MOLECULAR AND CELLULAR BIOLOGY, Nov. 1986, p. 4026–4030
0270-7306/86/114026-05$02.00/0

Vol. 6, No. 11

Constitutive RNA Synthesis for the Yeast Activator *ADR1* and Identification of the *ADR1-5*^{c} Mutation: Implications in Posttranslational Control of *ADR1*†

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Received 24 February 1986/Accepted 27 June 1986

The regulation of mRNA production for the yeast positive activator *ADR1*, a gene required for the expression of the glucose-repressible alcohol dehydrogenase (ADH II), was studied. *ADR1* mRNA levels did not vary when yeasts were switched from glucose- to ethanol-containing medium, while ADH II expression increased 100-fold. The mRNA for the *ADR1-5*^{c} allele, which augments ADH II expression 60-fold during glucose repression, was not present in greater abundance than *ADR1* mRNA. Additionally, the *ccr1-1* allele, which blocks *ADH2* mRNA formation and partially suppresses the *ADR1-5*^{c} phenotype, did not alter the levels of *ADR1* mRNA. These results indicate that *ADR1* is not transcriptionally controlled. To determine the character of the *ADR1-5*^{c} mutation, the region containing the mutation was identified and sequenced. At base pair +683 a G-to-A transition was detected in the *ADR1* coding sequence which would result in the substitution of a lysine residue for an arginine at amino acid 228. The location of the *ADR1-5*^{c} mutation in the interior of the *ADR1* coding sequences suggests that it enhances the activity of an extant but inactive *ADR1* protein rather than increases the abundance of *ADR1* by altered translation of its mRNA. The *ADR1-5*^{c} mutation occurs in a region of the polypeptide corresponding to a cyclic AMP-dependent protein kinase phosphorylation recognition sequence. The potential role of reversible phosphorylation in the posttranslational regulation of *ADR1* is discussed.

The transcription of glucose-repressible alcohol dehydrogenase (ADH II) of *Saccharomyces cerevisiae* is regulated by two pathways, one of which involves the positive activators *ADR1* (7) and *CCR1* (C. L. Denis, Ph.D. thesis, University of Washington, Seattle, 1982). A second pathway of ADH II control is mediated by both positive (*CCR4*) and negative (*CRE1* and *CRE2*) effectors and appears to be independent from *ADR1* (6). A link between *ADR1* and *CCR1* was postulated because the *ccr1-1* allele is capable of suppressing 3- to 10-fold the glucose-insensitive ADH II expression caused by the *ADR1-5*^{c} allele (5, 6). *ADR1* probably does not regulate *CCR1* mRNA transcription because *CCR1* is pleiotropic (4) and *ADR1* controls only ADH II. This relationship between *ADR1* and *CCR1* has been confirmed by other studies demonstrating that *CCR1* is not transcriptionally regulated (1) (studies used the *SNF1* gene which is allelic to *CCR1* [6]). It has not been ascertained, however, whether *CCR1* controls *ADR1* mRNA formation or is required for *ADR1* protein activity.

The cloning of the *ADR1* gene (9) has allowed *ADR1* mRNA levels to be assayed. By quantitating the levels of *ADR1* mRNA several questions about ADH II regulation can be answered. (i) Does *CCR1* regulate ADH II via control of *ADR1* mRNA formation? (ii) Do the changes in *ADH2* mRNA synthesis correlate with changes in *ADR1* mRNA formation? (iii) Does the *ADR1-5*^{c} mutation overcome the effects of glucose repression by augmenting *ADR1* mRNA levels? In this investigation, it was found that neither the *ccr1* or *ADR1-5*^{c} alleles nor different growth conditions affected *ADR1* mRNA synthesis. Furthermore, the identification of the *ADR1-5*^{c} mutation in the interior of the *ADR1* coding sequence suggests that *ADR1* is posttranslationally regulated.

