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Assignment of the mouse desmin gene to chromosome 1 band C3

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Summary

The chromosomal localization of the mouse gene coding for desmin, one of the muscle-specific intermediate filament subunits, was determined by *in situ* hybridization using a specific ^{3}H-labelled DNA probe. There is only one copy of the desmin gene and it is located on chromosome 1 in the band C3. This result adds an eleventh locus to a conserved gene cluster and confirms the partial homology that exists between the long arm of human chromosome 2 and chromosome 1 of the mouse.

1. Introduction

The desmin gene belongs to the family of intermediate filament (IF) genes whose expression is developmentally regulated. The different genes coding for the IFs can be divided into four groups according to their organization into intron-exon domains and nucleotide sequences (Steinert *et al.* 1985). These genes code for different cytoplasmic polypeptides forming the subunits of IFs which differ according to the tissue in which they are expressed: (I and II) keratins A and B being found in epithelial cells (Moll *et al.* 1982); (III) the neurofilament (Nf) triplet in neurones (Julien *et al.* 1987); (IV) glial fibrillary acidic protein (GFAP) in astrocytes (Lewis *et al.* 1984), desmin in muscle (Li *et al.* 1989), vimentin in mesenchyme-derived cells (Perreau *et al.* 1988), and peripherin in the peripheral nervous system (Landon *et al.* 1989). The isolation and characterization of individual IF genes has demonstrated that this diversity originates within the germ line and is not generated by somatic rearrangement. In the nonepithelial branch of the evolutionary tree the genes for Nf, vimentin, desmin and GFAP diverged from a common ancestor. Moreover, by comparison of protein sequences and gene organization it can be concluded that there must have existed a common ancestor for the α-helical domain, while the variable domains evolved through a series of tandem duplications and probably by gene conversion (Klinge *et al.* 1987).

Unlike the genes that encode the two other types of cytoskeletal structures, i.e. microfilaments represented by actins, and microtubules represented by tubulins, (Cleveland *et al.* 1981; Soriano *et al.* 1982; Hanauer *et al.* 1984) the genes for the IF polypeptides, such as desmin, vimentin and GFAP are single copy genes (Quax *et al.* 1983, 1984; Zehner & Paterson, 1983; Lewis *et al.* 1984; Lilienbaum *et al.* 1988). Whereas vimentin, desmin and GFAP are the expression products of single-copy genes, neurofilament proteins and cytokeratins are encoded by more than one gene. Nfs are encoded by three different genes. Cytokeratins are the most diverse of the IF proteins, since there are about 30 different subunits.

In mammalian skeletal muscle, the replicative presumptive myoblasts synthesize predominantly vimentin, these myoblasts fuse to form myotubes which synthesize high levels of desmin and, as the myotubes mature, vimentin and desmin gene expression becomes mutually exclusive, such that in mature muscle only desmin is found.

The organization of the mammalian vimentin and desmin genes is remarkably conserved between species such as human, mouse and hamster (Quax *et al.* 1983; Ferrari *et al.* 1986; Perreau *et al.* 1988) each of which contain 8 introns situated at identical positions. Using *in situ* radioactive hybridization, we have already been able to determine the location of the vimentin gene in region A2 of mouse chromosome 2 (Mattei *et al.*

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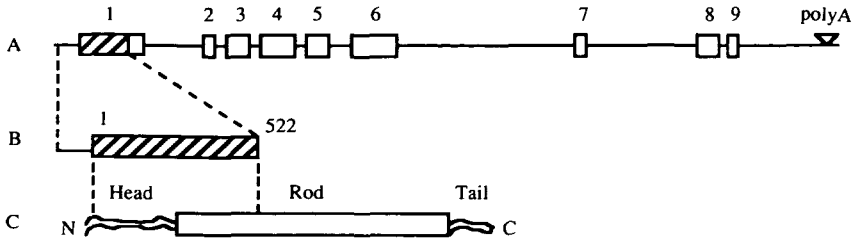

Fig. 1. Region of desmin gene used as probe for hybridization. (A) Sizes of exons and introns and their position. Open boxes represent the desmin exons numbered with arabic numerals, the hatched segment shows coding sequence contained in the probe. (B) The probe used in this study contains 552 bp coding sequence of exon1 and 180 bp 5′-upstream sequence. (C) Desmin subunit. The probe contains the sequence coding for amino acids from 1 to 174 of the human desmin.

