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Comparative Analysis of Mouse–Human Hybrids with Rearranged Chromosomes 1 by in Situ Hybridization and Southern Blotting: High-Resolution Mapping of *NRAS, NGFB,* and *AMY* on Human Chromosome 1

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Abstract—*The human protooncogene* NRAS *and the genes for the β-subunit of nerve growth factor* (NGFB) *and for amylase* (AMY) *have previously been assigned to the proximal short arm of chromosome 1, but their precise positions have not been unequivocally established. By in situ hybridization of DNA probes for the three genes, we have ascertained the location of complementary sequences in mouse–human somatic cell hybrids that contained translocations of chromosome 1. The results agreed with the presence or absence of the human sequences as determined by Southern blotting of hybrid cell DNA. The in situ data confirmed that the genes were present on the cytologically recognized rearranged chromosome. Compared to the autoradiographic silver grain distribution on normal human chromosome 1, our in situ results obtained with the translocation chromosomes allowed much greater precision of mapping. Both* NRAS *and* NGFB *map to band 1p22, and* AMY *was confirmed in band 1p21.*

INTRODUCTION

Cellular protooncogenes, and viral transforming genes derived from them, may represent genes for known proteins with growth regulatory function. Extensive sequence similarity between the transforming protein of the Simian sarcoma virus (v-sis) and human platelet-derived growth factor has been demonstrated (1, 2). In addition, the gene for epidermal growth factor (EGF) receptor and the avian erythroblastosis virus oncogene (v-erb-B) are apparently related (3). It is of interest to note that the human gene for EGF receptor and the human sequence homologous to the v-erb-B oncogene had both been mapped independently to the same region of chromosome 7 (4, 5). Thus, chromosomal mapping of protooncogenes and of genes for growth factors can facilitate the identification of those genes that are evolutionarily related.

Another rationale for mapping genes for growth factors and transforming proteins is to compare their chromosomal band localizations with the sites of nonrandom chromosome breaks that lead to translocations or deletions in human malignant tumor cells. The common DNA rearrangements near the *c-myc* gene resulting from translocations between chromosome 8 and one of the chromosomes carrying immunoglobulin genes in human Burkitt's lymphoma and mouse plasmacytoma represent prototypes for this kind of association (6, 7). Any correlations between breaks lead-

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ing to nonrandom chromosome rearrangements in human tumors and localization of protooncogenes or growth factor genes may allow the formulation of hypotheses regarding the activation of specific genes in certain tumor types. Such hypotheses can be tested experimentally.

Two approaches are currently available for the mapping of cloned DNA sequences to chromosomal sites: Southern blot analysis of interspecies somatic cell hybrids with defined chromosome content (8) and in situ hybridization of radiolabeled probes directly to metaphase chromosome preparations (9). In the current studies we have compared both approaches in the mapping of human *NRAS, NGFB,* and *AMY* on chromosome 1.

The *NRAS* protooncogene, a distant relative of the viral *ras* oncogenes, has been identified as the transforming sequence isolated from human tumor cell lines, HL-60 promyelocytic leukemia (10), SK-N-SH neuroblastoma (11) and HT 1080 fibrosarcoma (12) by transfection of mouse NIH-3T3 fibroblasts. *NRAS* has been mapped to human chromosome 1, independently, by four different groups of investigators using somatic cell hybrids (12–15). By Southern analysis of Chinese hamster–human and rat–human hybrids containing defined parts of chromosome 1, our laboratory has previously assigned *NRAS* to the proximal short-arm region 1p3200→cen (13).

The same somatic cell hybrids that contained NRAS sequences were also positive when probed with part of the genomic sequence of *NGFB* (β-subunit of nerve growth factor), although the hybridizing restriction fragments were not identical in size to the NRAS fragments (16). Nerve growth factor regulates growth and differentiation of sympathetic and certain sensory ganglia and consists of three different polypeptide chains. The β-subunit is responsible for the biological activity (17). It is apparently unrelated to the p21 protein product of *NRAS*.

