Alcohol dehydrogenase in *Drosophila*: isolation and characterization of messenger RNA and cDNA clone

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ABSTRACT

The mRNA for alcohol dehydrogenase (ADH) in *D. melanogaster* has been identified by translation in a cell-free system. The *in vitro* synthesized polypeptide, specifically precipitated by anti-ADH antibody, has identical subunit molecular weight (25,000 daltons) and tryptic peptide profile to the *in vivo* synthesized ADH. The poly A containing ADH-mRNA has been purified by specific precipitation of ADH-polysomes using anti-ADH antibody and *S. aureus*. Transformation of *E. coli* with the dA-tailed ADH-mRNA-complementary DNA hybrid annealed to the dT-tailed pBR322 yielded one plasmid which has been identified as the ADH-cDNA clone. The identification involved hybridization selection of ADH-mRNA and *in vitro* translation, *in situ* hybridization to the *Adh* locus on salivary gland polytene chromosomes and DNA sequencing. This ADH-cDNA plasmid contains 349 bases of the C-terminal protein coding and 180 bases of the 3' untranslated region.

INTRODUCTION

Alcohol dehydrogenase (ADH) in *Drosophila melanogaster* is a well studied gene-enzyme system with several interesting biochemical, genetic and regulatory features. Because of its relative abundance (it comprises 1-2% of the total soluble protein of mature adults) ADH protein has proven easy to purify (1,2). The availability of the pure protein has, in turn, allowed for the enzyme to be extensively characterized. These studies have revealed *Drosophila* ADH to be quite different from the mammalian liver and yeast ADH's; it has a smaller subunit molecular weight (25,000 vs. 39,800 for the liver enzyme and 35,300 for the yeast) and lacks the bound Zn^{2+} (3) required for catalysis by the other ADH's. The completed sequence of the protein (4,5) has shown very little homology between *Drosophila* ADH and either liver or yeast enzymes.

From a genetic point of view, *Drosophila* ADH is virtually unique among genes in higher organisms because chemical selection procedures have been developed that allow screening for flies that lack enzyme activity (6,7). The large number of homozygous viable mutants recovered that lack active

enzyme indicate that enzyme activity is not required for survival or fertility (8). These mutants have also made it possible to precisely map the location of the Adh structural gene both genetically (9,10) and cytologically (10,11,12).

Finally, the expression of the Adh gene appears to be regulated. Activity is high at certain stages of development (in mature adults and late third instar larvae) and low at others (in pupae). Moreover, certain tissues, such as fat body, intestine and Malpighian tubules have relatively high enzymatic activity; others, like salivary glands and imaginal discs have little or none (13).

We have been interested in the regulation of gene expression of the Adh gene. Our strategy has been to use the selective procedures that we developed to secure mutants at the Adh locus. Among these mutants we hope to find some whose lesion is in the regulatory machinery the gene. In order to carry out this type of analysis we required a probe for the Adh gene. In this report, we first describe the isolation and characterization of ADH-mRNA. This mRNA was then used to produce an ADH-cDNA clone which we in turn identified and characterized. The cloned cDNA will allow us to analyze wild type and mutant strains of Drosophila for the presence and structure of the Adh gene and ADH-mRNA.

MATERIALS AND METHODS

Organisms. The ADH-positive strains, Adh^F and Adh^D pr cn which are homozygous for the ADH^F and ADH^D electrophoretic variants respectively, were the sources of Drosophila melanogaster RNAs. The symbols pr (purple) and cn (cinnabar) refer to eye color markers. E. coli K-12, strain HB101, was obtained from Dr. H. Smith of the Microbiology Department, Johns Hopkins University.

DNA and Enzymes. pBR322 DNA was used as the vector to transform E. coli HB101. Plasmid DNA was purified according to Clewell (14). Avian myeloblastosis virus reverse transcriptase was provided by Dr. J. W. Beard, the Office of Program Resources and Logistics, NCI. Bacterial alkaline phosphatase and terminal transferase were purchased from Bethesda Research Labs. Restriction enzymes were from Bethesda Research Labs and New England BioLabs. Polynucleotide kinase, nucleotides and p(dT)₁₂₋₁₈ were from PL Biochemicals.

