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The molecular cloning and characterisation of cDNA coding for the α subunit of the acetylcholine receptor

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ABSTRACT

A rare cDNA coding for most of the α subunit of the Torpedo nicotinic acetylcholine receptor has been cloned into bacteria. The use of a mismatched oligonucleotide primer of reverse transcriptase facilitated the design of an efficient, specific probe for recombinant bacteria. DNA sequence analysis has enabled the elucidation of a large part of the polypeptide primary sequence which is discussed in relation to its acetylcholine binding activity and the location of receptor within the plasma membrane.

When used as a radioactive probe, the cloned cDNA binds specifically to a single Torpedo mRNA species of about 2350 nucleotides in length but fails to show significant cross-hybridisation with α subunit mRNA extracted from cat muscle.

INTRODUCTION

The acetylcholine receptor (AChR) in the electric organ of the fish Torpedo is an oligomeric glycoprotein consisting of the α (40,000 daltons (D)), β (49,000 D), γ (57,000 D) and δ (65,000 D) subunits in the molar ratio $\alpha_2 \beta_1 \gamma_1 \delta_1$ (1,6). There is evidence that the α subunit contains the acetylcholine binding site (1,18) and upon binding ligand, the ion channel is opened in the cell membrane at the neuromuscular junction. Although Torpedo AChR has been a subject of extensive investigation because of its relative abundance in the electric organ compared with mammalian muscle, it is still a rare protein, has been difficult to purify in undegraded form and only short N-terminal polypeptide sequences of its subunits have been reported (6). Furthermore, little is known regarding the functional roles of the β, γ and δ subunits and also of the mechanisms involved in assembling the oligomeric receptor and in opening the ion channel following ligand binding. Clearly, it is likely that the application of recombinant DNA technology to genes encoding this neurotransmitter

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receptor will be valuable in investigating these questions and many others, including the relationship and evolution of the genes encoding the different subunits. The cloning of the Torpedo γ subunit cDNA was reported during the preparation of this manuscript along with a short N-terminal protein sequence derived from the corresponding DNA sequence (29). We now report an extensive characterisation of the mRNA and cloned cDNA coding for the Torpedo α subunit.

MATERIALS AND METHODS

Purification of AChR mRNA by Sucrose Gradient Centrifugation

150μg of poly A^+ RNA extracted (4) from the electric organ of Torpedo marmorata was heated to 65°C for 5 min and then sedimented through a 10-31% ($^w/_w$) isokinetic sucrose gradient containing 10mM Hepes-Na, pH 7.5, 1mM EDTA and 0.1% Lithium dodecyl sulphate in a Beckman SW41 rotor at 40,000 r.p.m. for 17 hrs at 2°C. Fractions (~0.34ml) were collected, precipitated with ethanol and dissolved in 10ul 5mM Hepes-Na, pH 7.5.

Assay of AChR mRNA

Poly A^+ RNA was injected into Xenopus oocytes and cultured at 21°C as described (5). The oocytes were then extracted in 50mM phosphate buffer, pH 7.2/1% Triton X-100/1mM EDTA/1mM EGTA/0.1mM phenylmethylsulphonyl flouride and analysed for α-bungarotoxin (α-BuTX) binding activity using $\{^{125}I\}$-α-BuTX as described (5).

Priming Reverse Transcription of α Subunit mRNA

The oligodeoxyribonucleotide primers were synthesised using triester methodology (8). cDNA was synthesised with reverse transcriptase from 1.4μg of the 18S mRNA from a sucrose gradient using 4 p mole of the primer that had been phosphorylated using T4 polynucleotide kinase and $\{\gamma\text{-}^{32}P\}$ ATP as described previously (4).

Cloning of AChR α Subunit cDNA

Single stranded cDNA was synthesised by reverse transcription of the 18S mRNA fractions from sucrose gradients using oligo (dT) as primer. Double stranded cDNA was prepared again with the use of reverse transcriptase. After S1 nuclease treatment followed by incubation with E.coli DNA polymerase I, the blunt-

ended double stranded cDNA was fractionated on a 5% acrylamide gel and cDNA (>700bp in length) was eluted, joined to Hind III specific decanucleotide linkers with DNA ligase and then restricted with Hind III endonuclease. The plasmid cloning vehicle pAT 153 (10) was digested with Hind III, treated with bacterial alkaline phosphatase and then ligated to the cDNA.