Using in situ hybridization as well as Southern analysis of additional mouse–human somatic cell hybrids that contained spontaneous de novo rearrangements of 1p (18, 19), we have found that the genes coding for NRAS and NGFB are not only in the same chromosomal region but even in the same band (1p22). Both of them are distal to the gene coding for amylase (AMY) that has previously been assigned to region 1p21-p22 (20, 21). The in situ hybridization data presented here confirm our previous interpretation of the spontaneous rearrangements of human chromosome 1 in the two mouse–human hybrids and exclude the possibility that the human NRAS and NGFB sequences detected by filter hybridization have been translocated elsewhere in these cells. In situ hybridization of single-copy sequences to chromosomes with well-characterized rearrangements results in far greater precision of mapping than hybridization to metaphase spreads from normal human donors.

MATERIALS AND METHODS

Somatic Cell Hybrids with Rearranged Chromosome 1. From a fusion of mouse 3T3 TK^- cells with human leukocytes, two independent hybrid clones have been isolated, each of which contained a different de novo translocation involving the short arm of human chromosome 1, interpreted as der(7)[t(1;7) (1pter→1p22.1::7q22→7pter)], henceforth called 7^T, and der(14)[t(1;14) (1pter→1p31.1::14q23.2→14pter)], called 14^T (18, 19). A schematic representation of the rearranged chromosomes and G-banded examples are shown in Fig. 1A and B.

The 7^T translocation chromosome was present in 54% (73 of 134) of metaphase spreads of the primary hybrid clone XVI-10C. The reciprocal product of the t(1;7) translocation and normal chromosomes 1 and 7 were not observed. Seven other human chromosomes were present at variable copy numbers. A GTG-banded karyotype of this hybrid has been published (19).

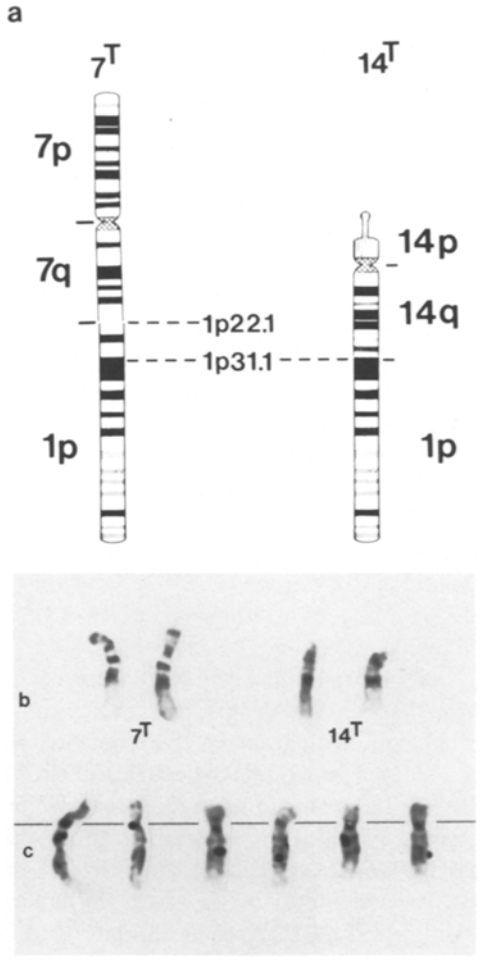

Fig. 1. (A) Schematic representation of G-banded rearranged chromosomes (49). The der(7),t(1;7) translocation chromosome 7^T of hybrid XVI-10C with breakpoints in 1p22.1 and 7q22 (left) and the der(14),t(1;14) translocation chromosome 14^T of hybrid XVI-18A with breakpoints in 1p31.1 and 14q23.2 (right). The short arm regions of chromosome 1 are upside down. (B) G-banded translocation chromosomes 7^T and 14^T. (C) 7^T chromosomes after in situ hybridization with labeled probes containing sequences of the human *NRAS* protooncogene (left three) and the human *NGFB* gene (right three).

The primary hybrid clone XVI-18A contained the 14^T translocation chromosome in 90% (49 of 54) of the metaphases examined. The reciprocal translocation product and normal chromosomes 1 and 14 were not observed. Six other human chromosomes were retained. For a complete karyotype of this hybrid, see George and Francke (18).

Culture and chromosome preparation of hybrid and peripheral blood cells were carried out as described previously (18, 19, 22).

