Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1651–1655, March 1986
Biochemistry

Enhanced c-Ki-*ras* expression associated with Friend virus integration in a bone marrow-derived mouse cell line

(proviral activation/oncogene/transformation)

DONNA L. GEORGE*†‡, BEATRIZ GLICK†, STEPHEN TRUSKO†, AND NANCYANNE FREEMAN†

*Howard Hughes Medical Institute Laboratory and †Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Communicated by Gertrude Henle, November 14, 1985

ABSTRACT **We have investigated the molecular basis for a 25- to 30-fold overexpression of the c-Ki-*ras* oncogene in a mouse bone marrow-derived, early myeloid cell line, 416B. Southern blot hybridizations revealed that the 416B cells contain a rearranged c-Ki-*ras* gene in addition to an apparently normal gene. Molecular cloning and DNA sequence analyses demonstrated that the rearrangement involves the insertion of a 3.5-kilobase-pair segment of Friend virus that includes the envelope gene (*env*) and 3′ long terminal repeat. The Friend provirus is positioned between a 5′ nontranslated exon (exon ϕ) and the first coding exon (exon 1) of the c-Ki-*ras* gene in the same transcriptional orientation. Results of RNA blot analyses indicate that transcription from the rearranged gene initiates at a promoter that excludes sequences in exon ϕ. The data support the hypothesis that enhanced c-Ki-*ras* expression in the 416B cells results from integration of a Friend provirus within this gene.**

The cellular c-Ki-*ras* protooncogene was originally identified by homology to the transforming gene (v-Ki-*ras*) of the Kirsten murine sarcoma virus (Ki-MuSV) (1, 2). The Ki-MuSV is a rat-derived virus that can cause sarcomas and erythroleukemias in susceptible mice as well as transform fibroblasts in culture (1, 2). Although its normal cellular function has not been identified, an altered or activated c-Ki-*ras* gene has been implicated as a transforming gene in a large number of human and animal tumors of diverse origin (3–10). In most of the cases described to date, activation of c-Ki-*ras* involves a single nucleotide change affecting amino acid 12 or 61 of the 21,000-dalton, GTP-binding protein (p21) encoded by this gene (7, 9, 10–12). Appropriately elevated levels of normal c-*ras* genes also can transform certain recipient cells (13, 14). In the case of the Y1 mouse adrenal tumor cell line, there is a 25- to 30-fold amplification and overexpression of this gene (15) and no evidence for the presence of point mutations previously associated with the activation of its transforming potential (16).

A 25- to 30-fold overexpression of c-Ki-*ras* also has been reported in the 416B mouse cell line (17). This early myeloid cell line was isolated from a BDF_1 (C57BL/6 × DBA/2) bone marrow culture infected with Friend virus complex consisting of a replication-competent type C retrovirus (Friend murine leukemia virus, Fr-MuLV) and a replication-defective spleen focus-forming virus (SFFV) (18). 416B cells produce Fr-MuLV but not SFFV (17). The elevated c-Ki-*ras* expression in the 416B cells is not associated with amplification of the gene (17), and our cytogenetic analysis does not support the hypothesis that a chromosome translocation involving this locus has occurred in these cells (19).

We have explored the molecular basis for the enhanced c-Ki-*ras* expression in the 416B cells. Here we report that in the 416B cells a segment of Friend proviral DNA, including the *env* gene and 3′ long terminal repeat (LTR), is inserted in a c-Ki-*ras* gene upstream of the first coding exon in the same transcriptional orientation. The data support the hypothesis that elevated c-Ki-*ras* expression in the 416B cells results from the integration of Friend proviral DNA.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Lines. The 416B and 427E cell lines (kindly provided by R. Goldberg and E. Scolnick) were derived from the same Friend virus-infected female BDF_1 bone marrow cells (18). A9 is an unrelated, C3H-derived mouse cell line. The properties of the Y1-HSR cells, in which there is a 25- to 30-fold amplification and overexpression of the c-Ki-*ras* gene, have been presented (16, 20).

