

Localization of the Human NCAM Gene to Band q23 of Chromosome 11: The Third Gene Coding for a Cell Interaction Molecule Mapped to the Distal Portion of the Long Arm of Chromosome 11

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Abstract. cDNA clones containing sequences coding for the murine neural cell adhesion molecule (N-CAM) were used in Southern hybridizations on human genomic DNA and demonstrated ~90% homology between human and murine NCAM genes. In situ hybridization with one of these clones was performed on human metaphase chromosomes and allowed the localization of the human NCAM gene to band q23 of chromosome 11. The genes for two other cell surface molecules believed to be involved in cell-cell interac-

tions, Thy-1 and the δ chain of the T3-T cell receptor complex, have recently been localized to the same region of chromosome 11 in man. Moreover, this region of the human chromosome 11 appears to be syntenic to a region of murine chromosome 9 that also contains the *staggerer* locus: *staggerer* mice show abnormal neurological features which may be related to abnormalities in the conversion of the embryonic to the adult forms of the N-CAM molecule.

THE cell surface glycoprotein called N-CAM¹ (neural cell adhesion molecule) is involved in the formation of cell-cell bonds, probably by a like-with-like interaction mechanism (for reviews, see Rutishauser, 1983; Edelman et al., 1983; and Goridis et al., 1983). This molecule was initially identified in chick retina and brain (Rutishauser et al., 1976); it is also present in muscle cells (Grumet et al., 1982). Similar molecules have been described in other vertebrate species including man (McClain and Edelman, 1982; Rougon et al., 1982; Hoffman et al., 1984; and Lyles et al., 1984). In all cases different forms of N-CAM are found which differ in the degree of sialylation and in polypeptide chain length during development.

The isolation of cDNA clones for chicken (Murray et al., 1984) and murine (Goridis et al., 1985) N-CAMs has made possible Southern and Northern blotting experiments (Goridis et al., 1985; Gennarini et al., 1986) as well as isolation of genomic clones in the mouse (Goridis, C., and M. Steinmetz, unpublished results). These experiments indicate the existence of a single NCAM gene which gives rise to the different forms of the molecule through a combination of transcriptional, posttranscriptional, and posttranslational events.

Although direct cloning of the human NCAM gene has not

yet been performed, the fact that monoclonal antibodies against mouse N-CAM react with a similar molecule in human cells (McClain and Edelman, 1982) suggested close relationships between these products and possibly their genes. As shown in this paper, murine N-CAM probes can be successfully used both for Southern hybridization with human DNA and for localization by in situ hybridization on human metaphase chromosomes. The in situ hybridization data allow the assignment of the human NCAM gene to the distal portion of the long arm of chromosome 11.

Materials and Methods

Probe Labeling and Hybridization Conditions for Southern blots

The pM1.3 and pM3.7 N-CAM cDNA clones (0.6 and 0.7-kb insert, respectively) (Goridis et al., 1985) were subjected to oligolabeling with ³²P nucleotides. The resulting specific activity of the probes was $\sim 2 \times 10^8$ dpm/ μ g. EcoRI cut human DNA (from peripheral blood lymphocytes) and mouse DNA (from the C1300-derived N2a neuroblastoma cell line) was fractionated on a 0.5% agarose gel (10 μ g/lane) and transferred to nitrocellulose filters along with end-labeled markers (HindIII cut DNA labeled by the exchange reaction using the Klenow fragment of DNA polymerase I). The filter was prehybridized overnight at 68°C in 2 \times standard saline citrate (SSC [1 \times SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4]), 0.1% SDS, 2 \times Denhardt's mix, and 100 μ g/ml denatured salmon sperm DNA. The hybridization mix (5 ml total volume) contained in addition 10% Dextran sulphate and 2 $\times 10^7$ dpm (0.1 μ g) of probe. Hybridization was for 24 h at 68°C and was followed by successive washes in 2 \times SSC, 1 \times SSC, and 0.5 \times SSC at 68°C before autoradiographic exposure (48 h). An

¹ Abbreviations used in this paper: N-CAM, neural cell adhesion molecule; NCAM, the corresponding gene (according to the Nomenclature Committee of the 8th Human Gene Mapping Workshop, 1985); SSC, standard saline citrate (1 \times SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4).

additional wash in 0.2× SSC (results not shown) abolished the signal with human (but not with murine) DNA.

