Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 2471–2475, March 1993
Neurobiology

Expression of a human acetylcholinesterase promoter–reporter construct in developing neuromuscular junctions of *Xenopus* embryos

REVITAL BEN AZIZ-ALOYA*, SHLOMO SEIDMAN*, RINA TIMBERG*, MEIRA STERNFELD*, HAIM ZAKUT†, AND HERMONA SOREQ*‡

*Department of Biological Chemistry, The Life Sciences Institute, The Hebrew University of Jerusalem, Israel 91904; and †Department of Obstetrics and Gynecology, The Sackler Faculty of Medicine, Tel Aviv University, The Edith Wolfson Medical Center, Holon 58100, Israel

Communicated by George B. Koelle, November 16, 1992

ABSTRACT **We have employed *Xenopus* embryos to express human acetylcholinesterase (AcChoEase; EC 3.1.1.7) in developing synapses. Transcription of AcChoEase mRNA was driven by a 2.2-kb sequence upstream from the initiator AUG in the *ACHE* gene encoding AcChoEase, with multiple potential sites for binding universal and tissue-specific transcription factors. These included clustered MyoD elements, E-box, SP1, EGR1, AP-2, and the development-related GAGA motif. A DNA construct composed of this sequence linked to a 2.1-kb sequence encoding human AcChoEase was designated human AcChoEase promoter–reporter (HpACHE). HpACHE but none of its several 5′-truncated derivatives was transcriptionally active in developing *Xenopus* embryos. Furthermore, PCR analysis using chimeric PCR primers revealed usage of the same 1.5-kb intron and 74-bp exon within the HpACHE sequence in microinjected embryos and various human tissues. Cytochemical staining revealed conspicuous accumulation of overexpressed AcChoEase in neuromuscular junctions and within muscle fibers of apparently normal 2-day *Xenopus* embryos injected with HpACHE. The same reporter driven by the cytomegalovirus promoter was similarly efficient in directing the heterologous human enzyme toward neuromuscular junctions, attributing the evolutionary conservation of AcChoEase targeting to the coding sequence. Our findings demonstrate that a short DNA sequence is sufficient to promote the exogenous transcription and faithful splicing of human AcChoEase mRNA in developing *Xenopus* embryos and foreshadow their use for integrative studies of cholinergic signaling and synapse formation.**

Development of a functional neuromuscular junction depends on the regulated expression and localization of numerous pre- and postsynaptic proteins (1, 2). Several of these proteins have been characterized by molecular cloning and *Xenopus* oocyte microinjection (for a recent review, see ref. 3). However, the static nature of the oocyte restricts its utility as a heterologous expression system for studies aimed at dissecting the physiological role(s) of important synaptic proteins. Furthermore, the lack of convenient transgenic organisms where the levels of key proteins can be manipulated within neuromuscular junctions has precluded direct investigations into the coordinated interactions between these proteins in synapse formation and neurotransmission. Early *Xenopus* embryos may provide such a system. Morphologically distinct neuromuscular contacts develop in *Xenopus* during the first 48 h after fertilization, accompanied by maturation of synaptic potentials, acquisition of neuromuscular function, and developmentally regulated increases in acetylcholinesterase [AcChoEase (4, 5); EC 3.1.1.7]. Thus, the well-characterized rapid development of their neuromuscular system makes *Xenopus* embryos highly suitable for probing synaptogenesis *in vivo* by using cloned AcChoEase as a model synaptic protein.

The acetylcholine hydrolyzing enzyme AcChoEase has long been noted for its expression in nervous system and muscle, where it is involved in terminating cholinergic neurotransmission (for reviews, see refs. 6 and 7). The *ACHE* gene encoding human AcChoEase has been cloned, expressed in *Xenopus* oocytes (8), and mapped to a single chromosomal position (7q22; refs. 9 and 10). Here, we report the characterization of a 2.2-kb genomic DNA fragment upstream of the human AcChoEase coding sequence§ and demonstrate the ability of this region to promote transcription in microinjected embryos of *Xenopus laevis*. Furthermore, we demonstrate that coupling of this promoter to the AcChoEase coding sequence may be employed to introduce high levels of recombinant human AcChoEase into developing *Xenopus* neuromuscular junctions.

