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The complete coding sequence of the human *raf* oncogene and the corresponding structure of the c-*raf*-1 gene

Tom I.Bonner, Hermann Oppermann[1], Peter Seeburg[1], Stephen B.Kerby[2], Mark A.Gunnell[2], Alice C.Young and Ulf R.Rapp[3]

Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205, [1]Department of Molecular Biology, Genentech Inc., South San Francisco, CA 94080, [2]Program Resources Inc., Frederick Cancer Research Facility, Frederick, MD 21701 and [3]Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701, USA

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ABSTRACT

The complete 648 amino acid sequence of the human raf oncogene was deduced from the 2977 nucleotide sequence of a fetal liver cDNA. The cDNA has been used to obtain clones which extend the human c-raf-1 locus by an additional 18.9 kb at the 5' end and contain all the remaining coding exons.

INTRODUCTION

Oncogenes are genes which have been identified because they cause cellular transformation either when naturally incorporated into a retrovirus or when introduced into tissue culture cells by transfection. Since these genes are evolutionarily well conserved, they are generally thought to have a normal role in the cell which is subverted by their reintroduction into the cell in a form which alters their function or regulation. A normal function has been identified for 3 of the approximately 25 known oncogenes. The erbB gene is derived from the receptor gene for epidermal growth factor (EGF) (1), the sis oncogene corresponds to a portion of the gene for platelet derived growth factor (PDGF) (2) and the fms gene product has been shown to be related to the CSF-1 receptor (3). The raf oncogene was originally isolated from a murine transforming retrovirus, 3611-MSV (4). Subsequently the mil (or mht) oncogene of the avian virus MH2 was identified as being the avian homologue of raf (5, 6). The deduced amino acid sequences of these oncogenes are distantly related to a number of oncogenes which encode tyrosine specific kinases, as well as others which apparently do not (6, 7). Although the viral raf and mil gene products do not have tyrosine specific kinase activity, they have recently been shown to be associated with a serine-threonine-specific kinase (6, 8). We have recently reported that there are two human genes related to raf and mil (9). The functional gene, c-raf-1 contains eleven exons which are homologous to mil, nine of which are also homologous to raf. The second gene, c-raf-2, is a processed pseudogene. In order to

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                                                                                                                        99
CCG AAT GTG ACC GCC TCC CGC TCC CTC ACC CGC CGC GGG GAG GAG GAG CGG GCG AGA AGC TGC CGC CGA ACG ACA GGA CGT TGG GGC GGC CTG GCT CCC
exon 2
  *                                                                                                                    198
TCA GGT TTA AGA ATT GTT TAA GCT GCA TCA ATG GAG CAC ATA CAG GGA GCT TGG AAG ACG ATC AGC AAT GGT TTT GGA TTC AAA GAT GCC GTG TTT GAT
                                        MET Glu His Ile Gln Gly Ala Trp Lys Thr Ile Ser Asn Gly Phe Gly Phe Lys Asp Ala Val Phe Asp

                                                                                                                       297
GGC TCC AGC TGC ATC TCT CCT ACA ATA GTT CAG CAG TTT GGC TAT CAG CGC CGG GCA TCA GAT GAT GGC AAA CTC ACA GAT CCT TCT AAG ACA AGC AAC
Gly Ser Ser Cys Ile Ser Pro Thr Ile Val Gln Gln Phe Gly Tyr Gln Arg Arg Ala Ser Asp Asp Gly Lys Leu Thr Asp Pro Ser Lys Thr Ser Asn
                                                exon 3
                                                  *                                                                    396
ACT ATC CGT GTT TTC TTG CCG AAC AAG CAA AGA ACA GTG GTC AAT GTG CGA AAT GGA ATG AGC TTG CAT GAC TGC CTT ATG AAA GCA CTC AAG GTG AGG
Thr Ile Arg Val Phe Leu Pro Asn Lys Gln Arg Thr Val Val Asn Val Arg Asn Gly MET Ser Leu His Asp Cys Leu MET Lys Ala Leu Lys Val Arg
                                                                        exon 4
                                                                          *                                            495
GGC CTG CAA CCA GAG TGC TGT GCA GTG TTC AGA CTT CTC CAC GAA CAC AAA GGT AAA AAA GCA CGC TTA GAT TGG AAT ACT GAT GCT GCG TCT TTG ATT
Gly Leu Gln Pro Glu Cys Cys Ala Val Phe Arg Leu Leu His Glu His Lys Gly Lys Lys Ala Arg Leu Asp Trp Asn Thr Asp Ala Ala Ser Leu Ile
                                                                            exon 5
                                                                              *                                        594
GGA GAA GAA CTT CAA GTA GAT TTC CTG GAT CAT GTT CCC CTC ACA ACA CAC AAC TTT GCT CGG AAG ACG TTC CTG AAG CTT GCC TTC TGT GAC ATC TGT
Gly Glu Glu Leu Gln Val Asp Phe Leu Asp His Val Pro Leu Thr Thr His Asn Phe Ala Arg Lys Thr Phe Leu Lys Leu Ala Phe Cys Asp Ile Cys

