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Induction and Repression of the Urea Amidolyase Gene in *Saccharomyces cerevisiae*

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The *DUR1,2* gene from *Saccharomyces cerevisiae* has been isolated on recombinant plasmids along with all DNA between the *DUR1,2* and *MET8* loci. *DUR1,2* was found to encode a 5.7-kilobase transcript, which is consistent with our earlier suggestion that the *DUR1* and *DUR2* loci are two domains of a single multifunctional gene. Steady-state levels of the *DUR1,2* transcript responded to induction and nitrogen catabolite repression in the same way as urea amidolyase activity. *dal81* mutants (grown with inducer) contained barely detectable amounts of *DUR1,2* RNA, whereas *dal80* mutants (grown without inducer) contained the same amount as a wild-type induced culture. These observations support our earlier hypothesis that *DUR1,2* is transcriptionally regulated, with control being mediated by the *DAL80* and *DAL81* gene products. We cloned the *DUR1,2-O^h* mutation and found it to be a *Ty* insertion near sequences required for complementation of *dur1,2* mutations. The ROAM phenotype of the *DUR1,2-O^h* mutation is sharply different from that of *cis*-dominant, *DUR80* mutations, which enhance *DUR1,2* expression but do not affect the normal control pattern of the gene. There is evidence that *DUR80* mutations may also be *Ty* insertions, which generate phenotypes that are different from those in *DUR1,2-O^h* mutations.

An appreciation for the molecular mechanisms involved in control and integration of procaryotic metabolic pathways has been gained by studying regulons with widely differing physiological functions (28, 31). Similar information is now beginning to accumulate for eucaryotic systems (42). Nitrogen catabolic systems are particularly useful for such investigations, because most are subject to multiple layers of regulation. Genes encoding the allantoin-degradative system in *Saccharomyces cerevisiae*, for example, respond to both induction and nitrogen catabolite repression (15, 25, 38). The five enzyme activities of this system are present at relatively low basal levels unless compounds that can be degraded to allophanate are added to the culture medium (15). In the presence of this native inducer or its gratuitous analog oxalurate the levels of these enzymes dramatically increase (33). Induction requires participation of the *DAL81* gene product, as shown by the observation that *dal81* mutants are unable to increase enzyme production in the presence of an inducer (37). Mutation of a second, putative regulatory locus appears to affect induction in the opposite way, i.e., mutations at this locus (*dal80*) result in high level production of the allantoin-degrading enzymes even when an inducer is absent (8).

Nitrogen catabolite repression is observed when cells are provided with readily used nitrogen sources such as asparagine, ammonia, or glutamine (13). Under these conditions, enzyme activities associated with the degradation of poor nitrogen sources are not observed. For example, the allantoin-degrading enzymes are decreased approximately 100-fold in the presence of asparagine. This loss of allantoin system function derives from at least two processes. All of the active transport systems associated with allantoin metabolism become inoperative after the addition of a readily used nitrogen source to the medium. Loss of transport function involves at least one rapid process, as evidenced by its 3-min half-life after the addition of asparagine to the medium (12). In contrast, enzyme activity is not lost after the addition of a repressive nitrogen source, but continued enzyme synthesis ceases (12). The lack of enzyme induction does not result from inducer exclusion, because *dal80* mutants, which do not require the presence of an inducer for enzyme production, are similarly devoid of allantoin-degrading enzymes when grown in glucose-asparagine medium (8).

Earlier kinetic studies are consistent with the hypothesis that both induction and nitrogen catabolite repression are exerted at gene expression (1–4, 16, 24–26). This hypothesis predicts that steady-state levels of *DUR* and *DAL* gene mRNAs respond to genetic and environmental variation in a manner qualitatively similar to the enzyme levels previously reported. The purpose of this work was to test this hypothesis. Most of our past kinetic experiments utilized urea amidolyase as the representative enzyme activity. This large (204-kilodalton), multifunctional protein catalyzes the urea carboxylase and allophanate hydrolase reactions, which are responsible for synthesis and degradation of the pathway inducer, respectively (33). Therefore, we chose to clone the gene encoding this protein (*DUR1,2*) and used an internal fragment of it to measure the steady-state levels of mRNA present in wild-type and mutant cells grown under conditions of induction or repression or both.

(Preliminary accounts of this work have already appeared [F. S. Genbauffe, G. E. Chisholm, and T. G. Cooper, Abstr. Cold Spring Harbor Symp., p. 267, 1983; T. G. Cooper, D. Platoniotis, H. S. Yoo, R. A. Rai, G. Chisholm, and F. Genbauffe, Abstr. Cold Spring Harbor Symp., p. 247, 1983].)

MATERIALS AND METHODS

Strains and culture conditions. The genotypes of *S. cerevisiae* and *Escherichia coli* strains used in this work are listed in Table 1. Strain constructions and linkage analyses were performed by standard genetic techniques (19, 29). All autonomously replicating vectors were derived from plasmids yRP7 or yRP17 containing *ARS1*.

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TABLE 1. Strains used in this work

Strain designation	Genotype
S. cerevisiae	
M1-2b	*MATa trp1-289 ura3-52*
M693-9R	*MATa dur1-E145 met8-1*
M1014-1c	*MATa dur1-E145 met8-1 trp1-289*
M1417-1c	*MATa dur1-E145 met8-1 trp1-289 ura3-52*
M1417-8b	*MATa met8-1 ura3-52*
M1417-16a	*MATα met8-1* **ura3-52**
M1418-23b	*MATα ade6 dur2-N116 tyr1 ura3-52*
M1666-6a	*MATa durO^h ura3-52*
M1666-11a	*MATα durO^h ura3-52*
RH218	*MATa CUP1 gal2 SUC2 trp1-289*
VT51	*MATa dur1,2 trp1-289*
Σ12786	*MATα* wild type
M970	*MATa lys5* / *MATα lys2*
M1081	*MATa dal80-1 lys5* / *MATα dal80-1 lys2*
M1407	*MATa dal81-2 lys2* / *MATα dal81-2 lys5*
M1667	*MATa durO^h ura3-52* / *MATα durO^h ura3-52*
E. coli	*hsdR hsdM recA13 proA2 supE44 lacZ24* Sm^r *leuB thi-1*

Culture conditions for growth and transformation were those described by Sumrada and Cooper (35, 36). All single nitrogen sources were supplied at a final concentration of 0.1%, except for urea, whose final concentration was 10 mM. The medium on which methionine and uracil prototrophic transformants were selected was yeast nitrogen base (without amino acids or ammonium sulfate; Difco Laboratories) supplemented with 0.5% ammonium sulfate. The medium used to test complementation of various *dur* mutations was Difco yeast nitrogen base medium (without amino acids or ammonium sulfate) provided with 0.1% allantoin as the sole nitrogen source.

Enzyme assay procedures. Urea amidolyase activity was determined with nystatin-permeabilized cells by the method of Whitney et al. (38).

