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# The human MyoD1 (MYF3) gene maps on the short arm of chromosome 11 but is not associated with the WAGR locus or the region for the Beckwith-Wiedemann syndrome

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**Summary.** The human gene encoding the myogenic determination factor myf3 (mouse MyoD1) has been mapped to the short arm of chromosome 11. Analysis of several somatic cell hybrids containing various derivatives with deletions or translocations revealed that the human MyoD (MYF3) gene is not associated with the WAGR locus at chromosomal band 11p13 nor with the loss of the heterozygosity region at 11p15.5 related to the Beckwith-Wiedemann syndrome. Subregional mapping by in situ hybridization with an myf3 specific probe shows that the gene resides at the chromosomal band 11p14, possibly at 11p14.3.

## Introduction

Myogenic determination factors are muscle-specific regulatory proteins that have been identified by their capacity to initiate the myogenic program in mouse C3H 10T1/2 embryonic fibroblasts, thereby converting them into myoblasts (Davis et al. 1987). We have recently reported the identification of four distinct human cDNA clones encoding the factors myf3 to myf6, which individually or in concert are capable of generating the myogenic phenotype in non-muscle cells (Braun et al. 1989a, b and 1990). Nucleotide sequence analysis revealed that all four proteins shared a highly conserved sequence motif coding for a putative helix-loop-helix structure. This sequence element required for biological activity was also present in other regulatory genes, such as the immunoglobulin enhancer binding proteins E12 and E47 (Murre et al. 1989) and the proto-oncogenes of the myc family (Caudy et al. 1988). The cDNA clone myf3 constitutes the human homolog of the mouse MyoD1 cDNA, and the corresponding human gene was shown to be localized on the short arm of human chromosome 11 (Tapscott et al. 1988; Braun et al. 1989b).

Restriction fragment length polymorphism (RFLP) analysis of childhood tumors such as rhabdomyosarcoma, Wilms tumor, adrenocortical carcinomas and hepatoblastomas has suggested that the appearance of these tumor types is associated with tumor cell-specific loss of heterozygosity for chromosome 11 (Koufos et al. 1984, 1985; Henry et al. 1989a). The Beckwith-Wiedemann syndrome, a rare condition of growth abnormalities, has also been cytogenetically located on chromosome 11p (Waziri et al. 1983) and physically and genetically mapped to 11p15.5 (Koufos et al. 1989, Henry et al. 1989b; Ping et al. 1989). Individuals with Beckwith-Wiedemann syndrome carry an increased risk for the same three embryonic tumors (Sotelo-Avila and Gooch 1976). Furthermore, it has been recognized that mothers of children with rhabdomyosarcoma frequently develop breast cancer (Li and Fraumeni 1969; Strong et al. 1987). A second region on chromosome 11p13 has been implicated in the development of Wilms' tumor based on constitutional deletions seen in individuals with the WAGR syndrome (*W*ilms tumor, *a*niridia, *g*enitourinary anomalies, and mental *r*etardation) (Riccardi et al. 1978; Francke et al. 1979). Similar deletions of chromosome 11p13, limited to tumor cells, have been detected in sporadic Wilms' tumors (Kaneko et al. 1981). More recently, long range physical maps of the WAGR region have been established; these lead to a precise localization of the genes for Wilms' tumor (WT) and aniridia (AN2) at 11p13 (Compton et al. 1988; Gessler and Bruns 1989; Gessler et al. 1989b).

The human MyoD gene based on its chromosomal localization, its biological activity in cell differentiation

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and growth control, and its structural similarity to the myc proto-oncogenes, could well play a significant role in the development of the different abnormalities and tumor types mentioned above. In addition, few expressed sequences specific to this area of chromosome 11 have been described. We therefore undertook the detailed regional mapping of the human MyoD gene.

## Materials and methods

### *Cell lines and Southern blot analysis*

Somatic cell hybrids with WAGR deletions (N.W., M.J.) and a translocation involving 11p13 (POR4 from V.v. Heyningen) have been described previously (Porteous et al. 1987). Cell lines GM5518 and GM3808 (NIGMS Human Genetic Mutant Cell Repository, Camden, N.J.) carry hemizygous deletions of the WAGR region and were analyzed by gene dosage analysis (Gessler et al. 1989a). The cell hybrid PEL16 contains 11p15.4–pter lacking the telomeric part of the short arm, and PEL40 constitutes a reciprocal translocation carrying 11p15.4–pter (Henry et al. 1989a). Bid7 cells carry the deletion 11p12–11p14.dist. (Couillin et al. 1989). Extraction of DNA, preparation of Southern blots, and hybridization conditions have previously been described (Gessler et al. 1989a). A 830-bp cDNA fragment specific for MYF3 (Braun et al. 1989b) was used as a probe.

