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FMS mutations in myelodysplastic, leukemic, and normal subjects

(colony-stimulating factor 1 receptor/polymerase chain reaction/constitutional and somatic mutation)

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ABSTRACT **The *FMS* gene encodes the functional cell surface receptor for colony-stimulating factor 1, the macrophage- and monocyte-specific growth factor. Codons 969 and 301 have been identified as potentially involved in promoting the transforming activity of *FMS*. Mutations at codon 301 are believed to lead to neoplastic transformation by ligand independence and constitutive tyrosine kinase activity of the receptor. The tyrosine residue at codon 969 has been shown to be involved in a negative regulatory activity, which is disrupted by amino acid substitutions. This study reports on the frequency of point mutations at these codons, *in vivo*, in human myeloid malignancies and in normal subjects. We studied 110 patients [67 with myelodysplasia (MDS) and 48 with acute myeloblastic leukemia (AML)], 5 patients being studied at the MDS and the later AML stage of the disease. There was a total incidence of 12.7% (14/110) with mutations in codon 969 and 1.8% (2/110) with mutations in codon 301. Two patients had mutations in the AML stage of the disease but not in the preceding MDS and one had a mutation in the MDS stage but not upon transformation of AML. This is consistent with the somatic origin of these mutations. *FMS* mutations were most prevalent (20%) in chronic myelomonocytic leukemia and AML type M4 (23%), both of which are characterized by monocytic differentiation. One of 51 normal subjects had a constitutional codon 969 mutation, which may represent a marker for predisposition to myeloid malignancy.**

The *FMS* gene encodes for the receptor of the macrophage- and monocyte-specific growth factor, colony-stimulating factor 1 (CSF-1) (1–3). The protein product of the gene is a cell surface glycoprotein that is expressed on cells of the monocyte/macrophage lineages and possesses ligand-dependent tyrosine-specific kinase activity (3, 4). Binding of CSF-1 to its receptor is required for survival, proliferation, and differentiation of these cells *in vitro*. The *FMS* gene is the cellular homologue of the v-*fms* gene of the Susan McDonough feline sarcoma virus (5, 6). The v-*fms* gene product exhibits ligand-independent tyrosine kinase activity (7, 8) and will transform cells *in vitro*, whereas the normal human *FMS* gene will not (9, 10). DNA sequence analysis (11) has shown that the *FMS* and v-*fms* genes differ by a number of point mutations and by the replacement of the 50 amino acids at the carboxy C terminus of the human gene with 11 unrelated amino acids in the v-*fms* gene. This C-terminal deletion removes a tyrosine residue at codon 969 that negatively regulates the response of the gene product to CSF-1 stimulation (9, 12). *In vitro* studies have shown that substitution of the tyrosine residue at codon 969 to phenylalanine up-regulates the stimulation of the receptor to CSF-1 but is insufficient to confer transforming activity on the gene (9). Alterations in addition to Tyr-969 must therefore be necessary to fully activate the *FMS* gene *in vitro*.

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In the light of the sequence differences between the v-*fms* and *FMS*, studies of chimeric v-*fms*/FMS proteins have highlighted codon 301, in the extracellular domain, to be functionally important. The human, feline, and murine *FMS* genes all encode leucine at this position, whereas the viral gene encodes a serine residue (13). Substitution of Ser-301 for Leu-301 in the human *FMS* gene rendered the gene transforming in an *in vitro* assay (10). The mutation is believed to lead to a conformational change that mimics ligand binding, resulting in a constitutive tyrosine kinase activity. Mutant genes with Ser-301 and Phe-969 have increased transformation efficiency.

The involvement of the *FMS* gene in myeloid malignancy has been implicated previously. It is known that the *FMS* gene is located on chromosome 5q33 (14), a region frequently altered in myelodysplasia (MDS) patients (15–17). Loss of one *FMS* allele has been demonstrated in some MDS patients with a 5q- refractory anaemia (14, 18). Expression of the *FMS* gene has been demonstrated in leukemia cells from acute myeloblastic leukemia (AML) patients but not in patients with acute lymphocytic leukemia. The highest levels were detected in AML type M5, which is characterized by a monocytic phenotype (19, 20). Coexpression of *FMS* and CSF-1 in the same leukemia cells has also been demonstrated in 5 of 15 AML cases studied (21), implicating autocrine stimulation of the receptor.

