Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 2182–2186, March 1990
Genetics

Rhabdomyosarcoma-associated locus and *MYOD1* are syntenic but separate loci on the short arm of human chromosome 11

(muscle-associated genes/mapping)

HEIDI J. SCRABLE*, DABNEY K. JOHNSON†, EUGENE M. RINCHIK†, AND WEBSTER K. CAVENEE*‡

*Ludwig Institute for Cancer Research, 687 Pine Avenue West, Montreal, PQ Canada H3A-1A1; and †Biology Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077

Communicated by Liane B. Russell, December 29, 1989 (received for review November 1, 1989)

ABSTRACT **The *MYOD1* locus is preferentially expressed in skeletal muscle and at higher levels in its related neoplasm, rhabdomyosarcoma. We have combined physical mapping of the human locus with meiotic and physical mapping in the mouse, together with synteny homologies between the two species, to compare the physical relationship between *MYOD1* and the genetically ascertained human rhabdomyosarcoma-associated locus. We have determined that the myogenic differentiation gene is tightly linked to the structural gene for the M (muscle) subunit of lactate dehydrogenase in band p15.4 on human chromosome 11 and close to the *p* and *Ldh-1* loci in the homologous region of mouse chromosome 7. Because the rhabdomyosarcoma locus maps to 11p15.5, *MYOD1* is very unlikely to be the primary site of alteration in these tumors. Further, these analyses identify two syntenic clusters of muscle-associated genes on the short arm of human chromosome 11, one in the region of rhabdomyosarcoma locus that includes *IGF2* and *TH* and the second the tightly linked *MYOD1* and *LDHA* loci, which have been evolutionarily conserved in homologous regions of both the mouse and the rat genomes.**

A major effort in contemporary cancer research is to identify loci that contribute to tumorigenesis only when both cellular alleles are inactivated (1). The paradigm of this approach is the childhood eye tumor, retinoblastoma. In this case, cytogenetic (2, 3) and molecular genetic (4) analyses targeted attention to the 13q14 region of the human genome and resulted in isolating a gene of aberrant (*i*) genomic structure (5), (*ii*) expressed transcript (6), or (*iii*) protein product (7) in tumors. Further, the introduction of a wild-type copy of the gene into tumors could suppress tumorigenecity (8).

Another tumor to which this approach may be extended is embryonal rhabdomyosarcoma, a pediatric tumor that resembles embryonic striated muscle and that may be clinically associated with the congenital malformation disorder known as the Beckwith–Wiedemann syndrome (BWS, refs. 9, 10). Some patients with BWS have constitutional cytogenetic alterations of chromosome 11 band p15 (11, 12), and subsequent genetic linkage studies indicated that predisposition to BWS resides in the same region (13, 14). This map location overlaps precisely with the smallest region of somatic reduction to homozygosity accomplished by mitotic recombination (11p15.5–pter) in embryonal rhabdomyosarcoma tumors (15). Thus, it appears that mutations that either cause or contribute to the appearance of generalized hyperplasia (BWS) or tissue-specific neoplasia (rhabdomyosarcoma) colocalize to this same limited region.

The striking histological resemblance of rhabdomyosarcoma to fetal striated muscle extends to the genes they express. Several genes have been isolated that appear to play pivotal roles in the establishment and maintenance of the myogenic phenotype. Two of these are *Myod-1* (16) and myogenin (17, 18), which are preferentially expressed in skeletal muscle in the mouse and are uniquely expressed in human rhabdomyomatous neoplasms (19). In most of these tumors, the abundance of corresponding RNA transcripts from both genes is considerably larger than at any stage of human fetal striated-muscle development so far analyzed. Thus, it was of interest to learn that one of these genes, *MYOD1*, mapped to human chromosome 11 (20).

We have recently suggested (21) that one step in the etiology of embryonal rhabdomyosarcoma may involve inactivation of loci on the short arm of chromosome 11, perhaps by gamete-specific modification. One consequence of such an inactivation process might be deregulation of expressed genes in this chromosomal region. This recognition raised the possibility that overexpression of the myogenic gene *MYOD1* and loss of function by homozygosis at the rhabdomyosarcoma locus (RdL) might be two aspects of the same step in tumorigenesis and that *MYOD1* and RdL might be the same locus. As a first test of this hypothesis, we sought to determine the physical relationship between the two genes by using a combination of physical mapping to human chromosomes coupled with meiotic and physical mapping in the mouse. We report here the identification of an evolutionarily conserved muscle-specific linkage group comprising *MYOD1* and the gene for the M (muscle) subunit of lactate hydrogenase (LDHA), which in humans is syntenic with, but distinct from, the RdL and discuss the possible implications of muscle-specific gene clusters on the same chromosome.

