

# Regulation of Acetylcholine Receptor Transcript Expression during Development in *Xenopus laevis*

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**Abstract.** The level of transcripts encoding the skeletal muscle acetylcholine receptor (AChR) was determined during embryonic development in *Xenopus laevis*. cDNAs encoding the alpha, gamma, and delta subunits of the *Xenopus* AChR were isolated from *Xenopus* embryo cDNA libraries using *Torpedo* AChR cDNAs as probes. The *Xenopus* AChR cDNAs have >60% amino acid sequence homology to their *Torpedo* homologues and hybridize to transcripts that are restricted to the somites of developing embryos. Northern blot analysis demonstrates that a 2.3-kb transcript hybridizes to the alpha subunit cDNA, a 2.4-kb transcript hybridizes to the gamma subunit cDNA, and that two transcripts, of 1.9 and 2.5 kb, hybridize to the delta subunit cDNA. RNase protection assays demonstrate that transcripts encoding alpha, gamma, and delta subunits are coordinately expressed at late gastrula and that the amount of each transcript increases

in parallel with muscle-specific actin mRNA during the ensuing 12 h. After the onset of muscle activity the level of actin mRNA per somite remains relatively constant, whereas the level of alpha subunit and delta subunit transcripts decrease fourfold per somite and the level of gamma subunit transcript decreases >50-fold per somite. The decrease in amount of AChR transcripts per somite, however, occurs when embryos are paralyzed with local anaesthetic during their development. These results demonstrate that AChR transcripts in *Xenopus* are initially expressed coordinately, but that gamma subunit transcript levels are regulated differently than alpha and delta at later stages. Moreover, these results demonstrate that AChR transcript levels in *Xenopus* myotomal muscle cells are not responsive to electrical activity and suggest that AChR transcript levels are influenced by other regulatory controls.

THE skeletal muscle acetylcholine receptor (AChR)<sup>1</sup> is the most thoroughly studied and well understood neurotransmitter receptor. The AChR is a complex composed of four different, but structurally related, subunits (alpha<sub>2</sub>, beta, gamma, delta) that form a ligand-gated channel (Karlin, 1980). During synapse formation the cell surface distribution, metabolism, and channel kinetics of AChRs are altered and these alterations have important functional consequences for synaptic transmission (Fambrough, 1979). Nevertheless, the steps and mechanisms that control these biochemical and physiological changes are poorly understood.

To understand more about how AChR expression is controlled during muscle differentiation, we investigated whether transcripts encoding different subunits of the AChR are expressed coordinately during early embryonic development in vivo. We studied AChR expression in *Xenopus laevis* so that initial expression of AChR transcripts could be determined and so that results from these studies could be placed in context with the wealth of information regarding muscle differentiation and synapse formation in *Xenopus* (Anderson and

Cohen, 1977; Kullberg et al., 1977; Chow and Cohen, 1983). Furthermore, study of AChR expression in the mononucleated myotomal muscle cells of *Xenopus* offers a unique system to investigate mechanisms underlying synaptic control of AChR expression; as described below, we hypothesize that control of AChR expression in these muscle cells is dominated by synaptic factors and not influenced by muscle cell electrical activity.

It is well established that nonsynaptic AChR number in multinucleated myofibers is controlled by the rate and pattern of myofiber electrical activity (Lomo and Westgaard, 1975). An increase in myofiber electrical activity causes a decrease in AChR synthesis and a decrease in electrical activity causes an increase in AChR synthesis (Brockes and Hall, 1975; Devreotes and Fambrough, 1975b; Reiness and Hall, 1977). Although the mechanism by which electrical activity regulates AChR biosynthesis is not clear, AChR mRNA levels are greater in denervated than innervated adult muscle (Merlie et al., 1984; Goldman et al., 1985); moreover, AChR mRNA levels are greater in electrically quiescent than electrically active muscle cells grown in cell culture (Klarsfeld and Changeux, 1985).

Although electrical activity causes a reduction in the num-

1. Abbreviation used in this paper: AChR, acetylcholine receptor.

ber of nonsynaptic AChRs, AChRs remain concentrated at adult synapses despite electrical activity and continual turnover of AChR molecules (for review, see Salpeter, 1987). It is not clear how such a high level of synaptic AChRs is maintained, but it is possible that components of the synapse have a local influence on the biosynthetic machinery of the myofiber so as to maintain AChR synthesis at the synaptic site. Support for this hypothesis is provided by experiments that demonstrate that AChR mRNAs are enriched in the central innervated zone of adult skeletal muscle (Merlie and Sanes, 1985).

Thus, it is possible that the level of AChR transcripts is reduced throughout the myofiber by electrical activity and increased locally at the synaptic site. We are interested in studying this dual control of AChR expression.

*Xenopus* myotomal muscle cells may provide a simplified system to study how AChR biosynthesis is controlled by synaptic factors. The somitic muscle cells that form early in *Xenopus* development do not fuse to form multinucleated myotubes but rather remain mononucleated (Hamilton, 1969). These myotomal muscle cells are initially 100  $\mu$ m in length with a single nucleus near the center and synapses at each end of the cell (Lewis and Hughes, 1960; MacKay et al., 1960; Kullberg et al., 1977). Thus, the geometry of the single nucleus in the myotomal muscle cell with respect to synaptic sites resembles the geometry of synaptic nuclei to the synaptic site in multinucleated myofibers. Therefore, it is possible that the single nucleus in the myotomal muscle cell is regulated in a manner similar to that of the synaptic nuclei in multinucleated myotubes.

Moreover, study of AChR regulation in *Xenopus* offers a convenient developmental system in which to determine when AChR transcripts are first expressed during embryonic development and whether transcripts encoding different subunits are initially expressed coordinately.

This study demonstrates that transcripts encoding three subunits of the *Xenopus* skeletal muscle AChR appear coordinately during early embryonic development and increase in quantity as myogenesis and synaptogenesis proceed. The level of transcripts encoding alpha and delta subunits decreases to a modest extent during later stages of development, whereas the level of transcript encoding the gamma subunit decreases more than 50-fold. Identical changes in level of AChR transcripts occur in embryos that have been paralyzed during development with local anaesthetic. These results demonstrate that AChR transcripts are initially expressed coordinately, but that gamma subunit transcript levels are regulated differently than alpha and delta at later stages. Moreover, these results demonstrate that AChR transcripts are maintained in *Xenopus* myotomal muscle cells during development despite electrical activity and suggest that other regulatory factors control the level of AChR transcripts.

## Materials and Methods

### Isolation of *Xenopus* AChR cDNA Clones

cDNA libraries were prepared from *Xenopus* embryo poly(A)<sup>+</sup> RNA isolated from both stage 17 and stage 22–24 embryos. The RNA was copied into cDNA (Gubler and Hoffman, 1983), linked with Eco RI linkers, and cloned into either lambda gtl1 or lambda gtl0 (Kintner and Melton, 1987).

100,000 recombinant phage from both the stage 17 and the stage 22–24 library were screened with nick-translated (Rigby et al., 1977) full-length

cDNA <sup>32</sup>P-probes ( $\sim 10^8$  cpm/ $\mu$ g) encoding either the *Torpedo* alpha, gamma, or delta subunit. cDNA encoding the *Torpedo* AChR subunits were kindly provided by Dr. T. Claudio (Yale University School of Medicine, New Haven, CT). Filters were hybridized with <sup>32</sup>P-probes in 5 $\times$  SSPE, 5 $\times$  Denhardt's, 100  $\mu$ g/ml calf thymus DNA, and 0.1% SDS at 56°C and washed in 0.5 $\times$  SSC, 0.1% SDS at 56°C (Benton and Davis, 1977; Maniatis et al., 1982). These hybridization and wash conditions were established with genomic Southern blots.

