close association of actively transcribed genes with nuclear domains near the nuclear envelope (*32*) and is highly consistent with recent theoretical considerations of a defined 3-D structure of the cellular genome, specifically gated to a set of nuclear pores (*18*).

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33. We thank K. Norwich for assistance with computer graphics for the 3-D image analyses and V. Kalnins and D. Van der Kooy for criticism of the manuscript. Supported by the Medical Research Council and the Natural Sciences and Engineering Research Council of Canada. U.D.B. was supported by an associateship of the Ontario Mental Health Foundation.

20 May 1986; accepted 2 September 1986

Chromosomal Localization of Muscle Nicotinic Acetylcholine Receptor Genes in the Mouse

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The chromosomal localization of the genes encoding the four subunits of muscle nicotinic receptor was determined by analyzing restriction fragment length polymorphisms between two mouse species *Mus musculus domesticus* (DBA/2) and *Mus spretus* (SPE). Analysis of the progeny of the interspecies mouse backcross (DBA/2 × SPE) × DBA/2 showed that the α-subunit gene cosegregates with the α-cardiac actin gene on chromosome 17, that the β-subunit gene is located on chromosome 11, and that the γ- and δ-subunit genes cosegregate and are located on chromosome 1.

THE NICOTINIC ACETYLCHOLINE receptor (AChR) from fish electric organ and vertebrate skeletal muscle is a transmembrane protein composed of four different subunits synthesized from independent messenger RNA's (mRNA's) (*1*) and assembled into an $\alpha_2\beta\gamma\delta$ pentamer (*2*). Molecular cloning and nucleotide sequencing of complementary DNA (cDNA) and genomic sequences for the four genes from different species have disclosed a high degree of conservation of the protein structure throughout vertebrate evolution. Extensive homologies between subunits have suggested that the genes evolved by successive duplication of a common ancestor gene, with a first branch point for the α and β subunits and a second one for the γ and δ subunits (*3, 4*). Recently, Nef *et al.* (*5*) showed that, in the chick, the γ- and δ-subunit genes are separated by only 740 bp on the chromosome, and Shibahara *et al.* (*6*) isolated human genomic clones that hybridize to both the γ- and δ-subunit cDNA probes, thus suggesting a clustering of the AChR genes and a possible common control of their transcription.

We have made use of restriction fragment length polymorphisms (RFLP's) between two mouse species (*7*) to determine the chromosomal localization of the genes encoding AChR subunits and found that the four genes are not clustered in a single region but are distributed on three different chromosomes. The α-subunit gene cosegregates with the α-cardiac actin gene previously allocated to chromosome 17 by Czosnek *et al.* (*8*), the β-subunit gene is located on chromosome 11, and the γ- and δ-subunit

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Table 1. Segregation of the AChR subunit genes and other known chromosomal markers in the 42 offspring of the DBA/2 × (DBA/2 × SPE) backcross. Results of genomic blots for the AChR subunit genes are reported as (−) when mice are homozygous for the DBA/2 allele and as (+) when mice are heterozygous for the DBA/2 and SPE alleles. ND, not determined. MHC_F and $MLC1_F/MLC3_F$

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chromosome 17																
Glo-1	+	−	+	−	ND	−	−	+	+	+	−	+	+	−	+	−
α-Cardiac actin	+	−	−	−	+	−	+	−	+	−	+	+	−	+	+	+
α-Chain AChR	−	−	−	−	+	+	+	−	+	−	+	−	+	+	+	+
Chromosome 11																
MHC_F	−	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+
β-Chain AChR	−	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+
Chromosome 1																
Idh-1	−	+	+	+	+	−	−	−	−	+	+	−	−	+	+	+
$MLC1_F/MLC3_F$	−	+	+	+	+	−	−	−	−	+	+	−	−	+	+	+
γ-Chain AChR	−	+	+	+	+	−	−	−	−	+	+	−	−	−	−	+
δ-Chain AChR	−	+	+	+	+	−	−	−	−	+	+	−	−	−	−	+

genes cosegregate and are located on chromosome 1.