Recombinant Plasmids and Bacteriophage. Vector EMBL3 was obtained from Promega Biotech and its use in the construction of a recombinant library of 416B DNA followed protocols (21) described by the supplier. Subcloning of DNA fragments into the plasmid pBR322 and the isolation and growth of bacteria and bacteriophage have been described (16, 20). Plasmid pHiHi3 contains a 1-kilobase (kb) *Eco*RI insert fragment derived from Ki-MuSV clone KBE-2 (2) and includes sequences homologous to exons 1, 2, 3, and 4A of the cellular gene. Plasmid pY195 contains mouse c-Ki-*ras* exon 1 and adjacent flanking material in a 640-base-pair (bp) *Eco*RI fragment (16). Plasmid pFMuLV-57 contains an 8.5-kilobase-pair (kbp) subgenomic fragment of cloned Fr-MuLV DNA (22).

Southern Blot Analysis and DNA Sequencing. Conditions for the Southern blot analysis of DNA have been detailed (16, 20) but slightly modified to include the prehybridization of nitrocellulose filters in the presence of heparin (23). Nucleotide sequences were determined by the method of Sanger *et al.* (24) after subcloning of appropriate restriction fragments into phage M13 (25).

RESULTS

Rearrangement of the c-Ki-*ras* Gene. We explored the possibility that a DNA rearrangement might be associated with c-Ki-*ras* overexpression in the 416B cells. Southern blotting analyses were initially performed with a v-Ki-*ras* probe (pHiHi3) that contains sequences homologous to exons 1, 2, 3, and 4A of the cellular gene. When genomic DNA samples were digested with a number of restriction endonucleases, including *Bgl* II, *Pst* I, *Pvu* II, and *Xba* I, an additional hybridizing fragment was detected in the 416B DNA

Abbreviations: kbp, kilobase pair(s); Fr-MuLV, Friend murine leukemia virus; bp, base pair(s); kb, kilobase(s); LTR, long terminal repeat; Ki-MuSV, Kirsten murine sarcoma virus.

‡To whom reprint requests should be addressed at: Department of Human Genetics, 195 Medical Laboratory Building, University of Pennsylvania, Philadelphia, PA 19104.

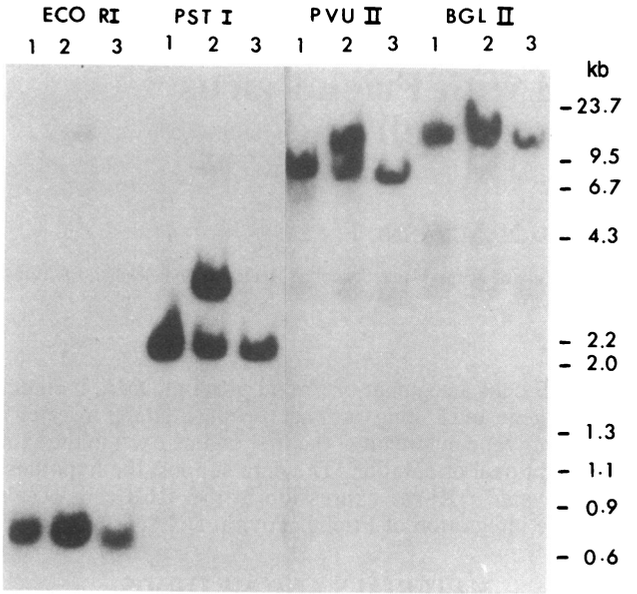

FIG. 1. Southern blot analysis of genomic DNA samples from mouse cell lines A9 (lane 1), 416B (lane 2), and 427E (lane 3). DNA samples digested with the restriction endonucleases indicated were hybridized to [^{32}P]DNA from a c-Ki-*ras* exon 1 probe (pY195).

compared to DNA from control mouse cell lines A9 and 427E (data not shown). When similar analyses were performed with molecular probes specific for different regions of the mouse c-Ki-*ras* gene (16), we found that the additional hybridizing fragment in each digest was detected with a probe (pY195) for the first coding exon (exon 1) of c-Ki-*ras* (Fig. 1). These results indicated that a DNA rearrangement associated with the 5′ portion of a c-Ki-*ras* gene has occurred in the 416B cells.

