reprogramming of the transcription machinery. However, the events in transcriptional regulation during diauxic shift and quiescence are incompletely understood. Because cells from multicellular eukaryotic organisms spend most of their life in G_0 phase, understanding transcriptional regulation in quiescence will inform other fields, such as cancer, development, and aging.

Diauxic Shift and Stationary Phase as a Stress Response

CELLS FROM MULTICELLULAR EUKARYOTIC ORGANISMS spend most of their life in a resting state referred to as quiescence or G_0 . Because quiescent cells do not divide and the entry and exit from quiescence are regulated processes, understanding the molecular mechanisms of quiescence will inform other fields, such as cancer, development, and aging.

Yeast cells, like metazoan cells, are able to exit the proliferating state and to enter quiescence. Although quiescent yeast and metazoan cells share many important characteristics, they differ in one important aspect. Unlike many types of quiescent metazoan cells, quiescent yeast cells display significantly reduced metabolic rate. However, despite this difference, yeast cells represent an excellent model for studying quiescence. This review focuses on transcriptional regulation during the diauxic shift and stationary phase in budding yeast *Saccharomyces cerevisiae*, from now on referred to only as yeast.

The preferred sources of carbon and energy for yeast cells are fermentable sugars, such as glucose. When yeast cells are grown in liquid cultures in rich media containing glucose,

they metabolize glucose predominantly by glycolysis, releasing ethanol in the medium. When glucose becomes limiting, the cells enter a diauxic shift characterized by decreased growth rate and by switching metabolism from glycolysis to aerobic utilization of ethanol. When ethanol is depleted from the medium and no other carbon source is available, cells enter quiescent or stationary phase G_0 . If yeast cells are starved for nitrogen or phosphate, they also enter quiescence; however, it is not known if the cells develop the same characteristics as if they are starved for carbon source (Gray et al., 2004; Herman, 2002; Werner-Washburne et al., 1993; Zhang et al., 2009). Nutrient deprivation of diploid cells does not necessarily lead to quiescence. When yeast diploid cells grow on nonfermentable carbon source such as acetate, and are starved for nitrogen, they enter meiosis and sporulate (Kupiec et al., 1997).

Cells in the diauxic shift and stationary phase are stressed by the lack of nutrients and by accumulation of toxic metabolites, primarily from the oxidative metabolism, and are differentiated in ways that allow to maintain their viability for extended periods without added nutrients. Some of the changes in the stationary phase cells resemble the changes in cells undergoing stress response, such as induction of

heat-shock genes *HSP26*, *HSP12*, *HSP82*, *HSP104*, *SSA3*, and accumulation of trehalose (Gray et al., 2004; Herman, 2002; Werner-Washburne et al., 1993).

Physiological Changes during the Diauxic Shift and Stationary Phase

Stationary-phase cells are unbudded and contain unreplicated nuclear DNA. The overall transcription rate is about three to five times lower than in cells growing exponentially (Choder, 1991), and translation is reduced to about 0.3% (Fuge et al., 1994). Stationary-phase cells exhibit thick cell walls (DeNobel et al., 2000) and contain more abundant mitochondria (Stevens, 1981) and morphologically distinct condensed chromosomes (Pinon, 1978). The chromatin compaction in stationary phase requires the linker histone Hho1p (Schafer et al., 2008). As cells approach the diauxic shift and stationary phase, they start accumulating storage molecules glycogen, trehalose, triacylglycerols, and probably also polyphosphate (Hosaka and Yamashita, 1984; Lillie and Pringle, 1980; Taylor and Parks, 1979; Thevelein, 1984) and induce autophagy (Noda and Ohsumi, 1998). Although glycogen accumulation occurs before depletion of glucose and peaks at the diauxic shift, trehalose is accumulated after the diauxic shift early in the stationary phase (Lillie and Pringle, 1980). The pattern of glycogen accumulation and mobilization suggest that it is the storage polysaccharide. Trehalose, on the other hand, is believed to protect cells against different stresses (Panek and Panek, 1990; Wiemken, 1990). Stationary cells are also more resistant to heat and osmotic shocks (Plesset et al., 1987), as well as to treatment with toxic drugs (DeNobel et al., 2000). Stationary cells, unlike exponential cells, are able to survive for extended periods of time without nutrients (Lillie and Pringle, 1980); however, they are able to mount a similar transcriptional response to different stresses

as exponential cells (Jelinsky et al., 2000), and they are able to rapidly induce transcription of many genes upon refeeding (Martinez et al., 2004). In order for cells entering the stationary phase to develop these "resistance" characteristics, they have to properly sense imminent depletion of a key nutrient and uniformly arrest in the unbudded state. Not all nutritional stresses elicit this developmental pathway into quiescence. When auxotrophic cells are starved for the required amino acid, they fail to arrest uniformly as unbudded cells and rapidly lose viability (Saldanha et al., 2004).