### *Preparation of chromosomes and in situ hybridization*

Human peripheral lymphocytes were stimulated for growth in culture with phytohemagglutinin (PHA). Chromosomes were labeled with bromodeoxyuridine (20 μg/ml) for 6.5 h and prepared according to standard procedures. For in situ hybridization, slides were pretreated with RNAse A (100 μg/ml) and chromosomes were denatured in 70% formamide, 2 × SSC at 70°C for 2 min. The myf3 cDNA fragment derived from the 3′ untranslated region of the cDNA (50–200 ng) was labeled with $^3$H-dTTP and $^3$H-dCTP (50–120 Ci/mmol) by nick-translation to a specific activity of $2–5 \times 10^7$ dpm/μg DNA. Hybridization were carried out in 50% formamide, 10% dextran sulfate, 2 × SSC, and 500 μg/ml salmon sperm DNA at 40°C for 16 h. Following hybridization, slides were washed three times in 50% formamide, 2 × SSC at 42°C for 15 min each, and six times in 2 × SSC 42°C for 2 min. Washing was continued for 2 h in 2 × SSC at room temperature. The slides were coated with Ilford L4 emulsion and developed after 2–4 weeks. Staining was performed according to a modified fluorochrome-photolysis-technique (FPG; Epplen et al. 1975). The chromosomes were identified and the grain distribution recorded at the same time (Mattei et al. 1985).

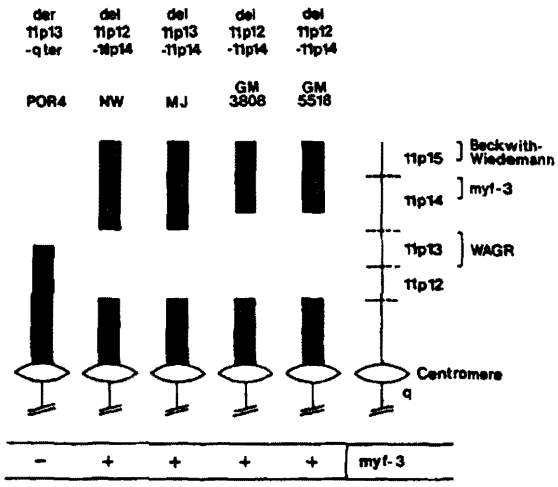


**Fig. 1.** Schematic representation of myf3 hybridization to DNA from the indicated somatic cell hybrids. The *stipled areas* indicate the regions of human chromosome 11 present in the various cell lines. *Plus* and *minus* symbols indicate the hybridization signals of the myf3 probe per hemizygous chromosome 11

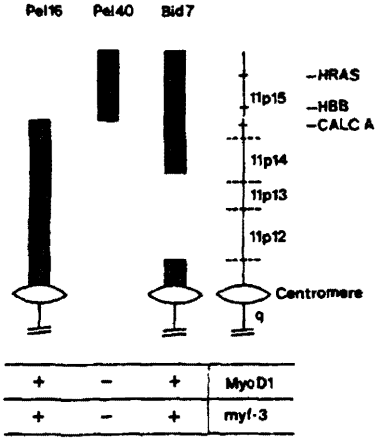


**Fig. 2.** Schematic representation of MyoD1 and myf3 hybridization to the indicated cell lines. The locations of few selected marker genes are shown. MyoD1 and myf3 represent the homologous gene products from mouse and human, respectively

## Results and discussion

In order to examine the precise localization of the human MyoD1 (MYF3) gene on the short arm of chromosome 11, we analyzed DNA from cell lines carrying various derivatives of chromosome 11. The deletions encompass the WAGR region in 11p13 and extend telomeric or centromeric to different degrees.

As shown in Fig. 1, specific hybridization signals were obtained with the radioactively labeled myf3 probe in DNA from cells carrying one chromosome 11 homolog of patients N.W. and M.J.; these homologs lack the regions from proximal 11p14 to distal 11p12 and from proximal 11p14 to 11p13, respectively. Gene dosage analysis of DNA from cell lines GM5518 and GM3808 containing hemizygous deletions of the WAGR region extending far into 11p14 and 11p12 showed no evidence for a deletion of myf3-specific sequences in both cases. In contrast, DNA from the POR4 hybrid that contained the 11p13–11qter part of an aniridia-associated translocation did not show hybridization with the myf3 probe. From these results, we conclude that the human MyoD gene is located in the region 11p14–11pter and can be excluded from the WAGR locus at 11p13.