MATERIALS AND METHODS

Strains, growth conditions, and RNA extractions. The following strains were used for these analyses: 79-72C, MATα *ura1 trp2*; R234, *Matα adh1-11 adh3 ADR1-5*^{c} *ura1 his4*; 53.3-1C, *MATα adh1-11 adh3 ccr1-1 ADR1 leu1 his4*; 43-2B, isogenic to R234 except *ADR1*; 500-16, *MATa ura1 his4 trp1 adh1-11 adr1-1 adh3*. Yeast transformations of strain 500-16 with reconstructed *ADR1-5*^{c} plasmids were conducted as described previously (9). Procedures used for overnight growth on YEP medium (2% Bacto-Peptone [Difco Laboratories, Detroit, Mich.], 1% yeast extract, 20 mg of adenine per liter, 20 mg of uracil per liter) supplemented with either 8% glucose or 3% ethanol and switching cells from glucose- to ethanol-containing medium have been described previously (7). Upon switching to ethanol-containing medium a lag in growth occurs for the first 4 h. Total yeast RNA was prepared as previously described (7). Poly(A)$^+$ RNA was prepared by applying total yeast RNA to an oligo(dT) column equilibrated with 0.5 M NaCl–10 mM Tris hydrochloride (pH 7.5) and selectively eluting the poly(A)$^+$ RNA with 10 mM Tris hydrochloride pH 7.5.

RNA analysis. Northern analysis was conducted exactly as described previously (18). The sizes of RNA species hybridizing to *ADR1* DNA were based on rRNA standards. Nitrocellulose blots were reprobed with Ty (5.8-kilobase [kb] *Xho*I fragment from *ADH2-2*^{c} [19]) and *ADH2* DNA segments to give additional RNA standards of 5.7 and 1.2 kb, respectively. The blots were treated with DNase (10 mg of DNase I per ml; Worthington Diagnostics, Freehold, N.J.) for 30 min in 10 mM Tris hydrochloride (pH 7.5)–1 mM $MgSO_4$; RNase treatment was for 30 min with 20 mg of RNase per ml–1% Tris base–0.55% boric acid–0.93% EDTA.

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† Scientific contribution 1443 from the New Hampshire Agricultural Experiment Station.

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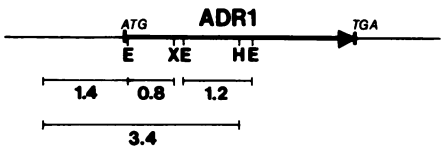

FIG. 1. *ADR1* probes used for Northern analysis. The *ADR1* open reading frame extends for 3,969 bp (10). The three probes derived from plasmid YRp7-ADR1-5^c-23A (9) include the upstream 1.4-kb probe that extends from bp +53 in the putative *ADR1* coding sequences through 1.3 kb of upstream *ADR1* sequences and 375 bp of pBR322 sequences to an *Eco*RI site, the 0.8-kb probe that extends from +53 bp to +842 bp, and the 1.2-kb probe that extends from +1010 to +2250 bp. The 3.4-kb probe extends from a *Hin*dIII site at bp +1971 through the *ADR1* sequences, 1.3 kb of upstream sequences, and 338 bp of pBR322 to a *Hin*dIII site. Routinely, 1×10^7 to 3×10^7 cpm of radioactive probe per μg were used for each hybridization. Restriction endonuclease designations are: E, *Eco*RI; X, *Xba*I; and H, *Hin*dIII.

DNase and RNase were removed by washing the blots for 30 min in 1% sodium dodecyl sulfate–50 mM Tris hydrochloride (pH 7.5) . RNA was quantitated by the densitometric method described previously (8) in which the amount of rRNA in each sample was used as a standard. Measurement of total RNA by using rRNA as a standard correlates well with other standards such as the total translatable mRNA and the 1.4-kb RNA upstream of the *ADR1* gene that is unaffected by growth conditions. The DNA probes were isolated from agarose gels after restriction digestion and separation by electrophoresis (9). Each probe was nick translated with [α-^{32}P]TTP as previously described (8).

