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In Vitro Transcriptional Activation by a Metabolic Intermediate: Activation by Leu3 Depends on α-Isopropylmalate

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In the absence of the leucine biosynthetic precursor α-isopropylmalate (α-IPM), the yeast LEU3 protein (Leu3p) binds DNA and acts as a transcriptional repressor in an in vitro extract. Addition of α-IPM resulted in a dramatic increase in Leu3p-dependent transcription. The presence of α-IPM was also required for Leu3p to compete effectively with another transcriptional activator, GAL4/VP16, for limiting transcription factors. Therefore, the addition of α-IPM appears to convert a transcriptional repressor into an activator. This represents an example in eukaryotes of direct transcriptional regulation by a small effector molecule.

An intricate feedback loop regulates leucine biosynthesis in fungi (*1*). Multiple controls of the first step, the synthesis of α-isopropylmalate (α-IPM), and the participation of a transcriptional activator are central features of the pathway. In yeast, the LEU4-encoded α-IPM synthase is subject to feedback inhibition by leucine, and the LEU4 gene is transcriptionally regulated by both GCN4 and LEU3 (*2*). The LEU3 gene product (Leu3p) is a 100-kD DNA binding protein that belongs to the $Zn(II)_2Cys_6$ binuclear cluster family of eukaryotic transcriptional activators (*3*). Leu3p activates transcription of the LEU1, LEU2, and LEU4 genes (*4*), and its binding site (UAS_{LEU}) is also found upstream of the ILV2, ILV5, and GDH1 genes of yeast (*5*). Genetic evidence suggests that activation by Leu3p requires the presence of α-IPM to complete the regulatory loop (*4*).

To analyze the components required for regulation, we have reconstituted Leu3p-dependent transcriptional activation in vitro. We prepared active whole cell transcription extracts (*6*) from LEU3 null, wild-type, and overexpressing yeast strains (*7*) and measured the amount of Leu3p by means of an electrophoretic mobility shift assay (*3*, *4*). The extracts from wild-type cells contained relatively little Leu3p, but high amounts of UAS_{LEU} binding activity were detected in extracts from the Leu3p-overproducing cells (*8*). The mobility shift obtained with the transcription extracts was indistinguishable from that of highly purified Leu3p, indicating that no auxiliary factors had been lost during the extensive purification of the protein (*8*, *9*). Complex formation by the extracts and by purified Leu3p was unaffected by α-IPM (*4*, *8*).

In vitro transcription reactions were performed as described (*6*). Templates contained various binding sites upstream of the CYC1 TATA box linked to a "G-less cassette," allowing the simple analysis of specific ribonuclease T1–resistant transcripts (*6*). Two templates, distinguishable by the lengths of their G-less cassettes, were used simultaneously to judge relative transcription efficiency. Transcriptional activation was attained with templates that contained either one or two UAS_{LEU} elements (Fig. 1). Activation was clearly dependent on the presence and concentration of Leu3p and absolutely required the addition of α-IPM. Tenfold to 20-fold activation was obtained with the extract from Leu3p-overproducing cells. Neither Leu3p nor α-IPM had any effect on transcription from the control template lacking the UAS_{LEU} sequence (Fig. 1).

We determined transcriptional activation as a function of α-IPM concentration (Fig. 2) (*7*). Saturation curves were identical over a twofold range of extract con-