Using these procedures we isolated cDNA clones encoding the *Xenopus laevis* AChR alpha, gamma, and delta subunits; characterization of these cDNA clones is presented in Results. We have isolated two *Xenopus* cDNA clones that hybridize to *Torpedo* beta subunit cDNA; these clones, however, have not been thoroughly characterized.

The cDNA inserts were subcloned into SP65 to produce RNA transcripts (Melton et al., 1984) and into M13 vectors for sequencing (Messing, 1983; Sanger et al., 1977).

All of the *Xenopus* cDNA clones encoding the alpha subunit had identical restriction maps with one exception. One clone that was isolated from the stage 22–24 library is deleted from nucleotide 175 through nucleotide 420, which produces an in-frame deletion of the alpha subunit corresponding to loss of amino acids 59–140 of the mature protein (Fig. 1). We used an antisense RNA probe from the full-length *Xenopus* alpha cDNA (Fig. 1) to determine whether a cellular RNA was present that corresponded to the deleted alpha cDNA. The <sup>32</sup>P-RNA probe was synthesized with SP6 polymerase from Bam HI-digested SP65-DNA, which harbored an Eco RI insert extending from the 5' linker (nucleotide –99) to the Eco RI site (nucleotide 237) of the full-length alpha subunit cDNA (Fig. 1). Hybridization with cellular RNA from either stage 20 or stage 35 embryos resulted in protection of a 336 nucleotide long fragment; shorter, protected fragments were not detected. Thus, we have no evidence that a truncated alpha subunit RNA is present in the embryo and therefore conclude that the deleted cDNA arose artefactually during cloning.

### Northern Blot Analysis and RNase Protection

Stage 20–41 *Xenopus laevis* embryos were obtained by mating gonadotropin-primed males with primed females. Stage 12–18 *Xenopus laevis* embryos were obtained from in vitro fertilizations (Newport and Kirschner, 1982). Total cellular RNA was isolated from *Xenopus* embryos using proteinase K/SDS (Krieg and Melton, 1984; Rebagliati et al., 1985). It was important to maintain the volume of extraction buffer in  $\sim 10$ -fold excess to the volume of embryos ( $\sim 100$  embryos/ml). Poly (A)<sup>+</sup> RNA was isolated from each stage separately by batch processing with oligo-(dT)-cellulose (Aviv and Leder, 1972).

RNA was fractionated on 1% formaldehyde-agarose gels (Maniatis et al., 1982) and transferred to Zetabind (AMF Cuno, Meriden, CT) as described for nitrocellulose (Thomas, 1980); the RNA was immobilized to the filter by UV crosslinking (Church and Gilbert, 1984) in addition to baking. Hybridization with nick-translated ( $\sim 10^8$  cpm/ $\mu$ g) full-length cDNA <sup>32</sup>P-labeled probes was in 5 $\times$  SSPE, 5 $\times$  Denhardt's, 100  $\mu$ g/ml calf thymus DNA, 0.1% SDS at 56°C. Filters were washed either in 2 $\times$  SSC, 0.1% SDS at 22°C (low stringency), 0.2 $\times$  SSC, 0.1% SDS at 56°C (moderate stringency), or at 0.1 $\times$  SSC, 0.1% SDS at 68°C (high stringency) and exposed to X-Omat AR x-ray film with an intensifying screen at –70°C.

Total cellular RNA was used in RNase protection experiments. AChR gamma subunit probe was synthesized with SP6 polymerase from SP65-gamma cDNA (Eco RI, 236, to 3' Eco RI linker) linearized with Hinc II. The delta subunit probe was synthesized with SP6 polymerase from SP65-delta subunit cDNA (5' Eco RI linker to 3' Eco RI linker) linearized with Bgl II. AChR alpha subunit probe was synthesized with T7 RNA polymerase from pGEM-2-alpha subunit cDNA (5' Eco RI linker to Pst I, 1,078) linearized with Hinc II. The cardiac actin probe was synthesized with SP6 polymerase from SP64-*Xenopus* cardiac actin AC100 cDNA (5' Eco RI to Xmn I, 1,250) linearized with Pvu II (Kintner and Melton, 1987). The alpha subunit probe was 449 nucleotides long (429 nucleotides protected); the gamma subunit probe was 273 nucleotides long (264 nucleotides protected); the delta subunit probe was 219 nucleotides long (210 nucleotides protected); the *Xenopus* cardiac actin probe was 591 nucleotides long (550 nucleotides protected). Preparation of <sup>32</sup>P-labeled probes, hybridization, digestion, and analysis of protected labeled products was as described (Melton et al., 1984). Probes, however, were purified by electrophoresis and elution from 8 M urea/5% polyacrylamide gels.

Hybridization of the delta subunit <sup>32</sup>P probe with both cellular and synthetic RNA was at 47°C; hybridization of all other <sup>32</sup>P probes with RNA was at 45°C. Hybridization of the delta subunit probe with cellular RNA resulted in protection of both a major fragment and several additional minor

fragments, whereas only the single major fragment was protected in hybridizations with synthetic delta subunit RNA. The amount of minor band protection was reduced slightly by increasing the hybridization temperature to 47°C; these results suggest that another cellular RNA can hybridize imperfectly to the delta subunit probe. Since Northern blots of cellular RNA demonstrate that this <sup>32</sup>P-labeled delta RNA probe hybridizes to both 1.9- and 2.5-kb delta subunit transcripts, it is likely that the additional protected fragments result from imperfect hybridization to one of the delta subunit transcripts. Since similar results were obtained with <sup>32</sup>P-labeled RNA probes derived from the coding region of the delta subunit cDNA, it is likely that the 1.9 and 2.5-kb transcripts differ not only in length but also in sequence within the coding region. Quantitation of delta subunit RNA levels was determined by including only the major protected band in the analysis.

The specificity of the protection assay was established by demonstrating that the anti-sense probes are protected only by their corresponding synthetic sense RNA and not by other subunit synthetic sense RNA. The level of each AChR mRNA was indexed to the level of muscle-specific *Xenopus* cardiac actin mRNA (Mohun et al., 1984; Dworkin-Rastl et al., 1986; Kintner and Melton, 1987). Between stages 20 and 41 there is a twofold increase in length and a fourfold increase in volume of muscle cells in the first 10 somites (Chow and Cohen, 1983); since actin mRNA increases twofold per somite (actin transcript per number of somites in whole embryo, Table I) between stages 20 and 41, the actin transcript level per muscle cell is relatively constant with development. Furthermore, the absolute quantity of each AChR subunit mRNA per embryo was determined with hybridizations to known amounts of synthetic sense RNA derived from the cDNAs added to egg RNA (Melton et al., 1984). The amount of protected radiolabeled RNA was quantitated both by densitometer scanning of autoradiograms and by measuring radioactivity in the gel slices with a beta counter.

### In Situ Hybridization

*Xenopus* embryos were fixed with 4% paraformaldehyde (in PBS) for 1 h at 4°C and embedded in paraplast. Sections (8 µm) were mounted on silated microscope slides and processed for in situ hybridization and autoradiography as described (Kintner and Melton, 1987). The <sup>35</sup>S-labeled anti-sense alpha subunit RNA probe (856 nucleotides long) was synthesized with SP6 polymerase from SP64-alpha cDNA (Eco RI, 237, to Pst I, 1,078) linearized with Eco RI. Exposures were for 4 d.

### Denervation of Adult *Xenopus* Muscle

The sciatic nerve to the left leg was cut at the greater sciatic foramen and ~1 cm of nerve was excised. 10 d after denervation both the left denervated and right normal triceps femoris muscles were removed and total RNA was isolated (Chirgwin et al., 1979). The anti-sense alpha subunit <sup>32</sup>P probe used in these RNase protection assays was synthesized with SP6 polymerase from SP65-alpha cDNA (Bgl II, 28, to Bgl II, 496) linearized with Hind III. The alpha subunit probe was 513 nucleotides long (468 nucleotides protected). The EF-lalpha probe was used to control for the amount of cellular RNA and was synthesized with T7 polymerase from SP72-*Xenopus* EF-lalpha cDNA (350 bp fragment; Kintner and Melton, 1987) linearized with Pvu II (Kintner and Melton, 1987).

