Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2370–2374, March 1995
Genetics

Identification of essential nucleotides in an upstream repressing sequence of *Saccharomyces cerevisiae* by selection for increased expression of *TRK2*

(transcriptional regulation/negative regulation)

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Communicated by Gerald R. Fink, Whitehead Institute for Biomedical Research, Cambridge, MA, October 31, 1994

ABSTRACT **The *TRK2* gene in *Saccharomyces cerevisiae* encodes a membrane protein involved in potassium transport and is expressed at extremely low levels. Dominant cis-acting mutations (*TRK2^D*), selected by their ability to confer *TRK2*-dependent growth on low-potassium medium, identified an upstream repressor element (URS1-*TRK2*) in the *TRK2* promoter. The URS1-*TRK2* sequence (5′-AGCCGCACG-3′) shares six nucleotides with the ubiquitous URS1 element (5′-AGCCGCCGA-3′), and the protein species binding URS1-*CAR1* (URSF) is capable of binding URS1-*TRK2 in vitro*. Sequence analysis of 17 independent repression-defective *TRK2^D* mutations identified three adjacent nucleotides essential for URS1-mediated repression *in vivo*. Our results suggest a role for context effects with regard to URS1-related sequences: several mutant alleles of the URS1 element previously reported to have little or no effect when analyzed within the context of a heterologous promoter (*CYC1*) [Luche, R. M., Sumrada, R. & Cooper, T. G. (1990) *Mol. Cell. Biol.* 10, 3884–3895] have major effects on repression in the context of their native promoters (*TRK2* and *CAR1*). *TRK2^D* mutations that abolish repression also reveal upstream activating sequence activity either within or adjacent to URS1. Additivity between *TRK2^D* and *sin3*Δ mutations suggest that *SIN3*-mediated repression is independent of that mediated by URS1.**

In *Saccharomyces cerevisiae*, cis-acting upstream repressing sequences (URSs) are of two types: sequences that repress expression of co-regulated genes, such as a-specific genes in *MATα* cells (1), and the elements that repress genes regulated by unrelated stimuli. The URS1 element identified in the *CAR1* promoter is the archetypal element of the latter class (2–4). URS1 binds to a protein(s) (URSF) proposed to function as a transcriptional repressor(s) (4–6). URS1-related sequences are present in the 5′ flanking region of a large set of apparently unrelated genes (3, 4). In several cases, these sequences were shown to be required for transcriptional repression in the context of their native promoters: *CAR1* (2), *CTT1* (7), *CYC7* (8), *ENO1* (9), *SPO13* (10), and *SSA1* (5, 11). Moreover, URS1-related sequences can compete with the *CAR1* URS1 for binding to URSF, suggesting that they represent cis-acting sites for a common negative transcription factor or a family of proteins with similar DNA-binding specificity (4).

In *S. cerevisiae*, *TRK1* and *TRK2* encode membrane proteins required for the uptake of K^+ (12, 13). In strains deleted for *TRK1* (*trk1*Δ), the expression of *TRK2* is too low to allow growth on low-K^+ medium (0.2 mM) (14). By selecting for mutants that allow *trk1*Δ *TRK2* cells to grown on medium containing 0.2 mM KCl, two trans-acting factors, products of *RPD1* (*SIN3*) and *RPD3* (15, 16), were identified. In this report we show that *TRK2* is also repressed by a URS1 element, URS1-*TRK2*, and that this repression occurs independently of *SIN3*-mediated repression. Our analysis of 17 independent spontaneous mutations (*TRK2^D*) that increase *TRK2* expression allowed a rigorous analysis of the key sequence specificities of URS1-mediated repression at this locus and revealed that conserved sequences in URS1 previously reported not to be required for repression when analyzed at a heterologous promoter (4) are, in fact, essential for URS1-mediated repression when tested within the context of native promoters.

MATERIALS AND METHODS

Strains and Growth Assays. The genotype of A159 is *MAT***a** *trk1*Δ *ura3-52 his3*Δ*200 trp1*Δ*1*. M398 and M476 strains (16) and strain RH218 (4) were described previously. Regular, low-salt, arginine, and asparagine media have been described (4, 12, 17). Growth on the low-K^+ plates was monitored after 24 hr of incubation at 30°C.

