The Journal of Experimental Biology 201, 1177–1195 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB 1453

OXYGEN SENSING AND THE TRANSCRIPTIONAL REGULATION OF OXYGEN-RESPONSIVE GENES IN YEAST

KURT E. KWAST*, PATRICIA V. BURKE† AND ROBERT O. POYTON‡

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA

*Present address: Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 407 South Goodwin Avenue, Urbana, IL 61801, USA

†Present address: Department of Physics, Whitman College, Walla Walla, WA 99362, USA

‡Author for correspondence (e-mail: poyton@spot.colorado.edu)

Accepted 4 December 1997; published on WWW 24 March 1998

Summary

The budding yeast *Saccharomyces cerevisiae* is a facultative aerobe that responds to changes in oxygen availability (and carbon source) by initiating a biochemically complex program that ensures that energy demands are met under two different physiological states: aerobic growth, supported by oxidative and fermentative pathways, and anaerobic growth, supported solely by fermentative processes. This program includes the differential expression of a large number of genes, many of which are involved in the direct utilization of oxygen. Research over the past decade has defined many of the *cis*-sites and *trans*-acting factors that control the transcription of these oxygen-responsive genes. However, the manner in which oxygen is sensed and the subsequent steps involved in the transduction of this signal have not been precisely determined. Heme is known to play a pivotal role in the expression of these genes, acting as a positive modulator for the transcription of the aerobic genes and as a negative modulator for the transcription of the hypoxic genes. Consequently, cellular concentrations of heme, whose biosynthesis is oxygen-dependent, are thought to provide a gauge of oxygen availability and dictate which set of genes will be transcribed. But the precise role of heme in oxygen sensing and the transcriptional regulation of oxygen-responsive genes is presently unclear. Here, we provide an overview of the transcriptional regulation of oxygen-responsive genes, address the functional roles that heme and hemoproteins may play in this regulation, and discuss possible mechanisms of oxygen sensing in this simple eukaryotic organism.

Key words: oxygen sensing, hypoxia, heme, hemoproteins, gene expression, transcription, yeast, *Saccharomyces cerevisiae*.

Introduction

Baker's yeast, *Saccharomyces cerevisiae*, is a facultative aerobe that responds to changes in oxygen availability by differentially expressing a large number of genes (reviewed by Zitomer and Lowry, 1992; Pinkham and Keng, 1994; Bunn and Poyton, 1996). This response, in conjunction with the regulation of gene expression by carbon substrates (e.g. repressing *versus* nonrepressing and fermentable *versus* nonfermentable sugars), is part of a biochemical and genetic program that regulates the efficiency of carbon-source utilization during aerobic growth, which is supported by both mitochondrial oxidative phosphorylation and glycolysis, and during anaerobic growth, which is supported exclusively by fermentative processes. The transcriptional regulation of genes by carbon substrates is complex and has been reviewed elsewhere (Trumbly, 1992; de Winde and Grivell, 1993; Thevelein, 1994).

Genes that respond to changes in oxygen availability can be placed into one of two broad categories: 'aerobic' genes, which are optimally expressed under normoxic conditions, and 'hypoxic' genes, which are optimally expressed under low-oxygen or anoxic conditions. Not surprisingly, many of the respiratory cytochromes and other proteins involved in strictly aerobic metabolism, including enzymes for controlling oxidative damage, are encoded by aerobic genes. However, most of the genes that are induced (derepressed) by oxygen deprivation also encode proteins that function in oxygen-utilizing pathways. These proteins include oxidases involved in electron transport and reductases and desaturases involved in the biosynthesis of heme, sterol and unsaturated fatty acids. Thus, the products of most hypoxic genes do not function in anaerobic (fermentative) metabolism *per se* and can be referred to in a functional sense as hypoxic genes rather than anaerobic genes. Why is the transcription of many of the hypoxic genes maximally upregulated (derepressed) during anaerobiosis, a condition in which their products serve no apparent function? Although direct experimental evidence is lacking in many

cases, the derepression of hypoxic genes probably serves to increase flux through these biosynthetic pathways during *oxygen-limiting conditions* – by increasing the efficiency of oxygen usage (e.g. hypoxic isoenzymes) or simply by increasing protein levels – because many of these genes encode enzymes (or enzyme subunits) that are rate-limiting in their respective pathways. The importance of maintaining flux through these biosynthetic pathways is underscored by an absolute nutritional requirement for sterols and unsaturated fatty acids during anaerobic growth (Andreasen and Stier, 1953, 1954).

Fig. 1 conceptualizes oxygen-sensing pathways in *S. cerevisiae*. The transcriptional control of nuclear-encoded, oxygen-responsive genes in this organism is mediated by at least three *trans*-acting factors: Hap1p (*h*eme *a*ctivated *p*rotein), which activates the transcription of many of the aerobic and some hypoxic genes; Hap2/3/4/5p, which activates the expression of several aerobic genes, typically in a carbon-source-dependent manner; and Rox1p (*r*egulation by *ox*ygen), which represses the transcription of the hypoxic genes under aerobic conditions (reviewed by Zitomer and Lowry, 1992; Pinkham and Keng, 1994; Bunn and Poyton, 1996). Heme acts as an intermediary in regulating the expression of oxygen-responsive genes; it is required for the activation of the Hap proteins and for the transcription of the *ROX1* gene (mediated by Hap1p). Because the biosynthesis of heme requires oxygen, it has been proposed that heme acts as a gauge of oxygen availability and dictates which set of genes will be transcribed. Under aerobic or heme-proficient conditions, transcription of the aerobic genes is activated (mediated, in part, by the Hap proteins), and under anoxic or heme-deficient conditions, transcription of the hypoxic genes is derepressed (owing to loss of repression by Rox1p).

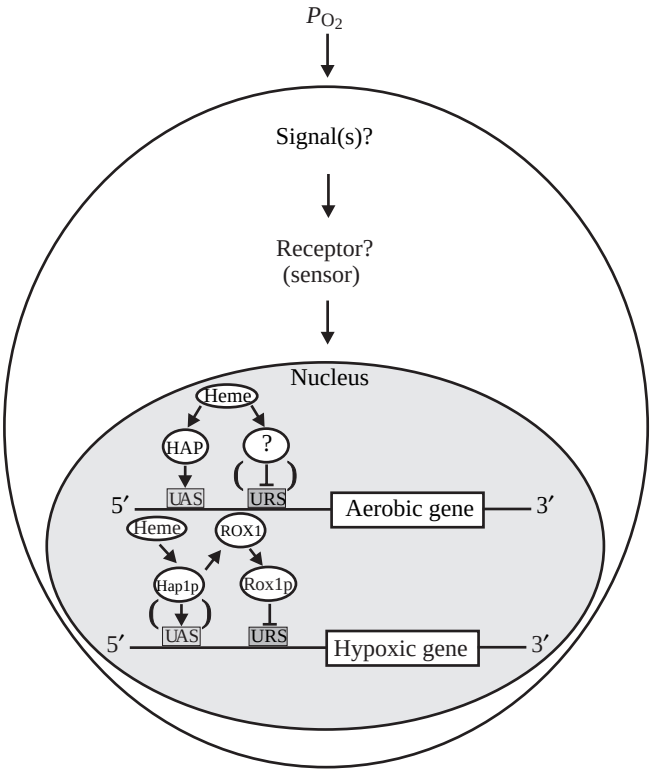

Fig. 1. An overview of oxygen-sensing pathways in *Saccharomyces cerevisiae*. The *trans*-acting factors that interact with upstream activator sites (UASs) or upstream repressor sites (URSs) are shown. HAP stands for Hap1p and/or Hap2/3/4/5p. The heme-mediated *trans*-acting factor(s) that represses the transcription of some aerobic genes has not been identified (?). Hap1p activates the transcription of some hypoxic genes, as indicated by the parentheses.

Previous studies have focused primarily on the *trans*-acting factors and *cis*-sites that regulate the transcription of oxygen-responsive genes, while upstream events have gone largely unstudied. Currently, the signals to which the cell responds are unclear. In addition to O_2 *per se*, byproducts and endproducts of oxygen-dependent metabolism (e.g. cellular redox, reactive oxygen species, heme) may act in signaling pathways that lead to changes in the transcription of specific genes. Similarly, little is known about the receptors (either their cellular location or identity) that are responsive to changes in these signals. Also unclear are how 'oxygen sensors' transduce this signal for the activation or repression of oxygen-responsive genes and how many pathways exist.

Currently, it is assumed that heme plays a central role in oxygen-sensing and signal-transduction pathways by acting as a redox-insensitive, metabolic cofactor or ligand for transcription factors (e.g. Hap1p) (reviewed by Zitomer and Lowry, 1992). However, the following recent findings are difficult to reconcile with such a model. First, transcript levels of many aerobic genes decrease with declining oxygen concentration over a range (200–1 μmol l^{-1} O_2) that is well above the K_m for oxygen of the rate-limiting step in heme biosynthesis (Burke *et al.* 1997). Cellular concentrations of heme are not thought to vary appreciably in this range of oxygen concentration (Labbe-Bois and Labbe, 1990). Similarly, transcripts of some of the hypoxic genes also respond (increase) over this range of oxygen concentration (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Second, after cells are shifted from aerobic to anaerobic conditions, the time course for induction and transcript profiles differ markedly among the hypoxic genes (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). These data are difficult to reconcile with models invoking the derepression of hypoxic genes simply by the loss of common repressor (e.g. Rox1p) or effector (e.g. heme) elements. Third, clamping the redox state of hemoproteins under different conditions of oxygen availability (e.g. carbon monoxide + anoxia, transition metals + air) modulates the expression of several hypoxic genes (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation), suggesting that one of the signaling pathways involves changes in the redox state of a hemoprotein oxygen sensor. Taken together, these data suggest that multiple mechanisms/pathways regulate the expression of these genes; some probably involve control by the *concentration* of heme

and others probably involve the *redox state* of hemoproteins. In this paper, we provide an overview of the transcriptional regulation of oxygen-responsive genes and discuss the role that both heme and hemoproteins play in oxygen-sensing pathways.

Oxygen- and/or heme-responsive genes

In *Saccharomyces cerevisiae*, the expression of a large number of proteins is oxygen-responsive, many at the level of transcription. Table 1 is a compilation of genes whose transcription is oxygen and/or heme-regulated. All of the genes listed are nuclear-encoded. Genes are grouped according to function, and known *trans*-acting activators and repressors are indicated. As can be seen, the effects of oxygen and heme act in parallel, i.e. those genes that are positively regulated by oxygen are also positively regulated by heme and *vice versa*.

The first group of genes (group I) in Table 1 contains a large number that encode proteins involved in mitochondrial respiration and oxidative phosphorylation. It includes many of the nuclear-encoded proteins that constitute the terminal portion of the mitochondrial respiratory chain, including complex III (ubiquinol cytochrome *c* reductase), complex IV (cytochrome *c* oxidase) and cytochrome *c*, the mobile electron carrier acting between these complexes. In addition, this group contains the gene encoding cytochrome b_2 and three genes encoding isoforms of the mitochondrial adenine translocase. Given the roles that these proteins play in oxidative metabolism, it is not surprising that all of their genes – with the exceptions of *COX5b*, *CYC7* and *AAC3* (discussed below) – are positively regulated by oxygen/heme. In addition to affecting the expression of the nuclear-encoded subunits of cytochrome *c* oxidase listed in Table 1, oxygen also positively affects the expression of the mitochondrial genes *COX1* and *COX2*, encoding the catalytic core of cytochrome *c* oxidase; this effect has been shown to be mediated post-transcriptionally (Groot and Poyton, 1975). Thus, the availability of oxygen/heme regulates the expression of a large portion of the mitochondrial respiratory chain.

COX5a/*COX5b*, *CYC1*/*CYC7* and *AAC2*/*AAC3*, as well as *TIF51a*/*ANB1* (see group IV genes below), are part of a family of genetically unlinked but functionally paired genes in which oxygen/heme activates the transcription of the aerobic isoform and represses the transcription of the hypoxic isoform. In all cases, the aerobic and hypoxic isoforms are functionally interchangeable. The primary sequence in the coding regions of these gene pairs is remarkably similar, for example 66 % for *COX5a*/*COX5b* (Cumsky *et al.* 1987) and 79 % for *CYC1*/*CYC7* (Montgomery *et al.* 1980). Given the high degree of sequence homology, it is likely that these pairs arose by gene duplication and have subsequently diverged to function in different oxygen environments. Indeed, both the *CYC1*/*CYC7* and *TIF51a*/*ANB1* gene pairs have been shown to be part of a large cluster of duplicated genes (Kang *et al.* 1992; Melnick and Sherman, 1993). Although functional differences have not been documented for some of these pairs, the products of the gene pairs *COX5a*/*COX5b* and *CYC1*/*CYC7* have been shown to influence the maximal turnover number of holocytochrome *c* oxidase, with the hypoxic isoforms increasing this rate several-fold (Allen *et al.* 1995; Burke and Poyton, 1998).

Group II (Table 1) includes several hypoxic genes whose products are involved in the synthesis of heme, sterol and unsaturated fatty acids. These biosynthetic pathways require molecular oxygen as an electron acceptor in redox reactions, and most of these genes encode enzymes that utilize oxygen directly. Unlike transcripts of the genes encoding hypoxic isoforms, transcripts of these hypoxic genes are detectable under normoxic conditions, and levels increase further in response to declining oxygen concentration (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Although it has been reported that *HMG1* and *HMG2* represent an aerobic and hypoxic (respectively) gene pair, whose transcription is regulated in opposite directions by oxygen/heme (Thorsness *et al.* 1989), we have found that both of these genes are optimally expressed in a number of wild-type strains under low-oxygen or anoxic conditions in both glucose-repressed and nonrepressed conditions (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Moreover, aerobic transcript levels of both *HMG1* and *HMG2* are derepressed in Δ*rox1* and *rox1* mutant strains, indicating that both of these genes are repressed by Rox1p (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). In support of this finding, a search of the promoter region of these genes reveals putative Rox1p-binding sequences (see Rox1 section below). Thus, it appears that both *HMG1* and *HMG2* are hypoxic genes that are regulated by Rox1p. There are striking differences in the post-translational regulation of these isoforms that indicate different functional roles for these isoforms (Hampton *et al.* 1996).

Group III (Table 1) includes a number of genes that encode enzymes involved in the oxidative stress response. Not surprisingly, their transcription is positively regulated by oxygen/heme as well as, for some, reactive oxygen species. Lastly, group IV (Table 1) contains an aerobic and hypoxic gene pair of the putative translational initiation factor eIF-5 encoded by *TIF51a*/*ANB1*. Functional differences between these isoforms have not been documented. In addition, group IV contains the aerobic gene *ROX1*, which encodes a transcriptional repressor of all of the hypoxic genes listed in Table 1.

