2 The environmental stress response: a common yeast response to diverse environmental stresses

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Abstract

Unicellular organisms require specific internal conditions for optimal growth and function, however sudden changes in the external environment can perturb the internal milieu, disrupting normal processes. Therefore, cells must maintain their internal system despite fluctuations in the external surroundings. One mechanism that yeast cells use to protect the internal system from the effects of environmental variation is to initiate a common gene expression program that generally protects the cell during stressful times. This program, referred to as the environmental stress response, includes ~900 genes whose expression is stereotypically altered when yeast cells are shifted to stressful environments. The coordinated expression changes of these genes is a common feature of the responses to many different environments, however the regulation of these expression changes is gene-specific and condition-specific, indicating that initiation of the program is precisely controlled in response to each new environment. This review will focus on recent developments in defining and characterizing the genes that participate in the environmental stress response and the regulatory mechanisms that the cell utilizes to orchestrate this program.

2.1 Introduction

Microorganisms must have specific and delicately balanced internal conditions for optimal growth and function. The internal milieu of the cell is maintained to promote proper operation of the cell, however fluctuations in the external environment can result in a variety of cellular perturbations that can disrupt the internal environment. These perturbations can prevent optimal enzyme activities, disrupt metabolic fluxes, destabilize cellular structures, perturb chemical gradients, etc., leading to overall instability. Thus, cells must be able to protect and maintain the

critical features of the internal homeostasis in the face of variable external conditions.

Yeast cells have evolved to be exceptionally proficient at surviving sudden and often harsh changes in their external environment. In the wild, yeast cells must contend with fluctuations in temperature, osmolarity, and acidity of their environment, the presence of radiation and toxic chemicals, and long periods of nutrient starvation. Growth under these various conditions requires maintenance of the internal system, however the cellular program required for its maintenance differs depending on the external challenges that the cell must deal with. Thus, when environmental conditions change abruptly, the cell must rapidly adjust its internal milieu to that required for growth at the new conditions.

Details regarding the mechanisms that the yeast *S. cerevisiae* uses to adapt to new environments have been emerging over the years. Yeast cells gain cross protection against different stresses, evident by the fact that cells exposed to a mild dose of one stress become resistant to large, normally lethal doses of other stresses (for example Mitchel and Morrison 1982; Blomberg et al. 1988; Wieser et al. 1991; Flattery-O'Brien et al. 1993; Lewis et al. 1995). This observation sparked the idea that yeast cells use a general mechanism of cellular protection that is provoked when cells are exposed to stressful stimuli. Concordant with this model was the realization that a set of so-called "heat shock" genes was induced not only by temperature shock but also by other stressful environmental changes, hinting that the genes played a more general role in protecting the cell in response to stressful environments (Kurtz et al. 1986; Werner-Washburne et al. 1989; Kobayashi and McEntee 1990; Susek and Lindquist 1990). Although these observations suggested a general stress response in yeast, the role and regulation of this response remained obscure.

Subsequently, it became apparent that the expression of the stress-induced genes was controlled by a common mechanism. A number of studies identified a sequence element common to the promoters of the stress-induced genes, referred to as the Stress Response Element (STRE), strongly suggesting that these genes were coregulated by a common factor (Kobayashi and McEntee 1990; Kobayashi and McEntee 1993; Marchler et al. 1993). The hypothetical STRE binding factor was proven to be either of two related zinc-finger transcription factors, Msn2p and Msn4p (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). Deletion of these factors renders cells sensitive to a variety of stressful conditions, and it was shown that Msn2p and Msn4p govern the induction of a large number of genes in response to many different stresses (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996; Boy-Marcotte et al. 1998; Moskvina et al. 1998). Thus, these factors became known as the "general stress" transcription factors and were proposed to be generically activated in response to cellular stress to induce a set of genes that defend against environmental insult. However, it was noted that under certain conditions the genes identified as targets of these factors were normally induced regardless of *MSN2* and *MSN4* deletion, thereby hinting that the regulation of the stress response was more complicated than the initial model suggested (Schuller et al. 1994).

The recent increase in popularity of whole-genome studies is expanding our definition and understanding of yeast stress responses. Studies characterizing genomic transcript abundance and global protein synthesis levels allow the exploration of these aspects of the cellular responses of yeast cells to environmental changes. Using DNA arrays, the relative transcript levels of all genes in an organism's genome can be rapidly quantified, and computational analysis of the resulting genomic expression data can implicate gene function and regulation while providing insights into the overall physiological response of the cell (Fodor et al. 1993; Pease et al. 1994; Shalon et al. 1996; Eisen et al. 1998; Brown and Botstein 1999). Large-scale changes in protein synthesis can be measured by two-dimensional electrophoresis of pulse-labeled proteins, complementing gene expression studies and adding additional levels of detail about the protein repertoire in the cell (Blomberg 1995; Norbeck and Blomberg 1996; Godon et al. 1998; Lee et al. 1999a; Appella et al. 2000). These types of global studies have provided insights into the mechanisms that yeast use to defend themselves against environmental insult. Many of the observed cellular responses are specifically triggered to counteract features that are unique to each environment. The reader is directed to other chapters in this book that review the specialized yeast responses to a number of environmental stresses that are prevalent in nature. In addition to these specialized responses, global studies have identified the players in a common response to environmental stresses while providing insights into the complicated regulation of this cellular program. This review will focus on recent advances in defining and studying the common yeast response to stressful environments while summarizing existing literature on the genes and proteins that participate in and regulate this program.

2.2 The environmental stress response

Characterization of the genomic expression programs in yeast responding to different environmental conditions revealed that a substantial fraction of each of the responses is not specific to the stimulus but instead represents a common response to all of the conditions tested. In a study conducted with colleagues, we used DNA microarrays to identify approximately 900 genes whose expression was stereotypically altered in *S. cerevisiae* responding to a variety of stressful environmental transitions (Gasch et al. 2000). (The complete list of the genes that participate in this response can be found at http://www-genome.stanford.edu/yeast_stress). These genes fell into two groups based on their expression patterns (Fig. 2.1): one group consisted of genes whose transcript levels increased in abundance in response to the environmental changes, and the other group was comprised of genes whose transcript levels decreased following environmental stress. (For the purposes of this review, genes whose transcript levels increase in response to environmental change will be referred to as induced, while genes whose transcript levels decrease will be referred to as repressed. It is important to note that the observed changes in transcript levels can be mediated by alterations in transcript

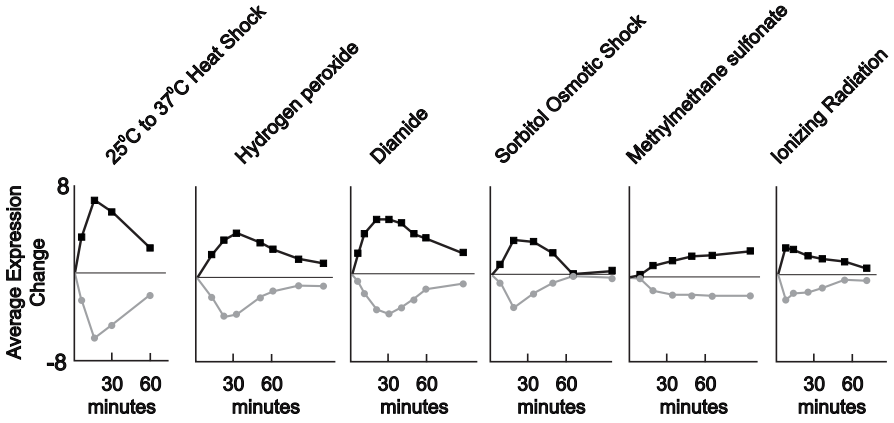

Fig. 2.1. Gene expression patterns in the ESR. The average gene expression changes of the genes whose expression is induced (—■—) and repressed (—●—) in the ESR in response to 25°C to 37°C heat shock, 0.3 mM hydrogen peroxide, 1.5 mM diamide, 1M sorbitol, 0.02% methylmethane sulfonate, and 170 Gray of ionizing radiation. The same scale is used for all of the plots shown. Data were taken from Gasch et al. 2000 and Gasch et al. 2001. The complete list of the genes that participate in this response can be found at http://www-genome.stanford.edu/ yeast_stress

synthesis as well as transcript degradation; therefore, the terminology used here to indicate the increases or decreases in gene expression is not intended to imply any mechanism in the alteration of transcript abundance.) The two groups of genes that participate in the common gene expression response displayed nearly identical but opposite patterns of expression in response to the environmental shifts. This strongly suggests that the expression changes were coordinately regulated. A similar common gene expression response was also identified in a study by Causton et al. (2001). Since then, these gene expression changes have been observed in the cellular response to many environmental conditions, corroborating the commonality of the program (Table 2.1). Remarkably, the genes that participate in this response amount to ~14% of the currently predicted genes in the yeast genome (Ball et al. 2000; Blandin et al. 2000).

Exploration of the genes involved in this response revealed that many of the induced genes are targets of Msn2p and/or Msn4p (Msn2/4p) and had already been implicated in a general stress response in yeast (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). However, characterization of the common gene expression program distinguished it from the previously described Msn2/4p-dependent response in a number of ways. First, the common gene expression program encompasses nearly 900 genes and includes not only induced genes but also hundreds of genes whose expression is repressed in response to environmental changes. Second, as discussed below in a subsequent section, although many of the induced genes are regulated by Msn2/4p under certain conditions, the coordinate expression changes of these genes extends beyond Msn2/4p control. Furthermore, detailed characterization of the regulation of this response revealed that

it is not controlled by a general regulatory mechanism, but rather is mediated by condition-specific signaling pathways. Despite similarities to the previously defined "general" stress response, the coordinate increases and decreases in the expression of the genes in this program were referred to as the environmental stress response (ESR) (Gasch et al. 2000). For consistency, the terminology will be maintained in this review.

2.3 Responsiveness of ESR gene expression

Each genomic expression program triggered by environmental change is unique to the specific features of the new conditions in terms of the genes affected and the magnitude and choreography of their expression, indicating that the cell precisely responds to the distinctive challenges of each new environment (see other chapters in this book). Nonetheless, the bulk of each genomic expression program is accounted for by the genes in the ESR. The ESR is initiated in response to a wide variety of environmental transitions, as indicated by the stereotyped alterations in expression of the genes in this response (Table 2.1). Although this program is commonly initiated in response to these diverse conditions, the precise levels and timing of the gene expression changes appear to be specific to the features of each new environment (Fig. 2.2), hinting at the sensitivity with which the program is regulated.

Like the overall genomic expression responses, initiation of the ESR is often transient: immediately after the shift to a new environment, the cell responds with large changes in the expression of genes in the ESR, however over time the differences in expression usually subside, and transcript levels return to near pre-stress levels (Fig. 2.1) (Gasch et al. 2000; Causton et al. 2001). This observation is in line with previous observations of transient gene expression changes in response to stress (for example Parrou et al. 1997; Parrou et al. 1999; Rep et al. 1999). The transient changes in gene expression may help the cell to rapidly adjust the concentrations of the corresponding gene products to the levels required for growth at the new conditions (discussed further below, see Fig. 2.9). According to this model, the transient pattern of gene expression represents an adaptation phase during which the cell initiates the optimization of its internal milieu before resuming growth. An important exception to this observation is the case of nutrient starvation, in which the cells do not resume growth but enter a quiescent state until nutrients become available (see Chapter 7); consistently, the gene expression response to nutrient starvation involves large gene expression changes that are not transient but instead persist until starvation is alleviated (Gasch et al. 2000).

The magnitude of the expression changes of genes in the ESR is graded to the severity of the environmental shock. Populations of cells experiencing larger doses of stress respond more strongly than cells experiencing subtle environmental changes (Gasch et al. 2000). For example, cells exposed to a 25°C to 37°C heat shock will show larger and more prolonged changes in gene expression before ad-

Table 2.1. Environmental transitions that lead to ESR initiation[a]

Environment	References
Temperature Shocks	
Heat shock	(Boy-Marcotte et al. 1999; Gasch et al. 2000; Causton et al. 2001)
Ethanol Shock	(Alexandre et al. 2001)
pH Extremes	
Acid	(Causton et al. 2001)
Alkali	(Causton et al. 2001)
Oxidative and Reductive Stress	
Hydrogen Peroxide	(Godon et al. 1998; Gasch et al. 2000; Causton et al. 2001)
Menadione	(Gasch et al. 2000)
Diamide	(Gasch et al. 2000)
Cadmium	(Momose and Iwahashi 2001)
DTT	(Gasch et al. 2000; Travers et al. 2000)
Hyper-Osmotic Shock	
Sorbitol	(Gasch et al. 2000; Rep et al. 2000; Causton et al. 2001)
Potassium Chloride	(S.M. O'Rourke and I. Herskowitz, personal communication)
Sodium Chloride	(Posas et al. 2000; Rep et al. 2000; Causton et al. 2001; Yale and Bohnert 2001; S.M. O'Rourke and I. Herskowitz, personal communication)
Starvation	
Stationary Phase	(Fuge et al. 1994; Gasch et al. 2000)
Amino Acid Starvation	(Gasch et al. 2000; Natarajan et al. 2001)
Nitrogen Starvation	(Gasch et al. 2000)
Phosphate Starvation	(Ogawa et al. 2000)
Zinc Starvation	(Lyons et al. 2000)
Respiration	
Petite mutants	(Traven et al. 2001)
Diauxic Shift Transition	(Fuge et al. 1994; DeRisi et al. 1997)
Nonfermentable Carbon Sources	(Kuhn et al. 2001)
Diverse Drug Treatments	(Hughes et al. 2000b)
Long-term Exposure to alpha Factor	(Spellman et al. 1998)
DNA-Damaging Agents	
Alkylating Agents	(Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Natarajan et al. 2001)
Ionizing Radiation	(Gasch et al. 2001)
Double-strand Breaks	(Lee et al. 2001b)

[a] This table lists conditions that trigger initiation of the ESR, as monitored in global studies of genomic expression and translation initiation.

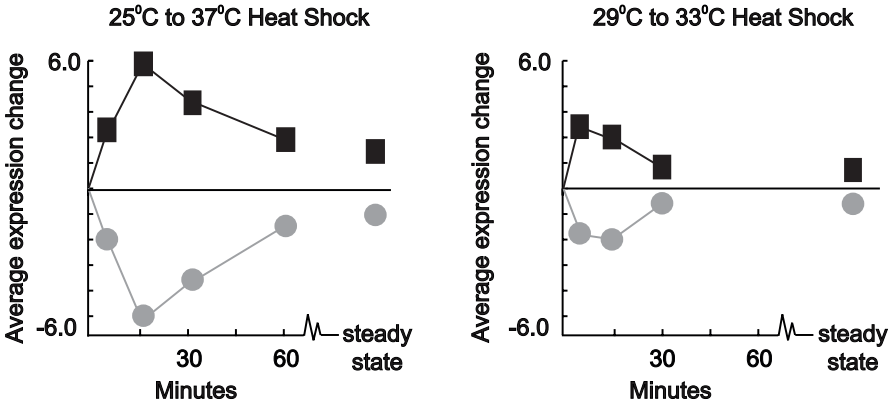

Fig. 2.2. Initiation of the ESR is often transient. The average gene expression changes of the genes whose expression is induced (—■—) and repressed (—●—) during ESR initiation in response to a 25°C to 37°C heat shock and a 29°C to 33°C heat shock. Data taken from Gasch et al. 2000

apting to their new steady-state expression program, relative to cells exposed to a mild temperature shift of 29°C to 33°C (Fig. 2.2). Furthermore, conditions that result in high levels of cell death usually provoke a substantial initiation of the ESR, with some of the ESR transcript levels changing more than 100-fold (A.P. Gasch and P.O. Brown, unpublished data). Thus, the ESR is initiated in response to a wi-

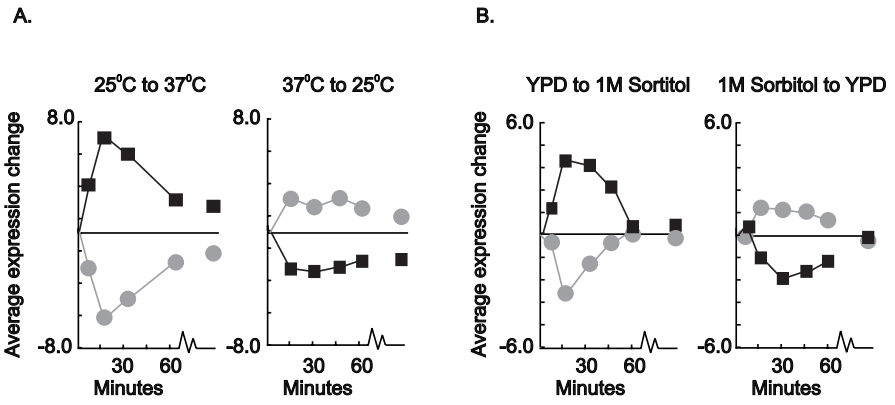

Fig. 2.3. Reciprocal expression of the ESR genes in response to reciprocal environmental changes. The average gene expression changes of genes whose expression is normally induced (—■—) and repressed (—●—) during ESR initiation are shown as cells responded to a 25°C to 37°C shock (A, left panel), 37°C to 25°C shock (A, right panel), a shift from YPD medium to YPD supplemented with 1M sorbitol (B, left panel), and YPD supplemented with 1M sorbitol to standard YPD medium (B, right panel)

de range of environmental transitions, from subtle changes in conditions to lethal environmental shocks, in a manner that is graded to the severity of the environment-al stress.

