standard protocols from an Mbo I partial digest human genomic library in EMBL3, provided by S. Orkin. Clone λHV-1 was isolated from a complete Bam HI digest library of H.V. DNA in EMBL3 and packaged with Gigapack plus (Stratagene). The λHBgl-1 clone was isolated from a Bgl II complete digest library of H.V. DNA in EMBL3 prepared without phosphatase treatment or size selection of the insert to ensure clonability of the 8-kbp rearranged fragment.
14. The segregation of pHV-Sac3 was completely concordant with that of chromosome 4 in 15 hybrids. Discordancy fractions for the other chromosomes ranged from 0.21 to 0.66.
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The Molecular Basis of Muscular Dystrophy in the *mdx* Mouse: A Point Mutation

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The *mdx* mouse is an X-linked myopathic mutant, an animal model for human Duchenne muscular dystrophy. In both mouse and man the mutations lie within the dystrophin gene, but the phenotypic differences of the disease in the two species confer much interest on the molecular basis of the *mdx* mutation. The complementary DNA for mouse dystrophin has been cloned, and the sequence has been used in the polymerase chain reaction to amplify normal and *mdx* dystrophin transcripts in the area of the *mdx* mutation. Sequence analysis of the amplification products showed that the *mdx* mouse has a single base substitution within an exon, which causes premature termination of the polypeptide chain.

THE *mdx* MOUSE WAS FIRST RECOGnized as a glycolytic X-linked mutant with myofiber necrosis and was proposed as a possible animal model for the X-linked human disease of dystrophin deficiency, Duchenne muscular dystrophy (DMD) (*1*). The absence of dystrophin (*2*) and reduced dystrophin RNA levels (*3*) later noted in the *mdx* mouse supported this concept, but the successful muscle fiber regeneration and reduced endomysial fibrosis (*4*) are in marked contrast to the DMD phenotype, casting doubt on its genetic homology (*5*). However, mapping of cDNA clones encoding the entire human DMD transcript showed that the *mdx* mutation is indeed located within the mouse dystrophin gene, which is the homologue of the human DMD locus (*6*). DMD has one of the highest mutation rates known (*7*), and more than 50% of affected males are known to have deletions (*8*).

To characterize the mutation in the inbred *mdx* mouse, we narrowed the limits of the mouse DNA sequence where the mutation had been predicted to be by human dystrophin cDNA probes (*6*). An adult mouse muscle cDNA library, constructed in the vector λgt10 with random primers, was screened with cloned cDNA sequences encoding human dystrophin to provide cDNA clones that encompassed almost the entire coding region of the mouse dystrophin transcript. Mouse clones spanning the known region of the *mdx* mutation were thus isolated and identified by DNA sequencing and by comparison with mouse (*9*) and human (*10*) dystrophin cDNA sequences (Fig. 1). These clones were used to probe Southern blots of Taq I–digested genomic DNA from three animals that showed recombination within the mouse dystrophin gene in a large interspecific mouse pedigree that contained the *mdx* mutation (*6*). By this method, we were able to identify by recombination analysis an even smaller region in which the mutation must lie.

The recombinational breakpoint in the 5′ end of the gene was found to lie between nucleotides 2083 and 3637 [numbers throughout correspond to those of the human cDNA sequence (*10*)] by using mouse clones MAD 18, MAD 19-2, and MAD 21 (Fig. 1). The breakpoint in the 3′ end of the gene was found to lie between nucleotides 6400 and 6783 by using overlapping fragments derived from clones MAD 25, MAD 26, MAD 28, MAD 29, and MAD 36-1 (not shown). Thus, the *mdx* mutation must lie between nucleotides 2083 and 6783. There was no evidence of any difference between normal and *mdx* DNA on Southern blots with any of the probes spanning this region, which indicated that a large insertion or deletion was not present.

Because of the low steady-state amounts of dystrophin transcript in normal muscle (*9*) and the still lower levels in *mdx* muscle (*3*), the polymerase chain reaction (PCR) (*11*) was applied to normal and *mdx* cDNA to amplify the region of the mutation. This 4.7-kb interval was amplified in six overlapping steps. We designed synthetic oligonucleotides (24 to 30 bases in length) on the basis of the normal mouse sequence, and we used these with total RNA in first-strand cDNA synthesis and subsequent PCR amplification. DNA blot analysis of the entire 4.7-kb interval showed no obvious difference in the size of the amplification products of *mdx* and normal mouse RNA (Fig. 2). However, DNA sequence analysis of normal and *mdx* cDNAs revealed a single base substitution in *mdx* mice when compared to normal animals. In *mdx* mice, a cytosine is