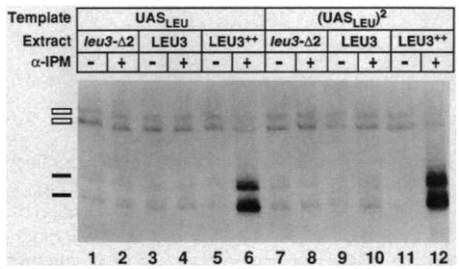

Fig. 1. Transcriptional activation from the UAS_{LEU} requires both Leu3p and α-IPM. In vitro transcription was carried out as described (*6*) with 1.2 μg of pUC18 and 0.3 ng of each template per 30-μl reaction. The longer pair of transcripts (open bars) are derived from a UAS−, basal template bearing the CYC1 TATA box linked to a G-less cassette (*6*). The shorter pair of transcripts (closed bars) is derived from plasmids with a 3′ truncation of the G-less cassette and with one (lanes 1 to 6) or two (lanes 7 to 12) UAS_{LEU} elements upstream of the TATA box (*14*). The α-IPM (natural isomer) (*15*) was added to a final concentration of 0.5 mM to reactions whose products were visualized in even-numbered lanes. Lanes 1, 2, 7, and 8, extract from the *leu3-Δ2* strain at a final concentration of 2 mg of protein per milliliter. Lanes 3, 4, 9, and 10, extract from the wild-type strain at a final concentration of 3 mg of protein per milliliter. Lanes 5, 6, 11, and 12, extract from the Leu3p-overproducing strain at a final concentration of 3 mg of protein per milliliter.

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centration (*10*) and yielded a value of approximately 0.2 mM α-IPM for half-maximal stimulation. Although a direct physical interaction remains to be proved, the effect was specific for both Leu3p and α-IPM: neither β-IPM nor malic acid stimulated transcription (Fig. 2, inset), and GAL4/VP16–dependent transcription from a UAS_{GAL} template was unaffected by α-IPM in the absence of Leu3p (*10*) (see below). We also obtained very strong activation by adding purified Leu3p (*9*) to a LEU3 null transcription extract (Fig. 3). Again, activation was completely dependent on the addition of α-IPM.

In addition to the dramatic activation seen when high concentrations of Leu3p and α-IPM are present in the transcription reactions, we also observed apparent Leu3p-dependent inhibition of transcription from templates containing the UAS_{LEU} element. In Fig. 3, lanes 3 and 5 contain high concentrations of Leu3p but lack α-IPM. UAS_{LEU} transcripts are reduced twofold to threefold when compared to UAS− transcripts in the same reactions or to UAS_{LEU} transcripts synthesized in the absence of Leu3p (Fig. 3, lanes 2 and 3; see also Fig. 1). To confirm that the repression was a function of Leu3p, we varied the amount of Leu3p and compared transcription from the two templates (Fig. 4). In the absence of α-IPM, Leu3p specifically inhibited transcription from the UAS_{LEU} template. Leu3p may therefore act both as a transcriptional repressor and as an activator, with α-IPM modulating the transition between the two states. This model is supported by in vivo analyses of LEU3 mutants (*4*, *11*).

Several previously described activators, including the recombinant GAL4/VP16 protein, have been shown to compete for transcription factors in vivo and in vitro (*12*). To determine whether Leu3p would compete with GAL4/VP16 for limiting transcription factors, we performed simultaneous transcription from UAS_{GAL} and UAS_{LEU} templates (Fig. 5). Leu3p was maintained at a constant concentration in the transcription extract from LEU3-overproducing cells, and increasing amounts of purified GAL4/VP16 were added to the reactions. Mutual interference between Leu3p and GAL4/VP16 occurred that was dependent on the presence of α-IPM. As the concentration of GAL4/VP16 was increased, transcription from the UAS_{LEU} template was diminished. Conversely, when α-IPM was present and Leu3p was active, GAL4/VP16–dependent transcription was reduced (more easily seen at lower GAL4/VP16 concentration; for example, compare Fig. 5, lanes 3 and 4). When the GAL4/VP16 concentration was held constant and the concentration of purified Leu3p was varied, similar results were obtained (*10*). Under all conditions tested, Leu3p interference was dependent on the presence of α-IPM.

The apparent lack of interference in the absence of α-IPM was not due to an inability of Leu3p to bind to DNA (*4*). Rather, we believe that a major (or the sole) effect of α-IPM on Leu3p-mediated transcription is to bring about a conformational change that converts Leu3p from a repressor into an activator configuration. In the latter form, Leu3p can activate