Some of the features of yeast cells in the stationary phase will be probably revisited in the future and characterized in more detail, because it was shown that stationary phase yeast cultures are heterogenous and contain at least two different cell types: quiescent and nonquiescent cells (Allen et al., 2006). Quiescent cells are dense, unbudded, replicatively younger, and are able to synchronously reenter mitotic cell cycle. Nonquiescent cells are less dense, accumulate more reactive oxygen species, and display reduced ability to reenter mitotic cell cycle. The term quiescence thus should not be equated with stationary phase and results obtained with stationary phase cells should not be automatically considered representative of quiescent cells.

Signal Transduction Pathways Regulating Entry into the Diauxic Shift and Stationary Phase

The exit of yeast cells from the exponential phase to the diauxic shift and stationary phase is regulated by protein kinase A (PKA), TOR, Snf1p, and Rim15p signaling pathways (Fig. 1). These pathways signal changes in availability of nutrients during transition from the exponential phase when glucose is abundant to the diauxic shift when cells utilize ethanol and to the stationary phase when no carbon source is available (Wilson and Roach, 2002). PKA and Tor pathways

FIG. 1. Overview of signaling pathways that control transcription during transition from exponential growth to diauxic shift and stationary phase. PKA and TORC1 converge and negatively regulate Rim15p and transition to diauxic shift and quiescence. The key targets of this pathway are transcriptional factors Msn2p/Msn4p and Gis1p. Snf1p is active when glucose is exhausted and regulates expression of genes involved in catabolism of nonfermentable carbon sources. Only the basic scheme is shown and the text provides more detail.

are negative regulators and Rim15p and Snf1p pathways are positive regulators of transition into the diauxic shift and stationary phase.

Protein kinase A pathway

PKA occupies a crucial position in response of cells to glucose and couples cell cycle progression and growth. PKA in yeast cells, like in all other eukaryotes, is a heterotetramer, composed of two catalytic subunits and two regulatory subunits. The catalytic subunits are encoded by three closely related genes, *TPK1*, *TPK2*, and *TPK3*. The regulatory subunit is encoded by *BCY1*. cAMP binds to the inhibitory subunit Bcy1p, and this interaction dissociates Bcy1p from catalytic subunits Tpk1/2/3p and thus activates them.

Adenylate cyclase (Cyr1p) and production of cAMP in *Saccharomyces cerevisiae* are regulated by two pathways (Santangelo, 2006; Zaman et al., 2008). The first pathway involves Gpa2p, a yeast homolog of the G α subunit of heterotrimeric G proteins. Gpa2p is coupled to a cell surface receptor Gpr1p and acts downstream from Gpr1p (Lorenz et al., 2000; Xue et al., 1998). Gpa2p is negatively regulated by two functionally redundant kelch proteins Gpb1p/Krh2p and Gpb2p/Krh1p (Harashima and Heitman, 2002, 2005). In addition, Gpa2p is negatively regulated by Rgs2p, a GTPase activating protein (Kraakman et al., 1999; Versele et al., 1999).

The second pathway that regulates Cyr1p and cAMP synthesis involves Ras1p and Ras2p (Powers et al., 1984). Ras2p is the major regulator of Cyr1p (Santangelo, 2006). Ras proteins are monomeric GTPases that are active in the GTP-bound state and inactive when GDP is bound. In the active state, the Ras proteins activate Cyr1p and stimulate cAMP synthesis. Ras proteins are negatively regulated by Ira1p and Ira2p, two GTPase-activating proteins (GAPs) that stimulate the intrinsic GTPase activity of Ras (Tanaka et al., 1990, 1991). On the other hand, Ras proteins are activated by Cdc25p and Sdc25p, two guanine nucleotide exchange factors (GEFs) that facilitate replacement of Ras-bound GDP for GTP (Boy-Marcotte et al., 1996; Broek et al., 1987; Damak et al., 1991).