The ESR is not initiated in response to any environmental shift but appears to represent a response to suboptimal environments. This is evident from the genomic expression program of cells shifted back and forth between two environments. For example, when cells adapted to growth at 25°C were transferred to 37°C, they responded with large and transient changes in the expression of the ESR genes (Fig. 2.3A) (Gasch et al. 2000). In contrast, when cells adapted to 37°C were shifted to 25°C, they showed reciprocal changes in the expression of these genes: genes whose expression is normally induced during ESR initiation showed decreased expression in response to the reverse temperature shift, and genes whose expression is normally repressed in response to stressful environments became induced under these conditions. This observation indicates that initiation of the ESR is relieved when cells that are adapted to 37°C are shifted to 25°C. Furthermore, the cells immediately (within 5 minutes) adjusted their transcript levels to the final steady-state required for growth at 25°C, with no observable transient features. Thus, while a shift from 25°C to 37°C triggered initiation of the ESR, the reciprocal shift rapidly relieved the ESR gene expression differences within a very short period (Gasch et al. 2000).

2.4 Transcript levels *versus* protein synthesis levels

The cell goes to great lengths to alter the expression of its genome, presumably to alter the abundance of the corresponding gene products. Indeed, many of the changes in ESR transcript levels correlate with changes in protein synthesis. Proteomic studies have identified proteins whose translation increases or decreases following starvation, osmotic shock, oxidative stress, and heat shock (Fuge et al. 1994; Norbeck and Blomberg 1997; Godon et al. 1998; Boy-Marcotte et al. 1999; Norbeck and Blomberg 2000). Although in each study, only a subset of these changes (<100) could be related to specific proteins, ~90% of the reported alterations in translation initiation correlate with the independently observed changes in transcript abundance (Fuge et al. 1994; Norbeck and Blomberg 1997; Godon et al. 1998; Boy-Marcotte et al. 1999; Norbeck and Blomberg 2000; Gasch et al. 2000).

Providing further evidence that changes in transcript levels correlate with changes in protein synthesis, it was recently shown that initiation of the ESR parallels the selective translation of transcripts induced in the program. Using DNA microarrays, Kuhn et al. (2001) identified yeast transcripts that displayed altered association with polyribosomes (polysomes) after cells were shifted from glucose- to glycerol-containing medium. Despite an overall reduction in translation initiation following the environmental shift (indicated by the decreased cellular incorporation of radioactive methionine), essentially all of the 600 transcripts that were increased after the transition, including the ~300 transcripts increased as part of the ESR, became more associated with polysomes, indicating that they were being

selectively translated (Kuhn et al. 2001). In contrast, ~100 transcripts became less prevalently associated with polysomes after the shift, almost all of which were ribosomal protein transcripts that are strongly decreased as part of the ESR. These results show a strong correlation between the induction of gene expression and increased association of transcripts with polysomes under the conditions studied, and it is likely that this correlation exists under other conditions as well. Therefore, despite the overall decrease in protein translation following a stressful environmental transition, the cell is still able to translate proteins that are critical for its survival. Altered polysome association is not a prerequisite for changes in protein synthesis, and thus it is interesting to note that the majority of transcripts that decrease when the ESR is initiated did not show changes in polysome association. The presumed decrease in these gene products may therefore be largely due to the decreased abundance of their transcripts.

2.5 Functions represented by genes repressed in the ESR

The precise role that the ESR plays in the adaptation to new environments is suggested by the functional roles of the characterized genes that participate in this response. The expression of approximately 600 genes is reduced as part of the ESR, and many of these genes can be directly related to protein synthesis (Table 2.2). More than 70% of the ~300 characterized genes in this group are annotated by the *Saccharomyces* Genome Database (SGD) as "involved in protein synthesis", and together these ESR genes account for almost 70% of the total number of genes in the yeast genome annotated as such (the exceptions consist almost entirely of genes involved in mitochondrial protein synthesis) (Ashburner et al. 2000; Ball et al. 2000). Given the similarity in the expression patterns of genes repressed in the ESR, many of the uncharacterized genes in this group are most certainly functionally related. The reduced expression of these genes correlates with the transient decrease in overall translation initiation, coupled with transient cell cycle and growth arrest, that has been observed in the responses to a number of stressful conditions (McAlister and Finkelstein 1980; Fuge et al. 1994; Blomberg 1995; Ashe et al. 2000; Kuhn et al. 2001; Teige et al. 2001). This transient drop in overall translation initiation may be largely caused by the temporary reduction in the levels of these transcripts, coupled with the disassociation of some of the transcripts from polysomes (Ashe et al. 2000; Kuhn et al. 2001); in addition, translation elongation is known to be inhibited during the response to osmotic shock (Teige et al. 2001)(see Chapter 4) and probably contributes to the translation arrest under other conditions as well. The combined effects of the decrease in transcript and protein synthesis may help to conserve mass and energy while the cell adapts to its new conditions.

In addition to genes involved in protein synthesis, other functional processes, represented by smaller numbers of repressed genes, include those related to cell wall biosynthesis, cytoskeletal and chaperonin functions, protein glycosylation and secretion, amino acid and pyruvate metabolism, nucleotide biosynthesis, DNA

Table 2.2. Functional categories of genes repressed in the ESR

Category[a]	Number of Genes in the ESR[b]	% of All Yeast Genes in Category[b]
Ribosomal Proteins	132	98
Ribosome Biogenesis	6	75
rRNA Processing	26	72
Nuclear Targeting (Excluding Nuclear Pore Components)	6	60
Transcription		
RNA Pol I Subunits	7	88
RNA Pol II Subunits	2	7
RNA Pol III subunits	8	89
Shared Polymerase Subunits	5	83
Cytosolic Translation Factors		
Initiation	20	83
Elongation	9	100
Termination	3	100
tRNA Processing	4	12
tRNA Synthetases (Cytosolic)	15	68
Nucleotide Metabolism	11	23
DNA Replication	6	10
mRNA Splicing	5	8
mRNA Decay	3	30

[a] Functional categories as listed in the *Saccharomyces* Genome Database (SGD) (Ball et al. 2000).
[b] The categories listed contain ~78% of all of the characterized genes that are repressed in the ESR.
[c] Percent of the total number of genes in each category, as listed in SGD, represented by genes in the ESR.

replication, nonsense mediated mRNA decay, and others. Some of the functional categories represented by larger numbers of genes are discussed below.

2.5.1 Ribosome synthesis

It is estimated that an actively growing yeast cell harbors ~200,000 ribosomes, each composed of four ribosomal transcripts and 78 ribosomal proteins (RP) (Warner 1999). Three of the four rRNAs are encoded by 100-200 tandem copies of the rDNA genes and are transcribed by RNA Polymerase (Pol) I to account for approximately 80% of the total RNA in the growing cell. The RP transcripts, generated through RNA Pol II transcription, are estimated to account for more than 60% of the RNA Pol II initiation events, and the synthesized proteins make up

15% of the total mass of cellular proteins under standard growth conditions (Warner 1999).

Because ribosome synthesis requires substantial mass and energy, it is not surprising that transcript levels of the rRNA and RP genes are sharply reduced under stressful conditions. As demonstrated by microarray studies, the RP genes are among the most tightly coregulated genes in the yeast genome. The transcript levels of essentially all of the RP genes that can be distinguished on microarrays are rapidly reduced following environmental stresses, in some cases more than 80-fold (Eisen et al. 1998; Gasch et al. 2000; Causton et al. 2001). Additionally, when cells are shifted to a nonfermentable carbon source (and perhaps other conditions as well), the existing RP transcripts become rapidly disassociated from polysomes, further suppressing RP synthesis (Kuhn et al. 2001). Expression of the rDNA is known to be repressed in response to a number of stresses, including heat shock, starvation, secretion defects, and drug treatments (Shulman et al. 1977; Veinot-Drebot et al. 1989; Klein and Struhl 1994; Zaragoza et al. 1998; Nierras and Warner 1999; Miyoshi et al. 2001). Because rRNA levels are infrequently monitored in whole-genome expression studies, rDNA expression in response to diverse conditions has been less thoroughly studied. However, given the tight coregulation of the RP genes and the known importance of the stoichiometry of the ribosomal components (Warner 1989), it seems logical to expect that the expression of the rDNA would parallel that of the RP genes during ESR initiation. This hypothesis is supported by the fact that the reduced expression of rDNA and the RP genes is coregulated by the Protein Kinase C pathway in response to secretion defects (Nierras and Warner 1999; Li et al. 2000).

2.5.2 tRNA synthesis

In addition to the reduced expression of components of the ribosome, expression of their tRNA substrates is also known to be repressed in response to some stresses. The tRNA transcripts, synthesized by RNA Pol III, are estimated to comprise 15% of the total RNA in a growing yeast cell (Warner 1999). Expression of the tRNAs is known to be repressed following a variety of stresses, including amino acid and nitrogen starvation, progression into stationary phase, defects in secretion, DNA damage, and treatment with rapamycin (Oliver and McLaughlin 1977; Shulman et al. 1977; Kief and Warner 1981; Zaragoza et al. 1998; Li et al. 2000; Ghavidel and Schultz 2001; Pluta et al. 2001). Analogous to the repression of rDNA and RP genes, repression of tRNA synthesis is likely to be a general feature of the ESR. Consistently, many of the genes involved in tRNA processing, and almost all of the genes encoding cytosolic aminoacyl tRNA synthetases, are repressed as part of this program.

2.5.3 General transcription

The decrease in rRNA, RP mRNA, and tRNA levels reveals that genes transcribed by RNA Pol I, II, and III are all repressed in response to stress. This decrease in expression might be expected to correlate with a decreased requirement for RNA Pol I, II, and III activity. Correspondingly, the expression of genes encoding RNA Pol I and III is reduced as part of the ESR: all of the genes encoding Pol I and Pol III subunits, as well as most of the shared polymerase subunits, are repressed with patterns similar to ESR gene expression. The decreased expression of these polymerase subunits may contribute to the decreased expression of their targets, however it should be noted that the rapid decline in rRNA and tRNA levels is likely also regulated at the level of silencing, as is the case of rDNA, and mRNA turnover (Smith and Boeke 1997; Smith et al. 1999). In contrast to the encoded Pol I and III subunits, genes encoding RNA Pol II subunits are generally not repressed in response to stress, nor are Pol II accessory factors such as TFII subunits and the mediator complex. This observation is consistent with the importance of Pol II-dependent gene expression for cell survival of stressful environments.

2.5.4 RNA splicing and export

Many transcripts, including rRNA and tRNA transcripts, are synthesized in the nucleolus but must get processed and exported to the cytosol for complete assembly and function (reviewed in Kressler et al. 1999). The expression of more than 75% of the genes in the SGD functional category “rRNA processing” (Ashburner et al. 2000; Ball et al. 2000) is repressed in the ESR (including RNA helicases, exoribonucleases, rRNA methyltransferases, and small nuclear ribonucleoproteins), and the expression of many genes involved in tRNA processing is also repressed as part of this general stress response. In contrast, only a handful of genes involved in mRNA splicing are repressed in the ESR, despite the fact that nearly half of all of the introns in yeast reside in the RP genes (Spingola et al. 1999; Warner 1999). Other genes whose expression is reduced as part of the ESR encode proteins that are known to be involved in the export of the pre-ribosome from the nucleolus, including karyopherins, nuclear transport proteins, and GTP/GDP binding proteins (Stage-Zimmermann et al. 2000), and their decreased expression is likely to be related to the decreased synthesis of ribosomes.

2.5.5 Translation

As with transcription, the expression of many genes encoding translation factors is reduced following environmental changes. This repression includes most of the yeast genes encoding translation initiation, elongation, and termination factors, as well as almost all of the cytosolic aminoacyl tRNA synthetases. This correlates with the observed decrease in cellular translation that occurs in response to stressful environmental transitions, as discussed above (McAlister and Finkelstein 1980;

Fuge et al. 1994; Blomberg 1995; Ashe et al. 2000; Kuhn et al. 2001; Teige et al. 2001). Together, these details indicate that the transient arrest in overall protein synthesis in response to stress likely results from a combination of cellular features, including the decreased levels of the transcripts described here, reduced synthesis of translation machinery, direct inhibition of translation elongation, and disassociation of many existing transcripts from polysomes (Ashe et al. 2000; Kuhn et al. 2001; Teige et al. 2001).

2.6 Functions represented by genes induced in the ESR

Unlike the genes repressed in the ESR, which can be largely related to protein synthesis and growth-related functions, the genes induced in the ESR are involved in a wide variety of processes. Of the more than 300 genes whose expression is induced in the ESR, roughly 45% are completely uncharacterized. This group is enriched for uncharacterized genes relative to the yeast genome, for which approximately 35% of the genes are of unknown function. The known functions of the characterized genes induced in the ESR have been related to carbohydrate metabolism, metabolite transport, fatty acid metabolism, maintenance of the cellular redox potential, detoxification of reactive oxygen species, autophagy, protein folding and degradation, cell wall modification, DNA-damage repair, secretion, vacuolar and mitochondrial functions, intracellular signaling, and others. The known functions of these genes hint to the cellular processes that may be affected in response to diverse environmental changes, and suggest mechanisms the cell uses to protect itself in the face of cellular stress. Discussed below are a number of functional categories represented by the characterized genes induced in this program, and the potential cellular effects mediated by the expression changes.

2.6.1 Carbohydrate metabolism

A critical component of cell survival is maintaining a viable energy source. Glucose is the preferred carbon source in yeast, and upon stress, the cell induces a variety of genes that affect glucose metabolism (Fig. 2.4). This includes genes encoding glucose transporters that serve to import external glucose into the cell and glucose kinases that activate the sugar for subsequent catabolism. In response to stressful environments, the fate of glucose is divided between trehalose synthesis, glycogen storage, ATP synthesis through glycolysis, and NADPH regeneration by the pentose phosphate shuttle.