An E.coli DH1 strain (kindly provided by D. Hanahan) was transformed with the resulting plasmids. Details of the above methods have been published elsewhere (11, 12). Colonies were then hybridised (13). The probe (3'CTTTGTGCAAACCAACGAT5') was radiolabelled with {γ-^{32}P}ATP and T4 polynucleotide kinase and hybridised to the filters at a concentration of 0.2nM (8 x 10^6 d.p.m./pmol) for 36 hours at 55^{o}C in 3 x SSC, 10 x Denhardts (14), 50ug/ml sonicated, denatured salmon sperm DNA, 0.1mM ATP and 0.1% SDS. The filters were then washed as described (15).

DNA Sequence Analysis

Restriction enzyme digestions were carried out following the instruction of the supplier. Nucleotide sequences were determined by the method of Maxam and Gilbert (9) using 5'-end and 3'-end labelled DNA fragments.

Northern Blot Analysis of Poly A$^+$ RNA

Poly A$^+$ RNA was denatured with glyoxal and electrophoresed through a 1.4% agarose gel and then transferred to nitrocellulose (23). After baking at 80^{o}C for 2 hours, the filter was pre-hybridised overnight in 40% (^{v}/v) deionised formamide, 35mM sodium phosphate, pH 6.5, 6 x SSc, 1 x Denhardts (14), 50ug/ml sonicated salmon sperm DNA at 30^{o}C and then hybridised with the nick-translated (25) cDNA plasmid (10^7 d.p.m./ml, 6 x 10^8 d.p.m./ug) in the above solution at 30^{o}C for 24 hours. The filter was then washed for 30 minutes in four changes of 6 x SSC containing 0.1% SDS at room temperature and exposed to X-ray film at -70^{o}C for 17 hours using an intensifying screen.

RESULTS

The mRNA coding for each Torpedo AChR subunit is present at only 0.1% - 0.4% of the total mRNA in the electric organ (2,3) and so as a first step towards cloning the α subunit, AChR mRNA was partially purified by sedimentation through sucrose gradients.

In order to assay the gradient fractions for AChR mRNA, the recovered mRNA was microinjected into Xenopus oocytes and the resulting translation products monitored for their ability to bind the radioactively - labelled snake venom toxin {^{125}I}α-BuTX, which is a well-known property of the native, active oligomeric AChR (5). We have previously described the synthesis and assembly of active AChR following the microinjection of total Torpedo poly A^+ RNA into Xenopus oocytes (5,30).

Fig 1A shows that active AChR, able to bind α-BuTX, was synthesised by mRNA sedimenting at about 18S and Fig 1B demon-

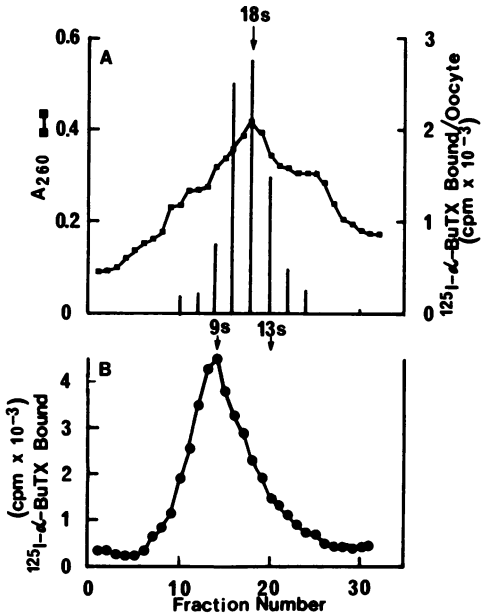


Fig. 1. A) Purification of AChR mRNA by sucrose gradient centrifugation. Every second fraction was microinjected into groups of 10 Xenopus oocytes (50nl per oocyte) and α-BuTX binding activity (5) found in the translation products of oocytes is shown. Human fibroblast ribosomal RNA was sedimented in a parallel sucrose gradient and the position of the 18S rRNA is indicated. B) Analysis of the molecular form of the AChR synthesised in oocytes following microinjection with 18S mRNA. The extract from 30 oocytes was loaded on to a 5-20% (w/w) sucrose gradient and centrifuged as described (5). The resulting fractions were analysed for α-BuTX binding activity using {^{125}I}-α-BuTX (5). The arrows indicate the positions to which the 9S (monomer) and the 13S (dimer) forms of the native AChR from Torpedo sediment in a parallel sucrose gradient (1,6).

strates that this AChR translation product sedimented on a separate sucrose gradient at about 9S, consistent with it corresponding to the native, monomeric receptor molecule containing the five subunits α_2 β γ δ (1,6). It is known that the different subunits are coded by different mRNAs (7) and so this result indicates that these mRNAs all co-sediment at about 18S.