Oligonucleotides. Oligonucleotides are shown in Table 1.

Construction of URS1-*CAR1* Mutants. URS1 mutations were made in the context of the native *CAR1* promoter by site-directed mutagenesis of the URS1 sequence contained in plasmid pRS46 (4), using the four-primer method (18).

Plasmids. *TRK2* and *TRK2^D* strains [isogenic with R1155 (12)] were crossed with A159 to generate *his3* recombinants. These were transformed with linearized pCK60-1 (13) to retrieve WT and mutant *TRK2* promoter regions by the method of integration and excision (19). The rescued plasmids from the *TRK2^D* strains conferred a $Trk1^+$ phenotype on A159. *TRK2*::*lacZ* fusions were generated by inserting *TRK2* 0.7-kb *Eco*RV–*Xba* I fragments (13) into the rescued plasmids and cloning the resulting *Xho* I–*Xba* I inserts into YEp357 and YIp357 (20). Upstream activating sequence 1 (URS1)-containing and UAS1-lacking *CYC1*::*lacZ* fusion plasmids [pLG312Z and pLG178, respectively (21)] were linearized with *Xho* I prior to insertion of the DE, FG, and HI oligonucleotides. Exonuclease III-mediated 5′ deletions of the *TRK2* promoters were generated in the *TRK2* rescued plasmids and tested upon integration into strain A159.

β-Galactosidase Assays. Plasmids were transformed in yeast as described (22). Cells were grown in glucose-containing medium at 30°C. β-Galactosidase assays were as described (21, 23).

Protein–DNA Binding. Protein extracts (from strain M398), protein–DNA binding assays, and electrophoresis were as described (4), except that 1 ng of γ-^{32}P-end-labeled DNA fragment, a 500-fold excess of calf thymus DNA, and a 50- or 100-fold excess unlabeled competitor DNA were used.

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Abbreviations: UAS, upstream activation sequence; URS, upstream repressing sequence; WT, wild type.

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Table 1. Double-stranded oligonucleutides

Name	Sequence (specificity)
DE	5′-TCGACGGCCGTGCGGCTGAAAAAGAGAAAG-3′
	3′-GCCGGCACGCCGACTTTTTCTCTTTCAGCT-5′ [(*TRK2* or wild-type (WT)]
FG	5′-TCGACGGCCGTGCGACTGAAAAAGAGAAAG-3′
	3′-GCCGGCACGCTGACTTTTTCTCTTTCAGCT-5′ (*TRK2^D-6*)
HI	5′-TCGACGGCCGTGCTAATGAAAAAGAGAAAG-3′
	3′-GCCGGCACGATTACTTTTTCTCTTTCAGCT-5′ (*TRK2^D-1,6,9*)
URS1	5′-TTCTAGCGCGCTCCTGCCGCACGCGGTAGCCGCCGAGGGGTCTAAAGAGTACTAGC-3′
	3′-TCGCGCGAGGACGGCGTGCGCCATCGGCGGCTCCCCAGATTTCTCATGATCGTCTT-5′

RESULTS

Structure of the *TRK2* Promoter. The *TRK2* promoter was defined by 5′ deletions that reduced *TRK2*-dependent growth on K^+-limiting medium (7 mM K^+) (Fig. 1). The position of a putative UAS element in the region identified by these deletions was further defined by using a constitutively derepressed *TRK2* promoter in which the URS1 element was mutated (*TRK2^D-1*; see below), which allowed a more sensitive growth test. Deletion to nt −922 retained a stronger growth phenotype than deletion to nt −910. Thus, the promoter region downstream of nt −922 is sufficient for WT *TRK2* expression and the sequence encompassing the −922 to −910 region may contain an UAS (Fig. 1).

TRK2 lacks a canonical TATA sequence in the immediate 5′ nontranslated region and, although two degenerate heat shock-responsive elements (24) are found at −426 and −133 (Fig. 2), *TRK2* expression was not increased in cells shifted to elevated temperatures (data not shown). A 6-nt element, 5′-GCGGCT-3′, at nt −105 to −100 (Fig. 2), is identical, although in the opposite direction, to the core of the upstream repression sequence (URS1) 5′-TCGGCGGCTA-3′ originally identified at *CAR1* (−148 to −157) and shown to mediate the repression of many yeast genes (3, 4).