Preparation and analysis of DNA. Plasmid DNA was isolated from transformed strains of *S. cerevisiae* and *E. coli* by the methods of Sumrada and Cooper (35, 36). Chromosomal DNA was isolated from *S. cerevisiae* by two different methods. Chromosomal DNA from strains carrying an integrated plasmid was prepared by the CsCl gradient technique of Hsiao and Carbon (23). This DNA was used to isolate DNA flanking the integration site. Chromosomal DNA used for Southern analysis was prepared as described by Winston et al. (41).

Restriction enzyme digestion, agarose gel electrophoresis, isolation of DNA fragments from agarose, ligation of DNA fragments, and *E. coli* transformation were all conducted by established procedures (35). The structure of each plasmid described in this report was verified by gel electrophoresis with a variety of diagnostic restriction enzyme digests.

Southern blot analysis and radioactive labeling of DNA fragments by nick translation and the T4 polynucleotide kinase reaction were performed by previously published techniques (43).

Preparation and analysis of RNA. Polyadenylated [poly(A$^+$)] RNA was isolated by the method of Carlson and Botstein (7). Cultures used for poly(A$^+$) RNA isolation were grown in Wickerham minimal medium (39) with proline or asparagine (0.1%) provided as the sole nitrogen source. Oxalurate (OXLU; 0.25 mM) was added as an inducer where indicated. Poly(A$^+$) RNA, passaged twice through an oligo(dT) column, was resolved on 1.3% agarose-formaldehyde gels as described by Sumrada and Cooper (35). The RNA was then transferred to nitrocellulose paper (HAHY; Millipore Corp.) by the method of Fryberg et al. (20). RNA species were sized by comparison to DNA standards run on the same gel.

RESULTS

Isolation of the *DUR1,2* gene. We initially attempted, without success, to clone the *DUR1,2* gene by the transformation-complementation method of Hinnen et al. (22). The large size of the polypeptide encoded by *DUR1,2* and our failure to obtain complementation prompted us to presume that the DNA fragment required for complementation was larger than that being routinely cloned into the drug resistant plasmid vectors available at the time (1981 to 1983). Therefore, we adopted the strategy of cloning a closely linked gene

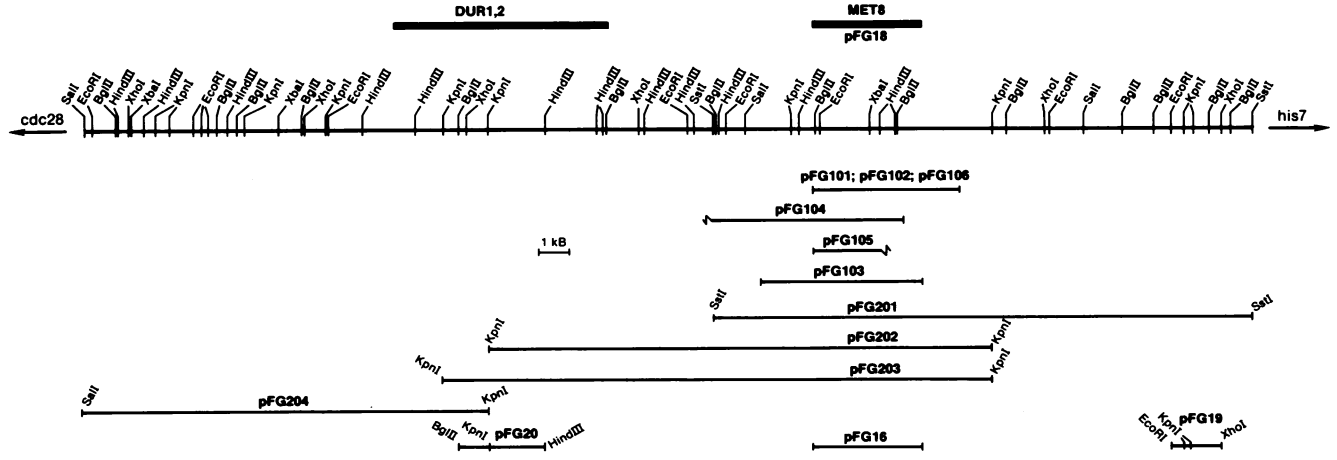


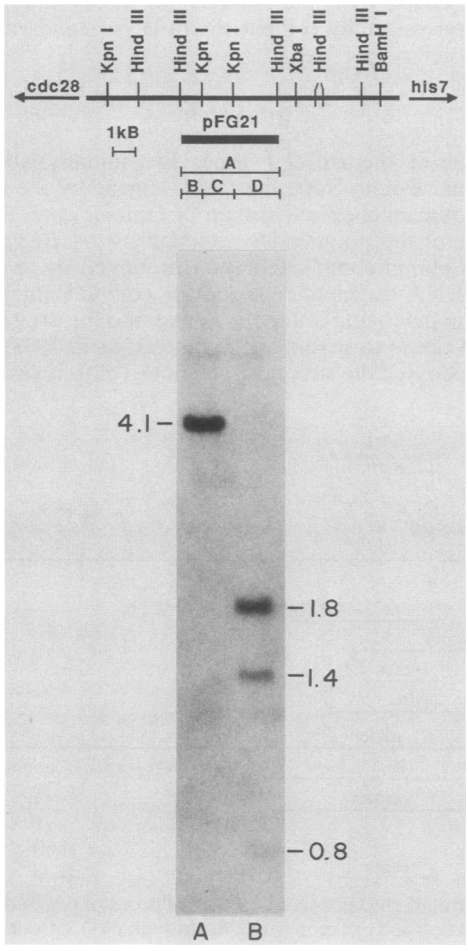
FIG. 1. Composite restriction enzyme map of the *DUR1,2-MET8* region (right arm) of chromosome II. The structures of plasmids isolated by transformation-complementation (pFG101 through pFG106), site-directed integration and excision (pFG201 through pFG204), or recloning (pFG16 through pFG20) are shown below the genomic restriction map.

TABLE 2. Linkage of integrated *URA3* alleles to various loci on chromosome II

Cross[a]	Integrated plasmid	Gene pair	No. of tetrads				Calculated genetic distance (cM)[b]
			Total analyzed	Parental ditype	Nonparental ditype	Tetratype	
I	pFG16	*dur1-met8*	64	57	0	7	5.4
		dur1-URA3		57	0	7	5.4
		met8-URA3		64	0	0	
II	pFG19	*dur2-met8*	71	63	0	8	5.6
		dur2-URA3		57	0	14	9.8
		met8-URA3		65	0	6	4.2
		tyr1-dur2		42	1	28	24
		tyr1-met8		34	1	36	30
		tyr1-URA3		29	2	40	37
III	pFG20	*dur2-met8*	75	68	0	7	4.7
		dur2-URA3		71	0	4	2.7
		met8-URA		64	0	11	7.3
		tyr1-dur2		39	0	26	17
		tyr1-met8		42	0	33	22
		tyr1-URA3		53	0	22	15

[a] Cross I was M1417-1C (pFG16) × M1417-16a, (*MATa dur1-E145 met8-1::MET8 trp1-289 ura3-52::URA3*) × (*MATα met8-1 ura3-52*). Cross II was M1417-8b (pFG19) × M1418-23b, (*MATa met8-1 ura3-52::URA3*) × (*MATα ade6 dur2-N116 tyr1 ura3-52*). Cross III was M1417-8b (pFG20) × M1418-23b, (*MATa met8-1 ura3-52::URA3*) × (*MATα ade6 dur2-N116 tryl ura3-52*).