PKA has a key inhibitory role in transition from exponential growth to diauxic shift and stationary phase. Cells with increased PKA activity fail to acquire the characteristics typical of stationary-phase cells, such as resistance to high temperature and osmotic stress, and die early in the stationary phase. Conversely, cells with decreased PKA signaling display these stationary phase characteristics even when glucose is abundant (Santangelo, 2006; Tatchell, 1986; Thevelein and de Winder, 1999; Zaman et al., 2008). The PKA targets most relevant for entry in the diauxic shift and stationary phase include transcriptional factors Msn2p (Görner et al., 1998, 2002; Smith et al., 1998) and protein kinase Rim15p (Reinders et al., 1998) (Fig. 1). The transcriptional complex Ccr4p–Not is also a likely target of PKA (Lenssen et al., 2002, 2005). In addition, Srb9p and perhaps other subunits of the Mediator complex of the RNA polymerase II complex as well as proteins that interact with the C-terminal domain (CTD) of Rpb1p are also targets of PKA signaling (Chang et al., 2004; Howard et al., 2001, 2002, 2003).

TOR pathway

Tor1p and Tor2p (target of rapamycin) in yeast are two partially redundant protein kinases similar to phosphatidyl-

inositol 3-kinase (PI3 kinase). All eukaryotes contain ortholog of TOR gene; however, higher eukaryotes possess only one TOR gene. In *S. cerevisiae*, Tor2p, together with five other proteins, comprise the TORC2 complex that regulates actin cytoskeleton and cell polarity (De Virgilio and Loewith, 2006; Wullschlegel et al., 2005). Tor1p or Tor2p and three other proteins comprise TORC1, which regulates cell growth and transition from logarithmic growth to the diauxic shift and stationary phase. TORC1, unlike TORC2, is inhibited by rapamycin. Rapamycin binds to peptidyl-prolyl *cis/trans* isomerase FKBP12, and the resulting complex then binds and inhibits TORC1. Activity of TORC1 responds to growth conditions and availability of nutrients, primarily a nitrogen source. Inhibition of TORC1 by rapamycin or nitrogen starvation results in decreased protein synthesis, induction of autophagy, and entry in the G₀ state. Interestingly, both treatments activate several stress-responsive transcriptional factors and cause very similar reprogramming of gene expression (Shamji et al., 2000). Both rapamycin treatment and nitrogen starvation inhibit expression of ribosomal protein genes (Shamji et al., 2000) but induce expression of stress-inducible genes, as well as genes of the nitrogen discrimination pathway (NDP) and retrograde (RTG) response pathway. The RTG pathway is induced by poor sources of nitrogen and allows expression of the first three enzymes of the citric acid cycle that are required for synthesis of α -ketoglutarate that is subsequently used for synthesis of glutamate (Zaman et al., 2008).

The two major targets of TORC1 are Tap42p, a regulator of protein phosphatase 2A (PP2A), and protein kinase Sch9p (Fig. 1). The binding of Tap42p to the catalytic subunits of PP2A and PP2A-like phosphatases is regulated by TORC1-mediated phosphorylation. When TORC1 is inhibited by rapamycin, Tap42p becomes dephosphorylated and dissociates from the catalytic subunits (Di Como and Arndt, 1996; Jiang and Broach, 1999). The current model posits that the Tap42–PP2A complex associates with TORC1 on cellular membranes, and nitrogen starvation or rapamycin treatment dissociates the Tap42–PP2A complex from TORC1 and releases it into the cytosol, where Tap42p gradually becomes dephosphorylated and dissociates from PP2A (Yan et al., 2006). Interestingly, Tap42 also dissociates from PP2A when cells enter the diauxic shift (DiComo and Arndt, 1996; Duvel and Broach, 2004; Jacinto et al., 2001). Inhibition of TORC1 function activates in a Tap42-dependent manner Gln3p and Gat1p transcriptional factors, resulting in upregulation of genes of the nitrogen discrimination pathway (NDP) and carbon discrimination pathway (CDP), and genes of the Krebs cycle (Shamji et al., 2000; Tate et al., 2002). NDP and CDP are induced by poor sources of nitrogen and carbon, respectively (Cooper, 2002). However, genome-wide expression analysis of *tap42* and PP2A mutants suggested that in addition to protein phosphatases TORC1 has an additional target (Duvell et al., 2003). This additional target was identified as the yeast ortholog of the mammalian PKB/Akt kinase Sch9 (Urban et al., 2007). Sch9 is one of the regulators of Start of the cell cycle and links ribosome biosynthesis and cell size (Jorgensen et al., 2002, 2004). Deletion of *SCH9* arrests cells at a very small size and carbon starvation or inhibition of TORC1 by rapamycin induces Sch9 dephosphorylation and inactivation (Jorgensen et al., 2004). Inactivation of Sch9 triggers nuclear translocation of protein kinase Rim15p that regulates the

entry into quiescence (Cameroni et al., 2004; Swinnen et al., 2006). The roles of Tap42p and Sch9p account for majority of TORC1 cellular signaling (Rohde et al., 2008).