Identification of the *ADR1-5*^{c} mutation. The *ADR1-5*^{c} mutation was localized by using hybrid *ADR1/ADR1-5*^{c} plasmids. Plasmid YRp7-ADR1-5^c-23A (9), which lacks *ADR1* sequences 3′ to base pair (bp) +2982, was cut with *Bcl*I to remove the region of *ADR1-5*^{c} from bp +441 to +1075. After religation, the deleted plasmid was recut with *Bcl*I, and the corresponding 634-bp *Bcl*I fragment from either an *ADR1* or an *ADR1-5*^{c} plasmid was inserted. The two resultant plasmids were then cut at an *Sst*I site at bp +1713, and the linearized plasmids were site specifically integrated (15) into the *adr1-1* locus by transforming yeast strain 500-16. Integration of the plasmid at the *adr1-1* locus creates a duplication of *ADR1* sequences. One copy would contain plasmid-derived sequences 3′ to the *Sst*I site at bp +2982 and genomic sequences 5′ to the *Sst*I site which includes the *adr1-1* mutation (L. Bemis, personal communication). This copy would be defective because of the *adr1-1* allele. The other copy would contain plasmid-derived *ADR1* or *ADR1-5*^{c} sequences 5′ to the *Sst*I site and complete genomic *ADR1* sequences 3′ to the *Sst*I site. Transformants carrying integrated copies of the plasmid were identified by the retention of the Trp$^+$ phenotype conferred by the YRp7 sequences after nonselective growth on YEP plates containing 2% glucose. Integration of a single copy of the plasmid was demonstrated by cutting genomic DNA isolated (9) from the integrants with *Hin*dIII and subjecting the cut DNA to Southern analysis with *ADR1* DNA as a probe. Plasmid integration would result in a 3.5-kb *Hin*dIII fragment, in addition to the usual genomic 5.3-kb *Hin*dIII fragment, hybridizing to the probe. An E-C 610 densitometer was used to quantitate the relative abundance of the 3.5-kb band to the 5.3-kb band. Sequencing was performed by chemical cleavage (14) and dideoxy chain termination methods (17). ADH assays were conducted as previously described (9).

RESULTS

Identification of *ADR1* mRNA. *ADR1* mRNA was initially identified by Northern analysis with a 3.4-kb segment of *ADR1* DNA as a probe (Fig. 1). An autoradiogram produced from a typical Northern analysis is shown in Fig. 2. Several different species were observed to hybridize to the probe: chromosomal DNA, 18S and 25S rRNA, and 5.1-, 1.5-, and 1.4-kb mRNAs. No hybridization was detected above the 5.1-kb band when the nitrocellulose was pretreated with DNase I. Conversely, treatment of the nitrocellulose with RNase A resulted in only chromosomal DNA hybridizing to the probe (data not shown). The hybridization of *ADR1* DNA to the leading edge of 25S and 18S rRNAs may have resulted from nonspecific hybridization. However, similar results were observed when Ty DNA was used as a probe (unpublished observations), suggesting that the large size of Ty (5.7 kb) and *ADR1* (5.1 kb) RNAs may facilitate their entrapment within rRNA.

Several additional probes were used to determine the location of the three RNA transcripts relative to the *ADR1* coding region. The 5.1-kb RNA was detected with three probes extending to +2220 bp in the *ADR1* sequence (Fig. 2; see Fig. 1 for probes) and a fourth probe extending from +1971 to +2982 bp (data not shown). These results suggest that the 5.1-kb RNA is colinear with *ADR1* coding sequences. Additional results have confirmed the 5.1-kb RNA to be *ADR1* RNA including 5′ and 3′ end mapping (T. Hartshorne, personal communication) and the identification of a mutation in *ADR1* coding sequences which blocks accumulation of the 5.1-kb RNA (L. Bemis and C. Denis, manuscript in preparation). The 1.4-kb RNA hybridized only to the probe containing 5′-flanking sequences (Fig. 2, lane d), indicating that it is a transcript derived from sequences upstream of *ADR1*. The 1.5-kb RNA hybridized very weakly to probes overlapping the N-terminal regions of *ADR1*