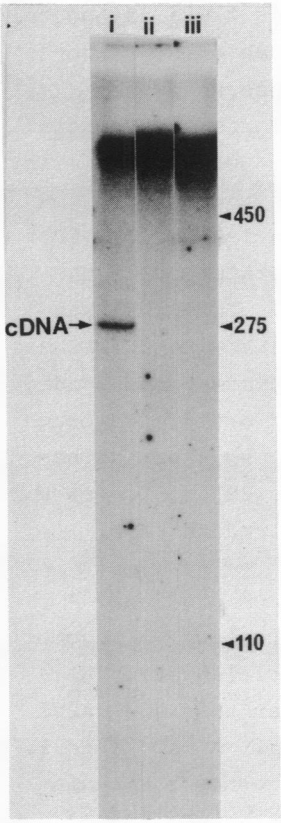
The N-terminal 54 residues of the α subunit from Torpedo californica have been identified by direct protein sequencing (6) and in an effort to obtain a sepcific oligonucleotide primer of reverse transcriptase on α subunit mRNA, we next synthesised six oligodeoxyribonucleotides (α1-α6) that are complementary to some of the potential coding sequences for amino acids 22-25 of the mature polypeptide (Fig 2A). We then tested these $\{^{32}P\}$ oligomers for their ability to prime the reverse transcription of ~18S mRNA extracted from the electric organ of Torpedo marmorata and purified by sucrose gradient centrifugation. Electrophoretic analysis of the resulting cDNA products revealed that the oligomer α2 primed a cDNA of about 275 nucleotides in length (Fig 2B) that was absent in the transcription products obtained with the other oligomers (K. Sumikawa; unpublished data). This cDNA was sequenced (Fig 2C) to show that it was complementary to the AChR α subunit mRNA, since the predicted protein sequence corresponded with that region previously determined by direct protein sequencing (6). Based on this α subunit cDNA sequence, we then synthesised an oligomer of 19 deoxyribonucleotides (Fig 2D) to act as an efficient and very specific probe for α subunit recombinant bacterial colonies.

A recombinant cDNA library was prepared from gradient-purified mRNA and screened by hybridisation to the $\{^{32}P\}$ oligomer shown in Fig 2D. Six positive clones were identified (Fig 2E). The frequency of positive colonies was much lower than the value (>50) we estimated from the abundance of α subunit mRNA. This is to be expected since the probe hybridises only to the α subunit cDNA containing the nucleotide sequence near the N-terminus of the α subunit. One containing the largest cDNA insert (of about 900 base-pairs (bp)) was used for sequencing studies. Included in Fig 3 is the mRNA structure deduced from this cDNA sequence. It exhibits a single open translation frame, thus enabling a

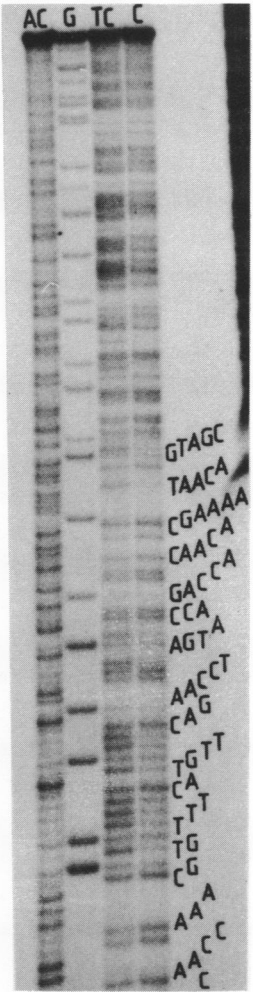
A.