MATERIALS AND METHODS

Hybridization Probes. Human *MYOD1* was isolated from a cDNA library constructed from rhabdomyosarcoma RNA by hybridization at reduced stringency to the mouse Myod-1 cDNA provided by A. B. Lassar (16). The source RNA was extracted from an alveolar rhabdomyosarcoma that expresses myogenic differentiation protein (MYOD1) at a high level. The poly(A)$^+$ RNA was reverse-transcribed, ligated to synthetic *Eco*RI linkers, and inserted into the *Eco*RI site of λgt11. The 1.9 kilobase (kb) human MYOD1 cDNA was subcloned into the *Eco*RI polylinker site of the plasmid vector, Bluescript (Stratagene).

Data for marker loci on human chromosome 11 were obtained by using the following recombinant DNA probes: pINT800 (22), which reveals alleles of 2.6 and 1.0 kb in *Msp* I-digested DNA at the catalase locus in 11p13 (our results); pLDH-1, with *Thermus aquaticus* (*Taq*) I alleles of 2.3 and 1.9 kb at *LDHA* (23); p20.36, with *Pst* I alleles of 2.6 and (1.9

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BWS, Beckwith–Wiedemann syndrome; RFLP, restriction fragment length polymorphism; RdL, rhabdomyosarcoma-associated locus; MYOD1, myogenic differentiation 1 protein; cM, centimorgan.

‡To whom reprint requests should be addressed.

+ 0.7) kb at the *PTH* locus (24); and JW151, which gives *Hin*dIII restriction fragment length polymorphisms (RFLPs) at both the γ^G (8.0 and 7.2 kb) and γ^A (3.5 and 2.7 kb) loci in the β-globin cluster in 11p15.5 (25). Probe pADJ-762, an arbitrary DNA segment, reveals RFLPs at the *D11S12* locus in *Bcl* I-, *Msp* I-, and *Taq* I-digested DNA (26). Alleles with *Bcl* I are 11.6 and 4.2 kb. Alleles at the *HRAS1* locus were determined by hybridizing *Taq* I-digested DNA with pTBB2 (27) and at the insulin locus by hybridizing *Rsa* I-digested DNA with the probe pHins310 (28).

Informative RFLPs (*Eco*RI; 16 kb in *Mus musculus* and 13 kb in *Mus spretus* DNA) for the mouse lactate dehydrogenase A (*Ldh-1*) locus were followed in a backcross by use of a 1.7-kb *Eco*RI–*Hin*dIII fragment derived from the 5'-end of the pUCLD14 clone containing the *Ldh-1* gene (29).

Southern Blotting. DNA (5 μg) was size fractionated, transferred, and hybridized as described (19). Probes were prepared by random priming (30, 31).

Somatic-Cell Hybrid Mapping Panels. The cell hybrids designated with GM prefaces were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ); those designated with A_3 were from P. Pearson (University of Leiden); and those designated with WC came from this laboratory (32). Hybrids H126 (chromosome 11 only), H127 [del(11)p15.5→pter], and H128 [del(11)p13→p15.3] were a gift from C. H. Scoggin (University of Colorado) (33). NYX3.1, a mouse myeloma/human lymphoblastoid hybrid, with del(11)p13→p15.3, was a gift from E. van Heyningen (MRC Unit, Edinburgh) (34). 517B-D3-D2a, a mouse T-cell lymphoma/human 8511 leukemia cell hybrid carrying del(11)p13→pter, was a gift from C. M. Croce (Temple University) (35). Before use, deletion hybrids were typed at all available marker loci on chromosome 11 to confirm the presence or absence of corresponding DNA segments.

Mice. All mice were bred at Oak Ridge National Laboratory. For the linkage-backcross analysis, *p* c^{ch}/*p* c^{ch} (*p*, pink-eyed dilution; c^{ch}, chinchilla) females of the 129/R1 − *p* c^{ch}/*p c* segregating inbred strain were crossed to males of a closed-colony outbred *M. spretus* (+ +/+ +) stock. [Nuclei of a breeding colony of *M. spretus* (chromosomes denoted by *SPT* superscripts) were provided in 1986 by V. Chapman, Roswell Park Memorial Institute.] F_1 females were then crossed to 129/R1 − *p* c^{ch}/*p* c^{ch} males; each segregant from this cross was classified for *p* and c^{ch} phenotypes encoded by the chromosome 7 loci *p* and *c* (albino), and DNA was prepared by standard methods from its spleen and/or liver.

The recessive-lethal *p*-locus mutation Df(*ru-2 p*)^{46DFiOD} elicits pseudodominance of *ru-2* (ruby eye-2), and, therefore, is likely to be a chromosomal deletion of *p* that extends proximally at least 3 centimorgans (cMs) (36). This mutation has also been shown to be deleted for molecular probes, including those at the *Ldh-1* and *Saa* loci (29, 36). Df(*ru-2 p*)^{46DFiOD} was placed opposite a *M. spretus* chromosome 7 (for rapid RFLP analysis) by a progeny-testing protocol. Female *M. musculus* deletion carriers [+ +/Df(*ru-2 p*)^{46DFiOD}] were crossed to a wild-type *M. spretus* male. F_1 females that carried the *M. musculus*-derived *p* lethal, as opposed to the *M. musculus*-derived wild-type (+) balancer chromosome, were identified by a progeny-test cross to males of the outbred T stock (*a/a, b/b, p* c^{ch}/*p* c^{ch}, *d se /d se, s/s*). F_1 females that produced pink-eyed progeny were considered to carry a *M. spretus* chromosome opposite the lethal Df(*ru-2 p*)^{46DFiOD} mutation.