## Results

### *Xenopus* AChR Alpha, Gamma, and Delta Subunits Are Homologous to *Torpedo* AChR Subunits

To measure skeletal muscle AChR transcript levels during *Xenopus* development and to subsequently isolate and characterize genomic clones encoding *Xenopus* AChR subunits, cDNA clones encoding AChR subunits from *Xenopus laevis* were isolated and characterized (see Materials and Methods).

We isolated several dozen alpha subunit cDNA clones, approximately one dozen gamma subunit clones and seven delta subunit clones. Several clones encoding each subunit were mapped with restriction endonucleases and the restriction maps of multiple clones encoding each subunit were identical (see Materials and Methods for one exception). Thus, the longest clone for each subunit was analyzed fur-

ther. Restriction map, sequencing strategy, cDNA sequence, and deduced amino acid sequence for each subunit are illustrated in Fig. 1.

We verified that the *Xenopus* cDNA clones encode AChR subunits by DNA sequencing and comparison of the amino acid sequence with the amino acid sequence of AChR subunits from other species. The *Xenopus* alpha subunit has 77% amino acid homology with the *Torpedo* alpha subunit and not more than 40% homology with other *Torpedo* AChR subunits (beta, 40%; gamma, 35%; delta, 37%). The *Xenopus* gamma subunit has 59% homology with the *Torpedo* gamma subunit and not more than 47% homology with other *Torpedo* AChR subunits (alpha, 33%; beta, 41%; delta, 47%). The *Xenopus* delta subunit has 61% homology with the *Torpedo* delta subunit and not more than 50% homology with other *Torpedo* AChR subunits (alpha, 33%; beta, 40%; gamma, 50%) (Noda et al., 1983).

Each of the *Xenopus* AChR subunits contains four hydrophobic stretches of amino acids that correspond to the putative membrane spanning regions (M1-M4) in AChR subunits of other species (Noda et al., 1983). In addition, the amino acid sequence corresponding to the putative amphipathic helix in AChR subunits of other species is similarly conserved in *Xenopus* (Finer-Moore and Stroud, 1984). Since immunological data suggest that the main immunogenic region of AChRs is highly conserved among different vertebrate species but different in *Xenopus* (Sargent et al., 1983), we examined the sequence of the NH<sub>2</sub>-terminal extracellular portion of the *Xenopus* AChR alpha subunit for amino acid substitutions that distinguish the *Xenopus* alpha subunit from alpha subunits of other species. Seven substitutions that produce nonconservative changes in the *Xenopus* alpha subunit were detected; the amino acid residues at these seven positions are indicated for *Torpedo*, bovine, mouse, human, and *Xenopus* (italicized) alpha subunits respectively: residue 30: asp,glu, gln,asp, val; residue 61: ile, val, val, val, arg; residue 83: asp, glu, glu, glu, ser; residue 87: leu, arg, arg, arg, ser; residue 103: val, val, val, val, ser; residue 105: met, phe, phe, phe, asp; and residue 183: gly, gly, gly, gly, cys. Since the main immunogenic region is constituted, at least in part, by several epitopes between amino acid residues 6 and 85, (Barkas et al., 1987), it is possible that several of these amino acid substitutions are involved in the structure of the main immunogenic region.

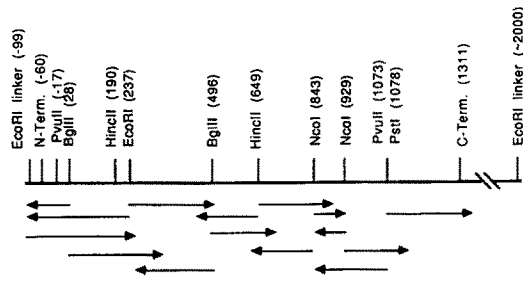
### *Xenopus* AChR Transcripts Are Restricted to Myotomes

We established that the *Xenopus* AChR cDNA clones encode skeletal muscle AChRs by in situ hybridization to tissue sections of *Xenopus* embryos. Alpha, gamma, and delta subunit transcripts are detected only in somites, as illustrated for the alpha subunit in Fig. 2. Hybridization above background was detected neither in neural tube nor in any other organ rudiments. Moreover, in situ hybridization with alpha and delta subunit probes to longitudinal sections of embryos from stages 26, 35, 41, and 48 demonstrates that there is a comparable level of AChR transcript at anterior and posterior somites at all stages (data not presented).

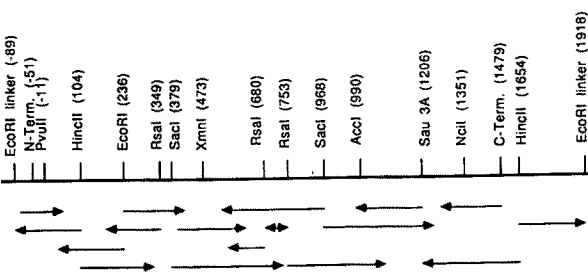
### Alpha and Gamma Subunit cDNAs Each Hybridize to a Single Transcript, whereas Delta Subunit cDNA Hybridizes to Two Transcripts