The transcription of most of the aerobic genes is activated by Hap1p, the Hap2/3/4/5p complex, or both Hap1p and Hap2/3/4/5p. For some of the aerobic genes (*COX6*, *COX7*, *COX9*, *COX8*, *CTA1* and *SOD1*), the *trans*-acting factor(s) responsible for their upregulation in response to heme/oxygen has not been identified. It makes intuitive sense that genes encoding enzymes that utilize oxygen are positively regulated by heme/oxygen and, in some cases, induced by growth on a nonfermentable carbon substrate (Hap2/3/4/5p activation) that must be oxidized in the mitochondrion. It is important to realize, however, that each of these genes (including the hypoxic genes) is independently regulated by these and

Table 1. *Oxygen- and/or heme-regulated genes in* Saccharomyces cerevisiae

Gene	Protein	Activator	Repressor	Heme effect	Oxygen effect	References
I. Mitochondrial respiration/oxidative phosphorylation						
	Cytochrome *c* oxidase					
COX4	Subunit IV	Hap2/3/4/5p		Induced	Induced	1–3
COX5a	Subunit Va	Hap2/3/4/5p		Induced	Induced	3–7
COX5b	Subunit Vb		Rox1p	Repressed	Repressed	3, 5–9
COX6	Subunit VI	Hap2/3/4/5p		Induced	Induced	3, 10–14
COX7	Subunit VII			Induced	Induced	3, 15
COX9	Subunit VIIa			Induced	Induced	3
COX8	Subunit VIII				Induced	3, 16
	Cytochrome *c*					
CYC1	Iso-1-cytochrome *c*	Hap1p, Hap2/3/4/5p		Induced	Induced	3, 17–26
CYC7 (*CYP3*)	Iso-2-cytochrome *c*	Hap1p (Cyp1p)	Rox1p	None/ Repressed	Repressed	3, 22, 24, 27–32
	Ubiquinol cytochrome *c* reductase					
COR1 (*QCR1*)	Subunit I	Hap1p, Hap2/3/4/5p		Induced	Induced	4
COR2 (*QCR2*)	Subunit II	Hap1p, Hap2/3/4/5p		Induced	Induced	33
QCR8 (*COR5*)	Subunit XIII	Hap2/3/4/5p			Induced	34–36
CYT1	Cytochrome c_1	Hap1p, Hap2/3/4/5p		Induced	Induced	26, 37, 38
	L-Lactate cytochrome *c* oxidoreductase					
CYB2	Cytochrome b_2	Hap1p, Hap2/3/4/5p		Induced	Induced	39, 40
	Mitochondrial adenine translocase					
AAC1	ADP/ATP translocator			None	Induced	41
AAC2	ADP/ATP translocator	Hap2/3/4/5p		Induced	Induced	42, 43
AAC3	ADP/ATP translocator		Rox1p	Repressed	Repressed	44, 45
II. Heme, sterol and unsaturated fatty acid synthesis						
HEM13	Coproporphyrinogen III oxidase	Hap1p	Rox1p	Repressed	Repressed	46–53
HMG1	3-Hydroxy-3-methylglutaryl CoA reductase	Hap1p	Rox1p	Induced	Induced	54–56
HMG2	3-Hydroxy-3-methylglutaryl CoA reductase		Rox1p	Repressed	Repressed	54–56
ERG11 (*14DM*)	Cytochrome P_{450} lanosterol 14α-demethylase	Hap1p	Rox1p	Repressed	Repressed	48, 57
CPR1 (*NCP1*)	NADPH cytochrome P_{450} reductase		Rox1p	Repressed	Repressed	57
OLE1	Δ-9 fatty acid desaturase		Rox1p	Repressed	Repressed	56, 58–60
III. Oxidative stress						
CTT1	Catalase T (cytosolic)	Hap1p		Induced	Induced	17, 61, 62
CTA1	Catalase A (peroxisomal)			Induced	Induced	17, 63
SOD1	Cu,Zn-superoxide dismutase			Induced	Induced	64, 65
SOD2	Mn-superoxide dismutase	Hap1p, Hap2/3/4/5p		Induced	Induced	64–67
YHB1	Flavohemoglobin	Hap1p, Hap2/3/4/5p		Induced	Induced	68, 69
IV. Translation and transcription factors						
TIF51A (*tr-1*)	eIF-5a	Hap1p		Induced	Induced	29, 70–73
ANB1 (*TIF51B*)	eIF-5b		Rox1p	Repressed	Repressed	8, 29, 70, 71, 74–76
ROX1 (*REO1*)	DNA-binding transcriptional repressor	Hap1p		Induced	Induced	29, 52, 70, 76–81

1, Saltzgaber-Muller and Schatz, 1978; 2, Forsburg and Guarente, 1989*b*; 3, Burke *et al.* 1997; 4, Myers *et al.* 1987; 5, Trueblood *et al.* 1988; 6, Poyton *et al.* 1988; 7, Hodge *et al.* 1989; 8, Trueblood and Poyton, 1988; 9, Hodge *et al.* 1990; 10, Trawick *et al.* 1989a; 11, Trawick *et al.* 1989b; 12, Trawick *et al.* 1992; 13, Wright *et al.* 1995a; 14, Wright *et al.* 1995b; 15, Gollub and Dayan, 1985; 16, Patterson, 1990; 17, Hortner *et al.* 1982; 18, Guarente and Mason, 1983; 19, Guarente *et al.* 1984; 20, Arcangioli and Lescure, 1985; 21, Pfeifer *et al.* 1987*a*; 22, Pfeifer *et al.* 1987*b*; 23, Olesen *et al.* 1987; 24, Cerdan and Zitomer, 1988; 25, Forsburg and Guarente, 1988; 26, Oechsner *et al.* 1992; 27, Wright and Zitomer, 1984; 28, Wright and Zitomer, 1985; 29, Lowry and Zitomer, 1988; 30, Verdiere *et al.* 1985; 31, Prezant *et al.* 1987; 32, Zitomer *et al.* 1987; 33, Dorsman and Grivell, 1990; 34, de Winde and Grivell, 1993; 35, de Winde *et al.* 1993; 36, de Winde and Grivell, 1995; 37, Schneider and Guarente, 1991; 38, Oechsner and Bandlow, 1996; 39, Guiard, 1985; 40, Lodi and Guiard, 1991; 41, Gavurnikova *et al.* 1996; 42, Betina *et al.* 1995; 43, Nebohacova *et al.* 1996; 44, Kolarov *et al.* 1990; 45, Sabova *et al.* 1993; 46, Zagorec and Labbe-Bois, 1986; 47, Zagorec *et al.* 1988; 48, Verdiere *et al.* 1991; 49, Keng, 1992; 50, Ushinsky and Keng, 1994; 51, Amillet *et al.* 1995; 52, Di Flumeri *et al.* 1996; 53, Amillet *et al.* 1996; 54, Thorsness *et al.* 1989; 55, Hampton *et al.* 1996; 56, Kwast *et al.*, in preparation; 57, Turi and Loper, 1992; 58, McDonough *et al.* 1992; 59, Choi *et al.* 1996; 60, C. Martin, personal communication; 61, Spevak *et al.* 1986; 62, Winkler *et al.* 1988; 63, Cohen *et al.* 1985; 64, Gralla and Kosman, 1992; 65, Galiazzo and Labbe-Bois, 1993; 66, Pinkham *et al.* 1997; 67, Flattery-O'Brien *et al.* 1997; 68, Crawford *et al.* 1995; 69, Zhao *et al.* 1996; 70, Lowry and Zitomer, 1984; 71, Lowry and Lieber, 1986; 72, Schnier *et al.* 1991; 73, Kang *et al.* 1992; 74, Mehta and Smith, 1989; 75, Mehta *et al.* 1990; 76, Lowry *et al.* 1990; 77, Zitomer and Lowry, 1992; 78, Balasubramanian *et al.* 1993; 79, Deckert *et al.* 1995*a*; 80, Deckert *et al.* 1995*b*; 81, Zitomer *et al.* 1997.

possibly other *trans*-acting factors. Thus, as a set, the aerobic (and hypoxic) genes do not constitute a coordinately expressed regulon or operon.

The negative effect of heme/oxygen on the transcription of the hypoxic genes has been shown to be mediated by the transcriptional repressor Rox1p. For a subset of these hypoxic genes (*CYC7*, *HEM13*, *HMG1* and *ERG11*), heme can act in both a positive (mediated by Hap1p) and a negative (mediated by Rox1p) manner. This combinatorial regulation by heme may allow for the fine-tuning of transcript levels of these genes in response to heme/oxygen. However, the overall effect of heme/oxygen proficiency on the transcription of hypoxic genes is negative. As stated above, given that most of the hypoxic genes encode products that are involved in the direct use of oxygen, their high levels of expression under strict anoxia are puzzling unless this upregulation (derepression) represents an adaptive response to make better use of a limiting substrate (oxygen).

Trans-acting factors and *cis*-promoter sites in oxygen-regulated genes

As discussed above, the expression of most of the oxygen-responsive genes is regulated by three transcription factors: Hap1p, Hap2/3/4/5p and Rox1p. In this section, we provide a more detailed review of transcriptional regulation by these factors. A discussion of earlier work on these components can be found in prior reviews (Forsburg and Guarente, 1989*b*; de Winde and Grivell, 1993; Zitomer and Lowry, 1992; Pinkham and Keng, 1994; Bunn and Poyton, 1996).

Hap1p (Cyp1p)

The *CYP1* gene was first identified as an activator of the hypoxic gene *CYC7* (Clavilier *et al.* 1969) and later found to be the same gene as *HAP1* (Verdiere *et al.* 1986), an activator of *CYC1* (Guarente *et al.* 1984). For simplicity, we shall use the *HAP1* designation here. Since the discovery of *HAP1*, the number of genes it has been found to regulate has steadily increased (Table 1). The *HAP1* gene has been cloned and sequenced (Creusot *et al.* 1988; Verdiere *et al.* 1988; Pfeifer *et al.* 1989) and found to encode a 1483-amino-acid protein with at least three functional domains. The first is a zinc-cluster DNA-binding domain in the amino terminus, between residues 1 and 148. This domain is responsible for sequence-specific DNA binding and the formation of the Hap1p homodimer (Verdiere *et al.* 1988; Pfeifer *et al.* 1989; Timmerman *et al.* 1996), which is the transcriptionally active form. This C6 zinc-cluster motif (Zn[II]2Cys6 binuclear cluster) has a high level of structural identity with other yeast regulatory proteins, including Gal4p, Ppr1p, Leu3p, Put3p and Cha4p (Schjerling and Holmberg, 1996). A second domain is found in the carboxy terminus, between residues 1309 and 1483. This acidic activation domain is required for the transcriptional activation of Hap1p (Pfeifer *et al.* 1989) and is similar to other transcriptional activation domains such as that in Gal4p. Lastly, there are two regulatory domains: one between residues 244 and 444, and the other between residues 1192 and 1197 (Pfeifer *et al.* 1989; Zhang and Guarente, 1995). Both of these regions contain a Lys/Arg-Cys-Pro-Val/Ile-Asp-His motif that has been implicated as a metal- or heme-binding site (Creusot *et al.* 1988; Zhang and Guarente, 1996). This sequence motif occurs six times in the first regulatory domain and once in the second domain of Hap1p, and it is found in a number of heme-dependent regulatory proteins (Lathrop and Timko, 1993).

The upstream activator sites (UASs) in the promoter region of Hap1p-regulated genes have been studied extensively. The Hap1p homodimer binds to two different classes of sequences: one represented by the UAS1 of *CYC1* and the other represented by the UAS of *CYC7* (Pfeifer *et al.* 1987*b*). The sequences within these UASs are divergent, and *hap1* mutants have been identified that bind to the UAS of *CYC7* but not to the UAS1 of *CYC1* (Kim and Guarente, 1989; Turcotte and Guarente, 1992; Verdiere *et al.* 1988). The DNA target for Hap1p binding has been further delineated through mutational analyses and DNAase I footprinting. The sequence consists of a direct repeat of a CGG triplet separated by a specific number of nucleotides (Zhang and Guarente, 1996); the consensus sequence has been determined to be CGG N3 TAN CGG N3 TA (Ha *et al.* 1996). This sequence motif is somewhat different from that recognized by other yeast regulatory proteins that contain the C6 zinc-cluster domain because most of these proteins recognize an inverted repeat rather than a direct repeat, as is the case for Hap1p. Recently, the three-dimensional structure of Hap1p bound to DNA has been examined using $^{1}H,^{15}N$ resonance spectroscopy (Timmerman *et al.* 1996). Interestingly, the specificity of DNA binding is attributable, in part, to a fine tuning between the structure of the Hap1p linker peptide and/or dimerization helix and the number of bases separating the two CGGs (Timmerman *et al.* 1996).

The role of heme in Hap1p activation has been the focus of considerable research. Although it was originally thought that heme was required for Hap1p to bind to its DNA target sequence (*via* a heme-dependent unmasking of the DNA-binding domain; Pfeifer *et al.* 1989), this model has been recently modified and refined (Fytlovich *et al.* 1993; Zhang and Guarente, 1994, 1995, 1996). Using *in vitro* DNA-binding assays with crude cellular extracts, it has been shown that Hap1p binds to its target DNA in a heme-independent manner, albeit probably more weakly, and forms a large complex with other, as yet unidentified, cellular protein(s) (Fytlovich *et al.* 1993; Zhang and Guarente, 1996). Residues 244–444, containing the heme regulatory motifs (HRMs) 1–6, are required for the formation of this larger complex (Fytlovich *et al.* 1993; Zhang and Guarente, 1996; Lodi *et al.* 1996). When hemin (oxidized heme) is added, a smaller complex is formed.

These observations have led to the following model of heme-regulated Hap1p activity. In the absence of heme, Hap1p is weakly bound to its target DNA and is transcriptionally inactive because of the binding of repressor proteins(s) to the region between residues 244 and 444. When heme is present, a smaller, presumably transcriptionally active, complex is formed because of heme binding to HRM1–6; the binding of

heme to this region may mask the binding site for the transcriptional repressor(s) (Zhang and Guarente, 1995). Heme also plays a role in Hap1p activation through its interaction with the second regulatory domain (residues 1192–1197), which probably indirectly influences the acidic activation domain of Hap1p (Zhang and Guarente, 1995). Thus, heme may regulate the activity of Hap1p by (1) masking the binding sites for repressor protein(s), and possibly regulating DNA-binding affinity, *via* residues 244–444, and (2) regulating the activation domain through binding of heme to the second regulatory domain (residues 1192–1197).

