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Regional Localization of Chromosome 3–Specific DNA Fragments by Using a Hybrid Cell Deletion Mapping Panel

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Summary

A series of human chromosome 3–specific DNA fragments isolated and characterized from a lamda phage genomic library were regionally localized on human chromosome 3. This was accomplished using filter hybridization blot analysis of a human chromosome 3 hybrid cell deletion mapping panel. Twenty-three new anonymous DNA fragments were assigned to one of four physical regions of chromosome 3. Seventeen DNA fragments were mapped to the long arm of chromosome 3, including one DNA fragment that demonstrated a restriction fragment length polymorphism (RFLP). Five DNA fragments were assigned to 3p14.2→pter, including one highly polymorphic fragment sublocalized at 3p25→pter by in situ hybridization. This DNA fragment is the second reported distal 3p polymorphic probe. One DNA fragment was localized to 3p14→p14.2. In addition, three fragments previously assigned to chromosome 3 were confirmed. Polymorphic DNA probes DNF15S2 (formerly D1S1) and D3S2 were mapped to 3p14.2→pter. The previous 3p25 in situ localization of the c-*raf*-1 oncogene was supported by deletion panel mapping. The physical localization of these twenty-three new DNA fragments has more than doubled the number of cloned DNA fragments assigned to chromosome 3. These and future regional assignments of DNA fragment probes will facilitate construction of both a physical and genetic linkage map of chromosome 3. They may also be useful in characterizing the chromosomal and molecular aberrations involved in small-cell lung cancer (SCLC), renal cell carcinoma, other malignancies, and the 3p14.2 common fragile site.

Introduction

A number of human malignancies have characteristic chromosomal abnormalities of the short arm of chromosome 3. Karyotypes of small-cell lung cancer (SCLC) cells demonstrate an interstitial 3p14→p23 deletion in about two-thirds of cell lines examined (Whang-Peng et al. 1982; Falor et al. 1985). Other SCLC tumor cells have various larger deletions or translocations involving this region. Deletion of a DNA sequence at the chromosomal region 3p21 has been shown in all major types of lung cancer (Kok et al. 1987). Both spontaneous and heriditary renal cell carcinomas have been found to exhibit alterations in the short arm of chromosome 3 (Cohen et al. 1979; Pathak et al. 1982; Wang and Perkins 1984). Translocations involving 3p21 and 3p25 have been demonstrated in mixed salivary-gland tumors (Mark et al. 1980). Variable deletions and balanced translocations involving the short arm of chromosome 3 have also been reported in carcinoid, ovarian carcinoma, rhabdomyosarcoma, and various hematologic malignancies (Berger et al. 1985). The c-*raf*-1oncogene has been regionally assigned to 3p25 by in situ hybridization (Bonner et al. 1984). The c-*erb*-A-*B* oncogene has been localized to 3p22–24.1 (Drabkin et al., in press). Chromosome band 3p14.2 is a locus of an inducible chromosomal fragile site which has been described as the most active in the human genome (Smeets et al. 1986).

The study of these malignancies and of the 3p14.2

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fragile site has been hampered by the lack of chromosome 3 probes. For instance, loss of constitutional heterozygosity has been demonstrated in SCLC tumor tissue compared with paired normal tissue (Naylor et al. 1987). The resulting homozygosity of 3p loci appers to occur in a fashion similar to that described for 13q loci in retinoblastoma and 11p loci in Wilms tumor (Solomon 1984). In most cases, however, determination of the mechanism of this loss of heterozygosity, i.e., chromosomal loss, loss with reduplication, mitotic recombination, or translocation, is not possible, owing to the paucity of DNA probes that reveal RFLPs. This predicament is analogous to that previously faced with Wilms tumor when proximal polymorphic short-arm chromosome 11 probes were not yet available.

A previously described chromosome 3–specific library constructed from a somatic cell hybrid containing only human chromosome 3 was used to derive a large number of chromosome 3–specific recombinants (Carlock et al. 1986). DNA was prepared from these, and restriction endonuclease–generated fragments were tested as unique-sequence, chromosome 3–specific hybridization probes. Hybrid cell deletion mapping of these fragments were utilized to rapidly map a large number of chromosome 3–specific probes. We have previously used a human chromosome 3–specific hybrid cell deletion mapping panel to regionally assign the polymorphic probe D3S3 to 3p14 (Gerber et al. 1986). Here we describe the filter hybridization blot analysis of this human chromosome 3–specific hybrid cell deletion mapping panel to regionally assign the first 23, including two polymorphic probes, of several hundred chromosome 3–specific, unique-sequence DNA fragments to physical regions of chromosome 3.