DNA Probes. Probe p52C⁻ is a pUC8 plasmid with a 1.1-kbp insert derived from the human *NRAS* oncogene that was activated in the HL-60 cell line (10). Plasmid N8C6 contains a 1.0-kbp *Pst I* fragment of the human *NGFB* gene (23). Probe λhaI, provided by Dr. Graeme I. Bell, carries a 14.5-kbp insert containing human genomic amylase sequences (21).

Southern Blotting. Three to five micrograms of human, mouse, and somatic cell hybrid DNA, digested to completion with *EcoRI* (New England Biolabs), were electrophoresed in 0.7% agarose gels and transferred to nitrocellulose filters as described (13). All probes were ^{32}P-labeled to high specific activity (3–4 × 10^8 cpm/μg) with two labeled nucleotides. Probes p52C⁻ and N8C6 were hybridized in 5 × SSC at 66–68° for 16–20 h, while higher stringency was used for λhaI (3 × SSC at 68–69°C for 10–12 h) because this probe appeared to contain repetitive sequences.

In Situ Hybridization. The three DNA probes were labeled by nick translation in the presence of 10–60 pg/μl DNase I (Sigma) and 0.3 units/μl of *E. coli* polymerase I (New England Biolabs) for 60 min at 15°C (24). The reaction mixture contained 10 ng/μl DNA; 10 μM each of three labeled nucleotides, [^{3}H]dATP at 59.1 Ci/mmol, [^{3}H]dCTP at 52.0 Ci/mmol, and [^{3}H]dTTP at 74.2 Ci/mmol (New England Nuclear; 1 Ci = 37 × GBq); 25 μM of unlabeled dGTP; 50 mM Tris HCl, pH 7.5; 5 mM $MgCl_2$; and 50 μg/ml of bovine serum albumin. The DNase

was inactivated by adding 40 mM EDTA and heating for 10 min at 70–80°C. The labeled DNA was recovered by centrifugation (4 min at 400 *g*) through a Sephadex G-50 medium column (Pharmacia) in 50 mM Tris HCl, pH 7.5, 1 mM EDTA. Specific activities of 2×10^7 cpm/μg DNA were achieved with approximately 30% incorporation.

Hybridization was carried out essentially as described by Harper and Saunders (9) at probe concentrations of 0.025–0.2 ng/μl. Washes after hybridization were at 40–42°C.

Autoradiography, Chromosome Banding, and Selection of Cells. Hybridized slides were prestained in 0.5% quinacrine dihydrochloride (Sigma) in distilled water (dH_2O) for 7 min, rinsed in dH_2O, and air dried (25). Prior to autoradiography, the slides were rinsed in a subbing solution containing 0.1% gelatin and 0.01% chromium potassium sulfate. Slides were then coated with NTB2 emulsion (Kodak), diluted 1:1 with dH_2O, at 43–45°C. After storage at 4°C for 10–24 days, the slides were developed as recommended by Kodak. The slides were stained for 20 min in 50 μg/ml quinacrine mustard (Sigma), in McIlvaine's buffer (0.1 M citric acid, 0.2 M Na_2HPO_4, pH 5.5), rinsed thoroughly, and soaked for 10 min in buffer (26).

Normal human metaphase spreads were selected on the basis of chromosome length and quality of Q-banding. All suitable cells were photographed in a Zeiss fluorescence microscope. For G-banding, the same slides were stained with aged (older than two months) 0.25% Wright's stain (MCB) diluted 1:4 with 0.06 M phosphate buffer, pH 6.8, for 10–12 min (27). The same mitotic spreads were rephotographed, and the grain locations were recorded on schematic representations of G-banded chromosomes (28). Only spreads with 46 chromosomes were included. In the mouse–human hybrids, only metaphases that contained the 7^T or 14^T translocation chromosome were selected for analysis.

Statistical Analysis. The numbers of grains on chromosomes 1, 7^T, and 14^T were compared to total grains over chromosomes per cell by chi-square analysis (one degree of freedom). The expected numbers on these chromosomes were calculated from their relative length as published (29), or, for 7^T and 14^T, as determined by measuring chromosomes of several metaphase spreads of the particular hybrid clones. The 7^T chromosome corresponded to approximately 0.83% of the total chromosome length per metaphase, and the relative length of 14^T was 1.53%.