                                                                                                                       693
CAG AAA TTC CTG CTC AAT GGA TTT CGA TGT CAG ACT TGT GGC TAC AAA TTT CAT GAG CAC TGT AGC ACC AAA GTA CCT ACT ATG TGT GTG GAC TGG AGT
Gln Lys Phe Leu Leu Asn Gly Phe Arg Cys Gln Thr Cys Gly Tyr Lys Phe His Glu His Cys Ser Thr Lys Val Pro Thr MET Cys Val Asp Trp Ser
                  exon 6
                    *                                                                                                  792
AAC ATC AGA CAA CTC TTA TTG TTT CCA AAT TCC ACT ATT GGT GAT AGT GGA GTC CCA GCA CTA CCT TCT TTG ACT ATG CGT CGT ATG CGA GAG TCT GTT
Asn Ile Arg Gln Leu Leu Leu Phe Pro Asn Ser Thr Ile Gly Asp Ser Gly Val Pro Ala Leu Pro Ser Leu Thr MET Arg Arg MET Arg Glu Ser Val
                  exon 7
                    *                                                                                                  891
TCC AGG ATG CCT GTT AGT TCT CAG CAC AGA TAT TCT ACA CCT CAC GCC TTC ACC TTT AAC ACC TCC AGT CCC TCA TCT GAA GGT TCC CTC TCC CAG AGG
Ser Arg MET Pro Val Ser Ser Gln His Arg Tyr Ser Thr Pro His Ala Phe Thr Phe Asn Thr Ser Ser Pro Ser Ser Glu Gly Ser Leu Ser Gln Arg
                                                                                                exon 8
                                                                                                  *                    990
CAG AGG TCG ACA TCC ACA CCT AAT GTC CAC ATG GTC AGC ACC ACG CTG CCT GTG GAC AGC AGG ATG ATT GAG GAT GCA ATT CGA AGT CAC AGC GAA TCA
Gln Arg Ser Thr Ser Thr Pro Asn Val His MET Val Ser Thr Thr Leu Pro Val Asp Ser Arg MET Ile Glu Asp Ala Ile Arg Ser His Ser Glu Ser
exon 9
  *                                                                                                                   1089
GCC TCA CCT TCA GCC CTG TCC AGT AGC CCC AAC AAT CTG AGC CCA ACA GGC TGG TCA CAG CCG AAA ACC CCC GTG CCA GCA CAA AGA GAG CGG GCA CCA
Ala Ser Pro Ser Ala Leu Ser Ser Ser Pro Asn Asn Leu Ser Pro Thr Gly Trp Ser Gln Pro Lys Thr Pro Val Pro Ala Gln Arg Glu Arg Ala Pro
                                            exon 10
                                              *                                                                       1188
GTA TCT GGG ACC CAG GAG AAA AAC AAA ATT AGG CCT CGT GGA CAG AGA GAT TCA AGC TAT TAT TGG GAA ATA GAA GCC AGT GAA GTG ATG CTG TCC ACT
Val Ser Gly Thr Gln Glu Lys Asn Lys Ile Arg Pro Arg Gly Gln Arg Asp Ser Ser Tyr Tyr Trp Glu Ile Glu Ala Ser Glu Val MET Leu Ser Thr
                                                            exon 11
                                                              *                                                       1287
CGG ATT GGG TCA GGC TCT TTT GGA ACT GTT TAT AAG GGT AAA TGG CAC GGA GAT GTT GCA GTA AAG ATC CTA AAG GTT GTC GAC CCA ACC CCA GAG CAA
Arg Ile Gly Ser Gly Ser Phe Gly Thr Val Tyr Lys Gly Lys Trp His Gly Asp Val Ala Val Lys Ile Leu Lys Val Val Asp Pro Thr Pro Glu Gln
                                            exon 12
                                              *                                                                       1386
TTC CAG GCC TTC AGG AAT GAG GTG GCT GTT CTG CGC AAA ACA CGG CAT GTG AAC ATT CTG CTT TTC ATG GGG TAC ATG ACA AAG GAC AAC CTG GCA ATT
Phe Gln Ala Phe Arg Asn Glu Val Ala Val Leu Arg Lys Thr Arg His Val Asn Ile Leu Leu Phe MET Gly Tyr MET Thr Lys Asp Asn Leu Ala Ile