METHODS

RNA–PCR Procedure and Primers. RNA–PCR analyses were as detailed elsewhere (11) using the thermal controller (Perkin–Elmer/Cetus) at 94°C for 1 min, at 65°C for 1 min, and at 72°C for 1 min and the following primers (primers 1–12), named according to their position in the human AcChoEase upstream and coding sequence (Fig. 1 and ref. 8) and noted as upstream (+) or downstream (−) according to their orientation: 1, 555+ (5′-CTGTGAGGCCCGGAGGACGCCG-3′); 2, 670+ (5′-GCTCGGCCGCCTCAGACGCCG-3′); 3, 705+ (5′-GGGACTCTCCTTAAGGCCCGGACGCC-3′); 4, 739+ (5′-TGCGCTCCCCGAGGGACGCC-3′); 5, 1000+ (5′-TACCCGAGCTGCGCAGACGCCG-3′); 6, 614+ (5′-CAGCCTGCGCCGGGGAACATC-3′); 7, 623+ (5′-GGGAACATCGGCCGCCTCCAG-3′); 8, 664+ (5′-GGCCCGGCTCGGCCGCCTCA-3′); 9, 590+ (5′-CGGCGGCTGTCAGAGTCGGCT-3′); 10, 2623− (5′-TCCTCGCTCAGCTCACGGTTGGG); 11, 1522+ (5′-CGGGTCTACGCCTACGTCTTTGAACACCGTGCTTC-3′); 12, 1797− (5′-CAGGTCCAGACTAACGTACTGCTGAGCCCCCGCCG-3′). Amplification products (20%) were electrophoresed as described (11) and were UV-photographed (320 nm). Control reactions, without reverse transcriptase, remained negative, proving the absence of contaminating cDNA sequences.

Microinjection of *Xenopus* Oocytes and Embryos. Female *Xenopus laevis* were induced to spawn by injection with human chorionic gonadotropin. *In vitro* fertilization was performed 0.1× MMR [1× MMR = 100 mM NaCl/2 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: AcChoEase, acetylcholinesterase.
‡To whom reprint requests should be addressed.
§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L06484).