Here we report on the frequency of mutations at potentially activating codons, 301 and 969. We have studied 67 patients with MDS, 48 with AML, and 51 hematologically normal individuals.

MATERIALS AND METHODS

Patient Material. Blood or bone marrow samples were obtained from 67 patients with MDS [13 with sideroblastic anemia (SA), 14 with refractory anemia (RA), 10 with RA with excess blasts (RAEB), and 30 with chronic myelomonocytic leukemia (CMML)] and 48 AML patients (9 type M1, 14 type M2, 22 type M4, 2 type M5, 1 unclassified). Diagnosis of AML and MDS was made according to the FAB (French–American–British) classifications (22, 23). Fifty-one normal blood samples were also studied, these being obtained from healthy blood donors, patients in the ophthalmology department, and healthy volunteers (17 aged 20–39, 13 aged 40–60, 21 aged 61–80). Fully informed consent was obtained from all individuals and the investigation was approved by the South Glamorgan Joint Ethics Committee.

DNA Extraction. High molecular weight DNA was extracted from cells as described (24).

Abbreviations: CSF-1, colony-stimulating factor 1; AML, acute myeloblastic leukemia; MDS, myelodysplasia; CMML, chronic myelomonocytic leukemia; SA, sideroblastic anemia; RA, refractory anemia; RAEB, RA with excess blasts; PCR, polymerase chain reaction.

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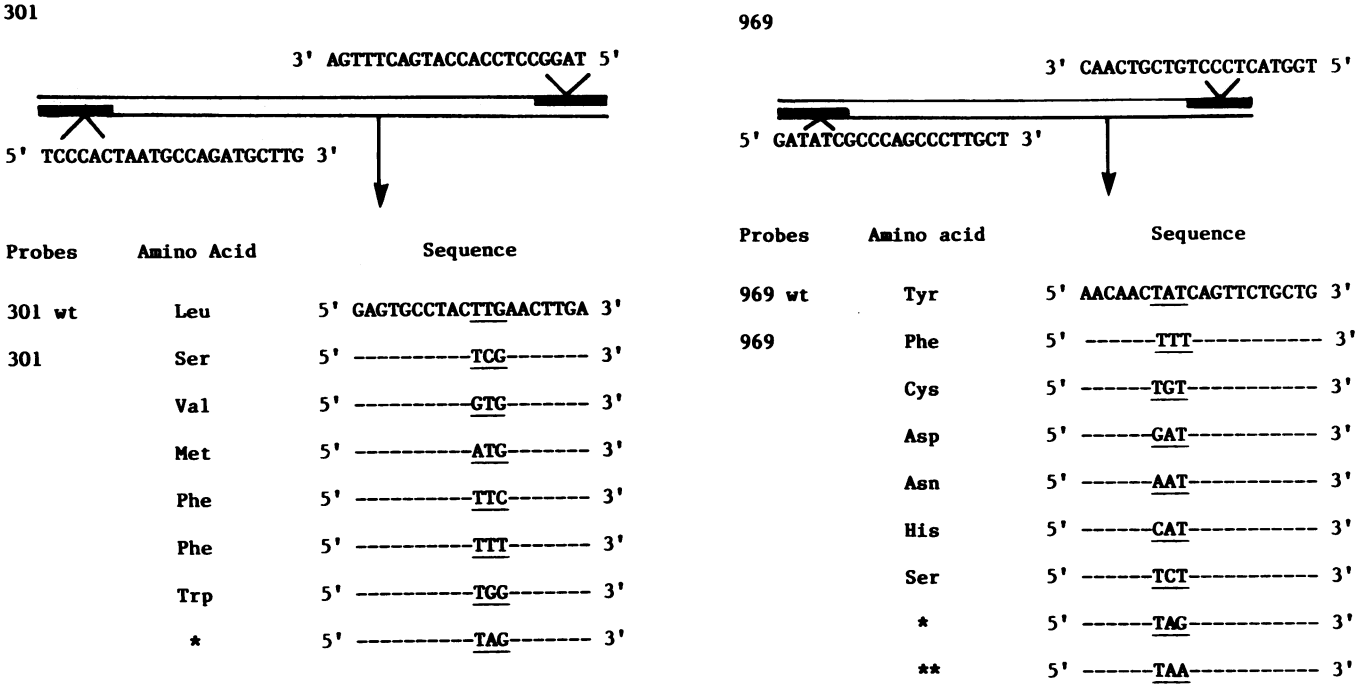