Radioactive materials were purchased from New England Nuclear and Amersham.

RNA. All glassware and solutions were either autoclaved, filter sterilized or diethylpyrocarbonate treated (15). For the preparation of total cytoplasmic RNA from Drosophila, four- to six-day-old imagoes (live or stored frozen in liquid N₂) were ground in liquid N₂ in a mortar to a fine powder. They were then homogenized in a loose-fitting Dounce homogenizer in 0.25 M sucrose, 0.25 M NaCl, 0.25 M NH₄Cl, 0.05 M MgCl₂, 0.025 M Tris-HCl, pH 7.5, 0.005 M EGTA, 0.005 M N-ethyl maleimide, 100 µg/ml heparin, 0.5% (v/v) β-mercaptoethanol; 0.001 M cytosine monophosphate and 50 µg/ml spermine (20 ml/g flies). (This buffer recipe was recommended to us by Dr. D. Sullivan, Syracuse University.) The homogenate was centrifuged (10 min at 10,000 rpm, 4°C in a Sorvall HB4 rotor) and the resulting supernatant made 1% NP40. This solution was extracted once with phenol (saturated with 0.01 M Tris-HCl, pH 7.5) and three times with chloroform. RNA was precipitated from the aqueous phase by adding 0.1 volume of 3 M sodium acetate and 2.5 volumes of cold ethanol and chilling in a dry ice/ethanol bath. The precipitate was collected by centrifugation washed once with 70% ethanol, air-dried and resuspended in distilled H₂O. The RNA was stored either in ethanol at -20°C or as an aqueous solution at -20°C.

For the isolation of polyadenylated RNA, poly U-sepharose (Pharmacia) was employed using deionized formamide containing buffers.

Partial purification of RNA was accomplished using 15-30% (w/v) sucrose gradients containing 0.01 M Tris-HCl, pH 7.5 and 0.5% SDS. Sedimentation was for 19 hr at 26,000 rpm at 20°C in a Beckman SW27 rotor. RNA was ethanol precipitated from the collected fractions and washed several times with 70% ethanol in order to eliminate sucrose.

Isolation and immunoprecipitation of polysomes. Flies were ground in liquid N₂, homogenized and the homogenate centrifuged as described above. After adding NP40 to 1%, polysomes were purified by sedimentation for 5 hr at 50,000 rpm at 4°C in a Beckman Ti 60 rotor through a cushion of 2 M sucrose in 0.3 M NH₄Cl, 0.05 M MgCl₂, 0.05 M Tris-HCl, pH 7.5 and 0.5% β-mercaptoethanol. The polysomes were resuspended in 0.3 M NaCl, 0.005 M MgCl₂ and 0.05 M Tris-HCl, pH 7.5 at 100-200 A₂₆₀/ml.

Immunoprecipitation of polysomes was done according to the methods of Gough and Adams (16) using goat anti-ADH antibody. RNA was then purified using the phenol/chloroform extraction procedure described above.

In vitro cell-free translation. Drosophila RNA was translated in a cell-free protein synthesizing system prepared from nuclease-inactivated

rabbit reticulocyte lysates (17). Tritiated leucine was used to follow incorporation. The translation products were electrophoresed in SDS-polyacrylamide gels (18) and visualized by fluorography (19).

To isolate cross-reacting material from the translation products, a procedure similar to that of Milman *et al.* (20) was used (21). The translation mixture was diluted 30-fold with 0.03 M KCl, 0.01 M Tris-HCl, pH 7.4, and 0.5% Triton X-100 and subjected to chromatography on a column of Sepharose linked to goat anti-ADH. The column was washed with 0.03 M KCl, 1 M NaCl, 1% Triton X-100, 1% sodium deoxycholate and 0.01 M Tris-HCl, pH 7.5 and then with deionized H₂O. ADH was eluted from the column with 0.2% SDS. The eluate was lyophilized and resuspended in SDS-gel sample buffer and electrophoresed as above.