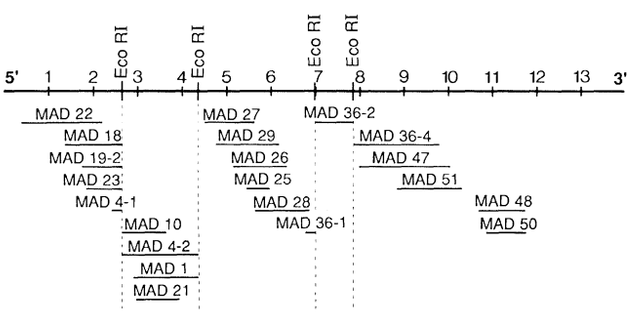

Fig. 1. Map of mouse dystrophin cDNA clones. The positions of cloned mouse dystrophin cDNA sequences are indicated. The entire coding region shown was cloned from a single library. Total RNA was isolated from normal leg muscle from 14-week-old C57B1/10 mice with guanidinium isothiocyanate (*19*), and polyadenylated RNA was selected (*20*). From this RNA, a size-selected random-primed cDNA library was constructed in λgt10, originally comprising approximately 2×10^6 independent recombinants. The library was plated and screened (*21*) with pCa1A (*22*) and with human cDNA probes 67, 73, 75, and 77 [which correspond to fragments 1a-2, 5b-7, 8, and 9-14, respectively (*8*)]. Between 3 and 20 positive clones were obtained from a screen of 500,000 unamplified recombinant phage. Inserts were subcloned into M13 vectors, sequenced by the dideoxy chain termination method (*23*), and positioned after comparison with the published sequences (*9, 10*).

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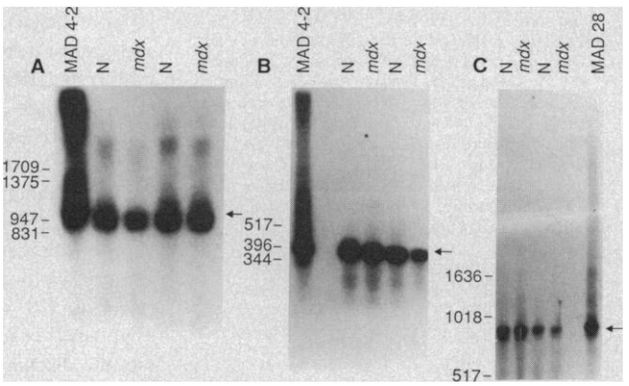

Fig. 2. PCR amplification of normal and *mdx* dystrophin transcripts. Amplified products of normal (N) and *mdx* dystrophin mRNA were separated on agarose gels and hybridized (*6*). (**A**) and (**B**) were probed with an Xba I–Pst I fragment of MAD 1 (position relative to published cDNA sequence, nucleotides 3748 to 4099); (**C**) was probed with a Bgl II fragment of human cDNA 73 (6295 to 6509). Amplification of cDNA clones MAD 4-2, MAD 4-2, and MAD 28 included as controls in (A), (B), and (C), respectively. Size standards are indicated in base pairs. Total RNA from muscle was prepared with guanidinium isothiocyanate (*21*) and reverse transcribed into first-strand cDNA with synthetic oligonucleotide primers P2, P5, and H4 and avian myeloblastosis virus reverse transcriptase. Second-strand synthesis, 35 cycles (*11*), on single-stranded cDNA with three different pairs of oligonucleotide primers: (A) B1 (3060 to 3083) and P2 (complementary to 4096 to 4119); (B) X2 (3727 to 3754) and P2; (C) K4 (5835 to 5864) and H4 (complementary to 6763 to 6786). The remainder of the 4.7-kb interval was amplified with three other pairs of primers: P6 (1949 to 1975) was used with P2; X2 and P5 (complementary to 4996-5020; E7 (4952 to 4981) and B7 (complementary to 6044 to 6067). Amplification was performed with 5 μg of cDNA and 3 units of Taq polymerase (Cetus). Annealing was at 37°C or 45°C for 2 min, polymerization at 72°C for 3 to 9 min, and denaturation at 95°C for 2 min.

