erated by the CA1 circuitry.

The high-frequency population oscillation (200 Hz) indicates precise temporal coherence of pyramidal cell firing. CA1 pyramidal neurons and interneurons are excited by the CA3 Schaffer collaterals during hippocampal sharp waves (9). We hypothesize that transient but powerful depolarization of the excited interneurons results in high-frequency firing of these cells which, in turn, determine the precise timing of pyramidal cell discharges. Inhibitory neurons may not cause the population oscillation but may control the timing of the action potentials of pyramidal cells in the background of an excitatory CA3 barrage. Feedforward and recurrent excitation of inhibitory cells, as well as specific membrane conductances in interneurons, are required for the generation of the population oscillation in the neocortex and may be operative in the CA1 region as well (*12*). When sufficiently activated, the CA1 network shifts into a fast, transient rhythmic activity, the frequency of which is determined by the time constants of the membrane conductances of the interneurons.

The remote populations of CA1 pyramidal cells with extremely sparse excitatory collaterals (*13*) became synchronized without a time lag. Spatial synchrony in this region may be brought about by interactive interneurons or by mutual effects of pyramidal cells (*14*). Fast entrainment of inhibitory interneurons at the observed frequency may be possible by mutual excitation (*15*) or by communication through interneuronal gap junctions (*16*). Synchronization of pyramidal cells in the absence of excitatory collaterals may be brought about by ephaptic effects (*17*). The voltage gradient across the pyramidal cell layer during the emergence of field oscillations will affect voltage-dependent transmembrane currents and increase coherent firing of pyramidal cells.

The coherent bursts of CA1 pyramidal cells can produce very large transient depolarizations on their postsynaptic targets in retrohippocampal areas. Transient postsynaptic depolarization is a prerequisite of long-term synaptic modification (*18*). Neuronal bursts associated with theta rhythm have been hypothesized to be a physiological candidate of long-term synaptic potentiation (*19*). The powerful synchrony of pyramidal cells during hippocampal sharp waves offers an alternative mechanism to theta-related bursting. We hypothesize that synaptic modification in hippocampal target structures is induced during population oscillatory activity of the CA1 pyramidal cells.