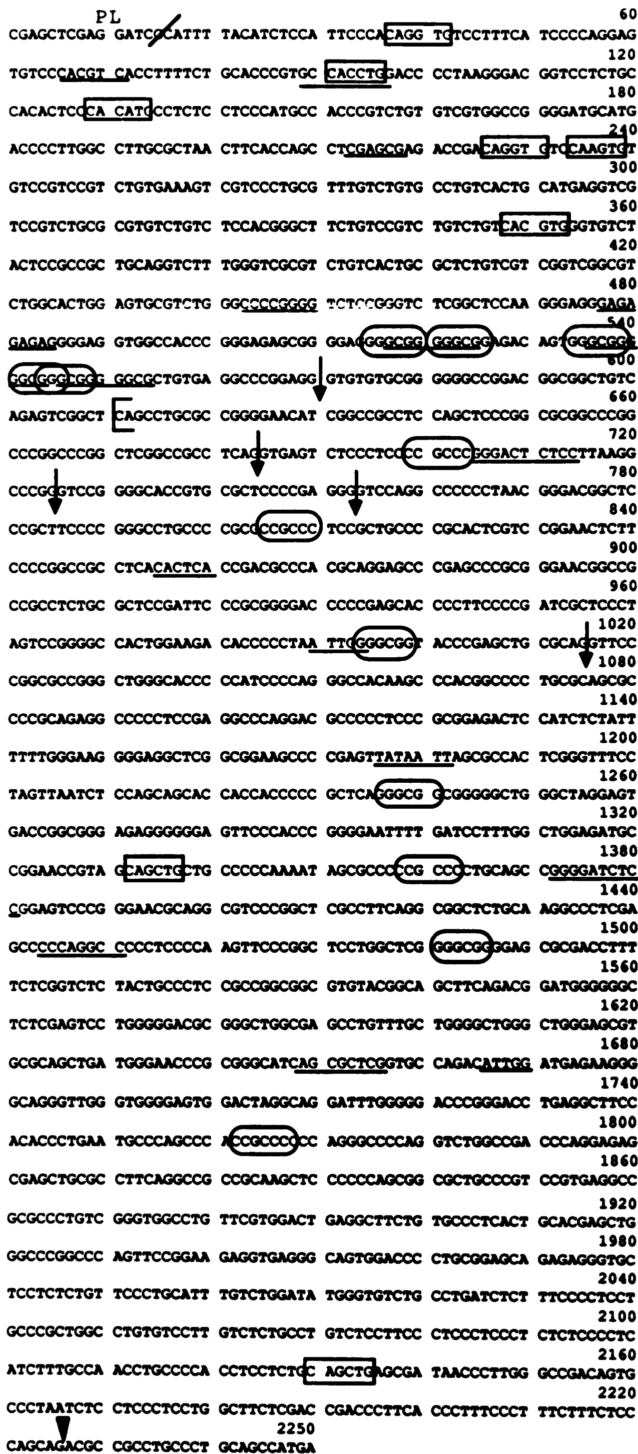

FIG. 1. *ACHE* 5′ upstream sequence. DNA sequencing was performed as detailed (8) on single- and double-stranded DNA by using commercial (Stratagene) or unique primers determined by sequencing. Data analysis (N-SITES program, University of Wisconsin) revealed potential binding sites for several transcription factors, including MyoD (nt 37, 91, 129, 226, 234, 348, 1332, and 2130; boxed), Sp1 (nt 515, 521, 534, 540, 544, 699, 805, 994, 1246, 1358, 1481, and 1762, circled), and additional sites (underlined): ATF/CREB (nt 67), E-box (nt 89), Zeste (nt 213 and 855), AP-2 (nt 443 and 1444), GAGA (nt 477), EGR1 (nt 517, 536, and 546), NF-κB (nt 705 and 1372), CCAAT box (nt 990 and 1666), and the TATA box (nt 1176). Arrows indicate potential sites for splicing at the 5′ acceptor site (G/GT). The consensus splicing motif (CAG/A) required at the 3′ boundaries of introns is noted by an arrowhead. One of several putative cap sites is noted by a [sign. PL /, polylinker including a *Sac* I site.

KCl/1 mM $MgSO_4$/2 mM $CaCl_2$/5 mM Hepes/0.1 mM EDTA, pH 7.4 (12)]. Fertilized eggs were dejellied, injected with 1 ng of DNA within the first two cleavage cycles and cultured overnight at 17°C. *Xenopus* oocyte microinjections and enzymatic activity assays were essentially as described (13) except that oocytes were subjected to a 10-min centrifugation (600 × *g*) prior to injection (50 ng per oocyte). Assays were performed in duplicate on whole cell extracts (150 μl per embryo) prepared in 0.01 M Tris/1.0 M NaCl/1% Triton X-100/1 mM EGTA, pH 7.4.

Cytochemical Staining and Electron Microscope Examination of *Xenopus* Embryos. Two-day embryos were fixed for 1 h in 1% paraformaldehyde/2.5% (vol/vol) glutaraldehyde/0.1 M sodium phosphate, pH 7.4, washed three times, and cut into three or four pieces. AcChoEase activity was detected by the thiocholine method (14, 15). After staining and rinsing, embryos were transferred to 1% osmium tetroxide for 1 h, dehydrated in ethanol, embedded in Epon, and sectioned. Thin sections were counterstained with uranyl acetate and lead citrate and analyzed with a Philips 300 electron microscope.

RESULTS

Promoter Composition. Expression of human AcChoEase in developing *Xenopus* embryos was tested using, as a potential promoter, a 2.2-kb DNA fragment upstream of the coding sequence in the human gene encoding AcChoEase (named *ACHE* by the Human Genome Committee). Sequencing of this clone [clone GNACHE (8); Fig. 1] revealed oligonucleotide motifs characteristic of binding sites for several known transcription factors (16). These included eight MyoD motifs, characteristic of myogenic expression, the E-box enhancer octamer controlling production of the heavy-chain immunoglobulin gene and compatible with hemopoietic expression, and 12 occurrences of the SP1 motif, predicting enhancement of productive transcription. The cAMP response element ATF/CREB, the EGR1 element (three sites, common in brain-specific genes subject to signal transduction pathways), and the AP-2 element (two sites, characteristic of genes expressed in embryonic neural crest lineages and presumed to interact with SP1 factors) were also found (16).

Elements indicating developmental control included the embryonically active Zeste sequences (two sites), and the GAGA motif important for the functioning of distant enhancer elements during early *Drosophila* development (16). Further downstream, this sequence included TATA and CCAAT elements and the NF-κB element (two sites) characteristic of genes active in the immune and other hemopoietic lineages (16) and implicated with the control of transition from G_0 to G_1 phases in the cell cycle (ref. 16; Fig. 1).

Transcription in *Xenopus*. The ability of the 2.2-kb AcChoEase upstream sequence to drive transcription was examined in microinjected *Xenopus* oocytes and embryos. This sequence was ligated, in its full form or after digestion with *Kpn* I, *Xho* I, or *Pvu* II (K, X, or P forms, Fig. 2) to AcChoEase cDNA containing exons 2, 3, 4, and 6 (Fig. 2; see also refs. 8 and 18 for gene structure) to create the HpACHE constructs. DNAs were injected into oocytes or early cleavage embryos and transcriptional activity was determined by reverse transcription and PCR amplification (RNA–PCR) using human AcChoEase cDNA-specific primers (Fig. 2). AcChoEase cDNA ligated to the cytomegalovirus enhancer-promoter region [CMVACHE (17)] was also injected. RNA from oocytes and embryos injected with the CMVACHE or the complete HpACHE construct, but not from those injected with any of the truncated HpACHE sequences, gave rise to the expected 275-bp PCR product (Fig. 2). Thus, the entire Hp sequence, but not the TATA and CAAT boxes included in the truncated *Kpn* I-digested construct, was sufficient to support transcription in *Xenopus*.