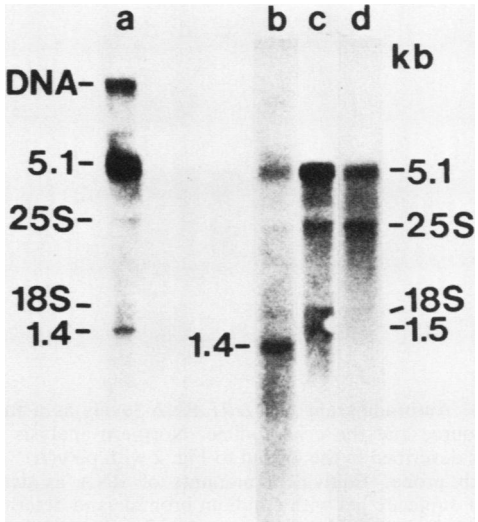

FIG. 2. Autoradiogram of RNA species hybridizing to *ADR1* DNA probes. Poly(A)$^+$ RNA was isolated from strain 43-2B after 18 h of growth on glucose-containing medium. A 30-μg sample of poly(A)$^+$ RNA was loaded in each slot. The 1.5-kb RNA while barely visible in lane a was detectable in other Northern blots with the 3.4- and 1.2-kb probes but not with the 1.4-kb probe (data not shown). Lanes: a, 3.4-kb probe; b, 1.4-kb 5′ probe; c, 0.8-kb probe; d, 1.2-kb probe.

(0.8-kb probe, Fig. 2, lane c; 1.2-kb probe, data not shown). The 1.5-kb RNA may represent weak homology between *ADR1* sequences and a heterologous transcript or, alternatively, a premature termination product of the *ADR1* transcript.

Expression of *ADR1* mRNA is independent of carbon source. The steady-state level of *ADR1* mRNA was analyzed after yeast cells were grown to the mid-logarithmic phase on medium containing either glucose or ethanol. Poly(A)$^+$ RNA isolated from strain 43-2B (*ADR1*) was subjected to Northern analysis as described above. The amount of 5.1-kb *ADR1* mRNA was equally abundant in cells grown on glucose-containing medium as in cells grown on ethanol-containing medium (Fig. 3). While the results in Fig. 3 (lane a and b) indicate that *ADR1* mRNA production is independent of the carbon source, it should be noted that *ADR1* mRNA was analyzed after 18 h of growth on ethanol-containing medium. Previous results have indicated that a peak of *ADH2* mRNA production occurs 2 to 4 h after cells are switched to medium containing ethanol (7, 8). It was therefore necessary to determine whether *ADR1* mRNA is synthesized at a constant rate when cells are grown under derepressed conditions. Cells from strain 79-72C (*ADR1*) were grown on medium containing glucose for 18 h, harvested, and switched to medium containing ethanol. Thereafter, total yeast RNA was isolated at selected times and subjected to Northern analysis with *ADR1* DNA as a probe (Fig. 4). Figure 5 compares the relative levels of *ADR1* mRNA to *ADH2* mRNA (8). It was observed that the steady-state level of *ADR1* mRNA did not alter appreciably during derepression, while *ADH2* mRNA levels increased by at least 70-fold (Fig. 5).

By reprobing the nitrocellulose filter (Fig. 4) with DNA sequences upstream of *ADR1*, we found the 1.4-kb RNA transcript to be present at a constant level throughout the time course of the experiment (0 to 6 h) (data not shown).

