affinity purification procedure using lysates from [^{35}S] methionine-labelled cells (data not shown). The GFK–ketorapamycin and GFK–isorapamycin complexes bound FRAP less effectively than GFK–rapamycin; at concentrations of 500 nM, the keto- and iso- complexes were unable to retain the 220K protein (Fig. 1*a*, lanes 4, 5), whereas at higher concentrations of the complexes (2.5 μM) detectable quantities of FRAP were retained (Fig. 1*a*, lanes 4, 5). This is consistent with the finding that these compounds are still strong cell-cycle inhibitors, albeit less potent than rapamycin itself. Thus, the binding of GFK–ligand complexes to FRAP correlates with the ability of the ligands to impede G1 progression in MG-63 cells. FRAP was also detected in Jurkat *T*-lymphocyte cells and rat basophilic leukaemia cells (Fig. 1*b*), two mammalian cell lines that are also sensitive to rapamycin[6,22]. No other rapamycin-specific bands were observed in each case.

FRAP purified from bovine brain (bFRAP) had a similar specificity for GFK-ligand (Fig. 2*a*). Microsequencing of bFRAP proteolytic fragments (298 amino acids in total, Fig. 2*b*) led to the design of a pair of degenerate oligonucleotides for use in the polymerase chain reaction (PCR). A 182 bp PCR product allowed for the isolation of overlapping clones from a human Jurkat T cell λZAP II cDNA library, yielding 7.6 kb of contiguous sequence. Using these cDNA sequences as probes, a band migrating at approximately 8.5 kilobases was detected by Northern blot analysis of oligo dT purified RNA isolated from a variety of human tissues and cell lines (Fig. 2*c*). The human cDNA sequence encodes an amino-acid open reading frame (ORF) and aligns with 99% identity to the bFRAP peptides (Fig. 2*b*). As N-terminal peptide sequence from purified bovine FRAP was not obtained, the initiating methionine shown in Fig. 2*b* is unconfirmed. The predicted molecular mass of this ORF (~300K) is greater than that inferred by the mobility of FRAP during SDS–PAGE (above).

Human FRAP is highly related to the *DRR1/TOR1* and *DRR2/TOR2* gene products. Overall it is 44% identical to DRR1/TOR1 and 46% identical to DRR2/TOR2. The region of greatest homology to DRR1/TOR1 and DRR2/TOR2 lies in the C-terminal 660 amino acids of human FRAP (57% and 59% identical, respectively). In addition, this region has homology to several known phosphatidylinositol kinases (21% identity on average), including mammalian phosphatidylinositol 3-kinase[17,18] (PI3K), a yeast PI3K *VPS34* (refs. 17 and 18) and *PIK1* (ref. 20). These similarities indicate that FRAP may also have phosphatidylinositol kinase activity.

Through the introduction of minute structural changes in rapamycin, this study implicates FRAP as a mediator of G1 cell cycle progression in mammalian cells. Identification of FRAP as the target of FKBP12-rapamycin together with the earlier demonstration of calcineurin as the target of FKBP12-FK506 (ref. 2) addresses a fascinating aspect of immunophilin research, namely that the immunophilin FKBP12 can bind two distinct natural products and thereby gain the ability to bind two distinct signalling molecules involved in cell cycle entry and progression. Further biochemical characterization of this unique mammalian protein should elucidate its role in propagating the mitogen-initiated signals that lead to the activation of p70^{S6k} and cyclin–Cdk complexes. □

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Functional dissection of the yeast Cyc8–Tup1 transcriptional co-repressor complex