RESULTS

Somatic Cell Hybrids with Rearranged Chromosome 1. Hybrid clone XVI-10C had on average 170 chromosomes per cell. Two hundred ninety-nine metaphase spreads with the 7^T translocation chromosome (Fig. 1) were analyzed; 28 of these (9.4%) contained two copies. The average chromosome number of hybrid XVI-18A was 80. Of 207 metaphase cells with the 14^T chromosome, 18 (8.7%) contained two copies.

Chromosome Banding and Grain Distribution after In Situ Hybridization. Chromosomes from synchronized lymphocyte cultures, stained with quinacrine dihydrochloride before and with quinacrine mustard and Wright's stain after autoradiography, displayed distinct G-banding patterns at the resolution of 400–550 bands per haploid karyotype (28). The human chromosomes in the two human–mouse hybrid cell lines (XVI-10C and XVI-18A) were recognized by a G-banding pattern equivalent to the 400-band stage (Fig. 1C). On mouse chromosomes all major bands, as defined by Nesbitt and Francke (30), were clearly discernible. In some metaphase plates the major bands split up into minor bands. Destaining and restaining with Wright's stain as suggested by Chandler and Yunis (27) did not improve the quality of banding.

Silver grains were more easily observed on G-banded than on Q-banded chromo-

somes. Only grains located directly on chromosomes or touching chromosomes were scored. The location of the center of a grain was recorded relative to actually visible chromosome bands (Fig. 1C). For all three single-copy probes, specific hybridization to consistent chromosome sites was observed in approximately 30% of metaphases with grains on chromosomes. Grains interpreted as specific label were distributed over a range of three to four bands on chromosomes at the 400 to 550-band stage with a peak at one band (Fig. 2).

In Situ Localization of Human NRAS *Oncogene.* Of 250 normal human prometaphase and metaphase spreads examined, 129 (51.6%) did not exhibit any grains on chromosomes, whereas 121 had 187 grains on chromosomes and 70 grains over the background within the metaphase plates (Table 1). Of the chromosomal grains, 27% were located on chromosome 1, and 77% of those were concentrated on the proximal short arm (1p13-1p31) with a peak at band 1p22 (Fig. 2).

