ing urea, Triton X-100, and acetate (*13*)—was assayed by fluorography (*14*). Figure 2A shows a fluorogram comparing globins synthesized in yolk-sac erythroid cells with those synthesized in erythroid cells of the spleen 1 day after birth. In accordance with the terminology established for mice (*1, 15*), we identify the embryonic α-like globin as band x and the two embryonic β-like globins as bands y and z (*16*). The same pattern of synthesis was obtained from liver erythroid cells 1 day after birth. No embryonic chain synthesis was detected in peripheral blood cells from any neonatal sampling. Five days after birth, synthesis of two embryonic globins (x and z) was barely detectable in spleen erythroid cells only.

Further confirmation of the synthesis of embryonic globin chains in neonatal erythroid cells was provided by coelectrophoresis. Tritiated hemoglobin from erythroid cells of the spleen 1 day after birth was mixed with unlabeled hemoglobin solution from yolk-sac erythroid cells. Bands 5 and 7 (Fig. 1B) were isolated and their globins were separated by electrophoresis in polyacrylamide gel (Fig. 2B). Each band resolved only into the two globin chains characteristic of that embryonic hemoglobin (Fig. 2B), and fluorography demonstrated synthesis of each of the three globins (Fig. 2C).

There are similarities between these findings for hamsters and observations in humans. Human adult-type β-globin chains first appear at a time in gestation (6 weeks) when yolk-sac erythropoiesis is being replaced by hepatic erythropoiesis, but the earliest site of synthesis has not been defined. Trace amounts of a hemoglobin whose α-like globin is embryonic have been detected in cord erythrocytes from human neonates (*17*). Our findings demonstrate conclusively the coexistence of embryonic and adult globin gene expression over a range of developmental stages from prehepatic to early postnatal (*18*). The currently accepted time span for the normal sequence of globin gene expression during mammalian ontogeny may be too narrow.

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9. Less than 0.1 percent maternal erythrocytes were detected on smears of purified yolk-sac erythroid cells stained by Wright's and Giemsa's stains and examined by light microscopy. At least 10 percent contamination by maternal erythrocytes in our purified yolk-sac cell preparations would have been required to provide the 4 percent of band 4 observed (Fig. 1B).
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Mammalian Muscle Acetylcholine Receptor: A Supramolecular Structure Formed by Four Related Proteins

Abstract. *The nicotinic acetylcholine receptor has been purified from fetal calf muscle. Amino terminal amino acid sequence data indicate that the mammalian receptor is formed from closely related but distinct subunits. A cytoskeletal component, actin, may be associated with the receptor.*

A human paralytic syndrome, myasthenia gravis (MG), is due to an autoimmune reaction against the nicotinic acetylcholine receptor (AcChR) (*1*). Elucidation of its structure is therefore crucial. So far, AcChR's from the electric organs of fish (*Torpedo*, *Narcine*, and *Electrophorus*) have been well characterized (*2*). Studies of mammalian AcChR have been less sophisticated; its subunit complement has been widely debated; subunit patterns containing from one to six polypeptides have been reported (*2*).

There are indications that mammalian muscle and piscine AcChR's are similar. Experimental autoimmune myasthenia gravis (EAMG) can be induced in mammals with the use of AcChR purified from electric fish (*3*), and polyclonal and monoclonal antibodies to *Torpedo* AcChR may precipitate mammalian muscle AcChR (*4, 5*).

We now report evidence that the mammalian AcChR is a complex of four homologous peptides, and that a fifth peptide that copurifies with the receptor is actin.