To characterize the nature of the rearrangement, we constructed a partial genomic library of the 416B DNA. After digestion with the restriction enzyme *Bgl* II, DNA fragments 7–22 kb in size were isolated from low-melting agarose and ligated to *Bam*HI-digested DNA from the vector EMBL3. The library was screened for recombinant phage having homology to c-Ki-*ras* exon 1. Two classes of recombinants were isolated, represented by clones λ104 and λ105. Clone λ104 has a restriction map consistent with that of the normal c-Ki-*ras* gene, whereas clone λ105 exhibits a pattern like that of the rearranged gene.

Because the 416B cell line had been derived from a culture of bone marrow cells infected with Friend virus complex, we used Southern blot analysis to determine if viral sequences were associated with the rearranged c-Ki-*ras* gene. As shown in Fig. 2, clone λ105, but not λ104, hybridized to a Friend virus probe (pFMuLV-57) demonstrating linkage between viral sequences and c-Ki-*ras* exon 1. Restriction endonuclease-digested DNA from clones λ104 and λ105 (or plasmids derived from them) allowed construction of a partial restriction map of the unaltered and of the rearranged c-Ki-*ras* fragments (Fig. 3). Restriction fragments hybridizing to the Friend virus probe correlated well with the published restriction map of the pFMuLV-57 clone for the region that includes the *env* gene and 3′ LTR (26, 27). The provirus insert is not full-size; a segment of approximately 5 kbp, encompassing the 5′ LTR–*gag*–*pol* region, is not present. The truncated Friend proviral fragment is inserted in the c-Ki-*ras* gene approximately 360 bp upstream of the first coding exon (Fig. 3) in the same transcriptional orientation. Limited DNA sequence analysis on a portion of the 850-bp *Bam*HI fragment of λ105 and comparison to the published sequence of the *env* gene of Fr-MuLV (26) supported the conclusion that the inserted sequences were derived from Fr-MuLV, although a few differences in sequence composition were noted (data not shown).

DNA Sequence Analysis on Regions of the Rearranged c-Ki-*ras* Gene. We also determined the DNA sequence at the provirus–cell junctions, using the strategy shown in Fig. 3. We compared the junction sequences to the corresponding c-Ki-*ras* cellular sequence in the ≈400-bp 5′ *Sal* I–*Stu* I fragment of clone λ104 as well as to the published sequence of pFMuLV-57 (26, 28, 29). Results of this DNA sequence analysis are shown in Fig. 4 and reveal that the proviral insert is bounded by a 130-bp direct repeat of cellular DNA. The

