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The human neurofilament gene (NEFL) is located on the short arm of chromosome 8

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Abstract. We have localized the gene coding for the human neurofilament light chain (NEFL) to chromosome band 8p2.1 by Southern blotting of DNA from hybrid cell panels and in situ hybridization to metaphase chromosomes.

Mammalian neurofilaments are composed of three neuron-specific proteins, NEFL, NEFM, and NEFH, with a molecular weight of 68,000, 145,000, and 200,000 Daltons, respectively. Although nerve-cell specific, they share homologous α-helical domains with other types of intermediate filaments. NEF proteins differ in size due to different-length COOH termini (Geisler et al., 1983; Julien and Mushynski, 1983), which all contain high levels of charged amino acids, as well as multiple phosphorylation sites in NEFL and NEFM (Julien and Mushynski, 1982; Geisler et al., 1983, 1985; Wong et al., 1984; Carden et al., 1985; Julien, unpubl. data; Myers et al., 1986). The cDNAs and genomic copies of each of the genes have been isolated (Julien and Mushynski, 1982; Lewis and Cowan, 1985, 1986; Myers et al., 1986; Julien et al., 1987), and murine NEFL (Lewis and Cowan, 1986) and human NEFL (Julien, unpubl. data) and NEFM (Meyers et al., 1986) have been completely characterized. All the genes are probably linked in a multigene family, because the murine NEFL and NEFM genes have been linked by cosmid cloning (Julien et al., 1987). The function of the NEF genes is unknown, but they presumably play an essential role in maintaining the neuronal cell. In this paper we report the chromosomal localization of the human NEFL gene by Southern blot hybridization of hybrid cell panels and in situ hybridization to metaphase chromosomes.

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Materials and methods

NEF recombinant clones. Human NEFL was isolated from a cosmid library, using blood-cell DNA and the vector pTCF (Grosveld et al., 1982). Positive colonies were detected after hybridization with mouse cDNA (Julien, unpubl. data). The human NEFL gene was positively identified by DNA-mediated gene transfer and DNA sequence analyses (Julien, unpubl. data).

In situ hybridization. Metaphase chromosomes were prepared from peripheral blood cells by standard procedures (Hagemeijer et al., 1979). The NEFL DNA probe was ^{3}H-labeled by nick translation and hybridized to RNAse-treated, denatured chromosomes for 16 h at 37 °C (Bertram et al., 1983). Slides were washed 3 times for 15 min each in 50% formamide, 2 × SSC at 39 °C, then for 2 min in each of five changes of 2 × SSC alone at 39 °C and, finally, for 6 h at room temperature with changes of 2 × SSC every 2 h. After dehydration in ethanol, slides were dipped in Ilford K2 nuclear track emulsion, diluted 1:1 with H_2O, at 42 °C. Slides were exposed for 18 days in a dessicated box at 4 °C and then developed. Chromosomes were subsequently heat-denatured and reverse-banded