                                                                                                                      1485
GTG ACC CAG TGG TGC GAG GGC AGC AGC CTC TAC AAA CAC CTG CAT GTC CAG GAG ACC AAG TTT CAG ATG TTC CAG CTA ATT GAC ATT GCC CGG CAG ACG
Val Thr Gln Trp Cys Glu Gly Ser Ser Leu Tyr Lys His Leu His Val Gln Glu Thr Lys Phe Gln MET Phe Gln Leu Ile Asp Ile Ala Arg Gln Thr
                exon 13                                                     exon 14
                  *                                                           *                                       1584
GCT CAG GGA ATG GAC TAT TTG CAT GCA AAG AAC ATC ATC CAT AGA GAC ATG AAA TCC AAC AAT ATA TTT CTC CAT GAA GGC TTA ACA GTG AAA ATT GGA
Ala Gln Gly MET Asp Tyr Leu His Ala Lys Asn Ile Ile His Arg Asp MET Lys Ser Asn Asn Ile Phe Leu His Glu Gly Leu Thr Val Lys Ile Gly
                                                                                                        exon 15
                                                                                                          *           1683
GAT TTT GGT TTG GCA ACA GTA AAG TCA CGC TGG AGT GGT TCT CAG CAG GTT GAA CAA CCT ACT GGC TCT GTC CTC TGG ATG GCC CCA GAG GTG ATC CGA
Asp Phe Gly Leu Ala Thr Val Lys Ser Arg Trp Ser Gly Ser Gln Gln Val Glu Gln Pro Thr Gly Ser Val Leu Trp MET Ala Pro Glu Val Ile Arg

                                                                                                                      1782
ATG CAG GAT AAC AAC CCA TTC AGT TTC CAG TCG GAT GTC TAC TCC TAT GGC ATC GTA TTG TAT GAA CTG ATG ACG GGG GAG CTT CCT TAT TCT CAC ATC
MET Gln Asp Asn Asn Pro Phe Ser Phe Gln Ser Asp Val Tyr Ser Tyr Gly Ile Val Leu Tyr Glu Leu MET Thr Gly Glu Leu Pro Tyr Ser His Ile
                    exon 16
                      *                                                                                               1881
AAC AAC CGA GAT CAG ATC ATC TTC ATG GTG GGC CGA GGA TAT GCC TCC CCA GAT CTT AGT AAG CTA TAT AAG AAC TGC CCC AAA GCA ATG AAG AGG CTG
Asn Asn Arg Asp Gln Ile Ile Phe MET Val Gly Arg Gly Tyr Ala Ser Pro Asp Leu Ser Lys Leu Tyr Lys Asn Cys Pro Lys Ala MET Lys Arg Leu
                                                                exon 17
                                                                  *                                                   1980
GTA GCT GAC TGT GTG AAG AAA GTA AAG GAA GAG AGG CCT CTT TTT CCC CAG ATC CTG TCT TCC ATT GAG CTG CTC CAA CAC TCT CTA CCG AAG ATC AAC
Val Ala Asp Cys Val Lys Lys Val Lys Glu Glu Arg Pro Leu Phe Pro Gln Ile Leu Ser Ser Ile Glu Leu Leu Gln His Ser Leu Pro Lys Ile Asn

