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Molecular basis of nutrient-controlled gene expression in *Saccharomyces cerevisiae*

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Abstract. The ability of a unicellular organism to alter patterns of gene expression in response to nutrient availability is essential to its survival in a changing environment. How is the cell able to identify individual metabolites amongst a myriad of other similar molecules, and convert the information of its presence into a concerted change in the transcription of the genes required for the response to that metabolite? There is increasing evidence that the activity of transcription factors can be influenced directly by interaction with metabolites. A variety of mechanisms have been identified by which this type of gene regulation by small molecules can occur.

Key words. Genetic switches; transcriptional control; metabolic regulation; Leu3p; Gal4p; Ppr1p.

Transcriptional control by metabolites

Transcription is a complex process. In eukaryotes, RNA polymerase II is composed of 12 different subunits, but these alone are not sufficient to direct accurate transcriptional initiation and efficient elongation [1]. A large number of auxiliary proteins, such as the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF and so on, and the Srb, Med and other proteins, are also required [2–5]. Many of these auxiliary proteins are associated with RNA polymerase II to form the polymerase holoenzyme. The process of transcription begins when an activator binds to specific sites, often present in multiple copies, upstream of the TATA box of a gene. The function of the activator has been the focus of much speculation. Initiation of transcription involves the isomerisation of the polymerase-DNA complex from a 'closed' to an 'open' form in which the DNA strands are locally opened to allow access to the polymerase [6]. Activators do not appear to function as mediators of this isomerisation event, but act rather more simply via a mechanism in which the polymerase and/or chromatin modification and remodelling complexes are recruited to a particular gene [7]. For example, the activation domain of Gcn4p, a yeast protein involved in the transcriptional response to amino acid starvation, has been shown to interact with components of the SAGA chromatin-modifying complex [8], the SWI/SNF chromatin-remodelling complex [9, 10], and various proteins of the RNA polymerase holoenzyme and TFIID complexes [11]. Thus, activation appears to be a combination of promoter clearance and polymerase recruitment, all of which are mediated through interactions with the activator. Activation is itself a highly regulated process, only occurring under specific conditions to ensure proper timing and levels of target gene expression.

Activators possess distinct and separable DNA binding and transcriptional activation domains. The DNA binding domain is required to target the activator to specific sequences of DNA located, in yeast, 50–200 bp upstream of the transcriptional start site of a gene. The activator functions as a site of recruitment for the polymerase machinery by interaction with either the RNA polymerase holoenzyme directly or with the TFIID complex, or both [7]. The DNA-binding properties of an activator or its ability to interact with the rest of the transcriptional machinery may be modulated in order to elicit a controlled transcriptional response, reviewed in [12].

Many yeast proteins that regulate the transcription of specific pathway genes in response to the availability of metabolites share a similar DNA binding domain—the

$Zn(II)_2Cys_6$ binuclear cluster motif [13]. Their protein sequence contains six conserved cysteine residues, often found at the amino-terminal end of the protein, which constitute the zinc binding and DNA interaction sites. Outside of this region the proteins are quite diverse, with the exception of a loosely defined middle-homology region (MHR) in some of the proteins. The role of the MHR has yet to be defined experimentally. Regulation of DNA binding activity of this family of proteins can occur either by regulating the amount of the protein in the cell or by sequestering the protein away from the DNA such that it cannot interact with its target genes [14]. There are, however, many examples of $Zn(II)_2Cys_6$ binuclear cluster proteins that bind constitutively to their target genes. Under these circumstances the transcriptional potency of the activation domain must be regulated. In the sections below, I will focus on transcriptional activation complexes (genetic switches composed of activators and associated regulators) in which there is evidence for direct control of activity through interaction with metabolites. A summary of $Zn(II)_2Cys_6$ binuclear cluster proteins, and others that are known to be regulated by metabolites, is shown in table 1.

Leucine biosynthesis

The genes required for the production of leucine (*LEU1*, *LEU2* and *LEU4*) are regulated as a consequence of the extracellular leucine concentration at the transcriptional level [15, 16]. A schematic illustration of the leucine biosynthetic pathway is shown in figure 1. The activation of the *LEU* genes is dependent upon a $Zn(II)_2Cys_6$ binuclear cluster containing a protein called Leu3p [17]. The overall architecture of Leu3p (fig. 2A) is similar to other members of this family of proteins in that it possesses an amino-terminal DNA binding domain and a carboxyl-terminal activation domain rich in acidic and hydrophobic amino acids, typical for yeast activators [18]. In the absence of DNA, Leu3p exists as a dimer [19] and contains a reasonably well defined coiled-coil dimerization element just to the carboxyl-terminal side of the binuclear cluster [13]. The consensus Leu3p DNA binding site is symmetrical ($CCGN_4CGG$), suggesting that the protein binds as a dimer to everted CGG triplets [20]. Purified Leu3p runs as a doublet on denaturing polyacrylamide gels [19]. This doublet can be resolved into a single band after treatment with phosphatases, indicating that Leu3p is a phosphoprotein. Both forms of Leu3p bound DNA equally well, and so far the functional significance of this phosphorylation is unclear [19]. The expression of Leu3p is itself controlled by Gcn4p and is therefore expressed at higher levels under conditions of amino acid starvation [21].