Material and Methods

Hybrid Cell Lines

Previously described is a human chromosome 3–specific hybrid cell deletion mapping panel that was produced using selectable genetic markers on the long arms of both chromosomes 3 and 8 (Gerber et al. 1986). The Chinese hamster ovary (CHO) parent cell line $Urd^{-}C$ is an auxotrophic mutant requiring exogenous uridine or complementation with the long arm of human chromosome 3 for growth (Patterson et al. 1983). UCTP-2A-3 is a hybrid containing a normal human chromosome 3 as its only nonhamster genetic material (Firnhaber et al. 1985). Hybrid Q314–1 contains another, seperately derived, intact normal chromosome 3. Somatic cell hybrids, which contain separately both derivative chromosomes from a hereditary renal cell carcinoma 3;8 translocation, have been described (Drabkin et al. 1985). Hybrid TL9542/UC2/12–8 contains the derivative 3 chromosome (3qter→14.2::8q24.1→8qter) as the only identifiable human genetic material. Hybrid 3;8/4–1 contains the derivative 8 chromosome (8pter→q24.1::3p14.2→pter) in the absence of the der(3) and the normal human chromosomes 3 and 8. Somatic cell hybrids Y-195-1 and Y-195-4 have been described (Miller et al. 1985). Hybrid Y-195-1 contains a del(3p14→pter) chromosome 3 from SCLC cell line NCl-H69 as its only human chromosome 3, in addition to several other human chromosomes. Y-195-4 contains two karyotypically normal chromosome 3's from SCLC cell line NCl-H69, in addition to other human chromosomes. Hybrid UCH-12 contains the 3qter→cen portion of a normal human chromosome 3 which has been translocated onto a hamster parent chromosome (Patterson et al. 1983). The isolation and maintenance of the various cell lines has been previously reported (Patterson et al. 1983; Drabkin et al. 1985; Gerber et al. 1986).

Hybrid Cell Cytogenetic and Biochemical Analysis

Metaphase chromosome spreads of hybrid cell lines UCTP-2A-3, Q314-1, TL9542/UC2/12-8, 3;8/4-1, Y-195-1, Y-195-4, and UCH-12 were analyzed by sequential Giemsa/trypsin banding and Giemsa 11 staining at the time of DNA preparation (Morse et al. 1982). The presence of the 3q portion of human chromosome 3 in hybrid cell lines UCTP-2A-3, Q314-1, Y-195-1, Y-195-4, and UCH-12 was confirmed by expression of the human transferrin receptor (Miller et al. 1983) and complementation of uridine deficiency. The presence of 3p was confirmed in hybrid cell lines UCTP-2A-3, Q314-1, 3;8/4-1, and Y-195-4 by aminoacylase-1 (ACY-1) assay (Voss et al. 1980). Hybrid lines TL9542/UC2/12-8, Y-195-1, and UCH-12 were ACY-1 negative. These various hybrids comprising the human chromosome 3–specific hybrid cell deletion mapping panel are represented in ideogram form in figure 1.

New Chromosome 3 Hybridization Probes

A human chromosome 3–specific genomic library was constructed in EMBL-4 from somatic cell hybrid HHW 423 that contains, as its only human DNA, chromosome 3 (Carlock et al. 1986). Recombinants containing human inserts were identified by hybridization to human-specific, highly repetitive DNA (Gusella et al. 1980). DNA prepared from these recombinants was digested with either *Eco*RI or *Hind*III. Subsequent *Alu-*