The disaccharide trehalose, comprised of α-(1,1) linked glucose molecules, has long been implicated in stress responses, particularly the response to hyperosmolarity, heat shock, and oxidative stress (reviewed in Singer and Lindquist 1998b; Francois and Parrou 2001). Cells unable to synthesize trehalose are sensitive to osmotic shock, which suggests that trehalose synthesis plays a role in the resistance to this stressful situation (Hounsa et al. 1998). During heat shock treha-

lose stabilizes protein structures and prevents aggregation of unfolded proteins (De Virgilio et al. 1994; Hottiger et al. 1994; Singer and Lindquist 1998a), while the sugar appears to protect cellular structures from oxidative damage in response to hydrogen peroxide (Benaroudj et al. 2001). It has also been observed that mutations in the trehalose synthase subunit Tps1p result in defective glycolytic flux, hinting that Tps1p and/or trehalose synthesis may play a role in modulating glycolysis (see more below) (Gonzalez et al. 1992; Van Aelst et al. 1993; Thevelein and Hohmann 1995).

The induced expression of genes affecting trehalose synthesis in response to diverse stresses is well documented, however it has also been observed that this induction does not always correlate with increased trehalose levels (Parrou et al. 1997; Zahringer et al. 1997; Francois and Parrou 2001). Furthermore, the concomitant induction of genes affecting the synthesis (*TPS1, TPS2, TSL1*) and degradation (*NTH1, ATH1*) of trehalose occurs in response to multiple stresses as part of the ESR (Fig. 2.4) (Parrou et al. 1997; Zahringer et al. 1997; Francois and Parrou 2001). The paradoxical nature of this coinduction was clarified by Zahringer et al. (1998), who showed that post-translational regulation of these gene products affects internal trehalose levels. Although enzymes synthesizing and degrading trehalose are coinduced, internal trehalose levels increase after the neutral trehalase Nth1p is phosphorylated and inactivated. (The increase in trehalose during heat shock is further promoted by the differences in the temperature optima of the trehalose synthase complex, which operates optimally at 40^{o}C, *versus* trehalase, which has a temperature optimum of ~25^{o}C (Neves and Francois 1992; Londesborough and Vuorio 1993.)) Regulating these enzymes by phosphorylation provides a method of rapid adjustment of enzymatic activity, and increasing the transcript and protein levels of these enzymes may further expedite the regulation. By coinducing synthetic and catabolic enzymes, the cell can continually modulate the counterproductive enzymatic activities to sensitively control the precise levels of trehalose within the cell (see more below). The importance of precise trehalose levels is underscored by the fact that aberrantly high trehalose concentrations can inhibit protein refolding during the recovery from heat shock (Singer and Lindquist 1998a; Wera et al. 1999).

Storage of glucose in the form of glycogen is known to be critical to cell survival of starvation, and glycogen likely plays an important role in response to a wide variety of stressful environments as well (Francois and Parrou 2001). Similar to the case with trehalose, genes encoding enzymes that promote the synthesis and degradation of glycogen are coinduced in the ESR (Fig. 2.4) and are post-translationally regulated (Hwang et al. 1989; Hardy et al. 1994; Parrou et al. 1997). Both the glycogen synthase Gsy2p and glycogen phosphorylase Gph1p are inactivated by phosphorylation, while Gsy2p is activated by high levels of glucose-6-phosphate. The activity of these enzymes is likely sensitively controlled during stressful situations to modulate glycogen stores. Interestingly, deletion of *GPH1* causes increased glycogen accumulation in response to multiple stresses, suggesting that glucose shuttles into and out of cellular glycogen stores during stress adaptation (Parrou et al. 1999). Glucose is catabolized through the glycolytic pathway to synthesize ATP while generating pyruvate for the TCA cycle;

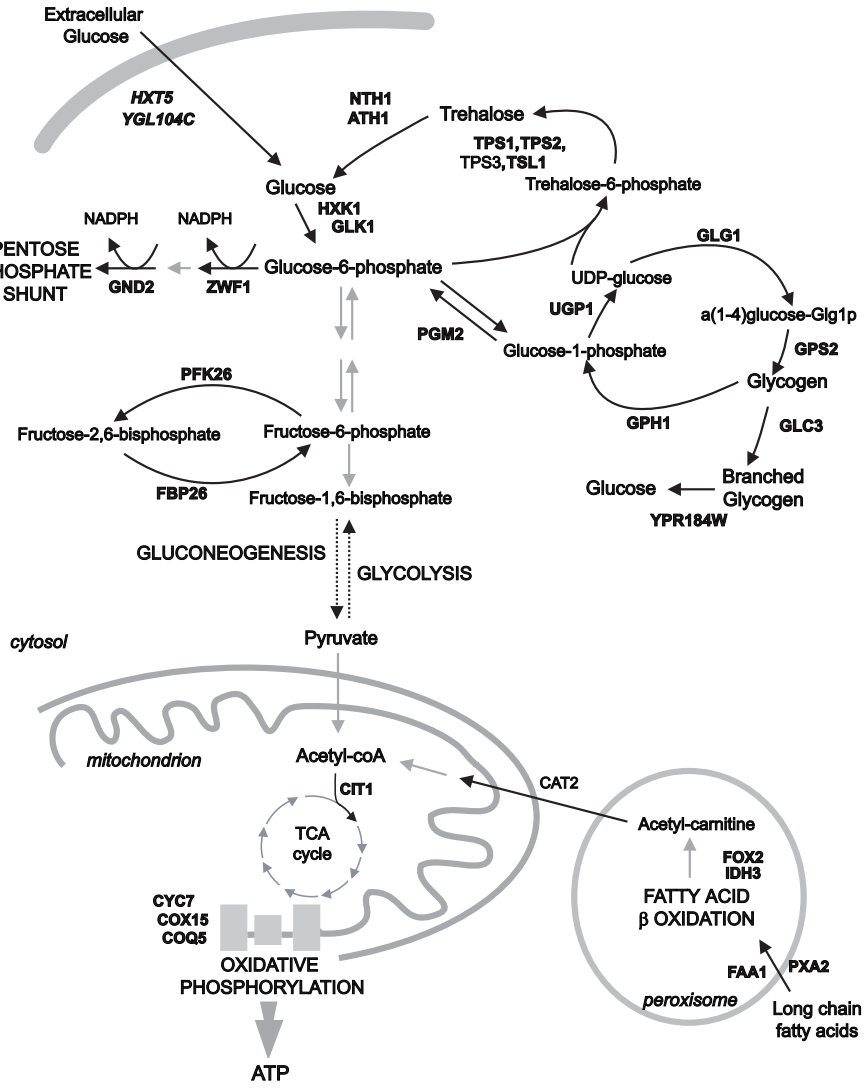

Fig. 2.4. ESR genes involved in carbohydrate metabolism. Genes whose expression is induced as part of the ESR that are involved in glucose import, trehalose and glycogen metabolism, the pentose phosphate shuttle, glycolysis, fatty acid metabolism, and respiration are shown in bold type

therefore, one might expect that expression changes in genes related to glucose metabolism might include the glycolytic genes. To the contrary, most of the genes encoding glycolytic or gluconeogenic factors do not show expression patterns cor-

related with the ESR. Instead, genes affecting the synthesis of fructose-2,6-bisphosphate, a key regulator of glycolytic flux, are induced. Both the kinase that synthesizes this allosteric regulator and the phosphatase that degrades it are coinduced as part of the ESR (*PFK26* and *FBP26*, Fig. 2.4). Again, the activity of these enzymes is regulated post-translationally by phosphorylation and by allosteric regulation of Pfk26p and Fbp26p by ATP and fructose-6-phosphate, respectively (Kretschmer et al. 1987; Manhart and Holzer 1988; Hofmann et al. 1989; Thevelein and Hohmann 1995). The concomitant induction of these synthetic and catabolic enzymes may allow the cell to sensitively modulate glycolysis without altering the expression of the entire pathway of genes.

2.6.2 Fatty acid metabolism

Yeast cells can consume metabolites other than glucose to generate energy, including fatty acids. Beta-oxidation of fatty acids occurs in the yeast peroxisome, and the resulting acetyl moieties are then transported to the mitochondria where they supply the TCA cycle for anabolic metabolism and respiration (reviewed in van der Klei and Veenhuis 1997; Hettema and Tabak 2000). Although most of the genes involved in fatty acid metabolism are not induced in the ESR, genes involved in importing (*FAA1, PXA2*) and exporting (*CAT2*) these metabolites into and out of the peroxisome are induced (Fig. 2.4). The induction of these genes may facilitate fatty acid oxidation simply by increasing the local concentration of fatty acid substrates while efficiently removing the metabolic products for further catabolism in mitochondria. This would allow the cell to take full advantage of existing peroxisomes, rather than expend significant energy required to proliferate the organelles.

2.6.3 Respiration

In the presence of glucose, yeast cells are unique among microbes in that they rely on fermentation to generate ATP, despite the fact that respiration generates many more ATP molecules per glucose. Genes involved in respiration are normally repressed in the presence of glucose, however they can be derepressed by a reduced ATP:AMP ratio in the cell, even in the presence of the sugar (Hardie et al. 1998; Carlson 1999; Hardie 1999). As many of the stress defense mechanisms consume ATP, it might be expected that many types of cellular stress would lead to the induced expression of respiration components. While this is true of the response to environmental changes that lead to substantial cellular stress, the expression of genes encoding respiration components is not induced under all circumstances, and these genes have an expression profile subtly distinct from the ESR expression pattern (Gasch et al. 2000). Furthermore, although 70% of the genes in this group contain the binding site for the general stress factors Msn2p and Msn4p, fewer than 10% are affected by *MSN2/MSN4* double deletion or overexpression. This is in contrast to the induced genes classified in the ESR, for which nearly

90% are affected by *MSN2/MSN4* deletion or overexpression (see below) (Gasch et al. 2000). The subtle differences in the expression patterns of the respiration components *versus* ESR genes suggest differences in the regulation of these two responses. Whether the respiration genes are induced as part of the ESR, or are induced concomitantly under some situations by a related regulatory system, remains to be characterized in detail.

However, as part of the ESR, the cell does induce a handful of genes that have been implicated in respiration. For example, in response to diverse stresses the cell induces genes encoding the rate-limiting step of the TCA cycle (*CIT1*), an alternate isoform of cytochrome c (*CYC7*), and two factors that affect the synthesis and assembly of the oxidative phosphorylation components cytochrome c oxidase (*COX15*) and ubiquinone (*COQ5*). Increasing the levels of these gene products may promote ATP synthesis by utilizing existing respiration components. Alternatively, the induction of factors that promote cytochrome c and ubiquinone synthesis may play a role in the defense against oxidative stress, as discussed below, rather than in ATP generation.

2.6.4 Oxidative stress defense

Reactive oxygen species (ROS), generated endogenously through oxidative phosphorylation and enzymatic activities or exogenously by environmental factors, can lead to a chain of oxidation reactions in the cell, which damage cellular structures such as proteins, lipids, and DNA and prevent proper enzymatic activity by perturbing the internal redox potential (reviewed in Scandalios, 1997 and Chapter 6). As a result, the cell has evolved a number of defenses against oxidative stress as well as mechanisms to maintain its internal redox potential.

The reducing environment of the yeast cytoplasm is affected in part by the balance of oxidized and reduced protein sulfhydryl groups, including those of the small peptide glutathione (Grant et al. 1996b; Grant 2001). This balance is modulated by thioredoxin and glutaredoxin, which mediate the NADPH-dependent reduction of disulfide groups. During oxidative stress, the oxidation of glutathione and other thiol-specific antioxidants, coupled with subsequent reduction by the thioredoxin and glutaredoxin systems, is thought to help buffer the reducing environment of the yeast cytoplasm while protecting cellular structures from oxidative damage (Kuge and Jones 1994; Grant et al. 1996a; Grant et al. 1996b; Luikenhuis et al. 1998). The importance of these cellular features likely explains the induction of many of these genes in response to diverse environmental stresses: as part of the ESR, the cell induces the expression of genes encoding isozymes of thioredoxin and glutaredoxin (*TRX2*, *TTR1*), the glutathione-biosynthetic enzyme gamma-glutamyl transferase (*ECM38*), and thiol-specific antioxidants (*PRX1*, *YDR453C*). Furthermore, genes encoding the NADPH-generating steps of the pentose phosphate shuttle (*ZWF1*, *GND2*) are also induced as part of the ESR, perhaps to help replenish NADPH reducing equivalents (Juhnke et al. 1996; Slekar et al. 1996).

Yeast cells also utilize a number of enzymes devoted to the detoxification of ROS, and many of these are induced not only by conditions that inflict oxidative damage but also in response to diverse stressful environments. Among those that get induced are genes encoding the cytosolic superoxide dismutase Sod1p and cytosolic catalase Ctt1p, which reduce superoxide and hydrogen peroxide, respectively. While catalase appears to specifically reduce hydrogen peroxide, the glutathione peroxidases Hyr1p and Gpx1p may act on organic peroxides as substrates, and the genes encoding these enzymes are also induced in the ESR (Inoue et al. 1999; Avery and Avery 2001).

Under standard growth conditions, endogenous ROS is thought to be generated primarily by electron leakage from the oxidative phosphorylation chain in mitochondria (Scandalios 1997), and therefore it is not surprising that this organelle harbors its own local ROS protection systems. Cytochrome b5 reductase (*MCR1*) and cytochrome c peroxidase (*CCP1*) both seem to protect against oxidative stress, as deletion of either of their genes increases the sensitivity of yeast cells to drugs that induce oxidative damage (Charizanis et al. 1999; Lee et al. 2001a), and both genes are induced with the ESR. Oxidized cytochrome c peroxidase is subsequently reduced directly by cytochrome c (Charizanis et al. 1999). With this in mind, the induction of the gene encoding the cytochrome c isoform Cyc7p as part of the ESR may be related to Ccp1p reduction instead of respiration. Another respiration component, ubiquinone, may also play a role in oxidative stress: this membrane-diffusible molecule can act as an antioxidant against lipid peroxides, and indeed, yeast cells defective in ubiquinone synthesis are sensitive to exogenously added peroxides (Schultz and Clarke 1999; Soballe and Poole 1999). One gene required for ubiquinone synthesis is induced in the ESR (*COQ5*), although other genes involved in its synthesis do not appear to be generally induced.

2.6.5 Autophagy and vacuolar functions

Autophagy is a process whereby the cell randomly engulfs its cytoplasmic material into vesicles destined for the yeast vacuole (reviewed in Abeliovich and Klionsky 2001). The engulfed materials, such as proteins, small molecules, and even organelles, are degraded in the vacuole so that their components can be recycled in the cell. Although autophagy shares many features with the constitutive process of cytoplasm-to-vacuole trafficking, which translocates specific cytoplasmic proteins, the process of autophagy is induced under conditions of starvation (Takeshige et al. 1992; Tsukada and Ohsumi 1993; Abeliovich and Klionsky 2001). Interestingly, the expression of genes involved in autophagy (*APG1, APG7, AUT1*), including the gene encoding the Apg1 kinase that may regulate the process (Tsukada and Ohsumi 1993; Matsuura et al. 1997; Kamada et al. 2000), is induced as part of the ESR in response to a wide variety of stresses, while genes specific to cytoplasm-to-vacuole trafficking are not commonly induced (Gasch et al. 2000). Although it is not known if the induced expression of these genes necessarily leads to increased autophagy, recent evidence from Wang *et al.* (2001) reveals that autophagy is active at the diauxic shift, with timing that parallels the in-

duction of the autophagy genes in the ESR (DeRisi et al. 1997; Wang et al. 2001a). Whether autophagy trafficking is activated in response to stresses beyond starvation is currently unknown.

In addition to autophagy genes, genes encoding vacuolar proteins are also induced in the ESR. These include genes encoding the vacuolar calcium pump Pmc1p, a protein implicated in vacuolar targeting (*VAB2*), a variety of vacuolar proteases (*PRC1, YPS6, LAP4, PEP4, PRB1*) as well as the protease inhibitors Pai3p and Pbi2p which are thought to inhibit the Pep4p and Prb1p proteases, respectively. The products of these genes may aid in the degradation of cargo delivered to the vacuole by autophagy as well as other proteins that arrive through endocytosis or through the secretory pathway (Van Den Hazel et al. 1996).