Control of DNA binding activity of Leu3p is not responsible for the controlled expression of the *LEU* genes. Leu3p DNA binding activity is found to be identical in cell extracts from induced or uninduced cells [22, 23]. Transcriptional control of Leu3p is mediated by α-isopropylmalate (α-IPM), an intermediate of the leucine biosynthetic pathway (fig. 1). The involvement of α-IPM in the regulation of leucine biosynthesis has

Table 1. Nutrient controlled genetic switches and their control elements in *Saccharomyces cerevisiae*.

Genetic switch	Activation conditions	Activator	Genes regulated	Ancillary proteins	Effector molecule(s)
Galactose metabolism	galactose as sole carbon source	Gal4p	*GAL1* *GAL2* *GAL7* *GAL10*	Gal80p (repressor) Gal3p (inducer)	galactose and ATP
Oxygen response	aerobic growth	Hap1p	*CYC1* *CYC7*	high molecular weight complex with other proteins disrupted upon induction	heme
Leucine biosynthesis	leucine starvation	Leu3p	*LEU1* *LEU2* *LEU4*		α-isopropylmalate
Lysine biosynthesis	lysine starvation	Lys14p	*LYS1* *LYS2* *LYS9*		α-aminoadipate semialdehyde
Phosphate metabolism	inorganic phosphate starvation	Pho4p	*PHO5* *PHO84*	Pho81p (CDK inhibitor) Pho80p (cyclin) Pho85p (CDK)	?
Proline utilisation	proline as sole nitrogen source	Put3p	*PUT1* *PUT2*		?
Uracil biosynthesis	uracil starvation	Ppr1p	*URA1* *URA3* *URA4* *URA10*		dihydroorotic acid

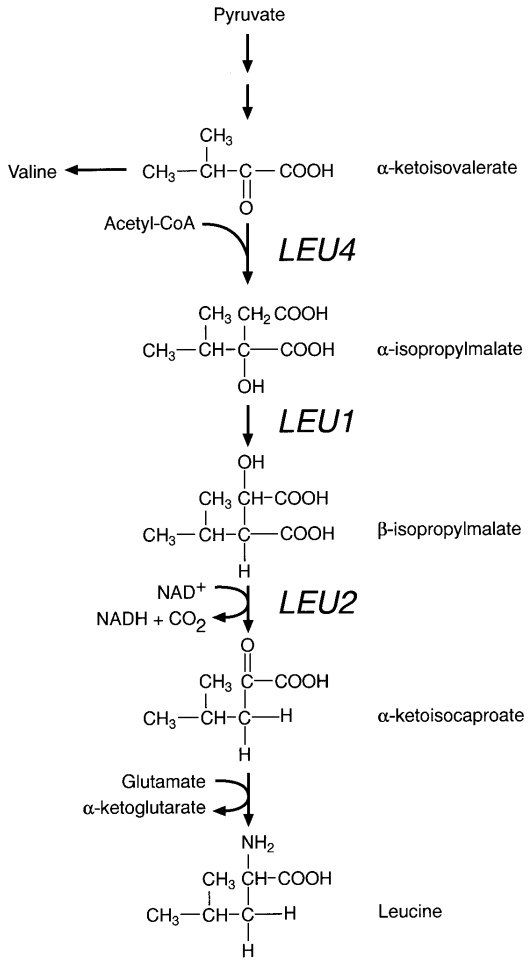

Figure 1. The leucine biosynthetic pathway. Leucine is synthesised de novo from pyruvate. The *LEU4* (encoding α-isopropylmalate synthase), *LEU1* (α-isopropylmalate isomerase) and *LEU2* (β-isopropylmalate dehydrogenase) genes are upregulated in response to leucine starvation.

been demonstrated on the basis of the following results: the levels of Leu1p and Leu2p are decreased dramatically in yeast strains which lack functional α-IPM synthase (Leu4p); mutations in the *LEU1* and *LEU2* genes, which would be expected to accumulate intermediates of the leucine biosynthetic pathway, have increased levels of Leu1p and Leu2p; and a yeast strain that contains a feedback-resistant mutant of α-IPM synthase, and consequently produces elevated amounts of α-IPM, also has increased levels of Leu1p and Leu2p [24].