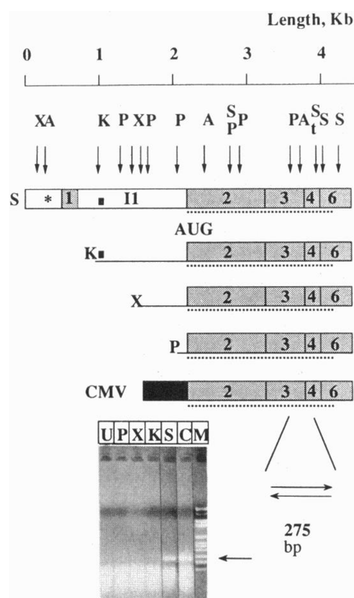

FIG. 2. Transcription of HpACHE in *Xenopus*. (*Upper*) Injected HpACHE DNAs included the 2.2-kb AcChoEase upstream sequence or its deletion constructs linked to the AcChoEase coding sequence. Characteristic restriction sites (S, *Sac* I; X, *Xho* I; A, *Acc* I; K, *Kpn* I; P, *Pvu* II; SP, *Sph* I; St, *Stu* I) included in the AcChoEase coding exons (exons 2, 3, 4, and 6, shaded boxes) and in the region upstream of the initiator AUG codon within clone GNACHE (8) were predicted according to sequence data and confirmed by blot hybridization of genomic DNA with PCR-amplified or subcloned probes from this sequence. Relative positions are noted on the length scale in kb, where the first sequenced nucleotide is numbered zero. A fifth construct, carrying the AcChoEase coding sequence downstream of the cytomegalovirus immediate early gene enhancer-promoter sequence (solid box), was as described (17). Putative TATA box and cluster of transcription factor binding sites (Fig. 1) are marked by a solid box and an asterisk, respectively. Open reading frames in exons are noted by dotted underlines. Positions of the first exon (exon 1) and intron (intron I1) were determined by RNA-PCR experiments (Fig. 3). (*Lower*) RNA was extracted from *Xenopus* oocytes 2 days after injection (50 ng per oocyte) with the noted linearized constructs. Residual injected DNA was eliminated by DNase I treatment [20 min at 37°C, 2 units per sample (Promega)] in 40 mM Tris·HCl/10 mM NaCl/6 mM $MgCl_2$ in the presence of RNasin (Boehringer Mannheim; 20 units per sample). DNase I was heat-inactivated (90°C, 8 min) and RNA-PCR amplification was performed using the primer pair 11/12. Lanes: U, uninjected; P, X, K, and S, constructs (named after the restriction enzymes used to prepare them) (*Upper*); C, CMVACHE; M, DNA size marker VI (Boehringer Mannheim). PCR products are indicated by the arrows.

Consensus 5′ Splice Site. To locate functioning splice sites within the upstream sequence, several consensus 5′ splice site motifs were tested by direct PCR amplification of cDNA from human tissues. Chimeric primer pairs were designed in which the 12 nt on the 5′ side of each upstream primer (+) terminated at one of the putative 5′ splice sites, and the common 3′-terminal 5 or 6 nt corresponded to the putative 3′ acceptor site at position 2227. The downstream primer (−) was located within the coding sequence at position 2623 (Fig. 3). In this fashion, a single 5′ splice site was identified at nt 685 (chimeric primer 2), delimiting a 1.5-kb intron designated I1 within the upstream sequence. The same chimeric PCR primer was active with RNA from fetal and adult brain and from the hemopoietic cell line K562 (data not shown), demonstrating similar splicing of I1 in various human tissues (Fig. 3). PCR primers designed with further upstream sequences delineated a length of 74 bp for the E1 exon, starting with a consensus cap site motif (Figs. 1 and 3). When RNA from oocytes and embryos injected with HpACHE was subjected to amplification using the chimeric PCR primer pair 2/10, the expected 400-bp band was also observed (Fig. 3).