2.6.6 Protein folding and degradation

The expression of many genes encoding protein folding chaperones is known to be induced specifically in response to heat-denatured proteins (see Chapter 3). However, it was early realized that a subset of chaperone genes is induced by a variety of stressful conditions (Kurtz et al. 1986; Werner-Washburne et al. 1989). The chaperone genes induced as part of the ESR include the so-called small heat shock proteins (*HSP12*, *HSP26*, and *HSP48*), members of the Hsp70 family of chaperones (*SSA4*, *SSE2*, *HSP78*), and *HSP104*.

The functions of the small heat shock proteins are not well characterized. However, Hsp26p was shown to protect proteins from heat-denaturation *in vitro* (Haslbeck et al. 1999), while Hsp42p has been implicated in repolarization of the actin cytoskeleton after adaptation to elevated temperatures (Gu et al. 1997). Individually, these chaperones appear to contribute little to thermotolerance in yeast, as deletion of any of the factors does not result in cellular sensitivity to heat shock or other conditions (Susek and Lindquist 1989; Gu et al. 1997). On the other hand, deletion of the *HSP104* chaperone gene does affect viability during heat shock (Lindquist and Kim 1996). Hsp104p appears to help disassociate aggregates of unfolded proteins to allow the Hsp70 chaperones, perhaps including those encoded by *SSA4* and *SSE2* which are induced in the ESR, to bind and refold the protein substrates (Parsell et al. 1994; Glover and Lindquist 1998). Similar to the Hsp104-Hsp70 system in the cytoplasm, the mitochondrial Hsp70 homolog Hsp78p has properties similar to both Hsp104p, to which it is 46% identical, as well as Hsp70 chaperones (Schmitt et al. 1995; Schmitt et al. 1996). Thus, chaperones that are localized to the cytoplasm and mitochondria are induced in the ESR, underscoring the importance to stress survival of proper protein folding in these subcellular locations.

Denatured proteins that cannot be properly refolded are targeted for degradation by ubiquitination, and thus it is not surprising that genes involved in ubiquitin targeting are also induced in the ESR. Genes encoding E1 and E2 proteins involved in ubiquitin ligation and conjugation participate in the ESR (*HUL4, UBC5*, *UBC8*), as do *UBI4*, which encodes polyubiquitin, and *UBP15,* which encodes a putative deubiquitinating enzyme. Deletion of *UBC5*, *UBI4*, or *UBP15* is known

to render cells sensitive to a variety of stresses (Finley et al. 1987; Seufert and Jentsch 1990; Spence et al. 1995; Amerik et al. 2000). Ubiquitination is important not only for removing misfolded proteins from the cell but also for targeting active proteins for turnover, and the induction of ubiquitin-mediated protein degradation may help the cell to rapidly alter the internal protein repertoire during stress adaptation. Monoubiquitination is specifically involved in targeting proteins for endocytosis and altering histone structure to affect chromatin remodeling (Hicke 1999; Hicke 2001), and thus the induction of some of the genes involved in ubiquitin metabolism may also play a role in these processes.

2.6.7 Cytoskeletal reorganization

When actively growing cells progress through the cell cycle, the arrangement of their actin cytoskeleton is polarized so that vesicles and materials can be delivered along the actin cables to the site of cell growth at the bud neck (reviewed in Pruyne and Bretscher 2000). However, in response to cellular stress, the actin cytoskeleton becomes rapidly depolarized and instead arranges isotropically, perhaps to deliver secretory vesicles and their cargo evenly within the cell (Chowdhury et al. 1992; Lillie and Brown 1994; Delley and Hall 1999). Soon after the adaptation to the new environment, the cytoskeleton becomes repolarized to promote localized growth and subsequent cell division. Reorganization of the cytoskeleton may be affected by factors encoded by genes in the ESR. The expression of a number of cytoskeletal genes is repressed as part of the ESR, including genes encoding Pac10p, a protein involved in microtubule assembly whose deletion renders cells sensitive to the actin destabilizing drug latrunculin (Geissler et al. 1998), and Sro9p which is proposed to affect the organization of actin fibers (Kagami et al. 1997). Two genes whose expression is induced in the stress program have also been implicated in cytoskeletal function. These include the genes encoding Arc18p, a member of the Arp2/3 complex that is thought to affect the mobility and polar distribution of cortical actin patches (Winter et al. 1997; Machesky and Gould 1999; Winter et al. 1999), and Hsp42p, which plays a role in cytoskeletal repolarization after heat shock (Gu et al. 1997). In addition, Msn2/4p have been specifically linked to cytoskeletal reorganization following cellular stress: repolarization of the cytoskeleton is partially controlled by Ras-dependent signaling, and the defective cytoskeletal reorganization seen in *ras2*Δ mutant cells can be suppressed by double deletion of *MSN2/MSN4* (Ho and Bretscher 2001). One explanation for this result is that genes induced by Msn2/4p inhibit cytoskeletal rearrangements, and therefore deletion of *MSN2/MSN4*, and reduced expression of their target genes, suppresses the *ras2*Δ cytoskeletal defect. However, an alternative function of Msn2/4p may be one of physical interaction: Msn2p and Msn4p were recently shown to bind Bmh2p (Beck and Hall 1999), a protein that has been linked to RAS/PKA-dependent signaling (Gelperin et al. 1995; Roberts et al. 1997; Roth et al. 1999) and affects the organization of the actin cytoskeleton (Roth et al. 1999). Although there is no direct evidence of a physical association be-

tween Msn2/4p and the actin cytoskeleton, the possibility exists that Msn2/4p play a more direct role in affecting cytoskeletal rearrangements.

2.6.8 Signaling

A variety of genes involved in cellular signaling are induced in the ESR, and many of these genes can be related to specific signaling pathways. Most notably, genes encoding both positive and negative regulators of Protein Kinase A (PKA) signaling are coinduced in the ESR (Fig. 2.5). The expression of the genes encoding two of the three catalytic subunits of PKA (*TPK1, TPK2*) is induced concomitant with the cAMP-dependent inhibitory subunit, *BCY1*, the phosphodiesterase *PDE1*, and the gene encoding the Yak1p kinase that is proposed to counteract PKA signaling (Garrett and Broach 1989; Hartley et al. 1994). The induction of PKA signaling components is intriguing, since activity of this pathway is known to suppress the stress response by triggering the cytoplasmic localization of Msn2/

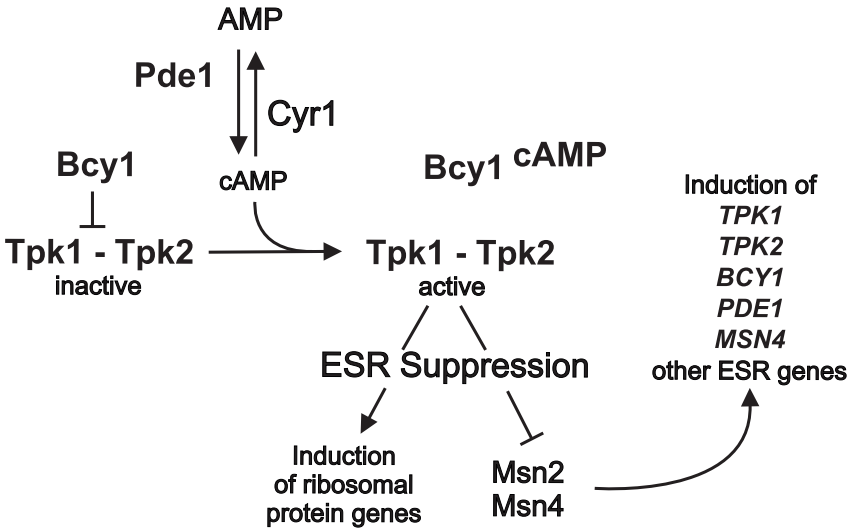

Fig. 2.5. Schematic diagram of PKA signaling in regard to ESR suppression. In response to increased cAMP levels, the inhibitor of PKA signaling (Bcy1p) binds cAMP to release the active complex, composed of two of three PKA subunits (Tpk1p, Tpk2p, Tpk3p) (Thevelein and de Winde 1999). PKA activity suppresses the ESR by affecting regulators that lead to the induction of protein synthesis genes and by triggering cytoplasmic relocalization of Msn2/4p and decreased expression of their targets. In contrast, following stressful environmental transitions, initiation of the ESR leads to nuclear localization of Msn2/4p and induction of their gene targets, including the genes affecting PKA signaling shown here

4p and by activating expression of the RP genes (Klein and Struhl 1994; Gorner et al. 1998). Even more interesting is the finding that the stress-dependent induction of some of the PKA subunits is dependent on Msn2p and Msn4p (Gaschet al. 2000). Analogous to PKA, the Tor1p kinase negatively regulates Msn2p and Msn4p activity by affecting their localization (Beck and Hall 1999), and the *TOR1* gene is also induced in the ESR in a manner dependent on Msn2/4p. As discussed below in a subsequent section, the induction of these genes as part of the ESR may provide a mechanism of feedback regulation to aid in the eventual suppression of the ESR after the cell has adapted to new conditions.

2.7 Functional themes in the ESR

2.7.1 Differential expression of isozymes

One notable feature of the ESR is the differential expression of isozymes: the expression of many genes involved in carbohydrate metabolism, respiration, protein folding, oxidative stress, and other processes is induced as part of the ESR, while expression of their paralogous counterparts is not induced (Table 2.3) (Gasch et al. 2000; Rep et al. 2000; Causton et al. 2001). This divergence in expression may point to different properties of the putative enzyme pairs, including biochemical function, substrate specificity, and physical location. For example, it has been shown that yeast glutathione peroxidases and thiol-specific antioxidants have different substrate specificities (Inoue et al. 1999; Lee et al. 1999b; Avery and Avery 2001), and thus the activity of some of these gene products may be critical for cell survival in response to certain conditions, while others are commonly involved in stress defense. In the case of yeast catalases, differences in their localization may explain the general induction of the gene encoding the cytosolic catalase Ctt1p as part of the ESR, while the gene encoding the peroxisomal catalase Cta1p is specifically induced under conditions of high peroxisomal activity (Filipits et al. 1993).

An alternative model for the differential expression of isozymes possessing similar properties is that it provides a mechanism of differential regulation of enzyme activity. Duplication of these genes and divergence of their regulatory mechanisms may have provided yeast a way to differentially regulate the encoded enzymatic activity. This might allow the cell to regulate ESR isozymes as part of this general program, while their counterparts are regulated by specialized signals.

2.7.2 Coinduction of genes with counterproductive functions

A recurring theme in the ESR is the induction of genes that encode counterproductive activities. This can be seen by the concomitant induction of genes leading to the synthesis and degradation of trehalose, glycogen, and fructose-2,6-bisphosphate, the coinduction of vacuolar proteases and their inhibitors, and the co

Table 2.3. Differentially Regulated Paralogs in the ESR[a]

ESR Genes	non-ESR Paralogs	Function
Metabolism		
HXK1	*HXK2*	Hexokinase
GLK1	*YDR516C*	Glucokinase
PGM2	*PGM1*	Phosphoglucomutase
PFK26	*PFK27*	2-phosphofructokinase
FBP26	*FBP1*	Fructose-2,6-bisphosphatase
GPM2	*GPM1 GPM3*	Phosphoglycerate mutase
GSY2	*GSY1*	Glycogen synthase
GLG1	*GLG2*	Glycogen initiator
NTH1	*NTH2*	Neutral trehalase
GND2	*GND1*	6-phosphogluconate dehydrogenase
GPD1	*GPD2*	Glycerol dehydrogenase
CYC7	*CYC1*	Cytochrome C
Oxidative Stress Defense		
TRX2	*TRX1 TRX3*	Thioredoxin
CTT1	*CTA1*	Catalase
SOD1	*SOD2*	Superoxide dismutase
HYR1 GPX1	*GPX2*	Glutathione peroxidase
GTT1	*GTT2*	Glutathione transferase
PRX1 YDR453C	*TSA1*	Thiol-specific antioxidant
Protein Folding and Degradation		
SSA3/SSA4[b]	*SSA1/SSA2*[b]	Hsp70 chaperones
SSE2	*SSE1*	Hsp70 chaperones
HUL4	*UBA1,2 RSP5 UBR1*	E1 Ubiquitin ligase
UBC5 UBC8	*UBC1,4,6,9,12,13*	E2 Ubiquitin conjugase
Signaling		
TOR1	*TOR2*	Tor kinase subunits
TPK1, TPK2	*TPK3*	PKA catalytic subunits
PDE1	*PDE2*	Phosphodiesterase

[a] Data taken from (Causton et al. 2001; Gasch et al. 2001).
[b] These genes are highly similar and prone to crosshybridization on the microarrays, and therefore their individual expression patterns cannot be distinguished.

regulated increase in transcripts encoding positive and negative effectors of cellular signaling. Post-translational regulation is known to regulate many of these gene products. Thus, the coinduction of gene products may render the cell poised to rapidly modulate enzymatic activity post-translationally. This would allow the cell a mechanism of rapid enzymatic regulation while alleviating the delay required for nascent protein synthesis.

Subtle modulation of the activities of positive and negative regulators may buffer concentrations of critical metabolites in the cell. A good example of this is the case of glucose. During stress conditions, the cell must regulate the flux of glucose into trehalose generation, glycogen synthesis, the pentose phosphate shunt, and glycolysis (Fig. 2.4). Inappropriate catabolism of glucose might deplete the sugar from critical metabolic pathways, while too much free phosphorylated glucose may lead to detrimental allosteric effects of enzymes regulated by this metabolite. Thus, the cell may buffer its intracellular glucose levels by the continued flux into and out of its trehalose and glycogen stores. This hypothesis is supported by the observation that cells lacking the catalytic subunit of trehalose synthase (Tps1p) have aberrantly high levels of fructose-1,6-bisphosphate, due to unregulated flux of glucose into glycolysis, and thus cannot grow on glucose-containing medium (Gonzalez et al. 1992; Van Aelst et al. 1993; Thevelein and Hohmann 1995). Although the mechanism of this Tps1p-dependent effect is incompletely explained, it is intriguing to consider that it results from unbuffered glucose pools (Thevelein and Hohmann 1995; Francois and Parrou 2001).

2.7.3 Regulation of control steps of metabolic processes

Another recurring feature of the ESR is the altered expression of genes whose products affect regulatory or rate-limiting steps of metabolic processes, rather than the altered expression of entire pathways of genes. For example, as part of the ESR the cell induces the expression of *CIT1* and *ZWF1*, encoding enzymes that perform the rate-limiting steps of the TCA cycle and pentose-phosphate shuttle, respectively, without significantly affecting other genes in these pathways. Similarly, the induction and subsequent regulation of two enzymes modulating fructose-2,6-bisphosphate may be enough to sensitively control glycolysis *versus* gluconeogenesis without altering the expression of most of the genes directly involved in these pathways. Increased expression of transporters, including plasma membrane glucose transporters, peroxisomal fatty acid transporters, and a mitochondrial acyl carrier protein, may affect metabolic flux simply by altering the local concentrations of the substrates and products of the pathways. These examples suggest that through initiation of the ESR the cell attempts to affect specific cellular processes through few and simple expression changes. This would not only reduce the effort and energy required to modulate those processes in response to stressful environments, but would also facilitate the rapid return to normal growth after the stress has been alleviated.

2.8 The role of the ESR

The ESR represents a common gene expression program that is initiated in response to a wide variety of sub-optimal growth conditions, in a manner that is graded to the severity of the environmental stress. Based on these observations, we

proposed that the ESR is initiated to protect critical features of the internal homeostasis while the cell adapts to new environments (Gasch et al. 2000). When the cell is shifted to an environment for which its internal system is not optimized, the specific cellular consequences resulting from the shift can lead to a series of secondary instabilities within the cell, potentially threatening many key physiological systems. To prevent such widespread internal damage, the cell has evolved to initiate the ESR in response to diverse signals of cellular stress in order to protect and maintain these critical features of the internal system.