In Situ Hybridization

The pM1.3 probe was labeled by oligolabeling (Feinberg and Vogelstein, 1984) with three ³H-labeled oligonucleotides to a specific activity of 8 × 10⁷ dpm/μg. The probe was redissolved in 2× SSC, 40 mM NaH₂PO₄, 0.1% SDS, 1× Denhardt's mix, 50% formamide, and 10× Dextran sulphate at a concentration of 0.1 μg/ml; 25-μl aliquots were placed on chromosome spreads under a coverslip and incubated at 42°C in a 50% formamide, 2× SSC saturated environment. After hybridization (16 h at 42°C), the coverslips were removed and the slides rinsed three times in 50% formamide, 2× SSC (pH 7.0) at 39°C, then twice in 2× SSC at 39°C, once in 0–1× SSC at room temperature, and once in 0–1× SSC at 4°C before dehydration and air drying. Autoradiography, staining, banding, and analysis were performed as previously described (Mattei et al., 1985a and b).

Results

Murine N-CAM Probes Reveal Distinct Bands in Southern Blots of Human DNA

The two cDNA clones used in this work were obtained by screening a λgt11 expression library with two monoclonal antibodies directed against sequence determinants on the murine N-CAM molecule (Goridis et al., 1985). Recent data from cosmid cloning and chromosome walking experiments (Goridis, C., and M. Steinmetz, unpublished observations) indicates that the pM1.3 (0.6-kb insert) and pM3.7 (0.7-kb insert) clones originate from a single murine NCAM gene and that pM3.7 corresponds to sequences 3' of those recognized by pM1.3. This is in agreement with the previously determined localization on the N-CAM molecule of the epitopes recognized respectively by monoclonals H28 (which reacted with products from the λgt11 clone containing pM1.3 sequence) and P61 (which reacted with products from the λgt11 clone containing pM3.7 sequences) (Gennarini et al., 1984), i.e., with the fact that P61 reacts with determinants closer to the COOH terminal than those recognized by H28.

Fig. 1 shows the pattern observed in Southern blots of Eco RI-cut human or mouse DNA probed with pM1.3 (*left*) and pM3.7 (*right*). The small number of bands suggests that in man (as in the mouse) there is a single NCAM gene; the different patterns observed with the two probes are consistent with the fact that the NCAM gene is larger than 50 kb (Goridis, C., and M. Steinmetz, unpublished experiments), therefore small probes corresponding to different exons will generally reveal different bands.

Results shown in Fig. 1 were obtained under conditions of moderate stringency (see Materials and Methods). When the blots are washed at higher stringency the signal on human DNA is essentially absent while the signal on mouse DNA remains. The level of divergence between the murine and human sequences can be estimated from this data to be of the order of 10% (Beltz et al., 1983), i.e., the homology between the murine pM1.3 and pM3.7 probe sequences and the human target N-CAM sequences is of the order of 90%. This high homology suggested that direct in situ hybridization on human chromosomes might be feasible.

In Situ Hybridization with a Murine Probe Localizes the Human NCAM Gene to Band q23 of Chromosome 11

In situ hybridization was performed on metaphase chromosomes using the pM1.3 probe labeled with [³H]thymidine.

