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A human acetylcholinesterase gene identified by homology to the *Ace* region of *Drosophila*

(acetylcholinesterase mRNA/hybridization-selection/*Xenopus* oocyte bioassay)

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ABSTRACT **The *Ace* locus of the *Drosophila* genome controls biosynthesis of the neurotransmitter-hydrolyzing enzyme acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7). We injected the mRNA species hybridizing with DNA fragments from this region into *Xenopus* oocytes, in which acetylcholinesterase mRNA is translated into active acetylcholinesterase. A 2.0-kilobase (kb) fragment of DNA from this region selectively hybridizes with *Drosophila* mRNA capable of inducing the biosynthesis of acetylcholinesterase in oocytes. This *Drosophila* DNA fragment cross-hybridized with human brain poly(A)$^+$ RNA. We therefore used this DNA fragment as a probe for homologous sequence(s) in a human genomic DNA library and thus selected a 13.5-kb human DNA segment. DNA blot-hybridization revealed that a 2.6-kb fragment of this human DNA segment hybridizes with the *Drosophila* 2.0-kb DNA fragment. Both *Drosophila* and human fragments hybridized with a human brain mRNA species of about 7.0-kb that was barely detectable in the acetylcholinesterase-deficient HEp carcinoma. A fraction containing mRNA of similar size, extracted from human brain, induced acetylcholinesterase biosynthesis in oocytes. The human DNA fragment also was used in hybridization-selection experiments. In oocytes, hybrid-selected human brain mRNA induced acetylcholinesterase activity that was completely inhibited by 1,5-bis[4-(allyldimethylammonium)phenyl]pentan-3-one dibromide but not by tetraisopropyl pyrophosphamide, a differential response to these inhibitors characteristic of "true" human brain acetylcholinesterase. These findings strongly suggest that both the *Drosophila* and the human DNA fragments are directly involved in controlling acetylcholinesterase biosynthesis.**

Acetylcholinesterase (AcChoEase, acetylcholine acetylhydrolase, EC 3.1.1.7) terminates synaptic transmission by rapidly hydrolyzing the neurotransmitter acetylcholine (AcCho). AcChoEase is found principally in brain and muscles, where it exists in multiple molecular forms that differ in their sedimentation coefficients, subcellular localization, and cell type of origin and probably in their regulation and physiological function (1, 2). AcChoEase is transiently expressed in various embryonic tissues (3), where its appearance has been correlated with cell migration (4). Changes in both concentration (5) and sedimentation pattern (6) of human brain AcChoEase have been reported in neurological disorders [e.g., Alzheimer disease (7)]. Pharmacological studies and enzyme kinetic analyses suggest that different molecular forms of AcChoEase, both within and between species, possess similar catalytic sites (8). Moreover, mRNA from several AcChoEase-expressing tissues, derived from phylogenetically remote species, can be translated in microinjected *Xenopus* oocytes to yield the active enzyme (9). AcChoEase has been purified by affinity chromatography from the electric organ of the electric eel (10) and can be isolated in fairly large quantities from human tissues (11, 12). However, mammalian brain AcChoEase has not been purified to homogeneity in amounts sufficient for amino acid sequence determination, due to its low concentration [0.001% of brain protein (13)]. Consequently, it is not known whether the multiple molecular forms of AcChoEases of the mammalian nervous system are attributable to multiple genes or to post-transcriptional or post-translational processing. Furthermore, because of the scarcity of the enzyme, the oocyte bioassay is still the only method for monitoring the level of mRNA that induces brain AcChoEase biosynthesis.