FIG. 1. Sequences of primers and mutant-specific probes for codons 301 and 969. A single asterisk (*) indicates the translational stop codon amber; a double asterisk (**) indicates the translational stop codon ochre.

Cloning and Sequencing. Four hundred thousand λ phage from a human leukocyte library in EMBL3 (Cambridge Bioscience, Cambridge, U.K.) were screened (24) using a 2.3-kilobase (kb) *Sst* I fragment of Susan McDonough feline sarcoma virus containing 5′ v-*fms* sequences (25). Two of 400,000 λ phage clones were plaque purified. Restriction enzyme analysis showed these clones to be identical. A 2.5-kb *Bam*HI fragment shown by hybridization to a codon 301 wild-type oligonucleotide probe (Fig. 1) to contain sequences around codon 301 was subcloned into a plasmid, pUC18. A sequencing primer (5′-TGAGGTTCTGCTCAGAGCTC-3′) 3′ to codon 301 was designed from the published cDNA sequence (11) and synthesized (Applied Biosystems). This was used to obtain intronic sequences 5′ to codon 301. Two-hundred fifty base pairs (bp) of sequence was obtained by the Sanger dideoxy chain-termination method (26) using Sequenase (Cambridge Bioscience). All restriction enzyme digestions were carried out according to the manufacturer's recommendations (GIBCO/BRL).

Polymerase Chain Reaction (PCR). Methods of amplification and hybridization were as described (27, 28) with modifications. Primers flanking codons 301 and 969 (Fig. 1) of the *FMS* gene were used. The primers were designed from cDNA sequences (11) and from sequences obtained by ourselves. Each sample was subject to two rounds of amplification each of 50 cycles with *Taq* polymerase (*Thermus aquaticus* DNA polymerase; Perkin–Elmer), using material from the first round as a template for the second. The samples were applied to the membrane without a vacuum. The filters were denatured in 0.5 M NaOH/1.5 M NaCl and neutralized in 1.5 M NaCl/0.5 M Tris, pH 7.5/0.001 M Na_2EDTA prior to baking at 80°C. Filters were hybridized to mutant-specific oligonucleotide probes (Fig. 1) 5′ end-labeled with [γ-^{32}P]ATP and polynucleotide kinase (Amersham). Autoradiography was carried out with intensifying screens at −70°C using XAR-5

Table 1. *FMS* mutations in MDS, AML, and normal subjects

Patient	Disease	Codon	Amino acid substitution
1	MDS (SA)	301	Phe/Ser
2	MDS (RA)	969	Cys
3	MDS (RAEB)	969	Cys
4	MDS (CMML)	969	Asn/Phe
5	MDS (CMML)	969	Ochre
6	MDS (CMML)	969	Cys
7	MDS (CMML)	969	Cys/Phe
8	MDS (CMML)	969	Cys
9A	MDS (CMML)	969	Cys
9B	AML (M5, post-MDS)	969	Cys
10	AML (M2)	969	Asp
11	AML (M4)	301	Phe
12	AML (M4)	969	His
13	AML (M4)	969	Asn
14	AML (M4)	969	Cys
15	AML (M4, post-MDS)	969	Cys
16	AML (M5, post-MDS)	969	His
NS		969	Cys (constitutional)

All mutations were confirmed by a second PCR and oligonucleotide hybridization analysis. NS, normal subject.