The protective nature of this program is consistent with the observation that a number of the gene products induced in the ESR are not necessarily active, supporting the notion that they are induced in preparation for their potential activity. Many of the gene products discussed here are regulated at the post-translational level, and thus induction of the genes does not necessarily correlate with activity of the gene products but rather renders the cell poised to rapidly regulate the synthesized proteins. Another indication that some of the ESR enzymes are not always highly active is suggested by the fact that other gene products required for the activity of ESR proteins are not increased as part of this common program but rather are induced only under specific conditions. For example, although genes encoding thioredoxin and glutaredoxin are induced in the ESR, the genes encoding the enzymes which are required for their processive activity, namely thioredoxin reductase and glutathione reductase which recycle their oxidized substrates, are only induced in response to conditions of severe oxidative stress. This happens when the activity of thioredoxin and glutaredoxin is required for cell survival (Kuge and Jones 1994; Grant et al. 1996a; Luikenhuis et al. 1998; Gasch et al. 2000). Finally, it has been observed for many of the induced ESR genes that their deletion does not result in a general sensitivity to all stresses but renders the cell sensitive to specific conditions only, revealing that those gene products are only required under some situations (for example Ramotar and Masson 1996; Parrou et al. 1997; Broomfield et al. 1998; Bruning et al. 1998; Luikenhuis et al. 1998; Thomson et al. 1998). Thus, initiation of the ESR serves to prepare the cell to rapidly utilize the synthesized gene products in the event that they are required to protect components of the physiological system.

Considering the known functions of characterized genes that participate in the ESR suggests the cellular processes that aid in protecting important features in the cell in response to stressful environments. Most of the genes whose expression is repressed as part of the ESR can be related to protein synthesis, and the reduction in their transcript levels, coupled with transient growth and cell cycle arrest, may help to conserve mass and energy while the cell adapts to the challenges of its new environment (Warner 1999; Gasch et al. 2000). The genes induced in the ESR are involved in a wide variety of processes, as discussed above, and the induced expression of these genes may prepare the cell to protect critical features of the internal homeostasis, such as internal glucose stores, ATP levels, internal osmolarity, the cellular redox potential, the integrity of cellular structures such as proteins and DNA, and other details. Indeed, the initiation of the ESR probably explains the observed cross protection against stressful environments, in which cells exposed to a low dose of one stress become resistant to an otherwise lethal dose of a

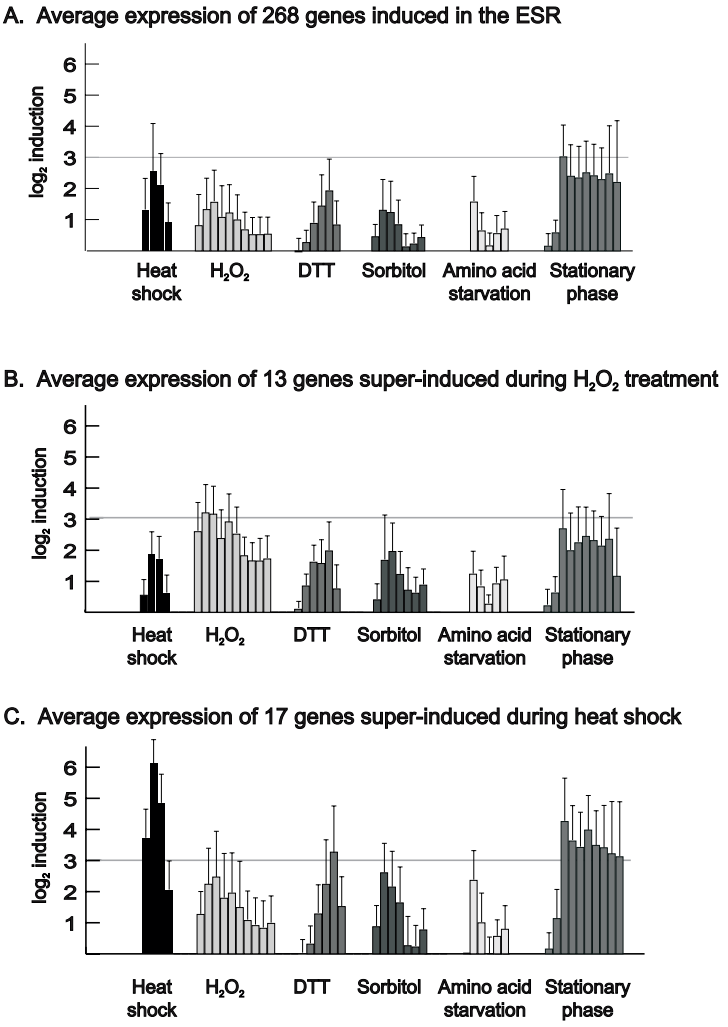

Fig. 2.6. Subsets of ESR genes are super-induced under certain conditions. (A) Each bar indicates the average expression and standard deviation of 286 genes whose expression is induced as part of the ESR as measured in one timepoint during the response to 25°C to 37°C heat shock (5, 15, 30, 60 min), 0.3 mM hydrogen peroxide (10, 20, 30, 40, 50, 60, 80, 100, 120, 160 min), 2.5 mM DTT (15, 30, 60, 120, 240, 480 min), 1M sorbitol (5, 15, 30, 45, 60, 90, 120 min), amino acid starvation (0.5, 1, 2, 4, 6 h), and progression into stationary phase (2, 4, 6, 8, 10, 12, 24, 48, 72, 120 h). (B) The average expression and standard deviation of 13 genes whose expression is super-induced in response to hydrogen peroxide and (C) of 17 genes whose expression is super-induced in response to heat shock are shown, as described for (A).

second, unrelated stress (Mitchel and Morrison 1982; Blomberg et al. 1988; Wieser et al. 1991; Flattery-O'Brien et al. 1993; Lewis et al. 1995).

2.9 Regulation of ESR gene expression

Although to a first approximation the ESR is commonly initiated in response to many stressful environments, several lines of evidence suggest that genes in this program are not controlled by a single system but are governed by different mechanisms in response to different environmental conditions. Subgroups of genes in the ESR display subtly different expression patterns, relative to other ESR genes, in response to specific stimuli. For example, the expression of genes involved in oxidative stress defense is induced as part of the ESR but is superinduced in response to conditions that result in oxidation (Fig. 2.6). Similarly, although genes encoding protein folding chaperones are induced in the ESR, their expression is more highly induced in response to conditions of protein unfolding, relative to other genes in this program. These subtleties also apply to genes whose expression is reduced as part of the ESR. In many of the experiments, transcripts of the RP genes decrease concomitantly with the other repressed ESR transcripts, however in response to certain experiments, such as exposure to the sulfhydryl oxidant diamide, expression of the RP genes is decreased with a delayed and muted profile, relative to the non-RP genes in the ESR. Thus, implications of condition-specific regulation are evident in the expression patterns of both the genes whose expression is induced and those whose expression is repressed as part of the ESR.

A variety of regulatory mechanisms have been implicated in the regulation of ESR gene expression. Although some of the regulatory factors involved are known (Table 2.4), the complexity of ESR regulation is only beginning to unfold. Discussed below are some of the key regulatory factors and systems that have been implicated in mediating ESR gene expression, followed by a model for the condition-specific regulation of the program.

2.9.1 Rap1p

The multipurpose factor Rap1p has been implicated in a variety of cellular processes, and as a transcriptional activator it is responsible for the high level of expression of the RP genes in actively growing cells (reviewed in Morse 2000). Because the turnover of these transcripts is relatively fast (5-10 minutes at standard conditions), constant Rap1p-mediated transcription helps to maintain these transcripts at high levels in the cell (Li et al. 1999). However, in response to stressful conditions, RP transcripts rapidly decline, in concert with other genes repressed in the ESR. The mechanism of repressed RP gene expression in response to heat shock appears to be mediated through silencing, coupled with the constitutively high rate of turnover of the RP transcripts (Li et al. 1999). However, the mechan-

Table 2.4. Factors implicated in regulating genes in the ESR

Factor [a]	Number of ESR genes affected [b]	Conditions [c]
Multifunctional proteins		
Rap1p	137 (23%) repressed ESR genes 13 (5%) induced ESR genes	
Silencing		
Tup1p-Ssn6p	30 (10%) repressed ESR genes 25 (4%) induced ESR genes	
Chromatin remodeling		
rpd3 mutant	164 (28%) repressed ESR genes	
Rpd3p/Isw2p	65 (23%) induced ESR genes	
Transcription factors		
Msn2/4p	251 (88%) induced ESR genes	Diverse conditions
Yap1p	17 (6%) induced ESR genes	Oxidative stress
Hot1p	9 (3%) induced ESR genes	Osmotic shock
Hsf1p	unknown	Heat shock
Msn1p	unknown	
Sko1p repressor	unknown	Osmotic shock
Protein Kinase Pathways		
MEC pathway	entire ESR	DNA-damaging agents
Ste11p/Ssk1p pathways	entire ESR	Osmotic shock
PKC pathway	entire ESR likely	Heat shock, Secretion Defects
PKA pathway	entire ESR likely	Nutrient repletion, Others?
TOR pathway	entire ESR likely	Nutrient repletion, Others?
SNF1 pathway	unknown	Glucose starvation
PHO85 pathway	unknown	Glucose repletion

[a] Data based on genomic expression studies. See text for references.
[b] Number and percent of the genes induced (out of 286 total) or repressed (out of 588 total) in the ESR.
[c] Conditions under which each factor is known to be active.

ism appears to be condition-specific and in some cases dependent on Rap1p: in addition to its role as a transcriptional activator, Rap1p also mediates gene silencing by interacting with a host of silencing factors through its carboxyl-terminal silencing domain (Morse 2000). Li et al. (1999) showed that the decrease in RP transcripts following DTT treatment required the silencing domain of Rap1p, while the drop in RP transcript levels after heat shock was independent of this Rap1p domain; a similar result was obtained by Miyoshi et al. (2001), who demonstrated that silencing of the rDNA was dependent on the Rap1p silencing domain in response to DTT but not during nitrogen starvation. This result reveals that, at least in some cases, Rap1p plays multiple roles in regulating the expression

of the RP genes, and that different mechanisms govern the reduced expression of these genes under different circumstances.

Many of the promoters of RP genes contain multiple binding sites for Rap1p, and these promoters were recently shown to be bound by Rap1p *in vivo*. Lieb et al. (2001) performed chromatin immuno-precipitation experiments to identify the genomic sites bound by the factor in living cells. In addition to the RP gene promoters, the promoters of roughly 15 other genes whose expression is repressed as part of the ESR, many of them involved in rRNA processing, were also bound by Rap1p. Most of the Rap1p-bound promoters contain binding sites for the factor; in addition, ~80 non-RP genes whose expression is repressed in the ESR also contain Rap1p binding sites in their promoters but were not observed to be bound by Rap1p under standard growth conditions. These data implicate Rap1p in regulating other repressed genes in the ESR; however, the expression pattern of most of these genes is subtly distinct from that of the RP genes, suggesting that their repression is mediated by a distinct mechanism.

In addition to promoters of repressed ESR genes, Rap1p was also detected bound to the promoter regions of a handful of genes whose expression is normally induced in the ESR (Lieb et al. 2001). The promoters of 13 induced ESR genes were bound by Rap1p, and included in this set was *GPD1*, whose induction in response to osmotic shock is Rap1p-dependent (Eriksson et al. 2000). Promoter analysis of this set of 13 genes revealed that 70% of them contain a novel sequence, AAAGGAG, which may be involved in the regulation of these genes. Although the putative binding factor of this sequence is not known, a candidate regulator is the recently identified transcription factor Hot1p. This factor appears to be active specifically in response to hyper-osmotic shock, and it is known to regulate a number of genes induced in the ESR under these conditions, including *GPD1* (Rep et al. 1999; Rep et al. 2000). Because Hot1p shares sequence homology with the transcription factor Gcr1p, which acts with Rap1p to regulate genes involved in glycolysis, it was suggested that Hot1p might also function with Rap1p to regulate its targets (Rep et al. 1999). That the sequence motif identified in the Rap1p-bound promoters differs from the known Gcr1p site at one position (GAAGGAG) (Huie et al. 1992; Huie and Baker 1996) raises the possibility that it may represent the unknown binding site of Hot1p. Regardless, the data of Lieb *et al.* clearly point to a role for Rap1p in the regulation of some of the genes induced in the ESR.

2.9.2 Chromatin remodeling

In addition to modulating transcription initiation, the cell can control the expression of its genome by altering chromatin structure to silence or desilence genes. In yeast, chromatin remodeling is affected by ATP-dependent chromatin remodeling factors as well as enzymes that chemically modify histones (reviewed in Perez-Martin 1999; Peterson and Logie 2000). The factors that mediate these processes are recruited to the sites of action by specific DNA binding proteins that bind to conserved sequences in the genome. The yeast corepressors Tup1p-Ssn6p have

been proposed to act as bridging factors between these specific DNA binding proteins and histone modifying factors, including the histone deacetylases Rpd3p and Hda1p (Smith and Johnson 2000; Watson et al. 2000; Wu et al. 2001). The resulting alterations in chromatin structure are thought to affect gene expression in the vicinity of the structural changes.

A number of lines of evidence suggest that the general repressors Tup1p-Ssn6p may contribute to the regulation of some of the genes that participate in the ESR. Under conditions of normal cell growth, the site-specific repressor Sko1p maintains the expression of genes required for growth at high osmolarity, including a number of genes that are normally induced in the ESR (such as *GRE2* and *YML131W*). The expression of these and other Sko1p target genes becomes derepressed after a hyper-osmotic shock in a manner dependent on the osmolarity-induced Hog1 pathway (Proft et al. 2001; Rep et al. 2001) (see Chapter 4). Numerous indications suggest that Tup1p-Ssn6p are involved in this repression: *ssn6* and *tup1* mutant cells show higher transcript levels of the Sko1p targets, and deletion of *SSN6* suppresses the sodium chloride sensitivity of *hog1* mutants (Garcia-Gimeno and Struhl 2000; Rep et al. 2001). Deletion of *TUP1* also leads to the derepression of many genes, including those that participate in the ESR, as measured in microarray experiments (DeRisi et al. 1997). Roughly 60 genes whose expression is normally induced (including *GRE2* and *YML131W*) or repressed (including the RP genes) under stressful situations were derepressed in the *tup1* mutant cells, including the gene encoding Msn4p. (It should be noted that a significant fraction of genes that are normally induced or repressed in the ESR were expressed at lower levels in the *tup1* cells, for reasons that are not understood but may be due to secondary consequences of *TUP1* deletion.) One caveat of these experiments is that deletion of the general repressor Tup1p may be stressful to cells and may therefore indirectly trigger ESR gene expression changes. An argument against this situation is that the genomic expression program in *tup1* mutant cells does not resemble the normal ESR initiation pattern: the expression of only a subset of the ESR genes was affected by *TUP1* deletion, and the genes that were affected did not display the stereotyped expression profile seen during ESR initiation. Therefore, these data suggest that Tup1p, likely along with Ssn6p, plays multiple, direct roles in affecting the expression of both induced and repressed genes in this program. These factors may participate in ESR initiation by contributing to the reduced expression of some of the repressed ESR genes, but they may also aid in ESR suppression under standard growth conditions by silencing the expression of genes that are induced when the program is initiated. Whether Tup1p-Ssn6p are commonly involved in regulating ESR gene expression following diverse environmental changes, or if the factors participate in the regulation of these genes only under specific conditions, is not known.