Several additional targets of TORC1 were identified. TORC1 inhibits the activity of Apg1p, a protein kinase involved in regulation of autophagy. This inhibition may occur by TORC1-mediated phosphorylation of Apg13p, a regulator of Apg1p (Kamada et al., 2000). Another possible downstream target of TORC1 is perhaps PKA (Schmelzle et al., 2004); however, other data suggest that TORC1 and PKA function in parallel pathways (Chen and Powers, 2006; Zurita-Martinez and Cardenas, 2005). Protein kinase C (Pkc1p) is inhibited by TORC1 and is required for viability in stationary phase (Krause and Gray, 2002).

Snf1p pathway

Snf1p is a yeast ortholog of mammalian AMP-activated protein kinase (AMPK). Snf1p is a catalytic subunit of a complex, including regulatory subunit Snf4p and one of three additional subunits: Gal83p, Sip1p, and Sip2p. The Snf1p complex is active in the absence of glucose and inactive during growth on glucose, and mutants lacking *SNF1* cannot grow on carbon sources other than glucose and die shortly after diauxic shift (Fig. 1). The regulatory subunit Snf4p binds Snf1p and alleviates its autoinhibition when glucose is absent (Hardie et al., 1998; Hedbacker and Carlson, 2008; Sanz, 2003).

It appears that Snf1p activates transcription by both regulating the transcription factors and by inducing chromatin remodeling. The downstream targets of Snf1p include transcriptional repressor Mig1p and activator Adr1p. Mig1p represses target genes in the presence of glucose and the repression is alleviated by Snf1p-mediated phosphorylation (Schuller, 2003). The transcriptional activator Adr1p activates transcription of genes involved in catabolism of non-fermentable carbon sources (Tachibana et al., 2005; Young et al., 2003). During exponential growth on glucose, Adr1p is negatively regulated by PKA. When glucose is exhausted at the diauxic shift, Snf1p activates Adr1p; however, the mechanism of Adr1p regulation by PKA and Snf1p is not well understood (Schuller, 2003). Snf1p also regulates transcriptional factors Hsf1p and Msn2p in response to carbon stress (De Wever et al., 2005; Hahn and Thiele, 2004; Sanz, 2003). In addition to regulating transcriptional factors, Snf1p also regulates the chromatin structure of target promoters. Upon glucose depletion, Snf1p phosphorylates serine 10 on histone H3 with concomitant recruitment of the SAGA complex and acetylation of histone H3 K14 (Lo et al., 2001). Snf1 is also required for glucose depletion-induced targeting of the histone acetyltransferase SAGA complex to promoters of glucose transporters *HXT2* and *HXT4* (van Oevelen et al., 2006).

Rim15p pathway

Rim15p is a member of PAS family of protein kinases. Deletion of *RIM15* leads to stationary phase defects, including failure to arrest at the G_1 phase of the cell cycle and to induce expression of stress-inducible genes, reduced thermotolerance, and decreased accumulation of trehalose and glycogen (Reinders et al., 1998). On the other hand, overexpression of *RIM15* results in appearance of cellular changes characteristic of cells in the stationary phase during exponential phase growth (Reinders et al., 1998). These results

suggest that Rim15p positively regulates entry into G_0 (Fig. 1). Rim15p's activity is inhibited by PKA-mediated protein phosphorylation (Reinders et al., 1998), and its nucleocytoplasmic distribution is regulated by the Sch9 branch of the TORC1 pathway and by the Pho80p–Pho85p pathway (Pedruzzi et al., 2003; Swinnen et al., 2006; Urban et al., 2007; Wanke et al., 2005). The nucleocytoplasmic distribution is mediated by Rim15p's binding site for the 14-3-3 protein Bmh2p, which retains Rim15p in the cytoplasm (Vidan and Mitchell, 1997). The cytoplasmic retention of Rim15p requires Pho80p–Pho85p-mediated phosphorylation of Thr1075 in the Rim15p's binding site for Bmh2p. Inhibition of TORC1 results in dephosphorylation of Thr1075 with concomitant nuclear translocation of Rim15p (Pedruzzi et al., 2003; Wanke et al., 2005). Sch9p is also required for anchoring Rim15p in the cytoplasm, presumably by phosphorylating an additional Ser/Thr residue in the binding site for Bmh2p (Wanke et al., 2005).