RESULTS

The *MYOD1* Locus Maps to Human Chromosome 11p15.4. To ensure that the chromosomal position of the human *MYOD1* locus detected by the 1.9-kb human cDNA was consistent with previous studies (20) using mouse Myod-1 cDNA, the former was hybridized to *Hin*dIII-digested DNA from several human–hamster somatic cell hybrids, each of which carried a different complement of human chromosomes. There was a perfect concordance of *MYOD1* hybridization with the presence of human chromosome 11. To sublocalize the locus, similar analyses were performed with other human–rodent cell hybrids, four of which carry chromosomes 11 with deletions of portions of the short arm. Fig. 1*A* shows a Southern blot of *Hin*dIII-digested DNA hybrid-

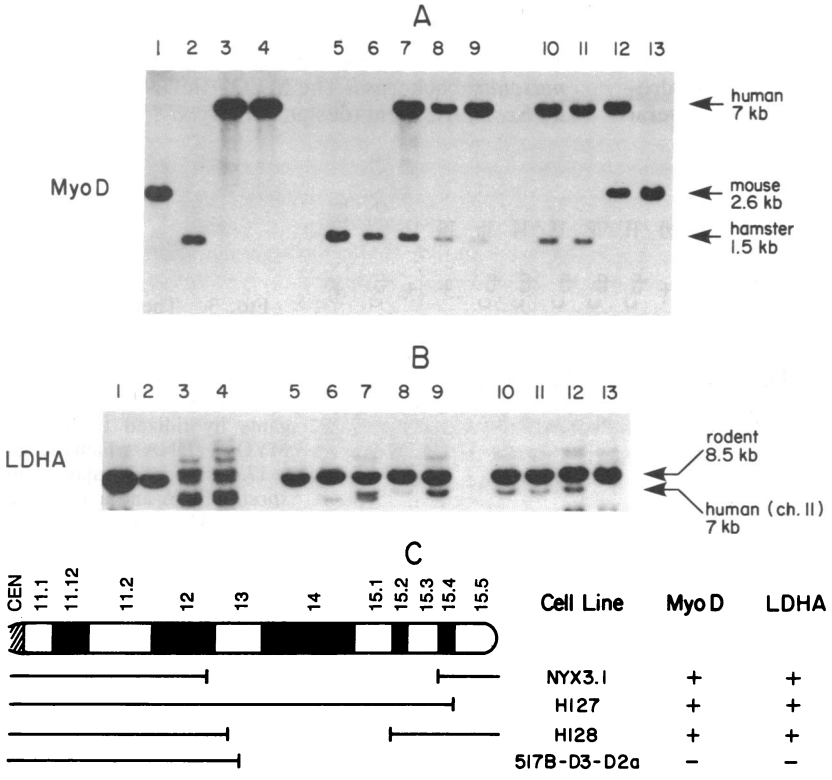

FIG. 1. The human *MYOD1* and *LDHA* loci map to chromosome 11 band p15.4. (*A*) Human–rodent hybrid mapping panel used to subregionalize the locus for *MYOD1* to the short arm of chromosome 11. Lanes: 1, mouse; 2, hamster; 3 and 4, human; 5 and 6, chromosome 11-deficient hybrids A_3ADA_1-D-12 and A_3G1; 7 and 8, chromosome 11-containing hybrids A_3G14 and GM7300; 9, chromosome 11-only hybrid H126; 10, deletion hybrid H127; 11, deletion hybrid H128; 12, deletion hybrid NYX3.1; 13, deletion hybrid 517B-D3-D2a. (*B*) Mapping panel rehybridized to the LDHA cDNA. (*C*) Diagram showing extent of 11p deletions in hybrids in lanes 10–13 of *A*, and concordance of chromosome (ch) 11 band p15.4 with the loci for *MYOD1* and *LDHA*. MyoD, *MYOD1*.