Despite considerable progress in our understanding of the regulation of Hap1p activity by heme, a number of questions remain. Although recent studies have shown that a synthetic peptide of the HRM (Ala-Lys-Arg-Cys-Pro-Val-Asp-His-Thr-Met) reversibly binds heme with an affinity in the micromolar range (Zhang and Guarente, 1995), it is still not known whether Hap1p is a hemoprotein *in vivo*. Also unclear is whether Hap1p binds redox-active metals (e.g. Fe). Although current models of Hap1p activation regard heme as a metabolic cofactor, it is possible that heme might function as a redox-sensitive group that either directly or indirectly controls the activity of Hap1p. The binding of heme to the HRM is qualitatively different from that in hemoproteins such as globins and cytochromes (Zhang and Guarente, 1995; Bock *et al.* 1978; Choma *et al.* 1994). Whereas the axial iron ligands in cytochromes are typically a histidine/methionine pair or bis-histidine, both of which bind heme tightly and shift the spectral Soret peak to a longer wavelength, the sulfhydryl group of the cysteine residue in the HRM is thought to bind heme iron and shift the Soret peak to a shorter wavelength (Zhang and Guarente, 1995). It has been suggested that this difference allows heme to bind reversibly to the HRM (Zhang and Guarente, 1995), but it is not known whether this difference in binding precludes the possibility that this heme is redox-active. In the absence of heme, Hap1p may actually *repress* the transcription of genes (e.g. *SOD2* and *ROX1*) that are activated in the presence of heme (Pinkham *et al.* 1997; Deckert *et al.* 1995*a*). It is not yet known how the larger, transcriptionally inactive complex that forms in the absence of heme may repress the transcription of these and possibly other Hap1p-regulated genes. Finally, it is interesting to note that the general transcription factors/mediators Tup1p and Ssn6p (Cyc8p), which are typically involved in repression, play a role in the *activation* of Hap1p (Zhang and Guarente, 1994); however, the mechanism for this activation is not known (see *Rox1p* section for a discussion of Tup1p/Ssn6p function). Undoubtedly, as additional cellular components of the Hap1p–DNA complex are identified, we will gain new insight into how the activity of this complex is regulated and how heme functions in the formation and activation of this transcription complex.

Hap2/3/4/5p

The other main transcriptional activator of oxygen-responsive genes in yeast is Hap2/3/4/5p. It is a highly conserved heteromeric complex that binds to CCAAT boxes in UASs containing the consensus sequence TNATTGTT (Forsburg and Guarente, 1988). This heteromer activates the transcription of genes primarily in response to heme/oxygen and/or growth on nonfermentable substrates (e.g. lactate, glycerol). Hap2/3/4/5p consists of four polypeptides: Hap2p, a 265-residue protein that contains a DNA-binding site consisting of 22 essential residues (Olesen *et al.* 1987; Pinkham *et al.* 1987; Forsburg and Guarente, 1989*a*; Olesen and Guarente, 1990; Xing *et al.* 1993); Hap3p, a 144-residue protein that is also required for DNA-binding (Xing *et al.* 1993); Hap4p, a 554-residue protein that contains an acidic activation domain (Forsburg and Guarente, 1989*a*; Olesen and Guarente, 1990); and Hap5p, a 216-residue protein that is required for both assembly and DNA-binding activity of the complex (McNabb *et al.* 1995). Hap2p and Hap3p are homologous to the mammalian transcription factors CPI-A and CPI-B (human) and CBF-A and CBF-B (rat) (Chodosh *et al.* 1988; Maity *et al.* 1990).

The Hap2/3/5p heterotrimer functions in DNA-binding, whereas Hap4p is a regulatory subunit required for the activation of the complex (McNabb *et al.* 1995). Transcription of both *HAP2* and *HAP4* is induced by nonfermentable carbon substrates, which may account for the increase in expression of Hap2/3/4/5p-regulated genes in response to these carbon substrates (Forsburg and Guarente, 1989*a*). There is some evidence that other regulatory proteins may substitute for Hap4p, suggesting that the Hap2/3/5p heterotrimer may be a general transcription factor whose activity is modulated by other regulatory proteins besides Hap4p (Forsburg and Guarente, 1989*a*; Olesen and Guarente, 1990; reviewed by Zitomer and Lowry, 1992). Moreover, additional cellular factors (e.g. Gcn5p) may be involved in the transcriptional activation of the Hap2/3/4/5p complex (Georgakopoulos and Thireos, 1992).

The function of heme in regulating the activity of Hap2/3/4/5p is unclear. Whereas the DNA-binding activity of Hap2/3/4/5p appears to be heme-independent, it is not known whether heme affects the overall abundance of the complex or its transcriptional activity. Forsburg and Guarente (1989*a*) suggested that heme could affect Hap4p post-translationally, but how this could influence the activity of the heteromeric complex is not known. Interestingly, the transcription of some of the Hap2/3/4/5p-regulated genes appears to be heme- and/or carbon-source-independent. These genes, which were not included in Table 1, fall into two classes: those regulated in a heme-independent, carbon-source-dependent manner (including *ACO1*, *CIT1*, *KGD1*, *KGD2* and *LPD1*, which encode tricarboxylic acid cycle enzymes), and those regulated in a heme-independent, carbon-source-independent manner (including *HEM1* and *HEM3*, which encode enzymes in the heme biosynthetic pathway) (reviewed by Pinkham and Keng, 1994). The differences in the regulation of these classes of genes by Hap2/3/4/5p are not understood, and they are mentioned here only for completeness. Finally, there are a number of Hap2/3/4/5p-regulated genes whose expression in response to oxygen, heme and carbon source is not fully

known. These genes encode a diverse array of proteins involved in cellular respiration (*SDH1*, *SDH3* and *SDH4*; Daignan-Fornier *et al.* 1994), glycolysis (*FBP1* and *PCK1*; Mercado and Gancedo, 1992), glutamate synthesis (*GDH1*; Dang *et al.* 1996), sporulation (*SPR3*; Ozsarac *et al.* 1995) and vacuolar function (*APE1*; Bordallo *et al.* 1995).

Given the diverse array of genes regulated by Hap2/3/4/5p, as well as the number of environmental and physiological factors that apparently modulate Hap2/3/4/5p activity, it is tempting to speculate that this transcription complex is modular and that additional elements (possibly subunits other than Hap4p) may regulate its activity. Further dissection of Hap2/3/4/5p should improve our understanding of the ways in which environmental and physiological factors, including heme and oxygen, regulate the activity of this complex.

Rox1p

The Rox1 protein represses the transcription of nearly all of the hypoxic genes. Its gene, *ROX1*, was originally identified by the characterization of mutations that resulted in the aerobic derepression of the oxygen- and heme-repressed gene *ANB1* (Lowry and Zitomer, 1984). Two classes of mutants were identified. One class, represented by the *rox1-a1* allele, is semidominant and pleiotropic, affecting both heme-repressed (*ANB1*) and heme-induced (*CYC1*, *TIF51A* and *SOD2*) genes. The second class, represented by the *rox1-b3* allele, is recessive and affects the expression of only heme-repressed genes (e.g. *ANB1*). The *rox1-b3* mutant strain was later shown to have the same phenotype as a *rox1 null* strain (Balasubramanian *et al.* 1993), which suggests that the *rox1-a1* strain may contain a second, as yet unidentified, mutation.

The *ROX1* gene encodes a 368-amino-acid protein whose N-terminal region shows homology to other high-mobility group (HMG) classes of nonhistone chromatin proteins (Balasubramanian *et al.* 1993). The region that accounts for the DNA-binding specificity is found within the HMG domain, which lies between residues 9 and 93 (Balasubramanian *et al.* 1993; Di Flumeri *et al.* 1996). *In vitro*, Rox1p synthesized in *Escherichia coli* or with an *in vitro* wheat-germ translation system forms oligomers that are dependent upon an intact HMG domain (Di Flumeri *et al.* 1996; Zitomer *et al.* 1997). The C-terminal domain is required for transcriptional repression and is presumed to interact with the Ssn6p/Tup1p complex (see below) (Balasubramanian *et al.* 1993; Zitomer *et al.* 1997). The function of a third region, containing a run of glutamines (residues 103–123), is unclear (Zitomer *et al.* 1997).

The DNA sequence to which Rox1p binds has been determined through a series of mutational analyses of the promoter regions of several Rox1p-regulated genes. It is found within the upstream repressor site (URS) and consists of the consensus sequence YYYATTGTTCTC (Y=pyrimidine) (Lowry *et al.* 1990; Balasubramanian *et al.* 1993). *In vitro* binding studies using partially purified Rox1p with synthetic oligonucleotides have verified that Rox1p binds specifically to this consensus sequence (Di Flumeri *et al.* 1996). This sequence is typically found within 500 base pairs 5′ of the TATA box and has been identified in all known Rox1p-regulated genes (Table 2). Several genes contain multiple copies of the consensus sequence, but one copy is apparently sufficient for repression. From a comparison of these sequences, as well as mutational studies, it appears that substitutions in the first three pyrimidines and the last three nucleotides do not severely inhibit Rox1p binding. There is some evidence that, in addition to the consensus sequence, a flanking T-rich region may also be important for Rox1p binding or activity (Sabova *et al.* 1993).

Although the precise mechanism of repression by Rox1p is unclear, a heme-dependent cofactor is not required: when transformed with a plasmid carrying the *ROX1* gene fused to the *GAL1* promoter, *hem1* null mutants grown on galactose in the absence of heme repress the transcription of hypoxic genes (Keng, 1992). However, heme is required for the transcription

Table 2. *Defined and putative Rox1p-binding sequences in the upstream repressor sites of hypoxic genes in* Saccharomyces cerevisiae

Gene	Sequence
COX5b	-228 TgTATTGTTCga[1]
	-63 TCTATTGTTtaa*
CYC7	-126 agaATaGTTCTC[6]
HEM13	-475 TCaATTGTTtag[2]
	-229 TgCtTTGTTCaa[2,*]
	-186 CCCATTGTTCTC[2]
HMG1	-622 CCgATTGTTCgt
HMG2	-282 CgCATTGTTtTg
ERG11	-358 CCTATTGTgCat[4]
CPR1	-211 gCtATTGTTCTC
OLE1	-130 TTTATTGTTCTa
	-260 ggCATTGTTaTC*
	-272 CCTATTGTTacg
ANB1	-316 TCCATTGTTCgt[3]
	-285 CCTATTGTTCTC[3]
	-218 TCCATTGTTCTC[3]
	-197 CTCATTGTTgct
AAC3	-197 TTCATTGTTtgg[5]
Consensus	YYYATTGTTCTC[6]

Sequences are presented 5′ to 3′ except those followed by an asterisk, which are oriented 3′ to 5′. Numbers indicate the position relative to the first nucleotide of the initiation codon (+1). Small letters indicate nucleotides that differ from the consensus sequence.

[1–5]Deletions or mutations in these sequences result in aerobic derepression of the gene: [1]Hodge *et al.* (1990); [2]T. Keng, unpublished observations (see Pinkham and Keng, 1994); [3]Lowry *et al.* (1990); Mehta and Smith (1989); [4]Turi and Loper (1992); [5]Sabova *et al.* (1993).

[6]See discussion in Lowry *et al.* (1990).

of the *ROX1* gene, which is mediated, in part, by Hap1p (Keng, 1992). As stated above, in the absence of heme, Hap1p may repress the transcription of *ROX1* (Deckert *et al.* 1995*a*). There is also some evidence that Hap1p is not the only transcriptional activator of *ROX1* expression. Indeed, *ROX1* transcript levels are only moderately depressed in Δ*hap1* mutants (Deckert *et al.* 1995*a*).

Two general transcription factors/mediators, Ssn6p (Cyc8p) and Tup1p, are required for Rox1p-mediated repression. When either protein is absent, Rox1p-regulated genes are expressed constitutively (Balasubramanian *et al.* 1993; Deckert *et al.* 1995*a*). Both Tup1p and Ssn6p are required for the activity of other DNA-binding transcriptional repressors that function to regulate a diverse array of cellular activities, including cell-type recognition and catabolite repression (Keleher *et al.* 1992; Schultz and Carlson, 1987; Trumbly, 1988; Williams and Trumbly, 1990). Although these factors do not bind DNA directly, they are thought to be recruited to form a complex with the DNA-binding repressors (Keleher *et al.* 1992; Varanasi *et al.* 1996; Tzamarias and Struhl, 1994, 1995). It has been proposed that Ssn6 provides the link to a pathway-specific DNA-binding protein (e.g. Rox1p), while Tup1p mediates repression (Tzamarias and Struhl, 1994, 1995). Indeed, Zitomer *et al.* (1997) recently collected evidence for a direct interaction between Ssn6 and Rox1p, and they suggest that Ssn6 may be involved in stabilizing the protein–DNA complex. The precise role of Tup1 in the formation of this complex and in repression is unclear.

The transcriptional regulation of genes by Rox1p can be summarized by the following model. In the presence of heme (e.g. aerobiosis), the *ROX1* gene is transcribed, mediated in part by Hap1p activation, and translated. Cellular levels of Rox1p are regulated *via* autorepression, in that Rox1p represses the expression of its own gene (Deckert *et al.* 1995*a*). Once translated, Rox1p enters the nucleus, binds to consensus sequences in the URS(s) of hypoxic genes, and forms a complex with Ssn6p/Tup1p, resulting in the transcriptional repression of these genes. There is some evidence that Rox1p may bind to URSs with different affinities, which may, in part, account for differences in the transcript levels of a number of hypoxic genes under identical conditions of heme and oxygen availability (see discussions in Zitomer *et al.* 1997). In the absence of heme (e.g. anaerobiosis), the transcription of *ROX1* is repressed, caused in part by Hap1p-mediated repression. Rox1p levels subsequently decline, repression is released, and the hypoxic genes are transcribed. A recent report suggests that Rox1p may be 'rapidly' degraded in the absence of heme/oxygen, although the mechanism responsible for this is not known (Zitomer *et al.* 1997).

Additional repressors of hypoxic genes

In addition to *ROX1*, *REO1* was identified as an aerobic repressor of the heme-mediated, hypoxic genes *COX5b* and *ANB1* (Trueblood and Poyton, 1988). However, complementation studies between *rox1* and *reo1* strains have yielded conflicting results concerning whether *REO1* and *ROX1* are the same or different genes (Trueblood and Poyton, 1988; Lambert *et al.* 1994). To resolve this confusion, we recently characterized phenotypic differences between *reo1* and *rox1* mutants, performed additional complementation studies, and sequenced the *ROX1* gene in *reo1* strains (Kwast *et al.* 1997). These studies revealed a similar degree of aerobic derepression for all of the hypoxic genes examined in Δ*rox1*, *reo1* and the resulting diploid strain. Sequence analyses of *ROX1* in *reo1* strains revealed a frame-shift mutation in the 5′-end of the *ROX1*-coding region. This mutation results in a nonsense codon in the sixth position of the coding region (Kwast *et al.* 1997). Thus, it appears that *reo1* is, in fact, an allele of *ROX1*.

In addition to *ROX1* (*REO1*), there are several other genes whose products are thought to repress the transcription of hypoxic genes. These include *ROX3*, *ROX5* and *ROX6* (Rosenblum-Vos, 1988; Rosenblum-Vos *et al.* 1991). These genes were identified in mutant hunts for factors regulating the expression of *CYC1*, *CYC7* or *ANB1*. Although mutations in *rox3* result in the aerobic derepression of *CYC7*, *ROX3* transcript levels increase during anaerobiosis in a heme-independent manner, and deletion of its product is lethal. These results suggest that Rox3p is probably a general transcription factor (Rosenblum-Vos *et al.* 1991). Indeed, recent studies have shown that Rox3p (synonymous with Ssn7p and Rmr1p) is a mediator and component of the RNA polymerase II holoenzyme (Gustafsson *et al.* 1997). Thus, through its involvement in the transcriptional regulation of *CYC7*, Rox3p contributes to the global stress response in *S. cerevisiae* (Gustafsson *et al.* 1997). The functional roles that *ROX5* and *ROX6* play in the repression of hypoxic genes are unclear and neither gene has been cloned.

