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Regulation of Gene Expression by Oxygen in *Saccharomyces cerevisiae*

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INTRODUCTION

Baker's yeast, *Saccharomyces cerevisiae*, is a facultative aerobe. In aerobic growth, energy can be generated through oxidative phosphorylation and molecular oxygen is available as an electron acceptor in a variety of enzymatic reactions. The use of oxygen in these processes generates oxidative radicals, and the cell requires a set of protective enzymes. In anaerobic growth, energy is generated solely through fermentation and at least some of the biosynthetic reactions requiring oxygen cannot be bypassed, resulting in requirements for sterol and fatty acids (1, 2). To efficiently regulate cellular metabolism in these two alternate physiological states, a large number of genes are differentially expressed in response to oxygen. Some of the target genes for regulation by oxygen are obvious; many of the genes encoding respiratory functions and functions involved in controlling oxidative damage are induced in aerobic growth. A recent review by Tzagoloff and Dieckmann (104) catalog more than 200 genes required for respiratory growth. The assembly of the respiratory apparatus is quite complex and requires the coordinate expression of many genes (see references 4, 30, and 31 for reviews). On the other hand, little is known about the functions specifically required for anaerobic growth, although, as will be seen, it is clear that there is a class of genes induced in the absence of oxygen.

In addition to differences in gene expression under the steady-state aerobic and anaerobic growth conditions, cells respond to decreases in oxygen tension. There is a class of genes which encode oxygen-dependent functions, such as alternate cytochrome subunits and oxidases and desaturases in heme, sterol, and fatty acid biosynthesis, that are induced at low oxygen tension. The expression of these hypoxic genes presumably allows the cell to utilize limiting oxygen more efficiently.

In this review, we will present the current knowledge of the classes of genes whose expression is sensitive to oxygen availability and of the regulatory circuitry that senses oxygen levels, transmits the signal to the transcriptional machinery, and perhaps integrates that information with the general physiology of the cell. One cautionary note must be struck at the outset. Oxygen plays a wide-ranging role in cellular metabolism, and genes that utilize common oxygen-dependent elements to regulate their expression are treated as a set here, but may also be regulated by very different signals to other ends. A number of examples of this cross-regulation will be given below. Consequently, it would be misleading to characterize any of the regulatory sets described below as similar to a bacterial operon or a regulon of coordinately expressed genes. The relative levels of expression of genes within the same set may vary widely under different growth conditions even as oxygen tension is held constant.

REGULATION OF GENE EXPRESSION BY HEME

Heme serves as the prosthetic group in the cytochromes and some oxygen-binding proteins such as catalases. Its function is intimately entwined with that of molecular oxygen, and its biosynthesis requires oxygen. Heme plays a regulatory role in many different processes in a wide variety of organisms (for a review, see reference 65), so it is not surprising that heme serves as an intermediate in the signaling mechanism for oxygen levels in yeast cells.

Heme as an Effector Molecule

A recent, comprehensive review by Labbe-Bois and Labbe discussed heme biosynthesis in *S. cerevisiae* (48). Several features of this pathway indicate why heme is an

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TABLE 1. Genes regulated by heme

Gene	Enzyme	Function	References
Induced by heme			
CYC1	Iso-1-cytochrome *c*	Electron transport	33, 40, 64, 66
CYT1	Cytochrome c_1	Electron transport	33
CYB2	Cytochrome b_2 [L$^-$(+)-lactate cytochrome *c* oxireductase]	Electron transport	35, 52
COR1	Subunit I of QH2:cytochrome c oxireductase	Electron transport	63
COR2	Subunit II of QH2:cytochrome *c* oxireductase	Electron transport	22
COX4	Subunit IV of cytochrome *c* oxidase	Electron transport	31, 82
COX5A	Subunit Va of cytochrome *c* oxidase	Electron transport	35, 100
COX6	Subunit VI of cytochrome *c* oxidase	Electron transport	99
CTT1	Catalase T (cytosolic)	Oxidative damage	40
CTA1	Catalase A (peroxisomal)	Oxidative damage	40
SOD2	Manganese superoxide dismutase	Oxidative damage	56
HMG1	3-Hydroxy-3-methylglutaryl CoA reductase[a]	Sterol synthesis	96
TIF51A	eIF5A	Translation initiation	54
ROX1	Heme-dependent repressor		57
Repressed by heme			
COX5b	Subunit Vb of cytochrome *c* oxidase	Electron transport	38, 100
ERG11	Cytochrome P450 (lanosterol 14α-demethylase)	Sterol synthesis	103, 109
CPR1	NADPH-cytochrome P450 reductase	Sterol synthesis	103
HMG2	3-Hydroxy-3-methylglutaryl CoA reductase	Sterol synthesis	96
OLE1	Δ9 fatty acid desaturase	Fatty acid synthesis	58a, 95
HEM13	Coproporphyrinogen III oxidase	Heme synthesis	109, 120, 121
ANB1	eIF5A	Translation initiation	54

[a] CoA, coenzyme A.

ideal effector molecule. First, there is an absolute requirement for oxygen for its biosynthesis (48, 60). Molecular oxygen serves as an electron acceptor in two steps; the enzyme coproporphyrinogen III oxidase, encoded by the *HEM13* gene (29, 62, 105), uses oxygen in the formation of protoporphyrinogen IX (8), and, in the next step, the enzyme protoporphyrinogen IX oxidase uses oxygen in the formation of protoporphyrin (71, 105). Second, all the enzymes of the pathway are present in anaerobically grown cells, so that the induction of heme biosynthesis requires only the addition of oxygen (47). Third, the coproporphyrinogen III oxidase step is the rate-limiting step in heme biosynthesis, suggesting that cellular heme levels would be dependent upon oxygen tension (48, 121). Nonetheless, it should be pointed out that this oxidase has a high affinity for oxygen (8, 48), and its induction at low oxygen concentrations (see below) results in significant heme accumulation even at low oxygen tensions (48).