(a)	Amino acid sequence		.....Val.Glu.His.His
			22 23 24 25
(b)	Possible codons		5'GU$^{A}_{C}$GA$^{A}_{G}$.CA$^{U}_{C}$.CA$^{U}_{C}$ 3'
			G
(c)	Primers	α1	3'CAT.CTT.GTG.GTG 5'
		α2	3'CAT.CTC.GTG.GTG 5'
		α3	3'CAG.CTT.GTG.GTG 5'
		α4	3'CAG.CTC.GTG.GTG 5'
		α5	3'CAA.CTT.GTG.GTG 5'
		α6	3'CAA.CTC.GTG.GTG 5'

B.



C.



D.

α2-primed cDNA sequence from (C) ...GAT GAC AAT AAA AGC ACA ACA

mRNA sequence deduced from 2-primed cDNA ...CUA CUG UUA UUU UCG UGU UGU

Amino acid sequence predicted ...Leu Leu Leu Phe Ser Cys Cys

Amino acid Sequence published ....

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CCA GAC CAT GAT CCA AGA CTT GTA CTT TGT GCA AAC CAA CGA TTA AAT AAT CTT

GGU CUG GUA CUA GGU UCU GAA CAU GAA ACA CGU UUG GUU GCU AAU UUA UUA GAA

Gly Leu Val Leu Gly Ser Glu His Glu Thr Arg Leu Val Ala Asn Leu Leu Glu

... ... Ser Glu His Glu Thr Arg Leu Val Ala Asn Leu Leu Glu

E.

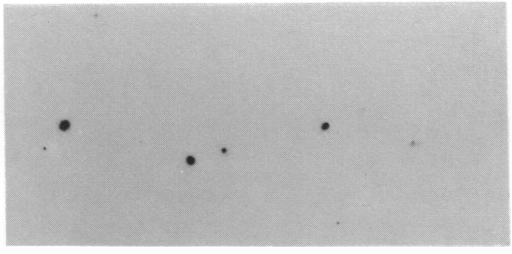
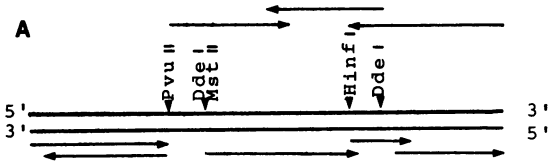


Fig. 2. Cloning strategy for the AChR α subunit cDNA. A) Deduction of synthetic oligonucleotide primers for amino acids 22-25 of the α subunit of Torpedo AChR (6). B) Electrophoretic analysis of the cDNA products obtained: i) With α2 primer and 18S mRNA. ii) With α2 primer and >18S mRNA (a sucrose gradient fraction that does not contain the α subunit mRNA). iii) With α1 primer and 18S mRNA. The migration of denatured Hind III restriction fragment markers of phage PM2 is indicated (sizes given in nucleotides). C) Nucleotide sequence of α2-primed cDNA. The cDNA arrowed in (B) was eluted and subjected to sequence analysis (9). Only part of the autoradiograph of the 8% polyacrylamide gel containing 7M urea, is shown. D) Comparison of the amino acid sequence deduced from the nucleotide sequence of α2 primed cDNA (C) with the published amino acid sequence (6). Asterisks denote the oligonucleotide synthesised as a hybridisation probe for recombinant bacteria containing the α subunit cDNA. E) Colony hybridisation with the ^{32}P labelled oligonucleotide probe. About 8,000 ampicillin resistant transformants were obtained from ~16ng of cDNA (~1,500 colonies/plate). An autoradiograph from two filters containing 3 positive clones is shown.

A



B

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5'------GGAGUGCAGAAGAUCUAACAAGUUCCUCGUUUAGUUAUUAGAAGUGGCAGAUUUGCUUGAAAAGCCAAUUAUUGAAAGCU
          -80       -70       -60       -50       -40       -30       -20       -10

                             -20                                       -10
      Met Ile Leu Cys Ser Tyr Trp His Val Gly Leu Val Leu Leu Leu Phe Ser Cys Cys
GAAGA AUG AUU CUG UGC AGU UAU UGG CAU GUC GGG UUG GUG CUA CUG UUA UUU UCG UGU UGU
   -1 1          10          20          30          40          50
                  -1  1                                      10
  Gly Leu Val Leu Gly Ser Glu His Glu Thr Arg Leu Val Ala Asn Leu Leu Glu Asn Tyr
  GGU CUG GUA CUA GGU UCU GAA CAU GAA ACA CGU UUG GUU GCU AAU UUA UUA GAA AAU UAU
    60          70          80          90          100         110