[b] Genetic distances were calculated by using the formula of Perkins (29).



(*MET8*) and "walking down the chromosome" to the *DUR1,2* locus by integration-excision techniques (41). Implementation of this strategy began with isolation of plasmids that were able to complement a *met8* mutation contained in strain M1014-1c. Twenty-five Met$^+$ transformants were recovered from a library constructed by Lacroute and his colleagues (18). Plasmid DNA prepared from 13 different transformants was used to transform *E. coli*; 7 of the transformations yielded positive results. Six plasmids (pFG101 through pFG106) derived in this manner were able to transform the original methionine auxotroph to prototrophy at high frequency. Restriction mapping of these plasmids revealed a common 3.5-kilobase (kb) region (Fig. 1). This fragment, which alone was able to complement the *met8* mutation, was transferred to an integrative vector (YIp5) (32), yielding plasmid pFG16. Plasmid pFG16 was integrated into the genome of strain M1417-1c in a directed fashion after its digestion with *Xba*I (Fig. 1) (30). Integrants derived from this procedure were isolated as Ura$^+$ colonies and crossed to strain M1417-16a. The resulting diploid strains were sporulated, and their meiotic products were analyzed for linkage of the Met, Dur, and Ura determinants (19, 29). The three determinants cosegregated in all 64 asci examined. Recombination frequencies indicated that integration had occurred approximately 5 map units from the *dur1* locus on chromosome II (cross I in Table 2), the previously reported location of *met8* (14).

Genomic DNA prepared from these integrants was digested with various restriction endonucleases, recircularized

FIG. 2. Verification of the structure of the chromosomal DNA spanning the insert of plasmid pFG21. Chromosomal DNA was prepared from strain M1417-8b and digested with restriction endonuclease *Hin*dIII (lane A) or a combination of *Hin*dIII and *Kpn*I (lane B). These digests were resolved on agarose gels, transferred to nitrocellulose, and probed with plasmid pFG21 that had been radioactively labeled by nick translation as described in the text. The solid bar beneath the chromosomal restriction map depicts the insert of plasmid pFG21.

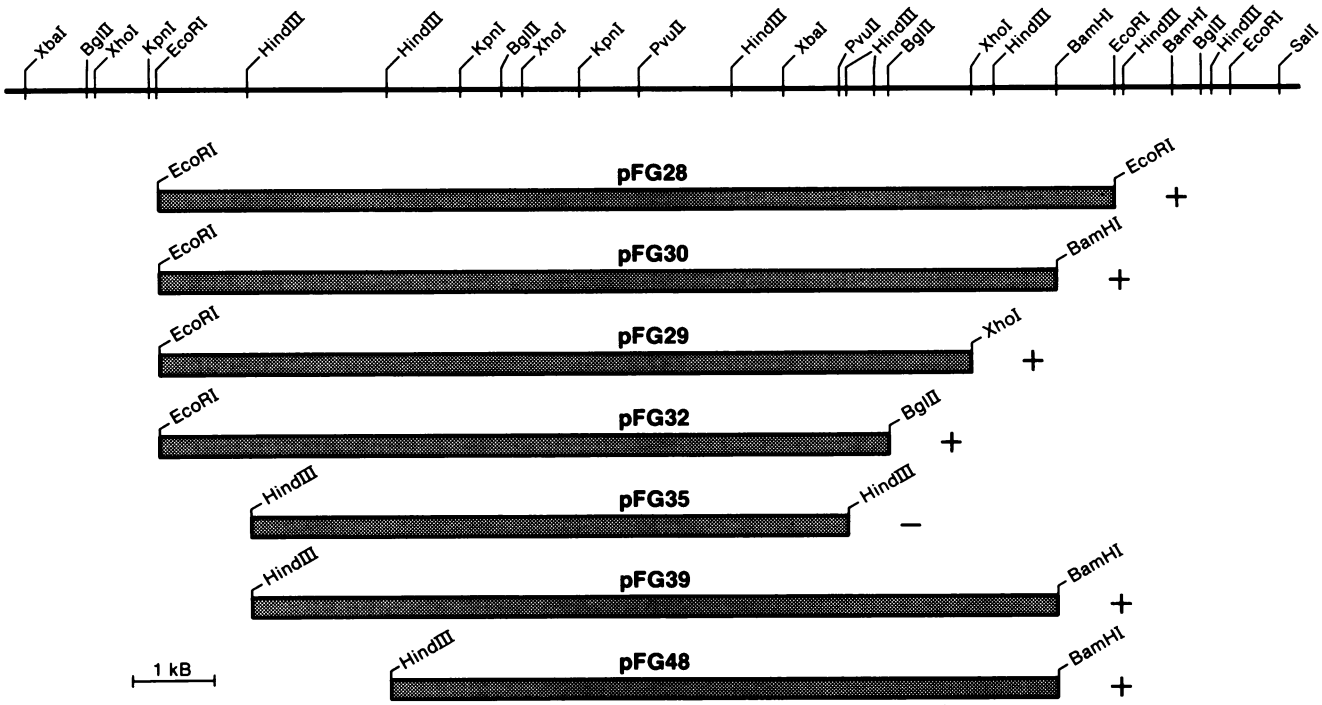


FIG. 3. Localization of the *DUR1,2* gene. The designated portions of plasmid pFG28 were recloned onto vector YRp17 (32). Structures of the resulting plasmids were verified by a series of diagnostic restriction enzyme digests of purified preparations of each plasmid. Each plasmid was then tested for its ability to transform yeast strain VT51 to a Dur^+ Trp^+ phenotype. A plus sign at the end of the insert indicates the capacity to support such high-frequency transformation when allantoin was provided as the sole nitrogen source. A minus sign indicates a lack of this ability.

with DNA ligase, and used to transform *E. coli* to ampicillin resistance. The purpose of this integration-excision procedure was to isolate genomic DNA that flanked the site of integration. Three of the largest plasmids recovered from *E. coli* were selected for further characterization. Plasmid pFG201 was isolated from an *Sst*I digest, whereas plasmids pFG202 and pFG203 were derived from a *Kpn*I digest. These plasmids were used to generate the chromosomal restriction map shown in Fig. 1. Also shown in Fig. 1 is the region covered by each plasmid insert.