The regulatory systems described below respond to heme in an oxygen-independent fashion. Although the concentration of free heme available to serve as a regulatory molecule is not easily measured owing to its high, nonspecific affinity for proteins and membranes, the use of heme biosynthetic mutants has clearly demonstrated its role in regulating gene expression (for examples, see references 33, 40, 54, and 82). A reliable paradigm involves growing heme biosynthetic mutants aerobically in the absence of heme or anaerobically in its presence. An oxygen-independent, heme-activated gene would be unexpressed in the first case and expressed in the second, whereas the pattern of expression for an oxygen-independent, heme-repressed gene would be the opposite. The only difficulty with this approach is that anaerobic growth of cells requires supplementation with sterol and fatty acids (1, 2), which inhibits uptake of heme. This difficulty can be circumvented by growing cells anaerobically for a short time (1 to 2 h) and monitoring gene expression by the induction or disappearance of the mRNA.

Nature of Heme-Regulated Genes

The nucleus-encoded genes known to be regulated by heme are listed in Table 1. Most of the heme-activated genes fall into two categories: those encoding respiratory functions such as the cytochrome subunits, and those encoding oxidative damage repair functions such catalase and manganese superoxide dismutase. The expression of a second set of genes, the hypoxic genes, is repressed by heme. For most, their functions involve the utilization of oxygen in electron transport (*COX5B*) or in membrane (*OLE1*, *ERG3*, *ERG11*, *HMG2*) or heme (*HEM13*) biosynthesis. Consequently, their gene products serve no purpose to cells growing in the complete absence of oxygen. Their induction under experimentally anaerobic conditions probably reflects induction at low oxygen tensions, at which increased enzyme levels would compensate for the limitation in the substrate.

An inspection of the list in Table 1 points out an interesting phenomenon. A number of functions are encoded in gene pairs, i.e., unlinked genes encoding identical functions, one of which is heme activated and the other is heme repressed. These include subunit V of cytochrome *c* oxidase (*COX5A* and *COX5B* [17, 18]), 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMG1* and *HMG2*), and the translational initiation factor eIF5A (*TIF51A* and *ANB1*). The gene pair encoding the cytochrome *c* protein contains the heme-activated *CYC1* gene and the heme-activated and repressed *CYC7* gene (57, 68, 123). Also, the gene pair for catalase consists of two heme-activated genes. One encodes the peroxisomal form of catalase, which is glucose repressed and fatty acid induced (*CTA1* [40]), and the other encodes the cytosolic form, which is repressed by cyclic AMP and the heat shock-induced (*CTT1*) gene (5, 6, 113). Finally, although not included in Table 1 because the role of heme in their regulation has not been determined, there are three genes that encode the ADP/ATP translocator protein: one is induced in aerobic cells, a second is induced in anaerobic

cells, and a third is expressed constitutively (46). In some cases, reasonable inferences can be made concerning the purpose for these differentially regulated gene pairs. The cytochrome *c* oxidase containing the hypoxic Vb subunit has been demonstrated to have a higher enzymatic turnover rate and a higher rate of heme *a* oxidation than its aerobic homolog (110). The *CYC7* gene, whose product has been shown to be induced late in exponential growth when oxygen becomes limiting (51), may also function more efficiently at low oxygen tension. Similarly, although high expression of any of the three ADP/ATP translocator genes can support growth under all conditions, one might speculate that the oxygen-repressed ADP/ATP translocator is more efficient at pumping ATP into anaerobic mitochondria.

For the gene pair encoding the translational initiation factor eIF5A, the reason for heme regulation is not clear; either gene can support both respiratory or anaerobic growth (72b, 84). One interesting feature of this gene pair is that they are part of the large duplication that gave rise to the *CYC1* and *CYC7* genes (88). *ANB1* lies upstream of *CYC1*, and the two genes are transcribed divergently (55). *CYC7* and *TIF51A* have the same relationship (9a). Is heme regulation of these duplicated translation factor genes an accident of evolution based upon a duplication of regulatory sites? Although not very pleasing, this possibility has not been ruled out.

Activation of Gene Expression by Heme

Table 2 lists the heme-dependent transcriptional activators for the heme-activated genes. As can be seen, two distinct heme activation protein (HAP) complexes have been identified: HAP1 and HAP2/3/4. At least four genes, *CYC1*, *COR2*, *CYT1*, and *CYB2*, are activated by both. The properties of these complexes have been reviewed by Guarente and Fosburg (31), and only the activities directly relevant to this review will be discussed. No other mechanisms for heme activation of transcription have been identified.

Transcriptional activation by HAP1. The *CYP1* gene was first identified in selections for increased expression of the iso-2-cytochrome *c* gene (13) and later as *HAP1* in a screen for decreased expression of the iso-1-cytochrome *c* gene (32). The two genes were found to be identical (106), and the *HAP1* designation has become more common. The gene has been cloned and sequenced (16, 67, 107, 108). The HAP1 protein contains 1,483 residues, with a number of interesting motifs (16, 108). A zinc finger motif is contained near the amino terminus within a domain (residues 1 to 148) which is responsible for sequence-specific DNA binding (67), and an acidic cluster characteristic of transcriptional activation domains is located at the carboxyl terminus (residues 1308 to 1483). Deletion of the large internal region converted the protein into a heme-independent, constitutive activator, leading to the conclusion that this region masks the DNA-binding domain in the absence of heme (31, 67). In support of this hypothesis, there is a repeat motif (residues 280 to 438) that resembles a metal- or heme-binding site (16), and heme stimulates DNA complex formation in vitro (66–68). However, the heme molecule is notorious for its nonspecific effects, appearing to act like a mild detergent in many cases, altering protein activity nonspecifically.

