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19. The gene for ribosomal protein S5 (*38*) was cloned by polymerase chain reaction from total *Escherichia coli* MRE600 genomic DNA, and cysteines were created at positions 21, 99, and 129 of S5 by site-directed mutagenesis (*39*). Mutant and wild-type genes were placed downstream from a T7 promoter in pEt-11a (*40*) and overexpressed in *E. coli* DH5a. S5 proteins were purified by chromatography on a Pharmacia Resource S fast protein liquid chromatography column with the use of a 50 to 300 mM sodium acetate (NaOAc) (pH 5.6) gradient in 6 M urea at 4°C. Ribosomes, 30*S* ribosomal subunits, and 16*S* rRNA were isolated as described previously (*41*). A complex between BABE and Fe(II), prepared as described previously (*8*), was conjugated with mutant S5 proteins by mixing of 20 μl of a 50 μM solution of S5 [in 80 mM K-Hepes (pH 7.7), 1 M KCl, and 6 mM β-mercaptoethanol] with 10 μl of a 10 mM solution of Fe-BABE in a buffer containing 1 M KCl, 80 mM Hepes (pH 7.7), and 0.01% Nikkol (Nikko Chemicals, Tokyo, Japan), in a volume of 100 μl, followed by incubation at 37°C for 15 min. Modified S5 was separated from excess reagent with Microcon3 vials (Amicon). Complexes between S5 or Fe(II)-derivatized S5 and 16S rRNA were formed, in a typical experiment, by adding 20 pmol of *E. coli* 16S rRNA [in 50 mM tris-HCl (pH 7.5), 20 mM $MgCl_2$, and 300 mM KCl] to buffer A [80 mM K-Hepes (pH 7.7), 20 mM $MgCl_2$, and 0.01% Nikkol]. After addition of 2.7 μl of a 30 μM solution of S5 or Fe(II)-derivatized S5 and 2 μl of a 40 μM mixture containing proteins S4, S7, S8, S15, S16, S17, and S20 [all proteins were stored in 80 mM K-Hepes (pH 7.7), 1 M KCl, and 6 mM β-mercaptoethanol], the salt concentration was adjusted to 330 mM to give a final reaction volume of 50 μl. Incubation took place at 40°C for 1 hour, followed by 10 min on ice. Reconstitution of 30*S* ribosomal subunits containing Fe(II)-derivatized S5 or unmodified S5 was done similarly, except that 10 μl of an 8 μM solution of Σ-S5 (a mixture containing all 30*S* ribosomal proteins except S5) was used to allow formation of complete subunits. S5-16*S* rRNP complexes and reconstituted 30*S* subunits were purified by sedimentation in a SW41 rotor for 18 hours at 35,000 rpm at 4°C, with the use of a 10 to 40% sucrose gradient in 50 mM tris-HCl (pH 7.5), 20 mM $MgCl_2$, and 100 mM KCl.
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42. Formation of hydroxyl radicals was initiated by addition of 1 μl of 250 mM ascorbic acid and 1 μl of 2.5% H_2O_2 to 25 μl of a solution of Fe(II)BABE-S5 RNP complex (0.2 mg/ml) in buffer A containing 330 mM KCl, or to 25 μl of a solution of Fe(II)BABE-S5 30*S* subunits (0.32 mg/ml), followed by incubation for 10 min at 4°C. The reaction was terminated by addition of 1/10 volume 3 M NaOAc (pH 5.2), 1 μl of a solution of glycogen (10 mg/ml), and 2.5 volume ethanol. The modified RNA was extracted and analyzed by primer extension as described (*7*).
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Activation of Gal4p by Galactose-Dependent Interaction of Galactokinase and Gal80p

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Yeast galactokinase (Gal1p) is an enzyme and a regulator of transcription. In addition to phosphorylating galactose, Gal1p activates Gal4p, the activator of *GAL* genes, but the mechanism of this regulation has been unclear. Here, biochemical and genetic evidence is presented to show that Gal1p activates Gal4p by direct interaction with the Gal4p inhibitor Gal80p. Interaction requires galactose, adenosine triphosphate, and the regulatory function of Gal1p. These data indicate that Gal1p-Gal80p complex formation results in the inactivation of Gal80p, thereby transmitting the galactose signal to Gal4p.