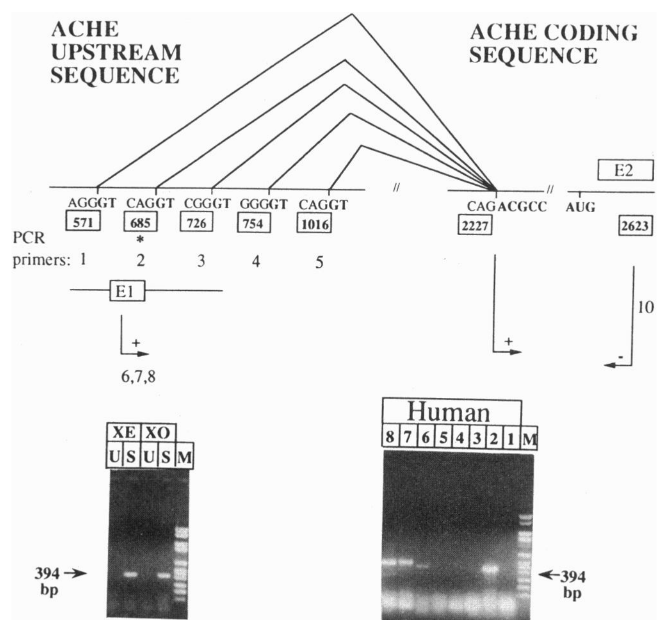

FIG. 3. RNA-PCR analysis of exon-intron boundaries. (*Upper*) Potential 5′ splice sites (consensus G/GT) were found in the AcChoEase upstream region at the noted positions in the sequence presented in Fig. 1. A potential 3′ splice site was known to be located at position 2226, 21 nt upstream from the first AUG (8). Chimeric downstream-oriented (+) PCR primers were computer-designed (20) to function only if their entire consecutive sequence would be present—i.e., wherever splicing occurred. These included 15–21 nt upstream from each potential 5′ splice site and an additional 5 or 6 nt from exon 2 (ACGCCG, nt 2227–2232) and were tested with a single upstream (−) primer (primer 10). The asterisk indicates the experimentally confirmed active 5′ splice site. (*Lower Right*) Total RNA was extracted from adult human (70 years old) brain basal nuclei. RNA-PCRs with the chimeric primer 2 and standard PCR primers 6–8 resulted in PCR products with increasing lengths (lanes 2 and 6–8). An additional primer at position 590 remained inactive (data not shown), delineating 74 bp for E1. Lane M contains molecular size markers (Boehringer Mannheim). Lanes 1 and 3–5 contain brain RNA and chimeric primers 1 and 3–5, which remained inactive when paired with primer 10. (*Lower Left*) RNA extracted from *Xenopus* oocytes (lanes XO) and 2-day embryos (lanes XE) injected with the HpACHE (lanes S) construct (Fig. 2) was used for RNA-PCR experiments using primers 2 and 10. Lane U, uninjected.

Table 1. Biochemical assessment of AcChoEase production in injected embryos

Group	Thiocholine release, A_{405} unit ($\times10^{-3}$)/min 1	2	3	Average	Net added activity, nmol per h per embryo
HpACHE-injected	3.25	3.26	3.49	3.33 ± 0.08 (6)	13.4
CMVACHE-injected	19.56	11.35	—	15.48 ± 4.11 (4)	228.1
Uninjected	2.85	2.24	—	2.55 ± 0.31 (4)	—

Acetylthiocholine hydrolyzing activity was determined (13, 19) in homogenates prepared from two or three groups (two embryos per group) from a single microinjection experiment with the specified AcChoEase encoding DNA. Raw data for individual groups are expressed as A_{405} unit ($\times10^{-3}$)/min and the net induced activity and standard deviation are presented as nmol of substrate hydrolyzed per embryo per h. Spontaneous substrate hydrolysis of 0.3 $\times10^{-3}$ A_{405} unit/min has been subtracted. Numbers in parentheses refer to the total number of embryos tested in the entire experiment.

Synaptic Targeting of Human AcChoEase. Whole cell extracts prepared from *Xenopus* embryos injected with HpACHE DNA displayed a small but significant ($P > 0.01$, Student's t test) increase in AcChoEase activity over native endogenous levels. In contrast, microinjected CMVACHE induced 20-fold greater levels of heterologous enzyme (Table 1). Up to 50% of the total AcChoEase produced in CMVACHE-injected embryos was extractable in a low salt buffer (data not shown), suggesting that a significant proportion of the heterologous enzyme may be secreted under conditions of high overexpression. The overexpressed enzyme was identified as human AcChoEase as it was efficiently inhibited by the organophosphorous inhibitor ecothiophate at 0.3 μM that, at this concentration, does not inhibit amphibian AcChoEase. Moreover, monoclonal antibodies specific to mammalian AcChoEases react with this enzyme but not with that of the frog (S.S. and H.S., unpublished data).

To locate the muscle-expressed heterologous human enzyme, we combined electron microscopy with the sensitive thiocholine technique for cytochemical activity staining (14)