Finally, recent studies have identified several genes, including *DAN1*, *GPD2* and *SRP1*, that are repressed by heme or oxygen in an apparently Rox1p-independent manner (Sertil *et al.* 1997; Ansell *et al.* 1997; Donzeau *et al.* 1996). The expression of *DAN1* is similar to that of other hypoxic genes in the following respects: its transcription is induced by anoxia, blocked by the addition of heme to anoxic cells and constitutive in heme mutants (Sertil *et al.* 1997). Because Rox1p does not influence its expression, these results suggest the existence of a parallel heme-dependent regulatory system. Similarly, the expression of *GPD2*, encoding an isoenzyme of NAD^+-dependent glycerol-3-phosphate dehydrogenase, is induced under anoxia in a Rox1p-independent manner (Ansell *et al.* 1997). However, its transcription appears to be modulated by the redox state of the cell, suggesting a regulatory pathway that is different from that of other hypoxically expressed genes (Ansell *et al.* 1997).

In addition to *DAN1* and *GPD2*, *SRP1* (Donzeau *et al.* 1996) and other genes, such as *SUT1* (Bourot and Karst, 1995) and *TIP1* (Donzeau *et al.* 1996), are hypoxic genes that are expressed under anaerobic conditions, but the *trans*-acting factor(s) that mediates their expression is not known. Finally, the URSs in a number of hypoxic genes also contain the core sequence AAACGA (Sabova *et al.* 1993; Turi and Loper,

1992), but a transcriptional repressor that may interact with this sequence has not been identified. Thus, at present, Rox1p is the only known transcriptional repressor of hypoxic genes. However, as suggested by these studies and others discussed below, additional *trans*-acting factors and regulatory pathways are probably involved in modulating the expression of some of the hypoxic genes.

Regulation of cellular heme levels

From the preceding discussion, it is clear that heme works together with a variety of *trans*-acting factors to regulate the transcription of oxygen-responsive genes in yeast. Further evidence for the central role of heme in the transcriptional regulation of these genes is provided by the following experimental observations. First, heme-deficient mutants derepress the transcription of hypoxic genes and repress the transcription of aerobic genes, irrespective of oxygen concentration (Hodge *et al.* 1989; Lowry and Lieber, 1986). Second, the addition of hemin to anoxic cells increases transcription of aerobic genes and decreases transcription of hypoxic genes (Hodge *et al.* 1989; Lowry and Lieber, 1986). Thus, heme can act either in a positive manner, activating transcription of primarily the aerobic genes, or in a negative manner, repressing the transcription of primarily the hypoxic genes. As a product of the mitochondrion and as a prosthetic group of respiratory cytochromes, heme may be ideally suited to coordinate the expression of the mitochondrial and nuclear genes involved in the biogenesis of the mitochondrial respiratory chain (Forsburg and Guarente, 1989*b*; Padmanaban *et al.* 1989). Indeed, heme is intimately entwined with energy production *via* its link as a prosthetic group in the mitochondrial cytochromes and other proteins directly involved in oxygen use and redox reactions. In the following sections, we provide an overview of the regulation of cellular levels of heme and discuss the functional roles of heme in transcription and oxygen-sensing pathways.

Heme biosynthesis

The heme biosynthetic pathway in yeast has been fairly well characterized and reviewed elsewhere (Labbe-Bois and Labbe, 1990; Pinkham and Keng, 1994). Two steps in its biosynthesis require molecular oxygen as an electron acceptor: the formation of protoporphyrinogen, catalyzed by coproporphyrinogen III oxidase, and the formation of protoporphyrin by protoporphyrinogen IX oxidase. Because of the requirement for oxygen in heme synthesis, it has been argued that cellular concentrations of heme reflect oxygen concentration (reviewed by Zitomer and Lowry, 1992). For this to be true, the rate-limiting step in heme synthesis must be oxygen-dependent. Except at very low oxygen concentrations, this does not appear to be the case. In aerobically growing cells, δ-aminolevulinate (ALA) accumulates as a result of low levels of ALA dehydratase (porphobilinogen synthase) and its low substrate affinity, suggesting that ALA dehydratase is rate-limiting under aerobic conditions (reviewed by Labbe-Bois and Labbe, 1990; Pinkham and Keng, 1994). However, under 'near-anoxic' (trace oxygen) conditions, coproporphyrinogen III oxidase, an oxygen-utilizing enzyme, is probably rate-limiting (reviewed by Labbe-Bois and Labbe, 1990; Pinkham and Keng, 1994). Moreover, the activity of coproporphyrinogen III oxidase increases in response to near-anoxic conditions (Miyake and Sugimura, 1968) and in strains with a defect in heme biosynthesis, regardless of the position of the block in the pathway (Labbe-Bois *et al.* 1980; Urban-Grimal and Labbe-Bois, 1981; Rytka *et al.* 1984). In addition, whereas other genes encoding enzymes in the heme biosynthetic pathway are thought to be expressed constitutively with respect to oxygen, the transcription of *HEM13*, which encodes coproporphyrinogen III oxidase, is repressed by heme/oxygen (mediated by Hap1p and Rox1p; Verdiere *et al.* 1991; Keng, 1992). This oxidase has a high affinity for oxygen, with an estimated K_m of below 0.1 μmol l^{-1} O_2 (see discussion by Labbe-Bois and Labbe, 1990), suggesting that heme levels would not reflect oxygen concentration until near-anoxic conditions. Supporting this view is the lack of evidence that cellular heme concentrations vary appreciably at higher oxygen concentrations (Labbe-Bois and Labbe, 1990). Therefore, if cellular concentrations of heme are controlling the expression of oxygen-responsive genes, heme probably acts as an on–off switch only at extremely low oxygen levels. Because of the difficulty in measuring free heme levels in cells, this hypothesis has not yet been tested.

Heme degradation and distribution

In addition to its effects on transcription, heme affects a large number of other cellular processes, including protein translation, transport, assembly and degradation (reviewed by Padmanaban *et al.* 1989). Because of the multitude of cellular activities that are influenced by heme, its synthesis, degradation and distribution are thought to be tightly regulated (Padmanaban *et al.* 1989). Although its synthesis is subject to feedback regulation that ensures adequate production, virtually nothing is known about the fate of heme in yeast once it is made in the mitochondrion (Labbe-Bois and Labbe, 1990). Yeast apparently lack heme oxygenase (Labbe-Bois and Labbe, 1990), which catalyzes the first step in heme degradation in higher eukaryotes. Moreover, heme degradation products (e.g. biliverdin, bilirubin) have not been found in yeast. Thus, there is no evidence that heme is degraded in yeast cells; under anoxic conditions, heme may simply be diluted as cell mass increases during anaerobiosis.

Similarly, little is known about the intracellular trafficking of heme. Once it is synthesized in the mitochondrion, heme must be distributed to other cellular compartments, including microsomes, peroxisomes, the nucleus and the cytosol. Given the high affinity of heme for proteins and lipids, it is likely that this distribution is carrier-mediated. There is some evidence in support of this view, at least in higher eukaryotes (Meier *et al.* 1978). Thus, it is unlikely that pools of free heme accumulate in these different cellular compartments. All of the steps in the synthesis, distribution and degradation or dilution of heme

could be important regulatory checkpoints. A thorough understanding of the role of heme in regulating cellular function, including transcription, awaits further characterization of these processes.

Functional roles of heme in oxygen-sensing and the transcriptional regulation of oxygen-responsive genes

There are at least three feasible pathways in which heme could be involved in the transcriptional regulation of genes; one involves control by the *concentration* of heme, and the other two invoke control by the *redox* state of hemoproteins (reviewed by Poyton and Burke, 1992; Bunn and Poyton, 1996). These different regulatory pathways are not mutually exclusive.

In the first type of pathway, heme would serve simply as a metabolic cofactor or ligand that binds to transcriptional components and regulates their activity, as has been proposed for the heme-dependent activation of Hap1p (Fytlovich *et al.* 1993; Zhang and Guarente, 1995). In this type of pathway, the concentration of heme, and not its redox state, would modulate the activity of transcriptional component(s). At very low oxygen concentrations, i.e. near the K_m of oxygen for coproporphyrinogen III oxidase, cellular heme concentrations could provide an effective on–off switch for the transcription of both the hypoxic and aerobic genes.

In the second type of pathway, heme would function as a redox-sensitive component of either a transcription factor or effector element that regulates the activity of transcriptional components (reviewed by Poyton and Burke, 1992; Bunn and Poyton, 1996). For example, the redox state of heme bound to Hap1p or other heme-mediated transcription factors could conceivably control their activity. Although Hap1p has been shown to bind heme *in vitro*, it is not known whether this heme is redox-active. Measurements of Hap1p activity in different redox environments would help clarify the role of heme in Hap1p activation.

In the third type of pathway, the redox (or spin) state of the iron in a hemoprotein oxygen sensor would modulate the activity of transcriptional components either directly or indirectly. For example, for mammalian cells, considerable evidence suggests that the redox state of a hemoprotein oxygen sensor controls the expression of a large number of hypoxic genes, acting through a transcriptional activator, hypoxia-inducible factor 1 (HIF-1) (reviewed by Bunn and Poyton, 1996; Ratcliffe, 1998; Huang *et al.* 1998). Perhaps the best-characterized hemoprotein oxygen sensor is FixL in nitrogen-fixing bacteria, *Rhizobium* sp.; the spin state of Fe in the heme moiety of FixL regulates its kinase activity, which controls the activity of a transcriptional component, FixJ (Gilles-Gonzalez *et al.* 1991, 1994; Gilles-Gonzalez and Gonzalez, 1993; reviewed by Bunn and Poyton, 1996). It is not known whether any of the oxygen-responsive transcription factors in yeast are differentially phosphorylated in response to oxygen or whether hemoproteins *per se* are involved in oxygen-sensing pathways in yeast.

Effect of oxygen concentration on the expression of oxygen-responsive genes

Recently, we completed a number of experiments addressing the role of heme and hemoproteins in regulating the transcription of oxygen-responsive genes in yeast. Previous experiments examining the transcription of these genes have been performed primarily using cells grown either aerobically or anaerobically, or with *hem1* mutants in the presence or absence of δ-aminolevulinate, which bypasses the *hem1* defect. While these approaches have identified genes that are oxygen- and/or heme-sensitive and have helped to define many of the *trans*-acting factors and *cis*-sites that control the expression of these genes, they do not address how oxygen is sensed or the functional role of heme in regulating their transcription. For example, one question raised by these studies is whether these genes respond in a graded fashion to oxygen concentration or in an all-or-none fashion to the presence or absence of oxygen. Moreover, measurement of their transcript levels as a function of oxygen concentration could provide insight into the functional role of heme in controlling their expression. For example, if cellular concentrations of heme were controlling the expression of both the aerobic and hypoxic genes, we would predict that their transcript levels would not vary with declining oxygen level until its concentration approached the K_m of coproporphyrinogen III oxidase. Below this oxygen concentration, transcript levels of the aerobic genes would decline, while those of the hypoxic genes would increase.

Fig. 2 illustrates the effect of oxygen concentration on transcript levels of an aerobic gene (Fig. 2A), an aerobic and hypoxic gene pair (Fig. 2B), and a hypoxic gene (Fig. 2C). Fig. 2A shows the relative mRNA levels of the aerobic gene *COX4* in cells grown at different oxygen concentrations (Burke *et al.* 1997). *COX4* transcript levels decline gradually between 200 and 1 μmol l^{-1} O_2, and then decline sharply below this concentration. Transcripts of a number of other subunits of cytochrome *c* oxidase, including *COX6*, *COX7*, *COX8* and *COX9*, show a similar trend with respect to oxygen concentration (Burke *et al.* 1997). Under nominally oxygen-free conditions (anoxia), mRNAs of all of these genes are detectable, varying from 8 to 40 % of their normoxic levels (Burke *et al.* 1997).

Fig. 2B shows the dose–response curve of transcripts of the aerobic and hypoxic gene pair *COX5a*/*COX5b* as a function of oxygen concentration. Unlike the other aerobic *COX* genes examined, *COX5a* transcript levels (solid line) vary little as a function of oxygen concentration between 200 and 10 μmol l^{-1} O_2, but decline sharply below approximately 5–1 μmol l^{-1} O_2 (Burke *et al.* 1997; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). In contrast, transcript levels of the hypoxic gene *COX5b* (dashed line) are undetectable, or nearly so, between 200 and 0.25 μmol l^{-1} O_2, and then they increase sharply below this concentration. Similar oxygen-dependent transcript profiles were found for the aerobic and hypoxic gene pairs *CYC1*/*CYC7*, *TIF51A*/*ANB1* and *AAC2*/*AAC3* (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). In all cases, a sharp break in their transcript levels occurs between

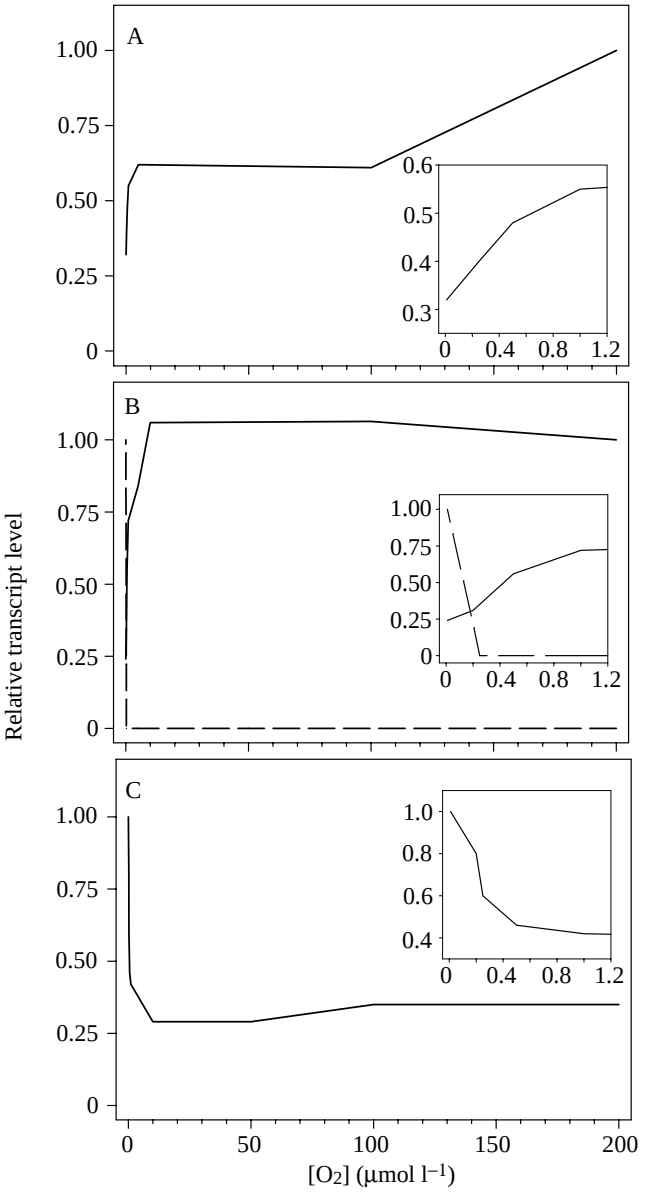

Fig. 2. Effect of oxygen concentration on transcript levels of an aerobic gene (*COX4*) (A), an aerobic and hypoxic gene pair (*COX5a*/*COX5b*) (B) and a hypoxic gene (*CPR1*) (C). *Saccharomyces cerevisiae* (strain JM43) was grown for at least six generations in semi-synthetic galactose medium containing Tween 80, ergosterol and antifoam (SSG-TEA) at oxygen concentrations of 200, 100, 50, 10, 5, 1, 0.50, 0.25 and 0.20 μmol l^{-1} and under anoxic conditions (O_2-free N_2 + 2.5 % CO_2) (see Burke *et al.* 1997, 1998; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Total RNA was isolated and subjected to northern-blot analyses. Transcript levels were normalized to the level of *ACT1* mRNA and are presented relative to their levels under normoxic conditions (for the aerobic genes) or anoxic conditions (for the hypoxic genes). The inset in each panel shows the change in the transcript levels of the genes between 0 and 1.2 μmol l^{-1} O_2. In B, the solid line represents *COX5a* transcript levels and the dashed line represents *COX5b* transcript levels.

approximately 1 and 0.5 μmol l^{-1} O_2, with the aerobic isoform being optimally expressed above this concentration and the hypoxic isoform expressed below it.