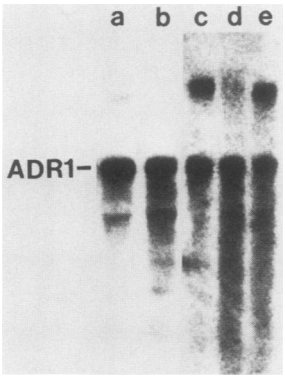

FIG. 3. Autoradiogram of *ADR1* RNA levels as a function of carbon source and the *ccr1-1* allele. Northern analysis was conducted as described in the legend to Fig. 2 with poly(A)$^+$ RNA and the 3.4-kb probe. Equivalent amounts of RNA as detected by staining a duplicate gel with ethidium bromide and determining the amount of rRNA which was present as previously described (8) were loaded into each slot. Lanes: a, RNA isolated from strain 43-2B after 18 h of growth on medium containing glucose; b, same as lane a except that RNA was isolated after growth on medium containing ethanol; c, RNA isolated from strain R234 (*CCR1*) after 18 h of growth on medium containing glucose; d, RNA isolated from strain 53.3-1C (*ccr1-1*) after 18 h of growth on medium containing glucose; e, same as lane d except that RNA was isolated after growth on ethanol-containing medium.

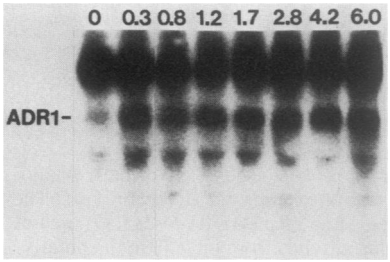

FIG. 4. Autoradiogram of *ADR1* RNA levels as a function of growth on medium containing ethanol. Northern analysis was conducted as described in the legends to Fig. 2 and 3 with total yeast RNA and the 3.4-kb *ADR1* probe. Cells from strain 79-72C (*ADR1*) were grown overnight on YEP medium containing 8% glucose, collected, washed once with H_2O, and suspended in fresh YEP medium containing 3% ethanol. The times at which RNA was extracted are given in hours.

Similarly, the 1.5-kb RNA was present under both glucose-repressed and -derepressed conditions (data not shown).

The *ADR1-5*^{c} mutation does not alter *ADR1* mRNA levels. The *ADR1-5*^{c} allele allows *ADH2* mRNA to be synthesized during glucose repression (7). There are several possible mechanisms by which the *ADR1-5*^{c} mutation could allow *ADR1* to overcome the effects of glucose repression. These include increasing the amount of *ADR1* RNA, affecting the efficiency of *ADR1* RNA translation, and altering the activity of the *ADR1* protein by a posttranslational modification. To differentiate among these possibilities, the levels of *ADR1* mRNA in an *ADR1-5*^{c} strain were analyzed and compared to those found in an *ADR1* strain, and the identity of the *ADR1-5*^{c} mutation was determined as described below. Cells from strain R234 (*ADR1-5*^{c}) were grown to the mid-