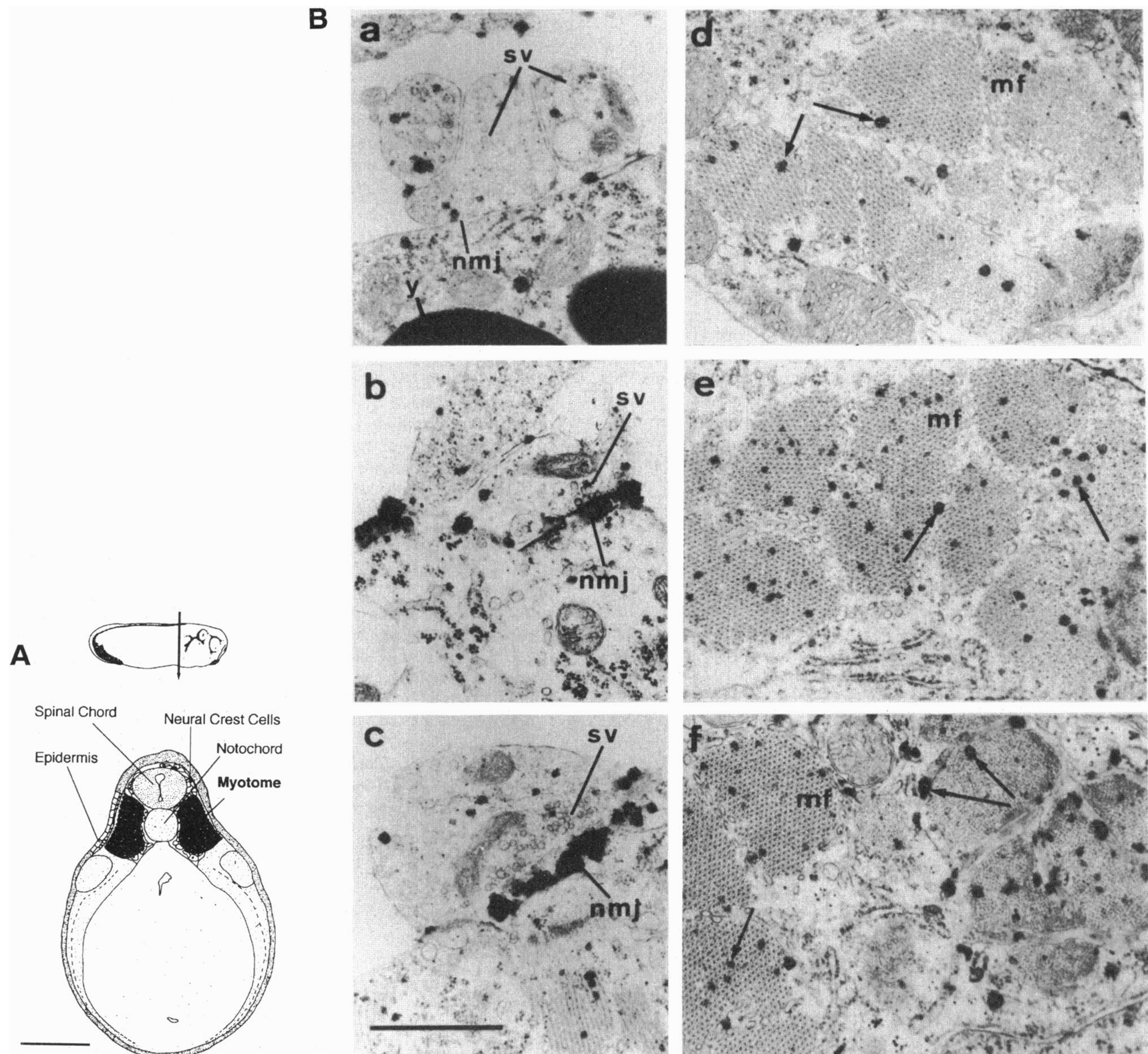

FIG. 4. AcChoEase expression in developing *Xenopus* embryos. (*A*) Schematic presentation of sectioned embryos. Thin sections (700 Å) were prepared from the anterior trunk region (*Upper*) of 2-day embryos. Cross sections (*Lower*) at this level reveal the close proximity of the muscle-forming myotomes and the principal components of the developing central nervous system. At this stage embryos displayed clearly differentiated muscle cells and the sporadic twitching that accompanies hatching. After cytochemical staining for catalytically active AcChoEase, electron microscopy was employed to detect high-density accumulations of thiocholine reaction product in myotome areas (shaded). (Diagrams are after ref. 21.) (Bar = 200 μm.) (*B*) Accumulation of AcChoEase in myotomes of transgenic *Xenopus* embryos. Two-cell cleaving embryos of *Xenopus laevis* microinjected with 1 ng of plasmid DNA were cultured for 2 days. Shortly after hatching [stage 26 (21)], embryos were fixed and stained for catalytically active AcChoEase by the thiocholine hydrolysis method (14, 15). Deposits of electron-dense reaction product appear as dark rectangular crystals that vary in size and intensity (arrows). Control sections incubated in reaction buffer lacking substrate displayed no reaction product. Panels a–c show neuromuscular junctions (nmj) demonstrating uncharacteristic synaptic accumulation of AcChoEase after microinjection of HpACHE (panel b) and CMVACHE (panel c) DNAs. Panels: a, uninjected; b, HpACHE injected; c, CMVACHE injected. Sections through somitic muscle cells from control uninjected embryos (panel d) or HpACHE-injected (panel e) or CMVACHE-injected (panel f) embryos illustrating overexpression of AcChoEase around and between the myofibrils (mf) of both DNA-injected groups. Arrows denote crystals of reaction product. sv, Synaptic vesicles; y, yolk platelets. (Bar = 1 μm.)