Tryptic peptide analysis. In order to show that the radioactive product synthesized *in vitro* and isolated by immunochromatography was ADH, the *in vitro* translation mixture was mixed with purified ¹⁴C-leucine labelled ADH^F (labelled *in vivo*), immunoprecipitated and subjected to SDS-polyacrylamide gel electrophoresis. The gel was fractionated and the ¹⁴C-ADH containing band TCA precipitated. The TCA was removed by ether extraction and the protein was digested with trypsin. The resultant peptides were fractionated on an Aminex A-5 ion exchange column (22).

Construction of recombinant plasmids. cDNA was synthesized from both sucrose gradient-enriched RNA and from antibody precipitated polysomal RNA. Each 50 μ l reaction mix contained 0.1 M Tris-HCl, pH 8.7, 6 mM MgCl₂, 60 mM KCl, 30 mM β -mercaptoethanol, 100 μ g BSA/ml, 20 μ g p(dT)₁₂₋₁₈/ml, 800 μ g RNA/ml (heated at 68°C, 10 min and quick-cooled before addition), 1 mM each of dGTP, dCTP, dATP and TTP and 10 μ Ci each of ³H-dNTPs as tracers. Reactions were started by the addition of 32 units of reverse transcriptase. Incubation was for 1 hr at 45°C at which point another 32 units of enzyme were added and the incubation continued for an additional hr. The reactions were stopped by centrifuging the incubation mixtures through packed Sephadex G-50 in microfuge tubes to eliminate unincorporated nucleotides and for desalting. The cDNA synthesized under these conditions were generally greater than 600 nucleotides in length, and represented about 5% of the input RNA.

The mRNA-cDNA hybrid was then "tailed" (23) in 50 μ l of a reaction mixture which contained 0.14 M potassium cacodylate, 0.03 M Tris base, 1 mM CoCl₂, 100 μ M β -mercaptoethanol, 100 mM dATP, 200 μ g BSA/ml, 200 units terminal transferase/ml and about 1 μ g of ³H-cDNA-RNA hybrid.

After 100 min of incubation at 37°C, the reaction was stopped by addition of EDTA to 5 mM. The incubation time was calculated to yield 50-75 added dA residues per 3' end. pBR322 DNA (8 µg) was linearized with Pst I and tailed similarly for 4 min at 37°C using 100 µM TTP. About 50-70 dT residues were added per 3' end.

Equimolar quantities of the dA-tailed mRNA-cDNA hybrids (average cDNA length = 1 kb) and the dT-tailed pBR322, both freed of low molecular weight components by centrifugation through Sephadex G-50, were combined in 0.1 M NaCl, 1 mM EDTA and 0.01 M Tris-HCl, pH 7.5. The mixture was heated at 68°C for 10 min and annealed at 43°C for 2 hr. The resultant hybrid molecules were used to transform E. coli K-12, strain HB101 by the transfection procedure of Mandel and Higa (24).

We obtained 74 tetracycline resistant, ampicillin sensitive transformants from 0.5 µg of cDNA synthesized from sucrose gradient enriched RNA. The DNA inserts range from 500 to 2500 bases with the majority larger than 900 bases. Forty-six transformants were obtained from 0.4 µg of cDNA synthesized from antibody selected polysomal RNA. The DNA inserts range from 200 to 1300 bases with the majority being 800 bases.

Hybridization-selection and translation. DNA (1 µg) from pBR322, bacteriophage λ and pBR322 containing Drosophila sequences was immobilized on nitrocellulose filters (Schleicher and Schull, BA85) and utilized in hybridization-selection and translation according to Ricciardi et al. (25).

In situ hybridization. Drosophila salivary gland chromosomes were prepared for in situ hybridization by a modification of procedure of Pardue and Gall (26) as described by Strobel et al. (27). Nick-translation was performed according to Rigby et al. (28) using tritiated dNTPs.