***TRK2* Is Repressed by URS1.** Dominant, cis-acting mutations at *TRK2* (*TRK2^D*) that increase K^+ uptake and thus confer growth of *trk1*Δ cells on K^+-limiting medium (14) were cloned and sequenced and found to reside in any of three adjacent nucleotides in this URS1-like sequence. WT and *TRK2^D* promoters, recovered by the integration–excision method (19), were fused to the *Escherichia coli lacZ* gene on integrative and multicopy plasmids. Cells expressing *TRK2^D*::*lacZ* exhibited 5- to 10-fold more β-galactosidase levels than cells expressing *TRK2*::*lacZ* (Fig. 3*A*). A 25-bp oligonucleotide encompassing the WT URS1–*TRK2* element, when inserted in either orientation into the *CYC1* UAS-containing promoter fused to *lacZ*, reduced expression by a factor of 7 (Fig. 3*B*). In contrast, a 25-bp oligonucleotide derived from the *TRK2^D-6* mutant failed to repress *CYC1* transcription (Fig. 3*B*).

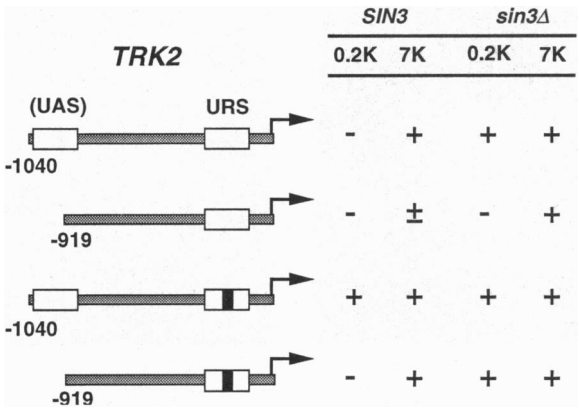

FIG. 1. Additive effects of *TRK2* UAS, *TRK2* URS1, and *SIN3* on *TRK2*-dependent growth. Plus and minus signs indicate optimal and no growth, respectively. KCl concentrations (0.2 and 7 mM) are indicated. The region required for the $Trk1^+$ phenotype is indicated (UAS). The URS boxes with a black line correspond to the *TRK2^D-1* allele.

URS1-*CAR1* and URS1-*TRK2* Bind Related Protein(s). Both the URS1-*CAR1* and the URS1-*SSA1* elements bind to a putative repressor-binding protein(s) (4, 5). Further, URS1-*SSA1* specifically competes with URS1-*CAR1* for binding, suggesting either that an identical protein (URSF) binds both sites or that different repressor proteins share similar DNA-binding specificities (4). To determine whether URS1-*TRK2* could bind a similar protein, radiolabeled double-stranded oligonucleotides carrying the different URS1 sequences were used as probes in gel mobility-shift assays (25) with protein extracts from a WT strain.

The URS1-*CAR1* oligonucleotide formed a stable DNA/protein complex with a protein contained in the yeast crude extract (Fig. 4*A*, lane 2). This complex formation was specifically inhibited by an excess of either unlabeled URS1–*CAR1* oligonucleotide (Fig. 4*A*, lanes 3 and 4) or URS1–*TRK2* oligonucleotide (Fig. 4*A*, lanes 5 and 6). The URS1-*TRK2*-dependent competition required nucleotides identical to URS1-*CAR1*, since the URS1-*TRK2^D-1,6,9* triple mutant failed to compete in the same conditions (Fig. 4*A*, lanes 7 and 8).

In the converse experiment, radiolabeled URS1–*TRK2* oligonucleotide formed a stable DNA–protein complex with mobility cmparable to that of the URS1-*CAR1*/URSF complex (Fig. 4*B*, lane 2). Formation of this complex was inhibited by the addition of unlabeled URS1-*TRK2* oligonucleotide (Fig. 4*B*, lanes 3 and 4) or URS1-*CAR1* oligonucleotide (data not shown), but not by oligonucleotide carrying either the URS1-*TRK2^D-6* mutant element corresponding to one of the mutations isolated *in vivo* or the triple mutant URS1-*TRK2^D-1,6,9* element (Fig. 4*B*, lanes 5–8). Thus, URS1-*CAR1* and URS1-*TRK2* bind to the same protein species or to different proteins sharing similar DNA sequence specificities.