Fig. 2C shows the dose–response curve of the hypoxic gene *CPR1* (*NCP1*). Unlike the hypoxic genes that encode isoforms, *CPR1* transcripts are detectable between 200 and 0.5 μmol l^{-1} O_2 (at approximately 30 % of their anoxic levels), but their levels also increase sharply below 0.5 μmol l^{-1} O_2. Similar transcript profiles were found for the hypoxic genes *HEM13*, *HMG1*, *HMG2*, *ERG11* and *OLE1*; transcript levels of all of these genes also show sharp changes, typically below 1 μmol l^{-1} O_2 (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation).

Another way to investigate how oxygen affects the transcription of these genes is to shift cells from anoxic to aerobic conditions and *vice versa*. We used such shift experiments to examine the kinetics of induction of aerobic genes and decline in transcript levels of hypoxic genes (Fig. 3) as well as the kinetics of induction of hypoxic genes and decline in transcript levels of aerobic genes (Fig. 4). The large change in oxygen concentration that occurs during the first 10 min after the shift is shown in the insets in Figs 3A and 4A. Under the experimental conditions used, the mass-doubling time of *S. cerevisiae* strain JM43 was approximately 4 h under anoxic conditions and approximately 2.4 h under aerobic conditions.

Fig. 3 shows the effect of shifting cells from steady-state anoxic conditions to aerobic conditions on transcript levels of *COX4* (Fig. 3A), *COX5a* and *COX5b* (Fig. 3B) and the hypoxic genes *CPR1*, *CYC7* and *HEM13* (Fig. 3C) (Burke *et al.* 1997; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). In general, transcript levels of aerobic genes, as illustrated by *COX4*, respond rapidly to reoxygenation and then increase slowly, reaching half of their aerobic steady-state levels ($t_{1/2}$) typically in less than 1 h (Burke *et al.* 1997). Transcript levels of aerobic isoforms, such as *COX5a* (Fig. 3B, solid line), typically increase more rapidly, with $t_{1/2}$ values ranging from 5 min (*TIF51A*) to 45 min (*AAC2*) (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Finally, transcript levels of most hypoxic genes, as illustrated by *COX5b* (dashed line, Fig. 3B) and *HEM13* (dashed line) and *CPR1* (solid line) in Fig. 3C, decline rapidly, with $t_{1/2}$ decay values ranging from 5 min (*HEM13*, *COX5b*, *AAC3*) to 70 min (*OLE1*) (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Currently, it is not known whether the decline in transcript levels of these hypoxic genes is due to the effect of oxygen on transcript synthesis, transcript stability or both. Surprisingly, upon reoxygenation, *CYC7* transcript levels (dash-dotted line, Fig. 3C) increase, reaching nearly five times their levels under anoxia, before declining (Burke *et al.* 1997; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation).

Fig. 4 shows the effect of shifting cells from steady-state aerobic conditions to anoxic conditions on transcript levels of these same genes. In general, transcript levels of aerobic genes (e.g. *COX4*, Fig. 4A), as well as the aerobic isoforms (e.g. *COX5a*, solid line in Fig. 4B), decline rapidly, with $t_{1/2}$ decay

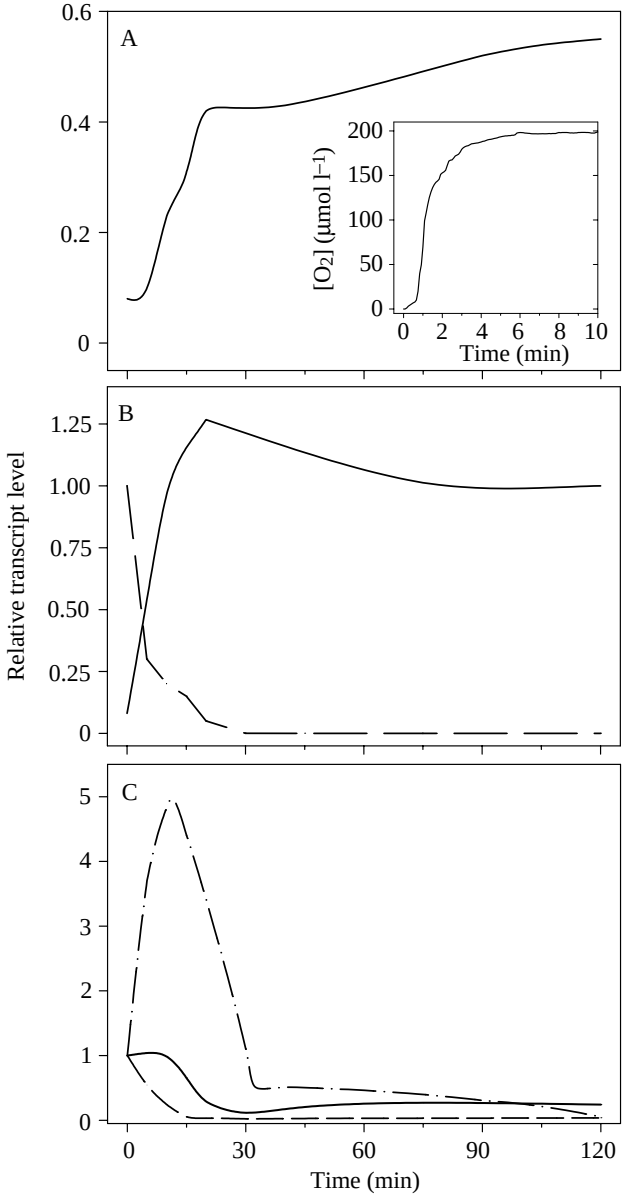

Fig. 3. Kinetics of induction of aerobic genes and decline in transcript levels of hypoxic genes after a shift from anoxic to aerobic conditions. *Saccharomyces cerevisiae* was grown for at least six generations under anoxic conditions. At time zero, the sparge gas was changed from O_2-free N_2 to air (see Burke *et al.* 1997; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Cells were harvested for RNA at different times after the shift, and the RNA was subjected to northern-blot analyses. Transcript levels were normalized as described in the legend to Fig. 2. The inset in A shows the change in oxygen concentration that occurs during the first 10 min after the shift. (A) Transcript levels of *COX4*. In B, the solid line represents *COX5a* transcript levels and the dashed line represents *COX5b* transcript levels. In C, the solid line represents *CPR1* transcript levels, the dashed line represents *HEM13* transcript levels and the dashed-dotted line represents *CYC7* transcript levels.

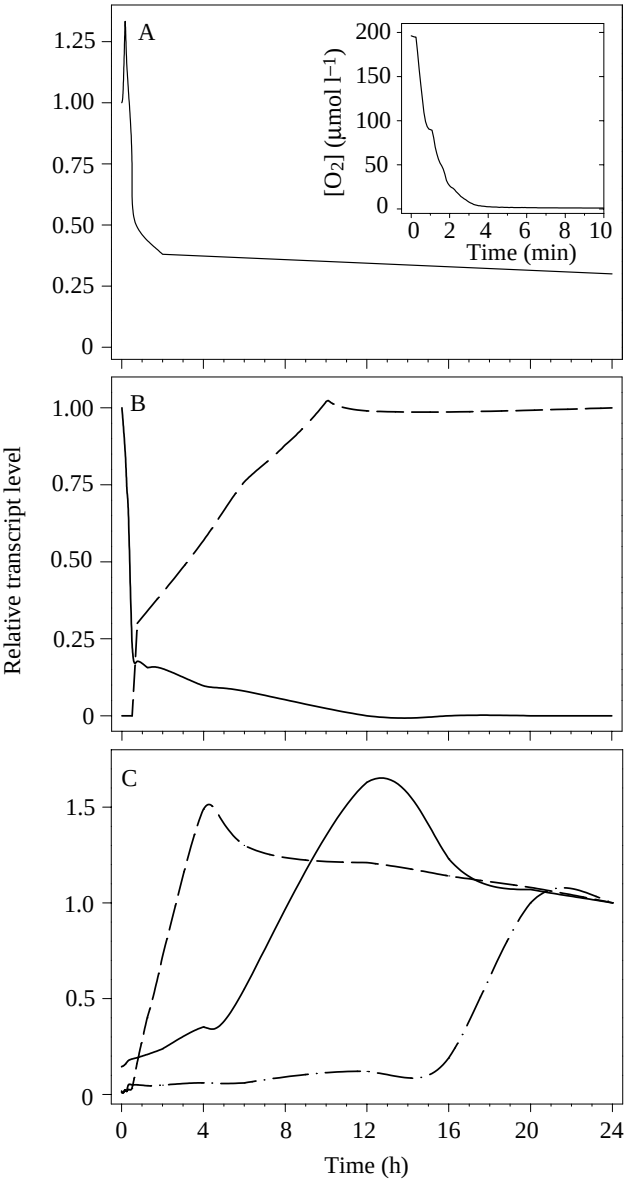

Fig. 4. Kinetics of induction of hypoxic genes and decline in transcript levels of aerobic genes after a shift from aerobic to anoxic conditions. *Saccharomyces cerevisiae* was grown for at least six generations under normoxic conditions. At time zero, the sparge gas was changed from air to O_2-free N_2. Cells were harvested for RNA at different times after the shift, and the RNA was subjected to northern-blot analyses (see Burke *et al.* 1997; K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). Transcript levels were normalized as described in the legend to Fig. 2. The inset in A shows the change in oxygen concentration that occurs during the first 10 min after the shift. (A) *COX4* transcript levels. In B, the solid line represents *COX5a* transcript levels, and the dashed line represents *COX5b* transcript levels. In C, the solid line represents *CPR1* transcript levels, the dashed line represents *HEM13* transcript levels and the dashed-dotted line represents *CYC7* transcript levels

values ranging from 5 to 60 min (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation). In contrast to the comparatively rapid induction of aerobic genes (Fig. 3), there is a remarkable variation in both the time course for induction of the hypoxic genes as well as their transcript profiles (Fig. 4B,C). Whereas

some hypoxic genes are induced fairly rapidly (e.g. *HEM13*, dashed line in Fig. 4C), others are induced much more slowly, not reaching fully anoxic levels until nearly 20 h (e.g. *CYC7*, dashed-dotted line in Fig. 4C). Moreover, many of the hypoxic genes exhibit large overshoots (e.g. *HEM13* and *CPR1*, solid line in Fig. 4C) above their steady-state anoxic levels, while others simply plateau (e.g. *COX5b*, dashed line in Fig. 4B, and *CYC7*) (K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation).

Taken together, these findings provide insight concerning a number of aspects of the transcriptional regulation of these genes. First, transcript levels of both the aerobic and hypoxic genes are determined by the *concentration* of oxygen, not merely by its presence or absence. Second, compared with the aerobic genes, the hypoxic genes are regulated much more tightly in terms of the oxygen concentrations over which they are optimally expressed. Third, the induction of the aerobic genes is much more rapid than that of the hypoxic genes. Fourth, there is a marked variation in both the time course for induction and transcript profiles of the hypoxic genes. And fifth, for nearly all of these oxygen-responsive genes, a sharp break occurs in their transcript levels at approximately $1\,\mu mol\,l^{-1}$ O_2, with transcript levels of the aerobic genes declining sharply and those of most hypoxic genes increasing sharply below this concentration. It is interesting that this sharp change occurs near the estimated K_m for O_2 of coproporphyrinogen III oxidase (approximately $0.1\,\mu mol\,l^{-1}$ O_2). Although we have not directly measured levels of free heme at these different oxygen concentrations, these data are consistent with models that invoke regulation by cellular concentrations of heme. However, the gradual decline in mRNA levels of a number of aerobic genes – and the increase in mRNA levels of some of the hypoxic genes (see K. E. Kwast, P. V. Burke and R. O. Poyton, in preparation) – over a range of oxygen concentration ($1–200\,\mu mol\,l^{-1}$) in which heme levels are thought not to vary (Labbe-Bois and Labbe, 1990) is difficult to reconcile with such a model. Instead, these data suggest that other regulatory mechanisms (e.g. the redox state of heme, hemoproteins or other metalloproteins) are controlling the expression of these genes at higher oxygen concentrations.

Evidence for the involvement of a redox-sensitive hemoprotein in the induction of a subset of the hypoxic genes

One way to test the hypothesis that the redox state of hemoprotein(s) is involved in regulating the transcription of oxygen-responsive genes is to clamp the redox state under different conditions of oxygen availability. An experimental approach that has been widely used to study the involvement of hemoproteins in oxygen-sensing pathways in higher eukaryotes is to use carbon monoxide (CO). CO is an inert gas that has a remarkably high specificity for ferrous (FeII) heme groups in hemoglobin, myoglobin, certain cytochromes and other hemoproteins that bind oxygen reversibly. Under hypoxic conditions, CO markedly reduces the transcription of a number of hypoxically induced genes in mammalian cells (Goldberg *et al.* 1988; Kietzmann *et al.* 1993; Goldberg and Schneider, 1994; reviewed by Bunn and Poyton, 1996) and inhibits the expression of several proteins in turtle hepatocytes (reviewed by Hochachka *et al.* 1996). Although the precise target of CO in these cells has not been determined, these results are consistent with CO binding to a ferrous hemoprotein oxygen sensor and preventing a redox change in the heme moiety that is required for the induction of these genes at low oxygen tension (Goldberg *et al.* 1988; reviewed by Bunn and Poyton, 1996). In mammalian cells, this hemoprotein oxygen sensor is proposed to function as an oxidase that reduces oxygen to superoxide, which acts as a second messenger.