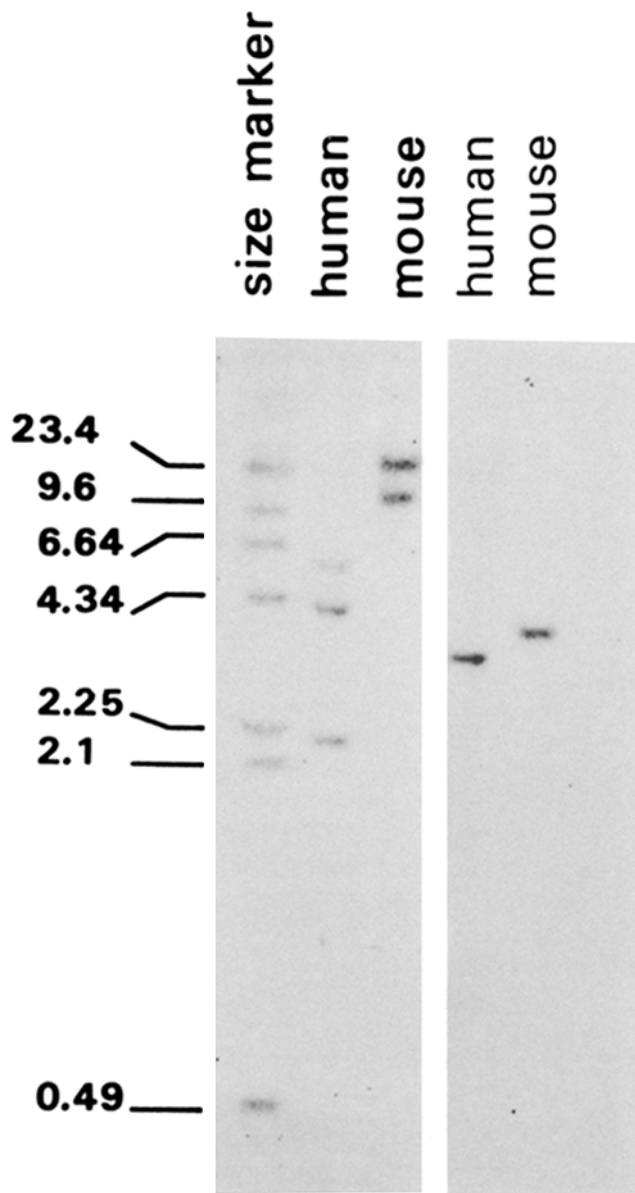


Figure 1. Southern hybridization of Eco RI cut human and mouse DNA with the murine N-CAM pM1.3 and pM3.7 probes. (*Left*) pM1.3 probe; (*right*) pM3.7 probe. Size markers (*extreme left*) are end-labeled Hind III-digested DNA. The blot was hybridized and washed under moderately stringent conditions (final wash 0.5× SSC at 68°C; see Materials and Methods).

After autoradiographic exposure and development the chromosomes were subjected to Giemsa staining and a photograph on which the silver grains are clearly visible was taken (Fig. 2A). To allow more precise localization, the chromosomes were then subjected to R-banding by the fluorochrome-photolysis-Giemsa technique and photographed again (Fig. 2B). Thus both precise localization and clear detection of grains (which often become invisible after R-banding) are possible. Fig. 2 shows a complete metaphase in which a single silver grain is seen on chromosome 11.

Fig. 3 shows the overall statistics from an experiment in which 200 metaphases were analyzed. In spite of the existence of a significant background on all chromosomes, a clear signal