Stationary Phase as a Model for Aging

The signal transduction pathways that are responsible for orchestrating the transition from exponential growth to the diauxic shift and stationary phase also affect aging of yeast cells. Aging in yeast is described either as chronological or replicative aging. Chronological aging is defined as the time nondividing cells can survive and is typically measured as viability of cells during stationary phase. Replicative aging is defined as the number of daughter cells a mother cell can produce (Blagosklonny and Hall, 2009; Chen et al., 2005; Gershon and Gershon, 2000). Downregulating the PKA, TORC1, and Sch9p pathways extends chronological as well as replicative life span (Fabrizio et al., 2001; Longo, 1999; Powers et al., 2006), partially by increasing cellular protection against oxidative stress through activation of *SOD2*, encoding mitochondrial superoxide dismutase (Fabrizio et al., 2003). The promoter of *SOD2* contains both STRE and PDS elements and is likely regulated by Msn2p/Msn4p and Gis1p (Fabrizio et al., 2003). Deletion of *RIM15* and *MSN2/MSN4* abolishes the increased life span of cells with mutations in the PKA and Sch9 pathways (Wei et al., 2008). These results indicate that the longevity of yeast cells is regulated by the signaling and transcription mechanisms that are instrumental for transition to quiescence.

Transcriptional Regulation at the Diauxic Shift and Stationary Phase

Transcriptional factors Msn2p/Msn4p and Gis1p

The targets of Rim15p include three transcriptional factors: Msn2p, Msn4p, and Gis1p. These factors mediate the Rim15p effect on transcription during a diauxic shift. The functions of Msn2p/Msn4p and Gis1p partially overlap, and deletion of all three genes causes synthetic growth defect during growth on nonfermentable carbon sources (Roosen et al., 2005).

Msn2p, together with its partially redundant homolog Msn4p, are zinc-finger transcription factors that play an important role in transcriptional response to starvation and other forms of environmental stress (Estruch, 2000; Estruch and Carlson, 1993; Martinez-Pastor et al., 1996). Transcriptional activation of Msn2p-dependent genes is very complex. Msn2p is regulated by nuclear translocation (Beck and Hall,

1999; Chi et al., 2001; Görner et al., 1998, 2002) or by increased binding of Msn2p to the STRE elements in the promoters of stress-responsive genes (Hirata et al., 2003). PKA negatively regulates transcription of Msn2p-dependent genes by phosphorylating Msn2p and keeping it in the cytosol (Görner et al., 1998, 2002; Smith et al., 1998). Low level of PKA activity is associated with increased nuclear localization of Msn2p and transcriptional activation of Msn2p-dependent genes, whereas Msn2p is translocated to the cytoplasm upon PKA activation. Subcellular localization of Msn2p is also regulated by the TORC1 pathway. TORC1 promotes interaction of Msn2p with the 14-3-3 protein Bmh2p that acts as a cytosolic anchor of Msn2p (Beck and Hall, 1999). Rapamycin treatment or growth conditions that result in TORC1 inactivation result in translocation of Msn2p to the nucleus. In addition, inactivation of *SRB10* compromises nuclear exclusion of Msn2p in unstressed cells (Chi et al., 2001). Four yeast orthologs of GSK-3 protein kinase encoded by *MCK1*, *RIM1*, *MRK1*, and *YGK3* do not affect nucleocytoplasmic distribution of Msn2p, but regulate binding of Msn2p to the STRE element (Hirata et al., 2003). Changes in chromatin structure also do not affect nucleocytoplasmic distribution, but may facilitate recruitment of Msn2p to target promoters (Lindstrom et al., 2006). It appears that Msn2p is also regulated by other, not so clearly understood, mechanisms (Durchschlag et al., 2004; Garreau et al., 2000; Lallet et al., 2004; Lenssen et al., 2005; Mayordomo et al., 2002), including nucleocytoplasmic shuttling (Garmendia-Torres et al., 2007; Jacquet et al., 2003).