To determine whether the redox state of hemoprotein(s) may also control the expression of oxygen-responsive genes in yeast, we recently examined the effects of CO on the induction of a number of hypoxic genes (*COX5b*, *CYC7*, *ANB1*, *AAC3*, *HEM13*, *HMG1*, *HMG2*, *ERG11*, *CPR1* and *OLE1*) and on the decline in transcription of several aerobic genes (*COX5a*, *COX6*, *CYC1*, *TIF51A*, *AAC2* and *ROX1*) after shifting cells from aerobic to anoxic conditions (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation). This set includes genes whose transcription is regulated by Hap1p, Hap2/3/4/5p and/or Rox1p. Interestingly, CO affected only a small subset of the hypoxic genes: CO totally blocked the anoxic induction of *CYC7* and *OLE1* and partially blocked the induction of *COX5b* (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation). The transcription of the other hypoxic genes and of all the aerobic genes examined was not affected appreciably by CO. These results define two classes of hypoxic genes: CO-sensitive and CO-insensitive. They also suggest that CO is not acting through any of the known heme-activated transcription factors or through Rox1p. Rather, these findings point to additional pathways (and probably *trans*-acting factors) that mediate the expression of these hypoxic genes.

Another experimental approach that has been widely used to address the involvement of hemoproteins in oxygen-sensing pathways is to examine whether transition metals induce the transcription of hypoxic genes under normoxic conditions. When added to cells, transition metals (e.g. Co, Ni) are thought to be incorporated into hemoproteins in place of Fe. Indeed, it has been shown that these metals can serve as substrates for ferrochelatase, and Co has been shown to be incorporated into heme both *in vivo* (Sinclair *et al.* 1979) and in cultured cells (Sinclair *et al.* 1982). Unlike Fe, these metals either cannot bind O_2 (Ni) or have an exceedingly low affinity for O_2 (Co). In mammalian cells, these metals typically induce the same set of hypoxic genes whose expression is blocked by CO under hypoxic conditions (reviewed by Bunn and Poyton, 1996).

In yeast, we find that Co and Ni induce the expression specifically of the CO-sensitive genes *OLE1* and, in some experimental conditions, *CYC7* under normoxic conditions in a concentration-dependent manner (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation). Our

findings with both CO and transition metals suggest that these hypoxic genes are induced *via* a pathway involving an oxidoreduction state change in a hemoprotein oxygen sensor. Although we have not definitively identified the hemoprotein involved in this oxygen-sensing pathway, a large body of evidence suggests that the mitochondrial respiratory chain, probably cytochrome *c* oxidase, is involved in this signaling pathway (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation); the anoxic induction of *OLE1* and *CYC7* is specifically blocked by inhibiting electron transport either with metabolic poisons (e.g. CN^-, antimycin A) or with mutations in the respiratory chain (cox^- or cob^- strains). The nature of the cross-talk between the mitochondrion and nucleus that is required for the transduction of this signal is being studied.

Multiple pathways/mechanisms regulate the transcription of hypoxic genes

Overall, our experiments examining oxygen thresholds, the induction/decline in transcript levels following a shift in oxygen concentration and the effects of CO and transition metals on the transcription of oxygen-responsive genes suggest that there are multiple pathways and mechanisms involved in regulating the oxygen-dependent expression of genes in yeast. With regard to the functional roles of heme, we have collected indirect evidence that cellular concentrations of heme regulate the transcription of these genes, which is probably mediated by the Hap proteins. However, the oxygen-dependent change in transcript levels of a subset of both the aerobic and hypoxic genes over a range of oxygen concentration in which heme concentration is thought not to vary suggests that other regulatory mechanisms are involved, possibly ones involving redox changes. Finally, of all of the oxygen-responsive genes examined, only a small subset of the Rox1p-dependent, hypoxic genes was affected by CO and transition metals. This suggests that these effects are exerted through pathways that are independent of Rox1p and Hap1p regulation. Moreover, it is interesting that the effect of these treatments apparently overrides the regulation by Rox1p on the transcription of these genes, given that *ROX1* transcript levels are not affected by these treatments (K. E. Kwast, P. V. Burke, B. Staahl, S. Fontaine and R. O. Poyton, in preparation). Taken together, these findings indicate that there are multiple regulatory and oxygen-sensing pathways involved in modulating the expression of oxygen-responsive genes in yeast.

Conclusions and future prospects

During the past decade, considerable progress has been made in our understanding of oxygen-sensing pathways in both prokaryotes and eukaryotes. These pathways share intriguing similarities, with hemoproteins playing a central role as proximal oxygen sensors. In yeast, the role of heme in oxygen-sensing pathways has been proposed to be somewhat different from that in higher eukaryotes; rather than being a redox-sensitive prosthetic group of a sensor, heme has been viewed primarily as a redox-insensitive, metabolic cofactor required for the function of transcription factors. However, our studies suggest that heme may act in both capacities in yeast.

This review provides testimony to the pivotal role that heme plays in the oxygen-dependent transcription of genes. Given that cellular levels of heme appear to control the transcription of a large set of both aerobic and hypoxic genes, it is possible that coproporphyrinogen III oxidase acts, in many respects, as an oxygen sensor. Our studies of the transcription of these genes as a function of oxygen concentration lend support to the view that heme may act as a redox-insensitive cofactor for transcription factors but, at the same time, the results suggest that additional regulatory mechanisms – possibly involving redox-sensitive sensors – are involved at higher oxygen concentrations. Furthermore, work with CO and transition metals indicates that multiple pathways are involved in the regulation of a subset of the hypoxic genes and points to the involvement of redox-sensitive hemoprotein(s). Indeed, these studies suggest that oxygen-sensing mechanisms involving hemoproteins may be universal in prokaryotes and both lower and higher eukaryotes. Further studies with yeast should define these pathways and identify oxygen sensor(s) in this simple eukaryotic organism and may provide insight into an area of research that has presented a considerable challenge to investigators working with higher eukaryotic cells.

We thank the *Company of Biologists* for the opportunity to present our work and for bringing together a diverse array of researchers studying many aspects of oxygen metabolism, as shown by the breadth of work in this volume. In addition, we thank the community of yeast researchers for providing preprints and reprints of recent work. The preparation of this article was supported, in part, by an American Heart Association Fellowship to K.E.K., and National Institutes of Health Grants GM-30228 and GM-39324 and Tobacco Research Council Grant 4557 to R.O.P.

References

ALLEN, L. A., ZHAO, X. J., CAUGHEY, W. AND POYTON, R. O. (1995). Isoforms of yeast cytochrome *c* oxidase subunit V affect the binuclear reaction center and alter the kinetics of interaction with the isoforms of yeast cytochrome *c*. *J. biol. Chem.* **270**, 110–118.

AMILLET, J. M., BUISSON, N. AND LABBE-BOIS, R. (1995). Positive and negative elements involved in the differential regulation by heme and oxygen of the *HEM13* gene (coproporphyrinogen oxidase) in *Saccharomyces cerevisiae*. *Curr. Genet.* **28**, 503–511.

AMILLET, J. M., BUISSON, N. AND LABBE-BOIS, R. (1996). Characterization of an upstream activation sequence and two Rox1p-responsive sites controlling the induction of the yeast *HEM13* gene by oxygen and heme deficiency. *J. biol. Chem.* **271**, 24425–24432.

ANDREASEN, A. AND STIER, T. (1953). Anaerobic nutrition of *Saccharomyces cerevisiae*. I. Ergosterol requirement for growth in a defined medium. *J. cell. comp. Physiol.* **41**, 23–36.

ANDREASEN, A. AND STIER, T. (1954). Anaerobic nutrition of

Saccharomyces cerevisiae. II. Unsaturated fatty acid requirement for growth in defined medium. *J. cell. comp. Physiol.* **43**, 271–281.

Ansell, R., Granath, K., Hohmann, S., Thevelein, J. M. and Adler, L. (1997). The two isoenzymes for yeast NAD$^+$-dependent glycerol 3-phosphate dehydrogenase encoded by *GPD1* and *GPD2* have distinct roles in osmoadaptation and redox regulation. *EMBO J.* **16**, 2179–2187.

Arcangioli, B. and Lescure, B. (1985). Identification of proteins involved in the regulation of yeast iso-1-cytochrome *c* expression by oxygen. *EMBO J.* **4**, 2627–2633.

Balasubramanian, B., Lowry, C. V. and Zitomer, R. S. (1993). The Rox1 repressor of the *Saccharomyces cerevisiae* hypoxic genes is a specific DNA-binding protein with a high-mobility-group motif. *Molec. cell. Biol.* **13**, 6071–6078.

Betina, S., Gavurnikova, G., Haviernik, P., Sabova, L. and Kolarov, J. (1995). Expression of the *AAC2* gene encoding the major mitochondrial ADP/ATP carrier in *Saccharomyces cerevisiae* is controlled at the transcriptional level by oxygen, heme and HAP2 factor. *Eur. J. Biochem.* **229**, 651–657.

Bock, K. W., de Matteis, F. and Aldridge, W. N. (1978). *Heme and Hemoproteins.* New York: Springer-Verlag.

Bordallo, J., Cueva, R. and Suarez-Rendueles, P. (1995). Transcriptional regulation of the yeast vacuolar aminopeptidase yscI encoding gene (*APE1*) by carbon sources [published erratum appears in *FEBS Lett.* 1995 Aug 7 **369**(2–3), 353]. *FEBS Lett.* **364**, 13–16.

Bourot, S. and Karst, F. (1995). Isolation and characterization of the *Saccharomyces cerevisiae* SUT1 gene involved in sterol uptake. *Gene* **165**, 97–102.

Bunn, H. F. and Poyton, R. O. (1996). Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* **76**, 839–885.

Bunn, E. F., Gu, J., Huang, J. E., Park, J.-W. and Zhu, H. (1988). Erythropoietin: a model system for studying oxygen-dependent gene regulation. *J. exp. Biol.* **201**, 1197–1201.

Burke, P. V., Kwast, K. E., Everts, F. and Poyton, R. O. (1998). A fermentor system for regulating oxygen at low concentrations in cultures of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* (in press).

Burke, P. V. and Poyton, R. O. (1998). Structure/function of oxygen-regulated subunit isoforms in cytochrome *c* oxidase. *J. exp. Biol.* **201**, 1163–1175.

Burke, P. V., Raitt, D. C., Allen, L. A., Kellogg, E. A. and Poyton, R. O. (1997). Effects of oxygen concentration on the expression of cytochrome *c* and cytochrome *c* oxidase genes in yeast. *J. biol. Chem.* **272**, 14705–14712.

Cerdan, M. E. and Zitomer, R. S. (1988). Oxygen-dependent upstream activation sites of *Saccharomyces cerevisiae* cytochrome *c* genes are related forms of the same sequence. *Molec. cell. Biol.* **8**, 2275–2279.

Chodosh, L. A., Olesen, J., Hahn, S., Baldwin, A. S., Guarente, L. and Sharp, P. A. (1988). A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. *Cell* **53**, 25–35.

Choi, J. Y., Stukey, J., Hwang, S. Y. and Martin, C. E. (1996). Regulatory elements that control transcription activation and unsaturated fatty acid-mediated repression of the *Saccharomyces cerevisiae OLE1* gene. *J. biol. Chem.* **271**, 3581–3589.

Choma, C. T., Lear, J. D., Nelson, M. J., Dutton, P. L., Robertson, D. E. and De Grado, W. F. (1994). Design of a heme-binding four-helix bundle. *J. Am. chem. Soc.* **116**, 856–865.

Clavilier, L., Pere, G. and Slonimski, P. P. (1969). Demonstration of several independent loci involved in the synthesis of iso-2-cytochrome *c* in yeast. *Molec. gen. Genet.* **104**, 195–218.

Cohen, G., Fessl, F., Traczyk, A., Rytka, J. and Ruis, H. (1985). Isolation of the catalase A gene of *Saccharomyces cerevisiae* by complementation of the *cta1* mutation. *Molec. gen. Genet.* **200**, 74–79.

Crawford, M. J., Sherman, D. R. and Goldberg, D. E. (1995). Regulation of *Saccharomyces cerevisiae* flavohemoglobin gene expression. *J. biol. Chem.* **270**, 6991–6996.

Creusot, F., Verdiere, J., Gaisne, M. and Slonimski, P. P. (1988). CYP1 (HAP1) regulator of oxygen-dependent gene expression in yeast. I. Overall organization of the protein sequence displays several novel structural domains. *J. molec. Biol.* **204**, 263–276.

Cumsky, M. G., Trueblood, C. E., Ko, C. and Poyton, R. O. (1987). Structural analysis of two genes encoding divergent forms of yeast cytochrome *c* oxidase subunit V. *Molec. cell. Biol.* **7**, 3511–3519.

Daignan-Fornier, B., Valens, M., Lemire, B. D. and Bolotin-Fukuhara, M. (1994). Structure and regulation of *SDH3*, the yeast gene encoding the cytochrome b560 subunit of respiratory complex II. *J. biol. Chem.* **269**, 15469–15472.

Dang, V. D., Bohn, C., Bolotin-Fukuhara, M. and Daignan-Fornier, B. (1996). The CCAAT box-binding factor stimulates ammonium assimilation in *Saccharomyces cerevisiae*, defining a new cross-pathway regulation between nitrogen and carbon metabolisms. *J. Bacteriol.* **178**, 1842–1849.

Deckert, J., Perini, R., Balasubramanian, B. and Zitomer, R. S. (1995*a*). Multiple elements and auto-repression regulate Rox1, a repressor of hypoxic genes in *Saccharomyces cerevisiae*. *Genetics* **139**, 1149–1158.

Deckert, J., Rodriguez Torres, A. M., Simon, J. T. and Zitomer, R. S. (1995*b*). Mutational analysis of Rox1, a DNA-bending repressor of hypoxic genes in *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **15**, 6109–6117.

de Winde, J. H. and Grivell, L. A. (1993). Global regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*. *Prog. Nucleic Acids Res. molec. Biol.* **46**, 51–91.

de Winde, J. H. and Grivell, L. A. (1995). Regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*. Intricate interplay between general and specific transcription factors in the promoter of the *QCR8* gene. *Eur. J. Biochem.* **233**, 200–208.

de Winde, J. H., Van Leeuwen, H. C. and Grivell, L. A. (1993). The multifunctional regulatory proteins ABF1 and CPF1 are involved in the formation of a nuclease-hypersensitive region in the promoter of the *QCR8* gene. *Yeast* **9**, 847–857.

Di Flumeri, C., Liston, P., Acheson, N. H. and Keng, T. (1996). The HMG domain of the ROX1 protein mediates repression of *HEM13* through overlapping DNA binding and oligomerization functions. *Nucleic Acids Res.* **24**, 808–815.

