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Chromosomal localisation of the mouse and human peripherin genes

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Summary

Using a mouse cDNA probe encoding for the major part of peripherin, a type III intermediate filament protein, we have assigned, by *in situ* hybridization, the mouse and human peripherin genes, *Prph*, to the E–F region of chromosome 15 and to the q12–q13 region of chromosome 12, respectively. These regions are known as homologous chromosomal segments containing other intermediate filament genes (keratins) and also other genes which could be co-ordinately regulated.

1. Introduction

Intermediate filament (IF) genes have been classified into several types according to the number and position of their respective introns (Steinert & Roop, 1988). These IF genes code for proteins which are built according to a common plan: a central α-helical rod domain whose amino acid sequence is highy conserved among IF proteins, flanked by head and tail domains which are more variable in sequence and length. Moreover, except for lamin genes which constitute the type V IF genes and which code for ubiquitous proteins (lamins A, B and C) assembled into the nuclear lamina located on the internal side of the nuclear membrane (Aebi *et al.* 1986), the other IF genes code for cytoplasmic polypeptides which are specific to the type of cell in which they are found: keratins, which constitute types I and II, are expressed in epithelial cells (Moll *et al.* 1982), type III genes code for vimentin detected in mesenchyme-derived cells (Franke *et al.* 1978), desmin in muscle cells (Lazarides & Hubbard, 1976), glial fibrillary acidic protein (GFAP) in astrocytes (Dahl & Bignami, 1973) and peripherin in well defined neuronal populations (Portier *et al.* 1984; Leonard *et al.* 1988; Parysek & Goldman, 1988; Escurat *et al.* 1990), type IV genes code for proteins found in neurons: the neurofilament triplet (Hoffman & Lasek, 1975) and α-anternexin (Patcher & Liem, 1985; Fliegner *et al.* 1990), and the single known type VI gene codes for nestin, a newly described class of IF expressed in central nervous system stem cells (Lendahl *et al.* 1990). Recent work shows that invertebrate IF are similar to vertebrate lamins whether they are expressed in neuronal or non-neuronal cells (Döring & Stick, 1990; Dodemont *et al.* 1990; Szaro *et al.* 1991); these authors thus propose that the different IF vertebrate genes evolved from a common ancestor which has some similarities with the lamin genes.

Chromosomal localization of several IF genes has already been achieved both in mouse and in man: the vimentin gene is located in region A2 of mouse chromosome 2 (Mattei *et al.* 1989*a*) and on human chromosome 10 (Quax *et al.* 1985); the desmin gene has been localized on mouse chromosome 1 band C3 (Li *et al.* 1990) and on human chromosome 2 (Quax *et al.* 1985) band 2q35 (Viegas-Péquignot *et al.* 1989); the NF-L gene has been assigned to mouse chromosome 14 in the region D1–E1 (Mattei *et al.* 1989*b*) and to human chromosome 8 band p21 (Hurst *et al.* 1987); interestingly, the NF-M gene has also been mapped to the same region as NF-L on human chromosome 8 (Hurst *et al.* 1987); the NF-H gene is located on mouse chromosome 11 (Dautigny *et al.* 1988) and on human chromosomes 22 and 1 (Lieberburg *et al.* 1989; Mattei *et al.* 1988). As for peripherin, its gene, *Prph*, has been mapped to the mouse chromosome 15 by the technique of somatic cell hybrids (Pendleton *et al.* 1991).

In this report, we describe the fine mapping of the peripherin gene in the mouse and human genomes using the *in situ* hybridization method and show that

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the mouse and the human peripherin genes map in regions of high homology, thus adding another locus to these conserved gene clusters.