                                                                                                                      2079
CGG AGC GCT TCC GAG CCA TCC TTG CAT CGG GCA GCC CAC ACT GAG GAT ATC AAT GCT TGC ACG CTG ACC ACG TCC CCG AGG CTG CCT GTC TTC TAG TTG
Arg Ser Ala Ser Glu Pro Ser Leu His Arg Ala Ala His Thr Glu Asp Ile Asn Ala Cys Thr Leu Thr Thr Ser Pro Arg Leu Pro Val Phe

                                                                                                                      2199
ACTTTGCACC TGTCTTCAGG CTGCCAGGGG AGGAGGAGAA GCCAGCAGGC ACCACTTTTC TGCTCCCTTT CTCCAGAGGC AGAACACATG TTTTCAGAGA AGCTCTGCTA AGGACCTTCT
                                                                                                                      2319
AGACTGCTCA CAGGGCCTTA ACTTCATGTT GCCTTCTTTT CTATCCCTTT GGGCCCTGGG AGAAGGAAGC CATTTGCAGT GCTGGTGTGT CCTGCTCCCT CCCCACATTC CCCATGCTCA
                                                                                                                      2439
AGGCCCAGCC TTCTGTAGAT GCGCAAGTGG ATGTTGATGG TAGTACAAAA AGCAGGGGCC CAGCCCCAGC TGTTGGCTAC ATGAGTATTT AGAGGAAGTA AGGTAGCAGG CAGTCCAGCC
                                                                                                                      2559
CTGATGTGGA GACACATGGG ATTTTGGAAA TCAGCTTCTG GAGGAATGCA TGTCACAGGC GGGACTTTCT TCAGAGAGTG GTGCAGCGCC AGACATTTTG CACATAAGGC ACCAAACAGC
                                                                                                                      2679
CCAGGACTGC CGAGACTCTG GCCGCCCGAA GGAGCCTGCT TTGGTACTAT GGAACTTTTC TTAGGGGACA CGTCCTCCTT TCACAGCTTC TAAGGTGTCC AGTGCATTGG GATGGTTTTC
                                                                                                                      2799
CAGGCAAGGC ACTCGGCCAA TCCGCATCTC AGCCCTCTCA GGAGCAGTCT TCCATCATGC TGAATTTTGT CTTCCAGGAG CTGCCCCTAT GGGGCGGGCC GCAGGGCCAG CCTGTTTCTC
                                                                                                                      2919
TAACAAACAA ACAAACAAAC AGCCTTGTTT CTCTAGTCAC ATCATGTGTA TACAAGGAAG CCAGGAATAC AGGTTTTCTT GATGATTTGG GTTTTAATTT TGTTTTTATT GCACCTGACA
                                                                  2906
AAATACAGTT ATCTGATGGT CCCTCAATTA TGTTATTTTA ATAAAATAAA TTAAATTTAA AAAAAA
AAATACAGTT ATCTGATGGT CCCTCAATTA TGTTATTTTA ATAAAATAAA TTAAATTTAG GTGTAATGGC TGGCTGTTAC CTCCTTTTAA AGTAATTCTG AGCTCACAAC TTGAATGCCC

CATTTGTTCA CCCTCTTCAG GATCAGAATT C
                           EcoRI
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further characterize the raf gene and its protein product, we have cloned a human cDNA which contains all the coding sequence of the raf gene and have used this DNA as a probe to identify the remaining 5 coding exons of the c-raf-1 gene.

MATERIALS AND METHODS

Library Screening: Two human cDNA libraries, one derived from fetal liver mRNA (provided by E.F. Fritsch) and one from placental mRNA, in the λgt10 vector were screened using a raf-specific portion of the cloned 3611-MSV virus (the 0.75 kb XhoI-SstII fragment) as probe (4). Filters were hybridized in 3X SSC at 60°C and washed at 1X SSC at 60°C. Genomic clones were obtained by screening partial EcoRI and HaeIII-AluI libraries in Charon 4A (10) using the 0.9 kb EcoRI-SalI fragment from the 5' end of the 2.9 kb liver cDNA clone.