Gis1 was identified as a high-copy number suppressor of the defect to express *SSA3* in *rim15* Δ cells. Epistasis analysis indicates that *Gis1p* acts in the PKA pathway downstream of *Rim15p*. *Gis1p* binds to the postdiauxic shift (PDS) element that is present in promoters of genes that are induced by glucose exhaustion at the diauxic shift (Pedruzzi et al., 2000). Msn2p/Msn4p and *Gis1p* are not functionally equivalent and are able to differentiate between similar STRE and PDS promoter elements (Pedruzzi et al., 2000). Expression of *Gis1p*-dependent genes is regulated by the PKA, Sch9, and TORC1 pathways and depends on *Rim15p* (Cameroni et al., 2004; Pedruzzi et al., 2000, 2003; Roosen et al., 2005; Zhang et al., 2009). The mechanism of *Gis1p* regulation by these or other pathways has not been elucidated yet. Recent data indicate that the activity of *Gis1p* is regulated by proteasome-mediated proteolysis to ensure that all *Gis1p*-dependent transcription is under *Rim1p* control (Zhang and Oliver, 2010).

General transcription machinery and chromatin structure

Yeast cells, like all other eukaryotes, contain three distinct DNA-dependent RNA polymerases that transcribe different sets of genes. RNA polymerase I (Pol I) transcribes rDNA into 35S precursor ribosomal RNA. RNA polymerase II (Pol II) transcribes protein-encoding genes, and RNA polymerase III (Pol III) synthesizes 5S ribosomal RNA and tRNAs (Thuriaux and Sentenac, 1992). Much of the total transcription in exponentially growing cells is devoted to biosynthesis of ribosomes: ribosomal RNA accounts for about 60% of total transcription and ribosomal proteins account for about 50% of Pol II transcription. The approximate distribution of RNA in exponentially growing yeast cells is 80% ribosomal RNA, 15% tRNA, and 5% mRNA (Venema and Tollervey, 1999; Warner,

FIG. 2. Comparison of the transcriptional effects of diauxic shift, histone H4 mutations (H4 K5,8,12R), and *MSN2* overexpression. For the comparisons we have used published data sets (Dion et al., 2005; Gasch et al., 2000; Radonjic et al., 2005). The Venn diagram shows the extend of overlap between genes that are upregulated at diauxic shift (Radonjic et al., 2005), genes that are upregulated by *MSN2* overexpression (Gasch et al., 2000), and genes that are upregulated in a strain harboring mutant form of histone H4 (K5,8,12R) (Dion et al., 2005). The numbers of transcripts that are increased twofold or more relative to a wild-type strain grown exponentially are shown. For the genes upregulated by diauxic shift we used the data set for culture grown for 26 h (Radonjic et al., 2005).

1999; Woolford and Warner, 1991), and synthesis of ribosomes consumes about 90% of the total cellular energy (Warner et al., 2001). The transcription by all three RNA polymerases is under the control of the signal transduction pathways discussed above, and during diauxic shift and especially stationary phase significantly decreases. Pol I-mediated transcription of ribosomal RNA is regulated by

FIG. 3. Comparison of the transcriptional effects of diauxic shift, stationary phase, and histone H4 mutations (H4 K5,8,12R). For the comparisons we have used published data sets (Dion et al., 2005; Radonjic et al., 2005). The Venn diagram shows the extend of overlap between genes that are upregulated at diauxic shift and stationary phase (Radonjic et al., 2005), and genes that are upregulated in a strain harboring mutant form of histone H4 (K5,8,12R) (Dion et al., 2005). The numbers of transcripts that are increased twofold or more relative to a wild-type strain grown exponentially are shown. For the genes upregulated by diauxic shift and stationary phase we used the data sets for cultures grown for 26 and 212 h, respectively (Radonjic et al., 2005).

TORC1 (Claypool et al., 2004; Li et al., 2006). Pol III-mediated transcription of tRNA and 5S ribosomal RNA is regulated by TORC1 by both the Sch9-dependent and Sch9-independent mechanism (Johnson, 2010; Wei and Zheng, 2009). The TORC1 regulation of Pol III transcription by the Sch9-dependent mechanism is mediated by modulating the phosphorylation state of a transcriptional repressor Maf1p. Phosphorylation of Maf1p is also regulated by other mechanisms, including PKA (Moir et al., 2006; Oficjalska-Pham et al., 2006; Roberts et al., 2006).

