Genet. Res., Camb. (1989), **53**, *pp.* 183–185 *With 2 text-figures Printed in Great Britain*

Chromosomal localization of the mouse gene coding for vimentin

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(*Received 23 November 1988*)

Summary

The chromosomal location of the mouse gene coding for vimentin, one of the intermediate filament subunits, was determined by *in situ* hybridization using specific H^3-labelled DNA probes. There is only one copy of the vimentin gene and it is located on chromosome 2 region A2.

1. Introduction

In recent years, intermediate filaments (IF) have attracted much interest, largely because their constitutive polypeptide units are specifically expressed in various cell types and thus represent excellent differentiation markers (Osborn & Weber, 1982).

Biochemical data has established the existence of five subclasses of polypeptides that can form filaments of about 100 A diameter in the cytoplasm of mammalian cells: two complex groups of acidic and basic keratins of 40–70 kDa; the 52 kDa protein desmin; the 55 kDa vimentin protein: the 50 kDa glial fibrillar acidic protein (GFAP) and three neurofilament (NF) proteins of 65, 100 and 135 kDa.

Cytokeratins A and B which represent the largest and most diverse classes of intermediary filament proeins are found in epithelial cells; desmin is synthesized in skeletal, visceral and certain vascular smooth cells, the GFAP in astroglia and the neurofilament protein triplet in neuronal cells. Vimentin has a widespread distribution among mesenchymal derivatives but is also present in progenitors of muscle and neural tissues (for review, see Traub, 1985).

Mammalian embryos at an early pre-implantation stage lack detectable IF; cytokeratins appear first, concomitant with the first epithelial differentiation of the outer cells of the blastocyst. (Paulin *et al.* 1980). Neurofilaments appear early in development and progressively replace vimentin which is expressed before NF in most, if not all, dividing neuroepithelial cells. *In vitro*, in culture conditions promoting rapid neuronal differentiation, the expression of IF follows a sequence very similar to that found in the embryo with neuroepithelial cells firt expressing vimentin and with NF then appearing and progressively replacing the vimentin (Cochard & Paulin, 1984).

In skeletal muscle, the replicative presumptive myoblasts predominantly synthesize vimentin. However, upon fusion, myotubes synthesize high levels of desmin. Then, in mammalian myotubes vimentin and desmin gene expression become mutually exclusive.

It has become clear from various structural analyses that all the IF subunits are built according to a common plan: each has a central helical rod domain flanked by end domains of variable size and chemical character. The differences in properties of the IFs are due to their end domains and the structural uniformity to the conserved structure of the rod central domains (see ref. in Steinert *et al.* 1985). The isolation and characterization of individual IF genes has allowed it to be demonstrated that this diversity originates within the germ line and is not generated by somatic rearrangement.

The organization of the mammalian vimentin and desmin genes is remarkably conserved between species such as man, mouse and hamster, (Quax *et al.* 1983; Ferrari *et al.* 1986; Perreau *et al.* 1988): each containing 8 introns occurring at identical positions. All the data point to the conclusion that these two genes arose from a common ancestor. Because of this, we are investigating the chromosomal location of the vimentin gene in the mouse.

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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 with 5-bromodeoxyuridine added for the final 6 h of culture (60 μg/ml of medium), to ensure a high quality chromosomal R-banding.

(ii) In situ *hybridization*

The DNA clones containing an insert of 700–100 base pairs in pUC18 were tritium labelled by nick-translation to a specific activity of 22×10^7 dpm μg_{-1}. The radiolabelled probe was hybridized to metaphase spreads at a final concentration of 500 ng per ml of hybridization solution as previously described (Mattei *et al.* 1985).

After coating with nuclear track emulsion (Kodak NTB_2), the slides were exposed for 8 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 metaphase photographed. R-banding was then performed by the fluorochrome-photolysis-giemsa (FPG) method and metaphases rephotographed before analysis.

(iii) *DNA blotting and hybridization*

DNA electrophoresis, transfer to nitrocellulose and hybridization were carried out as described by Southern (1975) using *E. cali* DNA in the place of salmon sperm DNA. Hybridization of 5×10^6 cpm/ml ^{32}P-labelled nick-translated probes to filter-bound DNA was performed at 42 °C for 15 h. Following hybridization, the filters were washed three times at room temperature with 2 × SSC, 0·1 % SDS, for 15 min each, twice at 65 °C with 1 × SSC 0·1 % SDS, then twice with either 0·2 or 0·1 × SSC, 0·1 % SDS at 65 °C for 30 min.

3. Results and discussion

(i) *The vimentin gene is present as a single copy*

The human vimentin cDNA probe has previously been characterized and its nucleotide sequence determined (Perreau *et al.* 1988). Comparison of the human and hamster coding sequences shows 90% homology at the nucleotide level.

The probe used was an insert of 1·1 kb in pUC18. The probe extends from nucleotide 331 corresponding to amino acid 111 through to the stop codon. Southern blot analysis of mouse genomic DNA after digestion

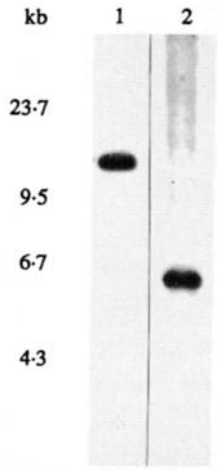

Fig. 1. Analysis of the vimentin gene in mouse DNA. All the slots were loaded with 20 μg of DNA digested to completion with *Bam* HI. After running overnight at 1 V/cm and Southern blotting, the filters were hybridized overnight with various ^{32}P-labelled probes (5×10^8 cpm/μg) 100 ng in 10 ml of hybridization buffer. Final washing was performed at 0·1 × SSC 30 min at 65 °C. Exposure was 15 h at −80 °C with an intensifying screen. Arrowheads indicate the sizes of bands. Lane 1, human DNA; lane 2, mouse DNA. Markers run on the gel were lambda phage digested with *Hin* dIII.

with *Eco* RI shows that the vimentin probe hybridized to a single restriction segment of mouse DNA, confirming that mouse vimentin is encoded by a single copy gene. Fig. 1 depicts a representative Southern Blot of mouse and human DNA. After hybridization to the nick-translated vimentin probe a single 12 kb band is observed for the human DNA and a 6·3 kb band for mouse DNA.