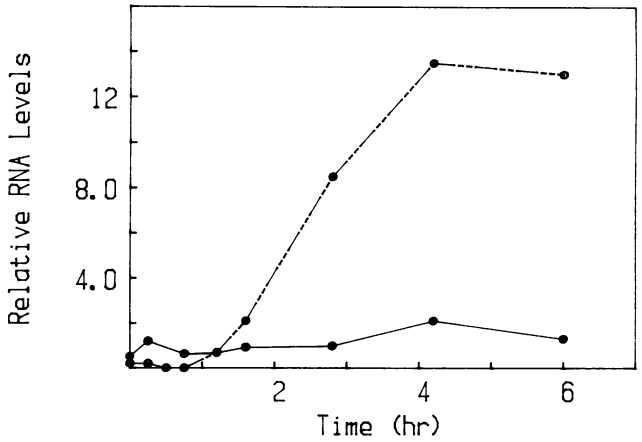

FIG. 5. Relative *ADR1* RNA levels during derepression. *ADR1* RNA levels were determined by densitometric analysis of the autoradiograms displayed in Fig. 4, using an E-C 610 densitometer. The amount of *ADR1* RNA was standardized against the amount of total rRNA as described in Materials and Methods. Use of the upstream 1.4-kb RNA or the total amount of translatable nRNA as a standard gave the same results as did using rRNA as a standard. The changes in *ADH2* RNA as a function of growth on ethanol-containing medium are given to put into perspective the slight changes observed in *ADR1* RNA levels. These values are derived from reference 8 in which the same RNA samples were analyzed. The ratio of *ADR1* to *ADH2* mRNA was estimated to be at least 1:10 by hybridizing each DNA, respectively, to the same nitrocellulose blot followed by densitometric analysis of the respective RNA bands (unpublished data). Symbols: ●, *ADR1*; ○, *ADH2*.

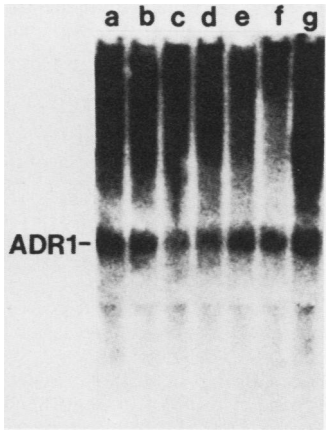

FIG. 6. Autoradiogram of *ADR1-5*^{c} RNA levels as a function of growth on medium containing ethanol. Northern analysis was conducted as described in the legend to Fig. 4 except that strain R234 (*ADR1-5*^{c}) was used for lanes a to f. RNA was extracted from R234 after switching cells from a medium containing glucose to one containing ethanol. Time points for extraction were: lane a, 15 min; lane b, 45 min; lane c, 1.25 h; lane d, 2.25 h; lane e, 4.25 h; and lane f, 6.25 h. Lane g, RNA was extracted from strain 79-72C (*ADR1*) 45 min after switching cells to medium containing ethanol, and at this time point only 20% more *ADR1* RNA is present than under glucose growth conditions (Fig. 4 and 5).

logarithmic phase on medium containing glucose. Derepression was initiated by transfer to fresh medium containing ethanol. At selected times after the transfer, total RNA was isolated and analyzed for *ADR1*-specific sequences by Northern analysis as described above. These results show (Fig. 6) that *ADR1-5*^{c} RNA levels do not change appreciably during derepression and are in no greater abundance than those found in cells containing the *ADR1* gene (lane g). After standardizing the amount of the 5.1-kb RNA species to the total amount of RNA in the sample, it was found that *ADR1-5*^{c} RNA levels did not vary by more than 1.6-fold (lowest level at 2.25 h) as a function of growth on medium containing ethanol (data not shown). In contrast, with the same RNA samples, *ADH2* mRNA levels increased four- to sixfold during the same interval (7). These results demonstrate that *ADR1-5*^{c} mRNA is synthesized independently of the carbon source and imply that the *ADR1-5*^{c} mutation exerts its effect on *ADR1* activity posttranscriptionally.

The *CCR1* gene does not regulate *ADR1* transcription. Under glucose growth conditions the *ccr1-1* allele partially suppresses the ability of *ADR1-5*^{c} to allow *ADH2* activation (5, 6). These data suggest that *CCR1* acts through *ADR1* to promote *ADH2* expression. The effect of *ccr1-1* on *ADR1* mRNA levels was analyzed to determine whether *CCR1* affects ADH II by control of *ADR1* mRNA production. Poly(A)$^+$ RNA was isolated from strain 53.3-1C (*ccr1-1*) grown on medium containing either glucose or ethanol and was subjected to Northern analysis with the *ADR1* DNA as a probe. The amount of *ADR1* mRNA was the same for both *CCR1* alleles analyzed (Fig. 3, lanes c and d). Furthermore, no alteration in *ADR1* mRNA levels was observed for the *ccr1-1* strain grown on the different carbon sources (Fig. 3, lanes d and e).