Northern blot analysis of poly(A)<sup>+</sup> RNA from *Xenopus* em-

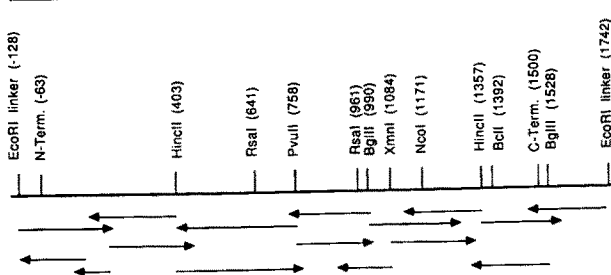
**ALPHA**



**GAMMA**



**DELTA**



**ALPHA SUBUNIT**

|  |   |
|--|---|
| <p>5'</p> <p>TGAAACCCCTGTAGGCAGATCAGAGAGAAGGAGCA</p> <p>ser phe leu ile phe leu phe ile ala ala gly thr val phe gly</p> <p>TCT TGC CTA ATT TTC CTC TTT ATT GCA GCT GGG ACA GTA TTC GGT</p> <p>1</p> <p>thr asp his glu thr arg leu ile gly asp leu phe ala asn tyr</p> <p>ACT GAC CAT GAA ACT CGC CTT ATT GGA GAT CTG TTT GCA AAC TAC</p> <p>20</p> <p>asn lys val val arg pro val glu thr tyr lys asp gln val val</p> <p>AAC AAA GTG GTT CGT CCT GTA GAA ACA TAT AAG GAT CAA GTA GTG</p> <p>40</p> <p>val thr val gly leu gln leu ile gln leu ile asn val asp glu</p> <p>GTA ACT GTT GGA CTT CAG CTC ATC CAG CTC ATC AAC GTG GAT GAA</p> <p>60</p> <p>val asn gln ile val ser thr asn ile arg leu lys gln gln trp</p> <p>CTG AAC CAA ATT GTT TCA ACA AAT ATC CGC CTA AAA CAG CAA TGG</p> <p>80</p> <p>arg asp val asn leu lys trp asp pro ala lys tyr gly gly val</p> <p>CGT GAT GTT AAC CTC AAG TGG GAC CCA GCA AAA TAT GGC GGT GTA</p> <p>100</p> <p>lys lys ile arg ile pro ser ser asp val trp ser pro asp leu</p> <p>AAG AAA ATT CGA ATT CCT TCC AGT GAC GTT TGG AGT CCA GAT TTG</p> <p>120</p> <p>val leu tyr asn asn ala asp gly asp phe ala ile ser lys asp</p> <p>GTT CTT TAT AAC AAT GCA GAT GGT GAC TTT GCT ATT TCC AAG GAC</p> <p>140</p> <p>thr lys ile leu leu glu tyr thr gly lys ile thr trp thr pro</p> <p>ACT AAA ATC CTT CTG GAA TAC ACT GGA AAG ATT ACA TGG ACA CCC</p> <p>160</p> <p>pro ala ile phe lys ser tyr cys glu ile ile val thr tyr phe</p> <p>CCA GCA ATT TTC AAA AGC TAC TGT GAA ATC ATA GTC ACA TAC TTT</p> <p>180</p> <p>pro phe asp gln gln asn cys ser met lys phe gly thr trp thr</p> <p>CCG TTT GAT CAG CAG AAT TGC AGC ATG AAG TTT GGC ACT TGG ACT</p> <p>200</p> <p>tyr asp gly ser leu leu val ile asn pro glu arg asp arg pro</p> <p>TAT GAT GGG AGC TTA CTA GTT ATA AAT CCA GAG AGG GAC CGA CCA</p> <p>220</p> <p>asp leu ser asn phe met ala ser gly glu trp met met lys asp</p> <p>GAT CTG AGC AAC TTC ATG GCG AGT GGA GAA TGG ATG ATG AAG GAC</p> <p>240</p> <p>tyr arg cys trp lys his trp val tyr tyr thr cys cys pro asp</p> <p>TAT CGT TGC TGG AAG CAT TGG GTT TAT TAT ACC TGT TGC CCA GAT</p> <p>260</p> <p>lys pro tyr phe ile val asn val ile ile pro cys leu leu phe</p> <p>AAA CCA TAT CTG GAT ATC ACC TAC CAT TTT GTT CTA CAA AGG CTT</p> <p>280</p> <p>pro leu tyr phe ile val asn val ile ile pro cys leu leu phe</p> <p>CCC CTC TAT TTC ATT GTC AAC GTT ATC ATT CCT TGT CTG CTC TTC</p> <p>300</p> <p>320</p> <p>340</p> <p>360</p> <p>380</p> <p>400</p> <p>420</p> <p>440</p> <p>460</p> <p>480</p> <p>500</p> <p>520</p> <p>540</p> <p>560</p> <p>580</p> <p>600</p> <p>620</p> <p>640</p> <p>660</p> <p>680</p> <p>700</p> <p>720</p> <p>740</p> <p>760</p> <p>780</p> <p>800</p> <p>820</p> <p>840</p> <p>860</p> <p>880</p> <p>900</p> <p>920</p> <p>940</p> <p>960</p> <p>980</p> <p>1000</p> <p>1020</p> <p>1040</p> <p>1060</p> <p>1080</p> <p>1100</p> <p>1120</p> <p>1140</p> <p>1160</p> <p>1180</p> <p>1200</p> <p>1220</p> <p>1240</p> <p>1260</p> <p>1280</p> <p>1300</p> <p>1320</p> | <p>met asp tyr thr ala</p> <p>ATG GAT TAC ACA GCC</p> <p>-1</p> <p>glu lys met thr leu ser ile ser val leu leu ser leu thr val</p> <p>GAG AAA ATG ACT CTG AGC ATT TCT GTA TTA CTG TCT CTG ACT GTG</p> <p>260</p> <p>phe leu leu val ile val glu leu ile pro ser thr ser ser ala</p> <p>TTC CTT CTG GTC ATC GTC GAA TTG ATC CCA TCA ACG TCA AGT GCT</p> <p>280</p> <p>val pro leu ile gly lys tyr met leu phe thr met val phe val</p> <p>GTC CCT TTA ATT GGG AAA TAT ATG TTA TTC ACC ATG GTC TTT GTA</p> <p>300</p> <p>ile ala ser ile ile ile thr val ile val ile asn thr his his</p> <p>ATC GCT TCT ATT ATT ATC ACA GTC ATT GTC ATC AAC ACC CAT CAC</p> <p>320</p> <p>arg ser pro ser thr his thr met pro pro trp val arg lys ile</p> <p>CGC TCC CCA AGT ACT CAC ACT ATG CCT CCA TGG GTG AGA AAG ATT</p> <p>340</p> <p>phe ile glu thr ile pro asn ile met phe phe ser thr met lys</p> <p>TTT ATA GAA ACC ATC CCA AAC ATC ATG TTC TTC TCT ACC ATG AAA</p> <p>360</p> <p>CGT pro ser gln glu lys gln pro gln lys thr phe ala glu glu</p> <p>CGT CCT TCA CAA GAA AAA CAG CCC CAG AAA ACA TTT GCA GAA GAG</p> <p>380</p> <p>met asp ile ser his ile ser gly lys leu gly pro ala ala val</p> <p>ATG GAC ATA TCA CAC ATT TCA GGA AAG CTC GGC CCA GCT GCA CTG</p> <p>400</p> <p>thr tyr gln ser pro ala leu lys asn pro asp val lys ser ala</p> <p>ACT TAT CAG TCC CCG GCT CTA AAG AAT CCT GAT GTA AAG AGT GCC</p> <p>420</p> <p>ile glu gly ile lys tyr ile ala glu thr met lys ser asp gln</p> <p>ATA GAG GGC ATT AAA TAC ATT GCT GAG ACC ATG AAG TCA GAC CAG</p> <p>440</p> <p>glu ser asn lys ala ser glu glu trp lys phe val ala met val</p> <p>GAA TCA AAT AAG GCT TCG GAG GAA TGG AAG TTT GTC GCT ATG GTG</p> <p>460</p> <p>leu asp his ile leu leu ala val phe met thr val cys val ile</p> <p>TTG GAT CAT ATC CTG CTC GCT GTT TTC ATG ACA GTC TGT GTC ATA</p> <p>480</p> <p>gly thr leu ala val phe ala gly arg ile ile glu met asn met</p> <p>GGA ACT TTA GCT GTG TTT GCT GGG CGT ATC ATT GAA ATG AAT ATG</p> <p>500</p> <p>gln glu</p> <p>CAG GAA TGA ACACITTT----3'</p> |
|--|---|

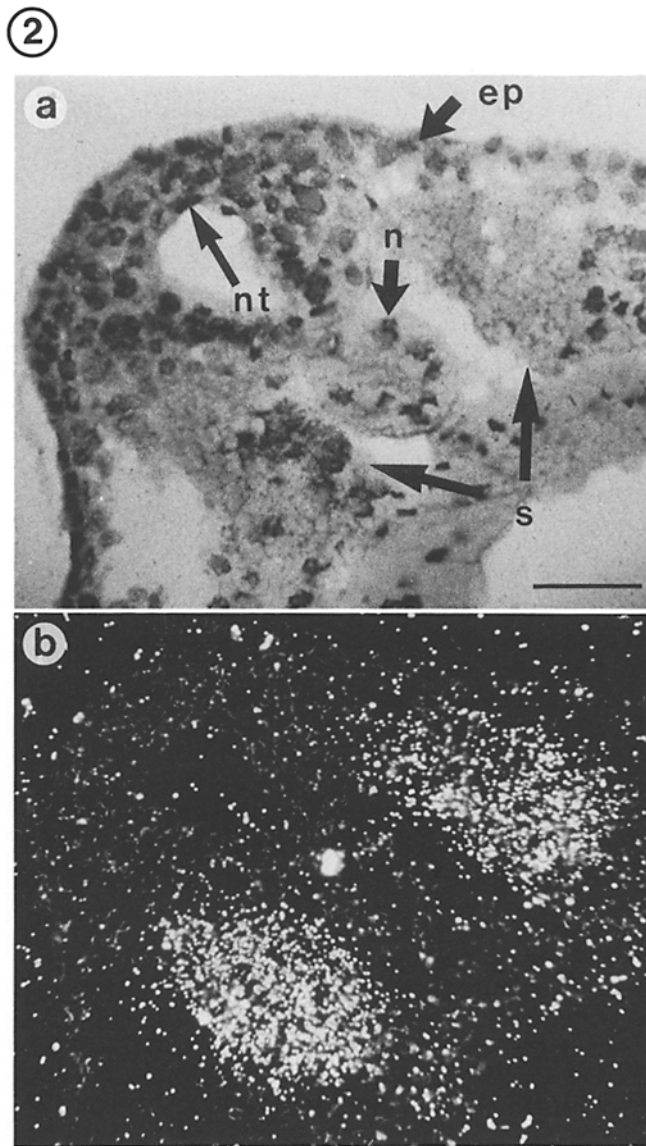
Figure 1. Restriction map, sequencing strategy, nucleotide sequence, and deduced amino acid sequence of the alpha, gamma, and delta subunits of the *Xenopus laevis* skeletal muscle AChR. (Left) Restriction map and sequencing strategy of the alpha, gamma, and delta subunits. Numbers indicate the 5' terminal nucleotide generated by cleavage. (Below and right) Nucleotide and deduced amino acid sequence of the alpha, gamma, and delta subunits. Nucleotide 1 indicates the first nucleotide of the codon encoding the amino terminal residue in the mature protein and nucleotides to the 5' side of this amino terminal residue are indicated with negative numbers. The number of the nucleotide residue at the end of each line is provided. The predicted amino acid sequence is shown above the nucleotide sequence. Amino acid residues are numbered beginning with the amino terminal residue of the mature protein and the preceding amino acids of the signal sequence are indicated with negative numbers. Since mRNA encoding each subunit is longer than the corresponding cDNA, these sequences are incomplete.