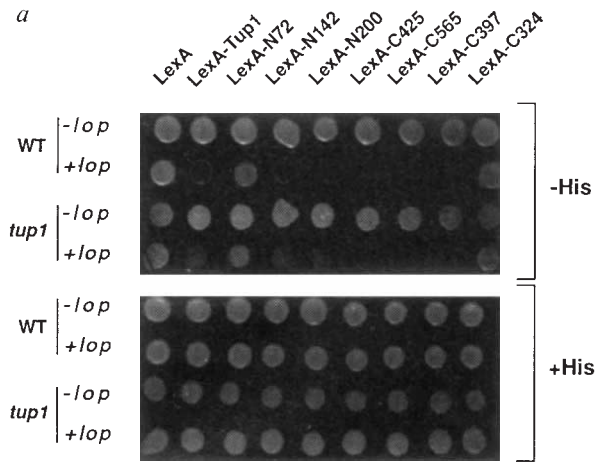
Dimitris Tzamarias & Kevin Struhl*

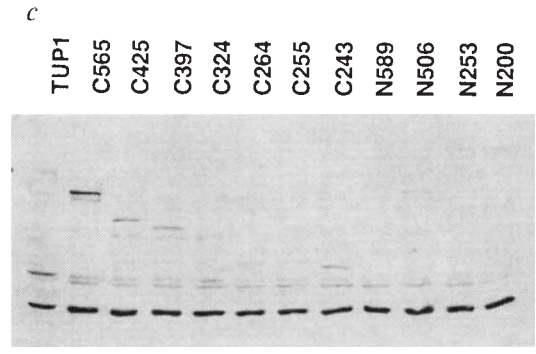
Department of Biological Chemistry and Molecular Pharmacology, 240 Longwood Avenue, Harvard Medical School, Boston, Massachusetts 02115, USA

DNA-BINDING repressor proteins mediate regulation of yeast genes by cell type (Mcm1/α2 and a1/α2), glucose (Mig1) and oxygen (Rox1) (refs 1–4 respectively). An unusual feature of all these regulatory pathways is that transcriptional repression requires two physically associated proteins[5] that do not bind DNA Cyc8(Ssn6) and Tup1. The Cyc8–Tup1 complex has been proposed to be a co-repressor that is recruited to target promoters by pathway-specific DNA-binding proteins[6], but the specific functions of the individual proteins are unknown. Here we show that when it is bound upstream of a functional promoter through the LexA DNA-binding domain, Tup1 represses transcription in the absence of Cyc8. Deletion analysis indicates that Tup1 contains at least two non-overlapping transcriptional repression regions with minimal primary sequence similarity, and a separable Cyc8-interaction domain. These Tup1 domains, which do not include the β-transducin motifs[7], are necessary and partially sufficient for Tup1 function. We suggest that Tup1 performs the repression function of the Cyc8–Tup1 co-repressor complex, and that Cyc8 serves as a link with the pathway-specific DNA-binding proteins.

It has been previously shown that Cyc8 can repress transcription in a Tup1-dependent manner when bound upstream of the intact *CYC1* promoter through the heterologous LexA DNA-binding domain[6]. Similarly, a LexA–Tup1 hybrid protein confers a 16-fold reduction of expression from a promoter containing four LexA operators upstream of the *CYC1* promoter (Table 1). LexA–Tup1 and LexA–Cyc8 also repress expression of a *his3* gene containing a single LexA operator upstream of the T_R TATA element (Fig. 1*a*), suggesting that they can inhibit basal transcription. Surprisingly, LexA–Tup1 retains almost its entire

* To whom correspondence should be addressed.





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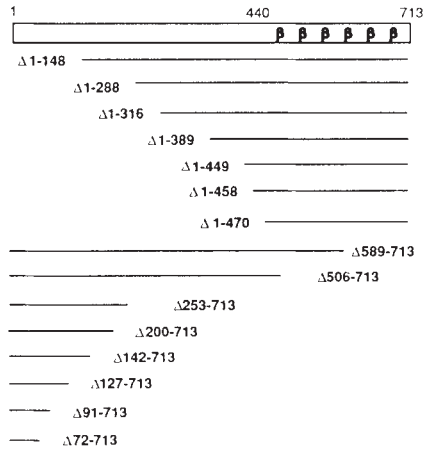