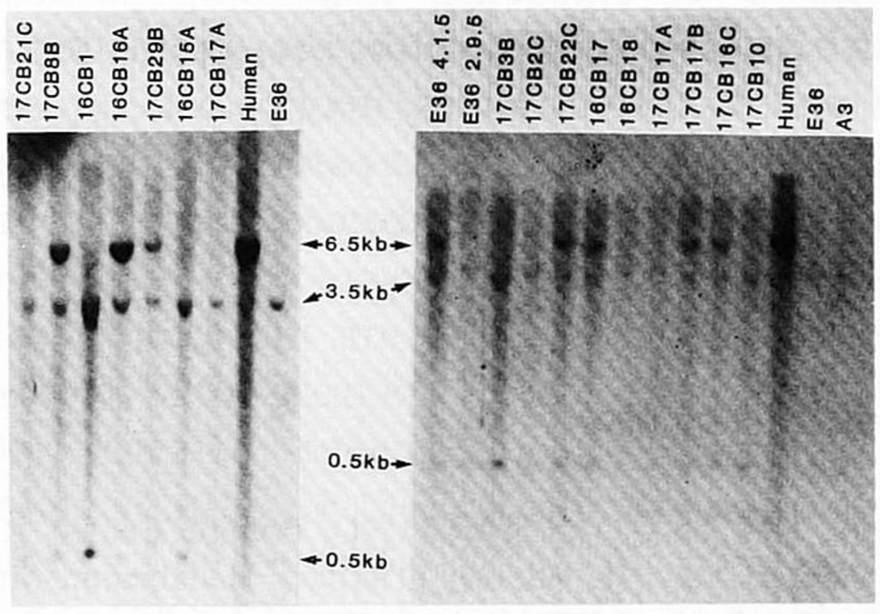

Fig. 1. Southern blot hybridization of a human *Sma*I-*Eco*RI NEFL probe to *Eco*RI-digested DNA from human-hamster hybrid cells. *Hin*dIII-digested λ DNA was used as size markers. The sizes of the hybridizing human (6.5 kb) and hamster (3.5 and 0.5 kb) bands are indicated. The numbers on top indicate the hybrid cell lines tested (Table I).

with acridine orange to show chromosomal bands and grains by exposing the same metaphases to UV and normal light.

Southern blotting. The human-hamster cell lines have been described (Bartram et al., 1983; Geurts van Kessel, 1984). All cell lines were analyzed for chromosomal content by identification of the chromosomes in at least 10 metaphase spreads. DNA (10 μg) from each cell line was digested with *Eco*RI, using λ DNA as an internal control marker for complete digestion. Digested DNA was electrophoresed in a 0.7% agarose gel, Southern blotted, and then hybridized by standard procedures (Southern, 1975; Jeffreys and Flavell, 1977).

Results and discussion

To map the neurofilament gene to a particular chromosome, a 4.3-kb *Sma*I-*Eco*RI fragment containing all four exons (Julien, unpubl. data) was isolated and used as a hybridization probe in two procedures: hybridization to Southern blots of DNA from a panel of human-hamster hybrid cell lines (Bartram et al., 1983; Geurts van Kessel et al., 1984) and in situ hybridization to fixed metaphase chromosomes. The Southern blots of *Eco*RI-digested DNA showed a