Ace, the genetic locus controlling AcChoEase activity in the *Drosophila* central nervous system, was mapped at region 87DE on the third chromosome by Hall and Kankel (14). The *Ace* locus was first delimited by two deletion breakpoints, Df(3R)ry^{1301} and Df(3R)Kar^{SZ11} (15). The region was cloned by "chromosome-walking" (16) and the breakpoints were localized in the DNA sequence (17). These breakpoints define a 50-kilobase (kb) DNA segment that encodes several transcripts (18). This large segment was further divided and the fragments obtained were tested by hybridization with labeled RNA isolated from ecdysone-treated *Drosophila* cells in culture, in which formation of AcChoEase is induced (19). A 10.5-kb fragment resulting from digestion of the 50-kb DNA segment with the restriction enzyme *Sal* I (designated Dro.S segment) was the only fragment hybridizing specifically to RNA that became more abundant in AcChoEase-induced cells (unpublished observation). We now report that a 2.0-kb fragment of DNA, produced by digestion of the Dro.S segment with the restriction enzyme *Eco*RI, hybridizes selectively to mRNA that induces AcChoEase synthesis in microinjected *Xenopus* oocytes. This fragment, designated Dro.SR, was used as a probe to select from a library cloned in λ Charon 4a a human genomic DNA sequence that appears to hybridize with mRNA species inducing the biosynthesis of human brain AcChoEase.

MATERIALS AND METHODS

All tissues were frozen in liquid nitrogen and stored at −70°C until used (20, 21). The Weizmann Institute's Review Board for Human Experimentation approved the study, and all experiments were performed in accordance with the National Institutes of Health guidelines for recombinant DNA work. Poly(A)$^+$ RNA was prepared by phenol extraction (22) followed by oligo(dT)-cellulose chromatography (23). RNA blot–hybridization was carried out as previously

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Abbreviations: AcCho, acetylcholine; AcChoEase, acetylcholinesterase; BW284C51, 1,5-bis[4-(allyldimethylammonium)phenyl]-pentan-3-one dibromide; iso-OMPA, tetraisopropyl pyrophosphamide; kb, kilobase(s).

described (24) with 9×10^6 cpm of ^{32}P-labeled DNA probe ($\approx 3 \times 10^5$ cpm/ng).

The human genomic DNA library was generated by cloning partial *Eco*RI cleavage fragments of peripheral blood leukocyte DNA in Charon 4a λ phages (25). Three genome equivalents (7×10^5 phages) were grown in *Escherichia coli* on 24 150-mm diameter Petri dishes, transferred to duplicate nitrocellulose filters (26), and hybridized with DNA probe ^{32}P-labeled by nick-translation (2.5×10^6 cpm per filter, 3.6×10^8 cpm/μg of probe DNA) under low stringency conditions (27). Positive plaques were picked, grown, and rescreened twice. Phage DNA was purified according to Maniatis *et al.* (28).

RESULTS

Identification of *Drosophila* mRNA That Induces AcChoEase in Oocytes. The Dro.S segment of the *Drosophila Ace* region, which previous work had indicated to be correlated with AcChoEase biosynthesis, was cloned in the pBR322 plasmid and then cut with *Eco*RI restriction enzyme. The 950-, 1650-, 2000-, 2300-, and 3000-base-pair fragments obtained were separated by agarose gel electrophoresis and recovered by electroelution. Each of the eluted fragments was then ^{32}P-labeled by nick-translation and used as a probe in blot–hybridization with poly(A)$^+$ RNA from *Drosophila* larvae. This analysis revealed that different fragments of the Dro.S segment hybridized with several different poly(A)$^+$ RNA species (Fig. 1*A*). The map of mRNA species assigned to the Dro.S segment is shown in Fig. 1*B*. To examine which of these mRNA species could be translated into active AcChoEase, poly(A)$^+$ RNA from *Drosophila* larvae was fractionated according to size by sucrose gradient centrifugation. The RNA fractions were microinjected into *Xenopus* oocytes and the enzymatically active AcChoEase formed (9, 20) was assayed radiometrically (32). The RNA fraction sedimenting at about 28 S was enriched in mRNA capable of inducing AcChoEase activity in oocytes (Fig. 2*B*). This fraction includes two RNA species that hybridize with the Dro.S DNA segment: a major species of 4.5 kb and a minor species of 5.2 kb (Fig. 2*A*). Both of these RNA species hybridize with the central part of the Dro.S segment, which includes the 2.0-kb *Eco*RI fragment Dro.SR (Fig. 1*B*). Taken together, the RNA blot–hybridization and microinjection of size-fractionated RNA indicated that Dro.SR might encode AcChoEase mRNA in *Drosophila* larvae.