                 20                                        30
  Asn Lys Val Ile Arg Pro Val Glu His His Thr His Phe Val Asp Ile Thr Val Gly Leu
  AAC AAG GUG AUU CGU CCA GUG GAG CAU CAC ACC CAC UUU GUA GAU AUU ACA GUG GGG CUA
   120         130         140         150         160         170

                 40                                        50
  Gln Leu Ile Gln Leu Ile Asn Val Asp Glu Val Asn Gln Ile Val Glu Thr Asn Val Arg
  CAG CUG AUA CAA CUC AUC AAU GUG GAU GAA GUA AAU CAA AUU GUG GAA ACA AAU GUG CGC
   180         190         200         210         220         230

                 60                                        70
  Leu Arg Gln Gln Trp Ile Asp Val Arg Leu Arg Trp Asn Pro Ala Asp Tyr Gly Gly Ile
  CUA AGG CAG CAA UGG AUU GAU GUG AGG CUU CGC UGG AAU CCA GCC GAU UAU GGU GGA AUU
   240         250         260         270         280         290

                 80                                        90
  Lys Lys Ile Arg Leu Pro Ser Asp Asp Val Trp Leu Pro Asp Leu Val Leu Tyr Asn Asn
  AAA AAG AUC AGA CUG CCU UCU GAU GAU GUU UGG CUG CCA GAU UUA GUU CUG UAC AAC AAU
   300         310         320         330         340         350

                100                                       110
  Ala Asp Gly Asp Phe Ala Ile Val His Met Thr Lys Leu Leu Leu Asp Tyr Thr Gly Lys
  GCU GAU GGU GAU UUU GCC AUU GUU CAC AUG ACC AAA CUG CUU UUG GAU UAU ACG GGA AAA
   360         370         380         390         400         410

                120                                       130
  Ile Met Trp Thr Pro Pro Ala Ile Phe Lys Ser Tyr Cys Glu Ile Ile Val Thr His Phe
  AUA AUG UGG ACA CCU CCA GCA AUC UUC AAA AGC UAU UGU GAA AUU AUU GUA ACA CAU UUC
   420         430         440         450         460         470

                140                                       150
  Pro Phe Asp Gln Gln Asn Cys Thr Met Lys Leu Gly Ile Trp Thr Tyr Asp Gly Thr Lys
  CCA UUU GAU CAA CAA AAU UGC ACU AUG AAG UUG GGA AUC UGG ACG UAU GAU GGG ACA AAA
   480         490         500         510         520         530

                160                                       170
  Val Ser Ile Ser Pro Glu Ser Asp Arg Pro Asp Leu Ser Thr Phe Met Glu Ser Gly Glu
  GUU UCC AUA UCU CCG GAA AGU GAC CGU CCG GAU CUG AGU ACA UUU AUG GAA AGU GGA GAG
   540         550         560         570         580         590

                180                                       190
  Trp Val Met Lys Asp Tyr Arg Gly Trp Lys His Trp Val Tyr Tyr Thr Cys Cys Pro Asp
  UGG GUA AUG AAA GAU UAU CGU GGA UGG AAG CAC UGG GUG UAU UAU ACC UGC UGU CCU GAC
   600         610         620         630         640         650

                200                                       210
  Thr Pro Tyr Leu Asp Ile Thr Tyr His Phe Ile Met Gln Arg Ile Pro Leu Tyr Phe Val
  ACG CCU UAC CUG GAU AUC ACC UAC CAU UUU AUC AUG CAG CGU AUU CCU CUU UAU UUU GUU
   660         670         680         690         700         710

                220                                       230
  Val Asn Val Ile Ile Pro Cys Leu Leu Phe Ser Phe Leu Thr Val Leu
  GUG AAC GUC AUC AUU CCU UGU CUG CUU UUU UCA UUU UUA ACU GUA UUA------3'
   720         730         740         750         760
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prediction of the encoded protein sequence. A comparison of this predicted polypeptide sequence with the previously determined N-terminal sequence of the α subunit (6) clearly reveals that this cloned cDNA encodes the AChR α subunit, since with only one exception (Asn instead of Ser) these two regions of sequence are identical (Fig 3). The cDNA also codes for an additional 24 residues at the N-terminus of the encoded protein and this sequence is very similar, both in length and in its hydrophobic character, to the pre-peptide signal sequences of secreted proteins (16) and viral membrane glycoproteins (17). These residues presumably constitute a cleavable signal peptide responsible for associating the protein with the endoplasmic reticulum as a first step towards insertion into the plasma membrane.