In hybrid XVI-10C, 124 metaphase spreads were analyzed that contained the 7^T

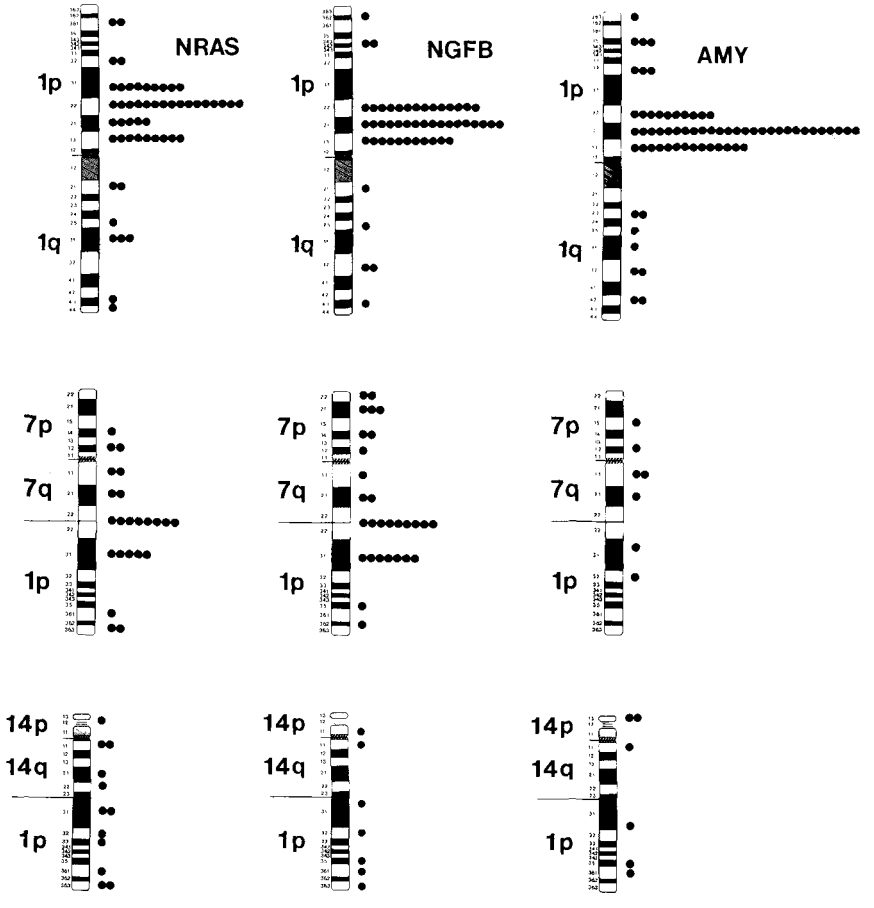

Fig. 2. Silver grain distribution after in situ hybridization of labeled probes to G-banded normal and translocation chromosomes on ISCN 1981 ideograms (29): probe p52C$^-$ (NRAS) in left column, probe N8C6 (NGFB) in middle column and probe λhaI (AMY) in right column. The ideograms include only low-resolution bands, consistent with the actual chromosomes in the in situ preparations, although the breakpoints have previously been defined at the subband level as t(1;7) (p22.1;q22) and t(1;14) (p31.3;q23.2) (18, 19).

Table 1. In Situ Hybridization of *NRAS*, *NGFB*, and *AMY* Probes to Chromosomes of Normal Human Cells and Human–Mouse Hybrid Clones XVI-10C and XVI-18A

	NRAS			NGFB			AMY		
	Human diploid	XVI-10C (7^T)	XVI-18A (14^T)	Human diploid	XVI-10C (7^T)	XVI-18A (14^T)	Human diploid	XVI-10C (7^T)	XVI-18A (14^T)
Experimental conditions									
Probe conc. (ng/μl)	0.05	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.2
Exposure (days)	15	15	20	20	15	20	11	13	11
Results									
Metaphases with grains	121	124	66	73	101	62	84	74	79
No. grains									
Total	187	539	486	152	461	405	227	472	471
Per cell	1.5	4.3	7.4	2.1	4.6	6.5	2.7	6.4	6.0
On chromosome									
1, 7^T or 14^T	51	23	13	50	29	7	66	7	6
% of total	27%	4%	3%	33%	6%	2%	29%	1%	1%
Over specific site	39	14	—	42	19	—	51	—	—
% of grains on 1, 7^T or 14^T	77%	64%	random	84%	66%	random	77%	random	random
Statistical analysis									
expected[a] on chromosome 1, 7^T or 14^T	16.8	4.8	8.5	13.7	4.3	6.4	20.4	4.2	7.7
Chi square	69.6	69.0	2.38	96.2	142.9	0.056	101.9	1.81	0.375
P	<0.0001	<0.0001	0.125	<0.0001	<0.0001	0.85	<0.0001	0.18	0.55

[a]Expected numbers of grains were calculated as described in the text.

chromosome. On average, 4.3 grains per metaphase were found; 23 of 539 grains were on 7^T which was significantly higher ($P < 0.0001$) than expected (Table 1). The grains on 7^T were nonrandomly distributed with a peak at the fusion band 1p22/7q22 (Fig. 2).

To further determine whether the *NRAS* oncogene was located in the bands 1p22 or 1p31, we used hybrid clone XVI-18A containing the 14^T chromosome with region 1pter→1p31.1 but without band 1p22. Sixty-six cells with 14^T had 7.4 grains per metaphase. The number of grains on 14^T was not significantly different ($0.5 > P > 0.1$) from the expected value (Table 1). The distribution of grains on the 14^T chromosome was random (Fig. 2).

In Situ Localization of Human NGFB *Gene.* The results were very similar to those obtained with *NRAS* (Table 1). Specific labeling occurred on the proximal short arm of chromosome 1 with 84% of the grains on chromosome 1 over bands 1p13 to 1p22 (Fig. 2). The level of nonspecific labeling of other chromosomes was relatively low with one to two grains per metaphase.