	Repression						Cyc8 interaction							
	wild-type		*tup1Δ::HIS3*		fold-repress		fold-activate		wild-type			*tup1Δ::HIS3*		
	-Lop	4 Lop	-Lop	4 Lop	WT	*tup1*	WT	*tup1*	Cyc8-VP16	Cyc8	VP16	Cyc8-VP16	Cyc8	VP16
TUP1	40	2.5	40	7.8	**16**	**5.1**	**15**	**7.0**	23	1.5	0.8	14	2.0	1.0
C565	80	9.0	85	12	**8.9**	**7.1**	**0.7**	**0.8**	0.8	1.2	0.8	1.2	1.5	1.0
C425	110	10	100	13	**11**	**7.7**	**0.9**	**1.0**	2.2	2.5	2.2	2.0	2.0	1.7
C397	120	22	120	30	**5.5**	**4.0**	**0.8**	**0.6**	2.5	3.0	2.5	2.5	4.0	3.2
C324	110	60	100	80	**1.9**	**1.2**	**1.1**	**1.2**	4.2	4.0	2.1	3.0	2.5	3.0
C264	120	65	120	80	**1.8**	**1.5**	**1.0**	**0.9**	4.0	4.0	2.5	1.8	2.0	2.1
C255	130	73			**1.8**									
C243	92	80			**1.2**									
N589	71	34			**2.1**									
N506	85	70			**1.2**		**4**		16	4.5	2.0			
N253	75	49			**1.5**		**5**		31	6.0	4.0			
N200	35	2.5	40	8.8	**14**	**4.6**	**41**	**12**	350	8.5	4.3	75	6.0	4.0
N142	22	2.2	33	11	**10**	**3.0**	**34**	**7.3**	290	8.5	4.0	29	4.0	2.5
N127	28	3.0	35	11	**7.0**	**3.2**	**17**	**8.2**	276	16	5.5	45	5.5	4.0
N91	45	19	60	50	**2.3**	**1.2**	**27**	**6.1**	90	3.4	1.4	11	1.8	1.2
N72	48	19	55	46	**2.5**	**1.2**	**21**	**6.0**	83	3.9	1.6	18	3.0	1.5
LexA	100	80	90	70	**1.2**	**1.3**	**0.9**	**1.0**	4.1	4.5	2.2	3.0	3.0	2.0
Vector	100	85	90	70	**1.2**	**1.3**	**1.0**	**1.3**	1.5	1.5	0.8	2.0	1.5	1.0

FIG. 1 Mapping the transcriptional repression and Cyc8-interaction domains of Tup1. *a*, Repression of basal transcription. YCp91 plasmids expressing various LexA-Tup1 derivatives were introduced into isogenic wild-type and *tup1Δ::HIS3* strains containing a *his3* promoter with (+lop) or without (−lop) a single LexA operator upstream of the T_R TATA element; the resulting cells were plated on minimal medium in the presence (+) or absence (−) of histidine (His). Repression of *HIS3* transcription was assayed by the inability of the resulting cells to grown on −His plates. LexA–Cyc8 also represses basal transcription in this assay (data not shown). *b*, Transcriptional repression and 2-hybrid assays. The structures of Tup1 (713 amino acids, including six copies of a β-transducin motif) and deletion derivatives (named by the number of N- or C-terminal residues contained in the protein) are indicated together with the sequences that have been removed. Repression assays were carried out by measuring β-galactosidase activities of wild-type and *tup1Δ::HIS3* strains harbouring the indicated LexA–Tup1 derivatives and *LacZ* reporter constructs that do or do not contain 4 LexA operators upstream of the *CYC1* promoter (Table 1 legends). The decreased *LacZ* expression from the *CYC1* promoter due to the various LexA–Tup1 proteins correlates well with the degree of Tup1 function and slower cell growth of the strains. 2-hybrid assays were carried out by measuring β-galactosidase activities of wild-type and *tup1Δ::HIS3* strains expressing the indicated combinations of LexA–Tup1 derivatives and activation (Cyc8–VP16) or control (Cyc8 or VP16) proteins. The strains all contain a plasmid derived from JK103 (ref. 22) in which *LacZ* is controlled by a promoter containing 4 LexA operators upstream of the *GAL1* TATA element. The ability of each LexA–Tup1 derivative to interact with Cyc8 is determined by the fold-activation (ratio of activity with Cyc8–VP16 compared to Cyc8 control). Weak activation of the N253 derivative might reflect lowered protein levels rather than functional activity. We do not understand why repression and two-hybrid interactions of all derivatives tested are reduced (typically ~3-fold) in the *tup1Δ::HIS3* strain in comparison to the isogenic wild-type strain. *c*, Western analysis of LexA–Tup1 derivatives. Electrophoretically separated proteins from yeast strains containing the indicated proteins were probed with the HA1 monoclonal antibody[23], which recognizes the 'flu epitope located at the N terminus of each protein. Bands appearing in all lanes represent yeast proteins that react with the HA1 antibody; they are observed in the parental strain lacking any LexA–Tup1 protein.