To study the more distal region of chromosome 11p in more detail, additional somatic cell hybrids defining the smallest region of overlap of the Beckwith-Wiedemann locus were used (Henry et al. 1989b). PEL16 lacking 11p15.4-pter, and PEL40 containing the telomeric fragment of a reciprocal chromosome 11 translocation

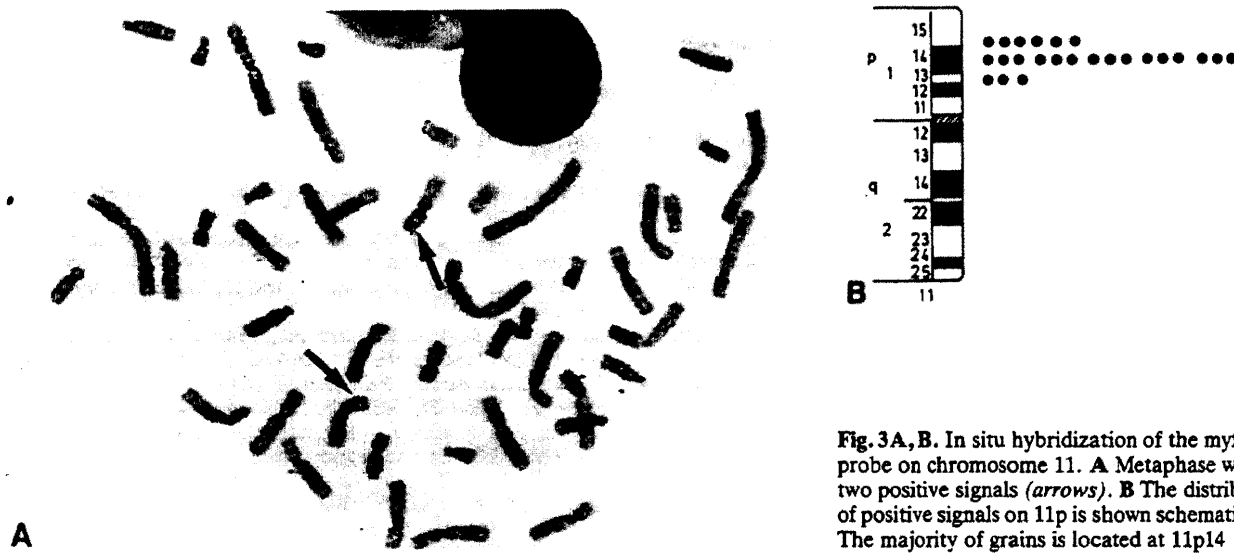


**Fig. 3A, B.** In situ hybridization of the myf3 probe on chromosome 11. **A** Metaphase with two positive signals *(arrows)*. **B** The distribution of positive signals on 11p is shown schematically. The majority of grains is located at 11p14

with the breakpoint in 11p15.4 were analyzed with the myf3 probe. As shown in Fig. 2, PEL16 containing the 11p15.4–11qter shows positive hybridization with myf3 and the mouse MyoD1 probe, whereas the reciprocal fragment of the same chromosome present in PEL40 cells cannot be detected by either probe. Another cell hybrid Bid7 (Coullin et al. 1989) with deletion breakpoints in 11p12 and 11p14.3 also shows myf3-specific hybridization, indicating that the myf3 gene maps to the region 11p14.3 to 11p15.4 and is certainly not associated with the Beckwith-Wiedemann region, which maps distal to the latter breakpoint.

Additional support for the localization of the MyoD gene at 11p14 came from in situ hybridization. From 24 observed hybridization signals, 15 were located around 11p14.3, the rest mapping in the adjacent areas (Fig. 3). This result, obtained by an independent technique, confirmed that the human MyoD gene encoding a myogenic determination factor maps to 11p14 and is therefore not linked to either of the two genetic loci associated with sporadic or hereditary forms of embryonic tumors in children. This is in general agreement with recent results published by Scrable et al. (1990) who mapped the human MyoD1 gene to 11p15.4, a localization that is slightly more distal than our sublocalization. It is interesting to note that the majority of rhabdomyosarcomas express detectable levels of myf3 (MyoD1) mRNA, irrespective of the losss or maintenance of heterozygosity at chromosome 11 (Scrable et al. 1989). Furthermore, it has been shown that the expression of the MyoD1 (Myf3) muscle determination gene in human rhabdomyosarcomas is closely related to the capacity of these cells to differentiate, but it does not define the malignancy of these tumors (Hiti et al. 1989).

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