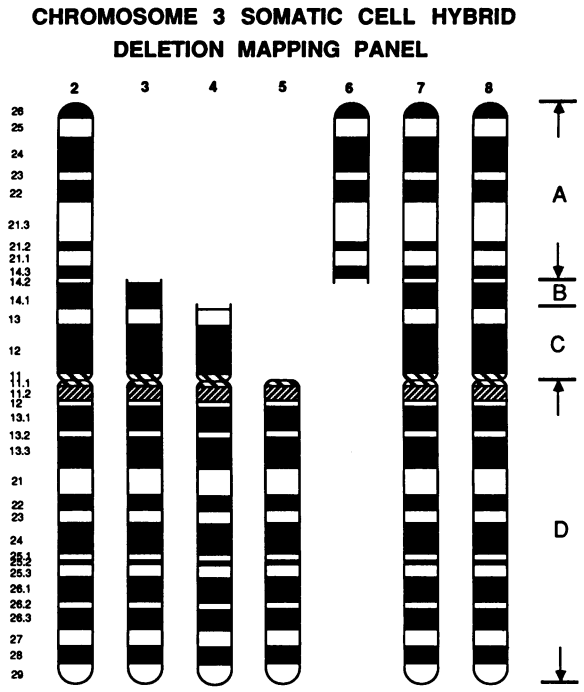


Figure 1 Ideograms of the human chromosome 3-specific hybrid deletion mapping panel. Lane 1, Chinese hamster ovary (CHO) parent cell line Udr⁻C (not shown). Lane 2, Hybrid UCTP-2A-3 containing a normal human chromosome 3 as its only nonhamster DNA. Lane 3, Hybrid TL9542/UC2/12–8 containing the der(3) chromosome (3qter→3p14.2::8q24.1→qter). Lane 4, Hybrid Y-195-1 containing the 3qter→p14 portion of a chromosome 3 from SCLC cell line NCI-H69. Lane 5, Hybrid UCH-12 containing the 3qter→cen portion of a normal human chromosome 3. Lane 6, Hybrid 3;8/4-1 containing the der(8) chromosome (8pter→q24.1::3p14.2→pter). Lane 7, Hybrid Y-195-4 containing two karyotypically normal chromosome 3's from NCI-H69. Lane 8, Hybrid Q314–1 containing a normal human chromosome 3 as its only nonhamster DNA. To the right of fig. 1 is a representation of the four physical mapping regions A–D delineated by the hybrid cell deletion mapping panel as described in Results.

negative fragments were then tested as unique-sequence, chromosome 3–specific hybridization probes. Restriction fragments were cut out of LMP-agarose and then radiolabeled with [^{32}P]-dCTP by using random oligonucleotide primers (Feinberg and Vogelstein 1984). The radiolabeled fragments were not purified from the random-primer labeling reaction but were directly hybridized to Nytran™ filters that contained *Eco*RI-digested DNA from CHO, HeLa, or hybrid HHW 423 (Carlock et al. 1986).

Existing Probes

The D1S1 locus (now designated DNF15S2) is defined by the phage lambda Ch4A-H3 which contains a 16-kb insert of human DNA (Harper and Saunders 1981; Donlon and Magenis 1984; Carrit et al. 1986; Goode et al. 1986). This probe was provided by the laboratory of Dr. G. F. Saunders. The phage DNA was isolated from infected *E. coli* LE392 by chloroform lysis of the cells and purification of the DNA on a cesium chloride gradient (Maniatis et al. 1982).

The probe D3S2 was provided by the laboratory of Dr. R. White. This polymorphic probe is a single-copy 0.54-kb human insert cloned into the *Bam*HI site of plasmid pACYC184 (Naylor et al. 1984).

A 3′ flanking sequence probe of the human c-*raf*-1 oncogene containing a 2.5-kb *Eco*RI DNA fragment was provided by Dr. T. Bonner. The D3S2 and c-*raf*-1 plasmid DNA were isolated by standard plasmid preparation (Maniatis et al. 1982).

For hybridization experiments D1S1, D3S2, and the plasmid containing the flanking sequence of c-*raf*-1 were labeled with [^{32}P]-dCTP to a specific activity of 10^8 cpm/μg DNA by nick-translation (Maniatis et al. 1975).

Filter Hybridizations Blot Analysis

High-molecular-weight DNA was isolated from each cell line, and peripheral blood leukocytes of normal adult volunteers by standard procedures (Kunkel et al. 1977). Samples (5–10 μg) of DNA were digested to completion with a restriction endonuclease according to the suppliers' specifications. The digested DNA fragments were separated by horizontal electrophoresis on 0.8% agarose gels. DNA was transferred from the gels to Nytran filters (Southern 1975). The Nytran filters were baked at 80 C for 2 h, prehybridized, and then hybridized with 1.0×10^6 cpm of probe/ml buffer at 42 C for 16–20 h. Hybridization buffer consisted of 6 × SSC, 1% SDS, 50 μg/salmon-sperm DNA/ml, and 50% formamide. The filters were washed in 2 × SSC and 0.1% SDS twice for 15 min at room temperature, in 1 × SSC and 0.1% SDS twice for 15 min at room temperature, then in 0.1% SSC and 0.1% SDS for 1 h at 55 C with shaking. Kodak XAR-5 film with a Dupont Chromex® intensifying screen was exposed to the Nytran filters for 1–7 days at −70 C.

RFLP Analysis

Many of the chromosome 3–specific hybridization probes had been previously screened for RFLPs by using 12 unrelated individuals' peripheral blood leukocyte DNAs digested with 14 different restriction endonucleases (Smith 1985). The polymorphic 3p fragment, designated 64 *Eco*RI band 2, was hybridized to filter hybridization blots of DNA from a total of 60 unrelated

individuals as described in the preceding section to more accurately assess the polymorphic allele frequency.