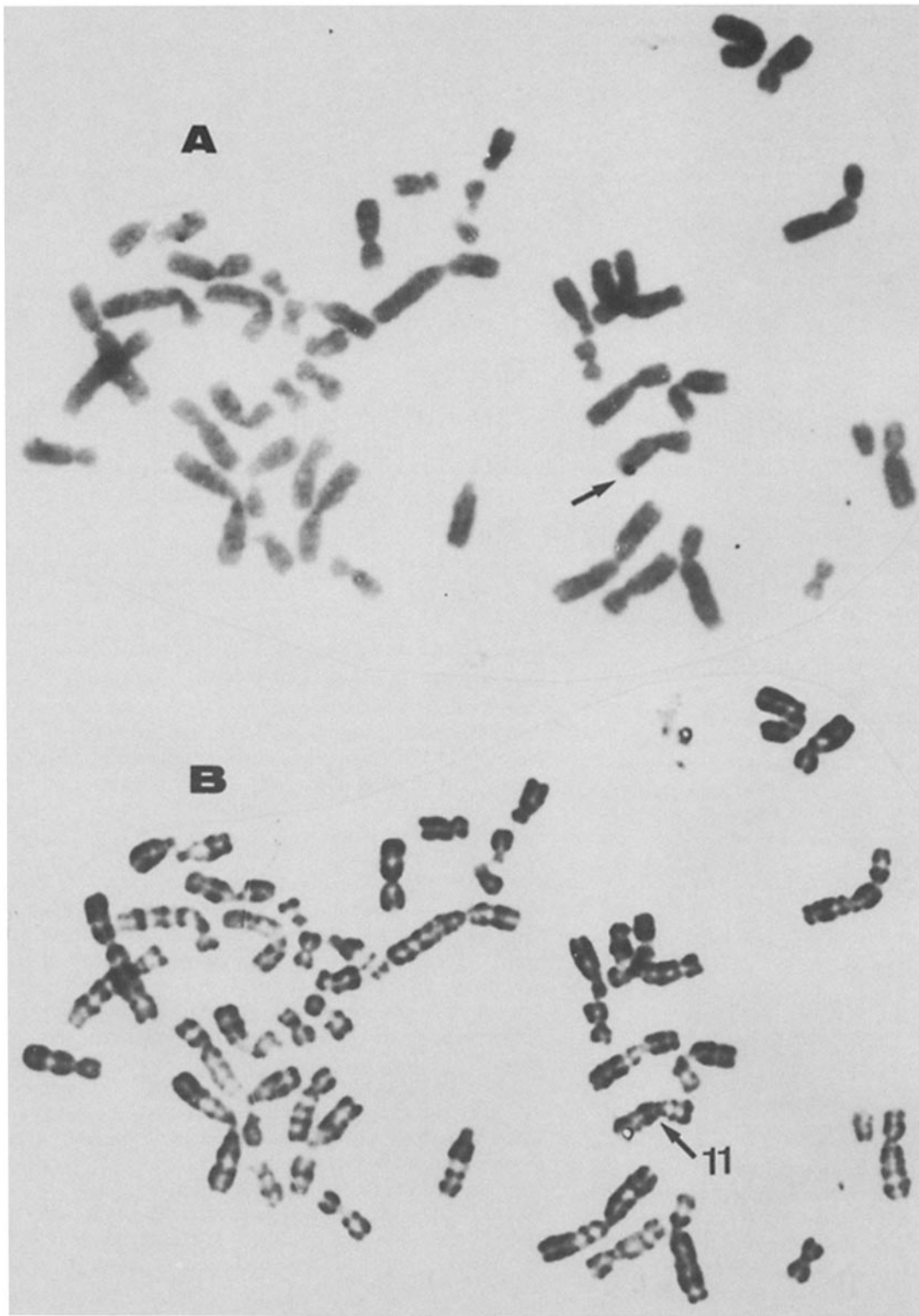


Figure 2. In situ hybridization of human mitotic chromosomes with the N-CAM pM1.3 probe. (A) Giemsa-stained chromosomes after autoradiography. (B) The same metaphase after subsequent R-banding to identify the chromosomes. A single grain is seen near the end of the long arm of chromosome 11 (top) identified by R-banding (bottom).

is visible for the long arm of chromosome 11. A more detailed indication of the regional localization of grains on chromosome 11 is shown in Fig. 4 (in which more different bands are scored). The distribution clearly peaks at band q23 and indicates this as the most probable localization for the human NCAM gene.