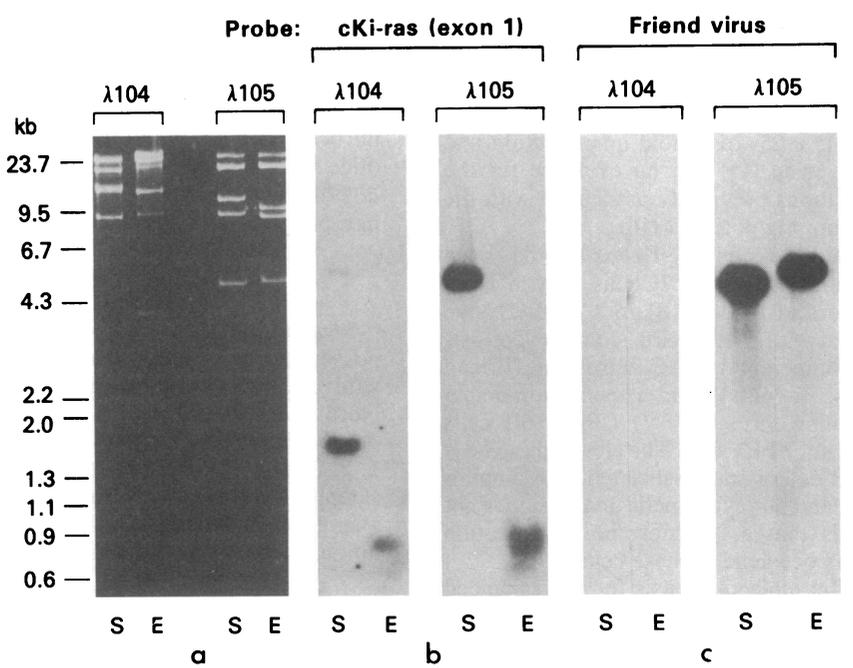

FIG. 2. DNA fragments generated by *Sal* I (S) or *Eco*RI (E) digestion of recombinants λ104 and λ105. (*a*) DNA stained with ethidium bromide. (*b*) Southern blot hybridized to c-Ki-*ras* exon 1 probe. (*c*) Southern blot hybridized to Friend virus probe pFMuLV-57.

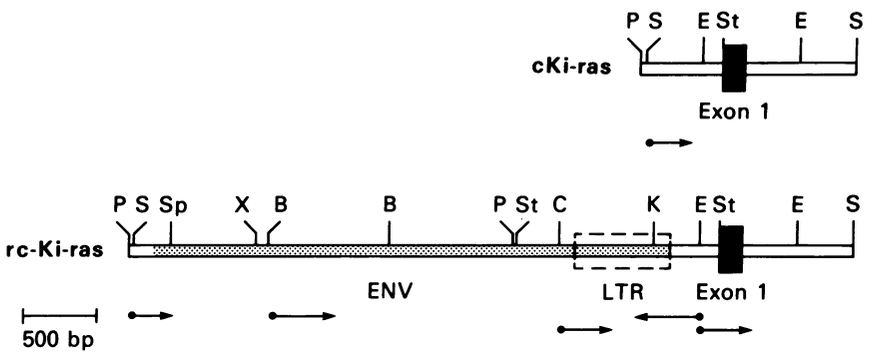

FIG. 3. Restriction maps of the normal and rearranged c-Ki-*ras* genes in the 416B cells. The exon 1 coding region is indicated by the solid box. Friend proviral DNA, including the *env* gene and 3′ LTR (dashed lines), is represented by stippling. The sequencing strategy is indicated by arrows. Abbreviations for restriction sites: B, *Bam*HI; C, *Cla* I; E, *Eco*RI; K, *Kpn* I; P, *Pst* I; S, *Sal* I; Sp, *Sph* I; St, *Stu* I; X, *Xba* I.

same sequence occurs only once in the *Sal* I–*Stu* I fragment of c-Ki-*ras* clone λ104 and presumably in the normal gene. Thus, it appears that a duplication of cellular DNA was generated some time during or after the viral integration event. However, we cannot rule out the possibility that the 130-bp sequence was duplicated in the normal gene and one copy of the sequence was deleted during the cloning of the *Sal* I fragment λ104.