Results

Human Chromosome 3 Hybrid Cell Deletion Mapping Panel

The chromosome 3 hybrid cell deletion mapping panel ideograms are depicted in figure 1. This panel allows mapping of DNA fragments and probes into one of four physical regions of chromosome 3, designated A–D as illustrated to the right of the ideograms in figure 1. Region A is 3p14.2→pter; region B is 3p14→3p14.2; region C is cen→3p14; and region D is 3q. Hybridization of any DNA probe with lanes 2, 7, and 8 containing the DNA of karyotypically intact chromosome 3's demonstrates that the probe of interest identifies a locus on chromosome 3. Probe hybridization with lane 5 containing the DNA of the 3q-only hybrid clearly assigns any given probe to the long-arm region D. Probes that hybridize with lane 6 containing the 3p14.2→pter region A are therefore localized to this region. Hybridization with lane 3 but not with lane 4 assigns the probe to 3p14→3p14.2 region B. Similarly, probes can be localized to the cen→3p14 region C by hybridization with all hybrid lanes except 5 and 6. Lane 1 contains the DNA of the CHO parent cell line Urd$^-$C.

Regional Assignment of Chromosome 3 DNA Probes and RFLPs

Twenty-three unique-sequence probes, including two that revealed polymorphic loci, were assigned to one of four physical regions of chromosome 3 (fig. 1). Seventeen DNA fragments were mapped to region D, the long arm of chromosome 3, as shown in figures 2*A* and 4. One of these 17 long-arm DNA fragments, 55 *Eco*RI band 2, demonstrated an RFLP. This fragment revealed an *Msp*I polymorphism of 3.0 kb–2.7 kb with allele frequencies of .83–.17, respectively, when probed against the DNA of 12 unrelated individuals (fig. 3*A*).

Five DNA fragments were regionally assigned to the 3p14.2→pter region A, including one highly polymorphic fragment, 64 *Eco*RI band 2 (figs. 2*B*, 4). It has been previously sublocalized at 3p25→pter by in situ hybridization (Gilbert et al. 1987). 64 *Eco*RI band 2 demonstrates an *Msp*I RFLP of 2.5 kb–3.0 kb with allele frequencies of .69–.31, respectively, when screened against the DNA of 60 unrelated individuals (fig. 3*B*). Besides a clone of c-*raf*-1 that demonstrates an RFLP, this is the only other distal 3p polymorphic DNA probe localized to date.

One DNA fragment, 214 *Eco*RI band 3, was assigned to region B 3p14→p14.2 Figure 2*C* shows that the probe hybridizes with lane 3, containing chromosome region 3qter→3p14.2, but not with lane 4, containing 3qter→3p14. Therefore, DNA fragment 214 *Eco*RI band 3 is located in the 3p14→p14.2 region B. No new probes were localized to region C, cen→3p14.