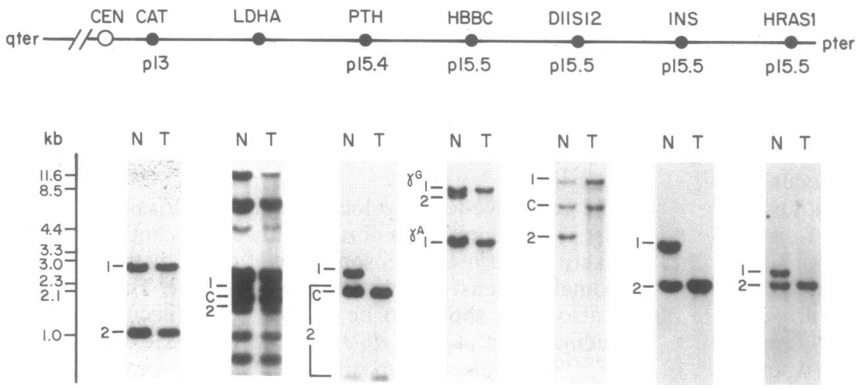

FIG. 2. Mitotic recombination mapping positions *LDHA* proximal to *PTH* in 11p15.4. RFLP analysis at marker loci on the short arm of chromosome 11 of normal (leukocyte) (N) and tumor (T) DNA from a 6-year-old boy with a retroperitoneal embryonal rhabdomyosarcoma. Alleles at each locus are designated 1 or 2; 1 is the longer allele. A common, invariant band is designated by C. γ^G and γ^A, loci in the β-globin cluster. The marker ladder at left is derived from standards run with each gel. Spacing of marker loci in the diagram at top is not reflective of the physical distances between them. CEN, centromere.

ized to the human MYOD1 cDNA. Lanes 1–4 are control DNAs from mouse (lane 1), hamster (lane 2), and human (lanes 3 and 4), which show species-specific *Hin*dIII restriction fragments of 2.6, 1.5, and 7 kb, respectively. Lanes 5–8 are analyses of portions of the hybrid panel used in Fig. 1: A_3ADA_1-D12 (lane 5) and A_3G1 (lane 6) are missing human chromosome 11, whereas A_3G14 (lane 7) and GM7300 (lane 8) contain it. H126 (lane 9) is a hybrid with chromosome 11 as its only human chromosome. H127, H128, NYX3.1, and 517B-D3-D2a (lanes 10–13) contain DNA from hybrids with deleted chromosomes 11, missing the regions shown schematically in Fig. 1*C*. The 7-kb human sequence is absent in the cells missing all of human chromosome 11 (lanes 5 and 6) or carrying the largest 11p deletion (lane 13; 517B-D3-D2a) but is present in each cell carrying an entire chromosome 11 (lanes 7 and 8) and in each of the smaller 11p deletions (lanes 10–12; H127, H128, and NYX3.1, respectively). The location of *MYOD1* on chromosome 11 that is consistent with these data is band p15.4, as illustrated in Fig. 1*C*.

A Second Muscle Locus (*LDHA*) Is Physically Colocated with *MYOD1*. The structural gene coding for the M (muscle) subunit of lactate dehydrogenase has been shown by genetic linkage studies to map between catalase in 11p13 and the β-globin complex in 11p15.5 (23). Because the location of *MYOD1* in 11p15.4 falls within this region, we tested whether these two muscle-related genes colocalized. We rehybridized the panel of somatic cell hybrids shown in Fig. 1*A* to an LDHA cDNA (23); the results are shown in Fig. 1*B*. This cDNA hybridizes to the structural gene for lactate dehydrogenase, muscle A subunit (LDHA) as well as to its several pseudogenes scattered throughout the genome (23). However, the band specific to chromosome 11 (7 kb) is clearly present, albeit at variable intensity, in all but the largest deletion, identical to the pattern for *MYOD1*, indicating that *LDHA* also cosegregates with band p15.4.

A *Taq* I polymorphism in *LDHA* (23) was used to refine the map position by mitotic recombination mapping (15). RFLP analysis of DNA from an embryonal rhabdomyosarcoma carrying a mitotic-recombinant chromosome 11 and DNA from the patient's normal peripheral blood leukocytes revealed that the recombinational event occurred in the tumor chromosomes between the marker loci *CAT* in 11p13 and *PTH* in 11p15.4, as reported (15), such that the tumor was isodisomic at all informative markers distal to and including *PTH*. *Taq* I-digested normal and tumor DNA from this patient was hybridized to the LDHA cDNA. Fig. 2 shows that both normal and tumor DNA were heterozygous at the *LDHA* locus, placing this locus proximal to the site of recombination in 11p15.4.

The Linkage Between *MYOD1* and *LDHA* Is Conserved in the Mouse and Maps to a Homologous Region on Chromosome 7. The map position for the human *MYOD1* locus suggested that the cognate gene in the mouse might map to one of several regions on mouse chromosome 7 known to exhibit significant conserved linkage homologies with human chromosome 11. To test this hypothesis, the human 1.9-kb MYOD1 cDNA clone was used as a hybridization probe in Southern blot analysis of segregants from a *M. spretus–M. musculus* backcross. The MYOD1 cDNA recognizes a 17.4-kb *Eco*RI fragment (designated *Myod-1*^{s}) in *M. spretus* DNA