2. Materials and methods

(i) *Preparation of chromosome spreads*

In situ hybridization experiments were carried out using metaphase spreads either from a WMP/Pas male mouse, in which all the autosomes, except 19, were in the form of metacentric robertsonian translocations, or from human lymphocytes. Mouse concanavalin A-stimulated or human phytohemagglutinin-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) *Probe preparation and* in situ *hybridization*

The mouse peripherin cDNA clone 5g, consisting of an insert of 1200 bp in pUC18 (Landon *et al.* 1989), was tritium labelled by nick-translation to a specific activity of $2{\cdot}2 \times 10^8$ d.p.m. μg^{-1}. The radiolabelled probe was hybridized to metaphase spreads at a final concentration of 25 ng/ml of hybridization solution as previously described (Mattei *et al.* 1985).

After coating with nuclear track emulsion (KODAK NTB2), the slides were exposed for 10–11 days at +4 °C, and 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 photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa (FPG) method, and metaphases were rephotographed before analysis.

(iii) *Southern hybridization of mouse genomic DNA*

The Southern blots were a generous gift of Dr Benoît Robert. Filters were hybridized with 4×10^6 cpm/ml ^{32}P-labelled nick-translated cDNA 3u probe (Landon *et al.* 1989) at 42 °C for 15 h. They were washed three times at room temperature with 2 × SSC, 0·1 % SDS for 15 min and once at 65 °C with 0·1 × SSC, 0·1 % SDS for 15 min.

3. Results and discussion

The peripherin probes, 5g and 3u, used in this study are cDNA inserts of 1·2 and 1·6 kb, respectively, in pUC18. They extend from the nucleotides corresponding to amino acids 183 and 63, respectively, through the poly(A) tail (Landon *et al.* 1989).

A Southern blot of mouse genomic DNA after digestion with BamHI shows that the peripherin cDNA 3u probe hybridizes to a single restriction fragment (Fig. 1). Other restrictions give a number of fragments compatible with the restriction maps of the mouse peripherin cDNA (Landon *et al.* 1989) and of the mouse peripherin gene (Karpov *et al.* in preparation). This suggests that the mouse peripherin gene is present only once in the haploid genome.

After *in situ* hybridization, 200 mouse and 100 human metaphase cells were examined. There were 497 silver grains associated with mouse chromosomes and 77 of these, i.e. 15·4%, were located on chromosome 15. The distribution of grains was not random: 87% mapped to the [E–F] region of chromosome 15 with a maximum in the 15F band (Fig. 2 (*a*) and (*b*). Similarly, there were 398 silver