The cDNA clones encoding the α, β, and γ subunits of the nicotinic AChR were isolated from mouse muscle cell lines and characterized by their nucleotide sequences (legend to Fig. 1). The mouse δ-subunit probe used was part of the 6H fragment (*9*). These probes were used to detect allelic forms of each gene in two mouse species: *Mus musculus domesticus* (DBA/2) and *Mus spretus* (SPE) (*7*). DNA preparations from these two inbred parental lines were digested independently with several restriction enzymes and hybridized to each of the AChR subunit cDNA probes chosen as the most 3′ fragments to reveal a single exon. In each case, the probe detected a single band, consistent with the presence of a single gene encoding each AChR subunit (*10, 11*). Results for the β- and γ-subunit probes are shown in Fig. 1. After systematic screening, RFLP's were found by digestion with Hinc II for the α- and β- subunit genes: the α-subunit probe detected a 6-kb band in DBA/2 and a 3-kb band in SPE. The β-subunit probe revealed a 2.5-kb band in DBA/2 and a 1.5-kb band in SPE (Fig. 1). The γ-subunit probe detected one RFLP between the two parental strains with Eco RI, a 9-kb and a 4-kb band in DBA/2 and SPE, respectively. Finally, for the δ-subunit probe, digestion of the DBA/2 and SPE DNA's with Pvu II gave a 5-kb band in DBA/2 and a 4.2-kb band in SPE (Fig. 1).

The Mendelian segregation of the four genes was followed by means of these RFLP's in the progeny of the mouse backcross DBA/2 × (DBA/2 × SPE) as described (*7*). DNA samples from 42 offspring were digested with Hinc II, Eco RI, or Pvu II, transferred to nylon membranes, and hybridized with the corresponding probe as illustrated for the DNA's from progeny 29 to 36 with the β- and δ-subunit probes in Fig. 1. Results of all the RFLP analyses are

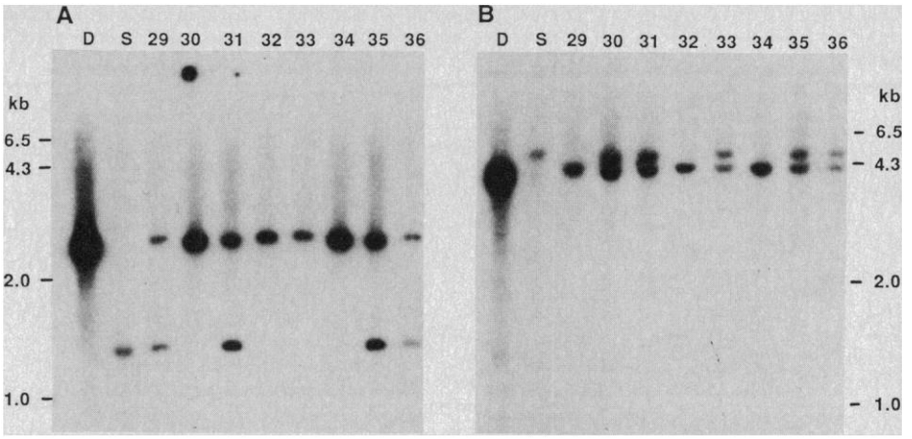

Fig. 1. Southern blots of (A) Hinc II digests (β probe) or (B) Pvu II digests (δ probe) of DNA from *Mus musculus* (D = DBA/2), *Mus spretus* (S = SPE), and 8 of the 42 offspring of the (DBA/2 × SPE) × DBA/2 backcross (progeny 29 to 36). High molecular weight spleen DNA (5 μg) was digested with the appropriate restriction enzyme and size-fractionated on 0.8% agarose gels. DNA's were blotted onto nylon membrane according to Southern's technique (*17*) and fixed by ultraviolet irradiation (5 minutes). Filters were hybridized with M13 derived single-stranded radioactive probes and washed in 0.2× SSC containing 0.1% SDS at 65°C before autoradiography. Complementary DNA clones for α (*18*), β, and γ subunits (*19*) were isolated from a library prepared from the mouse muscle cell line BC3H-1 using λgt11. Radioactive probes were made from cDNA inserts subcloned in the Eco RI site of M13 mpI8 in the orientation giving the message strand in the virus. Primed vectors were elongated with radioactive nucleotides and digested with a restriction enzyme which cut 300 to 600 bp from the 3′ end of each insert (Xmn I for α, Pst I for β, Bgl I for γ, and Pvu II for δ). Radioactive single-strand fragments (corresponding to the 3′ end of each insert) were isolated on conventional sequencing gels. None of the probes gave cross-hybridization with heterologous subunit restriction fragments.

presented in Table 1, and the linkage data are compiled in Table 2. We found perfect cosegregation (100%) of the γ- and δ-subunit genes; in contrast only 50% cosegregation occurred between the α- and β-subunit genes, 48% (or 46%) between the α- and γ- (or δ-) subunit genes, and 60% (or 61%) between the β- and γ- (or δ-) subunit genes. Thus, these results indicate that the γ- and δ-subunit genes map very closely on the same chromosome, whereas the α- and β-subunit genes are unlinked with the γ-δ locus.