DNA sequencing. 5' terminal labelling of restriction fragments by polynucleotide kinase and DNA sequencing were performed as described by Maxam and Gilbert (29). Polyacrylamide gels of 0.4 mm thick were used. The sequences were analyzed by the program of the Stanford Molgen Project and the NIH SUMEX-AIM facility.

Northern hybridization. RNA was glyoxalated and separated on agarose gels according to McMaster et al. (30). The RNA was transferred to diazo-benzoyloxymethyl (DBM)-paper and hybridized with ³²P-labelled nick-translated DNA (31).

Results

Identification of ADH-mRNA. Mature adult flies have been observed to

synthesize ADH (32) indicating that they possess functional ADH-mRNA. To identify this ADH-mRNA we isolated RNA from wild-type flies and translated it in a mRNA dependent rabbit reticulocyte cell-free system. The resultant ^3H -labelled translation products were separated by SDS-gel electrophoresis and visualized by fluorography. Figure 1 shows the results of this experiment. The polypeptides synthesized when total cytoplasmic, poly A-containing and poly A-lacking RNA were translated in vitro are shown in lanes C, B and A respectively. The total cytoplasmic and poly A-containing preparations (these show the bulk of translational activity) code for a great variety of proteins, with one prominent band, accounting for 1-2% of the total translational activity, migrating to the same position on SDS-polyacrylamide gel as ADH monomer (corresponding to a molecular weight of 25,000).

Protein of this same size binds specifically to anti-ADH antibody coupled to Sepharose (Figure 2, lanes C, D and G). The results of a control experiment, showing that the antibody specifically binds ADH is shown in Figure 2, lane B.

Further confirmation that ADH can be synthesized in a mRNA dependent protein synthesizing system is shown in Figure 3. Here ^3H -leucine labelled protein synthesized in vitro was subjected to antibody precipitation and SDS-polyacrylamide gel electrophoresis. The material migrating at a position corresponding to a molecular weight of 25,000 was then subjected to tryptic digestion and the resultant peptides separated by ion exchange chromatography. Authentic ^{14}C -leucine labelled ADH was present during these manipulations to serve as a marker. As shown in Figure 3 the peptide profiles of authentic ADH matches almost exactly that of the in vitro synthesized product.

From these experiments we conclude that Drosophila adults contain a polyadenylated mRNA that can direct the synthesis of ADH. ADH was identified by antibody precipitation, by SDS-gel electrophoresis and by virtue of its tryptic peptide pattern.

ADH-mRNA purification. We used two approaches to enrich for ADH-mRNA. First, total cytoplasmic RNAs were fractionated by sedimentation through sucrose gradients containing SDS. ADH-mRNA was assayed by translation in a reticulocyte in vitro system and SDSgel electrophoresis. The results of a single round of enrichment yielded one fraction containing RNAs of 11-13S that coded for polypeptides of about 23,000 to 35,000 daltons (data not shown). A second round of purification by sucrose

Figure 1. In vitro translation products of *Drosophila* RNAs. *Drosophila* RNAs were translated in the inactivated rabbit reticulocyte lysate cell free system. The ^3H -labelled translation products were electrophoresed on a 12-1/2% SDS-polyacrylamide gel and displayed by fluorography. A: poly A-lacking RNA translation, 40 μg translated, B: poly A-containing RNA translation, 20 μg translated, C: total cytoplasmic RNA translation, 20 μg translated, D: mixture of ^3H -labelled purified protein molecular weight markers. All markers except in vivo ^3H -labelled ADH were labelled by the alkylation reduction procedure (42).

Figure 2. Specific binding of an in vitro translation product to anti-ADH antibody sepharose column. The diluted ^3H -labelled translation products were chromatographed on anti-ADH antibody sepharose columns as described in the Method Section. Specifically bound materials were eluted and electrophoresed on a 12-1/2% SDS-polyacrylamide gel and displayed by fluorography. A, E and F: ^3H -labelled protein molecular weight markers, B: purified in vivo ^3H -labelled ADH retained by the column, C: total cytoplasmic RNA from the Adh^F strain translation, D: poly A-containing RNA from the Adh^F strain translation, G: total cytoplasmic RNA from the Adh^D strain translation.

gradient sedimentation yielded a preparation 4-5 fold enriched for ADH-mRNA (Figure 4, lane D).