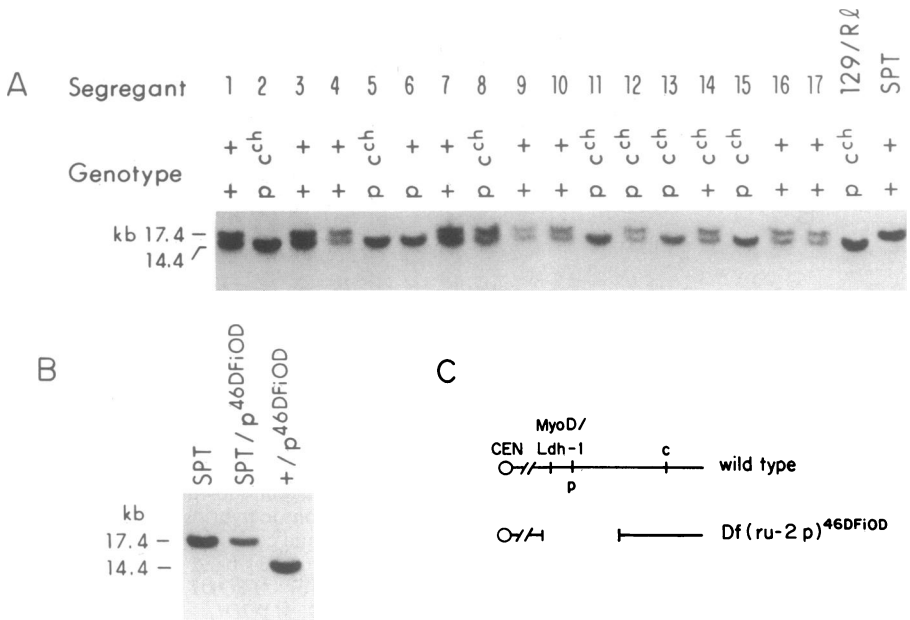

FIG. 3. The *Myod-1* locus is closely linked to the *p* locus on mouse chromosome 7. (*A*) Southern blot of DNA from *M. spretus–M. musculus* backcross segregants hybridized to the human MYOD1 cDNA, which recognizes a 17.4-kb *Eco*RI fragment in *M. spretus* DNA and a 14.4-kb fragment in *M. musculus* DNA. Each segregant carries a *p* c^{ch} chromosome in addition to the chromosome shown above each lane. (*B*) Hybridization of the human MYOD1 cDNA to *Eco*RI-digested DNA from a *M. spretus*-balanced lethal *p*-locus deletion. (*C*) The extent of the Df(*ru-2 p*)^{46DFiOD} deletion around the *p* locus on mouse chromosome 7. SPT, *M. spretus*.

and a 14.4-kb *Eco*RI fragment (designated *Myod-1*^{m}) in *M. musculus domesticus* 129/R1-*p* c^{ch}/*p* c^{ch} DNA. One hundred and thirty segregants from the cross (129/R1-*p* c^{ch}/*p* c^{ch} × *M. spretus*) F_1 × 129/R1-*p* c^{ch}/*p* c^{ch} were typed for the chromosome 7 anchor loci *p* and c^{ch} (an allele of the albino locus) by inspection and for *Myod-1* alleles by hybridization of *Eco*RI-digested splenic DNA with the MYOD1 cDNA clone. A representative blot demonstrating segregation of the *Myod-1*^{s} allele along with alleles at the *p* and *c* loci is presented in Fig. 3*A*. Genotypes for the entire panel of 130 segregants indicate that *Myod-1* is closely linked to *p*; only six *Myod-1–p* recombinants, in addition to 20 *p–c* recombinants, were identified among 130 segregants. The observed recombinant classes were consistent with the gene order: centromere–*Myod-1–p–c*, with a *Myod-1–p* distance of 4.6 ± 1.8 cM (and a *p–c* distance of 15.4 ± 3.2 cM).

An informative *Eco*RI RFLP for *Ldh-1* (*M. musculus* allele, 16 kb; *M. spretus* allele, 13 kb) was also followed in this cross. No recombinants were found between *Myod-1* and *Ldh-1* in this group of 130 segregants, implying a map distance of <1 cM (two-sided 95% confidence limits 0–2.8 cM).

These *M. spretus–M. musculus* backcross data were confirmed by deletion mapping. The MYOD1 cDNA clone was again used to probe *Eco*RI-digested DNAs from a *M. spretus*-balanced lethal *p*-locus mutation. This particular F_1 construction [+^{SPT} +^{SPT}/Df(*ru-2 p*)^{46DFiOD}] carries, opposite a *M. spretus* chromosome, a radiation-induced, prenatally lethal mutation of *p* (46DFiOD), which extends at least 3 cM proximal to *ru-2*. Fig. 3*B* demonstrates that the *Myod-1*^{m} allele contributed by the *M. musculus* + +/Df(*ru-2 p*)^{46DFiOD} parent is absent in +^{SPT} +^{SPT}/Df(*ru-2 p*)^{46DFiOD} F_1 DNA, indicating that *Myod-1* resides within the limits of the Df(*ru-2 p*)^{46DFiOD} deletion. [The *Myod-1* allele in the + +/Df(*ru-2 p*)^{46DFiOD} parental DNA is derived from the nondeleted *M. musculus* balancer chromosome.] Consequently, the map position for *Myod-1* within the proximally extending deletion Df(*ru-2 p*)^{46DFiOD} is consistent with the placement of *Myod-1* 4.6 ± 1.8 cM proximal to the *p* locus in mouse chromosome 7.