Gal4p, the key regulator of galactose metabolism in yeast, is a prototypical transcriptional activator that functions in fungal, plant, and animal cells (*1*). Genetic analysis revealed that the activity of Gal4p is controlled by the inhibitory protein Gal80p, and this system became a model to study eukaryotic gene regulation. However, it has been unclear how induction by galactose relieves the inhibitory effect of Gal80p on Gal4p activation function. Signal transmission requires the function of either the *GAL3* or the *GAL1* gene product. *GAL1* encodes galactokinase (Gal1p), the first enzyme of galactose metabolism. Gal3p shows homology to Gal1p but lacks galactokinase activity (*2, 3*). Early models proposing that both proteins catalyze the conversion of galactose into an inducer molecule that binds to and inactivates Gal80p [reviewed in (*4*)] have recently been questioned (*3, 5*).

The yeast *Kluyveromyces lactis* shares the regulatory mechanisms controlling expression of the galactose regulon with *Saccharomyces cerevisiae*. The transcriptional activator KlGal4p (also named Lac9p) and its inhibitor KlGal80p are exchangeable with their *S. cerevisiae* counterparts (*6, 7*). However, induction of galactose as well as lactose metabolism is entirely dependent on *K. lactis* Gal1p (KlGal1p) because *K. lactis* lacks a functional *GAL3* homolog. We have previously shown that induction requires a regulatory function of KlGal1p that is independent of the galactokinase activity and that complements a *S. cerevisiae gal3* mutation (*8, 9*).

Because in *K. lactis* Gal1p is only required for induction in the presence of Gal80p (*10*), we investigated whether KlGal1p inactivates Gal80p directly. Using a biochemical approach, we assayed for the formation of a complex between the two proteins. We tagged KlGal80p with six His residues (^{His}KlGal80p) (*11*) and then enriched the tagged protein from *K. lactis* cell extracts by binding to Ni–nitrilo-tri-acetic acid (NTA)–agarose (*12*). Protein immunoblot analysis with a polyclonal antiserum to KlGal80p (anti-KlGal80p) demonstrated that the epitope-tagged KlGal80p bound specifically to Ni-NTA-agarose (Fig. 1A). Cell extracts containing either of the two proteins, ^{His}KlGal80p or KlGal1p, were analyzed in binding assays as controls. Only when both protein extracts were mixed could KlGal1p be detected among the Ni-

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NTA–bound proteins with anti-KlGal1p (Fig. 1A), indicating that KlGal1p associated specifically with KlGal80p. KlGal1p was only found in the complex when galactose and adenosine triphosphate (ATP) were added to the binding reactions; the addition of galactose or ATP alone was not sufficient (Fig. 1B). In galactose-grown cells expressing both proteins, a small amount of KlGal1p was associated with KlGal80p without the addition in vitro of galactose and ATP, indicating that the complex also exists in vivo (Fig. 1C). No Gal1p-Gal80p complex was detectable in glycerol-grown cells, but complex formation after the addition of galactose and ATP demonstrated that the proteins present in these cells have the ability to interact.

If Gal1p regulates Gal80p activity by direct binding, a regulation-proficient mutant Gal1p protein should still bind to Gal80p. On the other hand, regulation-deficient Gal1p proteins may not bind to Gal80p anymore. Two *Klgal1* mutants have been characterized (8): *Klgal1-209* (reg^+kin^-) is regulatory-proficient but kinase-negative, and *Klgal1-r* (reg^-kin^-) lacks both activities. In addition, we isolated a regulatory-deficient allele, *Klgal1-m1* (reg^-kin^+), that still has galactokinase activity (13). Protein extracts were prepared from *K. lactis* cells expressing wild-type or mutant KlGal1p proteins, and the presence of the respective proteins in each of the extracts was verified by protein immunoblot analysis (Fig. 2A). Mixing of these extracts with a ^{His}KlGal80p extract showed that the regulatory-proficient proteins (Gal1p and Gal1-209p) bound to ^{His}KlGal80p, whereas the regulatory-deficient proteins (Gal1-rp and Gal1-m1p) did not bind (Fig. 2B). The kinase activity of Gal1-m1p suggests that the structure of this mutant protein was not drastically perturbed, and its failure to bind KlGal80p indicated that the regulatory function of KlGal1p was required for complex formation. The Gal1-209p variant, on the other hand, demonstrated that the galactokinase activity was dispensable.