Table 2. *FMS* mutations in disease subtypes

Disease	Total no. studied	No. of mutations (amino acid position)
SA	13	1 (301)
RA	14	1 (969)
RAEB	10	1 (969)
CMML	30	6 (969)
AML M1	9	0
AML M2	14	1 (969)
AML M4	22	5 (1 × 301, 4 × 969)
AML M5	2	2 (969)
AML UC	1	0
Total	115*	17†
None (NS)	51	1

UC, unclassified; NS, normal subjects.
*Five patients sampled twice in MDS and AML stages.
†One patient had an *FMS* mutation in MDS and AML stages.

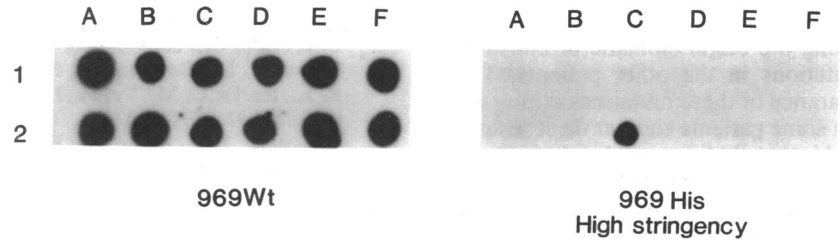

FIG. 2. Detection of a somatic *FMS* mutation. Hybridization of the AML sample of patient 16 (slot C2) to a His-969 probe at high stringency is shown. A previous MDS sample (slot B2) showed no evidence of the mutation. The same filter hybridized to the 969 wild-type probe is shown. This provides an estimate of quantitation of the DNA in each dot.

(Kodak) film for 2 hr to 3 days. Potential mutants were rescreened on independent filters and reamplified to confirm the presence of mutations. Only those that stably hybridized to the mutant probes with significant signals were scored positive. Each mutant was therefore assayed for two independent PCR reactions. This was a stringent screen as, unlike *RAS* mutations where a biological transformation assay could be employed to confirm the presence of the mutations (28), there is no similar biological assay for *FMS* mutations.

Statistical Analysis. Fischer's exact test (29) was employed to determine if the differences found between the groups were significantly different from the normal incidence.

RESULTS

A total of 110 patients with MDS or AML was investigated for the presence of *FMS* mutations in their peripheral blood leukocytes (Tables 1 and 2). DNA from 16 of 110 (14.5%) patients was found to have mutations. Fourteen of 110 patients had mutations at codon 969. A cysteine substitution for the wild-type tyrosine was the most prevalent alteration. Two of 110 patients had mutations at codon 301. The *FMS* mutation in a single patient (9) was shown to be present in leukocytes from the MDS (9A) and AML M5 (9B) stages but not in buccal epithelium (data not shown). Patients 15 and 16 had mutations in the AML stage of disease but not in the preceding MDS (representative filter in Fig. 2). Patient 3 possessed a mutation at the MDS stage of the disease, which was apparently lost upon leukemic transformation. These results indicate that *FMS* mutations can occur at early and late stages of disease and hence may not represent an initiating event in the generation of the abnormal clone. These results are consistent with the somatic origin of these mutations. No patients were found to possess a mutation at codons 301 and 969. However, three patients (1, 4, and 7) showed evidence of two mutations at the same codon. CMML and AML M4 samples, both characterized by significant monocytic differentiation, had the highest rate of *FMS* mutations [20% and 23%, respectively; Fisher's exact test, two-tailed analysis, $P < 0.02$ (29)].

In 1 of 51 (2%) normal subjects analyzed, a Cys-969 mutation was found to be present in peripheral blood. Analysis of buccal epithelial cells from this person showed the presence of the same mutation (Fig. 3). This finding suggests that the Cys-969 mutation is constitutional in this instance.