DISCUSSION

We have used a panel of somatic-cell hybrids to localize *MYOD1* to human chromosome 11 band p15.4. We then mapped *Myod-1* with respect to two anchor loci on mouse chromosome 7 by a standard three-point genetic cross to 4.6 ± 1.8 cM proximal to *p* and demonstrated that *Myod-1* is included in a radiation-induced, lethal-deletion mutation of *p*. By linking *Myod-1* genetically to *Ldh-1* in the mouse, we could use information from RFLP analysis of DNA from a mitotic recombinant rhabdomyosarcoma chromosome at the homologous locus (*LDHA*) in human DNA to position the *LDHA/MYOD1* linkage group with respect to other marker loci at the proximal side of 11p15.4.

We have taken advantage of conserved syntenic groups in mouse and man to integrate the results of these several interdependent mapping experiments, each of which provided some information about the chromosomal localization and genomic environment of the *MYOD1* locus. We found that *MYOD1* is tightly linked to the gene for the M (muscle) subunit of lactate dehydrogenase, which also maps to homologous regions of mouse and human genomes, ≈24 cM proximal to the β-globin cluster on mouse chromosome 7 and in band p15.4, ≈20 cM proximal to the homologous cluster on human chromosome 11. This places this locus adjacent to the subband of 11p that harbors the putative RdL (11p15.5).

MYOD1 has no known RFLP in human DNA that would allow its unequivocal separation from the putative RdL by mitotic recombination mapping. However, the colocalization

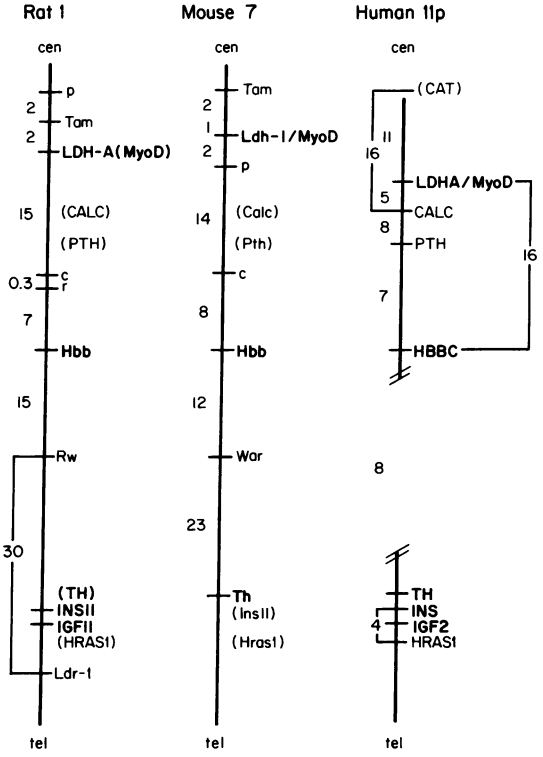

FIG. 4. Comparative maps of rat chromosome 1, mouse chromosome 7, and human chromosome 11p. *p*, pink-eyed dilution; *Tam*, tamase (rat), tosyl arginine methylesterase (mouse); *LDH-A/MyoD*, *Ldh-1/MyoD*, lactate dehydrogenase, muscle (A) subunit/*MYOD1* (ref. 23; G. V. Stolc, personal communication); *CALC*, *Calc*, calcitonin (37, 38); *PTH*, *Pth*, parathyroid hormone (37, 38); *c*, albino; *r*, *ru-2*, ruby eye-2; *Hbb*, *HBBC*, β-hemoglobin cluster; *Rw*, *War*, warfarin resistance; *TH*, *Th*, tyrosine hydroxylase (39, 40); *INS*, *Ins*, insulin; *IGFII*, *IGF2*, insulin-like growth factor 2 (41–43); *HRAS1*, *Hras1*, Harvey rat sarcoma virus oncogene; *Ldr-1*, lactate dehydrogenase regulatory gene. Unless otherwise noted, these maps are based on information in refs. 44 and 45 for rat chromosome 1, refs. 46 and 47 for mouse chromosome 7, and refs. 48 and 49 for human chromosome 11p. cen, centromere; tel, telomere; numbers indicate relative distance between loci in cM.

of *MYOD1* with *LDHA* to 11p15.4 on human chromosome 11, the reiteration of its association with *Ldh-1* in the mouse, where the genes are tightly linked genetically, and the physical separation of *LDHA* and RdL by mitotic recombination mapping in human tumor DNA (Fig. 2) make it highly unlikely that *MYOD1* is the primary site of alteration in rhabdomyosarcoma.