Dro.SR was isolated, bound to a nitrocellulose filter, and hybridized with poly(A)$^+$ RNA from *Drosophila* larvae. The hybridized mRNA fraction was eluted from the filter (33) and injected into oocytes to assay its capacity to induce AcChoEase activity (9, 20) (Table 1). Oocytes injected with hybrid-selected mRNA (≤20 ng) developed AcChoEase activity capable of degrading 150 nmol of AcCho per 24 hr under standard assay conditions. This activity included both AcChoEase induced by the selected *Drosophila* mRNA and endogenous oocyte AcChoEase activity (9, 20, 36). Oocytes injected in parallel with as much as 500 ng of unfractionated poly(A)$^+$ RNA from *Drosophila* larvae displayed AcChoEase which could degrade only 50 nmol of AcCho per 24 hr, including the contribution of the endogenous oocyte enzyme. Hence, the hybridization-selection procedure resulted in a considerable enrichment of AcChoEase-inducing mRNA. AcChoEase activity in oocytes injected with mRNA selected with filter-bound pBR322 DNA degraded 76 nmol of AcCho per 24 hr (Table 1), suggesting that some mRNA was bound nonspecifically to and then eluted from the pBR322 DNA and the nitrocellulose filter. However, the activity induced by Dro.SR-selected mRNA was considerably higher, in support of the conclusion that the Dro.SR fragment hybridizes specifically with AcChoEase-inducing mRNA from *Drosophila* larvae. Furthermore, use of Dro.SR in hybridization-selection of poly(A)$^+$ RNA from *Drosophila* pupae also resulted in enrichment of AcChoEase-inducing mRNA (Table 1). We therefore chose Dro.SR as a probe of the *Drosophila* AcChoEase gene.