METHODS. *CYC8* and *TUP1* sequences were obtained from yeast genomic DNA by PCR amplification using primers based on published sequences[7,24]. The YCp91 expression vector is a derivative of the *TRP1* centromeric plasmid pRS314 (ref. 25) that contains the *ADH1* promoter and 5′ untranslated region (nucleotides −410 to +10), followed by an ATG codon, sequences encoding the SV40 nuclear localization signal[26], the HA1 epitope from influenza virus[23], a polylinker, and the *CYC8* termination region (includes 410 bp beyond the stop codon). To express the LexA hybrid proteins, an *Eco*RI fragment containing the entire LexA coding region (residues 1–202) was inserted upstream of the nuclear localization sequence. LexA–Cyc8 contains the entire Cyc8 coding region including 9 bp from the 5′ untranslated region cloned between the *Bam*HI and *Nco*I sites, and LexA–Tup1 contains the Tup1 coding region cloned between the *Sma*I and *Kpn*I sites. The LexA–Tup1 deletion derivatives were made by Bal31 nuclease treatment of the Tup1 coding region with subsequent insertion into the *Sma*I and *Kpn*I sites of YCp91. The activation and control constructs used in the 2-hybrid experiment were cloned into YEp92, which was generated by inserting the entire expression cassette of the YCp91 (*Sph*I–*Sac*I fragment) into the 2μ/*LEU2* plasmid YEpLac181 (ref. 27). The Cyc8–VP16 activation construct contains the *Bam*HI-Asp718 fragment of *CYC8* (codons 1–300) fused to the *Bgl*II–*Bam*HI fragment harbouring the VP16 activation domain (codons 414–553)[28], whereas the control constructs contain the individual *CYC8* or VP16 fragments. The portion of Cyc8 in the hybrid protein complements a *cyc8* deletion allele[20] and represses transcription in a Tup1-dependent manner when bound to a promoter through the LexA DNA-binding domain (data not shown). The *his3* promoters used in the basal transcription experiment was generated by inserting GGATCCGTCATACCAACCATT-AACCCTACTGATGTACATACAGTAGTTGTTGG-TCACAGAAAATGGATCC (Lex operator underlined) into the *Bam*HI site of a derivative containing a mutated GCN4 site upstream of the T_R element[29]; the distance between the LexA operator and the T_R TATA element is 45 bp.

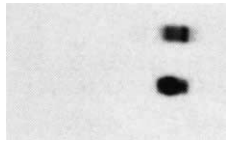

FIG. 2 Cyc8–Tup1 interaction *in vitro*. Each lane represents ^{35}S-labelled Cyc8 (residues 1–351, which contains 9 TRP motifs and complements a *cyc8* deletion allele[20]) stably bound to agarose beads containing GST, GST-Tup1-N250, and GST-Tup1-C324. The lane representing input ^{35}S-Cyc8 contains only 20% of the amount that was incubated with the agarose beads.

METHODS. The *Bam*HI–*Mlu*I fragment of Cyc8 was cloned downstream of the T3 promoter, and ^{35}S-protein was synthesized by transcription and translation *in vitro* using T3 RNA polymerase and rabbit reticulocyte lysates. The plasmids expressing GST–N250 and GST–C324 were generated respectively by cloning the *Sma*I–*Bam*HI fragment of Tup1 or the *Sma*I–*Nco*I fragment from C324 into the pGEX-2T vector, and the proteins were bound to glutathione agarose as described[30]. Agarose beads (50 μl) containing 1 μg of the GST proteins were pre-incubated for 30 min at 4 C with 0.25% BSA and then incubated with ^{35}S-Cyc8 for 2 h in binding buffer (20 mM Tris, pH 7.5, 100 mM NaCl, 0.1% NP-40, 2 mM PMSF, 0.25% BSA). Beads were washed 3 times with binding buffer (25 column volumes each) by microcentrifugation, and once with binding buffer lacking BSA. Samples were analysed by SDS–PAGE.

repression activity in a *cyc8* deletion. Thus unlike repression by LexA–Cyc8, which requires Tup1 (ref. 6), repression by LexA–Tup1 does not require Cyc8. This suggests that Tup1 mediates the repression function of the Cyc8–Tup1 co-repressor complex.