(ii) *The vimentin gene maps to mouse chromosome 2 region*

In situ hybridization experiments were carried out using metaphase spreads from a WMP male mouse in which all the autosomes, excepts 19, were in the form of metacentric roberstonian translocations.

In the 100 metaphase cells examined after *in situ* hybridization, there were 121 silver grains associated with chromosomes and 29 of these (23·9%) were located on chromosome 2; the distribution of grains on this chromosome was not random: 83% of them mapped to the proximal band of chromosome 2, with a maximum in the middle of this band, that is A2′ sub-band (Fig. 2).

These data unequivocally map the vimentin gene to the A2 band of mouse chromosome 2. In man, the vimentin gene is also unique (Lilienbaum *et al.* 1986) and has been localized to chromosome 10 (Quax *et al.* 1985), 10p13 (Ferrari *et al.* 1987) close to the interleukin-2 receptor gene (1L-2R) (Leonard *et al.* 1985). The two genes are growth-regulated in human lymphocytes and both vimentin mRNA and IL-2R mRNA are induced by interleukin-2 (Kaczmarek *et*

https://doi.org/10.1017/S0016672300028147 Published online by Cambridge University Press

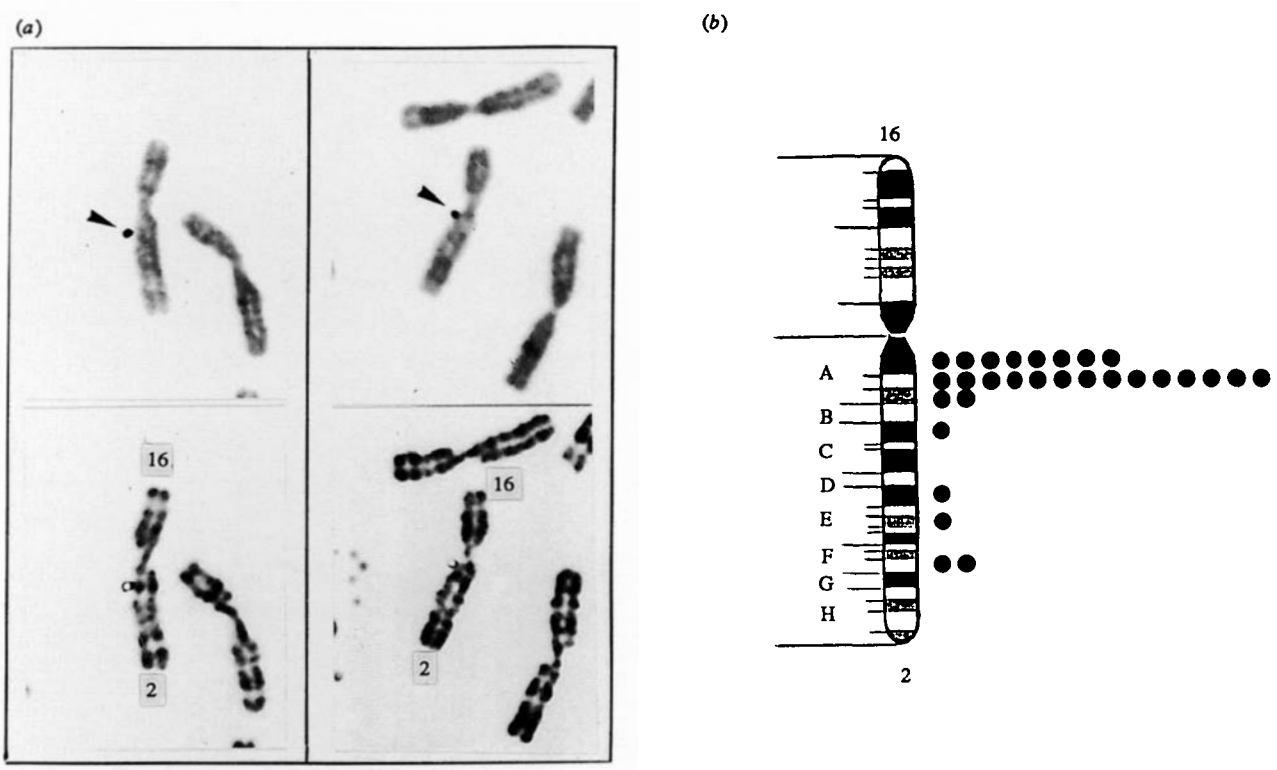

Fig. 2. (*a*). Localization of the vimentin gene to mouse chromosome 2 by *in situ* hybridization. A representative WMP mouse metaphase. *Top*; arrowheads indicate silver grains on giemsa-stained chromosomes, after autoradiography; *bottom*, chromosomes with silver grains were subsequently identified by R-banding. (*b*) Diagram of WMP mouse Rb (2;16) chromosome, indicating the distribution of labelled sites, with a maximum in the A2 sub-band.

al. 1985). It will be important to determine whether this location is conserved in both species.

This work was supported by grants from the Association Française contre les Myopathies. We are indebted to Dr Philip Avner for critical revision and Jeanne Freud for help in preparation of the manuscript.

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