Genetic evidence for a direct Gal1p-Gal80p interaction in vivo is presented in Fig. 3. The *S. cerevisiae GAL1* (*ScGAL1*), *ScGAL3*, or *KlGAL1* gene, each under control of the constitutive *ADH1* promoter, was introduced into a *K. lactis gal1* disruption mutant. Measurement of β-galactosidase activity (14) revealed that in single copy (sc), neither *ScGAL1* nor *ScGAL3* allowed for significant activation of KlGal4p (Fig. 3A), although, conversely, single-copy *KlGAL1* complemented the induction deficiency of a *S. cerevisiae gal3* mutant (8). Only in multicopy (mc) could *ScGAL3* confer some induction (30% of the amount of induction by single-copy *KlGAL1*).

To test whether the low efficiency of KlGal4p activation by the *S. cerevisiae* gene products was the result of incompatibility between ScGal1p or ScGal3p and KlGal80p, we replaced the *KlGAL80* gene in the *gal1* mutant strain by the corresponding *S. cerevisiae* gene, *ScGAL80* (15) (Fig. 3B). In such a strain, *KlGAL1* as well as *ScGAL3* and *ScGAL1* expression resulted in full activation of β-galactosidase (100%). The galactose dependence of induction was alleviated, indicating that a high concentration of Gal1p or Gal3p can compensate for the absence of galactose [compare also Fig. 3A, multicopy *KlGAL1*, and (3, 5)]. We concluded that in *K. lactis*, inactivation of KlGal80p but not of ScGal80p is achieved more efficiently with KlGal1p than with ScGal1p or ScGal3p.

Biochemical evidence supported the view that ScGal3p interacts only weakly with KlGal80p. In multicopy *ScGAL3* transformants, only a low amount of Gal3p was found associated with ^{His}KlGal80p (Fig. 2B), although ScGal3p was effectively recognized by anti-KlGal1p (Fig. 2A).

The allele specificity of the Gal1p or Gal3p interaction with Gal80p suggested that the Gal1p-Gal80p complex is formed by direct contact between the proteins; therefore, we investigated whether the individual purified components were able to interact. ^{His}KlGal80p was isolated with Ni-NTA-aga-

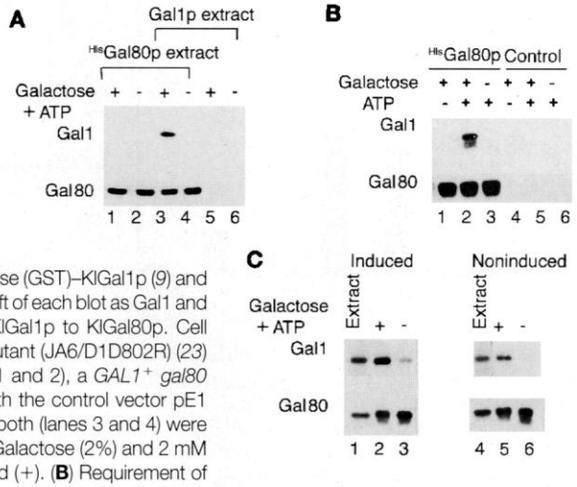

Fig. 1. Complex formation between KlGal1p and KlGal80p. The modified gene for ^{His}KlGal80p (11) was expressed from the constitutive *ADH1* promoter (24) from the high-copy pE1-based (25) plasmid pEAG80His. ^{His}KlGal80p was enriched from cell extracts by binding to Ni-NTA-agarose (12), and the bound protein fractions (26) were analyzed by immunoblotting with polyclonal antisera to the fusion proteins glutathione S-transferase (GST)–KlGal1p (9) and GST-KlGal80p (27) (indicated on the left of each blot as Gal1 and Gal80, respectively). (**A**) Binding of KlGal1p to KlGal80p. Cell extracts from a *K. lactis gal1 gal80* mutant (JA6/D1D802R) (23) transformed with pEAG80His (lanes 1 and 2), a *GAL1*$^+$ *gal80* strain JA6/D802R (7) transformed with the control vector pE1 (25) (lanes 5 and 6), and a mixture of both (lanes 3 and 4) were analyzed in Ni-NTA–binding assays. Galactose (2%) and 2 mM ATP together were added as indicated (+). (**B**) Requirement of both galactose and ATP for KlGal1p to bind to KlGal80p. Ni-NTA–bound fractions from strain JA6/D802R (*GAL1*$^+$ *gal80*) transformed with plasmid pEAG80His expressing ^{His}KlGal80p or the vector control pE1 were analyzed by protein immunoblotting. Galactose and ATP were added in binding reactions as indicated (+). (**C**) In vivo complex formation. Extracts from cells of JA6/D802R (pEAG80His) grown in the presence of galactose and glycerol (induced) or in the presence of glycerol (noninduced) were loaded directly (lanes 1 and 4) or after binding to Ni-NTA with (+) or without (−) the addition of both galactose and ATP.