GAMMA SUBUNIT

5' GCTTACTCCAC -73 leu phe leu val ala gln lys ile pro glu thr ser thr ser val pro leu ile 846
-17 met asp thr val leu leu leu val ser leu cys 300
CTCGACAGGACATGAAGAGCGCGC ATG GAT ACA GTA CTT CTG CTG GTG TCA CTA TGC 900
-1 1
ile ser ala gta phe cys asn asn glu glu glu arg leu leu asn asp leu met
ATC TCA ACT GCT TTC TGC AAC AAC GAG GAA GAG AGG CTC TTA AAT GAC TIG ATG 954
20
lys asn tyr asn lys asn leu arg pro val glu lys asp gly asp ile ile ser
AAG AAT TAC AAT AAG AAC TIG CGT CCT GTG GAA AAG GAT GGC GAC ATT ATC AGT 1008
40
val ser ile lys leu thr leu thr asn leu ile ser leu asn glu lys glu glu
GTC AGC ATA AAG TTG ACC CTT ACC AAC CTC ATT TCC CTG AAT GAG AAA GAA GAA 1062
60
ala leu thr thr asn val trp val glu met gln trp lys asp tyr arg leu ser
GCT CTG ACA ACC AAT GTC TGG GTT GAA ATG CAA TGG AAA GAC TAT CGC CTG AGC 1116
80
trp asp pro asn asp tyr his gly ile ser met met arg ile pro ser thr ser
TGG GAC CCC AAT GAT TAC CAT GGT ATT AGC ATG ATG CGA ATT CCC TCA ACC TGG 1224
100
val trp leu pro asp val gly leu glu asn asn val asp gly thr phe asp ile
GTG TGG CTT CCA GAT GTG GCG TTA GAA AAT AAT GTG GAT GGG ACA TTT GAT ATT 1278
120
ala leu tyr thr asn thr leu val ser ser asp gly ser met tyr trp leu pro
GCA CTA TAC ACC AAC ACG TTG GTA TCC TCA GAT GGT TCC ATG TAC TGG CTT CCF 1332
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pro ala ile tyr arg ser ser cys pro val val val thr tyr phe pro phe asp
CCC GCC ATC TAC CCG AGC TCA TGC CCT GTT GTA GTC ACC TAT TTT CCF TTT GAC 1386
160
trp gln asn cys ser ile val phe gln ser gln thr tyr ser ala asn glu ile
TGG CAG AAC TGT TCC ATA GGG TTT CAG TCA CAG ACT TAC AGC CTT AAT GAG ATT 1440
180
glu leu leu thr val asp glu gln thr ile glu trp ile glu ile asp pro
GAA TTA CTT CTT ACA GTT GAT GAA CAG ACA ATC GAA TGG ATA GAA ATA GAC CCT 1497
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GAA CCA TTT ACA GAA AAT GGG GAA TGG GCA ATA AAA CAG ATG CCA GCC AAG AGA 1565
220
ile ile asn his egg leu pro arg asp asp val asn tyr gln gln ile val phe
ATT ATA AAT CAC ACG CTC CCT CGT GAT GAT GTC AAT TAT CAG CAA ATT GTC TTC 1636
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tyr leu ile ile gln arg lys pro leu phe tyr ile ile asn ile ile val pro
TAT CTC ATC ATT CAA CGG AAA CCT CTC TTC TAC ATC ATC AAC ATC ATT GTA CCC 1708
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cys val leu ile ser phe val ser ile leu val tyr phe leu pro ala lys ala
TGT GTC CTT ATC TCC TTC GTC AGC ATT CTC GTC TAT TTC TTA CCT GCC AAA GCT 1849
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GGA GGA CAG AAG TGT ACT GTG TCC ATC AAC ATC CTA CTG GCC CAG ACT GTC TTC 1918
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320
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DELTA SUBUNIT

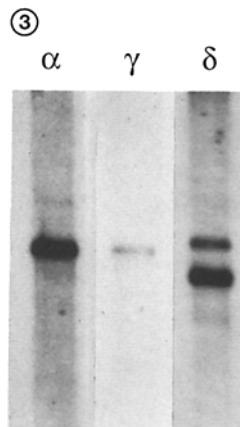
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-21 met ala trp ile trp ile ser leu leu leu pro ile
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-1 1
leu ile tyr phe pro gly cys phe ser glu ser glu glu glu arg 18
TTA ATC TAC TTT CCA GGA TGC TTT TCT GAG AGT GAA GAG GAG CGT 20
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CTG CTA AAT CAC ATT TTT GTT GAG CGA GGG TAT AGA AAG GAA CTT 300
arg pro val glu his thr gly glu thr val asn val ser leu ala 108
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val pro pro asp met val trp gln pro leu ile leu glu asn 288
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CAG AAC TGT TCT CTT AAG TGC AGT TCC TTG ACT TAC AAT GCA AAA 160
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arg tyr tyr pro val glu trp ile ile asp pro glu gly phe 558
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AAT ATA GAT AGA AGT TTG TCT CCT GAG AGC ACC AAG TAC CAG GAC 220
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ile asn ile leu ala pro cys val leu ile ala leu met ala asn 738
ATT AAC ATC CTG GCA CCT TGT GTG CTT ATT GCA CTA ATG GCT AAT 260



**Figure 2.** Alpha subunit transcript is confined to the somites of *Xenopus* embryos. (a) Bright-field optics of giemsa-stained transverse section of stage 26 *Xenopus* embryo illustrates the neural tube (nt), notochord (n), somites (s), and epidermis (ep). (b) Dark-field optics illustrates the position of the autoradiographic grains, which are highly concentrated over the somites and not present over other tissue at a level higher than background. Bar, 50  $\mu$ m.

bryos (stages 20 through 41) demonstrates that a 2.3-kb transcript hybridizes to the alpha subunit cDNA and that a 2.4-kb transcript hybridizes to the gamma subunit cDNA at both moderate and high stringency (Fig. 3, see Materials and Methods).