REFERENCES AND NOTES

1. J. O'Keefe and L. Nadel, *The Hippocampus as a Cognitive Map* (Clarendon, Oxford, 1978).
2. M. Baudry and J. L. Davis, Eds., *Long-Term Potentiation: A Debate of Current Issues* (MIT Press, Cambridge, MA, 1991).
3. G. Gerstein, D. H. Perkel, J. Dayhoff, *J. Neurosci.* **5**, 881 (1985); R. D. Traub, R. Miles, R. K. S. Wong, *Science* **243**, 1319 (1989); T. J. Sejnowski, C. Koch, P. S. Churchland, *ibid.* **241**, 1299 (1988).
4. G. J. Krüger and J. D. Becker, *Trends Neurosci.* **7**, 1001 (1991).
5. K. D. Wise and K. Najafi, *Science* **254**, 1335 (1991).
6. The rats were anesthetized with ketamine-xylazine. Four types of movable recording electrodes were chronically implanted [G. Buzsáki *et al.*, *J. Neurosci. Methods* **28**, 209 (1989)]: (i) tungsten microelectrodes (1- to 5-μm tips), (ii) arrays of eight tungsten microelectrodes separated by 300 μm, (iii) single shank silicon probes (15 μm thick by 30 μm wide) with five recording sites (5 by 15 μm each) and 100-μm recording site separation, and (iv) "comb" silicon electrodes with 24 recording sites. The vertical four recording sites (6 by 6 μm) were spaced 50 μm apart and the six shanks (100 μm wide at the base, narrowing to 15 μm at the tip) were 300 μm apart. In addition, two to four microwires (60 μm) were inserted into the left hippocampus with tips in the strata pyramidale or radiatum of CA1. A pair of stimulating electrodes was implanted into the right angular bundle.
7. Local field potentials and unit activity were recorded (wide band: 3 Hz to 8 kHz) on optical disks. We recorded up to 16 channels of data simultaneously, using a 10-kHz sampling rate on each channel and 12-bit resolution.
8. A K-means clustering algorithm was used for spike separation. The spikes were represented as points in a ten-dimensional feature-space, and the dissimilarity of their shapes was measured as the Euclidean distance between the corresponding points in that space. An iterative procedure was used to find the local minimum of the partition error, and the number of separate unit clusters was determined by variance ratios [D. H. Perkel, G. L. Gerstein, G. M. Moore, *Biophys. J.* **7**, 327 (1967)]. The negative peaks of the fast field oscillations were detected, and the resulting pulses served as reference signals for the construction of cross-correlograms. Power spectra, phase spectra, and coherence spectra were computed between 3 and 250 Hz.
9. G. Buzsáki, L. Leung, C. H. Vanderwolf, *Brain Res. Rev.* **6**, 139 (1983), G. Buzsáki, *Brain Res.* **398**, 242 (1986), *Neuroscience* **31**, 551 (1989).
10. S. S. Suzuki and G. K. Smith, *Electroencephal. Clin. Neurophysiol.* **67**, 438 (1987).
11. Bursts of spikes with decreasing amplitude (complex spikes) unequivocally identify pyramidal cells in the CA1 region [S. E. Fox and J. B. Ranck, Jr., *Exp. Brain Res.* **41**, 299 (1981), G. Buzsáki and E. Eidelberg, *J. Neurophysiol.* **48**, 597 (1982)] Interneurons responded with repetitive discharges to stimulation, and their spontaneous activity was always faster than 10 Hz.
12. R. R. Llinás, *Science* **242**, 1654 (1988). J. Lacaille and S. Williams, *Neuroscience* **36**, 349 (1990); W. W. Lytton and T. J. Sejnowski, *J. Neurophysiol.* **66**, 1059 (1991); P. C. Bush and R. J. Douglas, *Neural Comput.* **3**, 19 (1991).
13. E. P. Christian and F. E. Dudek, *J. Neurophysiol.* **59**, 110 (1988), D. M. Finch, N. L. Nowlin, T. L. Babb, *Brain Res.* **271**, 201 (1983), N. Tamamaki, K. Abe, Y. Nojyo, *Brain Res.* **452**, 255 (1988).
14. M. Kuperstein, H. Eichenbaum, T. VanDeMark, *Exp. Brain Res.* **61**, 438 (1986).
15. H. B. Michelson and R. K. S. Wong, *Science* **253**, 1420 (1991).
16. H. Katsumaru, T. Kosaka, C. W. Heizman, K. Hama, *Exp. Brain Res.* **72**, 363 (1988).
17. R. Traub, E. Dudek, R. W. Snow, W. D. Knowles, *Neuroscience* **15**, 947 (1985); J. G. R. Jefferys and H. L. Haas, *Nature* **300**, 448 (1982), C. P. Taylor and F. E. Dudek, *Science* **218**, 810 (1982).
18. G. L. Collingridge, S. J. Kehl, H. McLennan, *J. Physiol.* **334**, 33 (1983); T. V. P. Bliss and T. Lomo, *ibid.* **232**, 331 (1973).
19. J. Larson and G. Lynch, *Science* **232**, 985 (1986); G. M. Rose and T. V. Dunviddie, *Neurosci. Lett.* **69**, 244 (1986), C. Pavlides, Y. J. Greenstein, M. Grudman, J. Winson, *Brain Res.* **439**, 383 (1988), T Otto, H. Eichenbaum, S. I. Wiener, C. G. Wible, *Hippocampus* **1**, 181 (1991).

20. Supported by the National Institutes of Health (grants NS27058 and 02383), the Human Frontier Science Program, and the Whitehall Foundation. We thank J. B. Ranck, Jr., T. J. Sejnowski, R. D. Traub, D. W. Tank, and R. K. S. Wong for helpful discussions.

17 January 1992, accepted 26 March 1992

Acquisition of Myogenic Specificity by Replacement of Three Amino Acid Residues from MyoD into E12

Robert L. Davis and Harold Weintraub*

The basic helix-loop-helix (bHLH) protein MyoD is a transcription factor that is important for the induction of the myogenic phenotype. The DNA binding basic region (13 amino acids) is necessary for recognition of the consensus MyoD binding site, for transcriptional activation, and for conversion of fibroblasts to muscle. In contrast, the non-tissue–specific bHLH protein E12 can bind to the MyoD binding site but does not induce myogenesis. Here, it is shown that only two amino acids in the MyoD basic region and a single amino acid from the junction, which separates the basic region and helix 1, are sufficient for myogenic specificity when substituted into the corresponding region of E12. These findings suggest that the recognition of particular determinants in the basic region is required for conversion of fibroblasts to muscle.