Second, ADH-mRNA was purified from ADH containing polysomes specifically precipitated by anti-ADH antibody and *S. aureus* (16). Purification of at least fifty-fold was achieved; ADH polypeptide was the only in vitro translation product detected from such RNA preparations (Figure 4, lanes B and C).

cDNA cloning. The protocol we used to clone cDNA is described in the Materials and Methods section. A similar approach was reported by Wood and

Figure 3. Analysis of in vitro synthesized ADH. a) In vitro (^3H -labelled) synthesized protein was immunoprecipitated with anti-ADH antibody in the presence of in vivo (^{14}C -labelled) synthesized ADH. The immunoprecipitate was subjected to SDS-gel electrophoresis (10% gels) and the gels fractionated and counted. Electrophoresis was from right to left. b) Tryptic peptide profile of in vivo (^{14}C -labelled) and in vitro (^3H -labelled) synthesized ADH fractionated on the aminex A-5 ion exchange column.

Lee (33) and Zain et al. (34). The advantage of this procedure over the conventional double-stranded cDNA method is that fewer steps are required, thus reducing loss of material. However, as also noted by Zain et al. (34), the transformation efficiency in *E. coli* of such molecules is quite

Figure 4. SDS-polyacrylamide gel electrophoresis of in vitro translation products from antibody precipitated polysomal RNA. 5A260 units of polysomes per ml were immunoprecipitated as described by Gough and Adams (16) using 135 and 270 μ g of anti-ADH antibody and 375 and 750 μ l respectively of packed *Staphylococcus aureus* cells. The RNAs from phenol extracted polysomes were translated in a cell-free system, the translation products electrophoresed on a 12-1/2% SDS-polyacrylamide gel and fluorographed. A: 3 H-labelled protein molecular weight markers, B; translation of polysome precipitated by 135 μ g of anti-ADH antibody and 375 μ l of packed *Staphylococci*, C: translation of polysome precipitated by 270 μ g of anti-ADH antibody and 750 μ l of packed *Staphylococci* and D: translation of sucrose gradient enriched RNA.

low possibly due to the nuclease sensitive nature of the hybrid molecules. Both sucrose gradient enriched RNA and antibody selected polysomal RNA yielded the same efficiency--about 1×10^2 transformants per μ g of cDNA. Similar transformation efficiencies were reported (33,34).

Figure 5. SDS-polyacrylamide gel electrophoresis of in vitro translation products of RNAs hybridized by various DNAs. 1 μ g of each DNA was immobilized on nitrocellulose and hybridized to *Drosophila* RNA as described by Ricciardi et al. (25). The specifically bound RNA was eluted and translated in a cell-free system, the translation product electrophoresed on a 12-1/2% SDS-polyacrylamide gel and fluorographed. Lanes 1, 14: 3 H-labelled protein molecular weight markers, 2-7: translation of RNAs hybridized to six different plasmids containing *Drosophila* DNA inserts, 8: pBR322 DNA, 9: λ DNA, 10: translation of RNAs which do not bind to a blank filter in a mock hybridization, 11: translation of RNAs which bind to a blank filter in a mock hybridization, 12: sucrose gradient enriched RNA translation, 13: no exogenous RNA added.

From a transformation experiment using antibody selected polysomal RNA-cDNA hybrid molecules, one plasmid carrying ADH-cDNA (pADH-cDNA) has been identified by the three following criteria. First, using the technique of hybridization selection and translation (25), we demonstrated that DNA from this clone was capable of hybridizing to an RNA species that was translatable into ADH (Figure 5, lane 6). Control DNA from bacteriophage λ , pBR322 and other plasmids failed to do this.