The AcChR was purified from fetal calf muscle (*6*) and upon sodium dodecyl sulfate (SDS) gel electrophoresis resolved into five polypeptides having molecular weights (M_r) of 42, 44, 49, 55, and 58 K (*6*) (Fig. 1A, lane 3). A sixth polypeptide of M_r 53 K was present in some preparations (*6*). The polypeptides were characterized by different methods: the presence of carbohydrate was assessed by binding of ^{125}I-labeled conconavalin A (Con A) (*7*) which consistently labeled the peptides of M_r 42, 49, and 55 K. When the polypeptide of M_r 53 K was present it was consistently labeled while those of M_r 58 K and 44 K were consistently unlabeled. The amino acid composition of the latter is similar to that of actin (*6*), including the presence of 3-methylhistidine (*8*). We confirmed that

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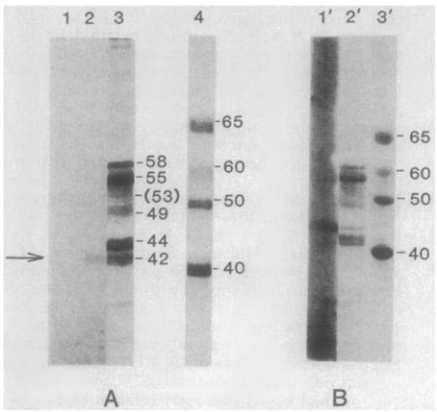

Fig. 1. (A) SDS gel electrophoresis of fetal calf AcChR (lane 3) and *Torpedo* AcChR (lane 4) stained with Coomassie blue. Fluorogram of the SDS gel of fetal calf AcChR labeled with [^{3}H]bromoacetylcholine (*11*) (lane 2). Lane 1 shows a control in which the fetal calf AcChR was first incubated with a 50-fold molar excess of *Naja naja siamensis* α-toxin prior to labeling with [^{3}H]bromoacetylcholine. The arrow indicates that the 42 K subunit was labeled (lane 2). (B) Coomassie blue staining of fetal calf (lane 2') and *Torpedo* AcChR (lane 3') after separation by SDS gel electrophoresis. The 42 K component of fetal calf AcChR [see lane 3 in (A)] was partially degraded to give a doublet. Lane 1' shows that antibody to actin binds to fetal calf AcChR polypeptides; the 44 K component and its degradation products of lower molecular weight were labeled by the antibody.

this peptide was actin by the binding of antibodies to actin (*9*) (Fig. 1B). The peptide of M_r 42 K was labeled by the cholinergic ligand [^{3}H]bromoacetylcholine (BrAcCh) (*10*) (Fig. 1A), which suggests that it corresponds to the lightest component ("α", $M_r \approx 40$ K) of electric organ AcChR (*2*).

Each polypeptide of fetal calf AcChR was subjected to amino terminal sequence analysis (*11*). The subunits of 42 and 49 K yielded distinct but homologous sequences (Fig. 2). The polypeptide of 53 K yielded a sequence that was homologous with those for the 42 and 49 K subunits, that is, ? E H E N K L Q A H L F D D Y A S H/K K/P F P ? E/R (*12*). The polypeptide with the M_r of 44 K did not yield any sequence, an indication of the presence of a blocked amino terminus, in agreement with its identification as actin (*13*). Because of the lack of identifiable sequences associated with the 55 and 58 K polypeptides (most likely due to blockage of their amino terminals during isolation) we used a different approach. As demonstrated for *Torpedo* (*14*) and *Electrophorus* (*15*) AcChR, it is possible to determine all four polypeptide subunit sequences simultaneously to obtain exact subunit stoichiometry. Sequence analysis of whole calf AcChR preparations yield four homologous sequences (*16*). Three were identical with those independently determined for the 42, 49, and 53 K polypeptides. The fourth sequence was deduced by difference, with the fourth amino acid being identified at each step.

These four sequences of calf AcChR are distinct but structurally related. At five positions out of the first 23 residues all four subunits contain the same amino acid residue (positions 4, 7, 12, 15, and 21). In several other positions two or three of the four amino acid residues are identical, and conservative substitutions (dotted circles in Fig. 2A) are frequent. Alignment of the subunits requires a single insertion of two amino acid residues in one of the subunits of higher M_r to bring all four sequences into register. The data strongly suggest that the four polypeptides are derived from a common ancestral gene. A comparison was made between the amino terminal sequences