The in vivo data described above have been subsequently supported using in vitro transcription experiments [25]. Using either yeast extracts overproducing Leu3p, or using purified protein, Sze et al. (1992) found that a DNA template containing Leu3p binding sites could only be transcriptionally active in the presence of Leu3p and α-IPM [25]. In the absence of α-IPM, Leu3p functioned as a repressor of basal transcription. It had previously been noted that Leu3p in the absence of induction repressed basal transcription in vivo [22]. This repression is solely dependent on the DNA binding domain of Leu3p [26]. The repression of activated transcription by uninduced Leu3p has not been shown. It is possible, therefore, that the binding of Leu3p to DNA physically blocks basal transcription by either bending the DNA such that it is refractory to the transcriptional apparatus or simply providing a block to read-through transcription. Some other $Zn(II)_2Cys_6$ binuclear cluster proteins have been shown to significantly bend DNA upon binding, e.g. Put3p bends DNA by approximately 40° [27, 28], and a similar apparent flexure angle has been calculated for Leu3p [G. Kohlhaw, personal communication].

How is α-IPM able to convert Leu3p into a transcriptional activator? Besides Leu3p and α-IPM, no other yeast factors are required for the induction. Leu3p expressed in mammalian cells will activate reporter genes bearing Leu3p binding sites only in the presence of exogenously added α-IPM [29]. The deletion of the central region of Leu3p (removal of amino acids 173–773) was found to convert the protein into a constitutive activator [30, 31], suggesting that the internal region of the protein may play a role in the control of induction. Also, a double point mutation within the activation domain of Leu3p (D872N/D874N) resulted in the formation of a protein that was unable to be activated by α-IPM [32]. The double mutation was shown not merely to possess a defective activation domain, since when combined with the large internal deletion described above, constitutive activation function was retained [32].

Using a modified two-hybrid experiment Wang et al. (1997) showed that the activation domain of Leu3p interacts with the central region of the protein [23]. In this experiment, Leu3p(1–773) was used as a bait to observe interactions with the activation domain (amino acids 775–883), which was itself fused to the activation domain of VP16. At low levels of α-IPM, an interaction between the activation domain and the rest of the protein was observed. High levels of α-IPM did not promote the association of the two parts of Leu3p [32]. Interestingly, the double mutation in the activation domain of Leu3p (D872N/D874N) interacted strongly with Leu3p(1–773) at low levels of α-IPM and did not interact at all at high levels of the inducer. This suggests that the activation domain containing the double mutation has an increased intramolecular affinity for the amino-terminal part of the protein. This interaction is still responsive to the presence of inducer, but the inducer is unable to activate the full-length protein [32].