Identification of *ADR1-5*^{c} mutation. While the *ADR1-5*^{c} mutation does not alter the level of *ADR1* RNA, this does not rule out the possibility that the translatability of *ADR1* RNA is affected. The mutation may, for instance, result in enhanced initiation of translation. This possibility was investigated by identifying the *ADR1-5*^{c} mutation relative to the start of the *ADR1* protein. The *ADR1-5*^{c} mutation was localized by transformation of yeast cells with hybrid *ADR1-5*^{c}/*ADR1* plasmids. A 634-bp segment of *ADR1-5*^{c} extending from bp +441 to +1075 (10) was removed and subsequently replaced with the corresponding *ADR1* or *ADR1-5*^{c} segment. A single copy of each hybrid plasmid was integrated into the *adr1-1* locus, and the resultant ADH II activity was determined for each integrant after growth on medium containing glucose or ethanol. Integrants derived from plasmids containing the *ADR1* segment between bp +441 and +1075 expressed an *ADR1* phenotype (5 mU of ADH II enzyme activity per mg on glucose; 3,100 mU/mg on ethanol). Integrants with *ADR1-5*^{c} material displayed an *ADR1-5*^{c} phenotype (260 mU of ADH II enzyme activity per mg on glucose; 5,800 mU/mg on ethanol) (2, 4). The *ADR1-5*^{c} mutation, therefore, occurs between bp +441 and +1075. Sequencing of this region from *ADR1-5*^{c} DNA as compared with *ADR1* DNA from an isogenic source detected a G-to-A transition at bp +683 (Fig. 7). This change would result in a lysine substitution for an arginine at amino acid 228 (10). The location of the mutation suggests that the *ADR1-5*^{c} mutation does not affect the translation of *ADR1* mRNA.

DISCUSSION

The mRNA transcripts in the region of the *ADR1* gene were analyzed. A 1.4-kb RNA is transcribed in regions upstream of the *ADR1* gene. A much less abundant RNA (the 1.5-kb transcript) was only observed in poly(A)$^+$ RNA samples. This transcript was shown, by using two different DNA probes, to be colinear with the 5′ end of *ADR1* and probably represents a low level of truncated *ADR1* RNA. The presence of truncated RNAs has been observed for another large RNA species (adenylate cyclase, 6.2 kb) (11). Neither the 1.5- nor the 1.4-kb RNA was found to be regulated by growth on different carbon sources. The 5.1-kb RNA is colinear with the *ADR1* coding sequences (Fig. 2) and is absent in strains carrying an *ADR1* nonsense mutation (Bemis and Denis, in preparation). Additionally, hybridization with a single-strand *ADR1* probe and 5′- and 3′-end

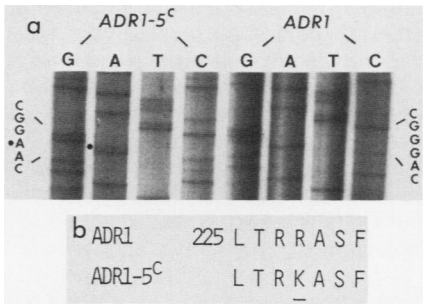

FIG. 7. Identification of *ADR1-5*^{c} mutation and location in a putative cyclic AMP-dependent protein kinase consensus sequence. The *Bcl*I fragment containing *ADR1* or *ADR1-5*^{c} sequences extending from +441 to +1075 bp was separately cloned into M13 vectors and sequenced by the dideoxy chain termination procedure (16). The primer was synthesized on an Applied Biosystems 380A DNA synthesizer and was complementary to *ADR1* sequences from bp +528 to +548. (a) Sequence of *ADR1* and *ADR1-5*^{c} DNAs in region of mutation. (b) Character of *ADR1-5*^{c} mutation in context of *ADR1* amino acid region homologous to cyclic AMP-dependent protein kinase phosphorylation consensus sequence; *ADR1-5*^{c} mutation is underlined.

mapping have shown that this 5.1-kb transcript is *ADR1* RNA (T. Hartshorne, personal communication).