Sequence of the *TRK2^D* Alleles. Key URS1-*TRK2* nucleotide sequence requirements were identified by determining the sequences of 17 independent *TRK2^D* mutations. Each was found to alter one of three adjacent nucleotides (5′-GCC-3′) within the core of the element (Table 2). Two of these nucleotides (C at −103 and G at −101) are essential for URS1-*TRK2* activity, since any substitution abolished repression (Table 2). Mutant URS1-*TRK2* elements with either a T or a G at −102 were also nonfunctional. Whether replacement of this nucleotide by an A had no effect or whether the mutation was simply not present in the mutant collection is unknown.

A comparison of our results with those of Luche *et al.* (4) revealed major differences in the structural features required for URS1-mediated repression (Table 2). First, although any mutation at positions 5 and 6 essentially abolishes repression by URS1-*CAR1* when tested at the heterologous *CYC1* promoter (4), none of these mutations was found among our *TRK2^D* mutants (Table 2). Although a bias inherent in the selection cannot be ruled out, the fact that all 17 *TRK2^D* mutations mapped to positions 2, 3, or 4 suggests that nt 5 and

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-1040 TATAGCCCATTGTTATAAAAATATGTATGTGCTTCAATATAGTGGTATATAGGTNGTTATTTGCGCTGAATGA
 -967 TGTACTTGTCAATGTCTGCGGCTCATTTCCCACCAACTTGTTTTCCCAGTCGACCCAAAGCGCCTCCTTTGGA
 -894 TTCCAAAGTCCCCTCTGGACACACTTCTGCAGGTCCATCCCGAACGTCGGGTCTGTGTGCTTGTATTTGAGCA
 -821 CTGAATCCAGCTTTGGAATCTGCCTTTGCAACGAAACTGGGCCTTGCGGGCACGAAGTTCTCATGTATTGCAG
 -748 CTGGTCCCACGTCGGGAAAGCGGTTGGCGATTTCCACGTCGATCGATGAGAGACGTCTCCCTTCAGCAACTGC
 -675 AGGCATTTCTGCGACTTGATATTGGACGCGATGTCATTGGTAAACAACGAGATGGTACGTGGCTGTCTCTGCA
 -602 ATGTTTTCCAAAACGACATTTATTGCGTTTGCGTTTGCGATCTCCCGGTCTGTTATTTTCATTTGCACTTTTC
 -529 TTAACGTTCTCTTTTGGTTTTATATGCCCCTTTCCAAGCTGGGTGGATACCCCGTCCATTGAGTGCCCGTGCC
 -456 CAATCAAGCTAAAGCCAGACTAAAAAGAGGAAGAGTTCAAGAACCATCAATGCCTCCAGCACAATTTCACAAC
 -383 AGCTTGACACTTTGGCAACGGTTATATCAGAGCATAGCGACGGGATTAGAGAGATGCAAGACGACGGAAGAAA
 -310 GAAGGGAGTGCCCGGCATCGGCTGAAAGCGCCCTGTAGTACAAGCGTACCGCCGTTGAATAGGCCATGGTACA
 -237 TCACACGATGATTGATAATTTGATGCAAGCTGCCATGCTCAGATGGTTGTTTCCCGTTTCTCTCTTTCACTTT
                                                              URS1
 -164 TGATCTCCTGCGAAGAATAGGATGAGATGTGAATTTTCGTTTCTAATCCCCTCGGCCGTGCGGCTGAAAAAGA
  -91 GAAATGATATTGGAATAACTAGGTTTCAGATGATGAGAAAAGAGGCTATTTTGTACTATTCACCGACGATAAG
  -18 AGGCTGTAAGAACCACTCATGCCAACAGCTAAGAGGAC
       1 MetProThrAlaLysArg
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FIG. 2. DNA sequence of the *TRK2* promoter. Nucleotides are numbered with +1 as the first ATG 3′ of the transcription start sites (see Fig. 3). Two regions resembling heat shock-responsive elements at −427 and −134 are indicated by arrows. The six nucleotides identical to the core of URS1 are boxed. The 25-mer oligonucleotide used in Figs. 3, 4, and 6 is indicated by stippled underlining. Transcription start sites at −48, −38, and −35 detected in the *TRK2^D-1* mutant (data not shown) are indicated by the bent arrows.