Regional Assignment of Existing 3p Probes

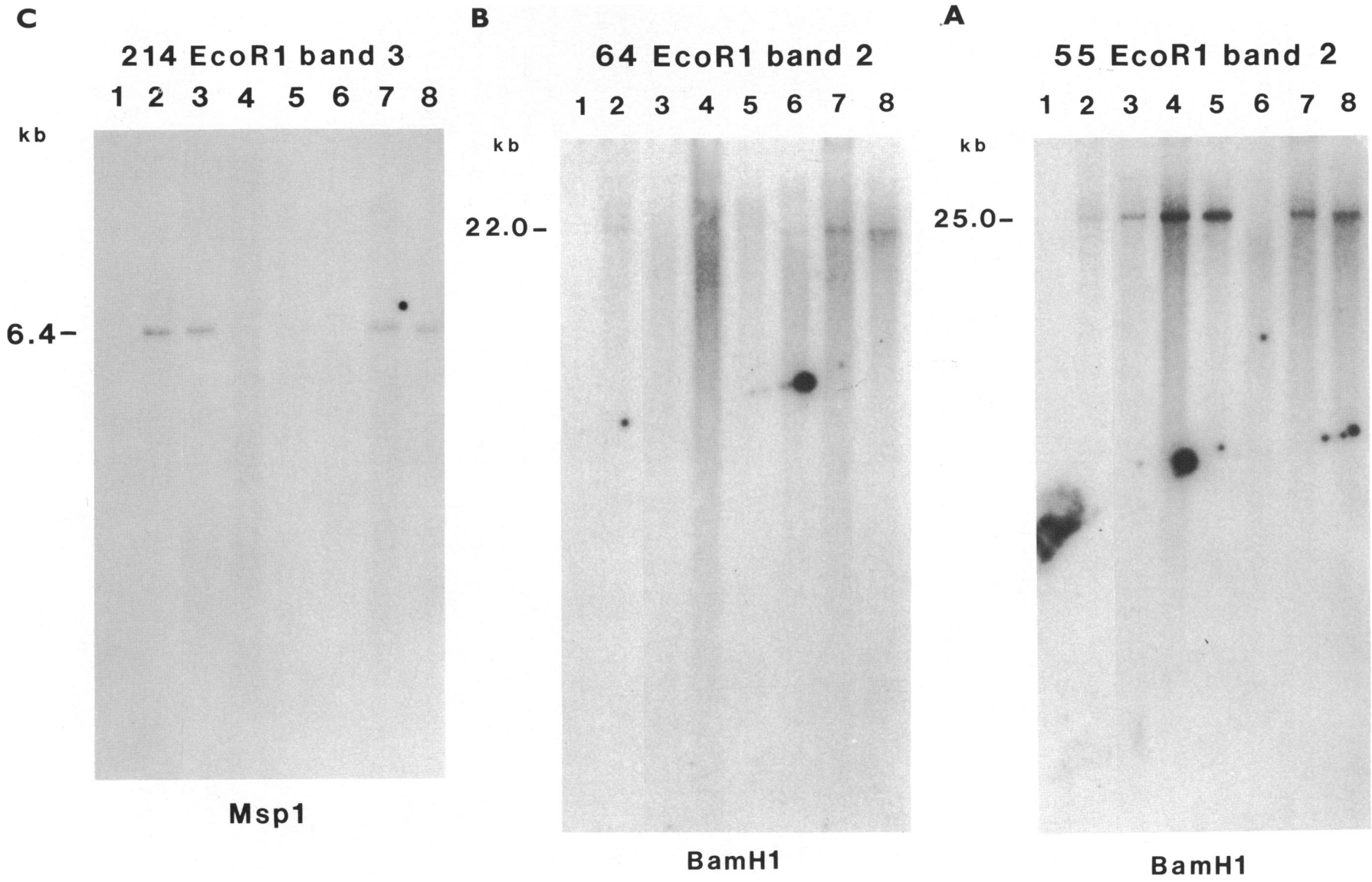
DNF15S2 (formerly D1S1) was the first nonrepetitive-sequence DNA clone mapped by in situ hybridization (Harper and Saunders et al. 1981). It was originally localized to 1p36. However, it was reassigned to chromosome 3 by using somatic cell hybrids and was found to be reduplicated on chromosome 1 (Carritt et al. 1986; Goode et al. 1986). The confirmatory in situ hybridization (Donlon and Magenis 1984) showed that the majority of the silver grains were located over 1p36; however, there were significant grains over 3p21. The D1S1 probe contains a sequence located on chromosome 3 and repeated on chromosome 1 with DNF15S2 at 3p14.2→pter and DNF15S1 at 1p36. By probing the human chromosome 3–specific hybrid cell deletion mapping panel with D1S1, we assigned the DNF15S2 locus to the 3p14.2→pter region A, thus supporting the chromosome 3 localization (fig. 2*D*).

D3S2 reveals an *Msp*I polymorphism of 2.9 kb and 1.3 kb with allele frequencies of 0.7 and 0.3, respectively (Naylor et al. 1984). Originally it was regionally localized to 3q21→qter by using hybrid cells constructed with human fibroblasts having chromosomal translocations at 3q21→qter (Naylor et al. 1984). It has since been provisionally reassigned to 3p14→p21 (Kidd and Gusella 1985; S. L. Naylor, personal communication). Figure 2*E* shows that the D3S2 probe hybridizes with the lane 6 containing the 3p14.2→pter portion of chromosome 3. This confirms that the D3S2 locus is in the 3p14.2→pter region A rather than in 3q.

The hybridization of the c-*raf*-1 oncogene flanking-sequence probe produces a filter hybridization blot autogradiogram that regionally assigns c-*raf*-1 to the 3p14.2→pter region A (fig. 4; autoradiogram not shown). This supports the prior in situ hybridization localization of c-*raf*-1 at 3p25 (Bonner et al. 1984).

Discussion

Filter blot hybridization using this chromosome 3–specific deletion mapping panel has enabled chromosome 3 probes to be regionally assigned to one of four specific physical regions of chromosome 3 (Fig.


C
214 EcoR1 band 3
1 2 3 4 5 6 7 8
kb
6.4–
Msp1
B
64 EcoR1 band 2
1 2 3 4 5 6 7 8
kb
22.0–
BamH1
A
55 EcoR1 band 2
1 2 3 4 5 6 7 8
kb
25.0–
BamH1

