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The gene coding for the p68 calcium-binding protein is localised to bands q32–q34 of human chromosome 5, and to mouse chromosome 11

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Summary. The gene coding for human p68, a membrane-associated calcium-binding protein, has been assigned to chromosome 5, using a cDNA clone to probe genomic DNA from rodent-human somatic cell hybrids by Southern hybridisation. The gene was localised, by in situ hybridisation, to 5q32–34. The murine gene was assigned to chromosome 11, using a murine cDNA clone to probe genomic DNA from rodent-rodent somatic cell hybrids.

Introduction

p68 is a member of a unique family of proteins that bind membrane/cytoskeleton in a Ca^{2+}-dependent manner; these proteins are characterised by homologous amino acid sequences that are present in multiple copies in each protein. The family, which is known variously as calelectrins, annexins, calpactins, endonexins and lipocortins, lacks the "E-F hand" Ca^{2+}-binding amino acid sequences ascribed to the calmodulin family of Ca^{2+}-regulated proteins (Tufty and Kretsinger 1975) and may bind Ca^{2+} at a common phospholipid-binding site (Geisow et al. 1986; Crompton et al. 1988).

p68 is an intracellular monomeric protein of approximately 68,000 molecular weight that was first identified as a major component of the non-ionic detergent-insoluble fraction of purified B lymphoblastoid cell plasma membrane (Davies et al. 1984; Owens and Crumpton 1984). It has since been shown to be present in other cell types and tissues, such as fibroblasts and liver, but not intestinal epithelium brush border membranes (Davies and Crumpton 1985). The primary structure of human and mouse lymphocyte p68, which has now been deduced by cDNA cloning and sequencing, is internally repetitive, being constructed from eight repeats of varying lengths, each of which contains a highly conserved sequence (Crompton et al. 1988; Moss et al. 1988). This paper describes the chromosomal location of the human and murine p68 genes using cDNA clones as probes.

Materials and methods

The rodent-human somatic cell hybrids used in this study have been described previously; the relevant references are given in Table 1. The p68 cDNA clone A2 was isolated from a λgt11 expression library of the human T-leukaemia cell line J6 and comprised 2 kb containing most of the coding sequence (Crompton et al. 1988).

The mouse-hamster and mouse-rat somatic cell hybrids were derived as described by Hoggan et al. (1988) and Killary and Fournier (1984). The murine cDNA clone CF4.2 contained the entire coding sequence (Moss et al. 1988).

Southern blot analysis

Genomic DNA (15 μg) from human, rat, mouse and hamster cell lines or genomic DNA (20 μg) from human-rat, human-mouse, human-hamster, mouse-hamster and mouse-rat somatic cell hybrids were digested in a final volume of 200 μl for 16 h at 37°C with *Bgl*II, *Bam*HI or *Pst*1 (4u enzyme/μg DNA) using the buffer provided by the manufacturer (BRL). Samples were stored at −20°C for 4 days, thawed, reduced to 20 μl volume using a Speed vac and separated on 0.8% agarose gels, which were run at 40 V for 18 h in 89 m*M* Tris-borate buffer pH 8, containing 2 m*M* diaminoethanetetra-acetate. Gels were denatured and transferred to Hybond-N (Amersham) by the method of Southern (1975). Filters were neutralised in 50 m*M* Na phosphate buffer, pH 6.5 for 2 min, baked at 80°C for 2 h and then irradiated with ultraviolet light for 2 min. They were then prehybridised for 2 h prior to hybridisation for 18 h at 67°C in a solution of 0.9 *M* NaCl, 90 m*M* sodium citrate buffer pH 7, 0.5% sodium dodecyl sulphate, Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidine, 0.1% bovine serum albumin) and denatured salmon sperm DNA (250 μg/ml). The hybridisation fluid (25 ml) also contained 2×10^6 cpm/ml of ^{32}P-labelled cDNA (10 ng) and 10% dextran sulphate. cDNA was labelled with ^{32}P-dCTP (Amersham) by random hexanucleotide priming (Feinberg and Vogelstein 1983). Filters were washed for 15 min at 67°C, twice with 0.3 *M* NaCl, 30 m*M* sodium citrate buffer pH 7, 0.1% sodium dodecylsulphate, and twice with 30 m*M* NaCl, 3 m*M* sodium citrate pH 7, 0.1% sodium dodecyl sulphate. Autoradiography was carried out with Kodak XAR 5 film and intensifying screens.