6 are not essential for URS1 repression of *TRK2*. In addition, identical mutations in the conserved core regions of URS1-*TRK2* and URS1-*CAR1* abolish repression at the former but have been reported to have little or no effect at the latter. For example, G → C and G → A mutations at position 2 disrupt repression at *TRK2* but retain 80% and 87% of repressing activity in URS1-*CAR1* when assayed at *CYC1* (4). More strikingly, whereas the C → T mutation at position 3 in URS1-*CAR1* retained 100% or repressing activity in the context of *CYC1* (Table 2), the corresponding URS1-*TRK2* mutation was shown to disrupt repression at *TRK2*; it not only conferred a strong *TRK2*-dependent growth phenotype and a 5-fold increase in *TRK2* transcription but also abolished the ability of the URS1-*TRK2* element to compete for binding to URSF *in vitro* (Fig. 4 and data not shown).

To assess structural differences between URS1-*TRK2* and URS1-*CAR1* as well as the possible effect of context on function, three site-directed mutations at position 3 (C → T, A, G) and one at position 4 (C → G) were made in URS1-*CAR1* at positions corresponding to mutations selected at URS1-*TRK2*, and their effects were tested in the native context of the *CAR1* promoter. The C → G mutation at position 4 [identical to the original *CAR1* mutation that defined URS1 (2)] abolished repression at both *CAR1* (Table 2 and Fig. 5) and *CYC1* (4). However, the other three mutations had significantly greater effects on repression at *CAR1* than at *CYC1*. In the most extreme case, the C → T mutation at position 3, which retained WT levels of repression at *CYC1* (4), essentially abolished repression at *CAR1* (Table 2 and Fig. 5). Structural differences between the URS1-*CAR1* and URS1-*TRK2* elements were strongly suggested by the observation that the C → T URS1-*TRK2* mutation at position 3 disrupted

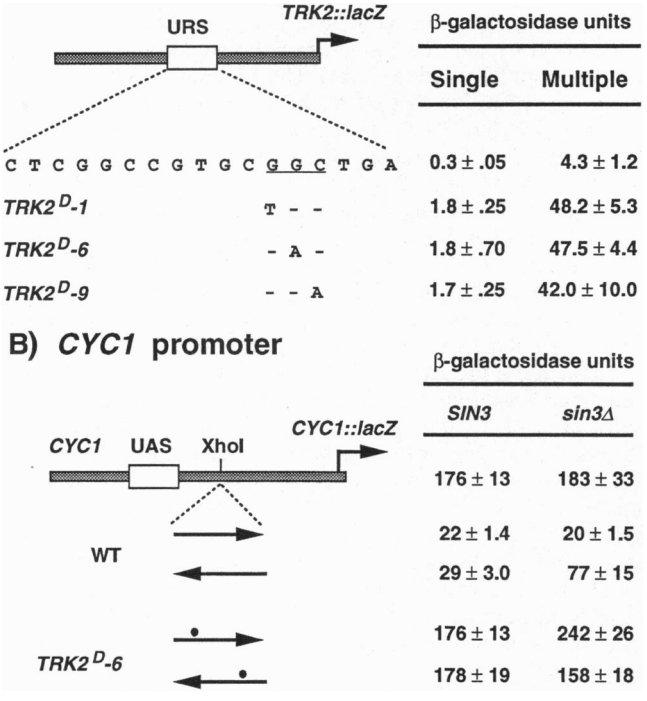

FIG. 3. URS1-*TRK2* is necessary and sufficient for repression. (*A*) WT and *TRK2^D* promoters fused to *lacZ* on an integrated (single) or multicopy (multiple) plasmids. The top line shows the *TRK2* promoter region. The sequence of the WT URS1-*TRK2* is shown with the respective nucleotide change in three *TRK2^D* alleles analyzed. (*B*) The top line shows the promoter region of *CYC1*::*lacZ* fusion contained in the multicopy plasmid pLG312Z (21) carrying the *CYC1* UAS1. Wild-type *TRK2* oligonucleotide and its *TRK2^D-6* derivative are represented by an arrow and an arrow with a single dot, respectively.

