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18. The limits of the position of the t(1;17) translocation breakpoint (cross-hatching in Fig. 2) were defined as follows: (i) the normal Sfi I site 10 kb telomeric to 17L1A is present on the translocation chromosome; (ii) in a Cla I + Not I double digest, the normal Cla I site 240 kb telomeric to 17L1A is replaced by a novel site 420 kb telomeric to 17L1A on the t(1;17) chromosome; (iii) despite restriction analysis with multiple other enzymes, no informative sites are present between Sfi I and Cla I to more accurately define this breakpoint. The Eag I and Nru I sites in chromosome 1 DNA shown in Fig. 2 apparently occur coincidentally in approximately the same location as their counterparts on the normal chromosome 17.
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Two NF1 Translocations Map Within a 600-Kilobase Segment of 17q11.2

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Balanced translocations, each involving chromosome 17q11.2, have been described in two patients with von Recklinghausen neurofibromatosis (NF1). To better localize the end points of these translocation events, and the NF1 gene (*NF1*) itself, human cosmids were isolated and mapped in the immediate vicinity of *NF1*. One cosmid probe, c11-1F10, demonstrated that both translocation breakpoints, and presumably *NF1*, are contained within a 600-kilobase Nru I fragment.

LOCALIZATION OF THE NEUROfibromatosis type 1 (NF1) gene (*NF1*) to chromosome 17 by linkage analysis (*1, 2*) was followed by description of a marker, pHHH202, that showed no recombination in the Utah panel of NF1 families (*3*). Close linkage of pHHH202 to NF1 was confirmed by an international consortium (*4*). Subsequent studies (*5, 6*) physically localized pHHH202 to band 17q11.2, the same band that is involved in the two known rearrangements associated with NF1: t(1;17) (p34.3;q11.2) (*7*) and t(17;22) (q11.2;q11.2) (*8*).

To locate *NF1* more precisely, we isolated a large number of cosmid clones as a source of additional polymorphic markers and probes. Because intact chromosome 17 is too large (150,000,000 bp) for development of probes specifically targeted to the NF1 region, we used pHHH202 and other elements of our chromosome 17 map (*9*) to characterize a large number of human × rat microcell hybrids (*10*) that had been created by fusion of a somatic cell hybrid containing a *neo*-marked human chromosome 17 and a rat cell line (*11*). To avoid confusion caused by undetected deletions or rearrangements, we chose to search for new probes in cosmid libraries prepared from the two microcell hybrids that best represented the NF1 region, 7AE-11 (*6*) and FTHB(17)L4 (*12*).

Human cosmids identified by screening the microcell hybrid libraries with labeled human DNA were physically mapped by means of a panel that included somatic cell hybrids (*5*), the two chromosome 17 microcell hybrids 7AE-11 and FTHB(17)L4, and two cell lines with *NF1* translocation breakpoints (Fig. 1A). One of the translocation cell lines, NF13 (*6, 8*), contains the derivative 22 [der(22)] chromosome from a sporadic NF1 patient who carries a balanced translocation between chromosomes 17 and 22, t(17;22)(q11.2;q11.2). The other hybrid, designated DCR1, was isolated from a fusion of lymphoblasts from an NF1 patient with a balanced translocation between chromosomes 1 and 17, t(1;17)(p34.3;q11.2) (*7*) and contains the der(1) chromosome from this patient (*13*). The results of the physical mapping are summarized in Fig. 1B.

We determined the relation of clones in the NF1 region to *NF1* by a combination of genetic and physical mapping. A high-resolution genetic map for the NF1 region (*6*) indicated that two probes, pTH17.19 and c11-2C11, flank *NF1* about 2 centimorgans apart. Given the density of cosmid probes within the target regions, we reasoned that it should be possible to detect abnormal pulsed-field fragments in the two NF1 patients with balanced translocations. DNA samples from normal individuals, patients with sporadic NF1, the two patients with balanced translocations, and somatic cell hybrids were prepared in agarose blocks, digested with a number of rarely cutting enzymes, and subjected to pulsed-field gel electrophoresis (PFGE) (*14*).

One region 3 clone, c11-1F10, hybridized to a 600-kb Nru I fragment. When tested on PFGE blots (*15*) prepared with DNA from the patients with translocations, this clone showed new, translocation-specific Nru I fragments. The t(17;22) cell line showed the normal 600-kb Nru I fragment plus a fragment of 390 kb. The t(1;17) cell line gave the normal 600-kb Nru I fragment plus a fragment of 450 kb.

A potential caveat is that these observations may reflect heterogeneous Nru I digestion of the translocation cell lines, caused by differences in methylation between the normal and translocation chromosomes. In fact, many lymphoblastoid cell lines, including the t(17;22) balanced translocation line, require a large excess (100×) of Nru I to reach complete digestion (Fig. 2). However, we believe these new c11-1F10–hybridizing Nru I fragments are associated with the translocation events because (i) both translocations revealed aberrant bands, each a different size, as would be expected for two independent translocation events; (ii) Nru I digests of 40 additional lymphoblastoid cell lines from normal people and from NF1 patients yielded only the 600-kb normal Nru I band; (iii) the two derivative fragments are smaller than the normal fragment, a finding inconsistent with a digestion artifact; (iv) the two derivative fragments are