Other data suggest that the expression of induced or repressed ESR genes in response to stressful environments is regulated by chromatin remodeling factors. The most complete dataset comes from a recent study by Fazzio et al. (2001) that characterized genomic expression in cells defective in the activities of a number of chromatin remodeling proteins. They observed expression in cells lacking the histone deacetylase Rpd3p and the chromatin-remodeling factor Isw2p, two proteins

thought to act in parallel pathways to mediate the repression of meiosis-specific genes (Goldmark et al. 2000). In addition, the group also observed genomic expression in cells harboring catalytically inactive mutants of Rpd3p or Isw2p, as inactive Rpd3p alleles provoke dominant-negative effects in cells (Kadosh and Struhl 1998). Double deletion of both factors resulted in the derepression of many genes, including ~30% of the genes normally induced in the ESR; the remaining ESR genes were largely unaffected in terms of expression. Most interestingly, cells harboring the catalytically inactive Rpd3p, but not the Isw2p inactive mutant, showed derepression of a different set of genes – most of these genes are normally repressed in the ESR, and together they amount to roughly 30% of the repressed ESR genes. Again, the data suggest that these effects were specific to these subsets of ESR genes, as opposed to a general initiation of the entire ESR as a secondary response to Rpd3p and Isw2p defects. The genes that were derepressed in cells harboring the inactive Rpd3p mutant largely overlap a set of non-RP ESR genes that contain the promoter sequence GATGAG, although it is unknown if this promoter element has anything to do with the Rpd3-dependent regulation of these genes (Gasch et al. 2000; Hughes et al. 2000a; Fazzio et al. 2001). Most of the genes that were derepressed by deletion of *RPD3* and *ISW2* did not correlate with the genes affected by *TUP1* deletion, indicating that, although Tup1p recruits Rpd3p under certain situations, the proteins can act independently of each other. Again, the conditions under which Rpd3p and/or Isw2p are involved in mediating ESR gene expression is currently unknown.

2.9.3 Regulated mRNA turnover

The coexpression of transcript levels is not only controlled by mRNA synthesis but also mediated by RNA decay, and the details of regulated mRNA degradation are beginning to unfold. Multiple pathways of mRNA turnover exist in yeast, including polyadenylation-mediated mRNA degradation (apparently the primary route of mRNA turnover in dividing cells), nonsense-mediated decay (which degrades aberrant as well as normal transcripts), and 3’ to 5’ transcript degradation (reviewed in McCarthy 1998; Wilusz et al. 2001). In the polyadenylation-mediated degradation pathway, upon removal of the polyA tail by specific yeast factors, the 5’ 7-methylguanosine cap of the transcript is removed to allow 5’ to 3' exonucleases to degrade the mRNA. Many factors have been implicated in mRNA stabilization and decay; a few examples will be presented here, as they pertain to the regulation of ESR gene expression.

In yeast, and likely in other organisms as well, transcripts that encode functionally related proteins are coordinately degraded. In a study by Wang et al. (2002), the decay rates of the genomic transcripts were characterized after rapid inhibition of nascent protein synthesis (Wang et al. 2002). Upon shifting cells to the non-permissive temperature required to inactivate an RNA Pol II mutant protein (*rpb1-1*), the mRNA decay rates were measured. The study showed that functionally related transcripts, especially those that encode proteins involved in multi-subunit complexes, shared highly similar decay rates, indicating that transcript degrada-

tion is coregulated. The vast majority of these transcripts appeared to be degraded by the polyadenylation-dependent pathway, as the disappearance of the polyA tail occurred before degradation of the rest of the transcripts (Wang et al. 2002). In terms of the genes that participate in the ESR, transcripts that are increased in this program *versus* those that are reduced showed differential decay rates: under the conditions studied, transcripts that normally decrease in abundance when the ESR is initiated were degraded on average 4X faster than transcripts that increase in abundance when the program is triggered. Because the experiment required a 37°C temperature shift to inactivate transcription, it is possible that the differential half-lives observed for the transcripts of genes whose expression is induced *versus* repressed in the ESR are specific for this stressful transition, which would normally trigger gene expression changes indicative of ESR initiation.

In mammalian cells, the half-lives of stress-induced transcripts can be altered by RNA binding proteins that affect transcript stability. A number of cytokines, including TNFα, contain AU-rich sequences known as AREs in their 3' untranslated region (UTR) that destabilize the transcripts (Chen and Shyu 1995). In response to specific conditions, the half-lives of these transcripts can be extended *via* binding of the ARE sequences by different proteins, as well as through an independent mechanism mediated by the stress-kinase p38 (Kontoyiannis et al. 1999; Winzen et al. 1999; Dean et al. 2001). ARE sequences also appear to play a role in yeast transcript stability. Vasudevan and Peltz (2001) showed that the transcript encoding the translation elongation factor TIF51A, which contains multiple 3' AREs, has different half-lives in glucose- *versus* glycerol-containing medium. Introduction of the TNFα 3'UTR to a reporter construct conferred carbon source-regulated mRNA turnover, suggesting that the 3' ARE sequences in the reporter, and perhaps also the TIF51A transcript, contribute to the regulated mRNA turnover. Stabilization of the reporter construct was proposed to be dependent on Pub1p, an RNA binding protein homologous to one of the mammalian ARE-binding factors (Anderson et al. 1993; Matunis et al. 1993): Pub1p bound to the 3' AREs of the reporter construct *in vitro*, and deletion of *PUB1* correlated with decreased levels of the transcript *in vivo* (Vasudevan and Peltz 2001). The repression of other ESR genes is probably also controlled by this mechanism, however because the ARE sequence is commonly found in 3' UTRs in the genome (~2 ARE sequences present in each gene's 3' UTR), it is difficult to predict additional ARE-regulated genes based solely on the presence of 3' ARE sequences.

Other transcripts reduced as part of the ESR show differential stability in response to stress, particularly during the diauxic shift transition. As demonstrated by Albig and Decker (2001), the half- lives of three transcripts that are reduced with the ESR (*RPL10*, *RPS15*, *ARO4*) were reduced as the cell went through the diauxic shift transition and when cells were exposed to rapamycin, an inhibitor of the Tor1p kinase. The degradation of a reporter transcript was abrogated in a rapamycin-resistant *tor1* mutant strain, indicating that Tor1p signaling affects mRNA degradation under these conditions. The mechanism of the Tor1p-mediated mRNA decay is unknown, however because the *ARO4* transcript showed an increased rate of polyA shortening, it was proposed that the regulation was through polyadenylation-mediated degradation. In response to rapamycin, the in-

crease in mRNA decay rates was transient: after ~60 min of rapamycin treatment the cells adapted to the drug and the half-lives of these transcripts returned to those seen in untreated cells. This observation is consistent with the transient changes in transcript levels of genes that participate in the ESR following stressful environmental changes.

2.9.4 Msn2p and Msn4p

In terms of the regulators of ESR gene expression, among the best characterized are the transcription factors Msn2p and Msn4p. Originally isolated as high copy suppressors of *SNF1* deletion (Estruch and Carlson 1993), these related factors were subsequently shown to bind the STRE promoter sequence that had been implicated in a general stress response in yeast (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). Indeed, Msn2/4p are involved in the transcriptional induction of many of the induced ESR genes in response to stressful conditions, and double deletion of these factors diminishes the induction of ESR gene expression and renders cells sensitive to a variety of environmental stresses (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996; Boy-Marcotte et al. 1998; Gasch et al. 2000; Amoros and Estruch 2001). Although the factors are often considered redundant, Msn2p *versus* Msn4p appear to affect gene induction differently, in a manner that is gene-specific and condition- specific, as discussed below. Because these factors play such a large role in regulating the expression of the genes induced in the ESR, significant discussion of their properties will be presented here.

Characterization

Msn2p and Msn4p share 32% amino acid identity, particularly in their DNA binding domains that are nearly identical, and they belong to the zinc-finger family of transcription factors (Estruch and Carlson 1993). Both factors bind the STRE sequence (CCCCT) with specificity *in vitro*, and both can mediate transcriptional induction from a plasmid construct containing the STRE promoter element, indicating that this sequence is sufficient for Msn2/4p-dependent induction of gene expression (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). *In vitro* binding of the STRE by either factor does not require stress treatment, as Msn2p and Msn4p were shown to bind to the STRE whether the proteins were recovered from cell extracts of stressed or healthy cells (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). This observation revealed that the proteins are activated in a manner that does not affect their DNA binding properties. It was subsequently realized that the activity of the transcription factors is dependent on their localization: Gorner et al. (1998) used epitope-tagged versions of these factors to demonstrate that in the absence of stress, Msn2p and Msn4p resided in the cytosol, however in response to stressful environments the factors were rapidly translocated to the nucleus (Gorner et al. 1998). Within minutes of exposure to temperature shock, hyper-osmolarity, salt stress, ethanol exposure, and glucose starvation, the majority of Msn2p and Msn4p within the cell accumulated in the nucleus. Af-

ter the cells were returned to their standard growth conditions, Msn2p and Msn4p relocalized to the cytosol within minutes, even in the absence of nascent protein synthesis, indicating that translocation of the proteins was reversible.

Regulation of Msn2/4p nuclear localization

That the nuclear translocation of Msn2p was shown to be independent of protein synthesis pointed to a post-translational regulatory mechanism governing the relocalization. PKA signaling is known to negatively affect induction of STRE-regulated genes (Marchler et al. 1993; Boy-Marcotte et al. 1998), and therefore Gorner et al. characterized the involvement of this pathway in mediating Msn2p and Msn4p localization. In cells defective in PKA signaling, Msn2p and Msn4p were shown to be primarily nuclear, whereas cells with abnormally high cAMP showed nuclear exclusion of the factors, even in the presence of environmental stress. These data revealed that PKA signaling negatively affects Msn2p and Msn4p-dependent signaling by restricting the factors to the cytosol. The central region of both Msn2p and Msn4p contain multiple putative phosphorylation sites, and indeed mutation of the serine residues in these sites on Msn2p results in a constitutively-nuclear protein (Gorner et al. 1998). Thus, it was proposed that PKA might directly phosphorylate Msn2p and Msn4p to trigger cytoplasmic relocalization of the proteins. Contradictory to this original model, it was subsequently observed by Garreau et al. (2000) that under standard growth conditions, both Msn2p and Msn4p exist in a phosphorylated state, but they become hyper-phosphorylated in response to multiple stresses. Addition of cAMP after cellular stress reversed the hyper-phosphorylation state and triggered relocalization of the factors to the cytosol. It now appears that the inhibitory effect of PKA signaling leads to the dephosphorylation of Msn2p and Msn4p to trigger their relocalization to the cytosol. The putative PKA-dependent phosphatase that acts on Msn2p and Msn4p is at present unknown.

The stress-induced relocalization of Msn2p was shown to be entirely dependent on a ~300 amino acid region of the protein that contains the putative phosphorylation sites, since fusion of this protein sequence onto an SV40 nuclear localization signal resulted in PKA- and stress-regulated translocation of the chimera, even in the absence of the remaining Msn2p protein sequence (Gorner et al. 1998). The corresponding domain in Msn4p was not extensively studied, however multiple, putative phosphorylation sites are conserved in this region of Msn4p. These details led Gorner *et al.* to propose that this domain may bind to a cytosolic protein that serves to anchor Msn2p and Msn4p in the cytosol under standard growth conditions. It was recently shown that in the absence of cellular stress, Msn2p and Msn4p interact with the constitutively-cytosolic protein Bmh2p (Beck and Hall 1999). Bmh2p is homologous to mammalian 14-3-3 proteins that mediate the localization of signaling proteins, and it and its paralog Bmh1p have been implicated in RAS-dependent signaling and pseudohyphal development in yeast (Gelperin et al. 1995; Roberts et al. 1997; Roth et al. 1999). Under standard growth conditions, Bmh2p was isolated in a complex with Msn2p or Msn4p, however in response to starvation or inhibition of the Tor1 kinase, the association be-

tween the proteins was not observed, and Msn2p accumulated in the nucleus (Beck and Hall 1999). Although it is not known whether the interaction with Bmh2p mediates the cytosolic localization of Msn2p or merely correlates with it, it is interesting to note that mammalian 14-3-3 proteins bind phosphoserine residues in proteins (Muslin et al. 1996; Bertram et al. 1998; Muslin and Xing 2000), hinting that Bmh2p may bind to Msn2p and Msn4p in a manner dependent on the precise phosphorylation state of these proteins.

Differential role and regulation of Msn2p versus *Msn4p*

Msn2p and Msn4p are often treated as 'redundant' transcription factors, however these proteins show subtly different behavior in cells. It has been observed that for many of the known STRE-regulated stress genes, deletion of *MSN2* results in decreased induction of gene expression in response to stress, whereas deletion of *MSN4* often has no result on gene expression (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996; Treger et al. 1998; Amoros and Estruch 2001). However, in the absence of Msn2p, Msn4p clearly contributes to transcriptional induction, as deletion of both *MSN2* and *MSN4* results in significantly weaker gene induction relative to that seen in the *MSN2* single deletion mutant (Treger et al. 1998; Garreau et al. 2000). These results indicate that Msn2p and Msn4p may have different functions in the cell. Consistent with this observation, the proteins are regulated differently. First, in response to stressful environments the expression of *MSN4* is induced as part of the ESR (DeRisi et al. 1997; Gasch et al. 2000), and at least in response to glucose starvation, the increased expression parallels increased levels of Msn4p, whereas neither *MSN2* nor its gene product increases in response to stressful environments (Garreau et al. 2000). Second, Garreau et al. (2000) showed that the stress-induced alterations in protein phosphorylation are different for Msn2p *versus* Msn4p, indicating that the proteins are differentially phosphorylated. Although Msn2p and Msn4p can both activate the induction of gene expression through the STRE promoter element, the proteins appear to be regulated differently and may play distinct roles in the regulation of specific genes induced in the ESR.

Targets of Msn2p and Msn4p

The role that Msn2p and/or Msn4p (hereafter referred to as Msn2/4p for simplicity) play in stress-responsive gene induction varies for each gene target as well as for the specific environmental conditions. For example, genes induced in the ESR show varying dependence on Msn2/4p, indicating that collectively these factors play different regulatory roles on a gene-by-gene basis. Genomic expression in *msn2msn4* mutant cells responding to heat shock or hydrogen peroxide treatment revealed that the expression of approximately 60% of the ~300 induced ESR genes was affected by the double deletion, and these genes fell into three groups (Gasch et al. 2000). One group of genes, including many of the previously known targets of the factors, was largely dependent on Msn2/4p for induction in response to both heat shock and hydrogen peroxide treatment, while a second group was

partially dependent on the factors in response to both stresses. The induced expression of a third group of genes was dependent on Msn2/4p in response to heat shock, but was unaffected by deletion of the factors in response to hydrogen peroxide, revealing that Msn2/4p are conditionally involved in regulating the expression of these genes. (It should be noted that for the vast majority of the genes whose expression was affected by deletion of the factors, residual induction occurred in the absence of Msn2/4p, indicating the involvement of additional regulators.) Most of the genes that were affected by deletion of *MSN2* and *MSN4* were also responsive to overexpression of the factors, and the expression of approximately 80 additional genes that were unaffected in the *msn2msn4* strain responding to heat shock or hydrogen peroxide was induced by *MSN2* or *MSN4* overexpression, revealing even more gene targets. These details show that Msn2/4p contribute to the induced expression of ESR genes to varying degrees, in a manner that is different for each gene and also dependent on the specific environmental features. The condition-specific involvement of Msn2/4p is supported by the observation that the pattern of hyper-phosphorylation of each protein is distinct in response to different environmental stresses, suggesting that the proteins are regulated in different ways in response to different stimuli (Garreau et al. 2000).