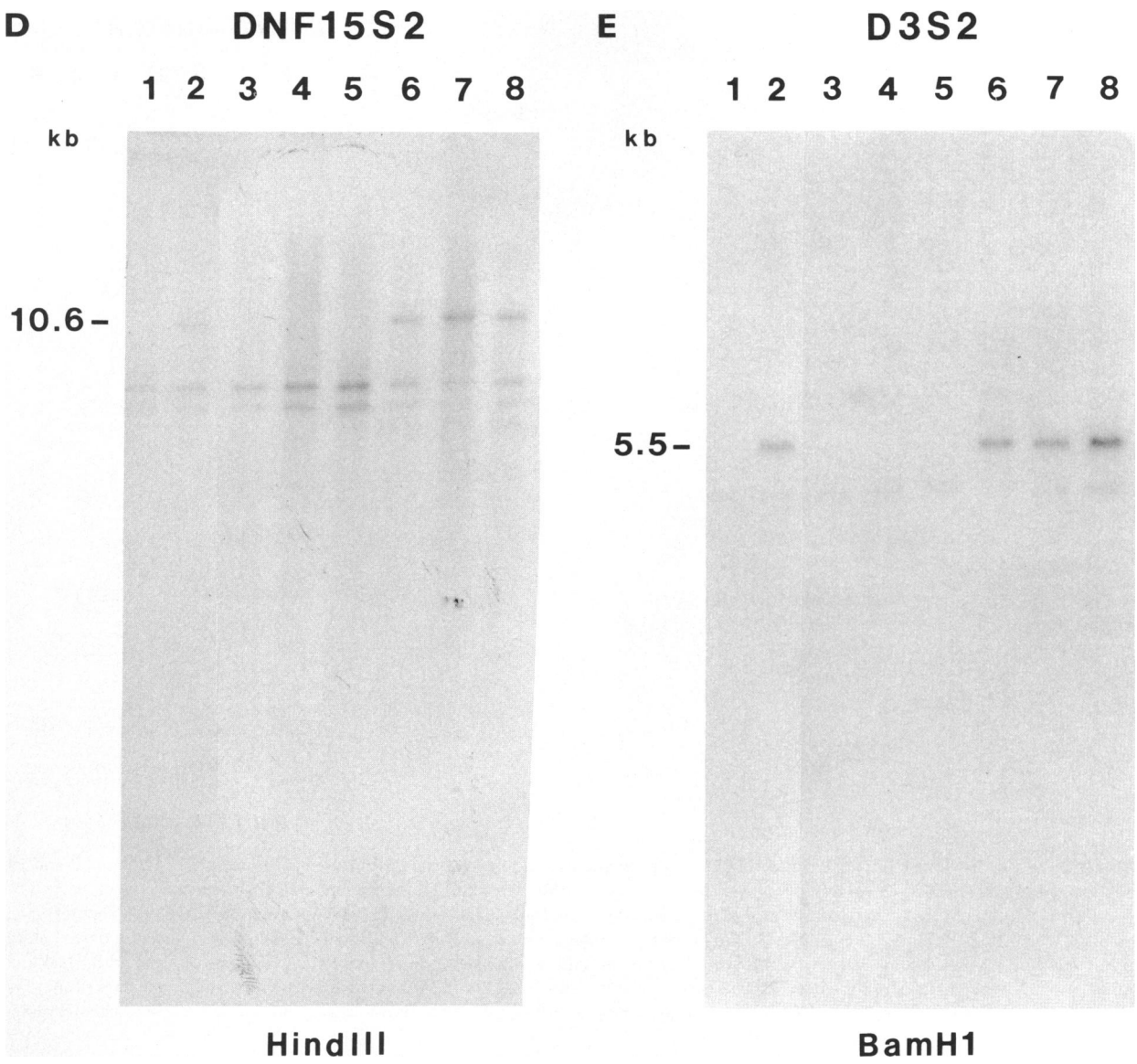


Figure 2 Filter hybridization blot autoradiograms of the hybrid deletion mapping panel digested with *Bam*HI (panels *A*, *B*, and *E*), *Msp*I (panel *C*), and *Hin*dIII (panel *D*). These Southern blots were probed with the following DNA fragments: 55 *Eco*RI band 2 (panel *A*), 64 *Eco*RI band 2 (panel *B*), 214 *Eco*RI band 3 (panel *C*), DNF 15S2 (panel *D*), and D3S2 (panel *E*). Lanes 1–8 correspond to the parent hamster cell line and hybrids as described in the legend for fig 1. In panel B, lane 4 demonstrates a smear but no 22.0-kb band. The smear likely represents repetitive sequence homology with other human chromosomes contained in the Y-195-1 hybrid. The autoradiogram of the deletion mapping panel probed with plasmid containing the 3′ flanking sequence of c-*raf*-1 is not shown.