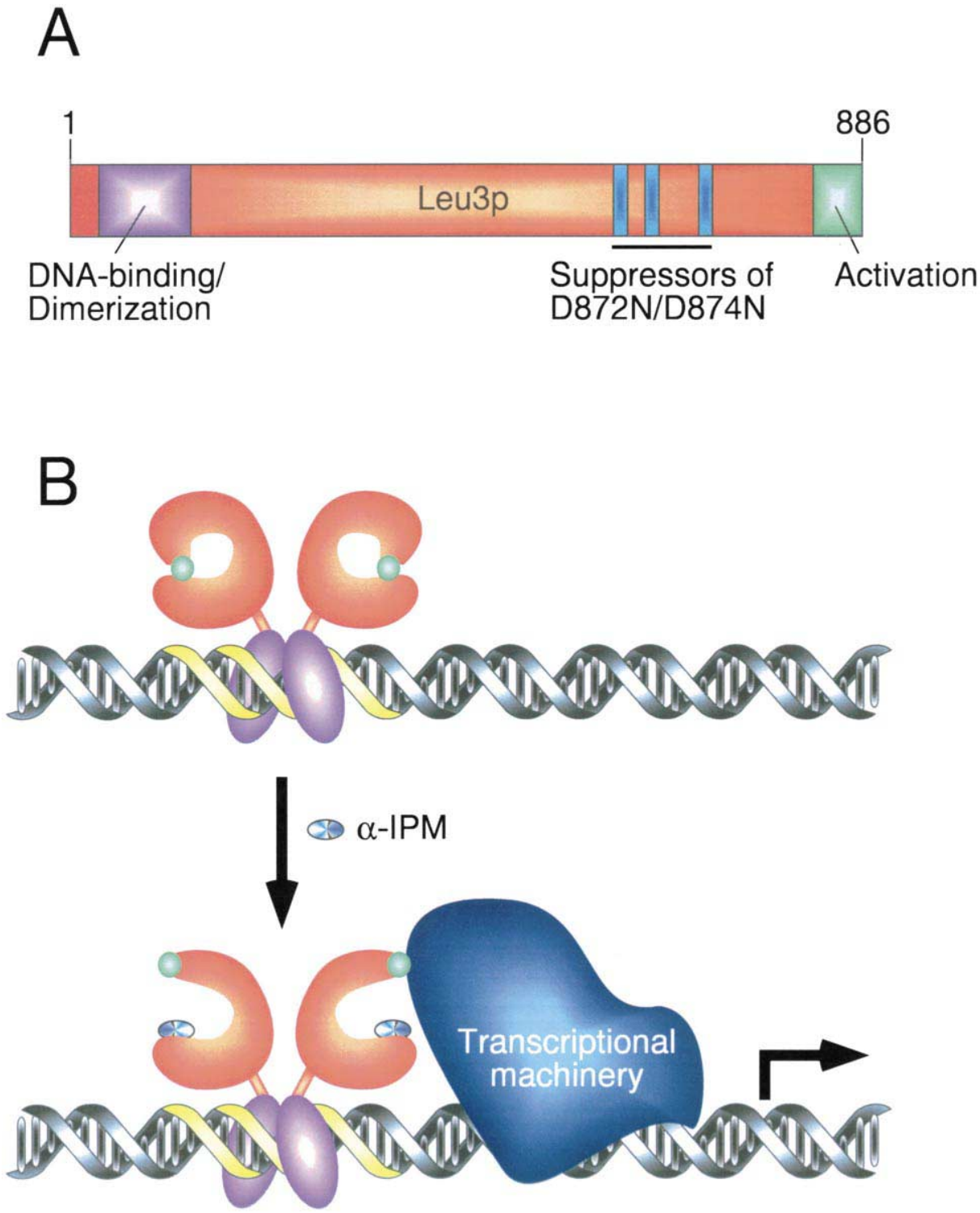

Figure 2. Leu3p and the mechanism of activation by α-isopropylmalate. (*A*) Schematic representation of Leu3p, which contains an amino terminal $Zn(II)_2Cys_6$ DNA binding and dimerization domain and a carboxyl-terminal transcriptional activation domain. The approximate location of suppressors of an activation domain defective mutation are also shown (see text for details). (*B*) A model for the activation of Leu3p by α-IPM. Leu3p binds to specific sites (yellow) upstream of the *LEU* genes, but it is transcriptionally inactive due to an intramolecular interaction between the activation domain and a central region of the protein. During leucine starvation, levels of α-IPM will build up and result in the disruption of this intramolecular interaction. The activation domain of Leu3p is thereby able to interact with the transcriptional machinery, and the gene is transcribed. The site of α-IPM interaction with Leu3p is unknown but, for clarity, is drawn here as occurring outside of the activation domain.

Intragenic suppressors of Leu3p containing the double mutation D872N/D874N have been sought [23, 32] and are found to be clustered within three short regions of the middle of the protein (at amino acids 604–611, 643–664 and 738–741), see figure 2A. When transferred to the wild-type protein sequence, these mutations resulted in the formation of constitutive activators, suggesting that the mutations cause the activation domain of Leu3p to be permanently unmasked [23]. The central region of Leu3p will not inhibit other activation domains, e.g. that of Cha4p [23], and thus appears to interact specifically with the activation domain of Leu3p.

A potential model for the induction of Leu3p is shown in figure 2B. DNA binding is not shown as a regulatory component of this model although as stated earlier the transcription of *LEU3* itself is under the general amino acid control of Gcn4p [21]. During conditions in which leucine is abundant, the intracellular concentration of α-IPM is low, and consequently Leu3p is transcriptionally inert. Under these conditions, the activation domain of Leu3p is masked by a physical interaction with a central region of the protein. As leucine becomes scarce, the concentration of early metabolic intermediates of the pathway (fig. 1) will increase. Although no direct biochemical interaction between α-IPM and Leu3p has been shown, it is highly likely that the two molecules physically associate. Perhaps the strongest evidence for this is the ability of Leu3p to activate transcription in mammalian cells in response to added α-IPM [29]. The interaction between Leu3p and α-IPM results in the unmasking of the activation domain, i.e. dissociation of the activation domain from the rest of the protein. The activation domain is presumably then able to interact with the RNA polymerase II transcriptional machinery to recruit the polymerase to the promoter and initiate transcription [7]. The precise details of the masking and unmasking process have still to be resolved. It is tempting to speculate that the suppressors of the Leu3p(D872N/D874N) mutation represent direct sites of intramolecular interaction that are disrupted upon interaction with α-IPM. Further biochemical and structural analysis will be required to elucidate their precise role.