DISCUSSION

We have described the finding of point mutations at codons 301 (2/110) and 969 (14/110) of the *FMS* gene *in vivo* in myeloid disease and in 1 of 51 normal subjects. Our results indicate that a mutation at codon 969, rather than codon 301, is the more common lesion in these patients. We speculate that in the hemopoietic environment, in the presence of CSF-1, mutations at codon 969 that alter a negative regulatory site may up-regulate the response of the receptor to ligand binding and thus confer a growth advantage to the cell. *In vitro* studies imply that mutations at codon 301 may be of little advantage in an environment with normal or increased levels of CSF-1 (10). In patients with reduced CSF-1 levels, however, a codon 301 mutation may confer a growth advantage on a clone of cells. Studies are necessary to examine the relationship between serum CSF-1 levels and mutational status of the *FMS* gene in these patients.

In the feline system, *FMS* mutations at codons 301 and 374 are required in addition to a C-terminal modification involving the loss of codon 969 for a fully transformed phenotype in Rat-2 cells (13), and it is possible that additional mutations may be found in myelodysplastic and leukemic patients. Cytogenetically, none of the present patients with *FMS* mutations has a gross deletion of chromosome 5q. We do not know, however, if a subpopulation of cells has microscopic deletions removing one allele, leaving only the mutant allele.

We have demonstrated the presence of constitutional and somatic point mutations in the *FMS* gene using PCR and oligonucleotide hybridization. The somatic origin of *FMS* mutations in four patients and the presence of a constitutional

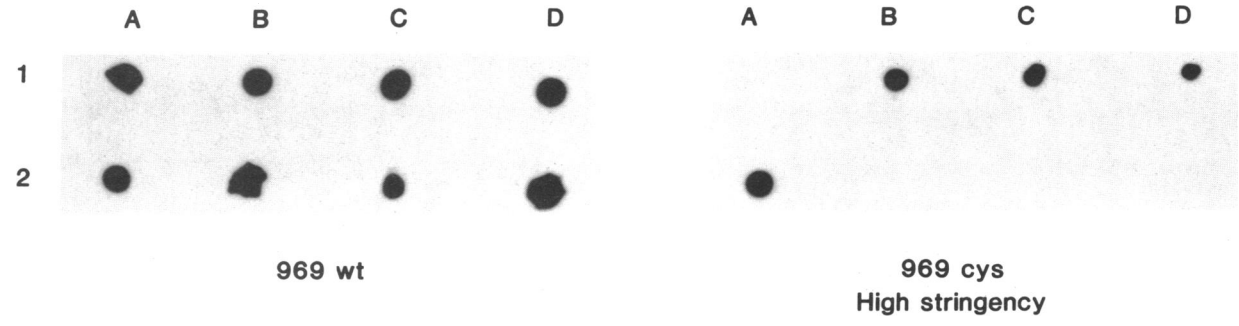

FIG. 3. Detection of a constitutional *FMS* mutation in a normal subject. Slots are DNA from a negative control (A1), original blood sample (B1), repeat blood sample (C1), and buccal mucosa (D1); A2 is a Cys-969 mutant from a patient used as a positive control; B2, C2, and D2 are negative controls. Hybridizations of the filter to the 969 wild-type and the Cys-969 probes are shown. B1, C1, D1, and A2 hybridize strongly to the Cys-969 probe.

mutation in one person have been shown. Lack of material prevents us from assessing the origin (somatic or constitutional) of the *FMS* mutations in the other patients. The appearance and disappearance of these mutations during the progression of disease in some patients suggest these lesions are not initiating events. Mutant *RAS* genes, also thought not to be an initiating lesion, are found in a high proportion of CMML patients (28, 30) possibly indicating that myelodysplastic or leukemic clones already showing potential monocytic differentiation may be especially susceptible to the transforming properties of these oncogenes.

A constitutional mutation has been demonstrated in one hematologically normal individual. This mutant allele may have been inherited from one patient or may have arisen during embryonic development. In view of the high percentage of CMML (20%) and AML M4 (23%) patients with a mutation at codon 969 this may represent a lesion involved in predisposition to these particular malignancies. To our knowledge, a constitutional point mutation in a protooncogene at a regulatory domain has not been reported previously. Further studies are necessary to determine the functional significance of these mutations.