The nucleotide sequence of c-Ki-*ras* exon 1, located downstream of the provirus–cell junction, was investigated to determine if it contained any changes implicated previously in activation of the transforming potential of c-Ki-*ras*. None was detected. We found that the DNA sequence of this exon 1 was indistinguishable from that reported previously for a c-Ki-*ras* exon 1 isolated from normal mouse cells (9) and from Y1 mouse adrenal tumor cells (16) that contain amplified copies of an apparently normal gene.

```
SalI
GTCGACAAGCTCATGCGCGTGTGTCCACAGGGTATAGCGTACTATGCAGAATATTTGTAC

TGAGTGAAGTCATGATACATTCCTTTGAGAGCCATTAGCTGCTACAAAACAGTAATCTGG

                                        Friend virus
CTGTTTAGATCAACAAGCTAAATGATAGAAGATGAAAGAAAGAAACTGCCAAAGTTGTAA
                                    SphI
CCAAGAAGCTACTAGAAGAAATCTTCCCTAGATTCGGCATGC....>3kb....AATTAA

CCAATCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTTCCCGAGCTCTATAAAAGA
                                                    KpnI
GCTCACAACCCCTCACTCGGCGCGCCAGTCCTCCGACAGACTGAGTCGCCCCGGTACCCG

TGTATCCAATAAATCCTCTTGCTGTTGCATCCGACTCGTGGTCTCGCTGTTCCTTGGGAG
                        Friend virus
GGTCTCCTCAGAGTGATTGACTACCGTCTCGGGGGTCTTTAGGTATAGCGTACTATGCAG

AATATTTGTACTGAGTGAAGTCATGATACATTCCTTTGAGAGCCATTAGCTGCTACAAAA

CAGTAATCTGGCTGTTTAGATCAACAAGCTAAATGATAGAAGATGAAAGTACTGGTTTCC

ATGTATTTTTATTAAGTGTTGATGAGAAAGTTGGTAACTGACTTACAGGTTACTCTGTAC
          EcoRI
ATCTGTAGTCACTGAATTCGGAATATCTTAGAGTCTTACACACAAAGGTGAGTGTTAAAA

TATTGATAAAGTTTTTGATAATCTTGTGTGAGACATGTTCTAATTTAGTTGTATTTTATT
                Exon 1
ATTTTTATTGTAAGGCCTGCTGAAAATGACTGAGTATAAACTTGTGGTGGTTGGAGCTGG

TGGCGTAGGCAAGAGCGCCTTGACGATACAGCTAATTCAGAATCACTTTGTGGATGAGTA

CGACCCTACGATAGAG
```

FIG. 4. Nucleotide sequence of provirus–cell junction regions. The sequencing strategy is indicated in Fig. 3. A 130-bp duplication of cellular DNA (underlined) flanks the provirus insert. Only one base-pair difference (†) was detected between the 5′ and 3′ copies of this sequence. Arrows indicate the beginning and end of the provirus insert and of c-Ki-*ras* exon 1. Putative control elements (28, 29) in the 3′ LTR include a "CAT" box (CCAAT) and "TATA" box (TATAAAA) (dashed lines). Nucleotides that differ from those of the published Fr-MuLV sequence (28, 29) are noted (*).

Analysis of c-Ki-*ras* Transcripts in the 416B Cells. From studies on the human c-Ki-*ras* gene, evidence has been obtained for the presence of an untranslated exon (exon ϕ) located upstream of exon 1 (6, 11). Because the organization and nucleotide sequence of the coding regions of the mouse and human genes are highly conserved (16), it was expected that a homologous exon ϕ is present in the mouse gene. Preliminary data supporting that conclusion have been obtained (unpublished results). The mouse exon ϕ is located >3 kbp upstream of exon 1, and in the 416B cells the Friend proviral insert is situated between the two exons. The c-Ki-*ras* transcripts (approximately 5.2 and 2.0 kb) in the 416B cells are similar in size to those in normal mouse cells and in the Y1 cells (15, 17). To determine if c-Ki-*ras* RNAs in the 416B cells contain sequences from exon ϕ we used as a probe in RNA blot analyses a homologous region (≈400-bp *Pvu* II–*Sst* II fragment) isolated from the Ki-MuSV clone pKBE-2 (2, 6, 11). In the Y1 sample, this exon ϕ probe hybridized to two RNA species the size of c-Ki-*ras* mRNAs; however, we detected no hybridization to the 416B RNA (Fig. 5*A*). When the same filter was subsequently hybridized to a probe containing exon 1 (pY195), c-Ki-*ras* transcripts were evident in the 416B cells (Fig. 5*B*). Therefore, the results of the RNA blot analyses indicate that transcription from the rearranged c-Ki-*ras* gene initiates at a promoter that excludes sequences in exon ϕ.