to examine developing myotomes from 2-day embryos (Fig. 4A). Neuromuscular junctions and the adjacent nerve and muscle structures in all of the examined embryos appeared morphologically normal (Fig. 4B, panels a–c). Synaptic staining for AcChoEase in both HpACHE-injected (Fig. 4B, panel b) and CMVACHE-injected embryos (Fig. 4B, panel c) resulted in significantly more conspicuous depositions of the electron-dense reaction product at the synapse than that observed in controls (Fig. 4B, panel a). In the neuromuscular junction of noninjected embryos, the average area covered by reaction product was in the range of 20 $nm^2/\mu m$ of contact length, whereas staining efficiency in HpACHE-injected embryos reached values of 195 $nm^2/\mu m$ and CMVACHE injections yielded labeling of up to 220 $nm^2/\mu m$. Uncharacteristic accumulations of reaction product were further observed in association with myofibrils of embryos injected with either the HpACHE or CMVACHE constructs (Fig. 4B, panels e and f).

Both the overall incidence and intensity of staining in sections prepared from embryos injected with HpACHE were enhanced compared to controls, but the levels were less than those observed in CMVACHE-injected embryos. Sections prepared from uninjected embryos displayed minimal staining around the myofibrils, and when present, staining was sparse and considerably less intense (Fig. 4B, panel d). No morphogenic or behavioral barriers to normal hatching, muscle twitching, or development were observed in microinjected embryos, and we have reared such embryos for up to 4 weeks, demonstrating that overexpression of heterologous AcChoEase in microinjected *Xenopus* embryos provides a viable model for developing neuromuscular junctions.

DISCUSSION

Xenopus embryos have been shown to transcribe a variety of heterologous promoters (22). However, to our knowledge, the present study may represent the first expression of a human promoter in *Xenopus* embryos. The same splicing pattern was observed in *Xenopus* oocytes and embryos and in human brain and myeloid K562 cells. The resultant reporter mRNA encodes the globular form of hydrophilic AcChoEase, which may remain soluble (23) or interact with the collagen-like subunit characteristic of asymmetric AcChoEase at the neuromuscular junction (24). The deposition of overexpressed enzyme in developing neuromuscular junctions indicates tissue-specific trafficking of recombinant AcChoEase to the correct extracellular compartment surrounding somitic muscle cells. Thus with the spatially restricted expression shown for AcChoEase (25) and the nicotinic acetylcholine receptor (26) in cultured muscle cells, these results may indicate the existence of a dedicated transport mechanism for localizing postsynaptic membrane proteins.

The human *ACHE* promoter was found to contain consensus recognition sites for transcription factors characteristic of genes expressed in muscle and nervous tissue, in hemopoietic cells, and during embryonic development. Its sequence further predicts responses to cAMP-inducing stimuli and signal transduction pathways in nervous system cell lineages as well as control by additional distant enhancer sequences, in good agreement with the multiplicity of human tissues and developmental stages where the AcChoEase protein has been observed (6). Furthermore, binding sites for early/immediate gene products (i.e., E-Box and Egr1) may explain AcChoEase expression in tumor tissues and may relate to the tumorigenic amplification of this gene (27, 28). The existence of an NF-κB element within the first intron could possibly imply that expression of the *ACHE* gene in lymphocytes is subject to regulation by transcription factors binding this intron and affecting cell cycle-related control, limited to the G_0–G_1 transition phase (16).

That the ectopic 10-fold overexpression of human AcChoEase in neuromuscular junctions of early *Xenopus* embryos imposed no overt barriers to apparently normal development is striking considering the high rate of hydrolysis and the important physiological function attributed to this enzyme (6, 17). The use of *Xenopus* embryos for coinjection experiments may reveal trans-activation of various promoter elements and/or other elements in the vicinity of the AcChoEase gene by tissue-specific mRNAs (for example, see ref. 29). Furthermore, coinjection with DNA encoding other important synaptic proteins may lend insight into the complex interactions between these molecules at their site of function. Transiently transgenic *Xenopus* embryos thus provide a convenient versatile system for integrative studies of the multileveled regulation of synapse formation and functioning.

We are grateful to Drs. J. Yisraeli and R. Karpel (Jerusalem), for help with experiments, B. Velan (Ness Ziona) for the CMVACHE construct, and E. Kedar (Jerusalem) for the K562 cells. This work was supported by the U.S. Army Medical Research and Development Command (Grant DAMD 17-90-Z0038) and the United States-Israel Binational Science Foundation (Grant 89-00205) (to H.S. and H.Z.) and by the E.W.H. British Research Trust, London (to H.Z. and H.S.).