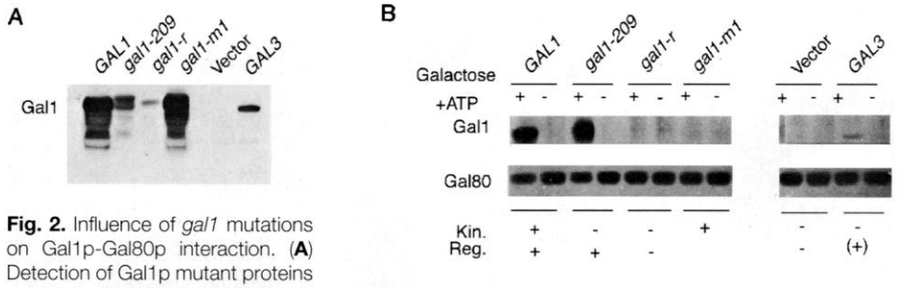

Fig. 2. Influence of *gal1* mutations on Gal1p-Gal80p interaction. (**A**) Detection of Gal1p mutant proteins and Gal3p in cell extracts. The cell extracts analyzed for complex formation in (B) were analyzed for the Gal1p or Gal3p content by protein immunoblotting with anti-KlGal1p. The KlGal1p wild-type and mutant proteins and Gal3p were expressed from the *ADH1* promoter from pE1-based multicopy plasmids. The host strain JA6/D1R (*gal1*) (23) transformed with pE1 (vector) served as a control. Transformants were grown in synthetic minimal medium with glycerol as a carbon source, and 30 μg of each protein extract was loaded in the gel. Differences in KlGal1p concentration are primarily caused by differences in plasmid stability (19). (**B**) ^{His}KlGal80p binding assay. Cell extract of the ^{His}KlGal80p-expressing strain (see Fig. 1A) was mixed with 500 μg of each of the extracts shown in (A). The Ni-NTA–bound fraction was analyzed by protein immunoblotting as in Fig. 1. The antiserum used to probe the immunoblots is indicated on the left. Kin. stands for galactokinase activity and Reg. for the regulatory function of the respective *GAL* alleles. The plus sign in parentheses indicates that the regulatory activity of *ScGAL3* is only observed in *S. cerevisiae* (compare data of Fig. 3).

rose and KlGal1p with glutathione-agarose as GST fusion protein GST-KlGal1p (*16–18*). The degree of purity of these fractions is shown by silver staining and protein immunoblotting (Fig. 4A). In the GST-KlGal1p preparation, some degradation products were detected (also visible in Fig. 4B), and ^{His}KlGal80p contained one major contaminant that also bound strongly to Ni-NTA-agarose in the absence of ^{His}KlGal80p (*19*), but it was not found in the complex (Fig. 4B). These purified fractions of ^{His}KlGal80p and GST-KlGal1p were able to interact in vitro as observed when glutathione-agarose was used as the binding matrix (Fig. 4B). Again, complex formation was dependent on both galactose and ATP. Although we cannot fully exclude the possibility that the interaction was mediated by minor contaminants, the data of Fig. 4 provide strong evidence for a direct protein-protein interaction between Gal1p and Gal80p. Binding assays performed with KlGal1p and KlGal80p produced in a *lac9* deletion mutant excluded the possibility that KlGal4p was required for the interaction (*19*).