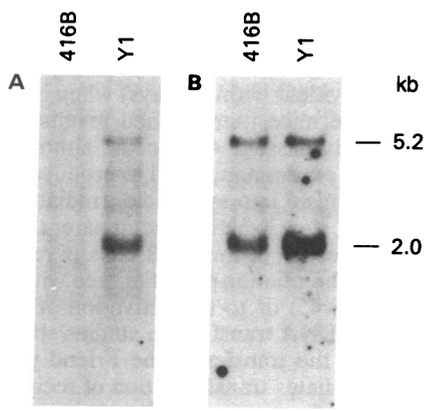

FIG. 5. RNA blot analysis. Samples (5 μg) of poly(A)$^+$ RNA isolated from 416B cells or Y1 cells were separated on formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized to [^{32}P]DNA from the 400-bp *Pvu* II–*Sst* II fragment from KBE-2, containing sequences homologous to c-Ki-*ras* exon ϕ (*A*), or the 640-bp *Eco*RI insert fragment from probe pY195, containing mouse c-Ki-*ras* exon 1 (*B*). [Under the hybridization conditions used in *A*, the lower level of c-Ki-*ras* expression (by a factor of 25–30) from a normal allele in the 416B cells would not be detected.]

DISCUSSION

Recent investigations have provided evidence that certain retroviruses that lack oncogenes may promote one or more steps of cellular transformation by insertion in the vicinity of a cellular protooncogene, activating the latter through the action of strong promoter and/or enhancer elements. For example, activation of the c-*myc* protooncogene via proviral integration has been described in tumors induced by murine leukemia virus (MuLV) (30–33) and avian leukosis virus (ALV) (34–37). Proviral activation of c-*erb* has occurred in ALV-induced erythroblastosis (38), activation of the cellular genes *int-1* (39, 40) and *int-2* (41, 42) occurs in some tumors induced by mouse mammary tumor virus (MMTV), and activation of the cellular gene *pim-1* has been described in MuLV-induced tumors (43, 44). Such activation of cellular genes by integrated proviruses can occur by transcription from the viral promoter (35, 36) and/or through proviral-mediated enhanced transcription from nearby cellular promoters (30, 32, 40–44). (For additional examples and discussion, see refs. 29 and 44).

Because Fr-MuLV does not have its own oncogene, it has been suggested that this retrovirus may also induce neoplasia, at least in part, by insertional activation of cellular genes. In this report, we have presented evidence that elevated expression of the c-Ki-*ras* protooncogene in the bone marrow-derived 416B cell line results from integration of Friend proviral DNA within this gene. Data we obtained in RNA blot analyses suggest that transcripts from the rearranged c-Ki-*ras* gene do not include sequences from a nontranslated exon ϕ located upstream of the integrated provirus. At present, little is known about the organization or nucleotide composition of the promoter elements and regulatory signals for the c-Ki-*ras* gene. Also, the possible cellular function of the 5′ nontranslated exon ϕ and the potential consequences resulting from its exclusion remain to be determined. Given that in the 416B cells the Friend provirus is present upstream of the c-Ki-*ras* coding region, transcription may be initiating within the proviral LTR. However, results obtained in an earlier study failed to provide evidence that such LTR sequences were linked to c-Ki-*ras* RNAs in the 416B cells (17). Investigations designed to determine the site(s) of transcription initiation in the rearranged and normal c-Ki-*ras* gene are necessary to resolve this issue.