To map the chromosomal sites of the four genes more precisely, we compared the segregation of each gene to the segregation in the same progeny of 25 previously mapped loci (*7*) distributed on 11 chromosomes.

represent the genes for the fast skeletal muscle isoforms of the myosin heavy chain and the myosin light chain, respectively (*7*). The segregation of the polymorphic markers *Idh-1* and *Glo-1* (*20*) was analyzed as described (*21*).

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
											Chromosome 17														
+	+	+	ND	−	+	−	−	+	−	−	+	−	−	−	+	+	+	−	−	+	−	ND	ND	ND	ND
−	−	+	−	+	+	−	+	+	+	−	−	−	−	+	+	+	−	+	+	+	−	−	+	−	+
−	−	−	+	+	+	−	+	−	+	−	−	−	−	−	+	+	−	+	+	+	−	−	+	−	+
											Chromosome 11														
−	+	+	−	+	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+
−	+	+	−	+	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+
											Chromosome 1														
+	−	−	ND	−	+	−	+	−	−	−	+	+	+	−	+	−	+	+	−	−	ND	ND	ND	ND	ND	
+	−	−	−	−	+	−	+	+	−	−	+	−	+	+	−	+	−	+	+	−	−	+	+	−	−	
+	−	−	−	−	+	−	+	+	−	−	+	−	+	+	−	+	−	+	+	−	−	+	+	−	−	
+	−	ND	−	−	+	−	+	+	−	−	+	−	+	+	−	+	−	+	+	−	−	+	+	−	−	

Such a backcross should cover half of the mouse genome. The α-subunit gene cosegregated with the α-cardiac actin gene (81%), which was previously assigned to chromosome 17 by Czosnek *et al.* (*8*), who used somatic cell hybrids with overlapping subsets of mouse chromosomes (Table 2). However, only 42% cosegregation occurred between the AChR α-subunit gene and the *Glo-1* marker, and 47% between the α-cardiac actin gene and *Glo-1*. The *Glo-1* marker has been genetically mapped on the proximal part of chromosome 17. In a backcross of this type, linkage should be detected for genes up to 30 centimorgans (cM) apart since the probability from a Gaussian distribution that two genes 30 cM apart would segregate independently by chance is less than 1% (*12*). Accordingly, the α-cardiac actin gene and the gene for the α subunit of AChR must be located in the very distal part of chromosome 17. Segregation of the β-subunit gene occurred in 100% of the offspring and is thus tightly linked with the locus encoding the different isoforms (embryonic, perinatal, and adult) of the myosin heavy chain genes, which has been localized on chromosome 11, near the *nude* locus (*13*). Finally, the γ- and δ-subunit genes showed 95% cosegregation with the gene of the fast skeletal muscle isoforms of myosin alkali light chain, $MLC1_F/MLC3_F$, which have been mapped about 2 cM from the *Idh-1* locus of the mouse (*7*). Since there are only 89% cosegregants between the *Idh-1*

Table 2. Analysis of the segregation of the AChR subunit genes and other known chromosomal markers in the backcross. Results are shown, in the lower left quadrant of the matrix, as cosegregants per total number of animals analyzed and, in its upper right quadrant, as percentages of cosegregants.