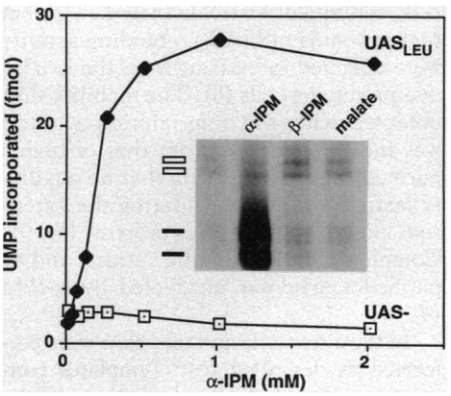

Fig. 2. α-IPM dependence of activation. Transcription reactions were performed as in Fig. 1 with extract from the Leu3p-overproducing strain. The concentration of α-IPM was varied as indicated. We determined the incorporation of [α-^{32}P]UMP (uridine 5′-phosphate) into specific transcripts by scintillation counting of bands excised from a dried urea–polyacrylamide gel (*6*). Closed bars, incorporation from the $(UAS_{LEU})^2$ template. Open bars, incorporation from the UAS−, basal template. (Inset) β-IPM and malate (2 mM) were also tested for their ability to activate Leu3p as indicated. The longer pair of transcripts was from the UAS−, basal template, and the shorter pair was from the $(UAS_{LEU})^2$ template.

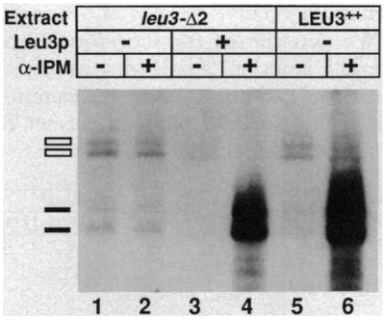

Fig. 3. In vitro transcription with highly purified Leu3p. Reactions were performed as in Fig. 1 with whole cell extract at a final concentration of 3 mg of protein per milliliter. α-IPM was included at 1 mM in the reactions whose products are displayed in even-numbered lanes. Lanes 1 and 2, extract from the *leu3-Δ2* strain. Lanes 3 and 4, extract from the *leu3-Δ2* strain plus 2.5 ng of affinity-purified Leu3p. Lanes 5 and 6, extract from the Leu3p-overproducing strain. The templates were the same as those used in Fig. 2.

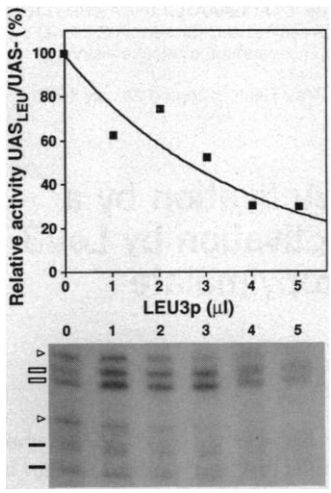

Fig. 4. Repression of UAS_{LEU} transcription by Leu3p in the absence of α-IPM. Reactions were performed as described in Fig. 1 with whole cell extract from the *leu3-Δ2* strain at a final concentration of 3 mg of protein per milliliter in the absence of α-IPM. The amount of Leu3p added to the reactions was varied as indicated (1.7 ng/μl) (*9*). Lower panel, autoradiograms of transcripts from the $(UAS_{LEU})^2$ (closed bars) and UAS− (open bars) templates. The transcripts indicated by triangles transit the entire length of the G-less sequence and may include read-through transcripts initiated upstream (*6*). Upper panel, transcripts were quantitated with a Molecular Dynamics Computing Densitometer, and the ratios of UAS_{LEU} to UAS− transcripts were plotted after normalization to the ratio in the absence of Leu3p.

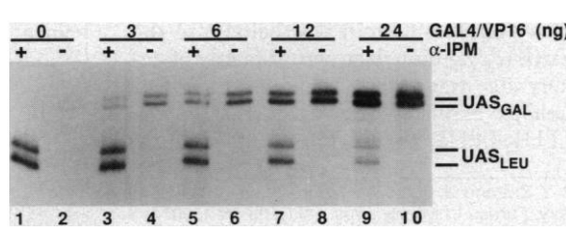

Fig. 5. Mutual interference of GAL4/VP16 and Leu3p–α-IPM–directed transcription. Reactions were performed as described in Fig. 2 with whole cell extract from the Leu3p-overproducing strain at a final concentration of 3 mg of protein per milliliter. The concentration of GAL4/VP16 was varied, and α-IPM was included at a final concentration of 1 mM as indicated. The longer pair of transcripts was from pGAL4CG−, a UAS_{GAL} template (*6*), and the shorter pair was from the $(UAS_{LEU})^2$ template.

transcription above a basal level and compete with GAL4/VP16 for transcriptional factors. This model is consistent with recent evidence indicating different conformational states of Leu3p and a relation between these states and transcriptional effectiveness (*11*).