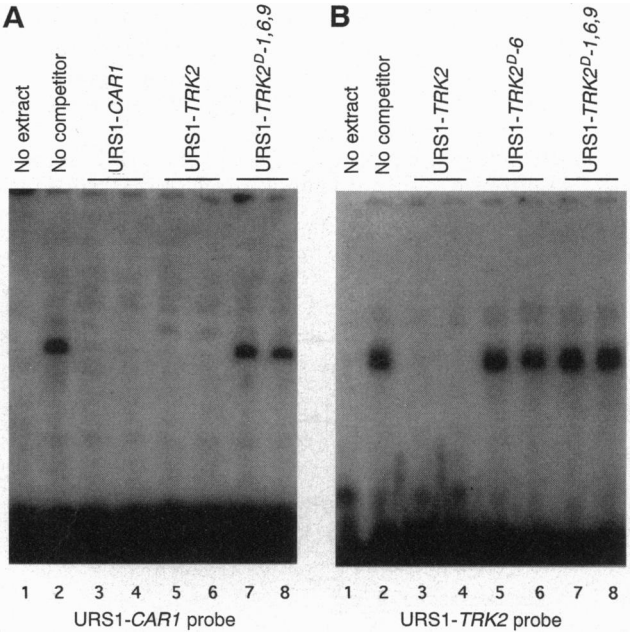

FIG. 4. URS1-*CAR1* and URS1-*TRK2* bind related protein(s). Radiolabeled URS1-*CAR1* and URS1-*TRK2* oligonucleotides were used in *A* and *B*, respectively. Lanes 1, probe only; lanes 2, absence of competitor DNA; lanes 3–8, 50-fold (odd numbers) and 100-fold (even numbers) molar excess of competitor DNA.

Table 2. Comparisons between essential nucleotides in URS1-*CAR1* and URS1-*TRK2* determined within native and heterologous promoter contexts

	Sequence position*									Repression	
USR1	1	2	3	4	5	6	7	8	9	Native context†	*CYC1* context‡
CAR1	A	G	C	C	G	C	C	G	A	100%	100%
			T							0%	100%
			A							0%	50%
			G							21%	67%
				G						0%	0%
TRK2	A	G	C	C	G	C	A	C	G	+	
TRK2^D-8		A								−	
TRK2^D-9		T								−	
TRK2^D-18		A								−	
TRK2^D-19		A								−	
TRK2^D-20		C								−	
TRK2^D-6			T							−	0%
TRK2^D-7			G							−	
TRK2^D-12			T							−	
TRK2^D-1				A						−	
TRK2^D-3				G						−	
TRK2^D-4				A						−	
TRK2^D-5				T						−	
TRK2^D-10				T						−	
TRK2^D-11				G						−	
TRK2^D-13				A						−	
TRK2^D-15				A						−	
TRK2^D-16				T						−	

*No. 1 was assigned to the first nucleotide of URS1-*CAR1* in its native orientation and corresponds to nt −157 and −99 at *CAR1* (4) and *TRK2*, respectively.

†Numbers indicate % repression activity measured at the *CAR1* gene for a mutant URS1, relative to the WT URS1 activity [% = (347 − *X*)/(347 − 33) × 100, where 347, 33, and *X* are the specific β-galactosidase activities measured under activation or repression conditions for the WT element and repression conditions for the mutant element, respectively (see Fig. 5)]. For URS1-*TRK2* function tested in the *TRK2* promoter by growth phenotype on low K^+ concentrations in *trk1*Δ cells, + and − indicate functional and nonfunctional elements, respectively.