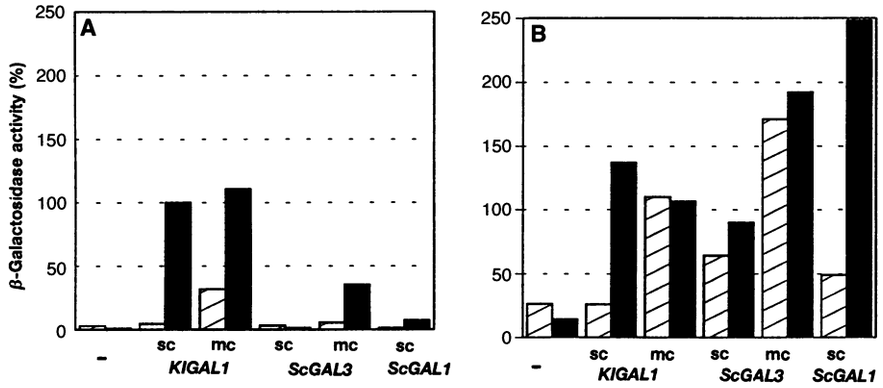

Fig. 3. Influence of *ScGAL1* and *ScGAL3* on (**A**) *KlGAL80*- and (**B**) *ScGAL80*-mediated repression of β-galactosidase activity in *K. lactis*. Expression of the KlGal4p-controlled β-galactosidase gene of *K. lactis* (*LAC4*) served as a measure for Gal80p activity. The isogenic strains JA6/D1R (*gal1 KlGAL80*) and JA6/D1Sc80 (*gal1 Klgal80::ScGAL80*) were used as host strains in (A) and (B), respectively. In JA6/D1Sc80 the *KlGAL80* structural gene had been replaced by that of *ScGAL80*. *GAL1* from *K. lactis* and *S. cerevisiae* (*KlGAL1, ScGAL1*) and *GAL3* from *S. cerevisiae* (*ScGAL3*) were introduced on single-copy (sc) or multicopy (mc) vectors. *KlGAL1*, *ScGAL1*, and *ScGAL3* genes were expressed under control of the *S. cerevisiae ADH1* promoter. β-Galactosidase activity was determined in extracts of cells grown under noninducing (3% glycerol, striped bars) or inducing (2% galactose and 3% glycerol, black bars) conditions. The mean of three to four independent measurements is given in relation to the induced JA6/D1R with single-copy *KlGAL1* grown in parallel (100%). The standard deviation was ~30%. β-Galactosidase activity of 100% is equivalent to 1940 ± 480 mU per milligram of protein.

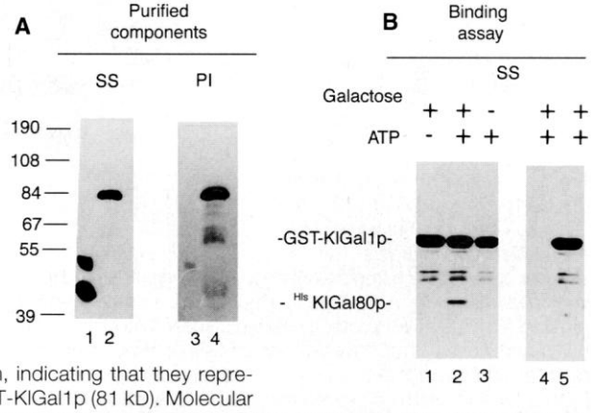

Fig. 4. Interaction of purified ^{His}KlGal80p and GST-KlGal1p. (**A**) Purified fractions (5 μg) of ^{His}KlGal80p and GST-KlGal1p were separated by SDS-PAGE and visualized by silver staining (SS, lanes 1 and 2) and protein immunoblotting (PI, lanes 3 and 4). Polyclonal antiserum to a GST-KlGal80p fusion protein was used for immunodetection. Protein bands with higher electrophoretic mobility than GST-KlGal1p cross-reacted with the antiserum, indicating that they represent degradation products of GST-KlGal1p (81 kD). Molecular sizes are indicated to the left in kilodaltons. (**B**) Binding reactions contained gluthathione-agarose, 5.5 μg of the ^{His}KlGal80p fraction, 17 μg of the GST-KlGal1p fraction (lanes 1 to 3), 2% galactose or 5 mM ATP (or both) where indicated (+), and binding buffer (*17*) in a total volume of 50 μl. As controls ^{His}KlGal80p (lane 4) and GST-KlGal1p (lane 5) were incubated separately in binding reactions. Samples were incubated for 1 hour at 4°C, washed with binding buffer, and denatured in sample buffer for SDS-PAGE. After electrophoretic separation, the proteins were visualized by silver staining.