Expression of MyoD in a large number of cell types leads to their conversion to muscle (*1*). Only 68 amino acids of MyoD, consisting of the bHLH domain, are necessary and sufficient for myogenic conversion of stably transfected 10T½ fibroblasts (*2*). MyoD is a muscle-specific transcription factor that binds to most muscle-specific enhancer sequences, through specific CANNTG motifs (N = specific subsets of nucleotides), to activate muscle-specific gene expression (*3*).

Howard Hughes Medical Institute Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

*To whom correspondence should be addressed.

Studies with related bHLH proteins and MyoD mutants that bind normally to DNA but fail to activate transcription indicate specific DNA binding is an event separable from subsequent transcriptional activation and that the basic region of MyoD is required not only for DNA binding but also for muscle-specific gene activation (*4–6*).

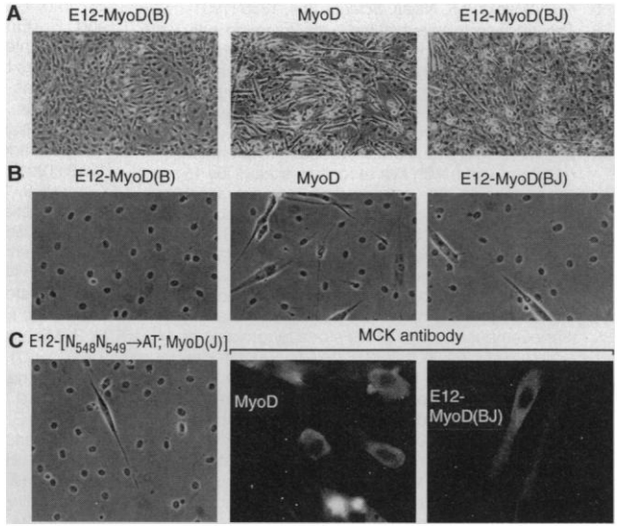

Fig. 1. Myogenic conversion with chimeric MyoD-E12 proteins. NIH 3T3 fibroblasts were transfected with expression vectors for E12-MyoD(B), wild-type MyoD, and E12-MyoD(BJ) and assayed 3 days later with phase-contrast microscopy at (**A**) low power (×50, high density cultures) or (**B**) high power (×200, low density cultures). In (**C**), E12 [$N^{548}N^{549} \rightarrow$ AT; MyoD(J)] is assayed similarly (left). In the two right-hand panels of (C), cultures were histologically stained with an antibody to creatine kinase for indirect immunofluorescence microscopy (×400). Because exposure time for the E12-MyoD(BJ) culture was three times that used for the MyoD culture, intensities of staining in the photos are comparable. Similar results were obtained with transfected 10T½ fibroblasts as before.

For example, the bHLH protein E12 (one of the differentially spliced products of the non-tissue–specific *E2A* gene) fails to support myogenesis on its own, although it too can bind to MyoD binding sites. With MyoD, E12 forms a hetero-oligomer that is a major species responsible for muscle-specific gene activation and myogenesis (*3, 7*). Similarly, a mutant MyoD protein containing the basic region of E12 also fails to support myogenesis even though this chimeric molecule (MyoD-E12Basic) dimerizes normally with wild-type E12 and binds well to MyoD binding sites (*4, 5*). Back mutation of only two residues of MyoD-E12Basic to corresponding amino acids in the basic region of MyoD ($N^{114} \rightarrow$ A; $N^{115} \rightarrow$ T) restores myogenic potential (*5, 8*). These two residues are conserved in all myogenic regulatory genes. It is thought that activation occurs when a buried, NH_2-terminal MyoD activation domain becomes released and that this is mediated by the specific interaction of the basic region to its cognate DNA binding site (*5*).