Deletion analysis of LexA–Tup1 indicates that at least two non-overlapping regions in the N-terminal half of Tup1 can mediate the repression function (Fig. 1*b*). The derivative containing the N-terminal 200 residues (N200) represses transcription to the same extent as LexA–Tup1, and the N142 and N127 derivatives are nearly as efficient; N91 and N72 are not functional. However, derivatives lacking the N-terminal 148 (C565) or 288 (C425) residues also repress transcription, although less efficiently than LexA–Tup1. C397 partially represses transcription, whereas more truncated derivatives do not, even though the hybrid proteins are expressed at levels comparable to LexA–Tup1 (Fig. 1*c*). In all cases, repression occurs in a *tup1* deletion strain, but for unknown reasons, the effect is less pronounced. Thus, the 200 N-terminal residues and a region(s) C-terminal to residue 288 can independently mediate repression.

To identify the region of Tup1 that associates with Cyc8, we used the 'two-hybrid' method for defining protein protein interactions[8]. We introduced the various LexA–Tup1 derivatives into strains expressing a Cyc8 VP16 hybrid protein. As Cyc8 and Tup1 are physically associated[5], the combination of Cyc8 VP16 and LexA–Tup1 confers 15-fold more expression than either protein alone (Fig. 1*b*). Similar results are obtained with LexA hybrid proteins containing 72, 91, 127 and 200 N-terminal residues of Tup1. These two-hybrid interactions are also observed to a lesser extent in a strain containing the *tup1Δ::HIS3* allele. Conversely, derivatives lacking 148 or more N-terminal residues do not stimulate transcription above the LexA control molecule. These results suggest that the N-terminal 72 residues of Tup1 are necessary and sufficient for the formation of the Cyc8–Tup1 complex. The Cyc8 interaction and transcriptional repression functions are separable because the N91 and N72 derivatives do not mediate repression even though they interact with Cyc8.

Affinity chromatography using glutathione-*S*-transferase (GST) fusion proteins suggests that the N-terminal region of Tup1 interacts directly with Cyc8. A radiolabelled Cyc8 derivative binds tightly to a GST column containing Tup1-N250, but it fails to bind to GST–Tup1-C324 (which contains the β-transducin repeats) or to a GST column (Fig. 2).

We also examined the Tup1 deletion derivates (LexA sequence removed) for two natural functions of the Cyc8–Tup1 complex, repression of *SUC2*, a glucose-regulated gene, and repression of *ANB1*, an oxygen-regulated gene (Fig. 3). N200, which contains the Cyc8 interaction and repression functions, reduces both *SUC2* and *ANB1* transcription, and it largely rescues the temperature-sensitive growth and clumpy-colony phenotypes caused by a *tup1* deletion. Repression of *SUC2* and *ANB1* by N200 requires Cyc8 (data not shown) but is not complete. In contrast, all derivatives lacking the Cyc8 interaction region (C565 to C243) fail to repress *SUC2* and *ANB1* transcription, even though some of them can mediate LexA-dependent repression. The N72 derivative, which interacts with Cyc8 but does not mediate LexA-dependent repression, also fails to reduce *SUC2* and *ANB1* transcription. These observations indicate that the Cyc8 interaction and transcriptional repression domain are necessary and partially sufficient for Tup1 function.

Our results suggest that Tup1 contains a domain that is responsible for the transcriptional repression function. Within this domain, short non-overlapping regions with minimal sequence similarity can independently mediate the repression

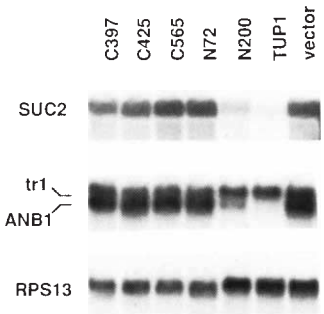

FIG. 3 Repression of *SUC2* and *ANB1* by Tup1 proteins. Total RNAs from *tup1Δ::HIS3* deletion strains transformed with the indicated derivatives were separated in a 1.4% agarose gel, transferred to nitrocellulose, and hybridized with radiolabelled *SUC2*, *ANB1* and *RPS13* probes prepared by nick-translation. The *ANB1* probe also hybridizes to TR1, a related gene that is not regulated by oxygen or by the Cyc8–Tup1 complex.