Galactose metabolism

Perhaps the most extensively studied genetic switch in yeast is that of the *GAL* genes. This pathway and its mechanism of control have been extensively reviewed [12, 33, 34], so here I will concentrate on recent advances only. Yeast cells activate the *GAL* structural genes (*GAL1*, *GAL2*, *GAL7*, *GAL10*, *MEL1* and *PGM2*) when grown on galactose as the sole carbon source. Multiple mechanisms exist to ensure that these genes are switched off in preferred carbon sources such as glucose [35].

The *GAL* genetic switch is composed of three proteins—an activator, Gal4p; an inhibitor, Gal80p; and an inducer, Gal3p. Gal4p is the prototypical $Zn(II)_2Cys_6$ binuclear cluster protein with an amino-terminal DNA binding domain and a carboxyl-terminal, predominately acidic activation domain. Gal4p and Gal80p physically associate with each other to form a transcriptionally inert complex [36, 37]. The activation domain of Gal4p colocalises with the site of Gal80p interaction [38, 39], suggesting that Gal80p may mask the activation domain of Gal4p such that it is unable to contact the rest of the transcriptional machinery. The nature and structure of the activation domain of Gal4p are unclear. Circular dichroism experiments of peptides representing the activation domain have shown it to be unstructured at neutral pH, but able to adopt a β-sheet conformation at low pH [40, 41]. Extensive mutational analysis has tended to suggest that structural constraints are required for the association of the activation domain with Gal80p, but that transcriptional activation occurs via an unstructured surface [42].

Recent advances in understanding the mechanism of the *GAL* genetic switch have stemmed from the observation that Gal3p and Gal80p associate with each other in a galactose- and ATP-dependent fashion [37, 43–46]. Rather than simply promoting disruption of Gal4p and Gal80p, the addition of Gal3p results in the formation of a tripartite complex of Gal4p, Gal80p and Gal3p that is transcriptionally active [37]. It has been suggested that Gal80p relocates to a separate region of Gal4p during the process of induction [47], but the location of this region is unclear. A schematic outline of how the *GAL* switch is thought to operate is shown in figure 3.

How does Gal3p induce the transcriptionally inert complex of Gal4p and Gal80p? Given that Gal3p does not promote the dissociation of Gal4p and Gal80p, it is likely that it induces a conformational change in Gal80p such that it remains associated, the activation domain of Gal4p is freed to allow interaction with the rest of the transcriptional machinery. Some clues to the nature of the association between Gal3p and Gal80p have been gained from the analysis of mutants of each protein. Constitutive and noninducible mutations in Gal3p have been isolated [44, 48]. The constitutive mutants show an uncoupled reliance on galactose and/or ATP in their interaction with Gal80p, whereas noninducible mutants interact with Gal80p extremely weakly. These data support, although not exclusively, the idea that induction of Gal80p occurs merely due to the binding of Gal3p, rather than a two-stage process in which binding is followed by an additional step in the

induction process. Gal3p mutants that still show normal interaction with Gal80p, but do not induce *GAL* gene expression have not been reported.

The phenotype of a *gal3* deletion is slow induction of the *GAL* genes [49]. Rather than being fully induced within 30 min of the addition of galactose to a culture, *gal3* yeast cells begin to express the *GAL* genes after 3–4 days, but will induce the *GAL* genes to the same final level as wild-type cells. The slow induction is thought to be due to leaky expression of *GAL1*, the gene-encoding galactokinase [50]. A *gal1*, *gal3* double mutation is not inducible by galactose [51]. Overexpression of either *GAL1* or *GAL3* results in constitutive expression of the *GAL* genes, suggesting that Gal1p can substitute for Gal3p in the induction process. Indeed, purified Gal1p will activate *GAL* gene expression in vitro, although approximately 60-fold less efficiently than Gal3p [37].

Gal1p and Gal3p are remarkably similar proteins, sharing approximately 90% homology and 70% amino acid identity across their entire length. Other yeasts, e.g. *Kluyveromyces lactis*, contain a single galactokinase molecule that is also able to efficiently induce *GAL* gene expression [46, 52]. Both Gal1p and Gal3p require galactose and ATP to function, but Gal3p has no galactokinase activity [53]. The interaction between Gal3p and Gal80p does not require ATP hydrolysis since, in the presence of galactose, a variety of nonhydrolysable ATP analogues will promote the association of the two proteins [43]. The insertion of just two amino acids from Gal1p into the corresponding position of Gal3p (directly after amino acid 164) imparts Gal3p with galactokinase activity [53a]. The role of galactose and ATP in the induction process is therefore likely to be similar to their role as part of the galactokinase mechanism. It is tempting to speculate that the binding of galactose and ATP to Gal3p uncover an interaction site for Gal80p, and that the changes required to do this form an integral part of the normal mechanism of galactokinase function. Further work on both the mechanism of the galactokinase reaction and the structural nature of the interaction between Gal3p and its small-molecule regulators is required to address this issue.