Our next objective was to orient the restriction map with respect to known genetic markers. The 1.2-kb *Eco*RI-*Xho*I fragment at the right end of plasmid pFG201 was subcloned onto vector YIp5 (Fig. 1). The resulting plasmid (pFG19) was digested with *Kpn*I and used to integratively transform strain M1417-8b. All integrants recovered were Met^- Ura^+ Dur^+, and one of them was crossed to strain M1418-23b. The linkage relationships derived from recombination frequencies and marker orientations observed among the meiotic products of this cross (Table 2, cross II) suggested the following gene order: *dur2*–5.6 cM–*met8*–4.2 cM–*URA3*. The 2.8-kb *Bgl*II-*Hin*dIII fragment from the opposite end of plasmid pFG203 was similarly subcloned to yield plasmid pFG20. This plasmid was digested with *Kpn*I and used to integratively transform strain M1417-8b. A similar genetic analysis (Table 2, cross III) suggested the following gene order: *URA3*–2.7 cM–*dur2*–4.7 cM–*met8*. The *dur2* marker of this cross was scored by complementation with *MAT***a** or *MAT*α *DUR1 dur2* tester strains. The transformants were urea amidolyase negative but $Dur2^+$, presumably because integration occurred in the 3′ *DUR1* domain of the *DUR1,2* gene. These genetic data suggested that the restriction and genetic maps shown in Fig. 2 are properly oriented with respect to one another. The observation that the insert of plasmid pFG20 was situated centromere proximal to the *dur2* locus prompted us to excise it from the genome of the integrant by digestion with *Sal*I (which cut once in chromosomal DNA and once in the vector). Plasmid pFG204 was isolated after recircularization of fragments generated by this digestion and was used to generate the remaining portion of the chromosomal restriction map shown in Fig. 1.

Deduction of the chromosomal restriction map (Fig. 1) with information derived from plasmids pFG202, pFG203, and pFG204 was potentially complicated in one important respect. The inserts of plasmids pFG202 and pFG203 differ by a 1.4-kb *Kpn*I fragment (Fig. 1). Since both plasmids were obtained by excision with *Kpn*I, the possibility existed that this small *Kpn*I fragment was derived from a location that was not contiguous with the desired DNA on chromosome II. Although the genetic analysis performed after integration of plasmid pFG20 and the structure of the plasmid excised (plasmid pFG204) argued against such a possibility, we wanted direct verification that the 1.4-kb *Kpn*I fragment was situated as shown in our restriction maps. Therefore, we determined the structure of wild-type chromosomal DNA which spanned the *Kpn*I fragment in question. The predicted fragments were found experimentally (Fig. 2), although the small 0.8-kb fragment in lane B is considerably less easily seen in the figure than it was in the original autoradiograph. A similar set of experiments verified the continuity of the chromosomal restriction map derived from the isolation and mapping of plasmids pFG201 and pFG202. In this case, the restriction site in question was the *Xba*I site in the *MET8* gene, and the probe used for verification contained the insert of plasmid pFG16 which spanned that site. Diagnostic digestions were carried out with restriction endonucleases

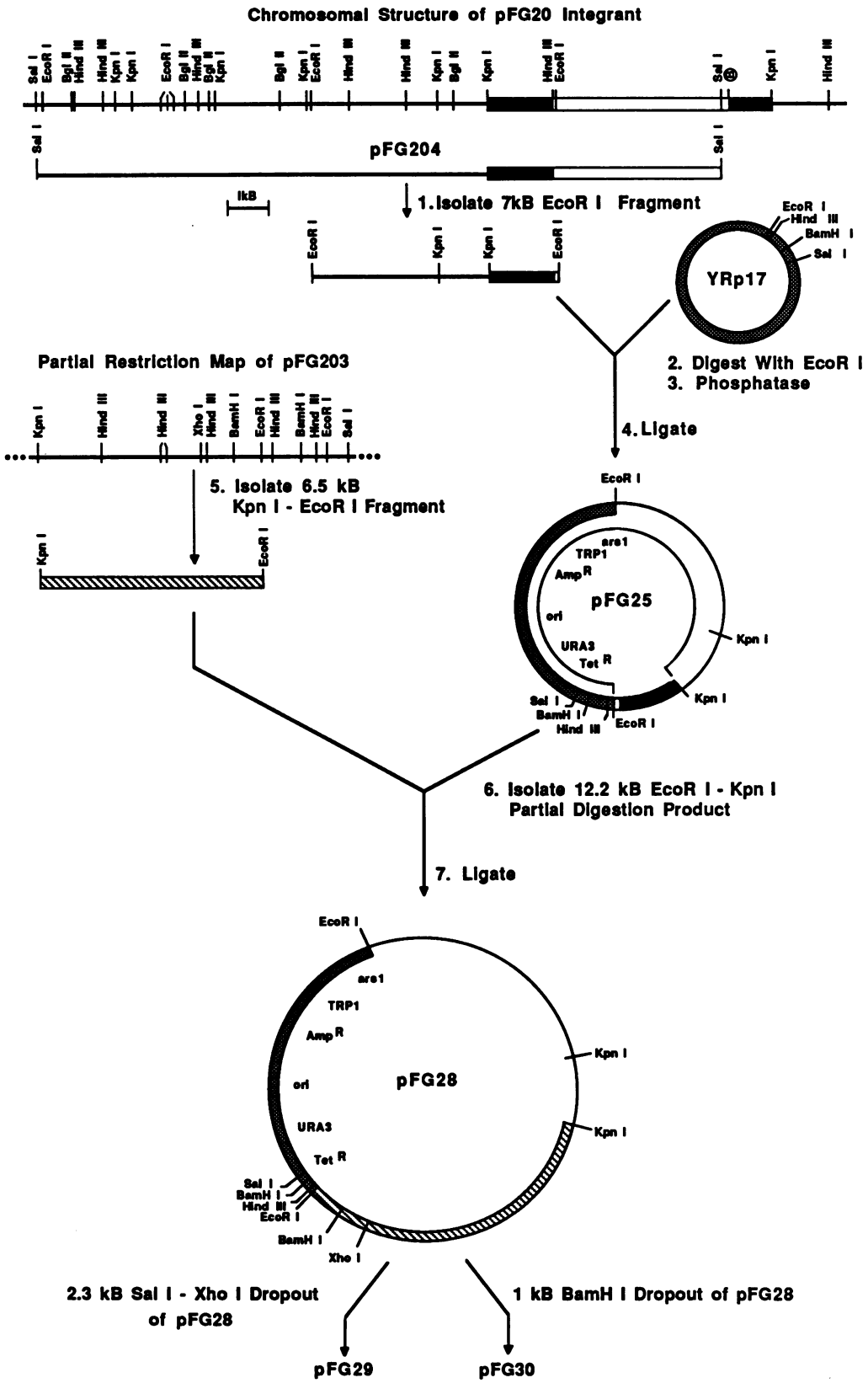


FIG. 4. Strategy used to construct plasmid pFG28. The chromosomal structure of plasmid pFG20 is depicted at the top. Dark bars represent the yeast DNA insert of plasmid pFG20, and the open bars signify YIp5 vector DNA. The 7-kb *Eco*RI fragment of plasmid pFG204 (containing the 3′ end of the *DUR1,2* gene) was cloned into the *Eco*RI site of vector YRp17 (dark hatched circle) in the orientation shown for plasmid pFG25. The 6.5-kb *Eco*RI-*Kpn*I fragment of plasmid pFG203 (containing the 5′ end of the *DUR1,2* gene, light hatched box) was then ligated to the 12.2-kb *Eco*RI-*Kpn*I partial digestion product of plasmid pFG25 to yield plasmid pFG28. Plasmids pFG29 and pFG30 were then constructed by dropping out the 2.3-kb *Sal*I-*Xho*I and 1-kb *Bam*HI fragments of plasmid pFG28, respectively.