There are striking structural similarities between mouse chromosome 7 and rat chromosome 1 that extend to regions of the short arm of human chromosome 11 and which are illustrated in Fig. 4. Homologous genes localized in humans and at least one of the other two species are shown in boldface; homologous genes that have been chromosomally assigned but for which precise map positions are only inferred from strong interspecies homologies are indicated in parentheses. Three conserved structural pillars are apparent: *HBBC* (*Hbb*), flanked by *LDH-A* (*Ldh-1*) towards the centromere, and the tightly linked *TH–INS–IGF2–HRAS* gene cluster towards the telomere.

The analyses described here cannot, of course, address the functional significance of the physical clustering of cell-type-specific genes such as these. It is tempting to speculate that the physical structure of these genomic regions may play a role in their specific expression. Perhaps this may function analogously to the transvection effect in *Drosophila melanogaster*, a clear example of an epigenetic mechanism in-

volving the physical pairing of alleles on homologous chromosomes that modulates gene expression at the locus (50, 51). Certainly, our finding (15) of frequent mitotic recombination between chromosome 11 homologues in embryonal rhabdomyosarcoma supports the idea that pairing does occur in somatic cells. Furthermore, the observation that heterozygous loci that undergo loss of an allele in pediatric tumors become homozygous (i.e., pseudodiploid) rather than hemizygous suggests that two copies of the locus, even if they are identical, must be maintained for a cell to remain viable. This is consonant with the recent suggestion that the maintenance of a normal diploid cell depends on the presence of two copies of specific genes (52). Whether this specificity varies according to cell type may be reflected in synteny relationships of genes that contribute to a specific phenotype and the maintenance of certain chromosomes in clones of mutant cells that mimic this phenotype but which have otherwise unstable karyotypes.

We thank Drs. A. Lassar, R. Davis, and H. Weintraub for the mouse Myod-1 cDNA; H. Kazazian, M. Wigler, R. White, G. Bell, G. Bruns, R. Gravel, and H. Mayer for recombinant DNA probes; C. Scoggin, J. Erikson, C. Croce, and E. van Heyningen for cell hybrids; and, L. Sapienza and R. Derval for artwork. This work was supported in part by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