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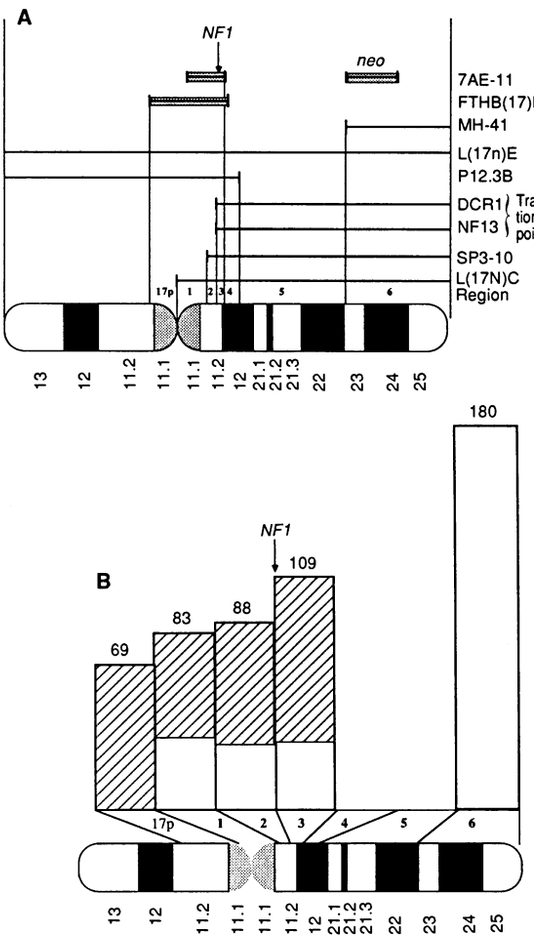

Fig. 1. (**A**) Mapping panel of somatic cell hybrids with approximate end points of the two microcell hybrids enclosing shaded blocks. The *NF1* region is defined by the breakpoint of hybrid SP3-10 (17q11.2) (*5*) and the distal breakpoint of microcell hybrid 7AE-11 (17q11.2–q12) (*6, 17*), regions 2 and 3, respectively. (**B**) Distribution of cosmids isolated from the microcell hybrids, as determined by somatic cell hybrid mapping. Procedures for mapping human cosmids from microcell hybrids are described elsewhere (*6*). Shading denotes cosmids derived from microcell hybrid FTHB(17)L4.

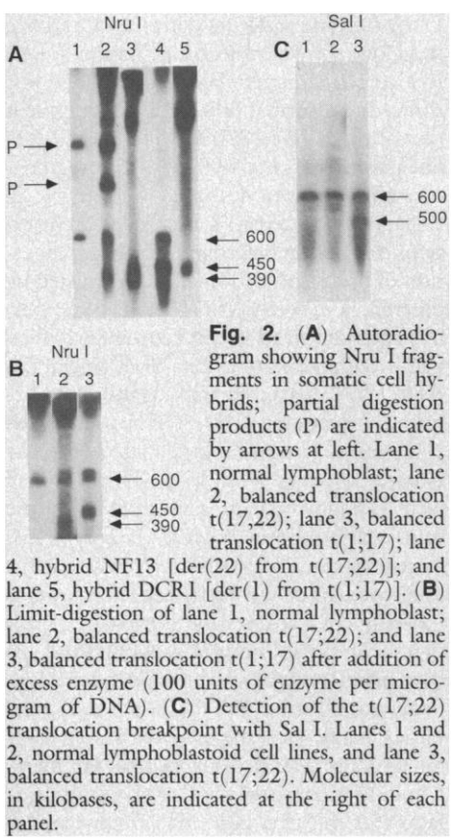

Fig. 2. (**A**) Autoradiogram showing Nru I fragments in somatic cell hybrids; partial digestion products (P) are indicated by arrows at left. Lane 1, normal lymphoblast; lane 2, balanced translocation t(17,22); lane 3, balanced translocation t(1;17); lane 4, hybrid NF13 [der(22) from t(17;22)]; and lane 5, hybrid DCR1 [der(1) from t(1;17)]. (**B**) Limit-digestion of lane 1, normal lymphoblast; lane 2, balanced translocation t(17;22); and lane 3, balanced translocation t(1;17) after addition of excess enzyme (100 units of enzyme per microgram of DNA). (**C**) Detection of the t(17;22) translocation breakpoint with Sal I. Lanes 1 and 2, normal lymphoblastoid cell lines, and lane 3, balanced translocation t(17;22). Molecular sizes, in kilobases, are indicated at the right of each panel.

the only Nru I fragments present in the NF13 and DCR1 somatic cell hybrids (both hybrids contain only the translocation chromosome and no normal chromosome 17); and (v) examination of the t(17;22) lymphoblastoid cell lines with the restriction enzyme Sal I shows a normal 600-kb fragment and a 500-kb, translocation-specific derivative fragment with c11-1F10 (Fig. 2C). The t(1;17) cell lines have not been successfully digested with Sal I. Digestion of DNA from normal cells and from both translocation cell lines with other enzymes (Mlu I, Not I, Sfi I, Bss HII, Fsp I, Nar I, and Cla I) yielded fragments smaller than 300 kb, too short to encompass the breakpoints and cosmid 11-1F10.

We do not yet have restriction maps of the new fragments derived from the translocation events involving chromosomes 1 and 22, respectively, so we cannot determine the order of the two translocation breakpoints on chromosome 17. Nevertheless, we can infer that both NF1 breakpoints and *NF1* are a maximum of 600 kb from c11-1F10 and may be much closer. We conclude that the NF1 translocation breakpoints, and *NF1* itself, are now within striking distance of conventional techniques of molecular genetics. We should now be able to use additional cosmids in regions 2 and 3, as well as recombinant DNA libraries prepared from the 600-kb Nru I fragment preparatively isolated from one of the microcell hybrid lines, to expedite the cloning of *NF1*.

Note added in proof: Probe p17L1A (*16*) has been tested on the same Nru I and Sal I PFGE filters used in this report. Both c11-1F10 and p17L1A identify the same 600-kb Nru I and Sal I fragments in normal individuals, supporting the premise that these markers and the two NF1 translocation breakpoints reside on identical large DNA fragments.

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