The delta subunit cDNA, however, hybridizes to two transcripts (1.9 and 2.5 kb) at high stringency (Fig. 3); at low stringency the delta cDNA also hybridizes to the 2.4-kb gamma subunit transcript. Both the 1.9- and 2.5-kb transcripts are expressed at stages 20 through 41 and the ratio of 1.9- to 2.5-kb transcript (5:1) is constant. It is likely that the delta subunit cDNA that we have isolated encodes the 1.9-kb transcript: the delta subunit cDNA is  $\sim$ 1.9 kb in length, con-



**Figure 3.** Northern blot analysis of poly(A)<sup>+</sup> RNA from *Xenopus* embryos. 1  $\mu$ g of poly(A)<sup>+</sup> RNA isolated from stage 41 *Xenopus* embryos was fractionated by electrophoresis in a formaldehyde agarose (1%) gel, transferred to Zetabind, and hybridized to <sup>32</sup>P-labeled AChR alpha ( $\alpha$ ), gamma ( $\gamma$ ) or delta subunit ( $\delta$ ) cDNA probes (Materials and Methods). This filter was washed at moderate stringency (Materials and Methods). The RNAs that hybridize to the alpha, gamma, and delta probes migrate at 2.3 ( $\alpha$ ), 2.4 ( $\gamma$ ), and 1.9/2.5 kb ( $\delta$ ), respectively. Washing at higher stringency did not reduce hybridization to AChR transcripts. The positions of RNA standards (4.4, 2.4, and 1.4 kb) are indicated at right. The pattern of Northern blots from earlier stages (stages 20 and 25) were identical to those from stage 41 embryo RNA except that gamma subunit transcript levels were comparable to those of alpha and delta at earlier stages. The filter was exposed to x-ray film with an intensifying screen at  $-70^{\circ}$ C for 4 d.

tains a consensus polyadenylation signal (AGTAAA) 17 nucleotides from a 3' poly (dA) tract, and encodes a synthetic RNA that nearly comigrates with the 1.9-kb transcript. Since mapping of the transcription start site in a genomic clone encoding this delta subunit demonstrates that the delta subunit cDNA lacks 6–12 bp from the 5' untranslated region (data not presented), the delta subunit cDNA encodes a transcript that is  $\sim$ 1.9 kb.

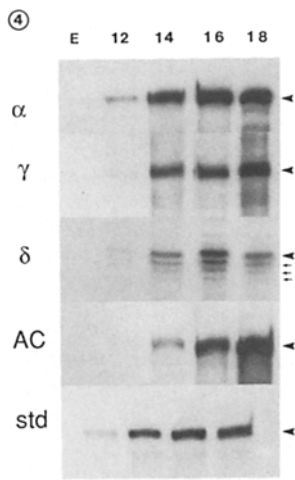
**AChR Transcripts Are Expressed Coordinately at Late Gastrula**

#### AChR Transcripts Are Expressed Coordinately at Late Gastrula

With radiolabeled anti-sense RNA probes (Fig. 1) complementary to *Xenopus* alpha, gamma, and delta AChR transcripts, we measured AChR mRNA levels during development by RNase protection analysis (see Materials and Methods).

Transcripts encoding alpha, gamma, and delta subunits are first detected at late gastrula (stage 12) after 13.75 h of development (Fig. 4, Table I). At this stage there are 500–1,500 molecules of each AChR subunit transcript per embryo (Table I). Alpha subunit transcript is two- to threefold more abundant than either gamma or delta subunit transcripts at stage 12. Actin expression is also first detected at stage 12 (Mohun et al., 1984; Fig. 4). During the next several hours somite rudiments form (Hamilton, 1969) and a burst in AChR mRNA levels is detected; between 13.75 and 16.25 h (stage 14) the level of each AChR subunit transcript increases  $\sim$ 30-fold per embryo (Fig. 4).

The level of AChR mRNA then increases more gradually. Between 16.25 and 19.75 h (stage 18) each subunit mRNA increases fourfold per embryo (Fig. 4 and Table I). The absolute levels of AChR subunit transcripts differ only modestly from each other during this period (Table I). The amount of actin mRNA increase four- to fivefold between stages 14 and 18. Thus, the initial expression of actin mRNA and AChR mRNAs occurs concurrently at late gastrula and the level of each transcript increases in parallel.



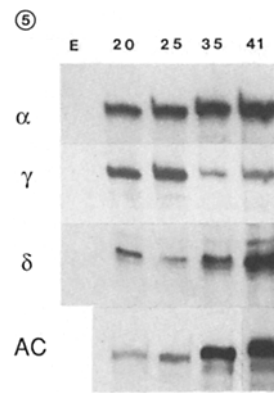
**Figure 4.** AChR transcripts are expressed at late gastrula. RNA from 25 embryo equivalents was included in hybridizations with <sup>32</sup>P-labeled AChR subunit probes, whereas RNA from 2.5 embryo equivalents was included in hybridizations with the <sup>32</sup>P-labeled cardiac actin probe (AC). A single batch of RNA isolated from each stage was used in protection assays with each probe, except that a different batch of stage 18 RNA was used in the protection assay with the delta subunit probe. This second batch of stage 18 RNA contained less total RNA per embryo and correspondingly less actin mRNA per embryo.

Quantitation of transcript levels is presented in Table I. The positions of protected fragments are indicated with arrowheads; the positions of the minor protected fragments in hybridizations with the delta subunit probe are indicated with arrows. The standard curve for delta subunit RNA is illustrated (*std*) and includes 0.5, 2.0, 5.0, and 10 pg of synthetic delta RNA. Developmental stages are indicated at the top of each lane; neither AChR nor actin mRNA was detected in eggs (lane E). Gels were exposed to x-ray films with an intensifying screen at -70°C for 70 h (actin) or 7 d (AChR).

#### After Innervation the Level of Alpha and Delta Subunit Transcripts Decreases Modestly, whereas the Level of Gamma Subunit Transcript Decreases 50-fold

Alpha, gamma, and delta subunit transcript levels were measured for the following stages of *Xenopus* development: stage 20 (21.75 h, initial stage of synapse formation, but before motor activity, 6 somites formed); stage 25 (27.5 h, onset of motor activity, 16 somites formed); stage 35 (50 h, hatching, 25 somites formed); stage 41 (76 h, tadpoles swim freely, 45 somites formed) (Nieuwkoop and Faber, 1967; Kullberg et al., 1977).

Between 21.75 (stage 20) and 76 h (stage 41) there is an elevenfold increase in the amount of actin mRNA per micro-



**Figure 5.** Gamma subunit transcript levels are regulated differently than alpha and delta subunit transcript levels. RNA from 10 embryo equivalents was included in hybridizations with <sup>32</sup>P-labeled AChR subunit probes, whereas RNA from 1 embryo equivalent was included in hybridizations with the <sup>32</sup>P-labeled cardiac actin probe (AC). A single batch of RNA isolated from each stage was used in protection assays with each probe, except that a different batch of stage 25 RNA was used in the protection assay with the

delta subunit probe. This second batch of stage 25 RNA contained less total RNA per embryo and correspondingly less actin mRNA per embryo. Quantitation of transcript levels is presented in Table I. Developmental stages are indicated at the top of each lane; neither AChR nor actin mRNA was detected in eggs (lane E). The positions of protected fragments are indicated with arrowheads. Gels were exposed to x-ray films with an intensifying screen at -70°C for 6.5 (actin) or 16 h (AChR).

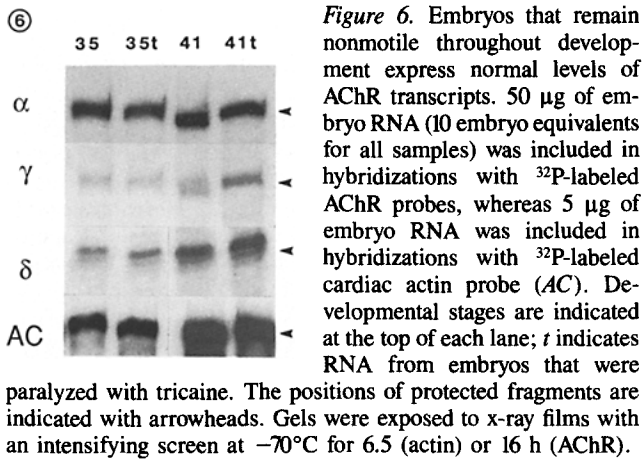
grams total embryo RNA (Fig. 5 and Table I). During the same period of development alpha and delta subunit transcripts increase two- to threefold, whereas gamma subunit transcript decreases five- to sixfold (Fig. 5 and Table I).