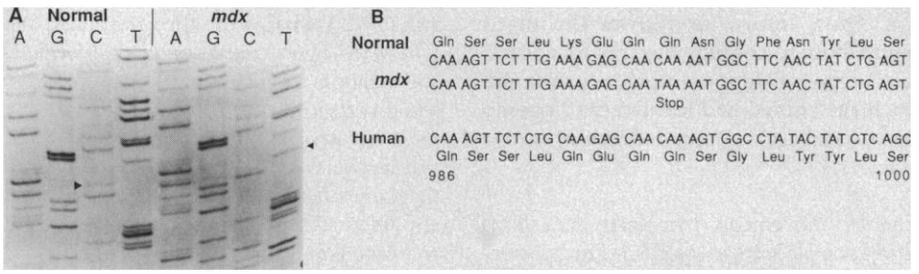

Fig. 3. (**A**) Sequence of the dystrophin cDNA from normal and *mdx* mice in the region of the mutation. The DNA sequence of part of the mouse dystrophin cDNA from inbred normal C57B1/10 and *mdx* C57B1/10 animals is shown. A single base transition (C→T) (arrowheads) is found in the *mdx* mouse when compared to the normal mouse. This change has been observed in all *mdx* animals tested ($n = 4$) and in different amplification reactions of the same *mdx* RNA. (**B**) The DNA sequences and the predicted amino acid sequences from normal and *mdx* mice and from normal humans in the region of the mutation. PCR amplification reaction products were separated by electrophoresis in an agarose gel, purified, digested with suitable restriction endonucleases (with natural or artificial restriction sites included within the oligonucleotide primers), and subcloned into M13 sequencing vectors. DNA sequence analysis was by the dideoxy chain termination method (*23*).

replaced by a thymine at nucleotide position 3185, resulting in a termination codon (TAA) in place of a glutamine codon (CAA) (Fig. 3). This change is the only one we have detected, and it has been consistently observed in separate PCR reactions on total RNA isolated from four *mdx* animals.

The molecular basis of the *mdx* mouse mutation has therefore been characterized and it is the first example of a point mutation in the dystrophin gene. The mutation in an exon to a stop codon results in premature termination of translation at 27% of the length of the dystrophin polypeptide. This would predict a truncated protein comprising the NH_2-terminal actin-binding domain and seven complete triple helical segments in the repeat domain of dystrophin (*9*). The protein would lack two-thirds of the repeat domain, the cysteine-rich and the COOH-terminal domains (*9*), and would have a relative molecular mass (M_r) of approximately 115,000. If stable, the protein should bind cytoskeletal elements and confer membrane stability, and thus could account for the benign pathology in the *mdx* mouse.

Since the cysteine-rich and COOH-terminal domains are more conserved than the actin-binding and repeat domains in human and chicken dystrophins (*12*), they are likely to have an important functional role, perhaps mediating the attachment of the protein to the cell membrane (*13*). Premature termination of translation could result in improper targeting of the truncated protein and, if not degraded, the protein could have a residual function with a beneficial role at the onset of muscle necrosis at 2 to 3 weeks of age in the *mdx* mouse (*4*).

Does, then, the truncated *mdx* dystrophin persist or is it immediately degraded? Polyclonal antibodies raised against part of the dystrophin protein that could be present in the truncated *mdx* polypeptide do not appear to recognize such a protein in *mdx* muscle (*2, 14, 15*). However, this question has not been settled, and Watkins *et al.* (*16*) mention that their high specificity antisera raised against the dystrophin protein cross-reacted strongly with components of the sarcoplasmic reticulum in *mdx* mice. This would be supportive evidence for persistence of a truncated species in *mdx* mice.

The reduced dystrophin steady-state mRNA level in *mdx* muscle (*3*) is probably a result of the premature termination codon. For example, nonsense mutations in the β-globin locus in man result in the absence of the gene product and a severe reduction in the amount of the corresponding transcript (*17*). This may be because mRNAs with nonsense mutations can be degraded in the nucleus (*18*).

The finding that a single base pair change in a DNA chain that is about 2 million base pairs in length can give rise to muscular dystrophy has implications for understanding of the human disease. Although about half of all DMD patients have deletions within the gene and a few have other rearrangements, many of the remainder may have point mutations. The nature of the mouse mutation could explain the high rate of new mutations associated with DMD. That is, the known frequency of mutation from cytosine to thymine, by methylation and deamination, and the preponderance of glutamine codons in the dystrophin gene (and other triplets that could easily mutate to a stop codon) provide a ready mechanism for new mutations.

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Spatial Selectivity of Rat Hippocampal Neurons: Dependence on Preparedness for Movement

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The mammalian hippocampal formation appears to play a major role in the generation of internal representations of spatial relationships. In rats, this role is reflected in the spatially selective discharge of hippocampal pyramidal cells. The principal metric for coding spatial relationships might be the organism's own movements in space, that is, the spatial relationship between two locations is coded in terms of the movements executed in getting from one to the other. Thus, information from the motor programming systems (or "motor set") may contribute to coding of spatial location by hippocampal neurons. Spatially selective discharge of hippocampal neurons was abolished under conditions of restraint in which the animal had learned that locomotion was impossible. Therefore, hippocampal neuronal activity may reflect the association of movements with their spatial consequences.