Locus	*Glo-1*	α Actin	α AChR	MHC_F	β AChR	*Idh-1*	MLC_F	γ AChR	δ AChR
Glo-1		47	42	50	50	39	44	44	46
α-Cardiac actin	17/36		81	60	60	51	57	52	54
α-Chain AChR	15/36	34/42		50	50	54	52	48	46
MHC_F	18/36	25/42	21/42		100	57	55	60	61
β-Chain AChR	18/36	25/42	21/42	42/42		57	55	60	61
Idh-1	14/36	19/37	20/37	21/37	21/37		95	89	89
$MLC1_F/MLC3_F$	16/36	24/42	22/42	23/42	23/42	35/37		95	95
γ-Chain AChR	16/36	22/42	20/42	25/42	25/42	33/37	40/42		100
δ-Chain AChR	16/35	22/41	19/41	25/41	25/41	32/36	39/41	41/41	

locus and the γ/δ-subunit genes, the three loci must be arranged in the following order: γ/δ-subunit genes—$MLC1_F/MLC3_F$ genes—*Idh-1*.

Our results are thus consistent with a distribution of the genes coding for the four AChR subunits on three different chromosomes in the mouse. The γ- and δ-subunit genes, which are linked on the same chromosome, are also the most homologous [57% at the amino acid level, compared to 36 to 43% for any other pair of the four genes in *Torpedo marmorata* (*4*)]. Either the γ/δ-subunit gene duplication took place later in evolution compared to the duplication of the α/β subunit, or, as in the case of the immunoglobulin genes, the close proximity of the two genes have maintained their high sequence homology via gene conversion (*14, 15*). However, evidence for this last possibility would require more extensive nucleotide sequence comparisons, in particular between allelic forms of the two genes. The partial dispersion of the four AChR subunit genes suggests that their expression is regulated by *trans*-activating factors rather than by a common *cis* regulatory mechanism. However, it is interesting that the genes for all four subunits of the AChR cosegregate with genes for contractile proteins that are expressed coordinately with them at the onset of myotube formation. Yet, the genetic distances between α-cardiac actin and AChR α-subunit genes (20 cM) and between $MLC1_F/MLC3_F$ and γ/δ-subunit genes (5 cM) correspond to considerable lengths of the DNA molecule that are not likely to form chromatin domains (*16*) responsible for the coordinate activation of these genes during myogenesis.

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22. Supported by grants from the National Institutes of Health, the Muscular Dystrophy Association of America, the Ministère de la Recherche et de la Technologie, the Collège de France, and the Centre National de la Recherche Scientifique. We are grateful to N. Davidson for providing the 6H fragment of the BC3H-1 δ subunit. A.B. was supported by a fellowship from Consejo Nacional de Ciencia y Technologia from Venezuela.

4 June 1986; accepted 30 October 1986

Flow Control Among Microvessels Coordinated by Intercellular Conduction

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Optimal distribution of blood flow requires coordination of vasodilation among resistance vessels. During hyperemia, blood vessels dilate upstream from the initiating stimulus. Spreading vasodilation independent of flow changes has not been previously demonstrated. In the present study, iontophoresis of acetylcholine adjacent to single hamster cheek pouch arterioles in situ (diameter, 20 to 37 micrometers) induced a rapid bidirectional dilation that was not attenuated when blood flow was eliminated with vascular occlusion. This finding indicates that a vasodilatory stimulus is conducted along the arteriole and demonstrates the existence of a mechanism of intercellular communication that is capable of coordinating diameter changes among resistance vessels.

VASODILATION MUST BE COORDINATED among various segments of the peripheral vasculature in order to accurately match blood flow with cellular metabolic demand. Coordination of vasodilation between feed arteries and microvessels is required because there is a substantial pressure drop across the small arteries (100 to 300 μm in diameter) feeding the microcirculation of many tissues (*1*). Thus both feed arteries and arterioles must dilate to achieve the observed increases in blood flow (*1*). An increase in vascular conductance initiated in arterioles intimately associated with the parenchymal cells can induce a complementary dilation of the feed arteries upstream through a flow-dependent mechanism (*2*), apparently mediated by an increase in shear stress acting on the endothelium (*3*).

Within the arteriolar network (vessel diameters less than 60 μm), resistance is also partitioned among and between vessel segments, and a spreading vasodilation has been observed in these vessels (*4, 5*). Whether this spreading dilation is dependent on flow changes or on some other form of conducted response is unknown. Since electrotonic conduction occurs in both isolated endothelial cells and vascular smooth muscle (*6, 7*), we examined the possibility that the coordination of vasodilation within arterioles is due to some form of intercellular communication. We report that acetylcholine (ACh) triggers an arteriolar dilation that is conducted along the vessel wall and is independent of blood flow.

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