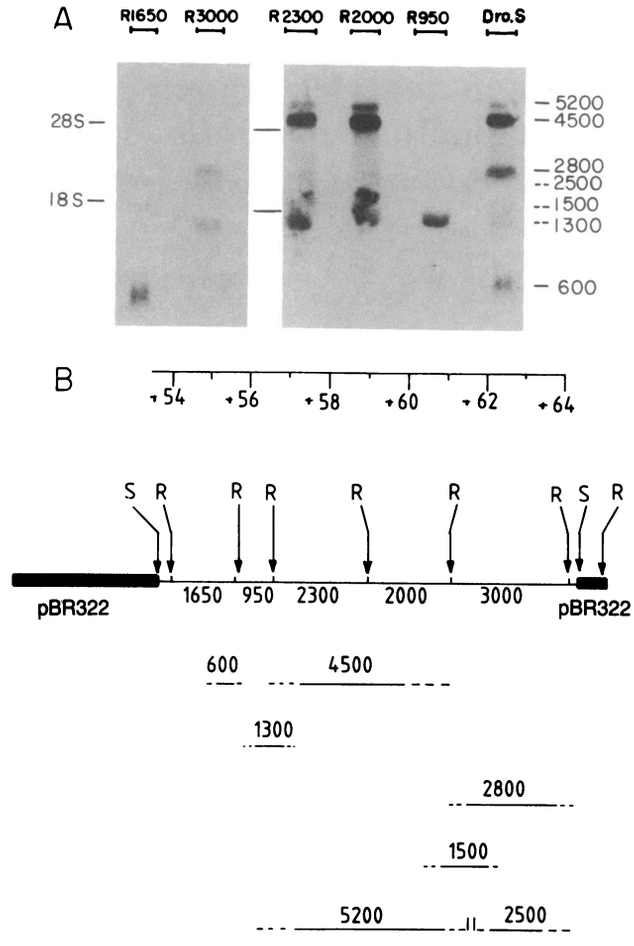

FIG. 1. Assignment of mRNAs to the *Eco*RI segments of the Dro.S fragment from *Ace* locus of *Drosophila*. Plasmid DNA carrying the Dro.S segment from the *Ace* locus was prepared from chloramphenicol-treated *E. coli* cultures carrying the Dro.S segment in the pBR322 plasmid, by using alkaline extraction (29) followed by equilibrium CsCl gradient centrifugation. Dro.S was excised from the plasmid DNA by *Sal* I digestion and then digested with *Eco*RI. Resulting DNA fragments were separated by 1% agarose gel electrophoresis and electroelution (30). Each fragment was ^{32}P-labeled separately by nick-translation (31). (*A*) RNA blot–hybridization. Samples (10 μg) of poly(A)$^+$ RNA from larvae of *Drosophila melanogaster* were fractionated by agarose gel electrophoresis and blotted onto nitrocellulose filters (24). The filters then were cut into single-lane strips, and each strip was hybridized with a different ^{32}P-labeled DNA probe (24) and then washed four times for 20 min at 50°C in 5 mM NaCl/1.5 mM Na citrate, pH 7/0.1% $NaDodSO_4$. The probes used were the entire 10.5-kb Dro.S segment and its *Eco*RI fragments (R) of 950, 1650, 2000, 2300, and 3000 base pairs, as shown at the top of the autoradiogram. Human 28S and 18S rRNAs served as markers. mRNA lengths, in nucleotides, are at right. (*B*) Restriction enzyme mapping and assignment of mRNAs to the *Eco*RI fragments of the Dro.S segment. Orientation and map coordinates of Dro.S are presented as previously reported (18). R and S refer to *Eco*RI- and *Sal* I-sensitive sites, respectively. Each of the mRNA species hybridizing with the various DNA segments was assigned an approximate length in nucleotides and was plotted according to its roughly estimated coordinate location.

Cross-Hybridization of Dro.SR with Human mRNA and DNA. Dro.SR was used for blot analysis of mRNA prepara-

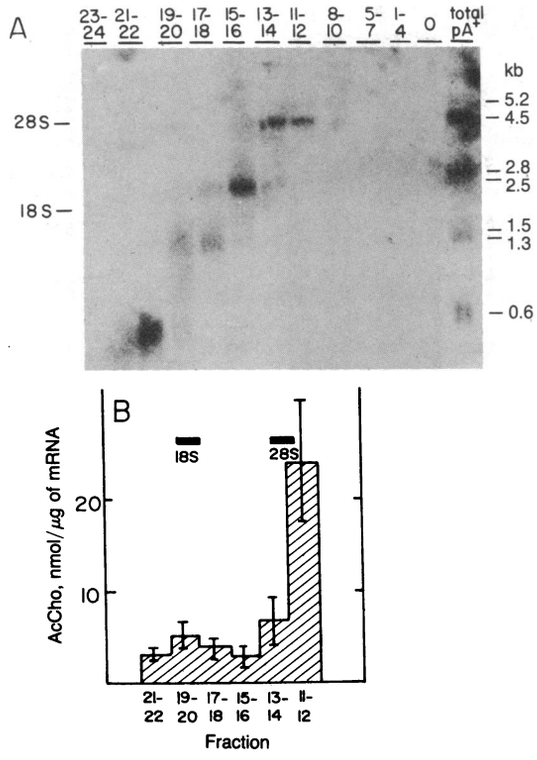

FIG. 2. Identification of *Drosophila* mRNA(s) capable of inducing AcChoEase activity in *Xenopus* oocytes. Dimethyl sulfoxide-denatured poly(A)$^+$ RNA (300 μg) from *Drosophila* larvae was size-fractionated by sucrose gradient centrifugation (20). (*A*) One-third of each mRNA fraction was subjected to agarose gel electrophoresis. The separated RNAs (numbered consecutively, as shown above the lanes) and the unfractionated poly(A)$^+$ RNA (total pA$^+$) were blotted onto nitrocellulose filters and hybridized with a ^{32}P-labeled DNA probe representing the entire Dro.S segment (see Fig. 1*A*). (*B*) One-sixtieth of each fraction was injected into each of 10 oocytes (9), and the resulting AcChoEase activity was determined by measuring the rate of hydrolysis of [^{3}H]AcCho (20). The background hydrolysis of AcCho in control, sham-injected oocytes was subtracted. The experiment was repeated five times, using oocytes from four different frogs. Average activities induced per μg of mRNA injected were calculated for each fraction (in nmol of AcCho degraded per 24 hr). Error bars represent mean deviations between the different experiments. Fractions whose rates of sedimentation corresponded to those of human 18S and 28S rRNAs are identified.