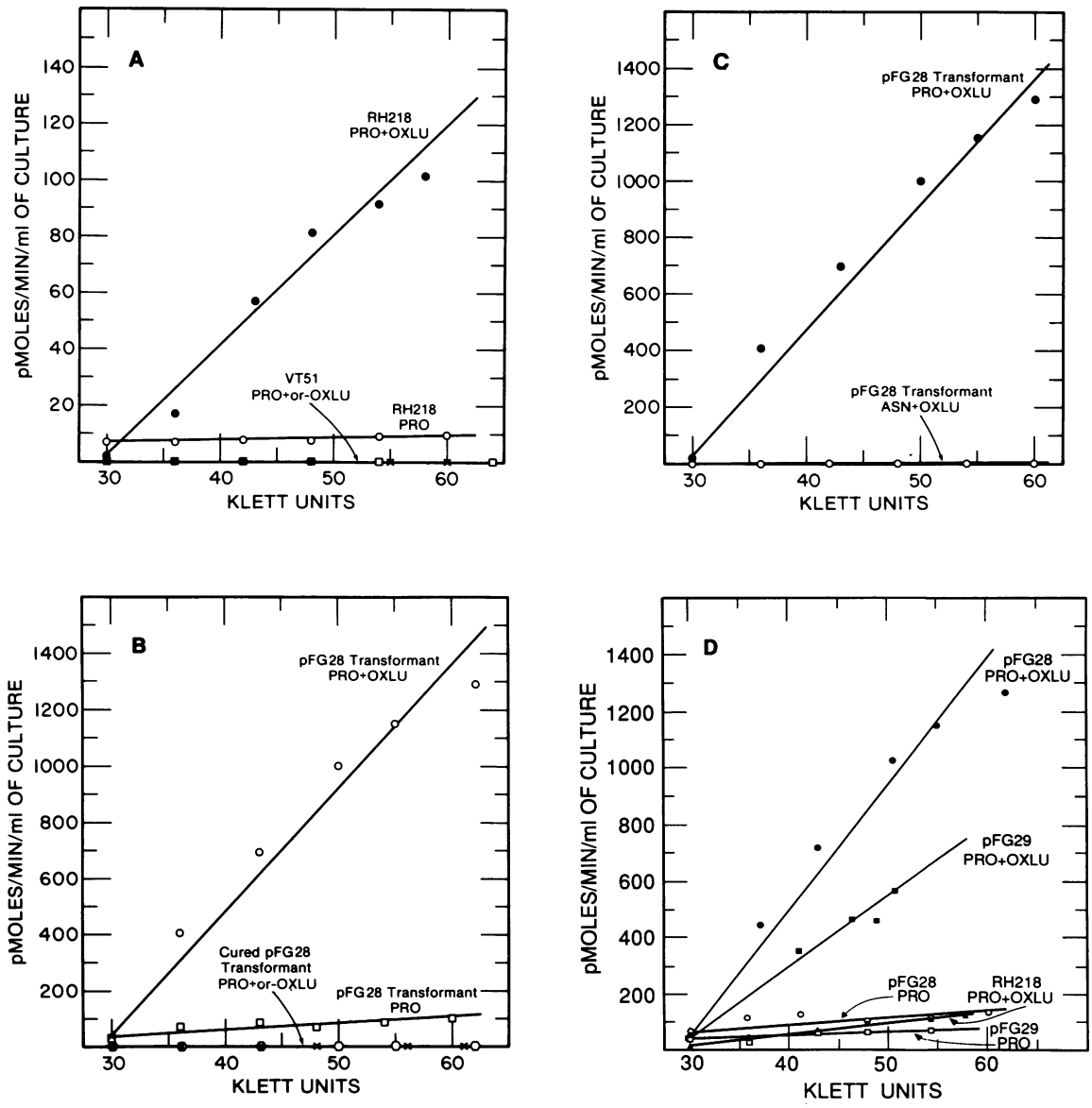


FIG. 5. Differential rate of urea amidolyase activity in a wild-type *DUR1,2* mutant and transformed mutant strains of yeast. Cultures were grown in minimal YNB medium containing the indicated nitrogen sources as described in Materials and Methods. Samples of the cultures were removed at the indicated cell densities and assayed for urea amidolyase activity. (A) Strain RH218 (wild type) provided with proline (a nonrepressive nitrogen source) or proline-OXLU and strain VT51 (*dur1,2 trp1*) grown in glucose-proline medium in the presence or absence of OXLU. (B) Strain VT51, transformed with plasmid pFG28, and a transformant that had been cured of this plasmid (by growth in nonselective medium) grown in proline or proline-OXLU medium. (C) Strain VT51, transformed with plasmid pFG28, grown in minimal YNB medium containing proline-OXLU or asparagine (a repressive nitrogen source)-OXLU. (D) strains VT51, transformed with plasmid pFG28 or pFG29 and a wild-type strain (RH218) grown in minimal proline medium in the presence or absence of OXLU.

*Bam*HI, *Bam*HI-*Xba*I, *Kpn*I, *Bam*HI-*Kpn*I, and *Bam*HI-*Xba*I-*Kpn*I (data not shown).

Localization of the *DUR1,2* gene by complementation. Our inability to clone the *DUR1,2* gene by transformation complementation methods prompted us to test whether complementation was possible. This was done by transforming strain VT51 (*dur1,2*) with various plasmids generated by subcloning DNA fragments of plasmids pFG203 and pFG204 (all plasmids in Fig. 3 were constructed similarly to pFG28 [Fig. 4] by using appropriate restriction sites in the vector). The largest segment tested was a 12-kb *Eco*RI fragment (plasmid pFG28) (Fig. 3 and 4). This fragment was able to complement *dur1*, *dur2*, and *dur1,2* mutations as assayed by growth on glucose-allantoin medium. Similar complementation was observed when inducible urea amidolyase activity was assayed (Fig. 5); urea amidolyase activity consists of the combined urea carboxylase (*DUR1*) and allophanate hydrolase (*DUR2*) activities. The recipient strain (VT51) used for these experiments did not possess detectable levels of enzyme activity (Fig. 5A). Transformation of this strain with plasmid pFG28 resulted in 10-fold higher levels of urea amidolyase activity than were observed in the wild-type parental strain (RH218). This was probably due to the increased copy number of the plasmid (*ARS1* vector)-borne gene. Although the levels of enzyme activity were higher in the transformant than in the untransformed wild type, the