Given the evidence demonstrating that the MyoD basic region was required for myogenesis, we tested whether the MyoD basic region was also sufficient for myogenesis by asking if the MyoD basic region can activate myogenesis in the context of E12. A chimeric E12 that contained the MyoD

Table 1. Summary of DNA binding, transactivation, and myogenic conversion activities of MyoD and E12. DNA binding of homo- and hetero-oligmers is shown as approximate relative affinity for the right and left MyoD binding sites of the MCK enhancer. DNA binding activity was measured as described below (*22*). For DNA binding, 100% is defined as the affinity of a hetero-oligomer of wild-type MyoD and wild-type E12 proteins for the right site (line 2). The "+++" represents 60 to 100% of maximal binding; the "++" 20 to 60% maximal binding; the "+" 10 to 20% maximal binding; the "±" 5 to 10% maximal binding; and the "−" no detectable binding under the conditions described here. Transactivation of the CAT reporter gene [4R-TK CAT (4R)] transiently cotransfected with the indicated MyoD and E12 expression vectors into 10T½ fibroblasts was as described (*5*). CAT enzyme activity was normalized to that of the EMSVscribe expression vector alone, which was set to one. We measured muscle conversion by observation of morphology with phase contrast microscopy and by immunohistochemical staining for muscle-specific gene products [myosin heavy chain and MGK; (*2*)]. The "++++" represents 90 to 100% of that obtained with wild-type MyoD; the "+++" 50 to 90% of that obtained with wild-type MyoD; the "++" 30 to 50% of that obtained with wild-type MyoD; the "+" 5 to 30% of that obtained with wild-type MyoD: the "±" 5% of that obtained with wild-type MyoD, but was still detectable; and the "−" no detectable myogenic conversion. Each transfection result is an average of three to five independent experiments for both transactivation and myogenic conversion assays. The SVE2-5 and E47 expression vectors are described in (*20*) and (*21*), respectively. E12MyoD(BJ)ΔN is an NH_2-terminal deletion of E12 sequences, lacking the activation domain, with the E12 sequence beginning at amino acid 217.

Protein	DNA binding		Transactivation (fold activation)	Muscle conversion
	Right CACCTG	Left CATGTG		
MyoD	±	−	750	++++
MyoD + E12	+++	+		+++
MyoD + E2-5	+++	±	1300	++++
MyoD + E12-MyoD(B)	+++	++	10	++
MyoD + E12-MyoD(BJ)	++	++	680	+++
MyoD + MyoD-E12(B)	ND*	ND	130	++
MyoD + MyoD-E12(BJ)	ND	ND	350	+++
E2-5	+	±	3	−
E12	+	±	210	−
E47	ND	ND	180	−
E12-MyoD(B)	++	++	210	−
E12-MyoD(B) + E12	++	++	ND	−
E12-MyoD(B) + E2-5	+++	+++	200	−
E12-MyoD(B) + MyoD-E12(B)	+	++	28	−
E12-MyoD(B) + MyoD-E12(BJ)	+	+	24	−
E12-MyoD(BJ)	++	++	390	++
E12-MyoD(BJ) + E12	+++	+++	ND	++
E12-MyoD(BJ) + E2-5	+++	++	310	++
E12-MyoD(BJ) + MyoD-E12(B)	+	+	26	+
E12-MyoD(BJ) + MyoD-E12(BJ)	+++	+	360	++
E12-MyoD(BJ)ΔN	++	++	3	−
E12-MyoD(BJ)ΔN + E2-5	+++	+++	8	−
MyoD-E12(B)	±	−	4	−
MyoD-E12(B) + E12	++	+	ND	−
MyoD-E12(B) + E2-5	+	±	6	−
MyoD-E12(BJ)	±	−	140	−
MyoD-E12(BJ) + E12	++	±	ND	−
MyoD-E12(BJ) + E2-5	+	±	120	−

*ND = not determined.