Donzeau, M., Bourdineaud, J. P. and Lauquin, G. J. (1996). Regulation by low temperatures and anaerobiosis of a yeast gene specifying a putative GPI-anchored plasma membrane protein (corrected) [published erratum appears in *Molec. Microbiol.* (1996) **21**, 431]. *Molec. Microbiol.* **20**, 449–459.

Dorsman, J. C. and Grivell, L. A. (1990). Expression of the gene encoding subunit II of yeast QH2: cytochrome *c* oxidoreductase is regulated by multiple factors. *Curr. Genet.* **17**, 459–464.

Flattery-O'Brien, J. A., Grant, C. M. and Dawes, I. W. (1997). Stationary-phase regulation of the *Saccharomyces cerevisiae SOD2* gene is dependent on additive effects of HAP2/3/4/5- and STRE-binding elements. *Molec. Microbiol.* **23**, 303–312.

Forsburg, S. L. and Guarente, L. (1988). Mutational analysis of

upstream activation sequence 2 of the *CYC1* gene of *Saccharomyces cerevisiae*: a HAP2-HAP3-responsive site. *Molec. cell. Biol.* **8**, 647–654.

FORSBURG, S. L. AND GUARENTE, L. (1989*a*). Identification and characterization of HAP4: a third component of the CCAAT-bound HAP2/HAP3 heteromer. *Genes Dev*. **3**, 1166–1178.

FORSBURG, S. L. AND GUARENTE, L. (1989*b*). Communication between mitochondria and the nucleus in regulation of cytochrome genes in the yeast *Saccharomyces cerevisiae*. *A. Rev. Cell Biol*. **5**, 153–180.

FYTLOVICH, S., GERVAIS, M., AGRIMONTI, C. AND GUIARD, B. (1993). Evidence for an interaction between the CYP1(HAP1) activator and a cellular factor during heme-dependent transcriptional regulation in the yeast *Saccharomyces cerevisiae*. *EMBO J*. **12**, 1209–1218.

GALIAZZO, F. AND LABBE-BOIS, R. (1993). Regulation of Cu,Zn- and Mn-superoxide dismutase transcription in *Saccharomyces cerevisiae*. *FEBS Lett*. **315**, 197–200.

GAVURNIKOVA, G., SABOVA, L., KISSOVA, I., HAVIERNIK, P. AND KOLAROV, J. (1996). Transcription of the *AAC1* gene encoding an isoform of mitochondrial ADP/ATP carrier in *Saccharomyces cerevisiae* is regulated by oxygen in a heme-independent manner. *Eur. J. Biochem*. **239**, 759–763.

GEORGAKOPOULOS, T. AND THIREOS, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J*. **11**, 4145–4152.

GILLES-GONZALEZ, M. A., DITTA, G. S. AND HELINSKI, D. R. (1991). A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. *Nature* **350**, 170–172.

GILLES-GONZALEZ, M. A. AND GONZALEZ, G. (1993). Regulation of the kinase activity of heme protein FixL from the two-component system FixL/FixJ of *Rhizobium meliloti*. *J. biol. Chem.* **268**, 16293–16297.

GILLES-GONZALEZ, M. A., GONZALEZ, G., PERUTZ, M. F., KIGER, L., MARDEN, M. C. AND POYART, C. (1994). Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. *Biochemistry* **33**, 8067–8073.

GOLDBERG, M. A., DUNNING, S. P. AND BUNN, H. F. (1988). Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. *Science* **242**, 1412–1415.

GOLDBERG, M. A. AND SCHNEIDER, T. J. (1994). Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. *J. biol. Chem.* **269**, 4355–4359.

GOLLUB, E. G. AND DAYAN, J. (1985). Regulation by heme of the synthesis of cytochrome *c* oxidase subunits V and VII in yeast. *Biochem. biophys. Res. Commun*. **128**, 1447–1454.

GRALLA, E. B. AND KOSMAN, D. J. (1992). Molecular genetics of superoxide dismutases in yeasts and related fungi. *Adv. Genet*. **30**, 251–319.

GROOT, G. S. AND POYTON, R. O. (1975). Oxygen control of cytochrome *c* oxidase synthesis in isolated mitochondria from *Saccharomyces cerevisiae*. *Nature* **255**, 238–240.

GUARENTE, L., LALONDE, B., GIFFORD, P. AND ALANI, E. (1984). Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. *Cell* **36**, 503–511.

GUARENTE, L. AND MASON, T. (1983). Heme regulates transcription of the *CYC1* gene of *S. cerevisiae via* an upstream activation site. *Cell* **32**, 1279–1286.

GUIARD, B. (1985). Structure, expression and regulation of a nuclear gene encoding a mitochondrial protein: the yeast L(+)-lactate cytochrome *c* oxidoreductase (cytochrome b_2). *EMBO J*. **4**, 3265–3272.

GUSTAFSSON, C. M., MYERS, L. C., LI, Y., REDD, M. J., LUI, M., ERDJUMENT-BROMAGE, H., TEMPST, P. AND KORNBERG, R. D. (1997). Identification of Rox3 as a component of mediator and RNA polymerase II holoenzyme. *J. biol. Chem.* **272**, 48–50.

HA, N., HELLAUER, K. AND TURCOTTE, B. (1996). Mutations in target DNA elements of yeast HAP1 modulate its transcriptional activity without affecting DNA binding. *Nucleic Acids Res*. **24**, 1453–1459.

HAMPTON, R., DIMSTER-DENK, D. AND RINE, J. (1996). The biology of HMG-CoA reductase: the pros of contra-regulation. *Trends biochem. Sci*. **21**, 140–145.

HOCHACHKA, P. W., BUCK, L. T., DOLL, C. J. AND LAND, S. C. (1996). Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc. natn. Acad. Sci. U.S.A.* **93**, 9493–9498.

HODGE, M. R., KIM, G., SINGH, K. AND CUMSKY, M. G. (1989). Inverse regulation of the yeast *COX5* genes by oxygen and heme. *Molec. cell. Biol.* **9**, 1958–1964.

HODGE, M. R., SINGH, K. AND CUMSKY, M. G. (1990). Upstream activation and repression elements control transcription of the yeast *COX5b* gene. *Molec. cell. Biol.* **10**, 5510–5520.

HORTNER, H., AMMERER, G., HARTTER, E., HAMILTON, B., RYTKA, J., BILINSKI, T. AND RUIS, H. (1982). Regulation of synthesis of catalases and iso-1-cytochrome *c* in *Saccharomyces cerevisiae* by glucose, oxygen and heme. *Eur. J. Biochem.* **128**, 179–184.

KANG, H. A., SCHWELBERGER, H. G. AND HERSHEY, J. W. (1992). The two genes encoding protein synthesis initiation factor eIF-5A in *Saccharomyces cerevisiae* are members of a duplicated gene cluster. *Molec. gen. Genet*. **233**, 487–490.

KELEHER, C. A., REDD, M. J., SCHULTZ, J., CARLSON, M. AND JOHNSON, A. D. (1992). Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68**, 709–719.

KENG, T. (1992). HAP1 and ROX1 form a regulatory pathway in the repression of *HEM13* transcription in *Saccharomyces cerevisiae*. *Molec. cell. Biol*. **12**, 2616–2623.

KIETZMANN, T., SCHMIDT, H., UNTHAN-FECHNER, K., PROBST, I. AND JUNGERMANN, K. (1993). A ferro-heme protein senses oxygen levels, which modulate the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. *Biochem. biophys. Res. Commun*. **195**, 792–798.

KIM, K. S. AND GUARENTE, L. (1989). Mutations that alter transcriptional activation but not DNA binding in the zinc finger of yeast activator HAPI. *Nature* **342**, 200–203.

KOLAROV, J., KOLAROVA, N. AND NELSON, N. (1990). A third ADP/ATP translocator gene in yeast. *J. biol. Chem.* **265**, 12711–12716.

KWAST, K. E., BURKE, P. V., BROWN, K. AND POYTON, R. O. (1997). *REO1* and *ROX1* are alleles of the same gene which encodes a transcriptional repressor of hypoxic genes in yeast. *Curr. Genet.* **32**, 377–383.

LABBE-BOIS, R. AND LABBE, P. (1990). Tetrapyrrole and heme biosynthesis in the yeast *Saccharomyces cerevisiae*. In *Biosynthesis of Heme and Chlorophylls* (ed. H. A. Dailey), pp. 235–285. New York: McGraw-Hill.

LABBE-BOIS, R., SIMON, M., RYTKA, J., LITWINSKA, J. AND BILINSKI, T. (1980). Effect of 5-aminolevulinic acid synthesis deficiency on expression of other enzymes of heme pathway in yeast. *Biochem. biophys. Res. Commun.* **95**, 1357–1363.

LAMBERT, J. R., BILANCHONE, V. W. AND CUMSKY, M. G. (1994). The *ORD1* gene encodes a transcription factor involved in oxygen regulation and is identical to *IXR1*, a gene that confers cisplatin sensitivity to *Saccharomyces cerevisiae*. *Proc. natn. Acad. Sci. U.S.A.* **91**, 7345–7349.

LATHROP, J. T. AND TIMKO, M. P. (1993). Regulation by heme of mitochondrial protein transport through a conserved amino acid motif. *Science* **259**, 522–525.

LODI, T. AND GUIARD, B. (1991). Complex transcriptional regulation of the *Saccharomyces cerevisiae CYB2* gene encoding cytochrome b2: CYP1(HAP1) activator binds to the CYB2 upstream activation site UAS1-B2. *Molec. cell. Biol.* **11**, 3762–3772.

LODI, T., PETROCHILO, E., GAISNE, M. AND VERDIERE, J. (1996). Characterization of a promoter mutation in the *CYP3* gene of *Saccharomyces cerevisiae* which cancels regulation by Cyp1p (Hap1p) without affecting its binding site. *Molec. gen. Genet.* **253**, 103–110.

LOWRY, C. V., CERDAN, M. E. AND ZITOMER, R. S. (1990). A hypoxic consensus operator and a constitutive activation region regulate the *ANB1* gene of *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **10**, 5921–5926.

LOWRY, C. V. AND LIEBER, R. H. (1986). Negative regulation of the *Saccharomyces cerevisiae ANB1* gene by heme, as mediated by the *ROX1* gene product. *Molec. cell. Biol.* **6**, 4145–4148.

LOWRY, C. V. AND ZITOMER, R. S. (1984). Oxygen regulation of anaerobic and aerobic genes mediated by a common factor in yeast. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6129–6133.

LOWRY, C. V. AND ZITOMER, R. S. (1988). *ROX1* encodes a heme-induced repression factor regulating *ANB1* and *CYC7* of *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **8**, 4651–4658.

MAITY, S. N., VUORIO, T. AND DE CROMBRUGGHE, B. (1990). The B subunit of a rat heteromeric CCAAT-binding transcription factor shows a striking sequence identity with the yeast Hap2 transcription factor. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5378–5382.

MCDONOUGH, V. M., STUKEY, J. E. AND MARTIN, C. E. (1992). Specificity of unsaturated fatty acid-regulated expression of the *Saccharomyces cerevisiae OLE1* gene. *J. biol. Chem.* **267**, 5931–5936.

MCNABB, D. S., XING, Y. AND GUARENTE, L. (1995). Cloning of yeast *HAP5*: a novel subunit of a heterotrimeric complex required for CCAAT binding. *Genes Dev.* **9**, 47–58.

MEHTA, K. D., LEUNG, D., LEFEBVRE, L. AND SMITH, M. (1990). The *ANB1* locus of *Saccharomyces cerevisiae* encodes the protein synthesis initiation factor eIF-4D. *J. biol. Chem.* **265**, 8802–8807.

MEHTA, K. D. AND SMITH, M. (1989). Identification of an upstream repressor site controlling the expression of an anaerobic gene (*ANB1*) in *Saccharomyces cerevisiae*. *J. biol. Chem.* **264**, 8670–8675.

MEIER, P. J., SPYCHER, M. A. AND MEYER, U. A. (1978). Isolation of a subfraction of rough endoplasmic reticulum closely associated with mitochondria. Evidence for its role in cytochrome P450 synthesis. *Exp. Cell Res.* **111**, 479–483.

MELNICK, L. AND SHERMAN, F. (1993). The gene clusters ARC and COR on chromosomes 5 and 10, respectively, of *Saccharomyces cerevisiae* share a common ancestry. *J. molec. Biol.* **233**, 372–388.

MERCADO, J. J. AND GANCEDO, J. M. (1992). Regulatory regions in the yeast *FBP1* and *PCK1* genes. *FEBS Lett.* **311**, 110–114.

MIYAKE, S. AND SUGIMURA, T. (1968). Coproporphyrinogenase in a respiration-deficient mutant of yeast lacking all cytochromes and accumulating coproporphyrin. *J. Bacteriol.* **96**, 1997–2003.

MONTGOMERY, D. L., LEUNG, D. W., SMITH, M., SHALIT, P., FAYE, G. AND HALL, B. D. (1980). Isolation and sequence of the gene for iso-2-cytochrome *c* in *Saccharomyces cerevisiae*. *Proc. natn. Acad. Sci. U.S.A.* **77**, 541–545.

MYERS, A. M., CRIVELLONE, M. D., KOERNER, T. J. AND TZAGOLOFF, A. (1987). Characterization of the yeast *HEM2* gene and transcriptional regulation of *COX5* and *COR1* by heme. *J. biol. Chem.* **262**, 16822–16829.

NEBOHACOVA, M., NOVAKOVA, Z., HAVIERNIK, P., BETINA, S. AND KOLAROV, J. (1996). Oxygen- and carbon source-dependent transactivation effect of ABF1 on the expression of the *AAC2* gene encoding mitochondrial ADP/ATP carrier. *Folia microbiol. (Praha)* **41**, 115–116.

OECHSNER, U. AND BANDLOW, W. (1996). Interactions of the yeast centromere and promoter factor, Cpf1p, with the cytochrome c_1 upstream region and functional implications on regulated gene expression. *Nucleic Acids Res.* **24**, 2395–2403.

OECHSNER, U., HERMANN, H., ZOLLNER, A., HAID, A. AND BANDLOW, W. (1992). Expression of yeast cytochrome c_1 is controlled at the transcriptional level by glucose, oxygen and haem. *Molec. gen. Genet.* **232**, 447–459.

OLESEN, J. T. AND GUARENTE, L. (1990). The HAP2 subunit of yeast CCAAT transcriptional activator contains adjacent domains for subunit association and DNA recognition: model for the HAP2/3/4 complex. *Genes Dev.* **4**, 1714–1729.

OLESEN, J., HAHN, S. AND GUARENTE, L. (1987). Yeast HAP2 and HAP3 activators both bind to the *CYC1* upstream activation site, UAS2, in an interdependent manner. *Cell* **51**, 953–961.

OZSARAC, N., BHATTACHARYYA, M., DAWES, I. W. AND CLANCY, M. J. (1995). The *SPR3* gene encodes a sporulation-specific homologue of the yeast CDC3/10/11/12 family of bud neck microfilaments and is regulated by ABFI. *Gene* **164**, 157–162.