TABLE 1 Transcriptional repression by LexA-Tup1 and LexA-Cys8 hybrid proteins

Protein	Strain	Promoter		Fold-repression
		-Lex op.	4 Lex op.	
LexA–Tup1	Wild type	40	2.5	16.0
LexA–Tup1	*cyc8-Δ9::HIS3*	34	2.7	12.6
LexA–Tup1	*tup1Δ::HIS3*	40	7.8	5.1
LexA–Tup1	*tup1Δ::HIS3 cyc8-Δ9::LEU2*	36	6.9	5.2
LexA–Cyc8	Wild type	62	2.3	26.9
LexA–Cyc8	*tup1Δ::HIS3*	27	10	2.7
LexA	Wild type	100	80	1.2
None	Wild type	100	85	1.2

β-galactosidase activities (average of 3 independent transformants) of yeast strains containing the indicated LexA proteins (see legend to Fig. 1), *CYC8* and *TUP1* alleles, and *LacZ* reporter constructs. Values are normalized to the absorbance at 600 nm (A_{600}) of cells at the time of collecting (clumped cells were dispersed by addition of EDTA to 2 mM) and are accurate to ±30%. The fold-repression is determined by the ratio of β-galactosidase activities driven by *CYC1* promoters (−324 to +141) that lack (-Lex op.) or contain four Lex A operators (4 Lex op.) 50 bp upstream of the *CYC1* UASs. Cells expressing LexA–Tup1 and LexA–Cyc8 grow slowly and contain only 30–50% of the total protein in control cells. Hence, the apparent decrease in expression from the *CYC1* promoter due to LexA–Tup1 and LexA–Cyc8 proteins reflects normalization of β-galactosidase activities to cell absorbance; when normalized to total protein, all six strains show similar levels of expression from the *CYC1* promoter. The promoter constructs were derived from pLGΔ312S (ref. 19) and pJK1621 (ref. 6) by removing the *Hin*dIII fragment that contains the 2μ origin, and they were integrated at the *URA3* locus. All yeast strains were derived from FT5 (*α ura3-52 trp1-Δ63 his3-Δ200 leu2::PET56*). The *cyc8-Δ9::HIS3* and *cyc8-Δ9::LEU2* alleles contains the *Pst*I *HIS3* or *LEU2* fragments between two *Pst*I sites in *CYC8* (codons 99–862) and are essentially identical to *ssn6-Δ9* (ref. 20). The *tup1Δ::HIS3* allele contains Sc4251, a *Bam*HI–*Eco*RI *HIS3* fragment[21], between an artificial *Bam*HI site 6 bp upstream of the ATG initiation codon and the *Eco*RI sites of *TUP1* (codons −2 to 672).

function. These properties are similar to those of transcriptional activation domains, and they suggest that the Tup1 repression domain interacts with a component(s) of the transcriptional machinery. The Tup1 repression domain is almost completely uncharged, and hence differs significantly from artificial repression domains selected from *Escherichia coli* sequences, which are highly basic[9]. Interestingly, it contains two alanine-rich regions (40% of residues 120–144 and 22% of residues 254–276) like those found in the repression domains of the *Drosophila* DNA-binding proteins, Kruppel[10], Engrailed[11,12] and Even-skipped[13].

We suggest that Cyc8 may serve as an adaptor between DNA-bound proteins (such as Mig1 and Rox1) and Tup1. Cyc8 and the Cyc8-interaction region of Tup1 are dispensable when the Tup1 repression function is artificially tethered to a promoter, but both are required for Tup1 to repress transcription of *SUC2* and *ANB1*, which is probably mediated by binding of Mig1 and Rox1 to the respective promoters. Conversely, Cyc8 does not repress transcription in the absence of Tup1, and the Cyc8-interaction region of Tup1 is insufficient for repression. Consistent with this adaptor hypothesis, we have made Cyc8 deletion derivatives that interact with Tup1 but differentially repress the pathway-specific genes. This suggests that Cyc8 might interact differently with the various DNA-binding proteins that mediate pathway-specific repression. Our results do not exclude the involvement of Tup1 and/or additional factors in the putative[6] association of the Cyc8–Tup1 complex with DNA-binding proteins.