HAP1 binds to the upstream activation sites (UASs) of a number of genes to activate transcription. These sites have been extensively characterized by mutational and footprinting analyses in the *CYC1* (two sites [49, 66, 90]) and *CYC7* (11, 68, 123) genes and by footprinting analysis in the *CTT1* (116) and *CYB2* (52) genes. A summary of the data is shown in Fig. 1. A consensus sequence can be derived from these data, although an important reservation remains. All the footprinting analyses were carried out with crude extracts, so although HAP1 binds to all these sequences, there is the possibility that other proteins differentially bind to these sites. In fact, a second protein, RC2, is known to bind to UAS1B of *CYC1* but not to UAS1A (3, 66, 90), and another protein binds to the *CTT1* site (116). Therefore, in trying to derive a consensus sequence, residues that were protected in one sequence but not the others were not considered significant. The major and minor groove contacts within the consensus sequence are based on methylation protection data. The redundancy in the A+T-rich central region recognized in the minor groove reflects the proposed inability of proteins to distinguish between A-T and T-A residues in this groove (86). Thus, on the simplest level, genes containing a UAS recognized by HAP1 are transcriptionally activated in the presence of heme.

However, this view is too simplistic. The affinity of HAP1 for DNA is not necessarily a measure of its transcriptional activation activity. HAP1 binds to the *CYC1* and *CYC7* UASs with similar affinity but activates transcription from the *CYC1* UAS much more strongly than from the *CYC7* UAS (52, 68, 123). Two types of experiments indicate that the two different UASs bind HAP1 equally: (i) in vitro competition studies between the *CYC1* and *CYC7* UASs

TABLE 2. Heme regulatory factors and target genes

Target gene	Regulatory factor		References
	Activator	Repressor	
Cytochrome subunits			
CYC1	HAP1, HAP2/3/4		26, 32, 36, 90
CYC7[a]	HAP1	ROX1	57, 68, 107, 123
CYT1	HAP1		31
CYB2	HAP1, HAP2/3/4		52
COR2	HAP1, HAP2/3/4		22
COX4	HAP2/3/4		31, 82
COX5A	HAP2/3/4		38, 100
COX5B		ROX1, REO1	38, 100
COX6	HAP2/3/4		99
Tricarboxylic acid cycle			
KGD1[b]	HAP2/3/4		73
Oxidative stress			
CTT1	HAP1		116
SOD2	HAP1		Unpublished
Heme, sterol, fatty acid biosyntheses			
HMG1	HAP1		96
ERG11	HAP1	ROX1	103, 109
OLE1		ROX1	58a
HEM1[a]	HAP2/3/4		43
HEM13	HAP1 (−heme)	ROX1	109
Translation factors			
TIF51A	HAP1		Unpublished
ANB1		ROX1	56, 57, 61
Regulatory factor			
ROX1	HAP1		Unpublished

[a] *CYC7*, encoding iso-2-cytochrome *c*, and *HEM1*, encoding Δ-aminolevulic acid synthetase, are not listed in Table 1 because the combination of regulatory elements makes their expression constitutive.

[b] Not demonstrated to be regulated by heme, but regulated by HAP2/3/4 complex. Encodes an α-ketoglutarate dehydrogenase subunit.

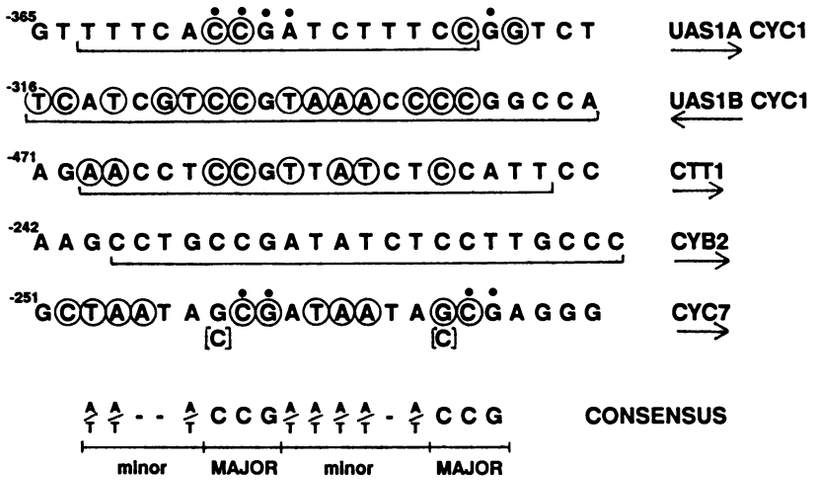

FIG. 1. Consensus sequence for HAP1-binding sites. The sequences are aligned to maximize homology. The underlined region represents the sequences protected against DNase digestion for the strand shown. The circled bases represent either residues protected against dimethyl sulfate attack when protein is bound or residues which, when methylated, prevent HAP1 binding. Dots over bases represent those in which mutations that cause decreased gene expression have been isolated. The bases in brackets represent changes in the *CYC7* sequence that cause HAP1-dependent increased gene expression. The arrow beneath the name of the site indicates the orientation of the site; all the sequences with the exception of UAS1B of *CYC1* represent the coding strand. The numbers at the left indicate the first base, with the A in the ATG translational initiation codon as number 1, and the upstream region numbered consecutively in negative integers.