The rate of *ADH2* mRNA synthesis increases dramatically upon switching yeasts from glucose-containing to ethanol-containing medium (7, 8). After 2 to 4 h of growth on ethanol-containing medium *ADH2* mRNA levels are at a peak, after which they decrease about 50 to 70%. These changes were shown not to result from an alteration in the steady-state levels of *ADR1* transcripts. Therefore, during glucose repression either the *ADR1* protein is present but not functional or the *ADR1* mRNA is not translated efficiently. In light of the fact that the *ADR1-5*^{c} mutation was found not to affect the level of *ADR1* transcription and that the *ADR1-5*^{c} mutation is in the interior of the *ADR1* coding sequences, it appears that during glucose repression the *ADR1-5*^{c} mutation enhances the activity of an already extant but inactive protein rather than the translational efficiency of the *ADR1* mRNA. This suggests that some part of the control over *ADR1* must be exercised posttranslationally.

CCR1 is shown herein not to be required for the transcription of *ADR1*. The *CCR1* protein product must be present during glucose growth conditions since *CCR1* is itself not regulated transcriptionally (2) and under glucose conditions the *ccr1-1* allele reduces the ability of *ADR1-5*^{c} to activate ADH II (5, 6). Therefore, if *CCR1* acts in concert with or through *ADR1*, then it probably does so posttranslationally.

The mechanism responsible for regulating *ADR1* protein activity cannot be deduced from this investigation. Evidence supporting the possibility that *ADR1* undergoes an inactive-to-active transition upon derepression derives from the identification of the *ADR1-5*^{c} mutation. As shown in Figure 7b, the *ADR1-5*^{c} mutation occurs in a region of *ADR1* homologous to a phosphorylation consensus sequence described for cyclic AMP-dependent protein kinase (R-R-X-S-X, where the serine is phosphorylated and X tends to be hydrophobic) (12). Of over 70 yeast proteins analyzed, only 5 others contain this sequence. Two of these five are known to be phosphorylated in this sequence (fructose bisphosphatase [16] and ribosomal S10 protein [13]), and another, pyruvate kinase, is presumed to be phosphorylated based upon the phosphorylation of an analogous sequence in its mammalian counterpart (1). These observations suggest that a protein kinase in yeasts does recognize the above sequence and that *ADR1* is phosphorylated. Additionally, an *ADR1-5*^{c} alteration might be expected to increase the K_m of phosphorylation. Mammalian protein kinase displays a decreased ability to phosphorylate a synthetic peptide containing an *ADR1-5*^{c}-like sequence as compared with its phosphorylation of an *ADR1*-like sequence (12). It is possible, therefore, that the *ADR1-5*^{c} mutation enhances *ADR1* activity by decreasing *ADR1* phosphorylation. Recent sequence analysis of the *SNF1* gene, which is allelic to *CCR1*, has shown homology at the amino acid sequence level between the *SNF1* gene product and protein kinases (20a). While *CCR1* does not affect the levels of *ADR1* mRNA (Fig. 3), it remains to be determined whether *CCR1* protein acts directly on *ADR1* protein or through another intermediate. The phosphorylation data do suggest, however, that if *CCR1* is a protein kinase and *ADR1* is activated by dephosphorylation, another protein (a phosphatase, perhaps) is involved. Phosphorylation of the phosphatase by *CCR1* would in turn result in the activation of *ADR1*.

ACKNOWLEDGMENTS

We wish to thank R. Vallari and D. Green for comments concerning the manuscript and S. Lucius for her secretarial skills.

Part of this research was conducted at the University of Washington. This research was supported by National Science Foundation grant PCM-8316271 and Hatch project 291.

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