For complex formation in cell extracts and with the isolated proteins, the cofactor requirement was specific for galactose; none of the other sugars tested supported Gal1p-Gal80p interaction (Table 1). However, GTP, UTP, and ADP (but not AMP) could substitute for ATP, as could ATP-γ-S. That the nucleotide analog could substitute for ATP indicated that ATP hydrolysis was not required. Although the other nucleotides are not galactokinase substrates, they may be able to bind to Gal1p and support a galactose-induced allosteric transition.

We propose that, in *K. lactis* as well as in *S. cerevisiae*, complex formation with Gal1p or Gal3p is the mechanism by which Gal80p is prevented from inhibiting the transcriptional activator Gal4p. If Gal80p remains bound to transcriptionally active Gal4p as proposed by the allosteric transition model (*20*), Gal1p or Gal3p binding could induce a transition of the Gal80p-Gal4p complex to a transcription-activating conformation. Alternatively, Gal1p or Gal3p could trap Gal80p or displace it from Gal4p. In any case, the Gal4p-activating function would be controlled by the relative affinity of Gal80p for the activating domain on Gal4p on the one hand and for Gal1p or Gal3p on the other hand.

The dual function of Gal1p, as a galactose- and ATP-dependent activator of Gal4p and as an enzyme using galactose and ATP as substrates, provides a means to couple *GAL* gene expression to the availability of the substrates. Through the dependence of the Gal1p-Gal80p interaction on galactose and ATP, the nutritional signal is transmitted to the transcription machinery

Table 1. Cofactor requirement for Gal80p-Gal1p interaction. ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; and ATP-γ-S, adenosine-5′-(γ-thio)triphosphate.

Sugar*	Nucleotide†	Complex formation‡
Galactose	ATP	+
Glucose	ATP	−
Fructose	ATP	−
Maltose	ATP	−
Mannose	ATP	−
Sucrose	ATP	−
Galactose	ADP	+
Galactose	AMP	−
Galactose	GTP	+
Galactose	UTP	+
Galactose	ATP-γ-S	+

*2% (w/v) of D-sugars was used. †Nucleotide concentration was 5 mM. ‡ Complex formation was tested in binding reactions as described in the legend to Figs. 1 and 4. No major difference in cofactor requirement was observed between binding reactions with cell extracts and purified components.

directly. It remains to be seen if substrate-induced protein-protein interactions also transmit other nutritional signals. Hexokinase-dependent glucose repression in yeast (*21*) and glucokinase-dependent regulation of glucose homeostasis in mammals (*22*) are possible candidates.