Tup1 contains six copies of a 'β-transducin' motif[7] which has been proposed[14] to interact with proteins containing 'TPR' motifs[15] such as Cyc8. But the properties of the N200 protein indicate that the β-transducin repeats are not absolutely required for Tup1 function. Interestingly several C-terminal deletions that remove one or more β-transducin motifs (N589, N506) are functionally impaired even though they contain the repression and Cyc8-interaction domains (Fig. 1; ref. 7). Thus, when truncated, the C-terminal region of Tup1 can interfere with the repression function and, to a lesser extent, the Cyc8 interaction function. This interference could reflect intramolecular masking or formation of non-productive complexes with other proteins, as appears to be the case with steroid receptors containing partial deletions of the hormone-response domain[16–18]. Although the β-transducin motifs are not essential for repression or interaction with Cyc8, they affect Tup1 function and are likely to be important. □

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Three-dimensional structure of β-galactosidase from *E. coli*

R. H. Jacobson, X-J. Zhang, R. F. DuBose* & B. W. Matthews†

Institute of Molecular Biology, Howard Hughes Medical Institute, and Department of Physics, University of Oregon, Eugene, Oregon 97403, USA

THE β-galactosidase from *Escherichia coli* was instrumental in the development of the operon model[1], and today is one of the most commonly used enzymes in molecular biology. Here we report the structure of this protein and show that it is a tetramer with 222-point symmetry. The 1,023-amino-acid polypeptide chain[2,3] folds into five sequential domains, with an extended segment at the amino terminus. The participation of this amino-terminal segment in a subunit interface, coupled with the observation that each active site is made up of elements from two different subunits, provides a structural rationale for the phenomenon of α-complementation. The structure represents the longest polypeptide chain for which an atomic structure has been determined. Our results show that it is possible successfully to study non-viral protein crystals with unit cell dimensions in excess of 500 Å and with relative molecular masses in the region of 2,000K per asymmetric unit. Non-crystallographic symmetry averaging proved to be a very powerful tool in the structure determination, as has been shown in other contexts[31,32].

Crystals of *E. coli* β-galactosidase with four tetramers (each of M_r 465.412K) per asymmetric unit (space group $P2_1$; a = 107.9 Å, b = 207.5 Å, c = 509.9 Å, β = 94.7°) were obtained as described[4]. To improve the multiple isomorphous replacement phases, which were of poor quality (Table 1), iterative cycles of 16-fold averaging and reconstruction were carried out initially at 5 Å resolution and ultimately extending to 3.5 Å resolution. The resulting map (Fig. 1*a*) exhibited extremely good connectivity throughout the entire polypeptide chain, was easily interpretable, and was consistent with the amino-acid sequence[2,3]. The current, partially refined[5], model has good geometry (Table 1), and includes all residues for the sixteen independent chains, as well as 148 water molecules and two bound magnesium ions per monomer. Representative electron density for the active site region of monomer A is shown in Fig. 1*b*.

The crystal structure shows β-galactosidase to be a 222-point symmetric tetramer with dimensions of roughly 175 × 135 × 90 Å along the respective two-fold axes. The constituent monomers form two different monomer–monomer contacts that we refer to as the 'activating' interface and the 'long' interface. The 'activating' interface, seen in Fig. 2*a* as the region of contact between the green/blue (or red/yellow) dimers, includes contacts between residues near the amino termini, and also includes two helices from each monomer that are packed together to form a four-helix bundle (Fig. 2*b*). In addition, as discussed below, an extended loop reaches across this interface to complete the active site of the neighbouring monomer (Fig. 2*b*). The 'long' interface is formed by contacts between red/green and blue/yellow dimers. This interface consists of two regions of contact, one of which includes residues near the middle of the sequence and

* Present address: Immunex Corporation, 51 University Street, Seattle, WA 98101, USA.
† To whom correspondence should be addressed.