tions extracted from human brain and tumor tissues (Fig. 3). A very highly ^{32}P-labeled Dro.SR probe hybridized with human poly(A)$^+$ RNA species of about 7 kb in blotted samples of RNA from postnatal and embryonic brain and from AcChoEase-positive meningioma and glioma (20, 21) but not of RNA from AcChoEase-deficient HEp carcinoma, in which the specific activity of the enzyme and the concentration of AcChoEase mRNA are both one-tenth of the corresponding values in the brain (20). The 950-, 1650-, and 3000-base-pair *Eco*RI fragments of the Dro.S segment showed no hybridization to brain poly(A)$^+$ RNA.

The Dro.SR fragment then was used as a probe to isolate homologous sequences from a human genomic library cloned in λ Charon 4a. Six plaques obtained by plating phages carrying a total of 3 human genome equivalents showed positive hybridization to the Dro.SR fragment under low stringency conditions (27). The DNA from one of these phages (containing a 13.5-kb human DNA fragment), designated Hu.Ache1, was further characterized by restriction enzyme digestion followed by DNA blot-hybridization (Fig. 4). The hybridization signals were rather weak, probably due to a high degree of mismatch between the *Drosophila* and the human DNA sequences. However, a 2.6-kb fragment of Hu.Ache1 DNA, produced by *Eco*RI digestion and designated Hu.Ache1R, was found to hybridize with Dro.SR (Fig. 4). This Hu.Ache1R fragment was used to rescreen the human genomic library. Twenty-four phages out of three genome equivalents gave strong hybridization signals. Preliminary investigations (data not shown) indicate that the 24 selected phages contain at least eight different sequences, as judged by restriction enzyme digestion and DNA blot–hybridization with the Hu.Ache1R fragment.

Table 1. Hybrid-selection of *Drosophila* and human AcChoEase-inducing mRNAs with filter-bound fragments of *Drosophila* and human DNAs

Source of mRNA	AcCho hydrolyzed, nmol/24 hr per 10 oocytes		
	Unfractionated	Hybrid-selected	Control
Drosophila			
Larvae	50	150	76
Pupae	120	170	95
Human			
Frontal cortex*	86	140	53
Hypothalamus*	84	86	47
Parietal cortex†	140	150	70
+ iso-OMPA	100	110	0
+ BW284C51	23	0	3

pBR322-cloned Dro.S and Charon 4a-cloned Hu.Ache 1 (100 μg of each) were digested with *Eco*RI. Resultant fragments were fractionated by agarose gel electrophoresis, transferred to nitrocellulose (34), and located by ethidium bromide staining. Filter regions to which these fragments were bound were excised and used for hybridizations with mRNA. pBR322 DNA and Charon 4a DNA lacking cloned inserts were similarly prepared and used as controls. Poly(A)$^+$ RNA was prepared from *Drosophila* larvae and pupae and from human frontal cortex (5 yr), hypothalamus (21 yr), and parietal cortex (21 yr). Samples of *Drosophila* poly(A)$^+$ RNA (20 μg) were hybridized with Dro.SR (hybrid-selected) or with pBR322 DNA (control). Similar samples of human RNA were hybridized with Hu.Ache 1R (hybrid-selected) or with Charon 4a DNA (control). Hybridization was according to Ricciardi *et al.* (33). Hybridization-selection was carried out once with each of the RNA preparations, and filters were reused once. After hybridization, filters were washed once with 100 μl of the hybridization buffer (15 min, 50°C), once with 1 ml of 0.15 M NaCl/0.015 M Na citrate, pH 7/0.2% $NaDodSO_4$ (15 min, 60°C), 10 times with 1 ml of 0.15 M NaCl/0.15 M Na citrate, pH 7, and 3 times with 1 ml of 2 mM EDTA, pH 7.0 (20 sec, 60°C). Bound mRNA was eluted and ethanol-precipitated (33). One-third (1 μl) of the eluted mRNA was microinjected into 10 oocytes (9). Unfractionated poly(A)$^+$ RNA (50 ng per oocyte, 10 oocytes per sample) from each of the sources was injected in parallel. AcChoEase activity was determined in oocyte homogenates and incubation medium (20). Background degradation of [^{3}H]AcCho (≈5 nmol) was subtracted. Therefore, the difference between the hybrid-selected and control values in each experiment reflects one-third of the AcChoEase-inducing mRNA that hybridized specifically [out of 20 μg of poly(A)$^+$ RNA], whereas the values for unfractionated poly(A)$^+$ RNA reflect the AcChoEase-inducing mRNA in 500 ng of the different mRNA preparations and include also the endogenous oocyte activities (up to 50 nmol/24 hr per 10 oocytes).