‡Percent repression activity measured at the *CYC1* gene for a mutant URS1, relative to the WT URS1 activity [% = (200 − *X*)/(200 − 13) × 100, where 200, 13, and *X* are the specific β-galactosidase activities measured from the *CYC1* promoter alone, *CYC1* combined with the WT URS1 element, and *CYC1* with mutated versions of URS1, respectively (see ref. 4)].

repression within the *CYC1* context (Fig. 3*B*) whereas the C → T URS1-*CAR1* mutation at position 3 had no effect within the *CYC1* context (4). Thus, both context and structural differences between the URS1 elements play a significant role in URS1-dependent repression.

Region Encompassing URS1-*TRK2* Has Endogenous UAS Activity. The URS1 element may be involved with transcriptional repression and activation of downstream genes (26, 27). To test for such activity at URS1-*TRK2*, the endogenous UAS1 element of the *CYC1* promoter fused to *lacZ* was replaced with WT or mutant URS1-*TRK2* sequences. β-Galactosidase activities measured in cells containing these fusions showed that replacement by one or three copies of the WT URS1-*TRK2* had no substantial effect on *CYC1* expression. However, when repression-defective URS1-*TRK2^D* elements were inserted, a 4-fold (single copy) to 40-fold (three copies) increase in expression was observed (Fig. 6). Thus, UAS activity associated with URS1-*TRK2* was detected only when the repressing function of URS1-*TRK2* was abolished by mutation.

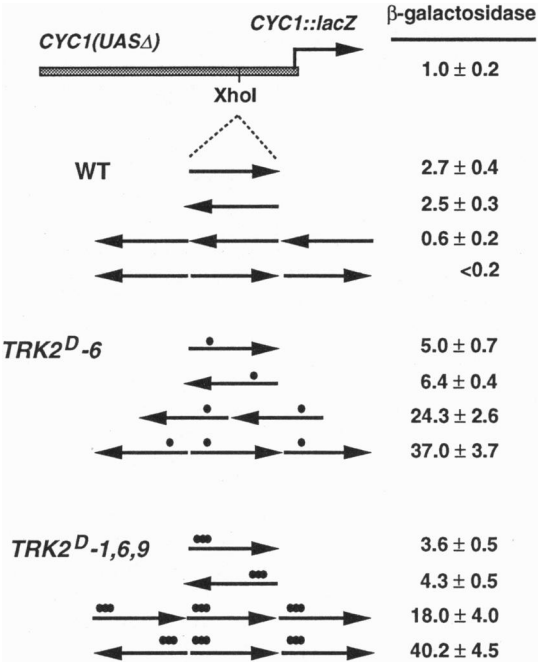

FIG. 6. The sequence encompassing URS1-*TRK2* has weak UAS activity. The promoter region of a *CYC1::lacZ* fusion which lacks an endogenous UAS is contained in the multicopy plasmid pLG178 at top. WT *TRK2* oligonucleotide and its *TRK2^D-6* and *TRK2^D-1,6,9* derivatives inserted in pLG178 are represented below.

Independence of *SIN3*- and *URS1*-Mediated Repression. *SIN3* has been identified in at least six independent screens on the basis of the loss of repression of various structural genes [*HO* (28, 29), *SPO13* (30), *IME2* (27), *INO1* (31), and *TRK2* (16)]. Interestingly, each of these *SIN3*-regulated genes contains a similar URS element (refs. 10, 27, and 31; D. Stillman, personal communication; and this report). The results of two complementary experiments suggest that *SIN3* mediates *TRK2* repression through a site independent of URS1-*TRK2*. First, WT URS1-*TRK2* repression of the heterologous *CYC1* pro-