PADMANABAN, G., VENKATESWAR, V. AND RANGARAJAN, P. N. (1989). Haem as a multifunctional regulator. *Trends biochem. Sci.* **14**, 492–496.

PATTERSON, T. E. (1990). Characterization of subunit VIII of cytochrome *c* oxidase in *Saccharomyces cerevisiae*. Doctoral dissertation, University of Colorado.

PFEIFER, K., ARCANGIOLI, B. AND GUARENTE, L. (1987*a*). Yeast HAP1 activator competes with the factor RC2 for binding to the upstream activation site UAS1 of the *CYC1* gene. *Cell* **49**, 9–18.

PFEIFER, K., KIM, K. S., KOGAN, S. AND GUARENTE, L. (1989). Functional dissection and sequence of yeast HAP1 activator. *Cell* **56**, 291–301.

PFEIFER, K., PREZANT, T. AND GUARENTE, L. (1987*b*). Yeast HAP1 activator binds to two upstream activation sites of different sequence. *Cell* **49**, 19–27.

PINKHAM, J. L. AND KENG, T. (1994). Heme-mediated gene regulation in *Saccharomyces cerevisiae*. In *Metal Ions in Fungi* (ed. G. Winkelmann and D. R. Winge), pp. 455–501. New York: Marcel Dekker.

PINKHAM, J. L., OLESEN, J. T. AND GUARENTE, L. P. (1987). Sequence and nuclear localization of the *Saccharomyces cerevisiae* HAP2 protein, a transcriptional activator. *Molec. cell. Biol.* **7**, 578–585.

PINKHAM, J. L., WANG, Z. AND ALSINA, J. (1997). Heme regulates *SOD2* transcription by activation and repression in *Saccharomyces cerevisiae*. *Curr. Genet.* **31**, 281–291.

POYTON, R. O. AND BURKE, P. V. (1992). Oxygen regulated transcription of cytochrome *c* and cytochrome *c* oxidase genes in yeast. *Biochim. biophys. Acta* **1101**, 252–256.

POYTON, R. O., TRUEBLOOD, C. E., WRIGHT, R. M. AND FARRELL, L. E. (1988). Expression and function of cytochrome c oxidase subunit

isologues. Modulators of cellular energy production? *Ann. N. Y. Acad. Sci.* **550**, 289–307.

PREZANT, T., PFEIFER, K. AND GUARENTE, L. (1987). Organization of the regulatory region of the yeast *CYC7* gene: multiple factors are involved in regulation. *Molec. cell. Biol.* **7**, 3252–3259.

RATCLIFFE, P., O'ROWKE, J. F., MAXWELL, P. H. AND PUGH, C. W. (1998). Oxygen-sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression. *J. exp. Biol.* **201**, 1153–1162.

ROSENBLUM-VOS, L. S. (1988). Isolation and characterization of mutations which cause overexpression of *CYC7* gene expression in *Saccharomyces cerevisiae*. Doctoral dissertation, State University of New York at Albany.

ROSENBLUM-VOS, L. S., RHODES, L., EVANGELISTA, J. C. C., BOAYKE, K. A. AND ZITOMER, R. S. (1991). The *ROX3* gene encodes an essential nuclear protein involved in *CYC7* gene expression in *Saccharomyces cerevisiae*. *Molec. cell. Biol*. **11**, 5639–5647.

RYTKA, J., BILINSKI, T. AND LABBE-BOIS, R. (1984). Modified uroporphyrinogen decarboxylase activity in a yeast mutant which mimics porphyria cutanea tarda. *Biochem. J.* **218**, 405–413.

SABOVA, L., ZEMAN, I., SUPEK, F. AND KOLAROV, J. (1993). Transcriptional control of *AAC3* gene encoding mitochondrial ADP/ATP translocator in *Saccharomyces cerevisiae* by oxygen, heme and ROX1 factor. *Eur. J. Biochem.* **213**, 547–553.

SALTZGABER-MULLER, J. AND SCHATZ, G. (1978). Heme is necessary for the accumulation and assembly of cytochrome *c* oxidase subunits in *Saccharomyces cerevisiae*. *J. biol. Chem.* **253**, 305–310.

SCHJERLING, P. AND HOLMBERG, S. (1996). Comparative amino acid sequence analysis of the C6 zinc cluster family of transcriptional regulators. *Nucleic Acids Res.* **24**, 4599–4607.

SCHNEIDER, J. C. AND GUARENTE, L. (1991). Regulation of the yeast *CYT1* gene encoding cytochrome c_1 by HAP1 and HAP2/3/4. *Molec. cell. Biol.* **11**, 4934–4942.

SCHNIER, J., SCHWELBERGER, H. G., SMIT-MCBRIDE, Z., KANG, H. A. AND HERSHEY, J. W. (1991). Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **11**, 3105–3114.

SCHULTZ, J. AND CARLSON, M. (1987). Molecular analysis of *SSN6*, a gene functionally related to the SNF1 protein kinase of *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **7**, 3637–3645.

SERTIL, O., COHEN, B. D., DAVIES, K. J. AND LOWRY, C. V. (1997). The *DAN1* gene of *S. cerevisiae* is regulated in parallel with the hypoxic genes, but by a different mechanism. *Gene* **192**, 199–205.

SINCLAIR, P., GIBBS, A. H., SINCLAIR, J. F. AND DE MATTEIS, F. (1979). Formation of cobalt protoporphyrin in the liver of rats. A mechanism for the inhibition of liver haem biosynthesis by inorganic cobalt. *Biochem. J.* **178**, 529–538.

SINCLAIR, P. R., SINCLAIR, J. F., BONKOWSKY, H. L., GIBBS, A. H. AND MATTEIS, F. D. (1982). Formation of cobalt protoporphyrin by chicken hepatocytes in culture: relationship to decrease of 5-aminolaevulinate synthase caused by cobalt. *Biochem. Pharmac.* **31**, 993–999.

SPEVAK, W., HARTIG, A., MEINDL, P. AND RUIS, H. (1986). Heme control region of the catalase T gene of the yeast *Saccharomyces cerevisiae*. *Molec. gen. Genet.* **203**, 73–78.

THEVELEIN, J. M. (1994). Signal transduction in yeast. *Yeast* **10**, 1753–1790.

THORSNESS, M., SCHAFER, W., D'ARI, L. AND RINE, J. (1989). Positive and negative transcriptional control by heme of genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in *Saccharomyces cerevisiae*. *Molec. cell. Biol*. **9**, 5702–5712.

TIMMERMAN, J., VUIDEPOT, A. L., BONTEMS, F., LALLEMAND, J. Y., GERVAIS, M., SHECHTER, E. AND GUIARD, B. (1996). ^{1}H, ^{15}N resonance assignment and three-dimensional structure of CYP1 (HAP1) DNA-binding domain. *J. molec. Biol.* **259**, 792–804.

TRAWICK, J. D., KRAUT, N., SIMON, F. R. AND POYTON, R. O. (1992). Regulation of yeast *COX6* by the general transcription factor ABF1 and separate HAP2- and heme-responsive elements. *Molec. cell. Biol.* **12**, 2302–2314.

TRAWICK, J. D., ROGNESS, C. AND POYTON, R. O. (1989*a*). Identification of an upstream activation sequence and other *cis*-acting elements required for transcription of *COX6* from *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **9**, 5350–5358.

TRAWICK, J. D., WRIGHT, R. M. AND POYTON, R. O. (1989*b*). Transcription of yeast *COX6*, the gene for cytochrome c oxidase subunit VI, is dependent on heme and on the *HAP2* gene. *J. biol. Chem.* **264**, 7005–7008.

TRUEBLOOD, C. E. AND POYTON, R. O. (1988). Identification of *REO1*, a gene involved in negative regulation of *COX5b* and *ANB1* in aerobically grown *Saccharomyces cerevisiae*. *Genetics* **120**, 671–680.

TRUEBLOOD, C. E., WRIGHT, R. M. AND POYTON, R. O. (1988). Differential regulation of the two genes encoding *Saccharomyces cerevisiae* cytochrome *c* oxidase subunit V by heme and the *HAP2* and *REO1* genes. *Molec. cell. Biol*. **8**, 4537–4540.

TRUMBLY, R. J. (1988). Cloning and characterization of the *CYC8* gene mediating glucose repression in yeast. *Gene* **73**, 97–111.

TRUMBLY, R. J. (1992). Glucose repression in the yeast *Saccharomyces cerevisiae*. *Molec. Microbiol*. **6**, 15–21.

TURCOTTE, B. AND GUARENTE, L. (1992). HAP1 positive control mutants specific for one of two binding sites. *Genes Dev.* **6**, 2001–2009.

TURI, T. G. AND LOPER, J. C. (1992). Multiple regulatory elements control expression of the gene encoding the *Saccharomyces cerevisiae* cytochrome P450, lanosterol 14 alpha-demethylase (*ERG11*). *J. biol. Chem.* **267**, 2046–2056.

TZAMARIAS, D. AND STRUHL, K. (1994). Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. *Nature* **369**, 758–761.

TZAMARIAS, D. AND STRUHL, K. (1995). Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. *Genes Dev*. **9**, 821–831.

URBAN-GRIMAL, D. AND LABBE-BOIS, R. (1981). Genetic and biochemical characterization of mutants of *Saccharomyces cerevisiae* blocked in six different steps of heme biosynthesis. *Molec. gen. Genet*. **183**, 85–92.

USHINSKY, S. C. AND KENG, T. (1994). A novel allele of *HAP1* causes uninducible expression of *HEM13* in *Saccharomyces cerevisiae*. *Genetics* **136**, 819–831.

VARANASI, U. S., KLIS, M., MIKESELL, P. B. AND TRUMBLY, R. J. (1996). The Cyc8 (Ssn6)-Tup1 corepressor complex is composed of one Cyc8 and four Tup1 subunits. *Molec. cell. Biol.* **16**, 6707–6714.

VERDIERE, J., CREUSOT, F., GUARENTE, L. AND SLONIMSKI, P. P. (1986). The overproducing CYP1 and the underproducing hap1 mutations are alleles of the same gene which regulates in trans the expression of the structural genes encoding iso-cytochromes *c*. *Curr. Genet*. **10**, 339–342.

VERDIERE, J., CREUSOT, F. AND GUERINEAU, M. (1985). Regulation of

the expression of iso 2-cytochrome *c* gene in *S. cerevisiae*: cloning of the positive regulatory gene *CYP1* and identification of the region of its target sequence on the structural gene *CYP3*. *Molec. gen. Genet.* **199**, 524–533.

VERDIERE, J., GAISNE, M., GUIARD, B., DEFRANOUX, N. AND SLONIMSKI, P. P. (1988). CYP1 (HAP1) regulator of oxygen-dependent gene expression in yeast. II. Missense mutation suggests alternative Zn fingers as discriminating agents of gene control. *J. molec. Biol.* **204**, 277–282.

VERDIERE, J., GAISNE, M. AND LABBE-BOIS, R. (1991). CYP1 (HAP1) is a determinant effector of alternative expression of heme-dependent transcribed genes in yeast (corrected) [published erratum appears in *Molec. gen. Genet.* (1991) **229**, 324]. *Molec. gen. Genet.* **228**, 300–306.

WILLIAMS, F. E. AND TRUMBLY, R. J. (1990). Characterization of TUP1, a mediator of glucose repression in *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **10**, 6500–6511.

WINKLER, H., ADAM, G., MATTES, E., SCHANZ, M., HARTIG, A. AND RUIS, H. (1988). Co-ordinate control of synthesis of mitochondrial and non-mitochondrial hemoproteins: a binding site for the HAP1 (CYP1) protein in the UAS region of the yeast catalase T gene (*CTT1*). *EMBO J.* **7**, 1799–1804.

WRIGHT, C. F. AND ZITOMER, R. S. (1984). A positive regulatory site and a negative regulatory site control the expression of the *Saccharomyces cerevisiae CYC7* gene. *Molec. cell. Biol.* **4**, 2023–2030.

WRIGHT, C. F. AND ZITOMER, R. S. (1985). Point mutations implicate repeated sequences as essential elements of the *CYC7* negative upstream site in *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **5**, 2951–2958.

WRIGHT, R. M., SIMPSON, S. L. AND LANOIL, B. D. (1995*a*). Identification of a low specificity, oxygen, heme and growth phase regulated DNA binding activity in *Saccharomyces cerevisiae*. *Biochem. biophys. Res. Commun.* **216**, 458–466.

WRIGHT, R. M., SIMPSON, S. L. AND LANOIL, B. D. (1995*b*). Oxygen regulation of the cytochrome c oxidase subunit VI gene, *COX6*, in *Saccharomyces cerevisiae*. *Biochem. biophys. Res. Commun.* **216**, 676–685.

XING, Y., FIKES, J. D. AND GUARENTE, L. (1993). Mutations in yeast HAP2/HAP3 define a hybrid CCAAT box binding domain. *EMBO J.* **12**, 4647–4655.

ZAGOREC, M., BUHLER, J. M., TREICH, I., KENG, T., GUARENTE, L. AND LABBE-BOIS, R. (1988). Isolation, sequence and regulation by oxygen of the yeast *HEM13* gene coding for coproporphyrinogen oxidase. *J. biol. Chem.* **263**, 9718–9724.

ZAGOREC, M. AND LABBE-BOIS, R. (1986). Negative control of yeast coproporphyrinogen oxidase synthesis by heme and oxygen. *J. biol. Chem.* **261**, 2506–2509.

ZHANG, L. AND GUARENTE, L. (1994). Evidence that TUP1/SSN6 has a positive effect on the activity of the yeast activator HAP1. *Genetics* **136**, 813–817.

ZHANG, L. AND GUARENTE, L. (1995). Heme binds to a short sequence that serves a regulatory function in diverse proteins. *EMBO J.* **14**, 313–320.

ZHANG, L. AND GUARENTE, L. (1996). The C6 zinc cluster dictates asymmetric binding by HAP1. *EMBO J.* **15**, 4676–4681.

ZHAO, X.-J., RAITT, D., BURKE, P. V., CLEWELL, A. S., KWAST, K. E. AND POYTON, R. O. (1996). Function and expression of flavohemoglobin in *Saccharomyces cerevisiae*. Evidence for a role in the oxidative stress response. *J. biol. Chem.* **271**, 25131–25138.

ZITOMER, R. S., LIMBACH, M. P., RODRIGUEZ-TORRES, A. M., BALASUBRAMANIAN, B., DECKERT, J. AND SNOW, P. M. (1997). Approaches to the study of Rox1 repression of the hypoxic genes in the yeast *Saccharomyces cerevisiae*. *Methods* **11**, 279–288.

ZITOMER, R. S. AND LOWRY, C. V. (1992). Regulation of gene expression by oxygen in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **56**, 1–11.

ZITOMER, R. S., SELLERS, J. W., MCCARTER, D. W., HASTINGS, G. A., WICK, P. AND LOWRY, C. V. (1987). Elements involved in oxygen regulation of the *Saccharomyces cerevisiae CYC7* gene. *Molec. cell. Biol.* **7**, 2212–2220.