Protein	DNA binding		Transactivation (fold activation)	Muscle conversion
	Alone	With E12		
MyoD	±	+++	1290	++++
E12-MyoD(BJ)	++	+++	360	++
E12[$N^{548}N^{549}$ → AT]	±	+	86	−
E12-MyoD(J)	±	+	3	−
E12[$N^{548}N^{549}$ → AT; MyoD(J)]	+	+	81	+
E12[$N^{548}N^{549}$ → AT; D^{558} → K]	+	+	220	+
E12[N^{548} → A; D^{558} → K]	+	+	31	−
E12[N^{549} → T; D^{558} → K]	+	+	22	−
MyoD	±	+++	590	++++
MyoD(K^{124} → D)	±	+	55	±
MyoD(A^{114} → I)	±	++	2	−
MyoD(A^{114} → D)	±	++	6	−
MyoD(A^{114} → K)	±	+	1	−
MyoD(A^{114} → V)	±	++	1	−
MyoD(A^{114} → T)	±	++	18	−

Table 2. Summary of DNA binding, transactivation, and myogenic conversion activities of wild-type MyoD, and various MyoD and E12 mutants. Experimental conditions are the same as those described in Table 1. The site used in the DNA binding assays was the right MyoD binding site of the MCK enhancer. Plus and minus signs are as in the Table 1 legend. E12-MyoD(J), MyoD (A → T), MyoD (A → I), and E12-MyoD(BJ)ΔN (Table 1) all act as dominant negative inhibitors of myogenesis and transactivation when cotransfected, at a two-to-one ratio with MyoD, into 3T3 cells, suggesting that they are efficiently expressed in these cells. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

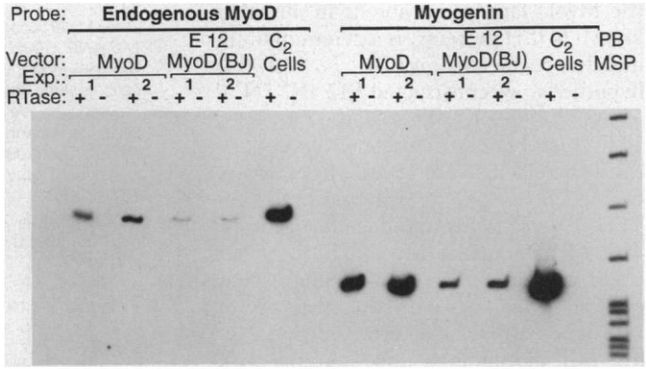

Fig. 2. Activation of endogenous MyoD and myogenin by E12-MyoD(BJ). Total RNA from 10T½ fibroblasts transiently transfected with MyoD or E12-MyoD(BJ) expression vectors was assayed by quantitative reverse-transcription polymerase chain reaction (RTPCR) as described (*19*), with the use of primers specific for endogenous mouse MyoD and myogenin. The figure shows results from two separate experiments. Positive controls were from C2C12 mouse myoblasts (C_2 cells) (*15*); negative controls included minus reverse transcriptase for RTPCR [RTase (−) lanes] and RNA from untransfected 10T½ cells as before. PB MSP represents Msp I–digested pBR322 size markers. The signals produced by the PCR reaction were in the linear range with 30 cycles used in the experiments shown. Similarly, all samples were normalized to the amount of signal obtained with an internal control PCR analysis of EF1α as before (*19*).