*Microinjection of the same selected RNAs was carried out twice using oocytes from different females, and the data presented are average values for the two injections.

†Effects of selective cholinesterase inhibitors on the induced activities were tested in parallel for determination of total cholinesterase activity. Iso-OMPA (tetraisopropyl pyrophosphamide) and BW284C51 (1,5-bis[4-(allyldimethylammonium)phenyl]pentan-3-one dibromide), which selectively inhibit butyrylcholinesterase and AcChoEase, respectively (35), were each used at 10 μM.

Hybridization of the Hu.Ache1R Fragment with Human AcChoEase mRNA. Poly(A)$^+$ RNA from human embryonic brain and HEp carcinoma was fractionated by agarose gel

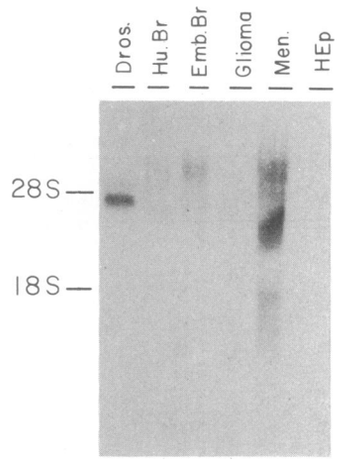

FIG. 3. Cross-hybridization between the Dro.SR fragment and human brain mRNA. Samples (20 μg) of poly(A)$^+$ RNA from human embryonic (Emb.Br.) and adult brain (Hu.Br.), human gliomas (Glioma) and meningiomas (Men.) (20.21), human epidermoid carcinoma [HEp, transferred in nude mice (20)], and *Drosophila* larvae (Dros.) were fractionated by agarose gel electrophoresis, transferred to nitrocellulose filters, hybridized with the Dro.SR probe, and washed four times for 20 min at 50°C in 75 mM NaCl/7.5 mM Na citrate, pH 7/0.1% $NaDodSO_4$. Subsequent hybridization with a cDNA probe complementary to the human mRNA coding for ribosomal protein L4 verified that all of the human poly(A)$^+$ RNAs contained high levels of full-size mRNA for L4 and were, therefore, not degraded.