The cloned cDNA is not a full-length copy of the α subunit mRNA and hence we were only able to deduce the mature polypeptide sequence as far as amino acid number 231, which represents about 65% of this sequence (19) and which establishes the identity of a further 177 residues of the mature α subunit. We have been able to identify 5 cysteine residues so far, which is interesting in view of the knowledge that a disulphide bridge exists in the immediate vicinity of the ACh binding site within the α subunit (1,18).

Another notable feature of the cloned cDNA sequence is that it indicates that the successful α2 primer was not totally complementary to the α subunit mRNA. There must have been a rG : dT mismatch, three nucleotides from the 3'oH priming terminus and a rU : dG mismatch nine nucleotides away from this terminus.

Fig. 3. A) Strategy employed for sequencing the cloned AChR α subunit cDNA. The restriction enzyme cleavage sites used to generate fragments for sequencing are shown. Arrows indicate the direction of sequencing and the approximate length of the determined sequence (arrows at the top indicate sequencing on the upper strand while arrows at the bottom indicate sequencing on the lower strand). B) Primary structure of the AChR α subunit mRNA. This was deduced from the determined cDNA sequence and is numbered in the 5' → 3' direction beginning with the first nucleotide of the initiator AUG codon. The 5' untranslated sequence is indicated by negative numbers. The encoded amino acid sequence is shown above the nucleotide sequence.

Clearly, these mismatches did not prevent specific priming with reverse transcriptase.

It is known that the Torpedo AChR subunits are glycoproteins (19,20) and the α subunit of muscle AChR is linked to a single oligosaccharide chain through an Asn linkage close to the N-terminus (27,28). Therefore, it is interesting in this connection that the cloned α subunit cDNA sequence codes for a potential N-glycosylation site (Asn-X-Ser/Thr (21)) at position 141 (Fig 3).

In order to determine the size of the mRNA coding for the α subunit of Torpedo AChR, the cloned cDNA was used as a radioactive probe for poly A^+ RNA separated by gel electrophoresis. Figure 4 (lanes 3 + 4) shows that a single mRNA species, corresponding to about 2350 nucleotides in length, specifically hybridised to the α subunit probe. Since the 5' untranslated mRNA sequence contains about 85 nucleotides (Figs 2B and 3B), the prepeptide signal sequence is coded by 72 nucleotides and the mature polypeptide must be coded by ~1068 nucleotides in order to specify the 356 amino acids estimated to be present in the mature α subunit (19), the 3' untranslated mRNA sequence must therefore

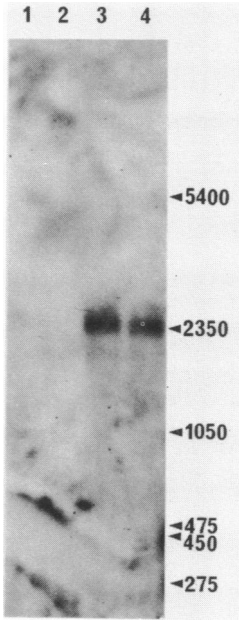

Fig. 4. Hybridisation of the radioactive, cloned AChR α subunit cDNA to size-fractionated poly A^+ RNA. Lane 1, 15ug of poly A^+ RNA extracted from cat innervated leg muscle. Lane 2, 9ug of poly A^+ RNA from cat denervated leg muscle. The extraction of active AChR mRNA from cat muscle is described elsewhere (26). Lane 3, 10ug of poly A^+ RNA from Torpedo electric organ. Lane 4, 6ug of poly A^+ RNA from Torpedo electric organ (from a different fish to the RNA in Lane 3). The parallel migration of glyoxal-treated denatured Hind III restriction fragments of phage PM2 (shown in nucleotides) is indicated. Details can be found in Materials and Methods.

be very long and of the order of ~1100 bases.

Mammalian AChR is of particular interest in view of its involvement in the disease myasthenia gravis. In order to ask whether the cloned Torpedo α subunit cDNA can be used as a probe for its mammalian counterpart, it was also hybridised in the same experiment to electrophoretically-separated AChR mRNA extracted from cat muscle (26). Fig 4 shows that although more cat muscle mRNA was loaded onto the gel than Torpedo mRNA, no cross-hybridisation was observed even though the stringency of this hybridisation was relatively low.