indicate equivalent affinities (52, 68), and (ii) a fusion of the HAP1 DNA-binding domain to the GAL4 transcriptional activation domain activated transcription from the *CYC1* and *CYC7* UASs equally well in vivo (31). Therefore, a model has been proposed in which allosteric interactions between the DNA-binding site and the activation domain are sensitive to the type of UAS (31). The determinant residues of the *CYC7* UAS may well be the two Gs (immediately above the two Cs indicated in brackets in Fig. 1). These vary from the consensus sequence, and changing these residues to Cs caused a dramatic increase in expression of the *CYC7* gene (11). Interestingly, reciprocal changes can be made in the protein. The initial identification of the gene was through mutations, *CYP1-16* and *CYP1-18*, that caused increased expression of the *CYC7* gene (13). These initial mutations were in the zinc finger region, changing serine 63 to arginine (108). This change caused a loss of expression of other HAP1-activated genes, including the *CYC7* mutants with the altered UASs described above (11, 13, 123). These data clearly show a difference in the way in which the protein binds to the wild-type *CYC7* and *CYC1* UASs which is not expressed in binding affinity.

A further layer of complexity in the function of the HAP1 protein has been added by Verdiere et al. (109). These workers found that a functional HAP1 is required for the transcription of the *HEM13* gene in the absence of heme. HAP1 could be acting indirectly, perhaps by repressing the expression of an activator, but if it acts directly, some very intriguing questions are raised. What is the nature of the UAS, and what makes it specific for heme-deficient activation? Also, if the absence of heme masks the DNA-binding site, how does the HAP1 protein bind in anaerobic cells? HAP1 is an intriguing protein; structure-function studies should provide exciting new insights into the relationship between DNA binding and transcriptional activation.

Transcriptional activation by the HAP2/3/4 complex. As can be seen in Table 2, a number of genes are transcriptionally activated by the HAP2/3/4 complex. This complex responds to two stimuli: heme and nonfermentable energy sources. *S. cerevisiae* preferentially ferments glucose even in the presence of oxygen, and many of the respiratory genes, including a number of those listed in Table 1, are repressed 10- to 20-fold when grown on glucose-containing media (see references 30 and 31 for reviews). The response of HAP2/3/4 to these two stimuli has been most extensively studied in the *CYC1* gene (32). In constructs lacking the HAP1-dependent UAS1, transcription was entirely dependent on HAP2/3/4. Expression was very low in the presence of glucose without heme, stimulated about an order of magnitude in glucose plus heme, and increased an additional 10-fold in heme plus lactate. The effect of glucose on activation by HAP2/3/4 may explain why the transcription of several genes listed in Table 2 are activated by both HAP1 and this complex. The dual activation would increase the level of gene expression in the presence of glucose plus heme, while still allowing substantial activation in the absence of glucose.

This complex is encoded by three distinct genes (27, 36, 37, 69, 70) and binds to a consensus sequence, TNATTGGT (26). This site contains the CCAAT motif common to many mammalian genes, and the homologies between HAP2 and HAP3 and their mammalian CCAAT-binding counterparts are sufficiently extensive to allow assembly of functional hybrids (12). All three of the yeast subunits are required for transcriptional activation; mutations in any one of the genes encoding these subunits cause a loss of function (27, 64). HAP4 appears to contain the transcriptional activation domain, whereas HAP2 probably contains the DNA-binding region (31). Although the nature of the heme effect is not known, the lactate induction appears to be the result of a lactate-dependent activation of *HAP4* transcription (27).

It has been suggested that the HAP2/3 heterodimer represents a general transcription factor involved in the regulation of a variety of cellular functions (31). For example, *hap2* or *hap3* mutants grow poorly on ammonia as a nitrogen source, whereas *hap4* mutants are not impaired. The implication is that the HAP2/3 complex can associate with different genes, perhaps by using alternate HAP4-like subunits as the regu-

latory circuitry requires. Also, although the DNA-binding site is within the HAP2/3 subunits, the different HAP4-like subunits must carry the specificity for the different UASs either by directly altering the HAP2/3 binding specificity or by interacting with other elements bound to the different UASs. As is the case with HAP1, dissection of this complex will prove extremely interesting.

Repression of Gene Expression by Heme

The set of genes known to be repressed by heme is listed in Table 1, and most of these are regulated through the action of the ROX1 repressor (Table 2). The *ROX1* gene was originally identified by mutations that caused constitutive expression of the heme-repressed *ANB1* gene (56). The site of action of the ROX1 protein in the *ANB1* gene has been mapped to two operator regions, each of which contains two copies of the operator consensus sequence YYYATTGT TCT (53). The operators lie between the TATA box and the UAS region which in this gene is diffuse, consisting in part of a number of T-rich (in the coding strand) stretches and a binding site for the ABF1 protein (23). The T-rich region is similar to the constitutive activation regions of a number of genes, including some encoding ribosomal proteins (50, 58, 78, 80, 93, 94). Similarly, the ABF1 protein is involved in the transcriptional activation of many genes, including, again, ribosomal protein genes (7, 21, 87). Since *ANB1* encodes a translation initiation factor, it is not surprising to observe this parallel between its activation and that of genes coding for other translational components. In a parallel study, deletion of a region containing an operator consensus sequence upstream from *COX5B* led to constitutive expression of this gene (39). This sequence is also present in the other genes regulated by ROX1 (53, 58a). Finally, when one of the *ANB1* operator regions was placed between the *GAL1* UAS and its TATA box, the galactose-induced expression of *GAL1* was repressed by ROX1 (53). Thus, the evidence strongly implicates this operator sequence as the ROX1 site of action and suggests that ROX1 is a repressor.