In hybrid XVI-10C, 101 metaphase spreads with the 7^T chromosome had 461 grains on chromosomes, 29 of which were located on 7^T. This was significantly higher ($P < 0.0001$) than expected. The specific grain distribution had a peak at the fusion band 1p22/7q22 (Fig. 2).

In hybrid XVI-18A, 62 cells with the 14^T chromosome had an average of 6.5 grains per metaphase plate. The observed number of grains on 14^T was almost identical to the number expected ($0.9 > P > 0.5$), and the grain distribution on 14^T was random (Fig. 2).

In Situ Localization of Human Amylase Genes (AMY). Whereas with *NRAS* and *NGFB* probe labeling was limited to only one specific grain per cell, the much larger amylase probe produced very different results. In 60% of the specifically labeled cells, there was more than one grain on one or both chromatids, or one grain on each of the homologs. On human prometaphase and metaphase preparations specific labeling occurred over the proximal short arm of chromosome 1 with 77% of all grains in chromosome 1 concentrated around a peak at band 1p21 (Fig. 2 and Table 1). The localization of *AMY* to 1p21 was confirmed by in situ hybridization to the human–mouse hybrid chromosomes. As detailed in Table 1, the numbers of grains on both translocation chromosomes were not significantly different from random ($0.5 > P > 0.1$ and $0.9 > P > 0.5$), and their distribution was uniform (Fig. 2).

Southern Analysis. Filters containing XVI-10C, XVI-18A, and control DNA samples were hybridized with the probes p52C$^-$ (NRAS), N8C6 (NGFB), and λhaI (AMY) (Fig. 3). For both *NRAS* and *NGFB* the single human-specific restriction fragments were present in hybrid XVI-10C (lanes 4) but not in hybrid XVI-18A (lanes 3), while mouse cross-hybridizing fragments were seen in both hybrids. The amylase probe reacted with several restriction fragments, forming an array of different intensity bands in the human control lane (lane 1). None of these bands were present in the hybrids (lanes 3 and 4), and no cross-hybridization with mouse DNA (lane 2) was detected. The stringency of hybridization was higher than NRAS and NGFB probes.

DISCUSSION

We have mapped the human *NRAS* protooncogene and the β-subunit of the nerve growth factor (*NGFB*) to the same region on human chromosome 1, segment 1p31.1→1p22.1, which includes essentially all of band 1p22. These data refine the *NRAS* map position within the previously reported region 1p3200→cen (13), but they are inconsistent with two reports in the literature. After in situ hybridization of labeled human NRAS sequences to human metaphase chromosomes,

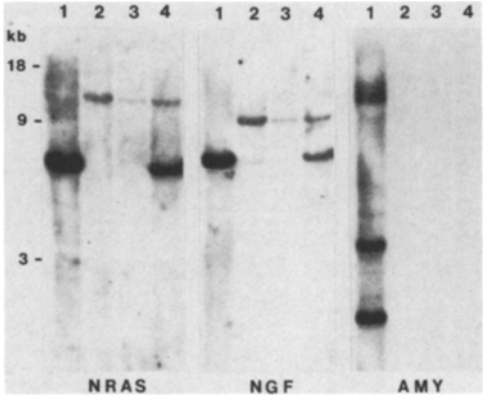

Fig. 3. Filter hybridization of human, mouse and hybrid DNA with NRAS, NGFB, and AMY sequences. EcoRI-digested DNA samples were applied as 5 μg/lane for all except lanes 3 that contained only about 3 μg. Lanes 1, human diploid control; lanes 2, mouse control; lanes 3, mouse–human hybrid XVI-18A with 14^T translocation; and lanes 4, mouse–human hybrid XVI-10C with 7^T translocation. The three identical filters were hybridized with ^{32}P-labeled probes p52C$^-$ (NRAS), N8C6 (NGFB) and λhaI (AMY). The hybrid in lanes 4 contained human *NRAS* and *NGFB* sequences, but was negative for *AMY*, while the hybrid in lanes 3 was negative with all three probes. Mouse cross-hybridizing fragments are present on filters NRAS and NGFB but not on AMY. The faint mouse band at the position of the human band visible in lane 2 was also seen in lane 3 on longer exposures.

both Davis et al. (31) and Rabin et al. (32) have observed silver grains on the proximal short arm of chromosome 1 (region 1p21-cen) with a major concentration at 1p13. They interpreted their data to indicate that *NRAS* is most likely located in band 1p13. This assignment differs significantly from ours as it would suggest a different gene order by placing *NRAS* on the centromere side of *AMY*. In order to resolve this inconsistency, we have carried out comparative studies using Southern blot analysis of somatic cell hybrids with rearranged chromosomes 1 and in situ hybridization of probes to normal and to rearranged chromosomes 1.