In situ hybridisation

Human lymphocytes were cultured with phytohaemagglutinin for 72 h at 37°C; bromodeoxyuridine was then added to a final concentration of 200 μg/ml (Zabel et al. 1983). The cells were

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Table 1. Correlation of the presence of p68 sequences with individual human chromosomes in rodent-human hybrids. *Bam*H1 and *Bgl*II digests of DNA from the hybrid cells were analysed by Southern blotting using the p68 cDNA probe. *Spaces* indicate cell line not tested for this chromosome; *tr*, trace, i.e. less than 10% of cells contain this chromosome; S^1, presence or absence of chromosome 5 using p68

S^1	Cell line	Human chromosome																							Reference
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
+	DUR4.3	−	−	+	−	+	−	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	Solomon et al. 1976
+	MOG34A4	+	−	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−	+	+	−	+	−	+	Solomon et al. 1979
+	FG10	−	+	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	+	−	−	+	−	+	Kielty et al. 1982
+	CTP34B4	+	+	+	−	+	+	+	+	−	−	−	+	−	+	−	+	+	+	−	−	−	−	+	Jones et al. 1976
+	SIR19A	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	Whitehead et al. 1982
−	TWIN19-D12	+	−	+	+	−	+	−	+	−	−	+	+	−	+	−	+	+	+	−	+	−	−	−	Philips et al. 1985
−	SIR74ii	+	+	tr	+	−	−	−	−	−	−	−	+	+	+	−	−	+	tr	−	−	+	tr	+	Whitehead et al. 1982
−	CTP41A2	−	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	Jones et al. 1976
−	SIF15	−	+	−	−	−	+	+	−	−	+	−	−	−	+	+	−	−	−	−	+	−	−	+	Edwards et al. 1985
−	3W4CL5	−	−	−	−	−	−	+	−	−	+	+	+	−	+	+	−	+	−	−	−	+	−	+	Nabholz et al. 1969
−	FST9/10	−	−	+	+	−	+	−	+		+	−	+	+	+	+	−	−	+	−	+	−	+	+	Kielty et al. 1982
−	DT1.2	−	−	+	−	−	−	−	−	−	+	+	−	+	−	+	−	+	+	−	+	+	+	+	Solomon et al. 1976
−	HORP9.5	−	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	−	+	+	van Heyningen et al. 1975
−	DIS20	−	−	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	+	−	Carritt and Povey 1979
−	F4Sc13C112	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	Heisterkamp et al. 1982
−	1AA9[a]	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	+	—
−	CLONE21E	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Croce and Koprowski 1974
−	C10b2BU	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	Carritt 1980
−	FIR5R3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	Hobart et al. 1981
−	HORL9X	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	Heisterkamp et al. 1982
−	MOG13/10	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	Povey et al. 1980
−	C10b	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	Carritt 1980
No. discordant		6	5	7	7	0	7	6	3	7	7	7	6	5	9	6	4	6	6	4	9	6	11	13	

[a] Gift from David Cox, San Francisco

washed 16 h later with fresh medium and then incubated in thymidine-rich (10^{-5} *M*) medium for a further 6 h. Harvesting of the cells and further procedures were carried out in subdued lighting.

In situ hybridisation was performed as described by Harper and Saunders (1981). The A2 probe was labelled using oligoprimers and ^{3}H deoxyribonucleotides to a specific activity of 4.8×10^7 cpm/μg, and hybridisation was carried out for 16 h at 37°C with probe concentrations of 0.02 and 0.1 μg/ml. Slides were washed in 0.3 *M* NaCl, 30 m*M* sodium citrate pH 7 at 39°C, dehydrated and immersed in Ilford K5 emulsion. They were developed after 1–3 weeks and then stained using Wright's stain.