Cellular transformation associated with elevated c-Ki-*ras* expression has been noted (13–16), and it seems likely that increased expression of this gene in the 416B cells contributes to their transformed properties. We have found that the 416B cells under investigation in our laboratory rapidly cause tumors (growth evident within 3 days) when injected subcutaneously in nude mice (unpublished results). It has been reported that early-passage 416B cells, although exhibiting elevated c-Ki-*ras* expression, failed to produce tumors when injected intravenously into syngeneic irradiated BDF_1 mice (18, 45). It is not clear whether the apparent change in the tumorigenic properties of the 416B cells is related to alterations in their differentiation potential seen with continued *in vitro* passage (18, 45) or to the activation of other cellular protooncogenes. DNA transfection studies should be useful in determining if the transfer of the Friend proviral-linked c-Ki-*ras* gene mediates transformation of recipient cells and if this occurs in a tissue-specific manner.

The expression of c-Ki-*ras* has been examined in other leukemic cells isolated from Friend virus-infected mice. In one study (46), two early myeloid cell lines and leukemia cells (stage 1) were found to have elevated levels of c-Ki-*ras* RNA compared to normal bone marrow. There was no report on possible rearrangements of the gene in these cells. In another analysis of preleukemia cells and leukemia cells obtained from Friend virus-infected mice, no evidence was obtained for altered c-Ki-*ras* RNA levels (47). Continued investigation of the causes and consequences of altered c-Ki-*ras* expression should provide a better understanding of the role of this gene in normal and neoplastic cells.

We thank Linda Cahilly-Snyder for valuable discussions and tumorigenicity assays and Lori Cole for help in the preparation of this manuscript. This work was supported by National Institutes of Health Grants CA34462 and GM32592.

1. Kirsten, W. H. & Mayer, L. A. (1967) *J. Natl. Cancer Inst.* **39,** 311–335.
2. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R. & Scolnick, E. M. (1981) *Nature (London)* **292,** 506–511.
3. Der, C. J., Krontiris, T. C. & Cooper, G. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3637–3640.
4. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. (1982) *Nature (London)* **297,** 474–478.
5. Pulciani, S., Santos, E., Lauver, A. V., Long, L. K., Aaronson, S. A. & Barbacid, M. (1982) *Nature (London)* **300,** 539–542.
6. Capon, D. J., Seeburg, P. H., McGrath, J. P., Hayflick, J. S., Edman, U., Levinson, A. D. & Goeddel, D. V. (1983) *Nature (London)* **304,** 507–513.
7. Eva, A., Tronick, S. R., Gol, R. A., Pierce, J. H. & Aaronson, S. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4926–4930.
8. Shimizu, K., Birnbaum, D., Ruley, M. A., Fasano, O., Suard, Y., Edlund, L., Taparowski, E., Goldfarb, M. & Wigler, M. (1983) *Nature (London)* **304,** 497–500.
9. Guerrero, I., Villansante, A., Corces V. & Pellicer, A. (1984) *Science* **225,** 1159–1162.
10. Santos, E., Martin-Zanca, D., Reddy, E. P., Pierotti, M. A., DellaPorta, G. & Barbacid, M. (1984) *Science* **223,** 661–664.
11. Nakano, H., Yamamoto, F., Neville, C., Evans, D., Mizuno, T. & Perucho, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 71–75.
12. Nakano, H., Neville, C., Yamamoto, F., Garcia, J. L., Fogh, J. & Perucho, M. (1984) in *Cancer Cells 2: Oncogenes and Viral Genes*, eds. Vande Woude, G. F., Levine, A. J., Topp, W. C. & Watson, J. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 447–454.
13. Chang, E. H., Furth, M. E., Scolnick, E. M. & Lowy, D. R. (1982) *Nature (London)* **297,** 479–483.
14. McCoy, M. C., Bargmann, C. I. & Weinberg, R. A. (1984) *Mol. Cell. Biol.* **4,** 1577–1582.
15. Schwab, M., Alitalo, K., Varmus, H. E., Bishop, J. M. & George, D. L. (1983) *Nature (London)* **303,** 497–501.
16. George, D. L., Scott, A. F., Trusko, S., Glick, B., Ford, E. & Dorney, D. J. (1985) *EMBO J.* **4,** 1199–1203.
17. Ellis, R. W., DeFeo, D., Furth, M. & Scolnick, E. M. (1982) *Mol. Cell. Biol.* **2,** 1339–1345.
18. Dexter, T. M., Allen, T. D., Scott, D. & Teich, N. M. (1979) *Nature (London)* **277,** 471–474.
19. Cahilly, L. A. & George, D. L. (1985) *Cytogenet. Cell Genet.* **39,** 140–144.
20. George, D. L. & Powers, V. E. (1981) *Cell* **24,** 117–123.
21. Frischauf, A., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170,** 827–842.
22. Oliff, A. I., Hager, G. L., Chang, E. H., Scolnick, E. M., Chan, H. W. & Lowy, D. R. (1980) *J. Virol.* **33,** 475–486.
23. Singh, L. & Jones, K. W. (1984) *Nucleic Acids Res.* **12,** 5627–5638.
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
25. Messing, J. & Vieira, J. (1982) *Gene* **19,** 269–276.
26. Koch, W., Hunsmann, W. & Friedrich, R. (1983) *J. Virol.* **45,** 1–9.
27. Oliff, A., Collins, L. & Mirnda, C. (1983) *J. Virol.* **48,** 542–546.
28. Koch, W., Zimmermann, W., Oliff, A. & Friedrich, R. (1984) *J. Virol.* **49,** 828–840.
29. Weiss, R., Teich, N., Varmus, H. & Coffin, J., eds. (1985) *RNA Tumor Viruses: Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
30. Corcoran, L. M., Adams, J. M., Dunn, A. R. & Cory, S. (1984) *Cell* **37,** 113–122.