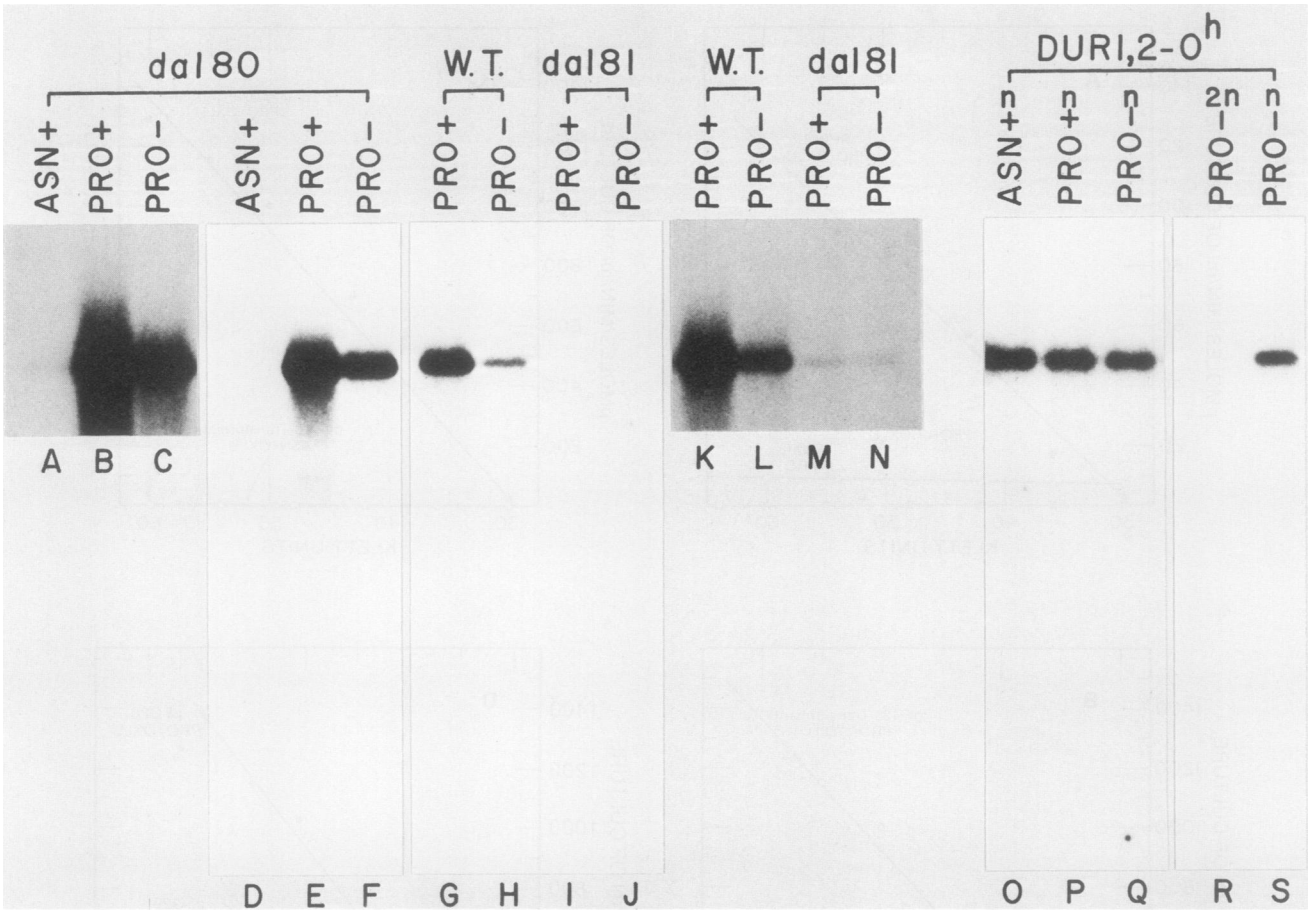


FIG. 6. Steady-state levels of *DUR1,2*-specific poly(A$^+$) RNA in wild-type and regulatory mutant strains of *S. cerevisiae*. Pro and Asn indicate the use of proline or asparagine as sole nitrogen source; + and − indicate the presence and absence, respectively, or oxalurate in the culture medium; n and 2n refer to haploid and diploid strains, respectively, homozygous for the *DUR1,2-O^H-1* allele. The following designations signify poly(A$^+$) RNA isolated from the strains listed: W.T., M970; *dal80*, M1081; *dal81*, M1407; *DUR1,2-O^h* n, M1666-6a; and *DUR1,2-O^h* 2n, M1667. Lanes A through C and K through N are overexposed photographs of the autoradiographs depicted in lanes D through J, respectively.

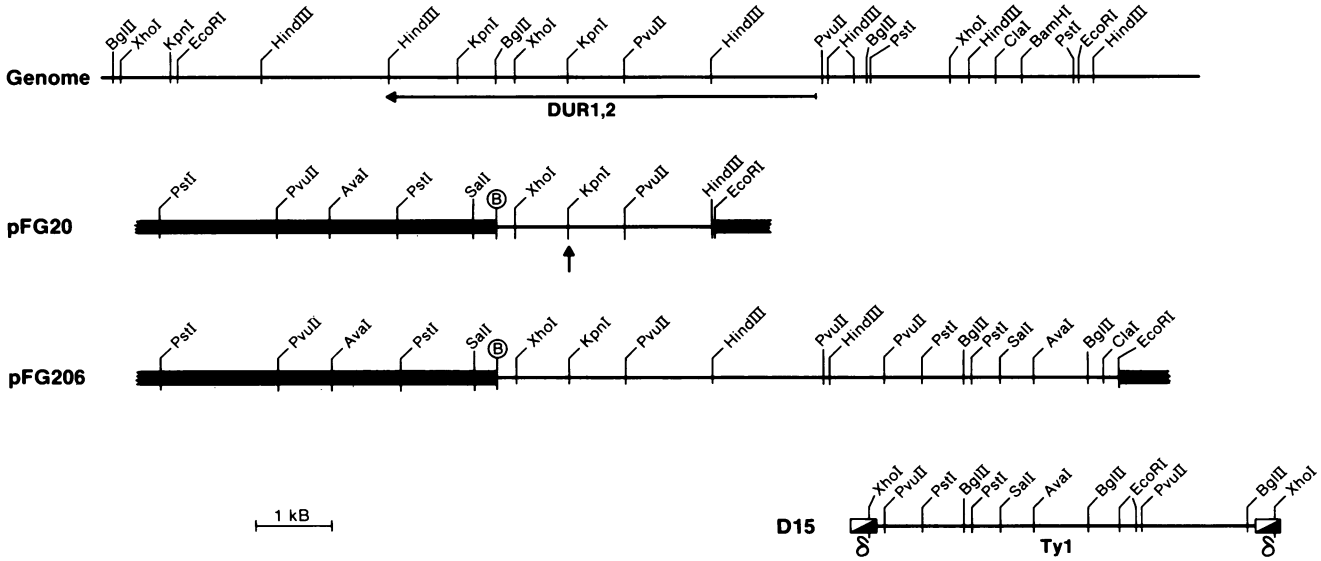


FIG. 7. Description of the integration-excision scheme used to isolate plasmid pFG206 from the genome of a *DUR1,2-O^h* mutant. The vertical arrow indicates the *Kpn*I site used to linearize the plasmid and thereby direct the integration of plasmid pFG20 (dark bars represent the vector YIp5). Restriction endonuclease *Eco*RI was used to excise the majority of the Ty element along with the DNA initially integrated. A restriction map of Ty1 is displayed (plasmid D15) (6) with the corresponding restriction enzyme sites aligned with those observed on plasmid pFG206.