Results and discussion

Localisation of the human p68 gene by Southern blot analysis

Human genomic DNA was digested with a variety of restriction endonucleases before analysis by Southern blotting. *Bgl*II and *Bam*HI produced restriction fragments of 11.2, 8.9, 5.9, 4.1, 3.9, 3.8 kb and 15.8, 11.7, 8.3, 5.0 kb respectively that were clearly defined and of high intensity as revealed by the A2 probe. The 11.2, 4.1, 3.9 kb and 3.8 kb *Bgl*II fragments and 11.7 and 5.0 kb *Bam*HI fragments proved to be diagnostic, as no bands of these sizes were detected when rat, mouse or hamster DNA was digested with these enzymes. The A2 probe also hybridised to restriction fragments from all three rodent cells. This was not unexpected as mouse and human cDNA sequences have been shown to have approximately 90% homology (Moss et al. 1988).

*Bgl*II was used for an initial screen of 14 human-rodent hybrids. Hybrids DUR4.3, MOG34A4 and CTP34B4 had the diagnostic pattern of restriction fragments clearly visible, while hybrids SIR19A and FG10 gave weakly positive results with most of the characteristic bands present. The 3 unequivocably positive hybrids all had human chromosomes 3, 5, 12, 14, 18 and X in common (see Table 1). A further nine rodent-human hybrids and FG10 were then digested with *Bgl*II and *Bam*HI and their pattern of restriction fragments analysed. This time FG10 gave a clearly positive pattern of bands with both enzymes. All other hybrids were negative and only had bands corresponding to the rodent digest controls. FG10 had human chromosomes 5, 18 and X in common with DUR4.3, MOG34A4 and CTP34B4. However, both of the hybrids FIR5R3, which retained human chromosomes 14 and 18, and HORL9X, which retained only human chromosome X, were negative. Table 1 summarises the correlation between the presence of p68 hybridising fragments in various somatic cell hybrids and the presence of human chromosomes. It can be seen that the highest degree of concordance is for chromosome 5. As all hybrids retaining human chromosome 5 gave positive signals and no positive signals were detected when chromosome 5 was absent, it was concluded that the human p68 gene probably resides on this chromosome.

Localisation of the human p68 gene by in situ hybridisation

Metaphase spreads (53) of human chromosomes were probed with the A2 probe, which had been labelled with tritium. Figure 1 shows a representative chromosome spread with a silver grain on band q32 of chromosome 5 (see arrow). A total of 153 grains were scored (see Fig. 2), 31 (20.3%) of which were located on the long arm of chromosome 5 and, of these, 20 grains (13.1%) were located over bands 5q32 to 5q34. The exact location of each grain on chromosome 5 can be seen in Fig. 3.

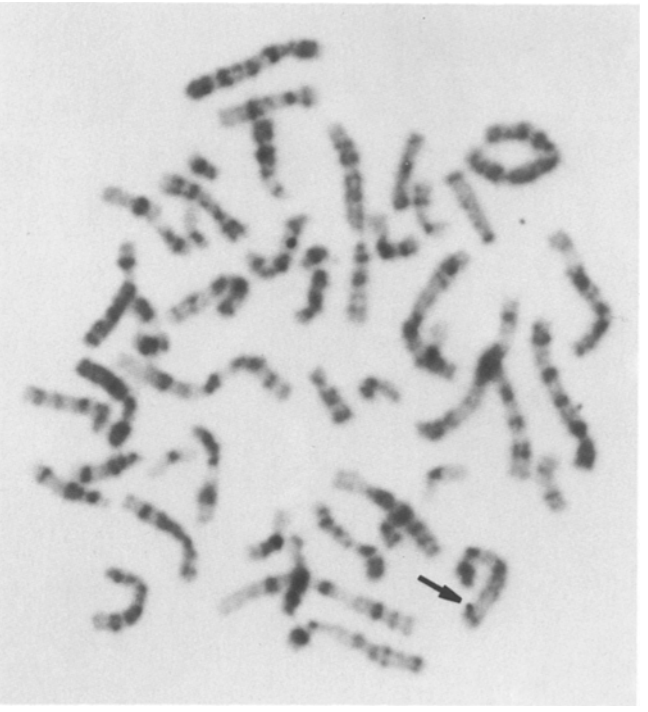

Fig. 1. In situ hybridisation of the p68-cDNA probe to human metaphase chromosomes after staining with Wright's stain to reveal silver grains