The events in regulation of Pol II transcription downstream of the signaling pathways that occur during transition from the exponential growth to the diauxic shift and stationary phase are beginning to emerge, primarily due to the DNA microarray expression profiling (Causton et al., 2001; De Risi et al., 1997; Gasch et al., 2000; Martinez et al., 2004; Radonjic et al., 2005; Shivaswamy and Iyer, 2008). The results demonstrate that during the diauxic shift, the cells reprogram their transcription machinery, which results in increased expression of hundreds of genes (De Risi et al., 1997; Radonjic et al., 2005); many of them, but not all, are targets of Msn2p/Msn4p (Fig. 2). When cells reach the stationary phase, the number of upregulated genes decreases to about 100, whereas the majority of genes are significantly downregulated (Radonjic et al., 2005). Most of the genes that are upregulated in the stationary phase experience increased transcription already at the diauxic shift, and only very few genes display increased transcription only after passage through diauxic shift (Fig. 3). Interestingly, detailed and careful genome-wide location analysis (ChIP on chip) showed that the general transcription machinery is present but inactive in stationary-phase cells (Radonjic et al., 2005). RNA polymerase II is loaded upstream of several hundreds genes, poised for transcriptional induction that is required for exit from the stationary phase when nutrients become available. However, the mechanism that targets RNA Pol II to the promoters of genes needed for exit from the stationary phase is not known (Radonjic et al., 2005).

Interestingly, we have noticed a significant correlation between the set of genes upregulated at the diauxic shift with the set of genes upregulated in a strain with mutations in histone H4 (Fig. 2). Histone H4 carries several acetylable lysine residues in its N-terminal tail that are targets of histone acetyltransferases. Mutational change of these lysine residues to nonacetylable arginines results in alterations of the transcriptional profile (Dion et al., 2005). The comparison of the transcriptional profile of cells at the diauxic shift with the profile of strain-bearing mutations in the acetylable lysines in histone H4 indicate that many of the genes that are induced at the diauxic shift are repressed during exponential growth by histone H4 acetylation, and the derepression at the diauxic shift is associated with a decreased level of histone H4 acetylation. This putative mechanism requires experimental verification; however, it can be potentially significant, because availability of nucleocytosolic acetyl-CoA regulates histone acetylation (Takahashi et al., 2006) and the level of nucleocytosolic acetyl-CoA may reflect metabolic changes during the diauxic shift (Friis et al., 2009; Ramaswamy et al., 2003; Sandmeier et al., 2002). This notion is in agreement with finding that the level of acetylated histone H3 and H4 decreases as cells enter the diauxic shift and stationary phase (Ramaswamy et al., 2003; Sandmeier et al., 2002), and that the addition of glucose to quiescent cells induces acetylation of

both histones H3 and H4. This acetylation is mediated by two major histone acetyltransferases: SAGA and NuA4 (Friis et al., 2009). Histone acetylation could thus provide an additional fine-tuning of the transcriptional reprogramming during the diauxic shift and connect the metabolic state of the cell with a chromatin structure and transcription (Friis and Schultz, 2009; Ladurner, 2009).

We can only speculate about the mechanism by which histone acetylation regulates expression of some of the genes that are induced at the diauxic shift. As suggested by Lindstrom et al. (2006), decreased histone acetylation in the corresponding promoters may create a chromatin environment that facilitates recruitment of Msn2p/Msn4p and assembly of the preinitiation complex. Alternatively, acetylation of histones H3 and H4 during exponential phase may facilitate recruitment of bromodomain-containing chromatin remodeling complexes, such as RSC, that establish repressive chromatin structure in the corresponding promoters (Mujtaba et al., 2007; Vignali et al., 2000). Decreased histone acetylation at the diauxic shift and stationary phase would then release the repressive chromatin structure and allow transcription.

The transition of yeast cells from exponential growth to the diauxic shift and stationary phase is orchestrated by a network of signaling pathways that reprogram the transcription machinery. Although many of the genes that are induced at the diauxic shift are regulated by Msn2p/Msn4p and Gis1p transcription factors, additional mechanisms probably exist. It is tempting to speculate that histone acetylation connects the metabolic state of the cell with the chromatin structure and contributes to transcriptional regulation during transition from exponential phase to quiescence.

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