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1. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Cook, A. T. & Stanley, E. R. (1985) *Cell* **41**, 665–676.
2. Sariban, E., Mitchell, T. & Kufe, D. (1985) *Nature (London)* **316**, 64–66.
3. Woolford, J., Rothwell, V. & Rohrschneider, R. (1985) *Mol. Cell. Biol.* **5**, 3458–3466.
4. Yeung, Y. G., Jubinsky, P. T., Sengupta, A., Yeung, D. C. Y. & Stanley, E. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1268–1271.
5. Donner, L., Fedele, L. A., Garon, C. F., Anderson, S. J. & Sherr, C. J. (1982) *J. Virol.* **41**, 489–500.
6. Hampe, A., Gobet, M., Sherr, C. J. & Galibert, F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 85–89.
7. Tamura, T., Simon, E., Niemann, H., Snoek, G. T. & Bauer, H. (1986) *Mol. Cell. Biol.* **6**, 4745–4748.
8. Barbacid, M. & Lauver, A. V. (1981) *J. Virol.* **40**, 812–821.
9. Roussel, M. F., Dull, T. J., Rettenmier, C. W., Ralph, P., Ullrich, A. & Sherr, C. J. (1987) *Nature (London)* **325**, 549–552.
10. Roussel, M. F., Downing, J. R., Rettenmier, C. W. & Sherr, C. J. (1988) *Cell* **55**, 979–988.
11. Coussens, L., Beveren, C., Smith, D., Chen, E., Mitchell, R. L., Isacke, C. M., Verma, I. M. & Ullrich, A. (1986) *Nature (London)* **320**, 277–280.
12. Browning, P. J., Bunn, H. F., Cline, A., Struman, M. & Nienhuis, A. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7800–7804.
13. Woolford, J., McAuliffe, A. & Rohrschneider, L. R. (1988) *Cell* **55**, 965–977.
14. Le Beau, M. M., Westbrook, C. A., Diaz, M. O., Larson, R. A., Rowley, J. D., Gasson, J. C., Golde, D. W. & Sherr, C. J. (1986) *Science* **231**, 984–986.
15. Jacobs, R. H., Cornbleet, M. A., Vardiman, J. W., Larson, R. A., Le Beau, M. M. & Rowley, J. D. (1986) *Blood* **67**, 1765–1772.
16. Heim, S. & Mitelman, F. (1986) *Clin. Haematol.* **15**, 1003–1021.
17. Borgstrom, G. H. (1986) *Scand. J. Haematol.* **36**, 74–77.
18. Nienhuis, A. W., Bunn, H. F., Turner, P. H., Gopal, T. V., Nash, W. G., O'Brien, S. J. & Sherr, C. J. (1985) *Cell* **42**, 421–428.
19. Dubreuil, P., Torres, H., Coarcoul, M. A., Birg, F. & Mannoni, P. (1988) *Blood* **72**, 1081–1085.
20. Preisler, H. D., Raza, A., Larson, R., Le Beau, M., Browman, G., Goldberg, J., Grunwald, H., Volger, R., Verkh, L., Singh, P., Block, A. M. & Sandberg, A. (1989) *Blood* **73**, 255–262.
21. Rambaldi, A., Wakamiya, N., Vellenga, E., Horiguchi, J., Warren, M. K., Kufe, D. J. & Griffin, J. D. (1988) *J. Clin. Invest.* **81**, 1030–1035.
22. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sultan, C. (1982) *Br. J. Haematol.* **51**, 189–199.
23. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sultan, C. (1985) *Ann. Intern. Med.* **103**, 620–625.
24. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
25. Roussel, M. F., Sherr, C. J., Barker, P. E. & Ruddle, F. H. (1983) *J. Virol.* **48**, 770–773.
26. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **73**, 5463–5467.
27. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
28. Padua, R. A., Carter, G., Hughes, D., Gow, J., Farr, C., Oscier, D., McCormick, F. & Jacobs, A. (1988) *Leukemia* **2**, 503–510.
29. March, D. L. (1972) *Commun. ACM* **15**, 991–992.
30. Yunis, J. J., Boot, A. J. M., Mayer, M. G. & Bos, J. L. (1989) *Oncogene* **4**, 609–614.