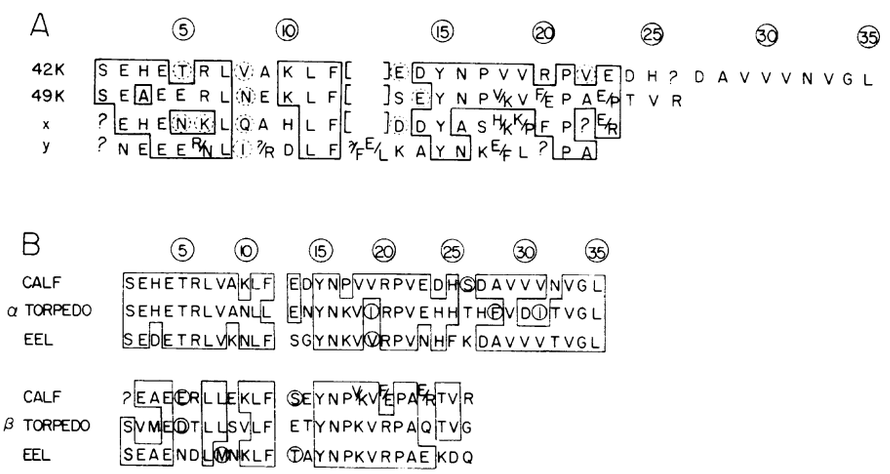

Fig. 2. (A) Amino terminal amino acid sequences in purified fetal calf AcChR. (B) Comparison between the α (molecular weight ~ 40 K) and β (~ 50 K) subunits of AcChR from fetal calf muscle and the electric organs of *Torpedo californica* and *Electrophorus electricus* (eel).

Table 1. Stoichiometry of fetal calf muscle AcChR subunits (Val, valine; Leu, leucine; Gln, glutamine; Ile, isoleucine).

Subunit	Residue (cycle 8)	Ratio
α	Val	2.16
β	Leu	.95
x	Gln	.92
y	Ile	.98

of the two lighter subunits of AcChR from fetal calf muscle, a cartilaginous fish (*Torpedo californica*) and a teleost fish (*Electrophorus electricus*), two highly diverged species (Fig. 2B). Among the lowest M_r subunits ("α"), 51 percent of the residues were identical, and in an additional 36 percent of the positions two of the three polypeptides had the same residue. In the case of subunits of M_r, approximately 50 K ("β"), 38 percent of the residues were identical. A comparison of all six polypeptide sequences showed that 23 percent of all positions were identical for the first 26 residues and an additional 19 percent identity was observed in five out of the six polypeptides at other positions. All the subunits forming the AcChR from these evolutionarily distant animal species must be derived from the same ancestral gene, probably by way of gene duplications occurring very early in animal evolution; this is likely since the degree of sequence identity is greater between subunits of similar molecular weight from different species than between the four subunits from a single species (*14*, *15*).

The stoichiometry of the subunits in the fetal calf AcChR molecule was obtained by simultaneous quantitative determination of the amino acid sequences present in intact preparations (*16*). A stoichiometry of 2:1:1:1 was found (Table 1), which demonstrates that mammalian muscle nicotinic receptor is a pentameric complex composed of two equivalent and three pseudoequivalent subunits (*17*).

From the stoichiometry and apparent molecular weight of fetal calf AcChR subunits, an M_r of 241,000 to 246,000 can be calculated, which is compatible with its observed size (*18*) and sedimentation behavior (*2*).

Actin consistently copurifies with the calf AcChR, suggesting a direct or indirect association of these two molecules in the intact cell. The known immobility of the AcChR in the postsynaptic membrane (*2*) could be due to AcChR-AcChR interactions or to interactions with cytoskeletal components such as actin (or both).