Since the amount of actin mRNA per embryo increases in proportion to the number of somites per embryo (Table I), we indexed the amount of AChR mRNA to the amount of actin mRNA to establish the amount of AChR mRNA per somite; alpha and delta mRNAs decrease fourfold per somite, whereas gamma mRNA decreases 55-fold per somite between stages 20 and 41 (Table I). The decrease in AChR mRNA occurs after stage 25 when motor activity has begun. These results demonstrate that AChR levels per somite decrease and that the regulation of gamma subunit transcript level is different than that for alpha and delta subunit after stage 25.

**Table I. Quantitation of AChR and Actin mRNA Levels during Development in *Xenopus laevis***

| Stage | RNA/embryo | No. of somites/embryo | Actin RNA/total RNA | Actin RNA/somite | Alpha RNA/total RNA | Gamma RNA/total RNA | Delta RNA/total RNA | Alpha/actin | Gamma/actin | Delta/actin |
|-------|------------|-----------------------|---------------------|------------------|---------------------|---------------------|---------------------|-------------|-------------|-------------|
|       | μg         |                       | U/μg                | U/somite         | pg/μg               | pg/μg               | pg/μg               | pg/U        | pg/U        | pg/U        |
| 12    | 0.5-1.0    | 0                     | 0.002               | -                | 0.003               | 0.001               | 0.001               | 2.5         | 1.5         | 1.5         |
| 14    | 0.5-1.0    | 0                     | 0.02                | -                | 0.10                | 0.07                | 0.03                | 5.0         | 3.5         | 1.4         |
| 16    | 0.5-1.0    | 0                     | 0.06                | -                | 0.30                | 0.20                | 0.10                | 5.0         | 3.3         | 1.3         |
| 18    | 0.5-1.0    | 3-4                   | 0.10                | 0.03             | 0.40                | 0.30                | 0.10                | 4.0         | 3.0         | 1.0         |
| 20    | 1.0-4.0    | 6-7                   | 0.20                | 0.03             | 0.70                | 1.10                | 0.40                | 3.5         | 5.5         | 2.0         |
| 25    | 1.0-4.0    | 16                    | 0.44                | 0.03             | 1.00                | 1.60                | 0.60                | 2.2         | 3.6         | 1.4         |
| 35    | 2.0-5.0    | 35                    | 2.00                | 0.06             | 1.50                | 0.20                | 0.90                | 0.8         | 0.1         | 0.4         |
| 41    | 3.0-7.0    | 45                    | 2.10                | 0.05             | 1.90                | 0.20                | 1.10                | 0.9         | 0.1         | 0.5         |
| 35t   | 2.0-5.0    | 35                    | 2.00                | 0.06             | 1.60                | 0.20                | 0.90                | 0.8         | 0.1         | 0.5         |
| 41t   | 3.0-7.0    | 45                    | 2.20                | 0.05             | 2.00                | 0.20                | 1.00                | 0.9         | 0.1         | 0.5         |

Quantitation of transcript levels was determined as described (Materials and Methods). RNA from each stage was isolated from two to three separate batches of embryos resulting from several different matings. Four separate protection assays were performed for each stage with each probe; averaged values are provided. Absolute values of actin RNA were not determined and the values for actin are expressed as arbitrary units. Values for animals that were reared in tricaine are indicated with a t following the stage at which they were collected for analysis. Actin mRNA per total RNA and AChR mRNA per total RNA varied by 10% in separate assays.



**Figure 6.** Embryos that remain nonmotile throughout development express normal levels of AChR transcripts. 50  $\mu$ g of embryo RNA (10 embryo equivalents for all samples) was included in hybridizations with  $^{32}$ P-labeled AChR probes, whereas 5  $\mu$ g of embryo RNA was included in hybridizations with  $^{32}$ P-labeled cardiac actin probe (AC). Developmental stages are indicated at the top of each lane; *t* indicates RNA from embryos that were paralyzed with tricaine. The positions of protected fragments are indicated with arrowheads. Gels were exposed to x-ray films with an intensifying screen at  $-70^{\circ}\text{C}$  for 6.5 (actin) or 16 h (AChR).

### Motor Activity has no Effect on AChR Transcript Levels in Myotomal Muscle

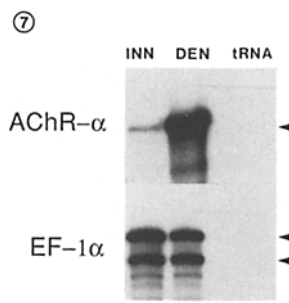
To determine whether the decrease in AChR mRNA levels per somite after stage 25 is caused by the onset of motor activity, embryos were allowed to develop in the presence of the local anaesthetic tricaine methanesulphonate from stage 20 to either stage 35 or 41. At a tricaine dose of 200  $\mu$ g/ml embryos developed normally up to stage 41, although they remained immobile (Cohen et al., 1984). Embryos that were removed from tricaine at stage 41 exhibited normal swimming behavior within 30 min. Moreover, paralyzed embryos expressed normal levels of actin mRNA (Fig. 6 and Table I).

Motor activity, however, had no effect on expression of AChR transcripts, since paralyzed and normal embryos expressed identical levels of AChR transcripts (Fig. 6 and Table I).

### Denervated Adult *Xenopus* Muscle Expresses 50–100-fold More Alpha Subunit Transcript than Innervated Muscle

It is well established that the number of nonsynaptic AChRs is regulated by the rate and pattern of electrical activity in multinucleated myofibers. An increase in AChR number is produced in muscle that has been paralyzed either by denervation or by pharmacological blockers of acetylcholine release, AChR function, or muscle and nerve action potentials (Lomo and Westgaard, 1975). Moreover, AChR mRNA levels increase 50–100 fold in adult mammalian muscle after denervation (Merlie et al., 1984).

Since it is more convenient to paralyze muscle in adult animals by denervation rather than by pharmacological blockers, we denervated adult *Xenopus* muscle to determine whether AChR transcript levels are regulated by myofiber electrical activity in multinucleated myofibers in adult *Xenopus* muscle. Fig. 7 demonstrates that the level of alpha subunit mRNA was low in normal innervated triceps femoris muscle and increased 50–100-fold 10 d after denervation. Thus, although AChR transcript levels in *Xenopus* myotomal muscle were not influenced by a reduction in electrical activity produced by local anaesthetic, AChR transcript levels in adult multinucleated myofibers of *Xenopus* were influenced by a reduction in electrical activity produced by denervation.



**Figure 7.** Alpha subunit transcript levels increase 100-fold in denervated adult muscle. The triceps femoris muscle of adult *Xenopus* was denervated and alpha subunit transcript levels in normal and denervated muscle were measured by RNase protection (Materials and Methods). The amount of alpha transcript in normal, innervated muscle (INN) is low and increases 100-fold in denervated muscle (DEN), whereas the amount of total mRNA as determined for Ef-1 alpha transcript in normal, innervated (INN), and denervated muscle (DEN) is indistinguishable. There is no protection of either probe in hybridizations with tRNA. The positions of protected fragments are indicated with arrowheads. The gels were exposed to x-ray films with an intensifying screen at  $-70^{\circ}\text{C}$  for 1 d.