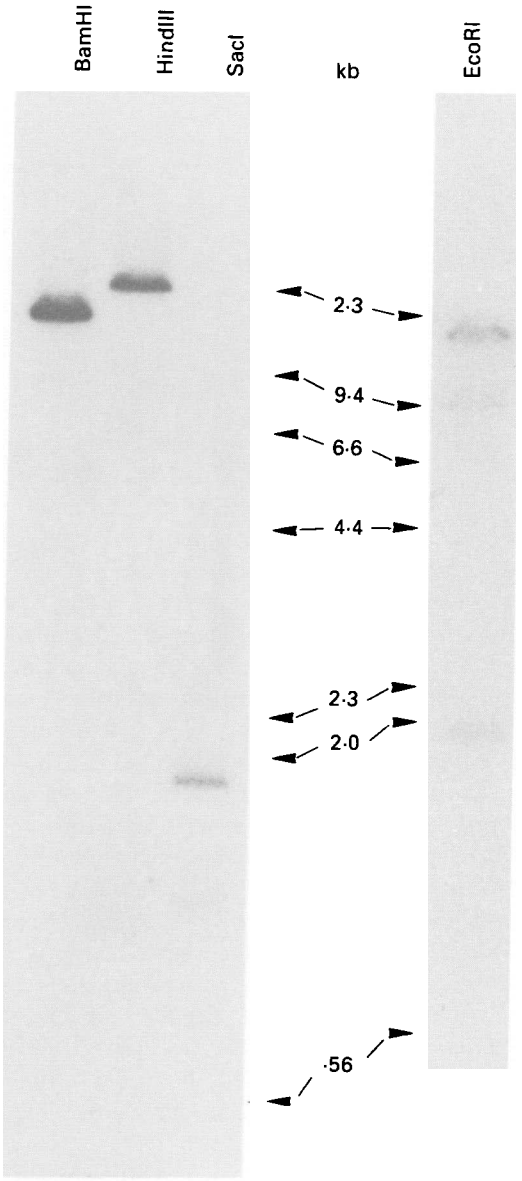

Fig. 1. Southern hybridization of mouse genomic DNA. Restriction fragments of the peripherin gene were probed with the labelled peripherin cDNA 3u. BamHI, one fragment of 12 kb; HindIII, two fragments of 13 and 1 kb; SacI, three fragments of 1·8, 1·1 and 0·7 kb; EcoRI, three fragments of 14·5, 10 and 2 kb.

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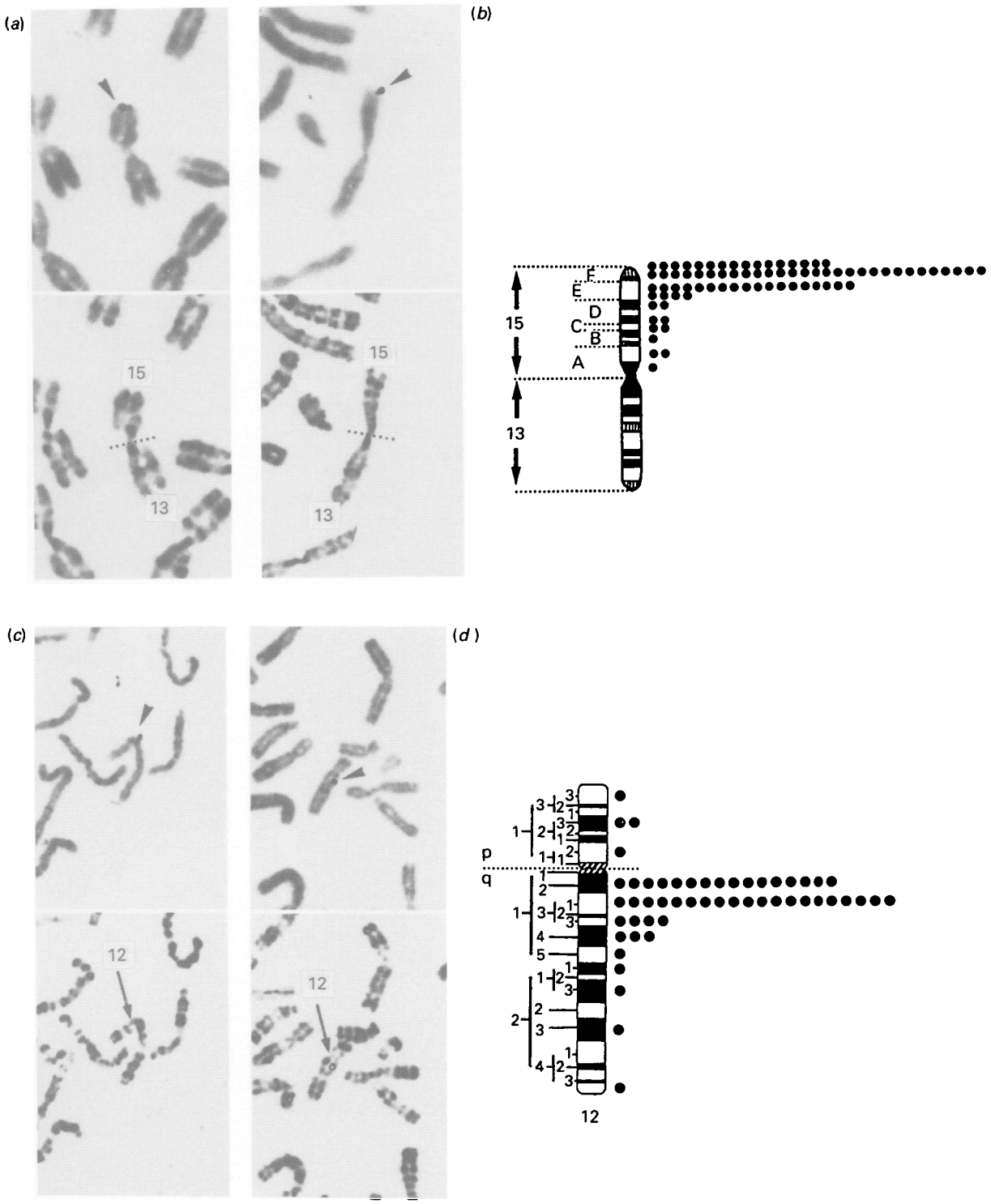