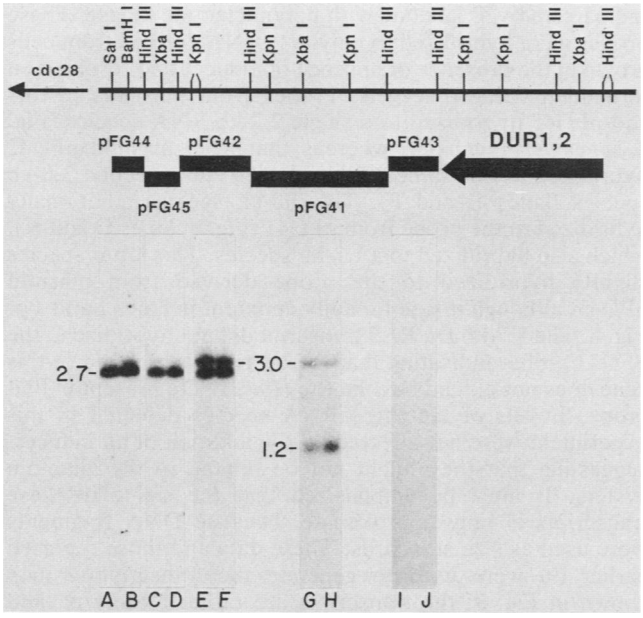


FIG. 8. Northern analysis of transcripts encoded by DNA carried on plasmid pFG204. The designated fragments of plasmid pFG204 were recloned onto vector pBR322, yielding plasmids pFG41 through pFG44 (solid bars). The inserts of these plasmids were radioactively labeled with T4 polynucleotide kinase and separately used to probe Northern blots of poly(A$^+$) RNA derived from wild-type strain M970. Lanes A, C, E, G, and I represent poly(A$^+$) RNA derived from cultures grown in minimal-proline medium containing OXLU, and lanes B, D, F, H, and J represent poly(A$^+$) RNA derived from cultures supplied with proline as the sole nitrogen source, but without OXLU.

regulation of activity was completely normal (compare the induction patterns in Fig. 5A and B). Induced urea amidolyase activity observed in the transformant was also normally sensitive to nitrogen catabolite repression (Fig. 5C). Finally, the ability of these cells to produce urea amidolyase activity was completely lost when they were cultured in nonselective medium, a condition favoring plasmid loss (Fig. 5C). These data would be expected if the entire *DUR1,2* gene, including regions associated with its normal regulation, were contained on the fragment tested. By subcloning portions of plasmid pFG28, we deduced that a 6.3-kb *Hin*dIII-*Bgl*II fragment was the smallest one capable of complementation (Fig. 3). Further localization experiments were not pursued, because we anticipated a coding region of about 5.7 kb based on the known monomer molecular weight and amino acid composition of the urea amidolyase protein (34).

During the subcloning experiments, we made two additional observations. First, we observed a 40% decrease in activity upon comparing the amounts of urea amidolyase activity supported by plasmids pFG28 and pFG29 (Fig. 5D). However, the ratio of enzyme activities observed in cells grown in the presence and absence of an inducer remained the same. Plasmid pFG29 was produced by deleting a 2.3-kb *Sal*I-*Xho*I fragment from pFG28 (Fig. 4). This fragment contained 650 base pairs of vector DNA (positions 1 through 650 of pBR322), which has been reported to interact with an uncharacterized yeast protein (5). Whether deletion of the binding site for the uncharacterized yeast protein accounts for the decrease in activity supported by plasmid pFG29 is not known at present. Second, we found that plasmid pFG204 was able to support high frequency transformation (Ura$^+$ was the selected phenotype). Plasmid pFG203 supported similar high-frequency transformation, but it appeared to be abortive since only a few of the colonies continued to grow (Ura$^+$ was the selected phenotype). The inserts carried on plasmids pFG20, pFG201, and pFG202 were unable to support high-frequency transformation when cloned into a vector lacking an *ars* sequence. The simplest interpretation of these results is to suggest the existence of an *ars* sequence situated within the 1.4-kb *Kpn*I fragment present in plasmids pFG203 and pFG204. It is probable, however, that the insert of plasmid pFG203 did not contain a complete set of the *ars* component sequences, thereby accounting for the observed abortive transformation.

Regulated expression of the *DUR1,2* gene. Early synthetic capacity measurements of urea amidolyase activity or protein levels pointed to transcription as the point of *DUR1,2* gene regulation (1–4, 16, 24–26). Isolation of the *DUR1,2* gene provided a probe to directly determine whether the observed pattern of enzyme regulation was congruent with that of RNA synthesis. This pattern exhibits the following major characteristics. Enzyme production is induced by allophanate or OXLU in wild-type cells, but not in *dal81* mutants. Constitutive enzyme production is observed in *dal80* cells grown in the absence of an inducer. Enzyme production in both wild-type and *dal80* mutant cells is repressed when cultures are provided with readily used nitrogen sources such as asparagine. Figure 6 depicts the results of a hybridization experiment in which RNA derived from each of the conditions described above was probed with a 4.1-kb *Hin*dIII fragment containing a major portion of the DNA required for complementation of *dur1,2* mutations.

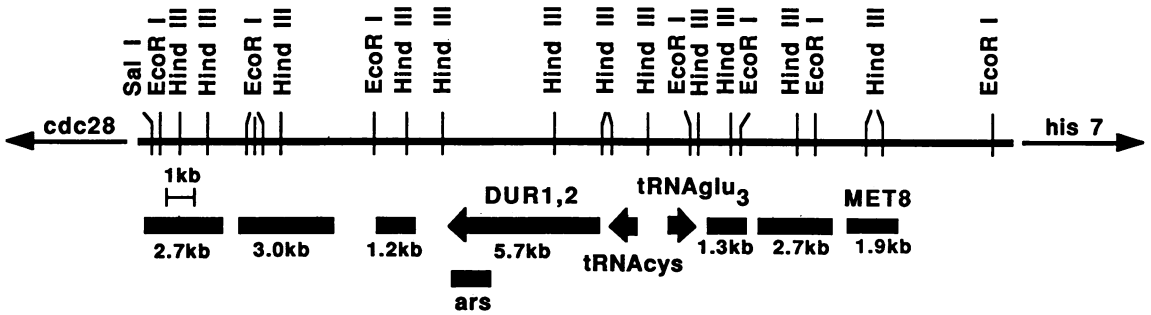


FIG. 9. Summary transcript map for the right arm of chromosome II in the region of the *DUR1,2* and *MET8* loci. The location of the transcripts between *DUR1,2* and *MET8* was derived from previously reported experiments (9). Arrows indicate the direction of transcription where it is known. *ars* indicates the location of an autonomously replicating sequence present on plasmids pFG203 and pFG204.