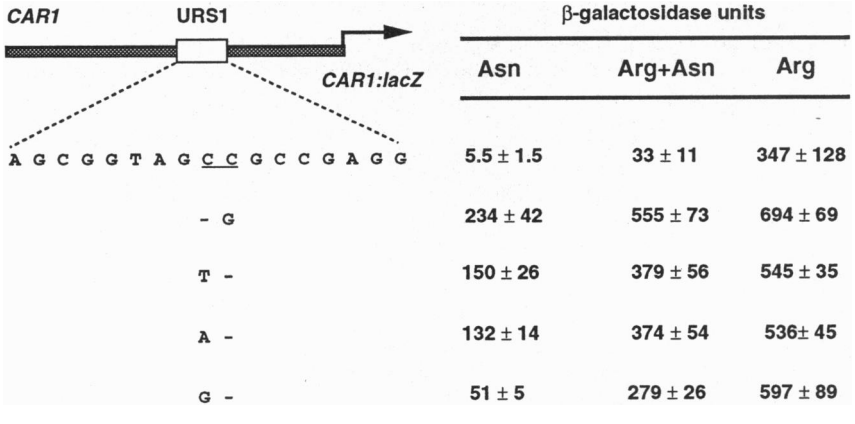

FIG. 5. URS1 nt 3 is essential in the context of the *CAR1* promoter. WT and mutant *CAR1* promoters were fused to *lacZ* on a multicopy plasmid. The sequence of the WT URS1-*CAR1* is shown with the respective mutations analyzed. Cells were grown under repression (Asn and Arg+Asn) or activation (Arg) conditions.

moter was not *SIN3*-dependent (Fig. 3*B*). Second, although *TRK2* expression was derepressed in cells deleted for *SIN3*, $TRK2^D$ mutations further increased derepression in *sin3*Δ cells (Fig. 1). Thus, the phenotypes of *sin3*Δ and $TRK2^D$ are additive.

DISCUSSION

URS1-*TRK2* acts as a cis-acting element both necessary and sufficient for repression: mutations selected *in vivo* relieve *TRK2* repression and the WT element is sufficient to repress the heterologous *CYC1* promoter. A six-nucleotide core (5′-AGCCGC-3′; Table 2) within URS1-*TRK2* is identical to that contained in URS1-*CAR1* and both elements can compete for binding to the same putative repressor protein, URSF.

URS1-*TRK2* masks the activity of an UAS element present in the same 25-bp region (−112 to −88) of the *TRK2* promoter. Expression levels from the *CYC1* promoter are increased ≈4-fold when a repression-defective mutant the URS1-*TRK2* element is present (40-fold with three elements). Conceivably this cryptic UAS activity is brought to bear under conditions that relieve URS1-mediated repression. Similar situations have been described at *SSA1* (26), *IME2* (27), and *CAR1* (32). We believe it unlikely that the $TRK2^D$ mutations create fortuitous activator binding-sites, since a mutant element containing substitutions at all three key URS1-*TRK2* nucleotides retains the UAS activity, as do insertions of multiple elements in different orientations.

The analysis of 17 independent cis-acting mutations selected *in vivo* identified three nucleotides crucial for URS1-dependent repression of *TRK2*. A disparity in effects of URS1 mutations, when tested at different promoters, revealed the possibility of strong context effects with regard to function of this element. For example, mutations at position 3 (Table 2) in URS1-*TRK2* disrupted repression when tested at *TRK2* or *CYC1*, and yet identical mutations in URS1-*CAR1* disrupted repression only at *CAR1*, with little or no effect at *CYC1* (4). These results suggest that the fidelity of some URS1-related elements varies when assayed outside their native contexts.

Luche *et al.* (4) reported that the sequence required for *CAR1* URS function is a "palindrome" and suggested that its symmetry (AGCCGCCGA) was related to functional constraints, since only the three central nucleotides were found to be essential for repression. The requirement for a cis-acting element to be palindromic by sequence is highly unusual, if not unique. The sequence encompassing the URS1-*TRK2* element differs markedly from such a palindrome: although nt 1–6 match the URS1 consensus, the three nucleotides at the 3′ end of the alignment differ (AGCCGCACG). Thus, the sequence of URS1-*TRK2* reveals that this element does not require a palindromic sequence or symmetry surrounding position 5 for function. Rather, the palindromic nature of URS1-*CAR1* may reflect the presence of overlapping core sequences within the element (minimally, GCCG). If so, unlike the URS1-*TRK2* sequence, the URS1-*CAR1* sequence might contain some structural redundancy, and this would explain why some mutations with strong effects in the former have little or no effect in the latter.

We thank L. Guarente, K. L. Ngai, and T. Cooper for plasmids and oligonucleotides and L. Ko for invaluable assistance with DNA–protein binding assays. This work was supported by grants from the National Institutes of Health (GM45739) and the National Science Foundation (DCB-8711346 and DCB-8657150) to R.F.G. and by a fellowship from the Belgian Fonds National de la Recherche Scientifique to M.V.

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