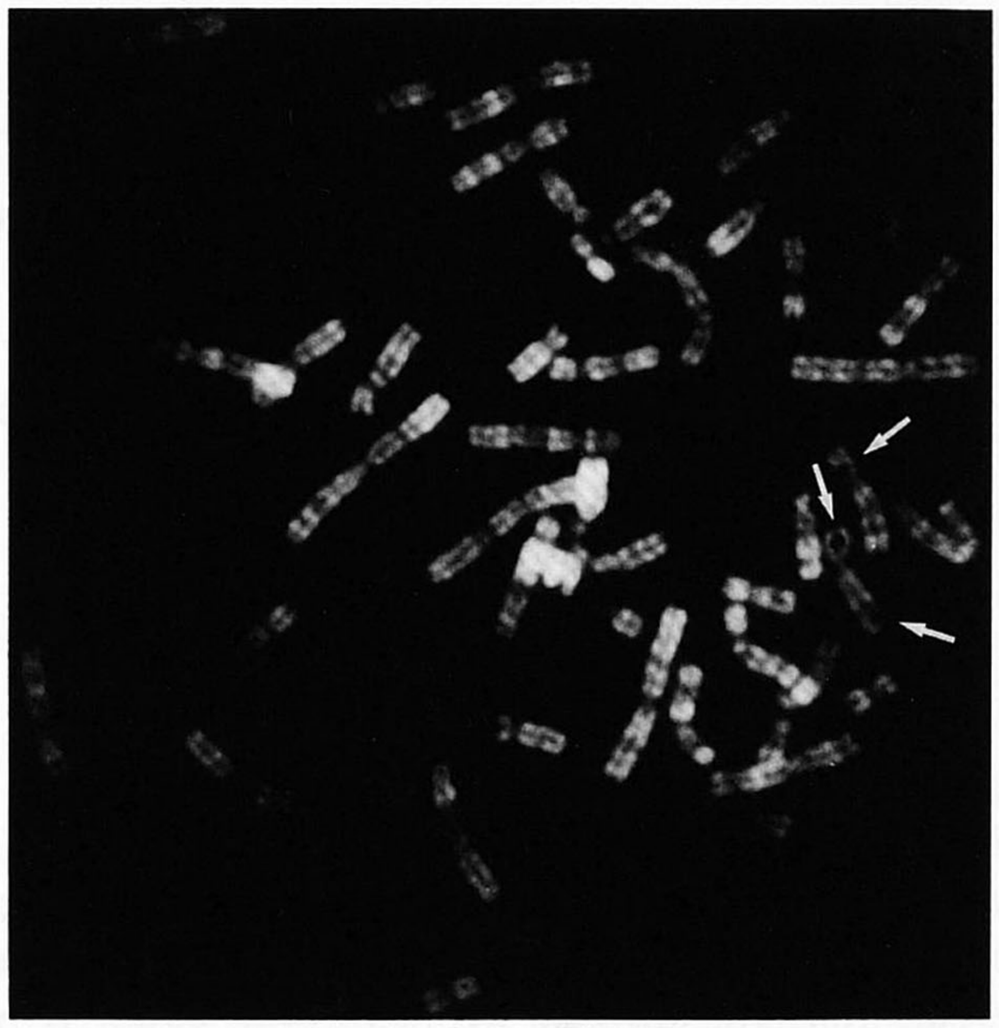

Fig. 2. In situ hybridization of the human NEFL probe to human metaphase chromosomes. UV-light image of a complete chromosomal spread (see Materials and methods). The arrows indicate grains at positions 8p2.1 (2×) and 8q (background).

Table I. Localization of human NEFL gene[a]

Hybrid	Human chromosome																							Presence of NEFL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
E36 4.1.5	+	+	+	±	+	+	±	+	+	+	+	+	±	+	−	+	+	+	+	+	−	+	±	−	+
E36 2.9.5	+	+	+	+	−	+	−	±	−	+	−	+	−	+	+	+	−	+	+	−	+	+	+	−	+
17CB3B	+	+	−	+	+	+	+	−	+	−	−	+	+	+	+	+	+	−	+	+	+	−	−	−	−
17CB2C	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−
17CB22C	−	±	+	±	−	+	+	+	+	+	−	−	+	−	−	+	±	−	−	+	+	+	+	−	+
16CB17	+	−	+	+	−	−	−	+	−	−	−	+	−	+	−	+	+	+	+	−	+	+	−	−	+
16CB18	−	±	+	+	−	−	±	−	+	−	+	−	−	+	+	+	−	−	−	+	±	+	+	−	−
17CB17A	−	−	−	±	−	−	−	−	−	−	+	−	−	−	−	+	±	−	+	−	−	−	−	−	−
17CB17B	−	−	+	+	±	+	−	+	+	+	+	+	−	−	−	−	+	+	+	−	+	+	−	−	+
17CB16C	+	−	+	+	−	+	−	+	+	−	−	+	−	±	−	−	+	+	±	+	−	−	−	−	+
17CB10	−	−	−	+	−	+	−	−	+	−	+	+	−	+	−	−	+	+	±	+	+	−	±	±	−
16CB15A	+	−	+	+	−	+	−	−	−	−	+	−	+	+	−	−	+	−	+	+	+	+	−	−	−
17CB29B	−	−	−	−	−	+	+	+	−	+	+	+	+	−	±	−	+	−	+	−	+	−	−	−	+
16CB16A	−	−	−	+	−	+	−	+	+	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	+
16CB1	+	−	+	+	+	+	−	−	−	−	+	−	−	+	+	+	+	+	+	+	−	+	−	−	−
17CB8B	−	−	−	+	+	+	−	+	+	+	+	+	±	−	−	+	+	−	+	±	+	+	±	−	+
17CB21C	−	−	−	+	−	−	+	−	+	−	−	+	−	+	−	−	+	−	+	+	+	−	±	−	−
17CB15B	−	±	+	+	+	−	−	−	−	+	+	+	+	−	−	+	+	±	±	+	+	+	−	+	−
Discordant	8	9	7	9	9	6	10	0	7	4	11	6	8	11	10	8	9	7	9	12	9	7	8	11	out of 18