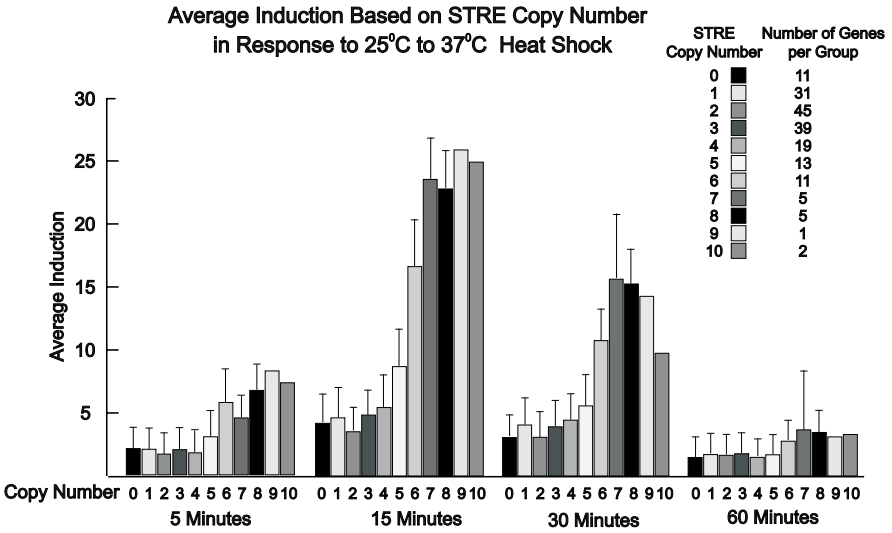

Fig. 2.7. Msn2/4p-dependent induction is proportional to the STRE copy number. Approximately 180 genes whose induced expression in response to heat shock was dependent on Msn2/4p were binned into ten groups based on the number of STRE (CCCCT) elements within 800 base pairs upstream of each open reading frame. The average induction and standard deviation of genes with 0 to 10 STRE promoter elements is shown at 5, 15, 30, and 60 minutes after a 25°C to 37°C heat shock. The key in the upper right corner indicates the number of genes present in each bin. Figure kindly provide by Alan Moses, based on the data from Gasch et al. 2000

Of the genes affected by Msn2/4p, roughly 95% contain at least one canonical STRE within 800 base pairs upstream of the open reading frame, and many of the genes contain multiple STREs. In fact, the level of transcriptional induction is roughly proportional to the STRE copy number in the genes' promoters: genes with multiple STREs show larger increases in expression in response to a number of environmental transitions, relative to Msn2/4p-regulated genes with one or zero STRE sequences (Fig. 2.7, A. Moses and M.B. Eisen, personal communication). The presence of one STRE promoter element is sufficient for Msn2/4p-regulated induction from a plasmid construct, however mere presence of this sequence in genomic promoter regions does not necessarily result in Msn2/4p-mediated transcriptional induction. Whereas ~3,600 genes in the yeast genome contain at least one STRE within 800 base pairs upstream of the open reading frame, only ~200 genes are affected by *MSN2/MSN4* double deletion or overexpression, and only ~300 genes display stress-responsive gene induction profiles. This is consistent with observations of other known regulatory sequences, for which only a fraction of those present in the genome are bound and presumably regulated by their respective factors (Ren et al. 2000; Iyer et al. 2001; Lieb et al. 2001; Simon et al. 2001). This suggests that many of the sequences potentially recognized by these DNA binding factors, including many of the genomic STRE sequences, are not bound by their respective factors, perhaps because those sequences are obscured in the genome by other DNA binding proteins or by the local chromatin conformation. Other genes whose expression appears to be affected by Msn2/4p do not contain STRE promoter elements; as no other similarities in these promoters are apparent, the effect of Msn2/4p on the expression of these genes be it direct or indirect, remains unclear.

Are Msn2p and Msn4p truly "general" stress factors?

That Msn2/4p are involved, albeit in different ways, in regulating gene expression in response to most of the stressful conditions observed to date raises questions about the role that these factors play in regulating genes whose expression is induced in the ESR. Together, the factors affect nearly 90% of the genes induced in this program, although the extent of their involvement varies under each set of conditions. Each of the factors is also differentially phosphorylated in response to different situations, indicating condition-specific activation. One possibility is that Msn2/4p are activated by many different stressful situations but play different roles in each case. This model implies that the condition-specific phosphorylation patterns of Msn2/4p lead to different activation states of the proteins and produce different gene expression outputs. An alternative model is that the different phosphorylation states of Msn2/4p all result in the same effect on Msn2/4p, for example relocalization of the factors to the nucleus, allowing Msn2/4p to be generally activated by many different upstream signaling pathways. The apparent differential effects of *MSN2/MSN4* double deletion in cells responding to different environments may be accounted for by the fact that the expression of each gene in the ESR is affected by many different factors (see below), and therefore the dependence of each gene's expression on Msn2/4p may vary, even though Msn2/4p are

similarly activated in each situation. As more details of the complete signaling networks that govern ESR gene expression unfold, the role of Msn2/4p in mediating ESR gene expression will become clearer.

2.9.5 Condition-specific transcriptional induction

Msn2/4p are not always required for induced expression of genes in the ESR, suggesting that other regulatory mechanism come into play under specific environmental conditions. Indeed, it appears that many different condition-specific transcription factors are involved in regulating the expression of subsets of genes in the ESR. This was clearly shown in a study by Rep et al. (1999), which characterized the involvement of four transcription factors, Msn1p, Msn2p, Msn4p, and Hot1p, in inducing the expression of three ESR genes (*GPD1*, *HSP12*, and *CTT1*) (Fig. 2.8) (Rep et al. 1999). In the case of *GPD1*, which encodes an isozyme of glycerol-3-phosphate dehydrogenase, the induced expression was mainly governed by Hot1p in response to osmotic shock, but controlled by Msn2/4p in response to heat shock in a manner unaffected by *HOT1* deletion. Induced expression of the cytosolic catalase gene *CTT1* was controlled by Msn2/4p and Msn1p following osmotic shock, evident by the fact that deletion of *MSN2/MSN4* or *MSN1* muted the induction of *CTT1*; however, in response to heat shock *CTT1* induction appeared to be almost entirely controlled by Msn2/4p, with little contribution from Msn1p. In contrast to these genes, the induction of *HSP12* expression was controlled by Msn2/4p in response to both osmotic and heat shock. These results reveal that *GPD1* and *CTT1* are regulated by different transcription factors under different conditions, and each of the genes displays a different dependence on the transcription factors studied.

Similar results have been observed for other genes and transcription factors. A number of genes whose expression is induced in the ESR, including those encoding protein folding chaperones, are super-induced in response to heat shock relative to other ESR genes. Many of these genes contain promoter elements recognized by the heat shock transcription factor Hsf1p as well as the STRE bound by Msn2/4p, implicating these factors in regulating the genes' expression. Indeed, Hsf1p and Msn2/4p are involved in regulating the expression of many of these genes, although the factors appear to play different roles in response to different stresses. Whereas the induced expression of *HSP26* and *HSP104* is primarily controlled by Hsf1p in response to heat shock, the induction is largely dependent on Msn2/4p in response to carbon starvation, oxidative stress, or osmotic shock (Treger et al. 1998; Amoros and Estruch 2001). Analogous to the protein folding chaperones, the expression of a subset of ESR genes that have been implicated in the response to oxidative stress is super-induced in response to conditions that inflict oxidative damage. The expression of these genes is induced by the oxidation-activated Yap1p transcription factor in response to hydrogen peroxide but is regulated by Msn2/4p following heat shock, independent of Yap1p (Gasch et al. 2000). Together, these data show that the expression of subsets of genes in the ESR are regulated by different transcription factors under different conditions, namely Hot-

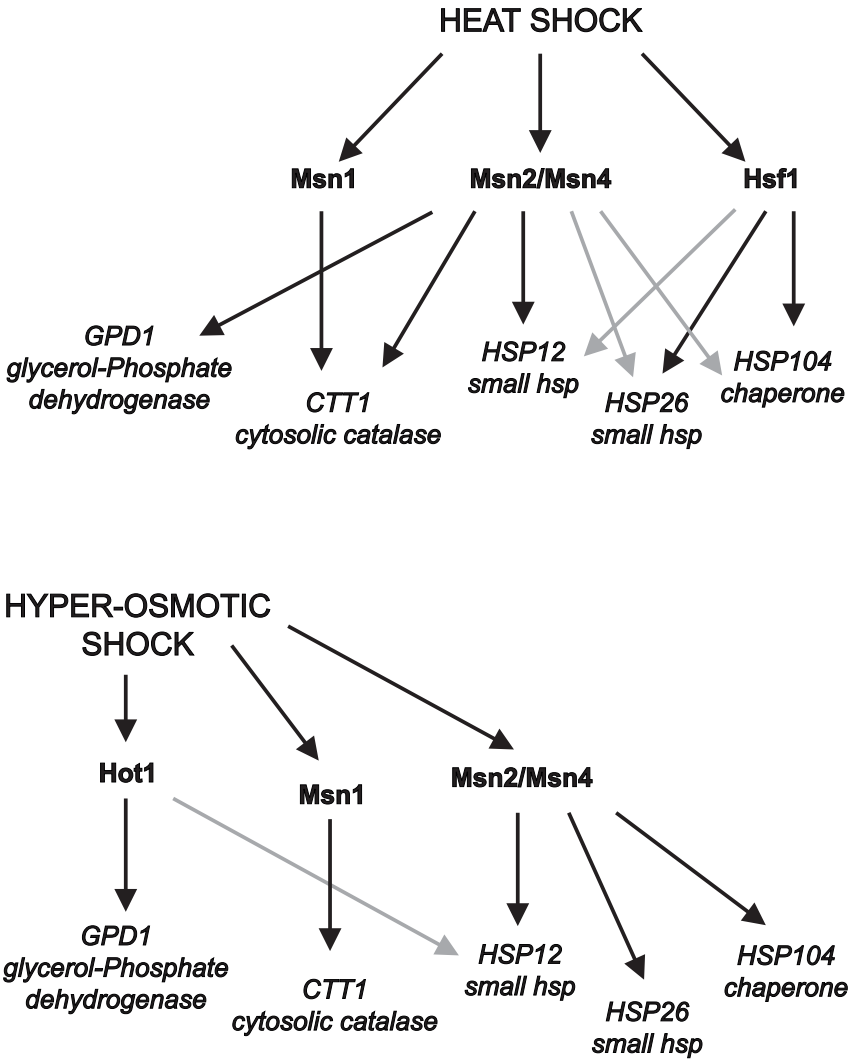

Fig. 2.8. Condition-specific transcriptional induction of genes in the ESR. Transcription factors that contribute to the regulation of genes in the ESR are shown in response to heat shock (top panel) and osmotic shock (bottom panel), as indicated by a bold arrow. Transcription factors that contribute to gene expression only in the absence of other factors (see text for details) are indicated by a gray arrow. Based on data from Treger et al. 1998; Rep et al. 1999; Amoros and Estruch 2001

1p during osmotic shock, Hsf1p following heat shock, and Yap1p in response to oxidative stress. In contrast, all of the genes tested were affected in some way by Msn2/4p, although the precise role of the factors varied for each gene in response to the different conditions tested.

In all of the studies mentioned above, residual induction was observed in strainslacking individual transcription factors, indicating that other regulators can supplement for the absent factors (Treger et al. 1998; Rep et al. 1999; Gasch et al. 2000; Amoros and Estruch 2001). For example, Rep *et al.* showed that in an otherwise wild type background, deletion of the transcription factor Hot1p had no effect on the induced expression of *HSP12* in response to either heat shock or osmotic shock. However, in the absence of the primary *HSP12* regulators Msn2/4p, Hot1p significantly contributed to the residual induction of its expression following osmotic shock. A quadruple mutant lacking *HOT1*, *MSN1*, *MSN2*, and *MSN4* still showed residual induction of *HSP12* expression in response to osmotic stress, suggesting still other regulators. In the absence of Msn2/4p, Hsf1p is known to induce the expression of *HSP12* (Treger et al. 1998), and perhaps this factor contributes to the residual induction of *HSP12* expression in the quadruple transcription factor mutant. Conversely, the involvement of Msn2/4p in regulating the induced expression of the ESR genes *HSP26*, *HSP78*, *HSP104* and *UBI4* following heat shock could only be detected in the absence of Hsf1p (Treger et al. 1998; Simon et al. 1999). Consequently, although certain transcription factors are not required for the induction of specific genes in wild type cells, those factors play a significant role in transcriptional regulation in the absence of the genes' primary regulators.

2.9.6 Condition-specific cellular signaling

Consistent with the condition-specific transcriptional regulation of genes induced in the ESR, the coordinate expression of genes induced and repressed in this program is controlled by different upstream signaling pathways depending on the environmental conditions. For example, characterization of the genomic expression pattern in wild type and mutant cells responding to DNA-damaging agents revealed that the Mec1 pathway is involved in regulating the ESR (Gasch et al. 2001). Mec1p is a protein kinase, related to phospho-inositol kinases, that is activated in response to DNA damage to phosphorylate downstream kinases that govern cell cycle arrest and gene expression changes (reviewed in Elledge 1996). In response to DNA-damaging agents including methylmethane sulfonate and ionizing radiation, wild type cells responded by inducing the expression of a subset of genes specifically enlisted to repair the resulting cellular damage and by initiating the ESR (Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Lee et al. 2001b). However, cells defective in Mec1 signaling failed to induce the DNA damage-specific genes and also failed to properly initiate the ESR: mutant cells lacking Mec1p or its downstream kinase Dun1p displayed muted expression changes of the genes that participate in the ESR, with an almost complete lack of ESR gene repression (Gasch et al. 2001). In contrast, deletion of *MEC1* or *DUN1*

had no significant effect on the initiation of the ESR in cells responding to heat shock. These results revealed that initiation of the entire ESR is controlled by the Mec1 pathway in response to DNA-damaging agents but by another mechanism following heat shock.

Other signaling pathways have also been implicated in the condition-specific regulation of ESR gene expression. The Protein Kinase C (PKC) MAP kinase pathway in yeast is activated by a number of conditions, including hypo-osmotic shock, heat shock, and secretion defects, although in each of these situations the signal that activates the PKC pathway may be related to cell wall defects (Levin and Bartlett-Heubusch 1992; Kamada et al. 1995; Heinisch et al. 1999; Nierras and Warner 1999). Activation of the PKC pathway has been linked to gene expression changes, reorganization of the actin cytoskeleton, and cell-cycle arrest, indicating that this pathway is involved in regulating many aspects of the cell's response to stressful environments (Gray et al. 1997; Delley and Hall 1999; Jung and Levin 1999; Harrison et al. 2001). In terms of gene expression, the PKC pathway is known to control the reduction in rRNA, tRNA, and RP transcripts following inhibition of secretion, however this pathway is uninvolved in the reduced expression of these genes in response to other stresses such as amino acid starvation (Mizuta et al. 1998; Li et al. 1999; Nierras and Warner 1999; Li et al. 2000; Miyoshi et al. 2001). Given the stereotypical expression patterns of genes in the ESR, it is likely that the PKC pathway governs initiation of the entire program in response to certain conditions. Indeed, cells lacking Slt2p, the terminal kinase on the PKC pathway, show muted induction of ~70% of the genes induced in the ESR following heat shock, although genes repressed in the ESR under these conditions appear to be unaffected by *SLT2* deletion (A.P. Gasch and P.O. Brown, unpublished data). That expression of the repressed ESR genes is unaffected by Slt2p is consistent with evidence by Li et al. (2000), which showed that in response to secretion defects, repression of the RP genes is controlled by the top kinase in the pathway, Pkc1p, but does not involve the known downstream kinases (including Slt2p), leading the group to propose a bifurcation of the PKC pathway. These preliminary details suggest that genes whose expression is induced in the ESR are regulated by a different branch of the PKC pathway than genes whose expression is repressed in the ESR.