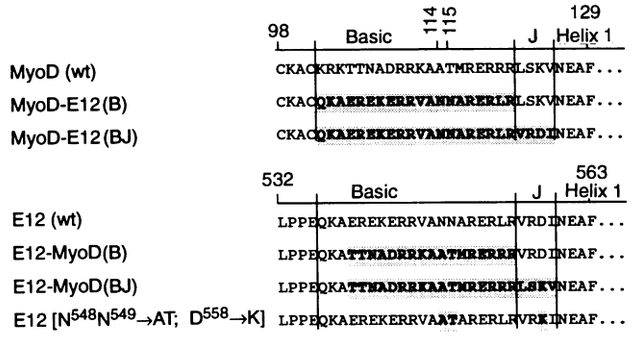

Fig. 3. Structure of chimeric MyoD and E12 proteins. The sequences of the basic and junction regions of wild-type and mutant MyoD and E12 are shown. Vertical lines indicate the borders (arbitrary) of the basic region, the junction [the first four residues of helix 1 as designated in (*4*)], and helix 1. Sequences altered in the mutant proteins are in bold. For the chimeric proteins, the letters in parentheses [(B), basic region; (J), junction] indicated the domains of the second protein that were substituted into the first protein listed. [For example, MyoD-E12(B) has the basic region of E12 inserted into MyoD in place of the MyoD basic region]. The amino acid numbers for the sequence shown are based on the mouse MyoD sequence (*15*) and the human E12 sequence (*16, 17*). Oligonucleotide-directed mutagenesis of both MyoD and E12 was performed as described (*4*). Although the domain swaps shown there involved a total of 20 amino acids and 17 amino acids of basic region substituted for those of MyoD and E12, respectively, previous results suggest that just the 13 amino acids adjacent to the junction are most critical for myogenesis (*4, 5*). A partial human E12 cDNA from pE12R was digested with Hind III and Eco RI, rendered blunt-ended, and cloned into the cDNA expression vector pEMSVscribe. And then mutagenesis was induced to substitute MyoD sequences. We reconstructed a full-length human E12 cDNA from these plasmids, in pEMSVscribe, by substituting an Eco RI to Sfi I fragment of E12 from pBD13-2 for the 5′ blunt to 3′ Sfi I fragment of E12 from pE12R, with regeneration of the 5′ Eco RI site.

basic region [E12-MyoD(B)] failed to activate myogenesis in transfected 10T½ cells but was able to activate a reporter gene, 4R-TK CAT, that contained four MyoD binding sites driving a minimal promoter choline acetyl transferase (CAT) gene fusion (Table 1). This substitution also enhanced the in vitro affinity of E12 binding to the MyoD binding sites in the muscle creatine kinase (MCK) enhancer by a factor of ~5 over wild-type E12 (*9, 10*).

Cotransfection of E12-MyoD(B) with its reciprocal partner MyoD-E12(B) failed to restore myogenic conversion. Similarly, cotransfection with an E12 expression vector or SVE2-5, an expression vector that expressed a differentially spliced E2A gene product similar to E12, also failed to restore myogenesis. Transfer of the basic region of myogenin into E12 or that of MyoD into rat achaete-scute also did not allow myogenesis (*8, 11*). Thus, although the basic region of MyoD is necessary for myogenesis, it is not sufficient when placed in another HLH protein framework.

Because the junction between the basic DNA binding domain and the HLH oligomerization domain is critical for myogenesis (*4*), we constructed an E12 mutant that contained the MyoD basic region and four adjacent residues (the junction) between the basic region of MyoD and helix 1 [E12-MyoD(BJ)]. This protein bound in vitro to MyoD DNA binding sites and when transiently transfected into 10T½ cells transactivated 4R-TK CAT (Table 1).

In contrast to E12-MyoD(B), E12-MyoD(BJ) converted either 10T½ or NIH 3T3 cells to muscle at about one-third to one-half the efficiency of wild-type MyoD (Fig. 1 and Table 1). These cells elongated, fused, and contained a typical serrated leading edge indicative of cells transfected with wild-type MyoD, although they were thinner and more spindly than cells converted by wild-type MyoD. In addition, they developed several prominent nucleoli, also

characteristic of myogenic conversion. Stable lines of myogenic cells could also be derived. Using immunofluorescence, we detected MCK and myosin heavy chain in these cells. When assayed 2 days after induction, the cells stained about one-third as intensely as cells transfected with wild-type MyoD; however, when assayed 5 days after induction, staining intensities were comparable. Analysis of RNA for endogenous MyoD and myogenin, which are inducible in 10T½ cells by exogenously supplied myogenic regulatory genes (*12*), showed induction with E12-MyoD(BJ) to be about one-third to one-fifth the amount of induction achieved with wild-type MyoD when the cells were assayed 2 days after induction. The basic region of MyoD was required in E12-MyoD(BJ) because E12-MyoD(J), which contains only the MyoD junction in an E12 background, failed to convert 10T½ cells to muscle (Table 2) (*13*). Increasing the amount of transfected E12-MyoD(BJ) plasmid failed to increase the efficiency of myogenesis, and cotransfection with SVE2-5 or E12 also failed. Cotransfection of E12-MyoD(BJ) with MyoD-E12(B) led to a decrease in myogenesis, whereas cotransfected of E12-MyoD(BJ) with MyoD-E12(BJ) showed neither synergism nor antagonism.