Second, ^3H -labelled nick-translated pADH-cDNA hybridized specifically to only the region 35 B1-5 on salivary gland chromosomes from D. melanogaster (Figure 6a, b and c). This region had previously been identified as the site of the Adh structural gene by genetic and cytological analyses (9, 10, 11, 12).

Third, the ADH.cDNA insert has been sequenced (Figure 7a and b). Translation of this sequence yields a protein sequence that corresponds to the C-terminal amino acid portion of ADH (4). The sequencing studies also show that pADH-cDNA contains an insert of about 710 bases; 348 code for 116 C-terminal amino acids (45% of the ADH protein), 180 represent nucleotides located at the 3' untranslated region of the ADH-mRNA, and the remainders are about 150 and 30 bases of dA residues added at the 5' and 3' ends of ADH-mRNA respectively.

Characterization of ADH-mRNA. ADH-mRNA was characterized further by "Northern" analysis using pADH-cDNA as a probe. RNA was denatured by glyoxalation and subjected to electrophoresis on agarose gels. After transferring to DBM-paper, ADH-mRNA was probed with ^{32}P -labelled nick-translated cDNA clone. Two RNA preparations, one prepared by sucrose gradient centrifugation, the other was antibody precipitated polysomes, each showed one hybridizable band of 1120 ± 20 bases (Figure 8). Since about 750 bases of the mRNA are involved with protein coding (ADH contains about 250 amino acids), and the 3' untranslated region is 180 bases long, and assuming the length of the 3' poly A segment is about 100 bases, the 5' untranslated region would consist of about 100 bases.

Figure 6. In situ hybridization of ^3H -labelled pADH-cDNA to salivary gland polytene chromosomes. Three different chromosome sets are shown in a, b' and c' at low magnification and their 2L (left arm of second chromosome) portions are shown at higher magnification in a, b and c respectively. Arrows point to the silver grains over the 35B1-3 region.

Figure 7. Nucleotide sequence of pADH-cDNA insert. a. Diagram of restriction map and sequencing strategy employed. The regions covered by sequencing are indicated by arrows. The solid dots correspond to the ³²P-labelled 5' ends. The orientation of pADH-cDNA inserted into the PstI site (between nucleotides 3612 and 3613) of pBR322 is as shown. b. 529 bases of the coding strand (mRNA like) of pADH-cDNA insert and corresponding amino acid sequence (Adh^F strain) are shown. The termination codon, TAA is in a dashed box. The AATAAA sequence, present near the 3' end in all polyadenylated eucaryotic mRNAs, is boxed. The 3' untranslated symmetric region is shown by arrows pointing away from the axis of symmetry. The 3' untranslated homologous region is underlined. The wavy line indicates the single amino acid difference between the Adh^F and Adh^S fly strains.

Figure 8. Autoradiogram of *Drosophila* RNA hybridized to pADH-cDNA. 8 μ g of RNA was glyoxalated and electrophoresed on 1.2% agarose gel, transferred to a DBM-paper and probed with 32 P-labelled nick-translated pADH-cDNA. A: sucrose gradient enriched RNA and B: immunoprecipitated poly-somal RNA. The size standards (in bases) are 32 P-uniformly labelled SV40 DNA digested with Hind III.

DISCUSSION

We have identified, isolated and characterized ADH-mRNA from *Drosophila melanogaster*. The message is about 1120 nucleotides long, is polyadenylated, has untranslated regions at the 5' and 3' ends, and accounts for about 1-2%

of the translational activity of mRNA from adult flies. Using the immunoprecipitation procedure of Gough and Adams (16), we have obtained at least 50-fold enrichment for this mRNA as assessed by *in vitro* translation.

From this mRNA, using dA-tailed mRNA-cDNA hybrid molecules annealed to dT-tailed pBR322, we have constructed a cDNA clone. The transformation efficiency of such hybrid structures was lower than that expected for double-stranded DNA, but the resultant pADH-cDNA clone proved to be a faithful copy of a portion of the ADH-mRNA; the translated amino acid sequence matched the known C-terminal amino acid sequence of ADH and the nucleotide sequence corresponded to the sequence of a genomic clone (5) isolated from the Maniatis library (35).