1). Molecular hybridization of somatic cell hybrid deletion panels allows for a large number of probes to be regionally assigned in a very short period of time. This is critical, owing to the increasing number of chromosome 3–specific DNA fragments isolated which require regional mapping (Golembieski et al. 1987). In addition, this hybrid deletion mapping panel can confirm the unique locus of a probe by comparing filter hybridization blots of single-chromosome hybrids to human genomic DNA. This mapping panel also poses another hypothetical characteristic. The hybrid Y-195-4 has two karyotypically normal chromosome 3's from

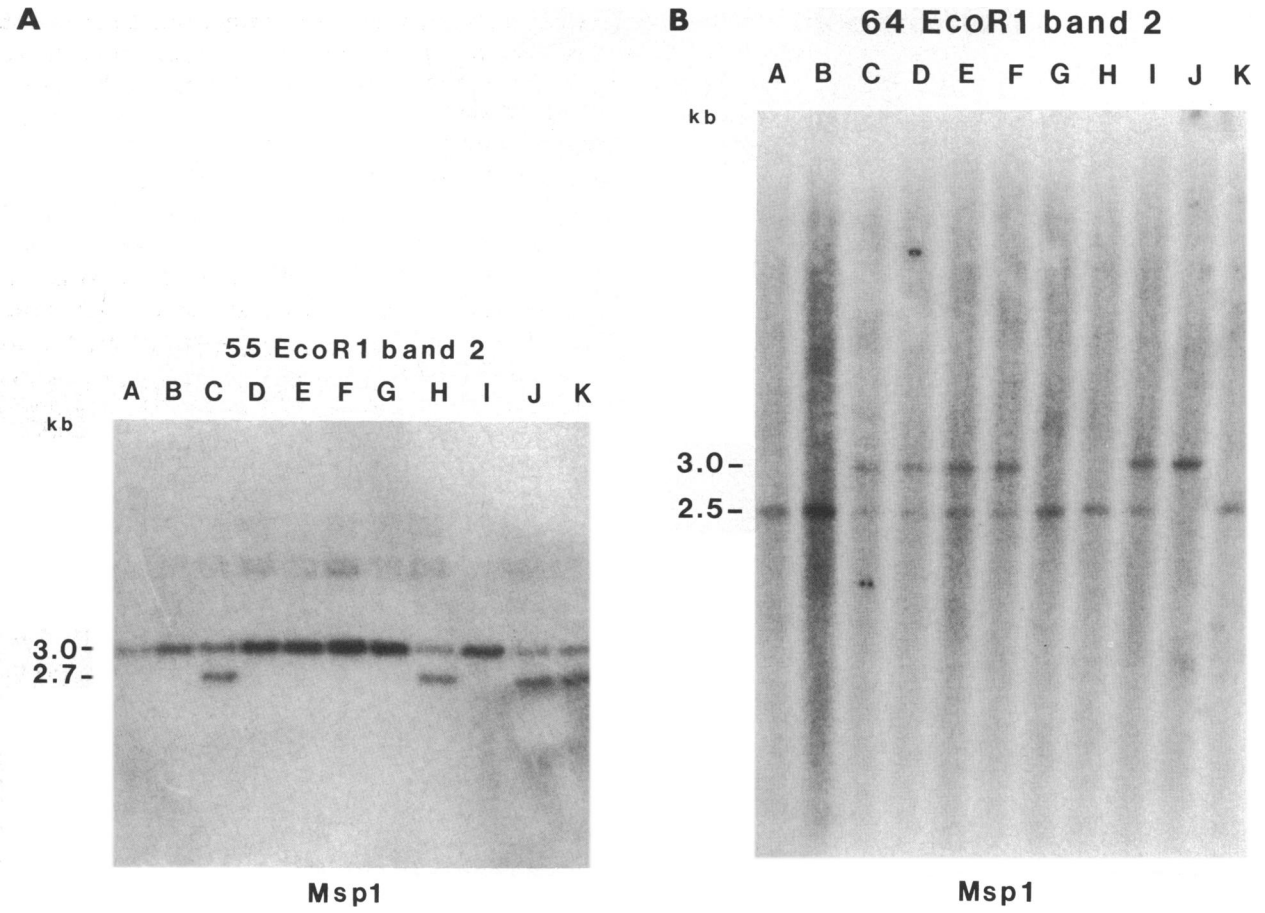


Figure 3 Representative filter hybridization blot autoradiograms demonstrating RFLPs revealed by probes *55 Eco*RI band 2 and 64 *Eco*RI band 2. Panel *A* shows the *Msp*I polymorphisms of 3.0 kb–2.7 kb with allele frequencies of .83–.17, respectively, when probed by the long arm DNA fragment *55 Eco* band 2 against 12 unrelated individuals (only 11 lanes shown). Lane A is a representative 3.0-kb homozygote; and lane C is a 3.0-kb/2.7 heterozygote. No. 2.7-kb homozygotes are demonstrated. The faint upper bands may represent a partial *Msp*I digest. Panel *B* is a representative blot of the DNA of 11 of the 60 random unrelated normal individuals that was digested with *Msp*I and probed with DNA fragment 64 *Eco*RI band 2. This probe demonstrates an RFLP of 3.0 kb and 2.5 kb with allele frequencies of .69–.31 respectively. Lane A is a representative 2.5-kb homozygote; lane C is a 3.0-kb/2.5-kb heterozygote; and lane J is a 3.0-kb homozygote.