31. Neil, J. C., Hughes, D., McFarlane, R., Wilkie, N. M., Onions, D. E., Lees, G. & Jarret, O. (1984) *Nature (London)* **308,** 814–820.
32. Selten, G., Cuypers, H. T., Zijlstra, M., Melief, C. & Berns, A. (1984) *EMBO J.* **3,** 3215–3222.
33. Steffen, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2097–2101.
34. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290,** 475–480.
35. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. (1981) *Cell* **23,** 323–334.
36. Payne, G. S., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295,** 209–214.
37. Westaway, D., Payne, G. & Varmus, H. E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 843–847.
38. Fung, Y. T., Lewis, W. G., Crittenden, L. B. & Kung, H. (1983) *Cell* **33,** 357–368.
39. Nusse, R. & Varmus, H. E. (1982) *Cell* **31,** 99–109.
40. Nusse, R., vanOoyen, A., Cox, D., Fung, Y. K. T. & Varmus, H. (1984) *Nature (London)* **307,** 131–136.
41. Peters, G., Brookes, S., Smith, R. & Dickson, C. (1983) *Cell* **33,** 369–377.
42. Dickson, C., Smith, R., Brookes, S. & Peters, G. (1984) *Cell* **37,** 529–536.
43. Cuypers, H. T., Selten, G., Quint, W., Zijlstra, M., Robanus-Maandag, E., Boelens, W., van Wezenbeek, P., Melief, C. & Berns, A. (1984) *Cell* **37,** 141–150.
44. Selten, G., Cuypers, H. T. & Berns, A. (1985) *EMBO J.* **4,** 1793–1798.
45. Scolnick, E. M., Weeks, M. D., Shih, T. Y., Ruscetti, S. K. & Dexter, T. M. (1981) *Mol. Cell. Biol.* **1,** 66–74.
46. Oliff, A., Oliff, I., Schmidt, B. & Famulari, N. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5464–5467.
47. Robert-Lezenes, J., Moreau-Gachelin, F., Wendling, F., Tambourin, P. & Tavitian, A. (1984) *Leuk. Res.* **8,** 975–984.