However, this cDNA clone was not of full length. It is possible that the incomplete length was due to some sequence and/or secondary structure peculiar to ADH-mRNA that might have affected the reverse transcription reaction. Others have observed that reverse transcriptase makes nonrandom stops along mRNA (36,37). We also cannot exclude the possibility that the tailing reaction and/or transformation of *E. coli* using the hybrid molecules might have adversely affected the final size of the cDNA insert.

Several observations can be made about the nucleotide sequence of the protein coding and the 3' untranslated regions of ADH-mRNA. Like other eucaryotic messages, the codon usage (Table 1) of this mRNA is nonrandom. There is underutilization of codons ending in A and preference for codons ending in C and G. This observation is true for the complete genomic ADH gene as well (5). Other eucaryotic mRNAs (for example, β -major globin mRNA (38)) exhibit different preferred codons. Thus, codon usage seems to be message specific.

The difference in electrophoretic mobilities of ADH^F and ADH^S from two naturally occurring Adh^{Fast} and Adh^{Slow} fly strains has been attributed to at least one amino acid change (threonine in ADH^F for lysine in ADH^S, (39)). This amino acid, underlined in Figure 9 by a wavy line, is threonine in pADH-cDNA which comes from the Adh^F strain. A single base change (ACG for threonine to AAG for lysine) has occurred at this position in the Adh^S strain.

The 3' untranslated region, which is AT rich, has several interesting features. The symmetric region (nucleotides 366-373; 375-382) contains the longest stretch of A residues (15 bases). The homologous region (nucleotides 499-509; 510-519) is 10 nucleotides from the putative poly A-addition site (the dinucleotide CC). The hexanucleotide AATAAA, which is usually

Table 1. Codon usage of the C-terminal portion of ADH.

0/TTT/PHE	0/TCT/SER	0/TAT/TYR	0/TGT/CYS
4/TTC/PHE	4/TCC/SER	3/TAC/TYR	1/TGC/CYS
0/TTA/LEU	0/TCA/SER	1/TAA/OC	0/TGA/OP
2/TTG/LEU	1/TCG/SER	0/TAG/AM	4/TGG/TRP
0/CTT/LEU	1/CCT/PRO	1/CAT/HIS	0/CGT/ARG
1/CTC/LEU	5/CCC/PRO	2/CAC/HIS	1/CGC/ARG
0/CTA/LEU	0/CCA/PRO	0/CAA/GLN	0/CGA/ARG
8/CTG/LEU	0/CCG/PRO	5/CAG/GLN	0/CGG/ARG
1/ATT/ILE	2/ACT/THR	1/AAT/ASN	0/AGT/SER
6/ATC/ILE	9/ACC/THR	6/AAC/ASN	1/AGC/SER
0/ATA/ILE	0/ACA/THR	2/AAA/LYS	0/AGA/ARG
0/ATG/MET	2/ACG/THR	4/AAG/LYS	0/AGG/ARG
2/GTT/VAL	3/GCT/ALA	1/GAT/ASP	0/GGT/GLY
4/GTC/VAL	9/GCC/ALA	2/GAC/ASP	5/GGC/GLY
0/GTA/VAL	0/GCA/ALA	0/GAA/GLU	2/GGA/GLY
5/GTG/VAL	1/GCG/ALA	5/GAG/GLU	0/GGG/GLY

found in other eucaryotic mRNAs about 10-20 nucleotides prior to the poly A-addition site and thought to be signal for transcription termination or poly A addition (40), is located 45 nucleotides from the putative poly A-addition site of this message. It is not known, whether any of these sequences play a role in gene expression and regulation.

We have used pADH-cDNA as a probe for identifying genomic clones of ADH. We have now completely sequenced one such clone that contains the entire coding sequence for the ADH polypeptide (41). There are two intervening sequences in the protein coding region. Both contain the 5' GT and 3'AG dinucleotides characteristic of those at the intervening sequence boundaries of eucaryotic genes.

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