Thus, the in situ hybridisation confirms the assignment of the gene for human p68 to chromosome 5 and further refines this assignment to bands 5q32 to 5q34. This region of chromosome 5 is rich in the genes that code for growth factor receptors (CSF1R and PDGFR) and growth factors (IL3, IL4, IL5, CSF1 and CSF2) (Olaisen et al. 1988; Sutherland et al. 1988). The location of the gene for p68 is interesting in view of the fact that it is a plasma membrane-associated intracellular protein and could therefore respond directly to rises in Ca^{2+} levels induced by these receptors; adjacent chromosomal locations may reflect some sort of functional association.

Diverse chromosomal locations have been found for the genes encoding other members of the family of Ca^{2+}-binding proteins that are structurally related to p68. Thus lipocortin I and IIb are on chromosome 9 (9q11–22 and 9pter–q34, respectively), whereas lipocortins IIa, IIc and IId are located on chromosomes 4q21–31, 10q21–22 and 15q21–22, respectively (Huebner et al. 1987). The genes for other "families" of proteins, such as the immunoglobulins and collagens, and even the genes for the different subunits of protein kinase C (Coussens et al. 1986) all have diverse chromosomal distributions. A clearer picture of the genetic relationships between various members of the family of Ca^{2+}-binding proteins will emerge as the chromosomal locations of their genes are established.

Localisation of the murine p68 gene by Southern blot analysis

Hamster, mouse and rat genomic DNAs were digested with a selection of restriction endonucleases and probed with clone

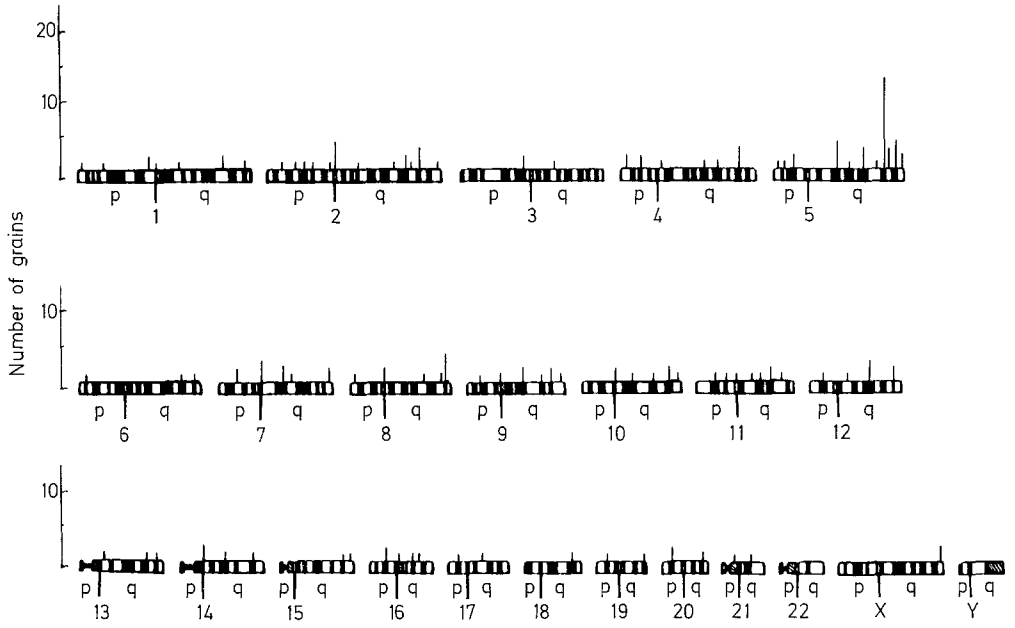

Fig. 2. Diagram showing silver grain distribution over chromosomes of normal lymphocytes following in situ hybridisation with the p68-cDNA probe