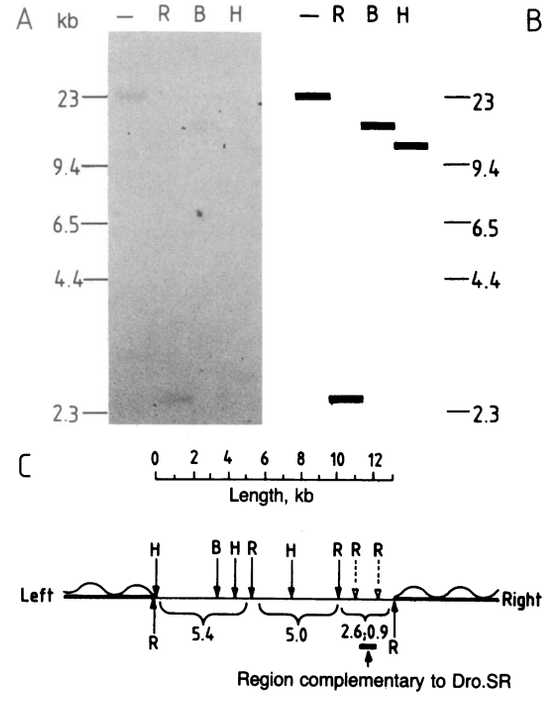

FIG. 4. Human DNA segment Hu.Ache1R is homologous to part of the *Drosophila* Dro.S fragment. (*A*) DNA extracted from the λ phage carrying the Hu.Ache1 segment was digested with *Eco*RI (R), *Bam*HI (B), or *Hin*dIII (H). Undigested (−) and digested DNA samples (2 μg/lane) were fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters by blotting (34). Filters were prehybridized at 65°C for 4 hr in 0.9 M NaCl/90 mM Na citrate, pH 7/0.1% Ficoll/0.1% bovine serum albumin/0.1% polyvinyl pyrrolidone/10 mM EDTA containing sheared, denatured salmon sperm DNA at 50 μg/ml. Hybridization was at 65°C for 18 hr under the same conditions, with 1.5×10^7 cpm ($\approx 3 \times 10^5$ cpm/ng) of the ^{32}P-labeled Dro.SR probe. After hybridization, filters were washed four times for 20 min at 50°C with 15 mM NaCl/1.5 mM Na citrate, pH 7/0.1% $NaDodSO_4$. Size markers were generated by *Hin*dIII digestion of normal λ DNA. (*B*) Enhancement of hybridization bands observed in *A*. (*C*) Restriction map of Hu.Ache1 and homology to the Dro.SR fragment. Dotted arrows represent two possible locations for an *Eco*RI site.

electrophoresis, transferred to nitrocellulose filters, and hybridized with the Hu.Ache1R DNA probe. The blot revealed an intense diffuse band of about 7 kb in poly(A)$^+$ RNA from embryonic human brain and a very faint, more slowly migrating but sharp band in mRNA from HEp carcinoma tissue (Fig. 5*A*). An intense diffuse 7-kb band also was observed in mRNA from AcChoEase-containing human leukemic cells (not shown), indicating a possible size heterogeneity of the RNA complementary to the Hu.Ache1R fragment. Dimethyl sulfoxide-denatured poly(A)$^+$ RNA from embryonic brain was size-fractionated by sucrose gradient centrifugation and microinjected into *Xenopus* oocytes to test for induction of AcChoEase synthesis (9, 20). The fraction sedimenting faster than 28 S ribosomal RNA was the most enriched in mRNA that induced AcChoEase activity in oocytes, as would be expected for a 7-kb mRNA (Fig. 5*B*). The major species of AcChoEase-inducing mRNA from primary gliomas and meningiomas, although of lower activities, also were found to be 7 kb long (20).

The Hu.Ache1R DNA fragment was tested for its ability to hybrid-select mRNAs capable of inducing AcChoEase in oocytes. Samples of 20 μg of poly(A)$^+$ RNA from various regions of human brain were hybridized with either Hu.Ache1R DNA or control λ Charon 4a DNA bound to nitrocellulose. Hybridized RNA was eluted and microinjected into oocytes, in parallel with unfractionated poly(A)$^+$ RNA (Table 1). One-third of the hybrid-selected mRNA (≤20 ng) induced AcChoEase activities capable of degrading 90–150 nmol of AcCho per 24 hr, whereas the activity detected in control oocytes degraded between 50 and 70 nmol, and the activity induced by 500 ng of the relevant unfractionated poly(A)$^+$ RNAs degraded 84–140 nmol. Since only minor amounts of translatable mRNA were selected by hybridization, it is clear that within each set of samples, translationally active AcChoEase-inducing mRNA was reproducibly retained on the nitrocellulose filters (Table 1).

Cholinesterase activity in the mammalian brain is mostly (>90%) of the "true" AcChoEase type, which can be distinguished by its susceptibility to the selective AcChoEase inhibitor BW284C51 and its resistance to the organophosphorous poison iso-OMPA (9, 20, 21, 35). In oocytes injected with unfractionated poly(A)$^+$ RNA from human parietal cortex, cholinesterase activity was composed of a major fraction resistant to iso-OMPA and a minor part resistant to BW284C51, (Table 1; see refs. 9 and 20). The enzyme in oocytes injected with hybrid-selected mRNA from this source was partially sensitive to iso-OMPA and completely inhibited by BW284C51. In contrast, the cholinesterase activity in control oocytes could be almost completely blocked by either inhibitor, implying that it was probably of amphibian origin and that unfractionated poly(A)$^+$ RNA from human parietal cortex induced in oocytes both "true" AcChoEase and (although to a much lower extent) BW284C51-resistant "pseudo"-cholinesterase. Finally, these results suggest that the Hu.Ache1R fragment selectively hybridized to mRNA that induced the synthesis of true brain AcChoEase, entirely resistant to iso-OMPA and completely susceptible to BW284C51.