Discussion

The Human NCAM Gene Can Be Located by In Situ Hybridization with Murine Probes

The data of Figs. 3 and 4, while not up to the standard of good in situ hybridizations with long homologous probes (see,

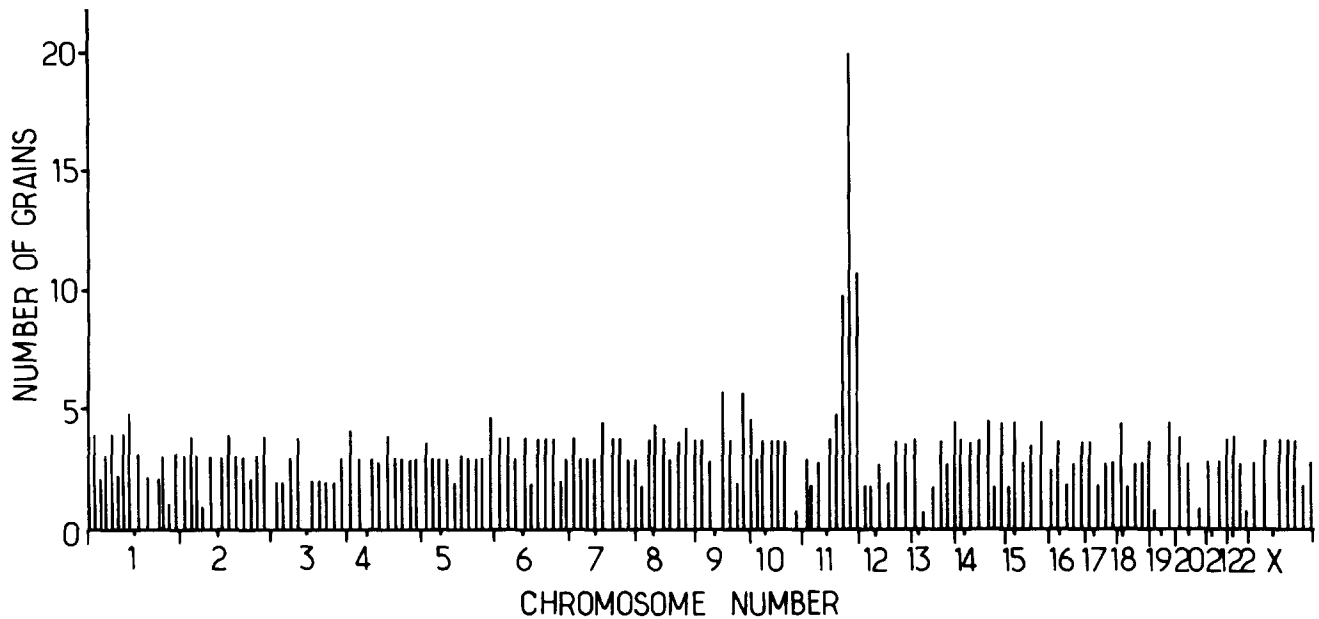


Figure 3. Distribution of silver grains in 200 metaphases. The diagram is shown at low resolution (3–10 regions per chromosome). A clear peak is evident at the long arm of chromosome 11. 11% of all grains observed lie on chromosome 11 and 71% of those are located on the distal part of the long arm (11q21 to 11q25).

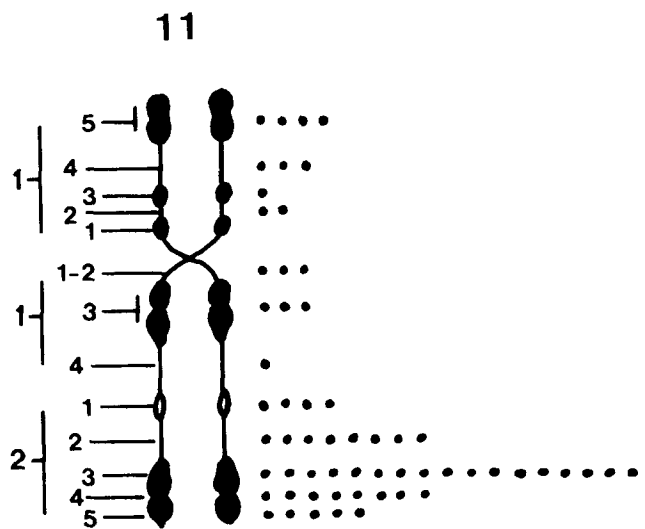


Figure 4. Regional distribution of silver grains on 59 labeled chromosome 11. The peak of hybridization occurs on band q23.