The structure of the mammalian AcChR has ramifications with regard to human pathology. In myasthenia gravis the neuromuscular AcChR is destroyed mainly by specific autoantibodies, which accelerate the catabolism of AcChR molecules as a result of cross-linking (*1*). Since mammalian AcChR is formed by similar subunits, one of which is present in two copies, repetitive homologous antigenic determinants should be present on its surface. Antibodies directed against such determinants would be inefficient in accelerating the degradation of the AcChR because, instead of cross-linking adjacent AcChR molecules, they should preferentially bind to two homologous determinants within the same AcChR molecule (*19*). In addition, both in myasthenia gravis (*20*) and in EAMG, its experimental model (*5*), a significant fraction of the antibodies present in the serum are directed against antigenic determinants on the "α" subunits. Such considerations would explain the existing lack of correlation between titers of antibody to AcChR and the severity of symptoms both in myasthenia gravis and in EAMG (*21, 22*), because only particular antibody subpopulations (that is, those directed against nonrepetitive antigenic determinants) should be able to induce acceleration of AcChR catabolism.

The highly conserved pentameric subunit structure of the AcChR complex strongly suggests that each of the subunits evolved to perform discrete, crucial functions in the physiological actions of the AcChR. In this respect, one could speculate about the existence of multiple binding sites for cholinergic ligands on the homologous domains of the AcChR molecules, whose binding could trigger different functions, such as activation, inactivation, or desensitization. The existence of an ancestral gene for this acetylcholine-binding protein raises the possibility of a shared ancestry with other acetylcholine-binding proteins, such as the muscarinic AcChR and cholinesterases, at least regarding the recognition domains of these molecules.

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12. One-letter abbreviations for amino acids: E, glutamic acid; H, histidines; N, asparagine; K, lysine; L, leucine; Q, glutamine; A, alanine; F, phenylalanine; D, aspartic acid; Y, tyrosine; S, serine; P, proline; and R, arginine.
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16. Purified AcChR (approximately 0.25 nmole) was electrodialyzed against 0.05*M* NH_4HCO_3, 0.1 percent SDS and subjected to amino terminal sequence analysis in a gas-phase microsequenator; the phenylthiohydantoin amino acids released at each cycle were quantified by reverse-phase high-performance liquid chromatography. The stoichiometry of the AcChR polypeptides was determined from the relative amounts of the amino acids released at selected steps, with sequence assignments for the individual amino acids to particular polypeptides made as described.
17. It is not possible at present to define the precise subunit composition of calf AcChR since, although it is clear that four homologous subunits comprise the AcChR, five polypeptides were present upon SDS gel electrophoresis that could give rise to these sequences in a variety of ways. There is no doubt that the peptides with molecular weights of 42, 49, and 53 K are AcChR subunits. However, the 53 K component could either be a subunit particularly susceptible to degradation or arise from either the 55 K or 58 K polypeptides as the result of either variation in the extent of glycosylation or by degradation. Alternatively, it is possible that the 55 K and 58 K components could represent different degrees of glycosylation of the same peptide.
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DNA Sequence of the Gene Encoding the E_α Ia Polypeptide of the BALB/c Mouse

Abstract. *A 3.4-kilobase DNA fragment containing the gene coding for the E_α chain of an Ia (I region–associated) antigen from the BALB/c mouse has been sequenced. It contains at least three exons, which correlate with the major structural domains of the E_α chain—the two external domains α1 and α2, and the transmembrane-cytoplasmic domain. The coding sequence of the mouse E_α gene shows striking homology to its human counterpart at the DNA and protein levels. The translated α2 exon demonstrates significant similarity to β_2-microglobulin, to immunoglobulin constant region domains, and to certain domains of transplantation antigens. These observations and those of others suggest that the Ia antigen, transplantation antigen, and immunoglobulin gene families share a common ancestor.*

The major histocompatibility complex (MHC) of the mouse encodes several families of cell-surface glycoproteins which regulate various aspects of immune responsiveness (*1, 2*). Certain products of the class I genes, the transplantation antigens, serve as restricting elements in T cell immunosurveillance. The products of the class II genes, the I region–associated (Ia) antigens, play a fundamental role in determining the effectiveness of cell-cell interactions between regulatory T cells, B cells, and macrophages. A variety of recent data from genetic, functional, and biochemical studies suggest that the Ia antigens are the products of the Ir (immune response) genes, which control the ability of an animal to respond to synthetic and naturally occurring antigenic determinants (*3–7*).

Two Ia antigens, I-A and I-E, have

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