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10. Whereas the *gal1* mutant is noninducible, a *gal1 gal80* double mutant has exactly the same constitutive phenotype as a *gal80* mutant (*9*, *23*).
11. The His tag of the sequence Arg-Ser-His_6 was inserted into the linker region of the *K. lactis* Gal80 protein at amino acid position 349. The linker region had been defined by sequence comparison with *S. cerevisiae* Gal80p (ScGal80p) as the only region without sequence and length conservation (*7*). When ^{His}KlGal80p was expressed from the *ADH1* promoter and introduced into a *K. lactis gal80*$^-$ mutant on a multicopy plasmid (pEAG80His), the mutation was fully complemented as assayed by the expression of the Lac9p-controlled β-galactosidase gene.
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13. The *Klgal1-m1* mutant was obtained by mutagenesis of a *GAL1*-containing plasmid with hydroxylamine. Regulatory-deficient mutant plasmids were obtained after transformation of a *gal1* strain by screening on X-Gal plates containing 3% glycerol, 2% lactate, and 2% galactose. The wild-type gene resulted in blue colonies because the *K. lactis* β-galactosidase gene was induced, whereas regulatory-deficient *gal1* mutant alleles were white. Among the latter, mutants retaining galactokinase activity were identified by their ability to complement the *gal1-209* allele (reg$^+$ kin$^-$) for growth on galactose [J. Meyer *et al.*, in preparation].
14. β-Galactosidase is encoded by the *LAC4* gene, which is one of the KlGal4p-controlled genes in *K. lactis*. Enzymatic activity is strictly controlled by transcriptional regulation.
15. A *gal80::URA3* disruption was replaced by the *ScGAL80* structural gene fused to the *KlGAL80* promoter.
16. A cell extract from a *gal1 gal80* deletion strain transformed with pEAG80His [compare (*11*)] was loaded on a Ni-NTA-agarose column (3 ml), washed with extraction buffer (50 mM Hepes buffered with NaOH to pH 8, 100 mM NaCl, 10% glycerol) containing 50 mM imidazole, and eluted with a 30-ml linear gradient of 50 to 200 mM imidazole.
17. The GST-KlGal1p fusion was purified from a *gal1* deletion strain transformed with a multicopy plasmid carrying the *Gst-KlGAL1* gene fusion under the *ADH1* promoter. The extract was loaded on a glutathione-agarose column (3 ml) and eluted with extraction buffer containing 10 mM glutathione. ^{His}KlGal80p and GST-KlGal1p fractions were pooled, concentrated by dialysis against 10% polyethylene glycol 20.000 in extraction buffer, and dialyzed against binding buffer (50 mM Hepes buffered with NaOH to pH 8, 100 mM NaCl, 10 mM $MgCl_2$, 7 mM β-mercaptoethanol, 10% glycerol).
18. The *Gst* gene was amplified by polymerase chain reaction from pGEX-3X [D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988)] and inserted into an *ADH1-GAL1* construct between the *ADH1* promoter and the *KlGAL1* gene. The gene fusion encodes a protein of 736 amino acids with a molecular mass of ~81 kD with 234 amino acids attached to the NH_2-terminal end of KlGal1p. The galactokinase activity of the fusion protein was comparable with that of the wild-type protein.
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26. Protein extracts from yeast transformants grown in synthetic minimal medium (SM) with 3% glycerol were prepared by glass bead lysis in binding buffer. Extracts were incubated for 1 hour with equilibrated Ni-NTA-agarose at 4°C. Galactose (2%) and 2 mM ATP were added during the binding and washing steps. The resin was washed with binding buffer containing 50 mM imidazole and heated for 10 min at 95°C in SDS sample buffer, followed by fractionation of the proteins by SDS–polyacrylamide gel electrophoresis (SDS-PAGE).
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Optical Imaging of Functional Organization in the Monkey Inferotemporal Cortex

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To investigate the functional organization of object recognition, the technique of optical imaging was applied to the primate inferotemporal cortex, which is thought to be essential for object recognition. The features critical for the activation of single cells were first determined in unit recordings with electrodes. In the subsequent optical imaging, presentation of the critical features activated patchy regions around 0.5 millimeters in diameter, covering the site of the electrode penetration at which the critical feature had been determined. Because signals in optical imaging reflect average neuronal activities in the regions, the result directly indicates the regional clustering of cells responding to similar features.

Columnar organization, that is, the clustering of cells with similar response properties in columns running orthogonal to the cortical surface, has been assumed to be a general architecture in the neocortex (*1*). However, the evidence for this is almost entirely limited to the lower stages of sensory pathways. The anterior part of the inferotemporal cortex (the anterior IT) represents the final stage of the visual pathway, which is critical for object recognition. Previous unit recording experiments have shown that cells in the anterior IT selectively respond to moderately complex visual features of objects (*2–4*) and that cells with similar selectivity cluster in columnar regions (*4*, *5*). To confirm the columnar organization in the anterior IT and to further study the spatial organization of the anterior IT columns, we used optical imaging (*6*). Optical imaging is complementary to unit recording for study of spatial organization because in optical imaging the local average of neuronal activities is measured simultaneously over a wide cortical region (*7*).

To find the visual stimuli that were effective for activation of the region of the anterior IT to be subjected to optical imaging and to establish the relation between neuronal activities and optical signals in the anterior IT, we performed unit recordings and optical imaging in two hemispheres (*8*). Unit recordings with electrodes were first conducted in several separate sessions, in which the features of objects critical for the activation of single cells ("critical features") were determined by a reduction method (*9*). In the subsequent optical imaging session, these critical features as well as some simpler stimuli that had been

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