The high osmolarity-responsive MAP kinase pathway involving Hog1p has also been implicated in regulating the ESR, and similar to the case of PKC, different branches of the pathway may control the induced *versus* repressed ESR genes. Hog1p is the terminal MAP kinase in a series of pathways that respond independently to high osmolarity, and activation of the kinase is involved in regulating cell cycle arrest, translation initiation, and gene expression changes critical to the survival of hyper-osmotic shock (Alexander et al. 2001; Teige et al. 2001). In response to salt- or sorbitol-mediated hyper-osmotic shock, many of the genes whose expression is induced in the ESR show muted but prolonged induction in cells lacking *HOG1* (Rep et al. 2000; S.M. O'Rourke and I. Herskowitz, personal communication). However, in contrast, the genes that are repressed in the ESR show stronger, prolonged patterns of repression in osmotic-shocked cells compared to the wild type (S.M. O'Rourke and I. Herskowitz, personal communica-

tion). Unlike the *hog1* mutant, O'Rourke and Herskowitz demonstrated that cells lacking *STE11* and *SSK1*, encoding two upstream components thought to act in parallel signaling pathways that activate Hog1p, showed muted expression changes for both the induced and repressed ESR genes (S. O'Rourke and I. Herskowitz, personal communication). Thus, genes whose expression is induced in the ESR *versus* those that are repressed in the program appear to be controlled by different branches of the Ste11p/Ssk1p-dependent signaling pathways in response to osmotic shock. The prolonged ESR gene expression changes seen in the *hog1* mutant suggest that these partially unresponsive cells may be experiencing a higher level of internal stress, consistent with the known sensitivity of the mutant to hyper-osmotic shock (Brewster et al. 1993).

Other regulatory systems have been tentatively linked to ESR regulation. The Snf1p kinase is activated by carbon starvation and leads to the derepression of many genes involved in respiration (reviewed in Carlson 1999). Snf1p also regulates a number of processes associated with the ESR, including glycogen accumulation and autophagy (Cannon et al. 1994; Wang et al. 2001a), raising the possibility that the kinase may play some role in ESR initiation. Furthermore, the effects of *SNF1* deletion are suppressed by high-copy expression of *MSN2* or *MSN4*, although the mechanism of the suppression is not entirely clear. Recently, a role for the Pho85 kinase, a proposed antagonist of Snf1p-dependent effects (Huang et al. 1998; Wang et al. 2001a; Wang et al. 2001b), in ESR regulation has been implicated. Carrol et al. (2001) demonstrated that chemical inactivation of the kinase lead to initiation of the ESR relative to control experiments, however whether the stress response was directly controlled or indirectly affected by rapid inactivation of Pho85p is not known. Despite these details, the precise role of Snf1p and Pho85p in ESR regulation remains to be decisively demonstrated.

The MEC-, PKC-, Ste11p/Ssk1p-, and likely other pathways are required for proper initiation of the ESR in response to specific environmental stresses, but other signaling systems have been implicated in the suppression of the ESR. The best characterized is the PKA system (see Fig. 2.5.). When starved cells are supplemented with glucose, a spike in intracellular cAMP levels (Mazon et al. 1982; Francois et al. 1988) triggers PKA activity (Jiang et al. 1998), and correlated with the resumption of cell growth is the increased expression of genes involved in protein synthesis and the decreased expression of Msn2/4p target genes (Marchler et al. 1993; Klein and Struhl 1994; Gorner et al. 1998; Thevelein and de Winde 1999; Norbeck and Blomberg 2000). These observations suggest that PKA activity suppresses the ESR in response to nutrient repletion to promote the resumption of normal cell growth. The negative effect of PKA signaling on ESR initiation is further evident by the fact that artificially high intracellular cAMP levels can suppress the ESR, even in the presence of environmental stress (Klein and Struhl 1994; Neuman-Silberberg et al. 1995; Gorner et al. 1998; Garreau et al. 2000). Whether PKA signaling provides a generalized mechanism of ESR suppression following stress relief, or is specific to nutrient repletion, is not known. An increase in intracellular cAMP is apparently not a general feature of stress relief, indicating that at least the cAMP spike seen following nutrient supplementation is specific to this condition (M. Jacquet, personal communication). However, that a

number of genes affecting PKA signaling are induced in the ESR in response to environmental stress argues for a more general role of the pathway. Because inappropriate activation of the ESR is likely detrimental to cell growth, one aspect of the ESR is to prepare for the suppression of the program once the cell has adapted to its new conditions. The induced expression of the PKA signaling components as part of the ESR may represent this preparation for ESR suppression. Alternatively, the induced expression of PKA genes may serve some other purpose: confusingly, cells lacking the catalytic subunits of PKA are unable to repress RP gene expression, a feature seen when the ESR is initiated, in response to glucose starvation (Ashe et al. 2000), arguing for a role for the PKA pathway in proper activation of the ESR. These complexities hint that PKA signaling components may have multiple roles in mediating ESR gene expression.

Similar to PKA signaling, activity of the TOR pathway has also been implicated in ESR suppression. Activation of the Tor1p kinase negatively regulates Msn2/4p by triggering cytosolic relocalization of the factors and is proposed to stimulate translation and RP gene expression in the absence of stress (Barbet et al. 1996; Beck and Hall 1999; Powers and Walter 1999). Again, exposure of cells to stressful environments leads to the induced expression of the gene encoding Tor1p as part of the ESR, in a manner dependent on Msn2/4p, raising the possibility that Tor1p-dependent signaling plays a more general role in mediating the ESR, perhaps as a mechanism of ESR suppression following adaptation to new environments.

2.9.7 Advantages of the complex regulation of ESR gene expression

The details presented above reveal the complexities of the regulation of ESR gene expression: changes in transcript abundance are mediated at the level of transcription initiation, chromatin structure, and mRNA stability, and these processes are controlled by different regulators in response to different conditions. Such a complicated system of regulation provides a number of advantages to the cell. First, this system allows specificity and precision in the cellular response to each new environment. By controlling ESR gene expression with many different factors, the cell can customize the overall initiation of the program to the exact features of the new environment. For example, different subsets of ESR gene products are particularly critical to survival in response to specific conditions, and therefore under the relevant circumstances those genes are controlled by specialized factors that guarantee their appropriate expression. Thus, the cell can sensitively control the precise levels of ~900 transcripts in the ESR by altering the context of a limited number of signaling factors which converge on the ESR genes to result in a unique overall pattern of ESR initiation.

A second advantage of this complicated regulatory system is that it provides a back-up mechanism of ESR initiation. In the absence of a primary regulator of a given ESR gene, other signaling factors can supplement for the regulator's activity to provide residual expression changes. Some of the genes discussed here are regulated by at least five transcription factors, and other mechanisms of transcript

regulation, such as regulated mRNA turnover, probably also contribute to the stress-induced expression changes. Whether the secondary factors that come into play are activated by the same upstream signal as the absent primary regulator, or become activated by a secondary cellular defect that arises due to the aberrant response of the mutant cells, is not known. Nonetheless, the cell has evolved this system to effectively guarantee initiation of the ESR in response to stressful environments, underscoring the importance of this program in surviving environmental fluctuations.

2.10 Orchestration of cellular responses to stress

The details presented in this review can be compiled into a model for the mechanism that cells use to orchestrate cellular responses to stressful environmental transitions. When a cell is transferred to a new environment for which its internal system is not optimized, specific cellular challenges arise. These features are detected by specific sensory systems (discussed in other chapters), which activate specialized signaling pathways to transmit a cellular signal. The upstream signaling factors activate multiple downstream protein kinases, each of which in turn likely activates a host of proteins that mediate cellular responses such as cell cycle arrest, translation inhibition, gene and protein expression changes, and enzymatic alterations. In terms of the gene expression changes, each specialized signaling pathway probably activates a variety of factors that affect gene expression. Some of these regulators trigger expression changes in genes that are specifically enlisted to combat the unique cellular challenges initially detected by the cell. At the same time, these and other factors collectively alter the expression of ~900 genes that participate in the ESR, initiated to protect critical features of the cell's internal homeostasis in times of stress. Thus, each cellular response is precisely orchestrated to result in a unique cellular program that will ensure survival of the cell in the new environment. Immediately after cells are shifted to a stressful environment, they respond by arresting growth and protein synthesis while triggering large changes in gene expression; however, over time the cell often adapts to the new conditions, and the alterations in genomic transcript levels subside as growth and translation resume. One model for the role of the transient gene expression changes is that large initial alterations in gene expression may promote the rapid adjustment in the corresponding gene products, and their activities, to new steady-state levels. A model for the order of events that occur during the adaptation phase is shown in Fig. 2.9, based on the details of the response to hyper-osmotic shock as an example. When cells are shifted to medium of high osmolarity, they trigger a cellular signal that leads to the phosphorylation and nuclear accumulation of Hog1p, followed by large changes in gene expression that are mediated by Hog1p and other factors (see Chapter 4 for further details; Parrou et al. 1997; Reiser et al. 1999; Rep et al. 1999). As transcripts critical to cell survival become translated, the activities of the corresponding gene products begin to counteract the cellular defect, reducing the amount of stress in the cell and diminishing cellular signaling

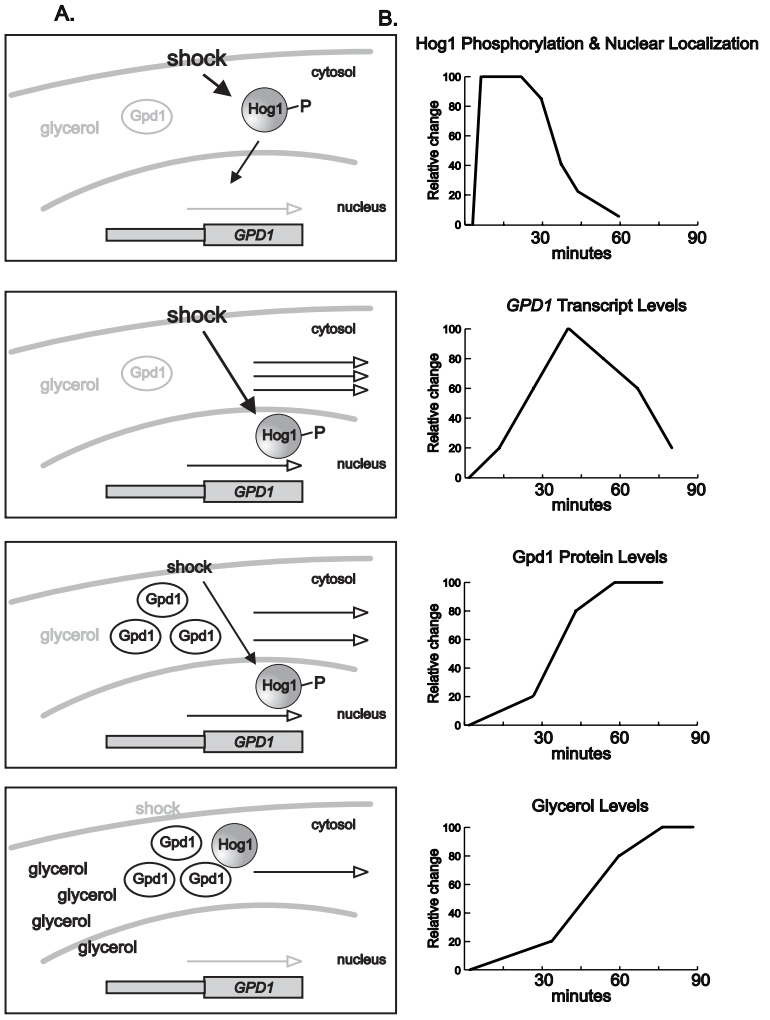

Fig. 2.9. Model for the sequence of events during stress adaptation. (A) A schematic diagram for the events that occur during stress adaptation, using Hog1p activation as a model. After initial detection of the stress signal, Hog1p becomes phosphorylated and accumulates in the nucleus (Reiser et al. 1999; Rep et al. 1999) to trigger a transient increase in transcript levels (arrows), which leads to a steady increase in protein levels (circles). See text for details. (B) Temporal profiles of the changes in kinase activation, transcript levels, protein levels, and metabolite levels, using Hog1p and Gpd1p as an example. In response to osmotic shock, transient changes in Hog1p activation and *GPD1* transcript levels lead to a steady increase in the amount of Gdp1p protein and the final product of the pathway it is involved in, glycerol (Reiser et al. 1999; Rep et al. 1999; Stefan Hohmann, personal communication)

related to the stress. Subsequently, Hog1p relocalizes to the cytoplasm and gene expression levels adjust to their new steady-state levels. Thus, although Hog1p activation and gene expression changes are transient, the levels and activities of many of the corresponding proteins are likely to steadily increase until they reach the new steady-state levels required for growth at the new cnditions. This is true for the glycerol-3-phosphate dehydrogenase Gpd1p, for which transient changes in *GPD1* transcript levels lead to a steady increase in Gpd1p protein abundance and internal glycerol levels (Rep et al. 1999; S. Hohmann, personal communication).

Two models can account for the mechanism of transient gene expression changes. One model is that after strong initiation of the ESR, one or more regulatory systems are activated to suppress the response until it reaches the appropriate state. This situation would require the initial activation of positive regulators of ESR initiation, followed by the activation of negative regulators of the program. Alternatively, adaptation of ESR gene expression may arise due to attenuation of cellular signaling that initiates the response, coupled with a constitutively-active mechanism of ESR suppression. In this model, stress-induced signaling immediately following a stressful environmental shift would override the mechanism that normally suppresses the ESR, leading to a strong initiation of the stress response. As the cell adapts to its new environment, the corresponding stress signal would abate, and the precise levels of ESR gene expression would be dictated by the balance of signaling through the positive (initiating) and negative (suppressing) signals. This model is consistent with the fact that cells lacking PKA activity have constitutively nuclear Msn2/4p, even under optimal growth conditions, suggesting that PKA is involved in mediating ESR suppression in the absence and presence of environmental stress.

2.11 Conclusions

Many details remain to be elucidated regarding the role and regulation of the ESR during the cellular adaptation to environmental changes. How initiation of the ESR contributes to cellular resistance to various stresses is a critical question in understanding the role that this program plays in the yeast's life cycle. The importance of the program is suggested by the complicated mechanism of regulation of ESR gene expression, indicating that the cell goes to great lengths to ensure that the expression of the program is delicately balanced to the demands of the cell. A variety of regulatory factors have been implicated in controlling the expression of subsets of ESR genes, and more will certainly emerge. Elucidating the conditions under which these factors are active and the interplay between the different regulators will contribute to our understanding of how the cell regulates ESR gene expression. Furthermore, detailed characterization of the signaling networks that govern these expression changes will provide insights into the mechanisms that the cell uses to precisely orchestrate the many features of its response. These include changes in genomic expression programs, inhibition of protein synthesis, ar-

rest of the cell cycle, and the subsequent resumption of these processes as the cell adapts to its new environment.

Another important consideration in the study of organismal responses to diverse environmental changes is the degree to which such a common stress response is conserved in other unicellular organisms. Preliminary results indicate that wild isolates of *S. cerevisiae* initiate the ESR in response to environmental changes, similar to the well-studied lab strains (S. Harel, B. Carlson, J.C. Fay, A.P. Gasch, M.B. Eisen, unpublished results). It will be interesting to learn whether the distantly related fission yeast *S. pombe* utilizes a similar response. Current understanding of the *S. pombe* response to different stresses suggests that this yeast, in contrast to *S. cerevisiae*, utilizes a single MAP kinase that is activated by a wide variety of stresses, and thus it will be especially interesting to compare the differences in the stress-dependent regulation of genomic expression in these organisms (Millar et al. 1995; Shiozaki and Russell 1995; Degols et al. 1996; Shieh et al. 1997). Answering these questions will help us understand the remarkable ability of yeast and other organisms to survive stressful variations in their environment.

Since this chapter was prepared, work by Gorner et al. (2002) has shown that phosphorylation of the nuclear localization signal of Msn2p, likely directly by PKA, controls the cytoplasmic relocalization of the protein specifically in response to glucose repletion, while the relocalization of Msn2p in response to other conditions is independent of this domain and may be primarily controlled by alterations in nuclear export.

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