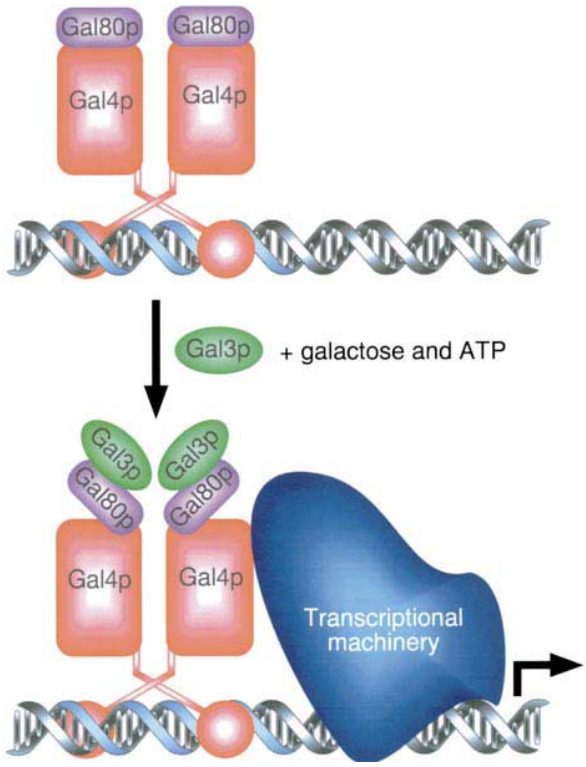

Figure 3. A model for the activation of the *GAL* genetic switch. In the absence of galactose, the transcriptional activity of Gal4p is inhibited by a physical association with Gal80p. In the presence of galactose and ATP, Gal3p associates with the Gal4p-Gal80p complex and results in the activation of the *GAL* genes.

Pyrimidine biosynthesis

Yeast cells utilise two mechanisms to increase the intracellular concentration of pyrimidines. The first is to simply import them from their growth media. During conditions of pyrimidine starvation, however, yeasts utilise a second mechanism in which pyrimidines are synthesised de novo from glutamine. The de novo pathway beginning with the condensation of carbamoyl phosphate, derived from glutamine, and aspartic acid is shown in figure 4. Regulation of this pathway occurs at several levels. First, UTP downregulates the enzymatic activity of Ura2p [54] and the transcription of the *URA2* gene [55]. Second, under conditions of pyrimidine starvation, the transcription of the *URA1*, *URA3*, *URA4* and *URA10* genes (the *URA* genes) is increased some 3–8-fold [56, 57]. Transcription of the *URA* genes, as exemplified by *URA3*, is controlled by two separate DNA elements. The first controls constitutive expression of the gene, and the activity of this element, via an as yet unidentified protein, is unaffected by pyrimidine levels in the media [56]. The second element is responsible for increased transcription during pyrimidine starvation, and is the binding site for a transcriptional activator, Ppr1p [58]. In the absence of Ppr1p, the *URA* genes are transcribed at a constitutive, basal level [56].

Ppr1p is a 904-amino-acid protein that contains a $Zn(II)_2Cys_6$ binuclear cluster DNA binding motif near its amino-terminal end—amino acids 29–123 [59]. The