E12-MyoD(BJ) could be activating endogenous MyoD (*12*), which then activates the muscle program. However, this is not the case because we observed that E12-MyoD(BJ) activates myogenesis, transiently and in stable transformants, in NIH 3T3 cells (Fig. 1), which in contrast to 10T½ cells (Fig. 2) fail to express endogenous MyoD when transfected with a MyoD expression vector (*12*).

We conclude that only the 21 residues of MyoD that contain the basic region and the junction with helix 1 are sufficient to confer myogenic specificity to E12. The first four basic region residues are nonessential for myogenic conversion (*4*), and, of the 17 remaining residues, 6 in the basic region and all 4 in the junction differ between MyoD and E12 (Fig. 3).

Substitution of Ala^{114} of MyoD (Fig. 3) with I, D, T, K, or V yielded proteins that bound specifically to DNA but did not activate myogenesis or 4R-TK CAT (Table 2). Given these results and previous data (see above) pointing to $A^{114}T^{115}$ being important for myogenic activation, we constructed (E12-$N^{548}N^{549}$ → AT); however, the resulting protein was unable to convert 10T½ cells to muscle. In contrast, when the MyoD junction was included {E12 [$N^{548}N^{549}$ → AT; MyoD(J)]} (Fig. 3), muscle cell–specific activation occurred. When stably transfected into NIH 3T3 cells, E12 [$N^{548}N^{549}$ → AT; MyoD(J)] gave stable myogenic lines with no activation of the endogenous MyoD gene.

These results show that only two residues in the basic region of MyoD in combination with several adjacent residues at the junction with helix 1 are sufficient for myogenesis when placed into the framework of E12. The junction residues are VRDI in E12 and LSKV in MyoD (Fig. 3). Differences in the first and fourth position are conservative; moreover, the last position, like E12, contains an I in herculin-mrf4 and the second position, like E12, has an R in *Caenorhabditis elegans* MyoD and *Drosophila* nautilus. These observations suggest that the third position K, which is conserved in all members of the MyoD family and absent in all other known bHLH proteins, is a determinant of muscle specificity (Fig. 3). To test this hypothesis, we constructed E12 ($N^{548}N^{549}$ → AT; D^{558} → K). This vector converted 10T½ fibroblasts to muscle at about one-third the efficiency of MyoD. In contrast, E12 [N^{548} → A; D^{558} → K] and E[N^{549} → T; D^{558} → K] failed to induce myogenesis. The MyoD mutant MyoD (K^{124} → D) could convert 10T½ cells to muscle but only at about one-tenth the efficiency of wild-type MyoD, as determined by assaying cell morphology and staining with antibodies to MCK and myosin heavy chain (Table 2). Thus, the K in the junction of MyoD is not absolutely necessary for myogenesis, and it is likely that in the context of the entire MyoD basic region and junction other residues can contribute to specificity.

Together with previous experiments, the data presented here suggest that a recognition process (*5*) must focus on specific amino acid residues located in specific positions within the basic region and junction region of MyoD to control whether subsequent muscle gene activation is permissible. In addition, many muscle gene enhancers contain binding sites for multiple ubiquitously expressed transcription factors that presumably cooperate with MyoD in building a regulatory machine capable of activating muscle-specific transcription. Whatever the nature of this multiprotein complex, it is apparently plastic enough to accommodate much of the framework of E12, which is not obviously related to MyoD outside the bHLH domain, in place of that of MyoD.