DISCUSSION

AcChoEase exists in closely related forms in various species, including *Drosophila* (37) and man (12). The results presented here show that a DNA fragment from the vicinity

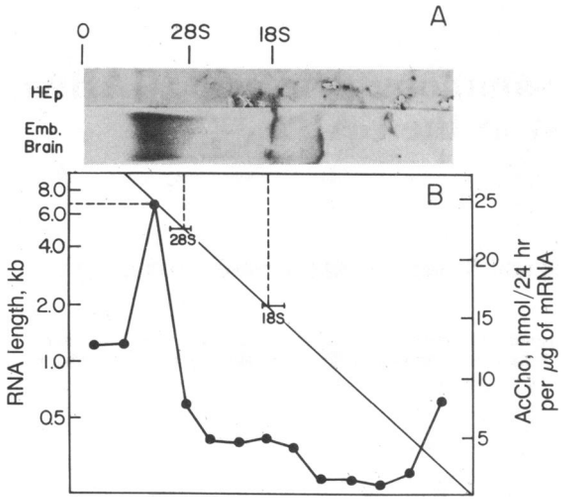

FIG. 5. Size analysis of AcChoEase-inducing human brain mRNA and the mRNA hybridizing with the human DNA fragment Hu.Ache1R. (*A*) Samples (10 μg) of poly(A)$^+$ RNA from human embryonic brain (17 weeks of gestation) and from HEp carcinoma (20) were fractionated by agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized with the purified Hu.Ache1R fragment (labeled by nick-translation). O, origin. (*B*) Dimethyl sulfoxide-denatured poly(A)$^+$ RNA (300 μg) from human embryonic brain (17 weeks) was size-fractionated by sucrose gradient centrifugation (20). One-sixth of each fraction was microinjected into 10 oocytes and the AcChoEase activity developed was determined in triplicate. The points indicate activities induced per μg of mRNA of various sizes, calculated for each fraction according to the measured RNA concentration.

of *Ace*, the genetic locus controlling AcChoEase biosynthesis in *Drosophila*, hybrid-selects RNA molecules that induce AcChoEase biosynthesis in microinjected *Xenopus* oocytes. We used this fragment as a probe for the equivalent of the *Ace* region in the human genome.

Our conclusion that the DNA fragments we isolated from *Drosophila* and man represent part of the AcChoEase genes of the two species is based on three pieces of evidence. First, in both cases these fragments hybridize with blotted RNA species in the same size range that shows greatest enrichment for mRNA capable of inducing AcChoEase. Second, this human mRNA is found in much lower concentration in the AcChoEase-deficient carcinoma than in brain and in AcChoEase-positive brain tumors. Finally, DNA fragments from both *Drosophila* and man hybridize specifically with RNA molecules that induce AcChoEase biosynthesis in oocytes. It should be mentioned that it is possible that the hybrid-selected mRNA is not translated directly into the AcChoEase protein but is involved in the regulation of expression of an AcChoEase gene endogenous to the oocytes.

Our preliminary finding of several independent DNA sequences in the human genome that are homologous to Hu.Ache1R suggests that the heterogeneity of cholinesterases extends from the levels of protein (2) and mRNA (20) to the genomic level. Specific AcChoEase-inducing gene(s) may be expressed in a tissue-, cell-type-, or developmental-stage-specific manner, by various mechanisms of transcription, to yield different forms of the mature enzyme.

Note Added in Proof. Recent genetic mapping data (unpublished) indicate that the 2.0-kb Dro.SR fragment is next to but not included within the *Ace* locus itself.

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