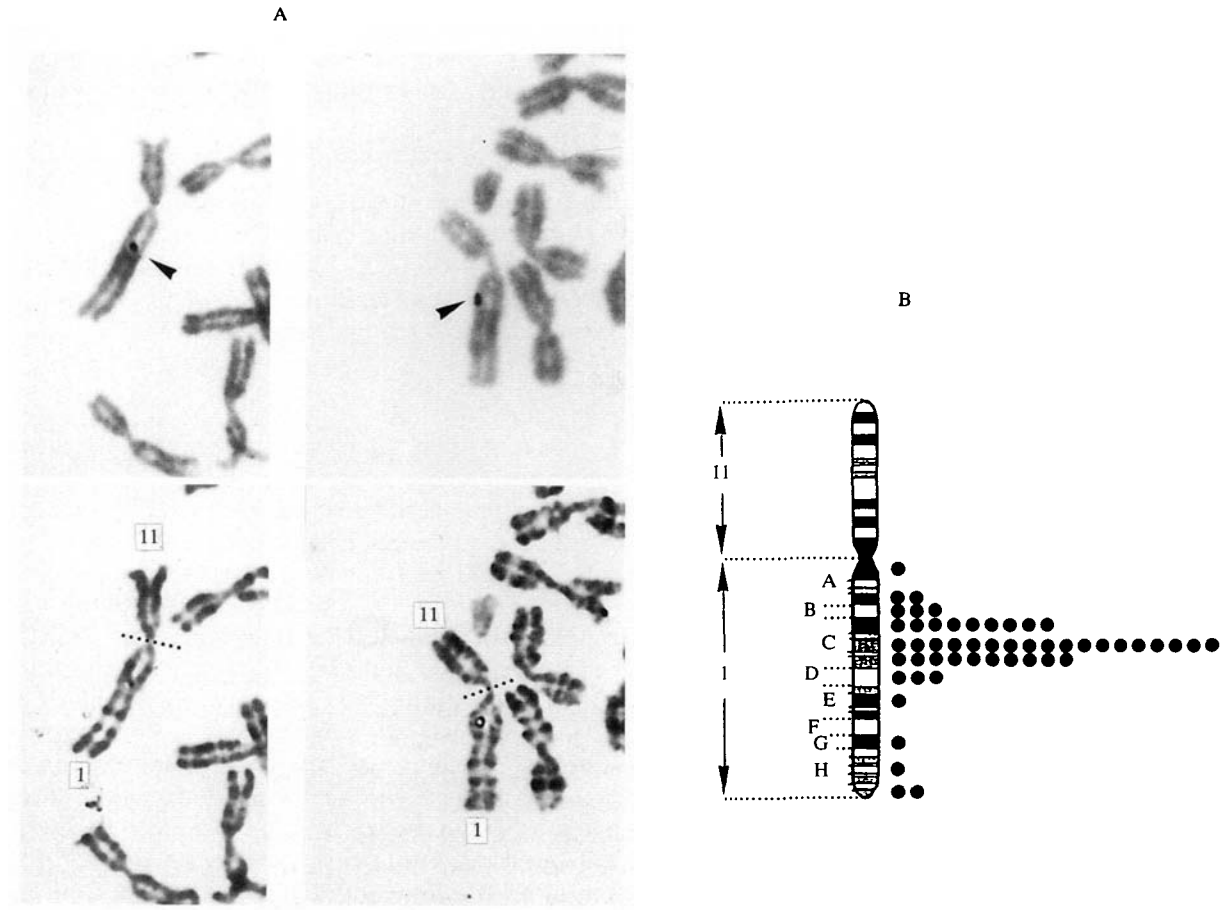

Fig. 2. Localization of the desmin gene to mouse chromosome 1 by *in situ* hybridization. (A) Two partial WMP mouse metaphases, showing the specific site of hybridization to chromosome 1. Top, arrowheads indicate silver grains on Giemasa-stained chromosomes, after autoradiography. Bottom, chromosomes with silver grains were subsequently identified by R-banding. (B) Diagram of WMP mouse Rb (1;11) chromosome, indicating the distribution of labelled sites on chromosome 1.

1989) and one of the neurofilament genes (Nfl) on to mouse chromosome 14 in the region D1-E1 (Mattei *et al.* in press). The Nfh gene has been assigned to chromosome 11 (Dautigny *et al.* 1988).

In humans both Nfl and Nfm genes have been mapped to chromosome 8 band p21 (Hurst *et al.* 1987), whereas the Nfh is localized on chromosomes 22 and 1 (Lieberburg *et al.* 1989; Mattei *et al.* 1988). In man, the vimentin gene has been localized to chromosome 10 (Quax *et al.* 1985), 10p13 (Ferrari *et al.* 1987) close to the interleukin-2 receptor gene (Il2r) (Leonard *et al.* 1985). The human desmin gene has been located to human chromosome 2 region q35 (Quax *et al.* 1985; Viegas-Pequignot *et al.* 1989).

In this study, using *in situ* hybridization with a radioactive probe, we have shown that the mouse desmin gene is located on chromosome 1 band C3. This localization of the desmin gene confirms the

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Table 1. *Homologous loci between human and mouse muscle-specific* genes*