1. Lu, B., Greengard, P. & Poo, M. (1992) *Neuron* 8, 521–529.
2. McMahan, U. J. (1991) *Cold Spring Harbor Symp. Quant. Biol.* 55, 407–418.
3. Soreq, H. & Seidman, S. (1992) *Methods Enzymol.* 207, 225–265.
4. Kullberg, R. W., Lentz, T. L. & Cohen, M. W. (1977) *Dev. Biol.* 60, 101–129.
5. Cohen, M. W. (1980) *J. Exp. Biol.* 89, 43–56.
6. Soreq, H. & Zakut, H. (1990) in *Human Cholinesterases Genes: Multileveled Regulation*, ed. Sparkes, R. S. (Karger, Basel), pp. 1–120.
7. Taylor, P. (1991) *J. Biol. Chem.* 266, 4025–4028.
8. Soreq, H., Ben-Aziz, R., Prody, C., Seidman, S., Gnatt, A., Neville, L., Lieman-Hurwitz, J., Lev-Lehman, E., Ginzberg, D., Lapidot-Lifson, Y. & Zakut, H. (1990) *Proc. Natl. Acad. Sci. USA* 87, 9688–9692.
9. Ehrlich, G., Viegas-Pequignot, E., Ginzberg, D., Sindel, L., Soreq, H. & Zakut, H. (1992) *Genomics* 13, 1192–1197.
10. Getman, D. K., Eubanks, J. H., Camp, S., Evans, G. A. & Taylor, P. (1992) *Am. J. Hum. Genet.* 51, 170–177.
11. Lapidot-Lifson, Y., Patinkin, D., Prody, C., Ehrlich, G., Seidman, S., Ben-Aziz, R., Benseler, F., Eckstein, F., Zakut, H. & Soreq, H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 579–583.
12. Ruiz i Altaba, A. & Melton, D. A. (1989) *Cell* 57, 317–326.
13. Neville, L. F., Gnatt, A., Loewenstein, Y., Seidman, S., Ehrlich, G. & Soreq, H. (1992) *EMBO J.* 11, 1641–1649.
14. Koelle, G. B. & Friendenwald, J. S. (1949) *Proc. Soc. Exp. Biol. Med.* 70, 617–622.
15. Karnovsky, M. J. & Roots, L. (1964) *J. Histochem. Cytochem.* 12, 219–221.
16. Faist, S. & Meyer, S. (1992) *Nucleic Acids Res.* 20, 3–26.
17. Velan, B., Kronman, C., Grosfeld, H., Leitner, M., Gozes, Y., Flashner, Y., Sery, T., Cohen, S., Ben-Aziz, R., Seidman, S., Shafferman, A. & Soreq, H. (1991) *Cell. Mol. Neurobiol.* 11, 143–156.
18. Li, Y., Camp, S., Rachinsky, T. L., Getman, D. & Taylor, P. (1991) *J. Biol. Chem.* 266, 23083–23090.
19. Soreq, H., Gnatt, A., Loewenstein, Y. & Neville, L. F. (1992) *Trends Biochem. Sci.* 17, 353–358.
20. Rychlik, W. & Rhoads, R. E. (1989) *Nucleic Acids Res.* 17, 8543–8550.
21. Hausen, P. & Riesebell, M. (1991) *The Early Development of Xenopus Laevis* (Springer, Berlin).
22. Vize, P. D., Melton, D. A., Hemmati-Brivanlou, A. & Harland, R. M. (1991) *Methods Cell Biol.* 36, 367–387.
23. Doctor, B. P., Chapman, T. C., Christner, C. E., Deal, C. D., De La Hoz, D. M., Gentry, M. K., Ogert, R. A., Rush, R. S., Smyth, K. K. & Wolfe, A. D. (1990) *FEBS Lett.* 266, 123–127.
24. Krejci, E., Coussen, F., Duval, N., Chatel, J. M., Legay, C., Puype, M., Vandekerckhove, J., Cartaud, J., Bon, S. & Massoulie, J. (1991) *EMBO J.* 10, 1285–1293.
25. Rotundo, R. L. & Gomez, A. M. (1990) *J. Cell Biol.* 110, 715–719.
26. Changeux, J. P. (1991) *New Biol.* 3, 413–429.
27. Lapidot-Lifson, Y., Prody, C. A., Ginzberg, D., Meytes, D., Zakut, H. & Soreq, H. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4715–4717.
28. Zakut, H., Ehrlich, G., Ayalon, A., Prody, C. A., Malinger, G., Seidman, S., Kehlenbach, R. & Soreq, H. (1990) *J. Clin. Invest.* 86, 900–908.
29. Benvenisty, N., Shoshani, T., Farkash, Y., Soreq, H. & Reshef, L. (1989) *Mol. Cell. Biol.* 9, 5244–5247.