Steroid hormone receptors and the yeast ACE1 metallothionein regulator are two examples of eukaryotic transcriptional activators that require small molecule cofactors (*13*). In these examples the ligand affects DNA binding; the effect on activation is indirect. For Leu3p, DNA binding is constitutive: the ligand affects activation directly. In addition to serving as a model system for the study of metabolite-dependent eukaryotic gene regulation in vitro, Leu3p–α-IPM activation is expected to facilitate the analysis of interactions between an activator and the components of the RNA polymerase II transcription complex.

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14. We constructed the UAS−, basal template, pJJ469, by subcloning the CYC1 TATA and G-less cassette sequences from pGAL4CG− as an Sma I fragment into the Sma I site of pUC18 (*6*). Templates with one (pJJ534) or two (pJJ482) UAS_{LEU} sites were derivatives of pJJ460 (*6*). The UAS_{LEU} sequence used in the templates was identical to that used in the band shift assays (*3, 4*).
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Bacteriophage λ*PaPa*: Not the Mother of All λ Phages

Roger W. Hendrix and Robert L. Duda

The common laboratory strain of bacteriophage λ—λ wild type or λ*PaPa*—carries a frameshift mutation relative to Ur-λ, the original isolate. The Ur-λ virions have thin, jointed tail fibers that are absent from λ wild type. Two novel proteins of Ur-λ constitute the fibers: the product of *stf*, the gene that is disrupted in λ wild type by the frameshift mutation, and the product of gene *tfa*, a protein that is implicated in facilitating tail fiber assembly. Relative to λ wild type, Ur-λ has expanded receptor specificity and adsorbs to *Escherichia coli* cells more rapidly.

The tail fibers of the double-stranded DNA bacteriophages are the organelles through which the virus contacts its host in the first steps of the infection process. By forming specific contacts with receptor molecules on the surface of the bacterial cell, the tail fibers provide a primary determinant of the host range of the bacteriophage that carries them. Mutations in tail fiber genes can lead to changes in receptor specificity that are thought to allow the phage to adapt to populations of bacteria with different or mutationally altered surface receptors. Some tail fibers show a remarkable plasticity in the range of receptor specificities they can access through small mutational changes (*1*).

A different evolutionary plasticity in the tail fiber structure in phage populations is suggested by recent comparisons of DNA sequences of phage tail fiber genes (*2, 3*). These studies revealed that tail fiber genes from different phages that were thought to be unrelated share stretches of sequence similarity. From these observations, there appears to be rather widespread and promiscuous sharing of tail fiber gene parts, and presumably receptor specificities, among populations of phages. Among the surprising observations from these sequence comparisons was that the tail fiber gene (gene *H*) of *Escherichia coli* phage P2 shares a sequence similarity with *E. coli* phage λ that extends across two adjacent open reading frames in the λ sequence. These open reading frames are located within the central "nonessential" region of the λ genome, near the end of the late gene operon. The P2 and λ sequences align with ~78% local identity if a single base gap is introduced into the λ sequence; if a base were to be inserted into the λ DNA at the position of the gap in the sequence alignment, the two open reading frames would fuse into one large open reading frame. These observations led Haggård-Ljungquist *et al.* (*2*) to suggest that there may exist versions of λ in which this hypothetical gene is intact and that it encodes a tail fiber not present on the laboratory version of λ. It had been recognized earlier that a region near the end of this hypothetical gene is similar in sequence to the corresponding part of the phage T4 distal half fiber gene, gene *37* (*4*), and that this part of the λ sequence (together with the downstream gene) can be substituted into T4 to produce a T4 phage with altered host range (*5*).

Phage λ was first reported in 1951 (*6*) as a phage derived from a prophage residing in *E. coli* strain K12, itself isolated from a clinical specimen in California in 1922 (*7*). However, the version of λ studied in laboratories around the world for most of the past 40 years is not identical to the version originally isolated from *E. coli* K12. Instead, nearly all strains of λ in current use are

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