The human–mouse hybrids used in this study had de novo translocations that resulted in chromosomes recognized by banding patterns and interpreted to contain region 1p22.1→1pter (7^T, hybrid XVI-10C) and region 1p31.1→1pter (14^T, hybrid XVI-18A) (18, 19). The NRAS and NGFB probes did not hybridize to DNA of hybrid XVI-18A on Southern blots. Thus, region 1p31.1→1pter is excluded as the possible site for these loci. However, both probes hybridized to DNA of XVI-10C.

Before accepting the conclusion that both loci are in region 1p22.1→1p31.1, that is present in 7^T and absent from 14^T, one has to rule out the possibility that the de novo rearrangement in XVI-10C is more complex and that other parts of chromosome 1, in particular any derived from region 1p21-cen, have been retained within another chromosome. Given the fact that these hybrid cell metaphase spreads contained ~170 chromosomes, such a subtle rearrangement might have escaped cytological detection. We have presented two independent sets of data that rule out this possibility and support our original interpretation. First, in situ hybridization of NRAS and NGFB probes to XVI-10C hybrid metaphase cells produced specific label on the 7^T chromosome but not on 14^T in hybrid XVI-18A. Second, human amylase (AMY) sequences, previously mapped to band 1p21 by in situ hybridization (20) and to region 1p21-pter by filter hybridization of somatic cell hybrid DNA (21) were not detected in either of our human–mouse hybrids by in situ or Southern analysis. The same cloned human amylase cDNA produced a clear signal on normal human chromosome 1 with a peak over band 1p21. The assignment of *AMY* to band 1p21 is thus confirmed, and the undetected presence of band 1p21 in XVI-10C is made very unlikely.

In situ and Southern analyses of the mouse–human hybrids have provided firm evidence that the breakpoint in band 1p22.1 of chromosome 7^T has separated *AMY* from *NRAS* and *NGFB*, placing *AMY* on the centromere side and *NRAS* and *NGFB* on the distal side. Surprisingly, the autoradiographic grain distributions on chromosome 1 from normal diploid donors (Fig. 2) would not have suggested the same gene localization. For *NGFB* the peak was clearly located at 1p21

and not 1p22. The distribution was similar to that for *AMY*. NRAS-specific label extended over four major bands (1p13-1p31) with a peak at 1p22 but also considerable labeling at band 1p13. On the 7^T chromosome, NRAS-specific label appeared to extend to the region derived from human chromosome 7.

Our comparative studies suggest that in situ hybridization to metaphase chromosomes may not generate information that is reliable enough for assignment of a DNA probe to a single chromosome band, although a primary chromosomal assignment and preliminary regional localization can be derived (34, 35). As demonstrated here, the peak of the grain distribution curve does not necessarily coincide with the location of the hybridizing sequence.

Factors that may contribute to the skewing of in situ hybridization data are related to chromosome morphology, state of condensation and quality of banding. In very short and tightly condensed metaphase chromosomes, DNA sequences may be more easily available for hybridization with probe when they are on the surface or when they are part of chromatin loops that have been pulled out during the chromosome preparation. Because of three-dimensional spatial limitations, such displayed loops could preferentially be located around the ends of chromosome arms and around the centromere. Thus, loops containing DNA sequences with a more distal location could protrude towards the centromere. This factor might explain the in situ localization of *NRAS* closer to the centromere reported by others (31, 32), and possibly the slight skewing of our *NRAS* and *NGFB* distributions in the same direction. Such a hypothesis could also explain the increased "background" label over telomere and centromere regions documented by Davis et al. (31). Furthermore, these authors have previously reported β-globin-specific label on the short arm of chromosome 11 near the centromere (36), while the globin region has now been mapped to the distal band 11p15 (37–39). The precision of mapping DNA sequences by in situ hybridization may be greatly increased with the use of prophase chromosomes at the 1000-band stage (20) or of meiotic pachytene chromosomes at similar levels of resolution (40).