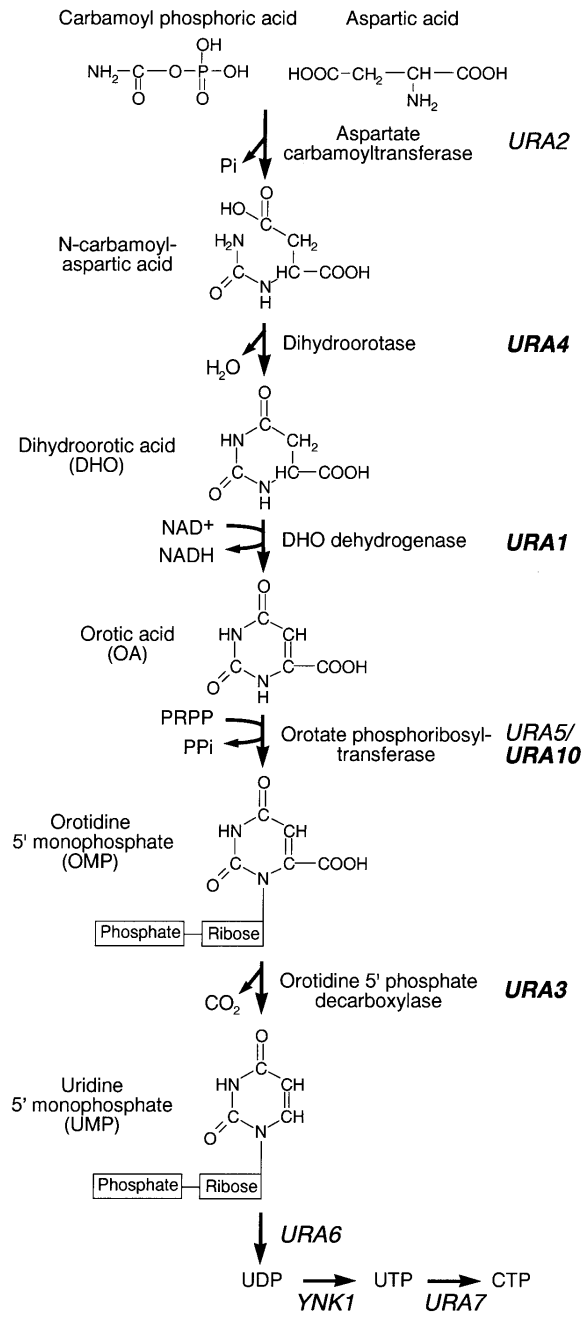

Figure 4. The de novo synthesis of pyrimidines in yeast. The pyrimidine ring is formed by the condensation of carbamoyl phosphoric acid and aspartic acid, followed by the elimination of a water molecule to form dihydroorotic acid (DHO). DHO is subsequently converted to orotic acid (OA) by the product of the *URA1* gene. A phosphoribose moiety, provided by 5-phosphoribose 1-pyrophosphate (PRPP), is added to OA to form orotidine 5′ monophosphate (OMP), which is then decarboxylated to form UMP by the product of the *URA3* gene. UMP is subsequently converted to UDP and UTP before its conversion into CTP. Ppr1p is a transcriptional activator of the genes shown in bold typeface during conditions of pyrimidine starvation.

crystal structure of the DNA binding domain of Ppr1p complexed with its cognate DNA site has been solved [60]. Ppr1p has an acidic and hydrophobic carboxyl-terminal domain which, by drawing comparisons with other yeast transcription factors, represents the activation domain [61]. The protein binds to defined sites ($CGGN_6CCG$) found approximately 100–200 bp upstream of the translational start sites of the *URA1*, *URA3*, *URA4* and *URA10* genes [56, 62].

Using mutant yeast strains, Lacroute [63] provided the first evidence that pyrimidine synthesis may be regulated by biosynthetic intermediates of the pathway itself. A yeast strain mutated in *ura2*, and thereby unable to synthesise dihydroorotic acid (DHO), was unable to induce the *URA* genes in response to pyrimidine starvation. A yeast strain mutated in *ura1*, which should result in an accumulation of DHO, results in constitutive induction [63]. The increase in activity of the pathway enzymes in response to pyrimidine starvation is due to increased expression of the *URA* genes themselves [57]. Altering the levels of DHO within the cell affects the induced levels of *URA* expression, but not the constitutive levels of gene expression [64].

Flynn and Reece [65] purified Ppr1p and found that the protein was only transcriptionally active in vitro in the presence of either DHO or orotic acid (OA). These structurally similar molecules (fig. 4) do not influence the DNA binding activity of Ppr1p [56, 65] and must therefore exert their influence through the activation domain. Construction of chimeric molecules in which parts of Ppr1p were fused to the DNA binding domain of Gal4p led to the identification of the carboxyl-terminal end of Ppr1p (amino acids 770–904) as the activation domain. This region of the protein retained its responsiveness to DHO and OA [65]. It is likely that DHO is the physiological inducer of the pathway and that the structural similarity of DHO and OA (see fig. 4) gives rise to transcriptional activation by both molecules.

How is the transcriptional activity of Ppr1p controlled by DHO? A number of constitutive and noninducible mutants of Ppr1p have been isolated. The noninducible mutations of the *PPR1* gene map to the $Zn(II)_2Cys_6$ binuclear cluster of the DNA binding domain of Ppr1p (amino acids 43, 57 and 64) [58, 66], presumably resulting in defective DNA binding. Constitutive mutations occur in two areas of the protein. The first, e.g. L233S, is located adjacent to the DNA binding domain (Roy and Losson, quoted in [65]), and the second, e.g. N884D, occurs within the DHO-dependent activation domain [M. C. Merlotti and R. J. Reece, unpublished observations]. The molecular basis for the constitutive activation of gene expression by these mutant proteins is not yet understood, and the presence of constitutive

mutations lying outside the DHO-dependent activation domain are difficult to explain.