The expression of the *ROX1* gene is transcriptionally activated by heme (57). This activation is partially dependent on HAP1 (119a), and the regulatory region also has a HAP2/3/4 consensus sequence which may account for the remainder of the heme-activation (122a), although *ROX1* expression is not catabolite repressed like that of other genes regulated by this complex. However, although the expression of the *ROX1* gene is heme dependent, the function of the ROX1 protein is not. The *ROX1* coding sequence was fused to the *GAL1* promoter, rendering the synthesis of the ROX1 protein galactose inducible and heme independent. In heme biosynthetic mutants carrying such a fusion, *ANB1* expression was repressed when galactose was added to the media even in the absence of heme (57a). Thus, heme repression of ROX1-regulated genes results from the heme-dependent transcriptional activation of the repressor gene.

The kinetics of *ANB1* mRNA induction at the onset of anaerobiosis is quite rapid; fully induced levels are reached within 90 min, less than one generation (57a). As indicated above, this induction is not due to the loss of a heme-dependent corepressor but, rather, must be due to the loss of *ROX1* gene expression and an accompanying loss of ROX1 protein. Therefore it appears that ROX1 is unstable. The system is probably geared to respond to rapid changes in oxygen tensions; the response to hypoxia might be considered a stress response.

The *ROX1* gene has been sequenced, and the protein sequence has been deduced (122a). It is 368 amino acids in length, and the amino-terminal one-third contains a DNA-binding motif termed the HMG box which is found in the HMG class of nonhistone chromatin proteins (45, 111), the human nucleolar transcription factor hUBF (41), mating-type determining proteins Mc from fission yeast (42) and a1 from *Neurospora crassa* (91), the LEF-1 lymphocyte regulatory factor (98), and the putative male-determining protein in mammals (34, 89). ROX1 has been expressed in *Escherichia coli* cells and purified, and in this pure form it bound specifically to the *ANB1* operator DNA in a gel retardation assay. Extracts prepared from wild-type cells did not form a complex on the operator site of *ANB1*, but extracts prepared from cells in which the *ROX1* gene was overexpressed did so. This complex was the same size as that observed with purified ROX1. Thus, ROX1 repression is mediated through its binding to the *ANB1* operator site. The mechanism of repression is still a mystery. ROX1 is capable of repression of a variety of different transcriptional activators as demonstrated already for the *ANB1* activator (57), HAP1 (as in *CYC* [57]), and GAL4 (from the artificial construct described above [53]). Therefore, we believe that the mechanism will provide some insight into general methods of repression of yeast transcription.

There are at least three genes that are listed in Table 2 as regulated both by HAP1 and ROX1. In *CYC7* these elements function antagonistically, resulting in low levels of aerobic expression (51, 57, 118, 123), and this may well be the case for *ERG11*. Also, as discussed above, *HEM13* represents an unusual case, in which the HAP1 regulation serves to activate transcription in the absence of heme. Although the pattern of expression for each of these genes is different, the regulation by both factors in three cases raises the possibility that these elements interact in a way which is as yet unclear and that more genes will fall into this class.

In selection for increased expression of the *COX5B* gene in aerobically grown cells, Trueblood et al. (100) isolated mutations in a gene designated *REO1*. These mutations also caused increased aerobic expression of *ANB1*. Although it was originally reported that *REO1* and *ROX1* were different genes (100), Cumsky (16a) recently found that a *ROX1*-containing plasmid can complement the *reo1* mutation, suggesting that *REO1* and *ROX1* are the same. Cumsky has identified a gene, *ORD1*, which does appear to be specific for the aerobic repression of *COX5b*; *ord1* mutations cause overexpression of *COX5b* but not of *ANB1* (16a). The expression of at least one heme-repressed gene, *HMG2*, is not affected in *rox1* mutants, indicating that there is at least one alternate heme repression system.

Other Factors Affecting Heme Regulation

In addition to the DNA-binding proteins which serve as activators or repressors in heme regulation, there are other factors which appear to modulate activity. In some cases, these factors do not appear to be specific to heme regulation, but affect a number of apparently unrelated cell processes.

Elements defined genetically. A number of these factors were initially identified in studies of the expression of the *CYC7* gene. As indicated above, this gene is regulated both positively, but weakly, by HAP1 and negatively by ROX1, resulting in low steady-state levels of expression. In the absence of a functional *CYC1* gene, the level of *CYC7* expression is insufficient to support cell growth on lactate, which provides a strong selection for increased *CYC7* expression (13). This selection initially identified the *HAP1*

(*CYP1*) gene through the allele that causes increased *CYC7* expression (13), and later selections identified the *CYC8* and *CYC9* genes (79), now referred to as *SSN6* and *TUP1*, respectively. More recent selections led to the reisolation of *rox1* and *tup1* mutants, as well as the identification of three new genes, *ROX3*, *ROX5*, and *ROX6* (76, 77).