DISCUSSION

The cloning strategy employed here consisted of elucidating the structure of a short synthetic oligonucleotide capable of specifically priming the reverse transcription of partially purified α subunit mRNA. The resulting α subunit cDNA could then be sequenced allowing the design and use of a larger, more efficient and highly specific probe for recombinant bacteria containing the α subunit cDNA. We have previously used a similar strategy to sequence (4) and clone (12) the human fibroblast interferon cDNA and more recently to sequence and clone a human epsilon immunoglobulin cDNA (31). Its ability to sequence and clone desired genes, based only on a partial polypeptide sequence, is quite clear.

In this case, it was of particular note that the successful mRNA primer contained a rG : dT mismatch, three nucleotides from the 3'oH priming terminus and a rU : dG mismatch, nine nucleotides away from this terminus. Although the relative stability of RNA : DNA hybrids containing such mismatches has been reported (22), we have extended this finding by showing the tolerance of two such mismatches in a primer of reverse transcriptase, even when one lies very close to the actual priming terminus. Although inevitably reducing the priming efficiency, this result further encourages the inclusion of such mismatches into oligonucleotide primers and probes which have been based on redundant protein sequences (22).

The successful cloning and sequencing of a partial cDNA copy of the Torpedo AChR α subunit mRNA has enabled us to ident-

ify the nature of a further 177 contiguous amino acids in the mature α subunit which were hitherto unknown. So far, we can identify five Cys residues within the mature α subunit sequence, which is relevant with respect to the structure of the acetylcholine binding site existing within the α subunit. It is known that a disulphide bridge exists in the immediate vicinity of the acetylcholine binding site within the α subunit (1,18) and primary sequence data such as that elucidated here, as well as improving our basic knowledge of the AChR, may also facilitate identification of the ligand binding site following secondary and tertiary structure predictions.

The 54 N-terminal residues of the mature α subunit, as deduced from our DNA sequence analysis, are identical to those previously established by direct protein sequencing (6) apart from one difference at position 42 (Asn instead of Ser). Although other possibilities exist, it is quite likely that this discrepancy reflects species differences (Torpedo californica versus Torpedo marmorata) or else is a consequence of genetic polymorphism within the same species.

Centrifugation of Torpedo poly A^+ RNA through a sucrose gradient resulted in the presence of AChR mRNA within the ~18S fractions. The latter were shown to code for active receptor, capable of binding α-BuTX, which sediments as the native, oligomeric structure. This means that although the sizes of the component subunits vary from 40,000 D to 65,000 D (1,6), the respective mRNAs of the four different subunits must be similar in size. Indeed, using the cloned α subunit cDNA as probe, we estimated that the α subunit mRNA consists of ~2350 nucleotides which is very similar to the recent estimate of 2100 nucleotides for the size of the Torpedo γ subunit mRNA (29).

Our cloned Torpedo α subunit cDNA failed to cross-hybridise with AChR mRNA extracted from cat muscle indicating the absence of extensive, largely uninterrupted homologies between the corresponding fish and cat α subunit mRNA sequences, even though both α subunits contain the ACh binding site (1,18) and similarities exist in their peptide maps (24). This suggests that Torpedo clones may not facilitate the isolation of mammalian AChR clones.

Amongst other applications, the cDNA clone now available will facilitate investigations into the regulation of AChR gene expression, the organisation of AChR genes within the genome and the purification of α subunit mRNA with a view to more detailed investigation into the function and properties of the α subunit and the mechanics of receptor assembly.

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Added in press: Another insert contained Fig.3B sequence and contiguous (Fig.5) region.

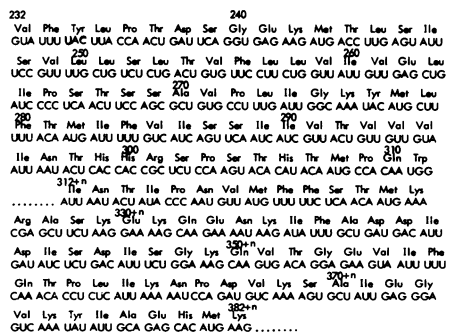

Fig. 5. mRNA sequence, obtained as for Fig. 3 on single cDNA strands. A few (n) residues were lost due to an internal Bgl II site. The mature subunit has > 400 amino acids (cf. 356 estimated from composition data (19)). Cysteine total remains at 5.