RESULTS

Characterization of cDNA Clones

Screening the fetal liver cDNA library with raf specific probe yielded two large clones (2.98 and 2.89 kb). These clones are only slightly smaller than the 3.4 kb raf message which has been observed in numerous tissues (J. Cleveland, personal communication) and contain the complete coding sequence. Similar but shorter clones were also isolated from the placental library. Upon analysis, two of the placental clones were found to contain intron as well as exon sequences.

The sequence of the long liver cDNA clones, as shown in Figure 1, contains a single large open reading frame from nucleotides 121 to 2073 followed by an untranslated region of 905 nucleotides which ends in a poly(A) stretch of 9 nucleotides. This poly(A) stretch is preceded by two AATAAA sequences which could serve as polyadenylation signals (11). To confirm that the apparent poly(A) sequence is not due to priming of the cDNA from an A-rich sequence of the gene, we have compared the cDNA sequence with the sequence of the corresponding region of the human c-raf-1 gene.

Figure 1. The nucleotide sequence of raf cDNA and the deduced amino acid sequence of the raf protein. The bottom two lines show the sequence of the 3' end of the human c-raf-1 gene (9) aligned with the 3' end of the cDNA. The EcoRI site in c-raf-1 is at 40.5 kb in Fig. 2. The presumptive ATP binding site is located near nucleotide 1200 and the majority of the amino acid homology with other kinases lies between nucleotides 1480 and 1773. The first nucleotide of each exon is indicated by an asterisk above the sequence.

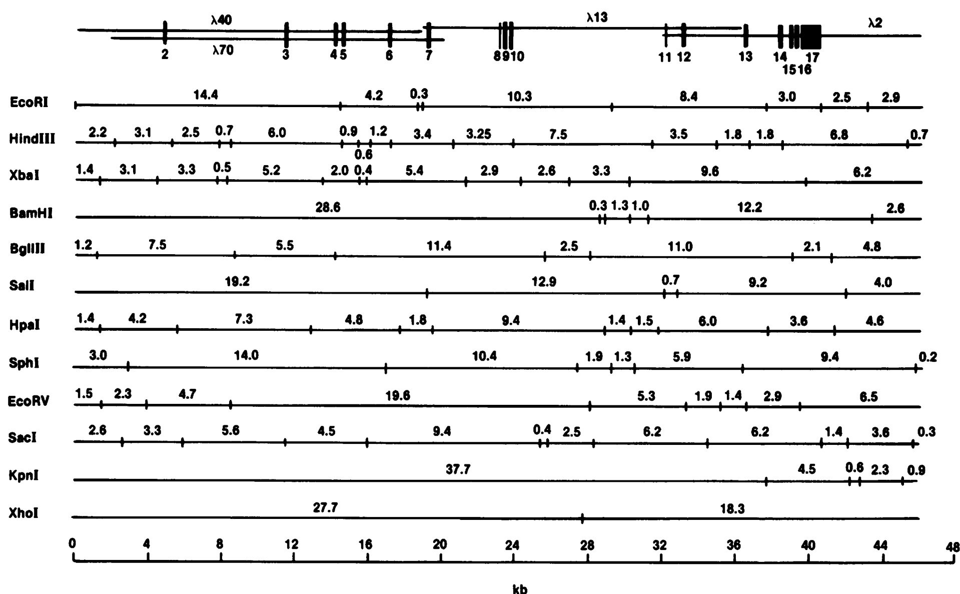

Figure 2. Restriction map of the human c-raf-1 locus derived from overlapping λ phage clones. Clones λ13 and λ2 have been previously described (9). The positions of four phage clones and the exons which they contain are shown above the map. The 14.4, 10.3, and 8.4 kb EcoRI fragments but not the 3.0 and 2.5 kb fragments hybridize to Alu family repeated sequences [BLUR8 (17)].

The sequence alignment shown in Figure 1 demonstrates that the gene has the sequence AGGTGTAAT in place of the poly(A) and thus the poly(A) stretch of the clone represents the true poly(A) of the message. The 1953 nucleotide open reading frame is the only open reading frame of more than 280 nucleotides and coincides with the reading frame of the viral raf and mil proteins. Its sequence has been confirmed by sequencing the corresponding 16 exons of the human c-raf-1 gene (see below). The cDNA thus encodes the complete 648 amino acid raf protein with a predicted molecular weight of 73023 daltons. This result extends the amino terminal of the sequence known from v-mil by 267 amino acids. However, this additional sequence has no significant homology to protein sequences in the National Biomedical Research Foundation Protein Data Bank. As judged by the criterion of Kyte and Doolittle (12), the complete protein contains no extensive hydrophobic regions which would be candidates for transmembrane regions. This characteristic is consistent with the observation of a 75000 molecular weight raf protein primarily in the cytosolic fraction of normal rat cells (13).