Mutations in the *TUP1* and *SSN6* genes are pleiotropic, causing increased *CYC7* expression, release from catabolite repression, flocculence, α-sterility and poor sporulation of homozygous recessives, and partial constitutive expression of the heme-repressed *ANB1* gene (9, 28, 79, 81, 83, 85, 101, 102, 114, 122). In addition, *tup1* mutants show increased plasmid stability (97) and can take up TMP from the medium (112). The similarity between the *ssn6* and *tup1* phenotypes suggests that the wild-type gene products function in a similar pathway or complex, and recently Williams et al. (115) have demonstrated that these proteins can be coprecipitated by antisera directed against one or the other. On the other hand, the *tup1 ssn6* double mutant accumulated twice as much invertase under repressing conditions as did either single mutant, indicating that each protein can function to some extent in the absence of the other (114). The role of the TUP1 and SSN6 proteins in catabolite repression is best characterized. It is part of a pathway involving the SNF1 protein kinase; *ssn6* and *tup1* mutations suppress the inability of *snf1* mutations to induce invertase in the absence of glucose (9, 10, 85, 114). This pathway also affects *CYC1* and *COX6* expression, which is decreased in *snf1* mutants and increased in *ssn6* mutants (119). These effects may well be mediated through the HAP2/3/4 complex, which, as mentioned above, is responsible for lactate induction of *CYC1* and *COX6*. There is as yet no demonstrated or obvious link between this catabolite repression pathway and the other phenotypes associated with *ssn6* and *tup1* mutations.

The effect of TUP1 on heme-regulated genes has also been studied (122). In *tup1* null mutants, the expression of heme-activated genes is increased in both the presence and absence of heme. As a member of this class, *ROX1* expression is also increased in both the presence and absence of heme, but the protein is not completely functional: *ANB1* is expressed constitutively. Thus, TUP1 function is required for the activation of the ROX1 repressor. This requirement is not for DNA binding. The *ROX1* gene is overexpressed in a *tup1* null mutant, and the ROX1-*ANB1* operator complex was observed in extracts prepared from this mutant (57a). These findings indicate that repression is not simply a function of DNA binding. TUP1 activation of ROX1 does not require heme, since ROX1 can function in heme-deficient cells. SSN6 was also found to be required for ROX1 repression, but not for DNA binding. These findings suggest two alternative functions for the TUP1-SSN6 complex (122). For the first, these proteins would serve as a general cofactor for repression and for keeping uninduced genes silent, perhaps by mediating the interactions of specific DNA-binding proteins with the transcriptional apparatus. In this model, the α-sterility of *tup1* and *ssn6* mutants might result from a requirement for the complex in α2 repression, and catabolite depression of HAP2/3/4-activated genes might result from a failure to keep the *HAP4* gene repressed or uninduced. The attractive aspect of this model is that the various cellular processes disrupted in *tup1* and *ssn6* mutants need not be functionally linked; they simply share a common transcription factor. For the second possibility, TUP1 and SSN6 would be members of a signal transduction pathway which coordinates a number of aspects of cellular metabolism. Further studies of both SSN6 and TUP1 are being conducted by a number of groups, and their functions should be elucidated soon.

The *ROX3* gene product also appears to be a general factor that mediates the response of heme-repressed genes. The original *rox3* mutants were isolated as causing increased aerobic *CYC7* expression and also caused dramatically increased anaerobic expression (76, 77). However, these mutations also caused decreased anaerobic expression of *ANB1*. These mutant phenotypes arose from ROX3 protein with either partial or modified function; the null allele is lethal. The *ROX3* gene is itself expressed at higher levels under anaerobic conditions, but its expression is heme independent. The gene product is localized in the nucleus. All these results suggest that *ROX3* plays some general role in transcription, but this role is as yet undefined.

The *ROX5* and *ROX6* genes have not been cloned, and little is known of the functions of their gene products. This catalog of genes modulating the expression of heme-regulated genes is probably incomplete. All the factors listed in this section, as well as the *HAP* and *ROX1* genes, were identified in mutant hunts by using only three target genes, *CYC1*, *CYC7*, and *ANB1*. Further analysis of other genes may well reveal other factors.

Elements defined biochemically. A discussion of modulating factors would not be complete without addressing the question of proteins which bind to the upstream region of some of these genes but whose role in gene expression has not yet been determined. For example, extensive analyses of the 5′ region of the *CYC1* gene revealed a number of complexes, as indicated in Fig. 2. Only the HAP1 and HAP2/3/4 complexes are described in detail above. The RC2 factor binds to UAS1B but not to UAS1A (3, 66, 90). HAP1 and RC2 binding is mutually exclusive, suggesting that RC2 serves as a repressor of HAP1 function. Complex A binds to the region immediately downstream of the HAP2/3/4 sites (64). The nature and function of this complex are not well defined.

Dorsman and coworkers discovered that the regulatory regions of a number of heme-activated genes encoding mitochondrial functions contained binding sites for multifunctional factors (23). GFI, identical to the ARS-binding factor ABF1 (also OBF1, SBF-B, TAF, and SUF), bound upstream of a number of genes, whereas GFII, identical to the centromere-binding factor CP1 (CBP-I), bound to a second, overlapping set. Intriguingly, the GFI-binding sites in the *COX6* and *COR2* genes are 1 bp from the HAP2/3/4 consensus sequence. Sites for these factors within regulatory regions are by no means unique to mitochondrial genes; these workers subsequently compiled an impressive list of sites within the upstream regions of genes encoding a wide range of functions (24). In some cases, these factors have been demonstrated to be important to transcriptional activation (7, 21, 87). However, there is no evidence for a specific role in the regulation of mitochondrial functions.

It is difficult to assign specific functions to these complexes simply on the basis of their binding to the upstream region. A genetic dissection of both the binding site and the genes that encode the particular proteins is required.