### Discussion

This study demonstrates that transcripts encoding alpha, gamma, and delta subunits of the AChR appear coordinately early during embryonic development in *Xenopus laevis*. Each AChR transcript is readily detected at 16.25 h of development (stage 14). At this time the presumptive somite mesoderm has thickened, though somite formation is not apparent until 18.75 h (stage 17). AChRs have been detected on the surface of myotomal muscle cells as early as 20.75 h (stage 19) and are likely to be present on the cell surface as early as 19.5 h (stage 18) (Chow and Cohen, 1983; Blackshaw and Warner, 1976). It is likely that the lag of 3–5 h between appearance of AChR transcripts in myotomal cells and presence of AChR protein on the cell surface is due in part to the  $\sim$ 3-h lag between synthesis of AChR subunits and incorporation of AChRs into the plasma membrane (Devreotes and Fambrough, 1975a).

Between stages 25 and 41 gamma subunit transcript levels decrease  $>50$ -fold per somite while alpha and delta subunit transcripts each decrease  $\sim$ fourfold per somite. Thus, initial expression of AChR transcripts is coordinate, but subsequent regulation of AChR transcript levels is not coordinate. These results raise the possibility that AChR subunit genes are initially activated coordinately, but regulated differently later in development.

The number of surface AChRs increases approximately sixfold per myotome between stages 25 and 41 (Chow and Cohen, 1983) despite a fourfold decrease in alpha and delta subunit transcript levels and a 50-fold decrease in gamma subunit transcript levels. Several explanations could account for these results: (a) a decrease in the rate of AChR degradation could allow AChRs to accumulate on the cell surface despite a diminishing synthetic rate; (b) the rate of AChR synthesis could be maintained by increased translational efficiency; (c) the rate of AChR synthesis could be maintained by replacement of the diminishing gamma subunit transcript with a second gamma subunit transcript. With regard to the later possibility, an epsilon subunit transcript that encodes a protein that can functionally substitute for the gamma subunit has been identified in bovine muscle (Takai et al., 1985). The temporal expression of the epsilon subunit transcript is different than the gamma subunit transcript and



this subunit substitution confers different AChR channel kinetics (Mishina et al., 1986).

The 2.5-kb transcript that hybridizes to the delta cDNA may encode a protein that can substitute for the gamma subunit. In this regard it is well established that AChR channel kinetics change during *Xenopus* development (Kullberg et al., 1981; Brehm et al., 1982) and that the transition from slow- to fast-channel kinetics occurs between 40 (stage 32) and 66 h (stage 40) of development (Kullberg and Kasprzak, 1985). Thus, the switch from slow- to fast-channel kinetics occurs at the same time during development that gamma subunit transcript levels decrease 50-fold. Moreover, both the change in AChR channel kinetics (Kullberg et al., 1984) and the decrease in gamma transcript levels occur in the absence of myofiber electrical activity. Isolation and characterization of cDNA encoding the 2.5-kb transcript will be necessary to determine which subunit this transcript encodes.

AChR transcript levels in *Xenopus* myotomal muscle are insensitive to changes in myofiber electrical activity. Since alpha subunit transcript levels increase 50–100-fold in denervated adult muscle, there appears to be electrical activity-dependent regulation of AChR transcript levels in multinucleated myofibers of adult *Xenopus* muscle. These results suggest that regulation of AChR genes in mononucleated myotomal muscle cells is different than that in multinucleated adult myofibers and are consistent with the model that we have described: that electrical activity has a repressive effect on AChR transcript expression and is exerted throughout a myofiber, whereas components of the synaptic site (e.g., soluble trophic factors or components of the synaptic extracellular matrix) exert a local activating effect on AChR transcript expression. Since this model also predicts that the synaptic activator is dominant, or epistatic, to the repressive effect of electrical activity, AChR expression in the mononucleated myotomal cells would be controlled by synaptic factors. Since only a small fraction (~1%) of the multinucleated myofiber would be controlled by the locally acting synaptic activator, the bulk of AChR regulation in a multinucleated myofiber would be controlled by electrical activity.

Since the number of genes encoding skeletal muscle AChR subunits in *Xenopus* is not yet clear, we cannot, however, exclude the possibility that different AChR genes are expressed in myotomal and adult muscle and that only the AChR genes expressed in adult muscle are regulated by electrical activity.

We have suggested that control of AChR transcript levels in *Xenopus* myotomal muscle is regulated by a dominant synaptic influence that allows AChR transcript levels to persist despite myofiber electrical activity. It is possible, however, that the regulatory pathway that is activated by electrical activity in multinucleated muscle cells is activated in *Xenopus* myotomal cells by signals other than electrical activity and that this pathway reduces AChR transcript levels in myotomal cells. In this regard the level of AChR gamma and delta subunit transcripts in C2 mouse muscle cells is 35 pg/ $\mu$ g cellular RNA (Yu et al., 1986) and the level of alpha and delta subunit transcripts in *Xenopus* embryos is 1–2 pg/ $\mu$ g total embryo RNA. Since myotomal muscle comprises <10% of the embryo, the specific activity of AChR transcripts in *Xenopus* myotomal cells is comparable to that in C2 cells. Thus, AChR transcript levels in *Xenopus* myotomal muscle cells are comparable to those in electrically quiescent multinucleated cells and not reduced to levels found in innervated

electrically active muscle. Similarly, the number of AChRs in *Xenopus* myotomal muscle cells is not responsive to changes in myofiber electrical activity, since nonsynaptic AChR density decreases only modestly after innervation of *Xenopus* myotomal muscle both in vitro and in vivo (Kido-koro and Gruener, 1982; Chow and Cohen, 1983; Goldfarb et al., 1984).

Thus, these results are consistent with the hypothesis that AChR genes in nuclei that are proximal to the synapse are constitutively activated despite myofiber electrical activity, whereas AChR genes in nuclei that are distal to the synapse are down-regulated by electrical activity.

Study of AChR expression in *Xenopus* has allowed us to analyze initial expression of AChR transcripts during development. Transcripts encoding three subunits of the *Xenopus* AChR appear coordinately at late gastrula. At this stage the presumptive somite mesoderm has not yet separated from the presumptive lateral mesoderm, yet differentiation among the mesodermal layers may have already begun. Study of AChR expression in cell culture is limited to the enhanced expression of AChR transcripts concomitant with myoblast fusion and myotube differentiation: since myoblasts already express AChR transcripts, this system is not suitable for the study initial expression of AChR transcripts (Merlie et al., 1983; Yu et al., 1986). Moreover, it is not yet clear whether the level of each AChR subunit transcript increases in parallel upon differentiation in these cell lines.

Both AChR and actin transcripts are first detected at late gastrula. Coordinate expression of AChR and actin transcripts suggests that a coordinate, rather than a sequential, program of muscle differentiation is executed during myogenesis in *Xenopus*.

In contrast to coordinate expression of AChR transcripts, transcripts encoding different subunits of other protein complexes such as immunoglobulin and globin are not expressed coordinately: heavy chain transcript and protein are present in pre-B cells before light chain transcript or light chain protein are synthesized (Levitt and Cooper, 1980; Perry et al., 1981; Siden et al., 1981) and alpha-globin transcripts precede beta-globin transcripts during fetal development (Nudel et al., 1977; Weatherall and Clegg, 1981). Coordinate expression of AChR transcripts in development suggests that a common pathway may be responsible for activation of the different subunit genes and that different subunit genes may contain common regulatory elements. Differential regulation of gamma subunit transcript levels at later developmental stages suggests, however, that different subunit genes may also contain unique regulatory elements. Analysis of the different AChR subunit genes may help us to understand how AChR transcripts are both coordinately and discoordinately controlled.

We would like to acknowledge the technical assistance of Ms. Katharine Alexander and Mr. Tristan Davies.

This work was supported by grants from the National Institutes of Health (NS 21579), the Muscular Dystrophy Association, and the Whitaker Health Sciences Fund. T. J. Baldwin is a postdoctoral fellow of the Muscular Dystrophy Association, C. M. Yoshihara is a postdoctoral fellow of the National Institutes of Health, and C. R. Kintner was a postdoctoral fellow of the Biomedical Foundation.

Received for publication 11 September 1987, and in revised form 3 November 1987.

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