Structure of the c-raf-1 Gene

The availability of a nearly full length cDNA clone allowed us to extend the characterization of the 5' end of the c-raf-1 gene beyond the region of homology to v-mil. Using the 5' portion of the cDNA as probe we have isolated two additional genomic clones, λHR40 and λHR70, from partial EcoRI and partial HaeIII-AluI libraries, respectively. These clones extend the 5' end of the restriction map of the locus by 18.9 kb (Fig. 2). The exons in these clones were located approximately by hybridization of cDNA probes to restriction digests of the clones and the appropriate regions were sequenced to precisely identify the exons. Five additional exons were identified which account for all the coding sequences. The intron boundaries all contain characteristic splice acceptor and splice donor sequences (13). Their positions have been indicated on the map of the genomic clones (Fig. 2) and the sequence of the cDNA (Fig. 1). The exon which contains the first 103 nucleotides of the cDNA sequence is not contained within these clones. Although there are about 500 nucleotides of mRNA unaccounted for in the genomic clones, we have assumed for the purpose of numbering the exons that there is only one additional exon which contains all but 26 nucleotides of the 5' untranslated sequence. The structure of the gene has been confirmed by the detection of the expected bands in genomic blots of human DNA using the cDNA as probe (not shown).

DISCUSSION

We have characterized a nearly fully length cDNA which contains the entire coding sequence of the human raf oncogene. Taking advantage of the HgiAI site which spans the second and third codons of the coding sequence we have begun an attempt to express the complete protein in E. coli. If substantial amounts of protein can be produced it should be very useful in characterizing the function of the gene and in verifying that the raf protein is a serine-threonine specific kinase. The cDNA has also allowed us to identify all the coding exons of the human c-raf-1 gene. This information should be useful in characterizing rearrangements of the gene in human tumors. Five transforming variants of the raf gene have been described, all apparently being truncated at the amino end. The viral raf and mil sequences in the transforming viruses 3611-MSV and MH2 begin within exons 9 and 7 of the mouse and chicken genes, respectively, and extend beyond the termination codon in exon 17. A promoter insertion activated form of the mouse gene has been described (14) in which a retroviral long terminal

repeat sequence was inserted between exons 5 and 6. In addition, two transforming raf DNAs have been identified by transfection of DNA derived from human humors (15, 16). In the first of these, obtained from a stomach tumor, the transforming DNA has been cloned and partially mapped with EcoRI and Bam HI. Comparison of the map of this DNA with Figure 2 indicates that the EcoRI site at 29.2 kb and the cluster of Bam sites at 28-32 kb (Fig. 2) is present. The 10.3 and 8.4 kb EcoRI fragments on either side of this site in our map agree well with the 10.0 and 8.0 kb fragments of the transforming DNA suggesting that exons 7-13 are present. However, the EcoRI site at 40.6 kb (Fig. 2) is absent in the transforming DNA suggesting a rearrangement near the poly(A) site. The EcoRI fragment immediately 5' of the 10.0 kb fragment in the transforming DNA is a 13.0 kb fragment containing two Bam sites while our clones have 0.3, 4.2, and 14.4 kb EcoRI fragments with no Bam sites. Thus, the transforming DNA is also rearranged at the 5' end. If the agreement between the 10.0 and 10.3 kb EcoRI fragments is not fortuitous, this would place the rearrangement near exons 6 or 7. The second transforming DNA, obtained from a glioblastoma, has only been characterized by hybridization to secondary and tertiary transformants. These transformants all contain human EcoRI fragments of 10, 8, 5, and 4.2 kb detected with a human Alu-family repeated sequence probe. Three of these fragments agree well with the 10.3, 8.4 and 4.2 kb fragment of our clones while the 5 kb fragment does not. Presumably the 5 kb fragment represents a rearrangement in the vicinity of exon 3 or 4. In addition a v-raf probe (containing exons 10-17) detected HindIII fragments of 3.5 and 1.9 as expected from Figure 2 but a 4.0 kb band instead of the 6.8 kb band of normal human DNA. The detection of a Pst 5.65 kb band which maps at 36.3 - 41.9 on Figure 2 (not shown), indicates that the rearrangement of the 6.8 HindIII band occurs 1.4 - 4.8 kb 3' of the poly(A) site (40.5 kb on the map). Thus both transforming DNAs appear to be rearranged at both the 5' and 3' ends. More precise characterization of the rearrangements should now be possible. Nevertheless, since all the transforming variants of the raf gene appear to be truncated at the amino terminus, it is tempting to speculate that this region (exons 2-9) represents a regulatory domain, the loss of which allows inappropriate activity of the apparent kinase domain (exons 10-16).