Fig. 2. Localization of the peripherin gene to mouse chromosome 15 and to human chromosome 12 by *in situ* hybridization. (*a*) and (*c*) Two partial WMP mouse (*a*) or human (*c*) metaphase spreads, showing the specific site of hybridization to chromosome 15 (*a*) or to chromosome 12 (*c*). Top: arrowheads indicate silver grains on Giemsa-stained chromosomes, after autoradiography. Bottom: chromosomes with silver grains were subsequently identified by R-banding. (*b*) Diagram of mouse Rb (13;15) chromosome (Lyon & Kirby, 1991), indicating the distribution of labelled sites on chromosome 15. (*d*) Idiogram of the human G-banded chromosome 12 (Craig & McBride, 1990) illustrating the distribution of labelled sites in the [12q12–12q13] region.

grains associated with human chromosomes and 52 of these, i.e. 13·1 %, were located on chromosome 12; of these 76·9 % mapped to the [q12–q13] region of the long arm of chromosome 12 with a maximum in the q13.1 band (Fig. 2 (*c*) and (*d*)). These results allow us to map the peripherin gene to the [15E–15F] region of the mouse genome and to the [12q12–12q13] region of the human genome.

The mouse peripherin gene, *Prph*, has been recently mapped to chromosome 15 (Pendleton *et al.* 1991).

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Table 1. *Homologous loci between human and murine genes*

Gene name	Chromosomes	
	Human	Mouse
Keratin 4 (type II acidic)*	12p12.2–q11	15 F
Homeo box region 3*	12q12–q13	15 F
Murine mammary tumor virus (v-int-1) oncogene homolog.*	12q13	15 F
Elastase 1, pancreatic*	12	15
Glycerol-3-phosphate deshydrogenase*	12	15 F
Phosphofructokinase, polypeptide X; phosphofructokinase-4*	12	15
Retinoic acid receptor gamma (Mattei *et al.* 1991)	12q13	15 F
Neuronal cell surface glycoprotein F3	12 (unpublished)	15 F (Gennarini *et al.* 1989)

* From Davisson *et al.* 1990.

The method that we have used shows that it maps to the region 15E–15F. We also show the mapping of the human peripherin gene to chromosome 12 in the [12q12–12q13] region. In fact, several genes that map to mouse chromosome 15 are located on the homologous chromosomal segment in human chromosome 12 (Davisson *et al.* 1990). These genes are listed in Table 1; interestingly, these conserved gene clusters include the type II cytokeratin 4 gene which is another IF gene and the homeobox-3 which is involved in regulation of vertebrate development as other Hox arrays. However, these homologous locations are not surprising since linkage groups have been shown to be conserved to a high degree in mouse and in man (Nadeau *et al.* 1989). Other IF genes have been mapped to human chromosome 12: keratin 18 (Waseem *et al.* 1990), type II keratin-like 1 and type II keratin-like 2 (Popescu *et al.* 1989). The co-localization of two gene families, IF and homeobox genes, may be important for co-ordinate regulation during embryonic development.

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