Another possible source of skewed in situ data involves the use of unbanded or poorly banded chromosomes. In this approach that has been described by Barton et al. (41), Davis et al. (31), and Rabin et al. (32), grain localizations are recorded by their relative positions between telomere and centromere. The distribution is then superimposed on a banded chromosome ideogram and regional localizations are estimated. However, depending on the state of chromosome condensation, the relative position of a grain on a chromosome arm will correlate with different chromosome bands. As the cell progresses through the stages of mitosis, the rates of chromosome contraction differ strikingly between regions that stain darkly and those that stain lightly with GTG techniques (22). The ISCN 1981 ideograms (28) do not reflect relative widths and band positions, and they ignore the relative movements of bands during transition from 850- to 400-band stages. In these ideograms, the relative positions of bands have been kept constant for didactic reasons, to illustrate the interpretations of how higher-resolution bands are derived from those of lower-resolution chromosomes. Thus, a grain over 1p22 may appear to be located near the centromere on a very condensed chromosome. When superimposed upon the ISCN 1981 ideogram, such a grain would be interpreted to be at band 1p13. In our studies, we have scored grain localizations only with respect to bands that were clearly visible in the particular cell analyzed. Recording relative distances from the centromere of grains on chromosomes at various stages of contraction, and superimposing the distribution on a published ideogram, is unlikely to generate precise gene localization data.

Localization of both *NRAS* and *NGFB* to the same band on the short arm of human chromosome 1 (1p22) may have relevance for

some specific chromosome abnormalities that have been observed in neural crest-derived tumors. Breaks in the short arm of chromosome 1, often leading to partial deletions, are the most consistent structural abnormalities reported in human neuroblastomas (42). More recently, Balaban and colleagues have identified nonrandom rearrangements with breaks in 1p (region p11-p31) in primary and metastatic melanomas (43). Furthermore, *NRAS* was found to be activated in three of 30 melanoma cell lines in the NIH/3T3 cell transformation assay (44). Thus it seems possible that in some neural crest-derived tumors, activation of *NRAS* or *NGF* sequences may be involved in tumor progression. Such activation could be associated with chromosome rearrangements involving breakpoints near the site of the genes.

The same human–mouse hybrids of this study (XVI-10C and XVI-18A) had been used to assign the human gene for phosphoglucomutase 1 (PGM1) to the short arm of chromosome 1 in segment 1p22.1→1p31.1 (19). Our data do not allow us to propose a linear order for the three loci in 1p22 as yet. Comparative mapping data, however, do provide some clues. *PGM1* is the most proximal member of a large conserved group of syntenic genes on the short arm of human chromosome 1 that includes genes for glucose dehydrogenase (*GDH*), enolase-1 (*ENO1*), phosphogluconate dehydrogenase (*PGD*), α-L-fucosidase (*FUCA*), and adenylate kinase-2 (AK2). On the male genetic map the distance between *GDH* and *PGM1* is greater than 50 centimorgans (cM) (33). In the mouse (MMU), the syntenic relationship has been conserved on chromosome 4 (45). *Pgm-2,* homologous to human *PGM1, Gpd-1,* and *Pgd-1* are on the linkage map, spanning a distance of 26 cM in the same centromere orientation as in the human. The other three loci have been assigned to MMU 4 by somatic cell hybrid studies, and their locations on the linkage map have not yet been determined (46). In contrast, the mouse genes homologous to human *NGFB* and *AMY* have been assigned to MMU 3 (47, 48).

These data suggest a gene order of *PGM1-NGFB–AMY* with an evolutionary breakpoint between *PGM1* and *NGFB* within subband 1p22.1. The murine homolog of *NRAS* could be predicted to be located on either MMU3 or MMU4. Mapping of *NRAS* to a mouse chromosome as well as in situ hybridization of NRAS and NGFB probes to rearranged human chromosomes 1 with breakpoints in band 1p22 should establish the precise localization of *NRAS* within the suggested gene order and will throw light on the evolutionary history of this region on human chromosome 1.

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