SCLC cell line NCI-H69. Hybrids UCTP2A-3 and Q314-1 contain single intact chromosome 3's from normal individuals. If any given probe hybridizes with the normal chromosome 3 hybrids but not with Y-195-4, then this putatively would suggest that the chromosome 3's derived from the SCLC tumor have a submicroscopic deletion. A probe with this characteristic could then focus the search for those 3p DNA segments and genes involved in SCLC.

The mapping panel used for these studies can only localize probes to large regions of the chromosome, although it can delineate an important area, 3p14→p14.2. Several recent hybrid additions to this panel have been constructed which expand the regional mapping capabilities (H. A. Drabkin, unpublished data). Also, this panel includes portions of chromosome 3 that were derived from tumor cells and therefore may have rearrangements not detected by cytogenetic analysis. However, studies to date using additional hybrids have shown no inconsistencies. Further in situ studies with selected probes are in agreement with our localizations (H. A. Drabkin, unpublished data).

Although the chromosome 3 library was constructed from a karyotypically intact human chromosome 3, only one new probe has been mapped to region B, 3p14→p14.2; and no new library DNA fragments have

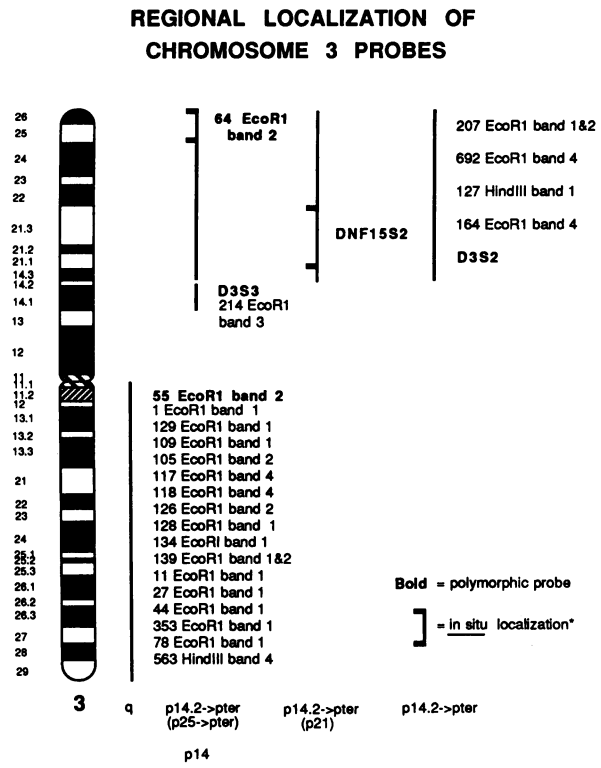


Figure 4 Physical location of the chromosome 3 DNA fragments and other existing probes on chromosome 3. Polymorphic probes are represented in boldface. The brackets indicate the regional localization of DNF15S2 and 64 *Eco*RI band 2. The DNF15S2 locus in situ hybridization (denoted by the asterisk [*] has been reported elsewhere (Donlon and Magenis 1984). The in situ hybridization of 64 *Eco*RI band 2 has been reported elsewhere (Gilbert et al. 1987).

yet been mapped to region C, cen→3p14. Rather, three-quarters of the probes map to 3q and one-quarter to region A (3p14.2→pter). Statistically, one would expect more fragments to be localized to regions B and C. There are several hypothetical explanations. These regions may be rich in repetitive sequences, and therefore, during the library construction, certain regions may have been eliminated during the cotting out of repeat sequences. Another possibility is that these regions are AT rich and are not easily cloned when endonuclease *Mbo*I is used to prepare genomic inserts. It has been suggested that the region surrounding the locus defined by D3S3, i.e., 3p14, may either be highly methylated or very AT rich (Gemmill et al. 1986). This may explain the paucity of region B or C probes derived from this chromosome 3 library. Similar observations have been made in the regional mapping of chromosome 21–derived DNA probes (Korenberg et al. 1987).

The physical localization of these 23 chromosome 3 library DNA fragments has doubled the number of cloned DNA fragments assigned to chromosome 3 (Kidd and Gusella 1985). Besides a clone of c-*raf*-1 that demonstrates an RFLP, the probe 64 *Eco*RI band 2 is the only other distal polymorphic 3p probe published to date. This probe will prove extremely useful not only in constructing a genetic linkage map of chromosome 3 but also in determining the distal extent of the 3p14–23 deletion in SCLC — and possibly the nature of the 3p25 translocation in mixed salivary-gland tumors. Similarly, DNA fragment 214 *Eco*RI band 3 is the only probe besides D3S3 that is localized to 3p14. This probe may help in delineating the 3p14.2 fragile site.

Acknowledgments

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