REFERENCES

1. Downward, J., Yarden, Y., Mayes E., Scrace G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J., and Waterfield, M.D. (1984) Nature 307, 521-527.

2. Doolittle, R.F., Hunkapiller, M.W., Good, L.E., Devare, S.G., Robbins, K.C., Aaronson, S.A., and Antoniades, H.N. (1983) Science 221, 275-277; Johnson, A., Heldin, C.H., Wasteson, A., Westermark, B., Deuel, T.F., Huang, J.S., Seeburg, D.H., Gray, E., Ullrich, A., Scrace, G., Stroobant, P., and Waterfield, M.D. (1984) EMBO J. 3, 921-928; Waterfield, M.D., Scrace T., Whittle, N., Stroobant, P., Johnson, A., Wasteson, A., Westermark, B., Heldin, C.H., Huang, J.S., and Deuel, T.F. (1983) Nature 304, 35-39.
3. Sherr, C.J., Rettenmier, C.W., Sacca, R., Roussel, M.F., Look, A.T., and Stanley, E.R. (1985) Cell 41, 665-676.
4. Rapp, U.R., Goldsborough, M.D., Mark, G.E., Bonner, T.I., Groffen, J., Reynolds, F.H., and Stephenson, J.R. (1983) Proc. Natl. Acad. Sci. USA 80, 4218-4222.
5. Jansen, H., Lurz, R., Bister, K., Bonner, T.I., Mark, G.E., and Rapp, U.R. (1984) Nature 307, 281-284.
6. Sutrave, P., Bonner, T.I., Rapp, U.R., Jansen, H.W., Patschiwsky, T., and Bister, K. (1984) Nature 309, 85-88.
7. Mark, G.E. and Rapp, U.R. (1984) Science 224, 285-289.
8. Moelling, K., Heimann, B., Beimling, P., Rapp, U.R., and Saunder, T. (1984) Nature 312, 558-561.
9. Bonner, T.I., Kerby, S.B., Sutrave, P., Gunnell, M.A., Mark, G., and Rapp, U.R. (1985) Mol. Cell Biol. 5, 1400-1407.
10. Lawn, R.M., Fritsch, E.F., Parker, R.C., Blake, G., and Maniatis, J. (1978) Cell 15, 1157-1174.
11. Proudfoot, N.J., and Brownlee, G.G. (1976) Nature 263, 211-213.
12. Kyte, J., and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105.
13. Mount. S.M. (1982) Nucl. Acids Res. 10, 459-472.
14. Molders, H., Defesche, J., Muller, D., Bonner, T.I., Rapp, U.R., and Muller, R. (1985) EMBO J. 4, 693-698.
15. Shimizu, K., Nakatsu, Y., Sekiguchi, M., Hokamura, K., Tanaka, K., Terada, M., and Sugimura, T. (1985) Proc. Natl. Acad. Sci. USA 82, 5641-5645.
16. Fukui, M., Yamamoto, T., Kawai, S., Maruo, K., and Toyoshima, K. (1985) Proc. Natl. Acad. Sci. (USA) 82, 5954-5958.
17. Jelinek, W.R., Toomey, T.P., Leinwand, L., Duncan, C.H., Biro, P.A., Choudary, P.V., Weissman, S.M., Rubin, C.M., Houck, C.M., Deininger, P.L., and Schmid, C.W. (1980) Proc. Natl. Acad. Sci. USA 77, 1398-1402.