In Fig. 6, lanes designated A to C and K to N are overexposures of the autoradiographs depicted in lanes D to F and G to J, respectively. Low levels of the 5.7-kb, *DUR1,2*-specific RNA were observed in wild-type cells grown in glucose-proline medium (Fig. 6, lanes H and L). This level increased when OXLU was included in the medium (PRO^+, lanes G or K). In contrast, barely detectable levels of *DUR1,2* RNA were observed when a *dal81* mutant was grown either in glucose-proline medium or glucose-proline medium supplemented with 0.25 mM OXLU (Fig. 6, lanes I and J or M and N). Note the strong signal observed in uninduced, wild-type cells (Fig. 6, lane L) compared with that derived from the *dal81* mutant samples (lanes M and N). *DUR1,2* RNA levels found in a *dal80* mutant grown in the absence of an inducer were equal to those observed in an induced, wild-type culture (Fig. 6; compare lanes F and G or C and K). Moreover, the *dal80* mutant strain remained superinducible when an inducer was added to the culture medium (Fig. 6; compare lanes F and E or C and B). Replacement of proline with asparagine, a repressive nitrogen source, resulted in a marked decrease of *DUR1,2* RNA in the *dal80* mutant (Fig. 6; compare lanes E and D or B and A). In sum, the steady-state levels of *DUR1,2* RNA qualitatively matched those reported earlier for urea amidolyase activity and protein concentration (4, 8, 25, 37).

In addition to the *dal80* and *dal81* mutant loci described above, two new classes of *cis*-dominant mutations have been isolated (9, 11, 27). The first class of mutations was designated *DUR80*. The phenotype of *DUR80* mutant strains, which is expressed both in haploid and *MATa*/*MAT*α diploids, does not appear to be an alteration of the control system for this gene because its expression remained fully inducible and sensitive to nitrogen repression. Rather, we found much higher levels of *DUR1,2*-specific RNA under both induced and uninduced conditions, i.e., enhanced expression appeared to be superimposed on normal regulation of the gene (9). The second class of mutants, isolated both in our laboratory and in that of Wiame, is designated *DUR1,2-O*^{h}. The phenotype of this mutation is similar to the ROAM phenotype reported by others and ourselves (11, 27, 40).

As a first step toward understanding the molecular basis of the *DUR1,2-O*^{h} mutations, we determined their effects on *DUR1,2* RNA levels. *DUR1,2* RNA is expressed constitutively in the *DUR1,2-O*^{h} mutants (Fig. 6; compare lanes Q and P), and expression was resistant to nitrogen catabolite repression (compare lanes P and O). Finally, *DUR1,2* RNA production was mating type dependent, i.e., high levels of RNA were observed in haploid but not diploid strains (Fig. 6; compare lanes S and R).

The phenotype of the *DUR1,2-O*^{h} mutants was that expected of a *Ty* insertion in the 5′ regulatory region of the gene. To test this expectation we isolated the *DUR1,2* flanking sequences from a *DUR1,2-O*^{h} mutant by integration-excision methods. Plasmid pFG20 was linearized by digestion with *Kpn*I and integrated into the genome of the *DUR1,2-O*^{h} mutant, strain M1666-6a (Fig. 7). The restriction map of DNA excised from this integrant by *Eco*RI digestion was found to be nearly identical to that of the *Ty1* element contained on plasmid D15 (Fig. 7). The point of *Ty* element integration appears to have been between the two closely spaced *Hin*dIII sites.

Transcription of the chromosomal region surrounding the *DUR1,2* locus. The availability of DNA probes in the vicinity of the *DUR1,2* gene allowed us to construct a crude transcription map of this region. We used five probes from plasmids pFG41 through 45 (Fig. 8); the probes were made radioactive by 5′ labeling with polynucleotide kinase. These probes were hybridized to poly(A^+) RNA derived from cells grown in the presence or absence of inducer and resolved on formaldehyde-agarose gels. Probes from plasmids pFG44 and pFG45 hybridized to a single 2.7-kb RNA species (Fig. 8, lanes A though D), whereas that from plasmid pFG42 hybridized to this same 2.7-kb species and a second 3.0-kb species (lanes E and F). The 3.0-kb species additionally hybridized to the probe from pFG41 (Fig. 8, lanes G and H), which also hybridized to a 1.2-kb species. This latter species slightly hybridized to the probe derived from plasmid pFG43, although it is not readily apparent in lanes I and J of Fig. 8. The 5.7-kb *DUR1,2* transcript did not hybridize to the pFG43 probe, indicating that the transcribed portion of this gene does not extend beyond the *Hin*dIII site present in that probe. Levels of the three RNA species detected in this experiment were not affected by the addition of an inducer, suggesting that they might not be related to the allantoin system. It must be emphasized that the sizing of these transcripts is only approximate, because DNA fragments were used as size standards. These data and those reported earlier (9) were used to generate the transcription map shown in Fig. 9; the transcripts are rather closely packed together. In some strains a repeated element, *tau*, has been found between the two tRNA genes situated distal to *DUR1,2* (10, 21). *tau* has been shown to be one member of a family of elements that also includes *sigma* and the *delta* sequences of Ty elements (6, 17, 21, 40).

DISCUSSION

The data presented in this work provide additional support for the hypothesis proposed in our early work. On the basis of experiments measuring synthetic capacity for enzyme synthesis, we suggested that the *DUR1,2* gene was controlled at the level of transcription (1–4, 16, 24–26). In this work we have shown that the steady-state levels of *DUR1,2*-specific RNA increase in response to the presence of an inducer. Whether this increase in *DUR1,2* RNA derives from an increased rate of synthesis or decreased degradation, however, is not yet known. The *dal80* and *dal81* mutants previously implicated in regulation of the allantoin pathway also affect *DUR1,2* RNA levels in a manner that parallels enzyme activities and protein levels reported earlier (8, 37). The constitutive production of *DUR1,2* RNA observed in *dal80* strains permitted us to ascertain the effects of nitrogen catabolite repression in the absence of an added inducer. The continued sensitivity of *DUR1,2* RNA production to nitrogen catabolite repression under these conditions eliminated inducer exclusion as a possible mechanism for this type of control. Again, in parallel with enzyme activity measurements, the addition of a preferred nitrogen source such as asparagine resulted in the loss of all detectable *DUR1,2*-specific RNA. This observation is consistent with repression being mediated by variations in the level of *DUR1,2* RNA. *dal81* mutants contained barely detectable levels of *DUR1,2* RNA, supporting our proposal that the product of this gene may serve as an activator of allantoin system gene expression.

Another important contribution of this work is the identification of the molecular basis for the *DUR1,2-O*^{h} mutations as a Ty insertion near the *DUR1,2* gene. The striking difference between the phenotype of this Ty insertion and those resulting in the DUR80 phenotype, which have also been shown to derive from Ty insertions, is provocative (G. Chisholm and T. G. Cooper, manuscript in preparation).

Why, for example, does one Ty insertion completely replace normal control of the *DUR1,2* gene with a control pattern normally observed for the Ty-encoded transcript, whereas another Ty insertion results in enhanced *DUR1,2* gene expression but leaves its normal control pattern untouched? Two possibilities come to mind: either the Ty elements inserted are different from one another, or insertion has occurred at different points. These possibilities are currently being investigated.

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