The simplest model for controlling the activity of Ppr1p based on the available data is as follows, and is shown diagrammatically in figure 5. The DNA-bound protein is transcriptionally inert at high pyrimidine levels in the media due to low cellular concentrations of DHO. High media levels of pyrimidine will result in the downregulation of Ura2p activity and *URA2* expression. As pyrimidines become depleted, flux through the pathway will increase, and concentration of intermediates will rise. Elevated levels of DHO activate Ppr1p, *URA* gene expression will increase, and the de novo pyrimidine biosynthetic pathway will proceed. It is tempting to speculate that DHO directly interacts with Ppr1p in order to activate it. So far, however, a direct interaction between the inducer and the activator has not been shown. The in vitro transcription experiments of Flynn and Reece [65] could be mediated through the action of another protein present in the nuclear extract required for the transcription reaction to occur. These experiments did, however, provide evidence for a feedback mechanism to shut off gene expression. Ppr1p was shown to be transcriptionally inert in the absence of DHO, but it was also noted that high levels of DHO inhibited its transcriptional activity while having no effect on other activators [65]. It is possible, therefore, that Ppr1p acts as a sensor of both low and high levels of DHO and adjusts the level of *URA* gene transcription accordingly.

Other systems

A variety of other yeast transcriptional activators have been implicated to be controlled by metabolic intermediates of the pathways they regulate, but less is known about the molecular details of regulation. For example, the transcriptional activity of Lys14p—the activator of genes required for lysine biosynthesis—is increased by the exogenous addition of α-aminoadipate semialdehyde, an intermediate in lysine production [67], and inhibited by the addition of lysine [68, 69]. Similarly, the transcriptional activity of Put3p, but not its DNA binding activity, is increased some 50-fold when yeast cells are grown on proline as the sole nitrogen source [70, 71]. By deletion analysis, the carboxyl-terminal activation domain of Put3p does not appear to be responsive to proline, suggesting that regulation occurs elsewhere in the protein [72]. The metabolic control of two other well-characterised systems, those involving Pho4p and Hap1p, have been reviewed recently [12] and will not be discussed further here.

Conclusions

The genetic analysis of yeast metabolic pathways has proved to be a powerful tool to understand the mechanisms by which these pathways are controlled. In combination with more recent biochemical data, we are now beginning to unravel the complex control networks required to sense nutrient availability and convert this information into a transcriptional response. In some cases, regulation is relatively straightforward with metabolites directly interacting with transcription factors, but more complex control of multiple component genetic switches is also observed. What we lack is a detailed structural understanding of the consequences of metabolite interaction with transcription factors.

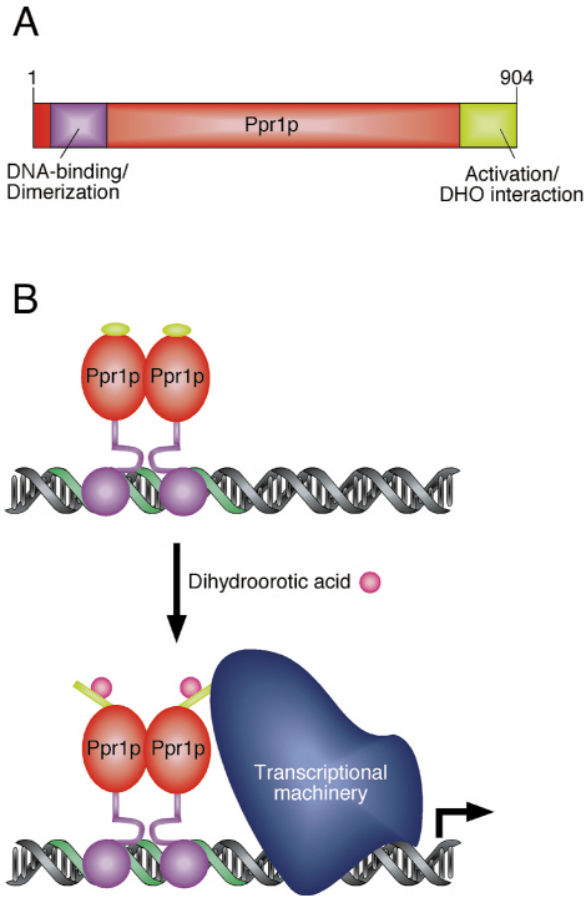

Figure 5. Ppr1p and the regulation of de novo pyrimidine biosynthesis. (*A*) Schematic representation of Leu3p, which contains an amino-terminal $Zn(II)_2Cys_6$ DNA binding and dimerization domain and a carboxyl-terminal transcriptional activation domain. (*B*) A potential model for the activation of Ppr1p by dihydroorotic acid. At high levels of external pyrimidines, the concentration of DHO will be relatively low, and Ppr1p will be transcriptionally inert. During conditions of pyrimidine starvation, the levels of DHO will increase, allowing it to interact with the activation domain of Pprp1 and convert the protein into a transcriptional activator.

This type of information is essential if we are to truly appreciate how these elegant genetic switches operate at the molecular level.

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