[a] Human chromosome content of human-hamster cell hybrids as determined by karyotype analysis: + and − indicate the presence or absence of the particular chromosome; ± indicates the presence of a chromosome but in <10% of the hybrid cells (which might result in very low human NEFL hybridization signals (as in, e.g., hybrid E36 2.9.5 [see Fig.1]). Right-hand column: presence of human NEFL gene as revealed by Southern blot hybridization (Fig. 1).

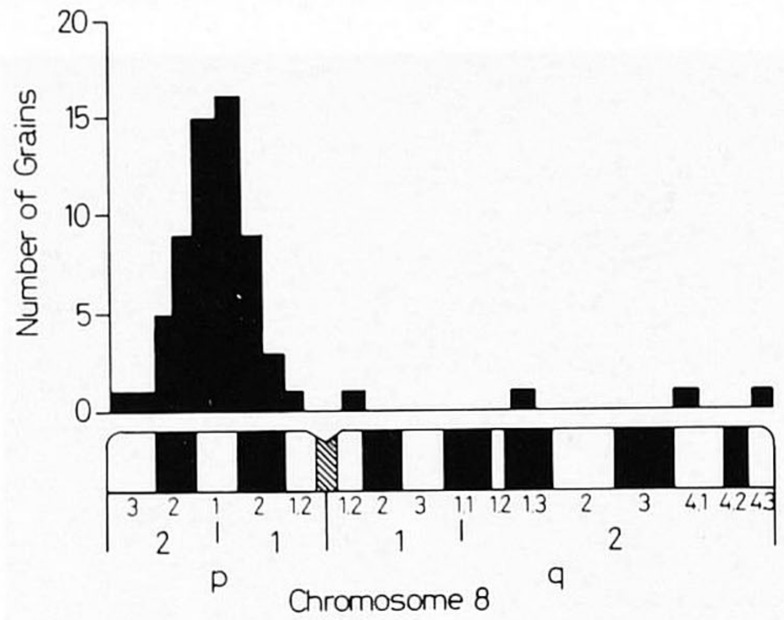

Fig. 3. A map of chromosome 8 with a histogram of the number of grains counted at each position.

positive 6.5-kb human NEFL hybridization band in nine of the DNA samples (Fig. 1). In addition, all lanes showed cross-hybridization to a 3.5-kb hamster NEFL fragment. Tabulation of these results (Table I) reveals that the human hybridization signal is obtained only when human chromosome 8 is present.

The same probe was used for hybridization to fixed metaphase chromosomes obtained from cultured blood cells (Hagemeijer et al., 1979). The probe was ^{3}H-labeled by nick translation and hybridized to RNAse-treated and denatured chromosomes (Bartram et al., 1983). After autoradiography and staining of the chromosomes (Fig. 2), 158 grains were counted on chromosomes in 47 metaphase spreads containing a full chromosome complement. Of these, 64 were found to be on chromosome 8 ($p < 0.0001$), while the remaining 94 were randomly distributed over all chromosomes. Of the 64 grains, 60 were distributed over the chromosome region 8p1→p2 (Fig. 3). We conclude that the human NEFL gene is located at 8p2.1. Other known markers in this region are glutathione reductase (8p2.1), plasminogen activator (8p12), and possibly luteinizing hormone releasing hormone (8p21→p11.2) (McKusick, 1986).

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