IN RATS, THE HIPPOCAMPAL FORMATION plays a major role in the encoding of spatial memory (*1*). Specifically, hippocampal pyramidal cells recorded in freely moving rats display both selectivity and memory for spatial location (*2*). In contrast, studies with restrained rabbits and primates emphasize hippocampal cellular involvement in associative learning (*3*), suggesting that neuron activity is related to learned stimulus-response contingencies (*4*). There is little sign of place-specific neuronal activation in such studies. However, the same cells that develop representations of conditioned responses also engage in spatial coding in extended environments (*5*), suggesting that the two types of activity may reflect processing of fundamentally similar kinds of information.

Two hippocampal cell types, complex spike (CS) and theta cells, can be identified electrophysiologically as pyramidal cells and interneurons, respectively (*6*). In the freely moving rat, both CS and theta cells discharge in phase with the rhythmic (theta) electroencephalogram (EEG) that accompanies orienting or translational movements, that is, movements that carry the animal from one place to another (*7*). Furthermore, the responsiveness of CS cells to spatial location is modulated by the velocity and direction of movement (*8*), indicating a possible influence of "motor set" (*9*).

We investigated the possible role of motor set for location-specific discharge activity in freely moving and restrained rats. Four animals were trained to tolerate restraint, which was implemented by snugly wrapping the body and limbs in a towel fastened with clips. This procedure allowed the animal to observe the environment by head movement and exploratory myostatial sniffing, while inhibiting attempts at displacement movements. Single units were isolated and recorded with "stereotrodes" (*10*) mounted in miniature manipulators. The manipulators were permanently implanted over the CA1 region of the hippocampus in rats under pentobarbital anesthesia. Several weeks after surgery, well-differentiated units were identified as CS or theta cells according to established criteria. In unrestrained, or free, rats the discharge specificity of the cells was repeatedly tested by manually transporting the animal alternately to an identified place field for 5 s, then to a neutral location for 5 s (*11*) (Fig. 1). The short interval between each transportation ensured that animals maintained an alert behavioral state. In some cases animals were left in one location long enough for us to examine unit activity during the large amplitude, irregular EEG activity (LIA) (voluntary prolonged immobility), which replaces the theta state after cessation of orienting or translational behaviors (*12*). Animals were then restrained and cells were again tested for place specificity and relation to EEG (Fig. 1). A recovery session was included to ensure the recording integrity. Behavior during the free, restraint, and recovery conditions was very similar in that animals engaged in head movements and myostatial sniffing. During free and recovery conditions animals also extended their limbs in anticipation of contact with the tabletop and made small shifts of posture. However, they were not actually locomoting during the 5-s sampling epochs.

Of 66 units recorded, 12 were classified as theta cells. The remaining 54 were identified as CS cells, of which 38 (70%) exhibited place specificity in the experimental environment. Thirty-one of these cells could be monitored during the conditions of free, restraint, and recovery. There was an almost

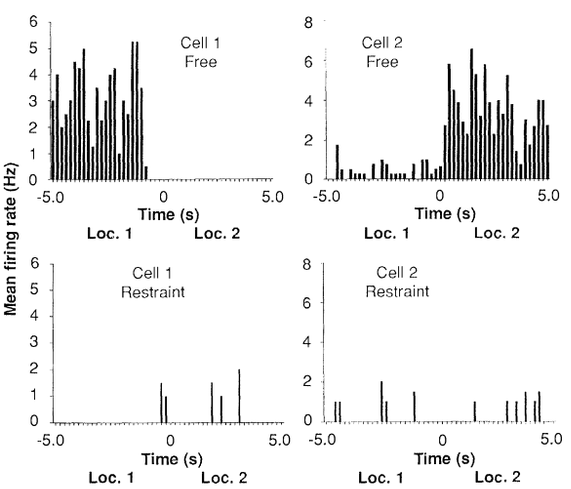

Fig. 1. Time histograms of two simultaneously recorded CS cells (cell 1 and cell 2) from one animal as it was manually transported from the place field of one cell (Loc. 1) into the place field of the other cell (Loc. 2) during the free (top panels) and restraint (lower panels) conditions; each place field thus served as a neutral location for the other place field (*11*). Spatially selective firing was abolished under the restraint condition.

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