1. Klein, G. (1987) *Science* **238,** 1539–1545.
2. Francke, U. (1976) *Cytogenet. Cell Genet.* **16,** 131–134.
3. Balaban, G., Gilbert, F., Nichols, W., Meadows, A. T. & Shield, J. (1982) *Cancer Genet. Cytogenet.* **6,** 213–221.
4. Cavenee, W. K., Dryja, T. P., Phillips, R. A., Benedict, W. F., Godbout, R., Gallie, B. L., Murphree, A. L., Strong, L. C. & White, R. L. (1983) *Nature (London)* **305,** 770–784.
5. Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapoport, J. M., Albert, D. M. & Dryja, T. P. (1986) *Nature (London)* **323,** 643–646.
6. Fung, Y.-K. T., Murphree, A. L., T'Ang, A., Qian, J., Hinrichs, S. H. & Benedict, W. F. (1987) *Science* **236,** 1657–1661.
7. Lee, W.-H., Bookstein, R., Hong, F., Young, L.-H., Shew, J.-Y. & Lee, E. Y.-H. P. (1987) *Science* **235,** 1394–1399.
8. Su Huang, H.-J., Yee, J.-K., Shew, J.-Y., Chen, P.-L., Bookstein, R., Friedmann, T., Lee, E. Y.-H. P. & Lee, W.-H. (1988) *Science* **242,** 1563–1566.
9. Beckwith, J. B. (1969) *Birth Defects Orig. Artic. Ser.* **5,** 188–196.
10. Sotelo-Avila, C. & Gooch, W. M. (1976) *Perspect. Pediatric Pathol.* **3,** 255–272.
11. Waziri, M., Patil, S. R., Hanson, J. W. & Bartley, I. A. (1983) *J. Pediat.* **102,** 873–876.
12. Turleau, C., de Grouchy, J., Chavin-Colin, F., Martelli, H., Voyer, M. & Charlas, R. (1984) *Hum. Genet.* **67,** 219–221.
13. Koufos, A., Grundy, P., Morgan, K., Aleck, K., Hadro, T., Lampkin, B., Kalbakji, A. & Cavenee, W. (1989) *Am. J. Hum. Genet.* **44,** 711–719.
14. Ping, A. J., Reeve, A. E., Law, D. J., Young, M. R., Boehnke, M. & Feinberg, A. P. (1989) *Am. J. Hum. Genet.* **44,** 720–723.
15. Scrable, H. J., Witte, D. P., Lampkin, B. C. & Cavenee, W. K. (1987) *Nature (London)* **329,** 645–647.
16. Davis, R. L., Weintraub, H. & Lassar, A. B. (1987) *Cell* **51,** 987–1000.
17. Edmondson, D. G. & Olson, E. N. (1989) *Genes Dev.* **3,** 628–640.
18. Wright, W. E., Sassoon, D. A. & Lin, V. K. (1989) *Cell* **56,** 607–617.
19. Scrable, H., Witte, D., Shimada, H., Seemayer, T., Wang-Wuu, S., Soukup, S., Koufos, A., Houghton, P., Lampkin, B. & Cavenee, W. (1989) *Genes Chromosomes Cancer* **1,** 23–35.
20. Tapscott, S. J., Davis, R. L., Thayer, M. J., Cheng, P.-F., Weintraub, H. & Lassar, A. B. (1988) *Science* **242,** 405–411.
21. Scrable, H., Cavenee, W., Ghavimi, F., Lovell, M., Morgan, K. & Sapienza, C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7480–7484.
22. Quan, F., Korneluk, R. G. & Gravel, R. A. (1985) *Am. J. Hum. Genet.* **37,** 171 (abstr.).
23. Gerhard, D. S., Bruns, G. A. & Housman, D. E. (1987) *Cytogenet. Cell Genet.* **46,** A619.
24. Schmidtke, J., Pape, B., Krengel, U., Langenback, U., Cooper, D. N., Brayel, E. & Mayer, H. (1984) *Hum. Genet.* **67,** 428–431.
25. Antonarakis, S. E., Boehm, C. S., Giardina, P. J. & Kazazian, H. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 137–141.
26. Barker, D., Holm, T. & White, R. L. (1984) *Am. J. Hum. Genet.* **36,** 1159–1171.
27. Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. (1982) *Nature (London)* **296,** 404–409.
28. Bell, G. I., Karam, J. H. & Rutter, W. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5759–5763.
29. Mendel, J. (1987) Dissertation (University of Tennessee, Memphis).
30. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
31. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
32. Griffin, C. A., Emanuel, B. S., Hansen, J. R., Cavenee, W. K. & Myers, J. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 512–516.
33. Fisher, J. H., Miller, Y. E., Sparkes, R. S., Bateman, J. B., Kimmel, K. A., Carey, T. E., Rodell, T., Shoemaker, S. A. & Scoggin, C. H. (1984) *Somatic Cell Mol. Genet.* **10,** 455–464.
34. Porteous, D. J., Bickmore, W., Christie, S., Boyd, P. A., Cranston, G., Fletcher, J. M., Gosden, J. R., Rout, D., Seawright, A., Simola, K. O. J., van Heyningen, E. & Hastie, N. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5355–5359.
35. Erikson, J., Williams, D. L., Finan, J., Nowell, P. C. & Croce, C. M. (1985) *Science* **229,** 784–786.
36. Russell, L. B. & Rinchik, E. M. (1987) *Banbury Rep.* **28,** 109–121.
37. Todd, S., Yoshida, M. C., Fang, X. E., McDonald, L., Jacobs, J., Heinrich, G., Bell, G. I., Naylor, S. L. & Sakaguchi, A. Y. (1985) *Biochem. Biophys. Res. Commun.* **131,** 1175–1180.
38. Lalley, P. A., Sakaguchi, A. Y., Eddy, R. L., Honey, N. H., Bell, G. I., Shen, L.-P., Rutter, W. J., Jacobs, J. W., Heinrich, G., Chin, W. W. & Naylor, S. L. (1987) *Cytogenet. Cell Genet.* **44,** 92–97.
39. O'Malley, K. L. & Rotwein, P. (1988) *Nucleic Acids Res.* **16,** 4437–4446.
40. Brilliant, M. H., Niemann, M. M. & Eicher, E. M. (1987) *J. Neurogenet.* **4,** 259–266.
41. Soares, M. B., Schon, E., Henderson, A., Karathanasis, S. K., Cate, R., Zeitlin, S., Chirgwin, J. & Efstratiadis, A. (1985) *Mol. Cell. Biol.* **5,** 2090–2103.
42. Soares, M. B., Turken, A., Ishii, D., Mills, L., Episkopon, V., Cotter, S., Zeitlin, S. & Efstratiadis, A. (1986) *J. Mol. Biol.* **192,** 737–752.
43. Bell, G. I., Gerhard, D. S., Fong, N. M., Sanchez-Pescador, R. & Rall, L. B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6450–6454.
44. Cramer, D. V. (1987) in *Genetic Maps 1987*, ed. O'Brien, S. J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 464–469.
45. Butcher, G. W., Clarke, S. & Tucker, E. M. (1979) *Transplanta. Proc.* **11,** 1629–1630.
46. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 286–298.
47. Nadeau, J., Eppig, J. & Reiner, A. (1989) *Linkage and Synteny Homologies Between Mouse and Man* (The Jackson Laboratory, Bar Harbor, ME).
48. Kittur, S. D., Hoppener, J. W. M., Antønarakis, S. E., Daniels, J. D. J., Meyers, D. A., Maestri, N. E., Jansen, M., Korneluk, R. G., Nelkin, B. D. & Kazazian, H. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5064–5067.
49. White, R., Leppert, M., Bishop, D. T., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1985) *Nature (London)* **310,** 101–105.
50. Lewis, E. B. (1950) *Adv. Genet.* **3,** 73–115.
51. Wu, C.-T. & Goldberg, M.-L. (1989) *Trends Genet.* **5,** 189–194.
52. Holliday, R. (1989) *Trends Genet.* **5,** 42–45.