Summary of Heme Regulatory Circuitry

A model for the HAP-dependent heme activation and ROX1-dependent heme repression is presented in Fig. 2 in a representation of the control region for the interval between the two divergently transcribed and oppositely regulated

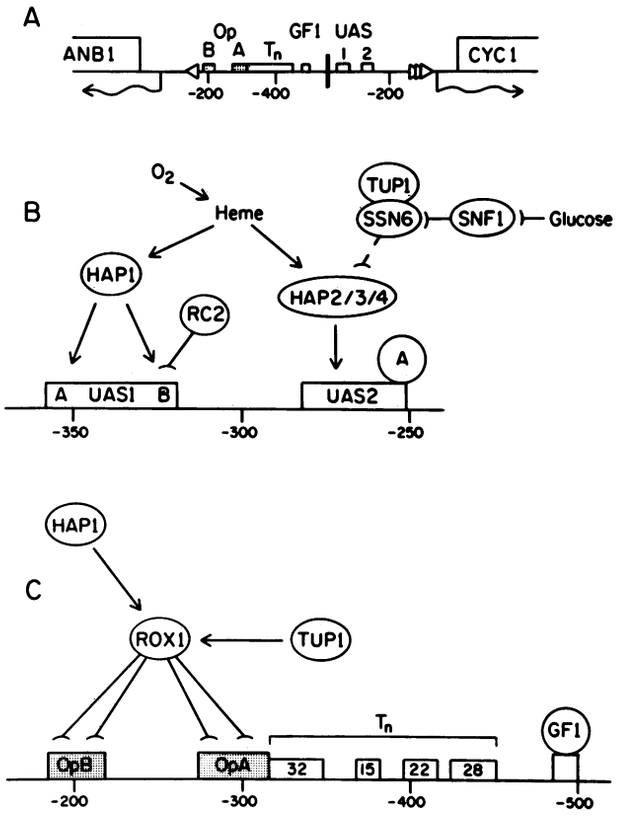

FIG. 2. Model for the expression of the *CYC1* and *ANB1* genes. (A) Intervening region between *CYC1* and *ANB1*. The large open boxes represent the coding sequences, the small open boxes represent activation sites, and the shaded boxes represent repression sites. Triangles represent the TATA boxes, and the wavy arrows represent the mRNA and indicate the direction of synthesis. The vertical bar represents the *Sma*I site which can be used to functionally separate the two regulatory regions. The bases are numbered in negative integers from the two coding sequences, converging at the *Sma*I site. (B) Regulatory region of *CYC1*, with the coding region to the right. The symbols are as in panel A. Straight arrows represent activation, and lines with concave bars represent repression. Dashed lines indicate more speculative interactions. These lines do not imply levels of interactions, i.e., transcription, protein activation, etc. (C) Regulatory region of *ANB1*, with the coding sequence to the left. The symbols are described above. The T_n region contains a series of runs of T in the coding strand. The numbers in the boxes represent the length of the runs from left to right as follows: 32 represents 24 T residues in 32 bases; 15 represents 15 of 15; 22 represents 21 of 22; and 28 represents 21 of 28. OpA and OpB represent the two operator sites, each containing two consensus sequences. The GF1 site was originally mapped to the *CYC1* upstream region (23).

CYC1 and *ANB1* genes (55). These two genes represent the best characterized of the heme-regulated genes, and nearly all the factors discussed above act within this region on either one or the other. As far as can be ascertained under laboratory conditions, although these genes are adjacent, their regulation is completely independent; separation of the two genes at the *Sma*I site designated by the vertical bar in Fig. 2 does not alter the expression of either (55). In this scheme, interactions for which there is direct evidence are represented by solid lines, while those which are more speculative are represented by dashed lines.

Heme levels reflect the availability of oxygen to the cell, manifest through the function of the *HEM13*-encoded oxidase and, possibly, the protoporphyrinogen IX oxidase. When free heme concentrations are high, HAP1 and HAP2/3/4 activate the transcription of the heme-activated genes as shown for *CYC1* in Fig. 2B. In addition, HAP2/3/4 function is probably modulated by the SNF1-SSN6/TUP1 pathway in response to the presence of glucose. The RC2 protein competes with HAP1 for binding to UAS1B, thereby preventing the proposed synergistic interactions between the two HAP1 complexes (90). The nature and function of complex A, which binds to UAS2 along with HAP2/3/4, have yet to be determined.

ANB1 expression, diagrammed in Fig. 2C, is activated through a regulatory region which contains both the T-rich region in the coding strand and an ABF1 (GF1)-binding site, both of which have been mapped to the regulatory regions of ribosomal protein genes which are functionally related to this translational initiation factor. This region activates transcription constitutively, but is antagonized by the ROX1 repressor. The *ROX1* gene is among the set activated by heme; therefore, the repressor is present in the cell only under limiting oxygen. TUP1 is required for activation of ROX1.

The effects of SSN6 and ROX3 on *ANB1* expression are not presented here because too little information is available to speculate on a site of action.

It should be stressed that this scheme cannot be directly generalized to all heme-regulated genes. As indicated in a previous section, heme regulation is not the sole regulatory input for many of the genes listed in Table 1. The catalase genes respond to a variety of inputs; *CYC7* expression is induced by heat shock (68a); and *HEM1* transcription is activated by HAP2/3/4, but is also regulated by other factors, resulting in constitutive expression (43). In addition, *HEM13* appears to be activated by HAP1 anaerobically, in the opposite fashion from the other HAP1-activated genes. Also, the ROX1-repressed genes do not all respond to the same activators. Thus, this scheme serves to illustrate how heme, and thereby oxygen, affect gene expression, but genes sharing common heme-dependent activators or repressors cannot be characterized as coordinately regulated. Rather, these genes belong to a common regulatory set, but in many cases they are also members of other sets.