Gene name	Chromosomes	
	Human	Mouse
Collagen, type III, alpha 1	2q31-32 Solomon *et al.* (1985)	1 Seldin *et al.* (1989)
* Acetylcholine receptor gamma	2q32-qter Cohen *et al.* (1987)	1 Heidmann *et al.* (1986)
* Acetylcholine receptor delta	2q32-qter Lobos *et al.* (1989)	1 Heidmann *et al.* (1986)
* Myosin light chain alkali, fast skeletal	2q32-qter Serero *et al.* (1987)	1 Robert *et al.* (1985)
Isocitrate dehydrogenase-1, soluble	2q32-qter Narahara *et al.* (1985)	1 Skow *et al.* (1987)
Lens crystallin, gamma	2q33-35 Shiloh *et al.* (1986)	1 Zneimer *et al.* (1988)
Inhibin, alpha subunit	2q33-qter Barton *et al.* (1987)	1 Barton *et al.* (1987)
Fibronectin-1	2q34-q36 Janwar *et al.* (1986)	1 Skow *et al.* (1987)
Villin	2q35-q36 Rousseau Merck *et al.* (1986)	1 Rousseau Merck *et al.* (1988)
Cytotoxic T lymphocyte associated protein-4	2q33-q34 Dariavach *et al.* (1988)	1C Brunet *et al.* (1987)
* Desmin	2q35-q36 Viegas-Péquignot *et al.* (1989)	1 C3 This article

partial homology of the long arm of human chromosome 2 and mouse chromosome 1, and adds an eleventh locus to the conserved gene cluster.

2. Materials and methods

(i) *Preparation of chromosome spreads*

In situ hybridization experiments were carried out using metaphase spreads from a WMP male mouse, in which all the autosomes except 19 were in the form of metacentric Robertsonian translocations. Concanavalin A-stimulated lymphocytes were cultured at 37 °C for 72 h, 5-bromodeoxyuridine was added for the final 6 h of culture (60 μg/ml of medium), to ensure a chromosomal R-banding of good quality.

(ii) *Probe preparation and* in situ *hybridization*

The human desmin clone BHudes07, containing an insert of 700 base pairs in Bluescribe plasmid (Stratagene), was tritium labelled by nick-translation to a specific activity of 1×10^8 d.p.m. μg^{-1}. The radiolabelled probe was hybridized to metaphase spreads at a final concentration of 100 ng/ml of hybridization solution, as previously described (Mattei *et al.* 1985).

(iii) *Autoradiography, staining and banding*

After coating with nuclear track emulsion (KODAK NTB2), the slides were exposed for 21 days at +4 °C, then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and the metaphases were photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa (FPG) method, and metaphases were rephotographed before analysis.

3. Results and discussion

The human desmin gene has been isolated and characterized and its complete nucleotide sequence has been determined (Li *et al.* 1989). Comparison of the human and hamster coding sequences shows 90% homology at the nucleotide level. The probe used in this study was a 0·7 kb fragment of the human desmin gene. This probe contains the nucleotide sequence exon1 coding for amino acids from 1 to 174 of the human desmin (Fig. 1). Hybridization of α-^{32}P-labelled BHudes07 probe to mouse genomic DNA detected only one band of 10 kb, 3·6 kb, 1·8 kb respectively in DNA digested with *Eco*R I, *Bam*H I and *Hin*d III. This suggests that there is only one copy of the desmin gene per mouse haploid genome.

In situ hybridization experiments were carried out using metaphase spreads from a WMP male mouse in which all the autosomes, except 19, were in the form of metacentric Robertsonian translocations. In the 100 metaphase cells examined after *in situ* hybridization, there were 164 silver grains associated with chromosomes and 51 of these (31·1%) were located on chromosome 1. The distribution of grains on this chromosome was not random: 72% of them mapped to the (C1–C5) band of chromosome 1 with

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a maximum in the middle of this band. These results allow us to map the desmin gene to the C3 band of the mouse chromosome (Fig. 2).

The single copy human desmin gene was located on human chromosome 2 (Quax *et al.* 1985). Regional mapping (Viegas-Péquignot *et al.* 1989) has located this gene to band q35 of human chromosome 2 by nonradioactive *in situ* hybridization. A translocation t(2;13) (q37;q13) has been found associated with alveolar rhabdomyosarcoma (Berger *et al.* 1985), raising the intriguing possibility that alterations in the pattern of desmin expression could be associated with cell transformation, as is known to be the case with vimentin (Chan *et al.* 1989). Many syntenic groups of homologous genes in both man and mouse have been reported to show conservation of some chromosomal segments during evolution (Buckle *et al.* 1984; Lalley *et al.* 1989). One of these conservation regions has been found on the long arm of human chromosome 2 and on mouse chromosome 1. Ten genes have been located to mouse chromosome 1 and to region q of human chromosome 2, and these genes have been listed in Table 1.

These results together with the present data add an eleventh locus to the conserved gene cluster encoding collagen type III, alpha 1, (Col3a), acetylcholine receptor delta (Acrd), acetylcholine receptor gamma (Acrg), isocitrate dehydrogenase-1 soluble (Idh-1), myosin light chain alkali, fast skeletal, (Mylf), lens crystallin gamma (Cryg-1), inhibin alpha subunit (Inha), fibronectin-1 (Fn-1), villin (Vil) and cytotoxic T lymphocyte associated protein-4 (Ctla-4), (Lalley *et al.* 1989), and confirm the partial homology which exists between the mouse chromosome 1 and the long arm of human chromosome 2 (Rousseau Merck *et al.* 1988). It is interesting to note that there are four muscle-specific genes: Acrd, Acrg, Mylf and desmin, in this conserved gene cluster.

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