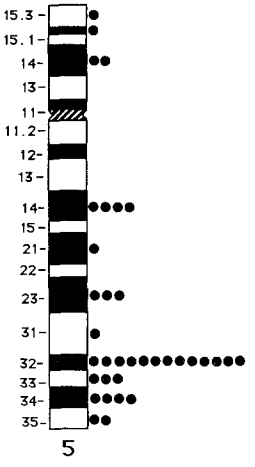

Fig. 3. Diagram showing a detailed analysis of silver grain distribution over chromosome 5 following in situ hybridisation with the p68-cDNA probe

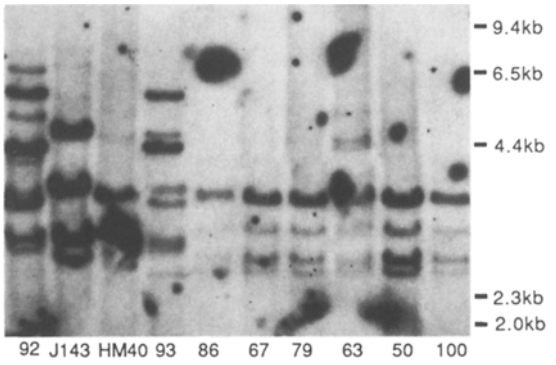

Fig. 4. Southern blot probed with murine cDNA of *Pst*1 digests of rat, mouse, and hamster genomic DNAs (*lanes 1–3* respectively), rat-mouse hybrid DNA (*lane 4*) and a series of hamster-mouse hybrid DNAs (*lanes 5–10*)

Table 2. Correlation of p68 cDNA hybridisation with specific mouse chromosomes in hamster-mouse hybrids. Not all hybrids were fully karyotyped

Mouse chromosome	No. of hybrid clones p68 gene/chromosome				Percent discordant
	+/+	−/−	+/−	−/+	
1	0	8	0	7	47
2	0	4	0	11	73
3	0	4	0	5	56
4	0	8	0	7	47
5	0	12	0	3	20
6	0	5	0	10	67
7	0	4	0	11	73
8	0	9	0	6	40
9	0	9	0	6	40
10	0	12	0	3	20
11	0	11	0	0	0
12	0	3	0	6	67
13	0	5	0	10	67
14	0	10	0	2	17
15	0	1	0	7	88
16	0	7	0	5	42
17	0	4	0	10	71
18	0	5	0	7	58
19	0	9	0	5	35
X	0	6	0	9	60

CF4.2 to identify an enzyme that would produce clearly definable fragments diagnostic of each species. On the basis of this criterion, a selection of hamster-mouse somatic cell hybrid DNAs, and a single rat-mouse hybrid DNA were digested with *Pst* 1, and analysed by Southern blotting (Fig. 4). The murine p68 gene fragments included two of 4.9 and 3.8 kb, which were clearly absent in the rat and hamster genomic DNAs. The species cross-reactivity of the murine CF4.2 probe was not unexpected in view of the high degree of sequence conservation (Moss et al. 1988). A total of 15 hamster-mouse hybrids were screened, none of which revealed the above two diagnostic murine bands (Table 2). However, a single rat-mouse hybrid (namely 93, Fig. 4), which retained murine chromosome 11 only, contained both of these bands. Since none of the hamster-mouse hybrids contained murine

chromosome 11, and the rat-mouse hybrid contained only chromosome 11, the murine p68 gene was therefore assigned to this chromosome. Mouse chromosome 11 has homologies on at least six human chromosomes, with one of the best known conserved linkage groups mapping to human chromosome 17 (Searle et al. 1987). Only two genes assigned to murine chromosome 11 have previously been assigned to human chromosome 5, namely GM-CSF (Gough et al. 1984; Huebner et al. 1985) and interleukin 3 (Ihle et al. 1987).

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