HEME-INDEPENDENT OXYGEN REGULATION

There are many genes and enzyme activities which have been demonstrated to be oxygen regulated but for which the effect of heme has not been determined. Obviously these cannot be discussed here. There are, however, a number of oxygen-regulated genes whose expression has been demonstrated to be heme independent. Effector molecules and, in most cases, regulatory elements have not been identified for these genes, and, as a consequence, it is not known how many different regulatory sets they represent. Below, we describe two systems for which heme independence has clearly been demonstrated: the mitochondrial translation factor PET494 and the anaerobic *ANB2* to *ANB15* genes.

Mitochondrial Gene Regulation

The mitochondrial DNA encodes both functions for its own maintenance, such as those involved in protein synthesis and RNA processing, and subunits of the oxidative phosphorylation apparatus including three subunits of cytochrome *c* oxidase, one of the cytochrome bc_1 complex,

and three of the ATP synthase (30). Efficiency dictates that these genes, like their nuclear counterparts, be regulated by oxygen, and such regulation has been demonstrated for the *coxI*, *coxII*, and *coxIII* genes encoding the cytochrome *c* oxidase subunits, although the kinetics of disappearance of these proteins from the cell upon anaerobiosis is much slower than that for the nucleus-encoded subunits (117).

One well-studied example is the regulation of *coxIII* expression, encoding subunit III. This gene is transcribed and the mRNA is processed at the same rates aerobically and anaerobically, but it requires at least three specific factors for its translation (14). These factors are encoded by the nuclear genes *PET494*, *PET122*, and *PET54* and exert their effect through recognition of the 5′ leader of the *coxIII* mRNA (15). Interestingly, the PET494 protein shares weak homology with the translational initiation factor eIF2α (30). The oxygen induction of *coxII* mRNA translation correlates with the induction of PET494 protein accumulation in aerobic cells (59). However, unlike the other nuclear genes encoding mitochondrion-related functions discussed above, *PET494* expression is regulated at the level of translation in an oxygen-dependent, heme-independent fashion. It is not clear whether the levels of the other two *coxIII*-specific translation factors are also regulated. Translation of the *coxI* and *coxII* mRNAs is also regulated by oxygen (72a), and each requires its own set of nucleus-encoded factors (19, 44, 72, 92). The PET111 protein required for *coxII* mRNA translation is itself translated from an mRNA with an unusually long 5′ leader, raising the possibility that, like the PET494 protein, it is under translation control (92).

This theme is repeated for the translation of the mitochondrial *cob* gene, encoding a subunit of the cytochrome bc_1 complex. Its translation also requires specific translation factors, the products of three nuclear genes, *CBS1*, *CBS2*, and *CBP6* (20, 74, 75). RNA blots demonstrated that the *CBS1* mRNA levels are regulated by oxygen (25), but whether this regulation is by heme and/or at the transcriptional level has not been determined. Furthermore, the data and the unusual length of the 5′ untranslated leader of the mRNA hinted at the possibility of a translational control component to *CBS1* regulation (25).

The pattern of regulation of the mitochondrial cytochrome subunits that is emerging suggests that the translation of each gene is independently regulated. Perhaps, as this story continues to develop, some insight will be gained as to why independent regulation evolved. Also, it should be noted that the initial transcripts of many of the mitochondrial genes require extensive processing which is mediated by intron-specific mitochondrial and nucleus-encoded genes (30). Although there is no solid evidence that processing is regulated by oxygen, the possibility cannot be formally eliminated.

Anaerobic Genes

In an attempt to better understand anaerobic metabolism, we cloned anaerobically induced genes by differential hybridization, using RNA prepared from aerobically grown cells and cells grown for 1.5 or 6 h after the onset of anaerobiosis (by perfusing cultures with nitrogen). Four "early" and 11 "late" genes were identified and confirmed as expressed exclusively in the absence of oxygen. Each gene was isolated only once, suggesting that the number of anaerobically expressed genes is much larger.

Although functional studies have not been carried out, preliminary characterization of the regulation of mRNA accumulation from several of these genes reveals some interesting features. First, despite their fast, *ANB1*-like induction, the expression of the three early genes tested, *ANB13*, *ANB14*, and *ANB15*, was heme and *ROX1* independent. None of these genes was expressed aerobically in heme-deficient cells or *rox1* mutants. Second, the induction of four of the late genes, *ANB2* through *ANB5*, required at least 3 h of anaerobiosis. The expression of the *ANB2* gene was tested and found to be heme and *rox1* independent. One interpretation of the long lag before the expression of these late genes is that they are sensitive to much lower oxygen concentrations than are the heme-repressed genes like *ANB1* or the early genes and may represent the best candidates for genes encoding true anaerobic functions. In any event, these genes appear to represent at least two distinct classes of oxygen-repressed, heme-independent genes.

CONCLUSIONS

As indicated in this review, there are a large number of genes whose expression is regulated to some extent by oxygen, and there are a number of different regulatory mechanisms. This is not surprising given the central and varied role of oxygen in cellular metabolism. There are a large number of groups studying various aspects of oxygen regulation, and the powerful tools of yeast genetics and molecular biology should allow rapid progress. Not only should important aspects of the regulation of cellular metabolism emerge, but also insights into the general mechanisms of gene regulation should be gained.

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