e.g., Mattei et al., 1985a and b) demonstrate that reasonable results can be obtained even with short (0.6 kb) heterologous probes. In fact, given the lack of stringency of in situ hybridization (washes in $2\times$ SSC, 50% formamide at 39°C and in $0.1\times$ SSC at 4°C), the relatively low signal-to-background ratio in this experiment is probably due mainly to the small size of the hybridizing sequence rather than to the limited divergence between the mouse probe and the human gene. Available murine genomic DNA clones could not be used since they contain mainly intron sequences which are likely to have diverged considerably between mouse and man. Human genomic NCAM clones are being isolated and will be used in particular to increase the precision of localization relative to other chromosome markers present in this region of chromosome 11 (see below).

Band 11 q23 and Genes for Cell Surface Molecules

In contrast to the abundance of genetic markers and cloned genes located on the short (p) arm of human chromosome 11 (including insulin, globin, calcitonin, and many others), the long arm of this chromosome was, until recently, relatively sparsely populated with markers. This is, however, changing rapidly. Recently the T3- δ subunit of the T3-T cell receptor complex has been mapped to the q23-qter region of human chromosome 11 (Van den Elsen et al., 1985); in addition, the gene for the Thy-1 molecule which has a variable tissue distribution in different species but is always expressed in brain (Williams and Gagnon, 1982) has been mapped to bands q23-q24 of the very same chromosome by in situ hybridization (Van Rijs et al., 1985). It may be a coincidence that two cell surface molecules expressed in neural cells (Thy-1 and N-CAM) are coded in the same subregion of chromosome 11, and of course the “small” chromosome region involved is still quite large since a single band may correspond to $\sim 10,000$ kb of DNA with coding potential for hundreds of genes. It is, however, remarkable that the three cell surface proteins encoded by genes on chromosome 11 q23 have either been shown (the T3 complex δ chain and N-CAM) or are likely (Thy-1) to be involved in specific cell surface interactions. Further in situ hybridization experiments should clarify the relative positions of these markers since use can be made both of a fragile site present at band q23 (FRA11 B) which effectively increases the resolution of in situ hybridization (as underlined, for example, by Mattei et al., 1985a and b) and of numerous translocations whose end point involve band 11 q23.

Interestingly, band 11 q23 lies within a region believed to be syntenic with a portion of the murine chromosome 9 where both the T3- δ subunit gene and the Thy-1 gene map (Antonucci et al., 1984). This region of murine chromosome 9 also contains the *staggerer* mutation, a neurological disorder in which structural abnormalities of the N-CAM protein have

been shown to occur during development (Edelman and Chuong, 1982). Although these appear to be due to changes in the carbohydrate side chains of N-CAM, this has not been definitely proved. In any case the possibility exists either that abnormal glycosylation is due to alterations in the N-CAM peptide chain or that enzymes involved in the N-CAM-specific addition of polysialic acid (Finne et al., 1983) are encoded in the same region of the murine chromosome 9. In situ hybridizations being performed on murine chromosomes should indicate whether the murine NCAM gene is indeed located at that position. At the present stage there are no human neurological disorders clearly mapped to the long arm of chromosome 11, and one might argue that in fact N-CAM is such a basic element in cell-cell interactions that most homozygous defects in the gene would be lethal early in development. Ongoing work on the human N-CAM gene will hopefully clarify these points.

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Note Added in Proof. The mouse N-CAM gene has now been mapped to chromosome 9 closely linked to the Thy-1 gene (D'Eustachio et al., 1985) showing that the genetic linkage of the loci coding for different cell interaction molecules has been phylogenetically conserved.

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