REFERENCES AND NOTES

1. J. Choi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7988 (1990), H. Weintraub *et al.*, *ibid.* **86**, 5434 (1989).
2. S. J. Tapscott *et al.*, *Science* **242**, 405 (1988).
3. A. B. Lassar *et al.*, *Cell* **58**, 823 (1989); C. Murre *et al.*, *ibid.* **56**, 777 (1989), C. Murre *et al.*, *ibid.* **58**, 537 (1989); B. M. Wentworth, M. Donoghue, J C. Engert, E. B. Berglund, N. Rosenthal, *Proc. Natl. Acad. Sci U.S.A.* **88**, 1242 (1991).
4. R. L. Davis, P.-F. Cheng, A. B. Lassar, H. Weintraub, *Cell* **60**, 733 (1990).
5. H. Weintraub *et al.*, *Genes Dev.* **5**, 1377 (1991).
6. C. V Cabrera, A. Martinez-Arias, M. Bate, *Cell* **50**, 425 (1987), J. Campos-Ortega and E. Knust, *FEBS Lett.* **190**, 1 (1990); M. Caudy *et al.*, *ibid.* **55**, 1061 (1988); A Garcia-Bellido, *Genetics* **91**, 491 (1979), H. J. Muller, *Brookhaven Symp. Biol.* **8**, 126 (1955).
7. A. B. Lassar *et al.*, *Cell* **66**, 305 (1991).
8. T. J Brennan, T. Chakraborty, E. N. Olson, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5675 (1991)
9 T Chakraborty, T J. Brennan, L. Li, D. Edmondson, E. N. Olson, *Mol. Cell. Biol.* **11**, 3633 (1991).
10. X.-H. Sun and D. Baltimore, *Cell* **64**, 459 (1991).
11 J. Johnson, S. Birren, T. Saito, D. Anderson, *Proc. Natl. Acad. Sci U.S.A.*, in press.
12. M J. Thayer *et al.*, *Cell* **58**, 241 (1989), T. Braun, E Bober, G. Buschhausen-Denker, S. Kotz, K. Grzeschik, H. H. Arnold, *EMBO J.* **8**, 3617 (1989); J H. Miner and B. Wold, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1089 (1990)
13. Background for spontaneous conversion of 10T½ cells is less than 10^{-8}. A. B. Lassar, B. M. Paterson, H. Weintraub, *Cell* **47**, 649 (1986).
14. H L. Beckmann, L. K. Su, T. Kadesch, *Genes Dev.* **4**, 167 (1990).
15. R. L. Davis, H. Weintraub, A. B. Lassar, *Cell* **51**, 987 (1987).
16. J. Nourse *et al.*, *ibid.* **60**, 535 (1990).
17. M. P. Kamps, C. Murre, X.-H. Sun, D. Baltimore, *ibid.*, p. 547.
18. R Benezra, R. L Davis, D. Lockshon, D. L. Turner, H Weintraub, *ibid.* **61**, 49 (1990).
19. R. A. W Rupp and H. Weintraub, *ibid.* **65**, 927 (1991).
20. P. Henthorn, M Kiledjian, T. Kadesch, *Science* **247**, 467 (1990).
21. C. Murre, A. Voronova, D. Baltimore, *Mol. Cell. Biol.* **11**, 1156 (1991)
22. In vitro–translated MyoD and E12 proteins were generated and used in mobility-shift assays as described (*4*). Reticulocyte lysate (6 μl of RNA-programmed lysate, plus 6 μl of unprogrammed lysate or 6 μl of a different RNA-programmed lysate) was incubated for 20 min at 37°C and then added to 12 μl of 2× binding buffer [40 mM Hepes (pH 7.6), 40 mM KCl, 6 mM $MgCl_2$, 2 mM EDTA, 2 mM dithiothreitol (DTT)] that contained 0 2 ng of double-stranded DNA probe (assuming 100% annealing of single-stranded oligonucleotides, unlabeled oligonucleotide was in threefold excess) and 1 μl of polydI-dC. The labeled oligonucleotide of the double-stranded probe contained the sequence 5′-GATCCCCCCAACACCTGCTGCCTGA-3′, which has the right MyoD binding site of the MCK enhancer (underlined) (*4*). The labeled oligonucleotide 5′-ATTAACCCAGACATGTGGCTGCCCC-3′ contained left site of the MCK enhancer (underlined). Binding reactions were performed at room temperature for 15 min. Under these conditions, the probe was in excess over MyoD and E12, with E12 being in two- to threefold excess over MyoD (*4*). After binding, reactions were immediately loaded onto 5% polyacrylamide gels in 1× TBE (50 mM tris, 50 mM boric acid, 1 mM EDTA) and separated by electrophoresis at 20 V/cm for 2.5 hours. The gel was dried and subjected to autoradiography.

23. We thank M. Groudine, H. Eisen, and members of the Weintraub lab for comments on the manuscript, numerous colleagues for reagents and plasmids; P.-F Cheng for technical assistance, and H. Devitt for typing the manuscript. R.L.D. is an associate and H.W. is an investigator of the Howard Hughes Medical Institute.

24 January 1992; accepted 31 March 1992