Structure and expression of c-fgr protooncogene mRNA in Epstein–Barr virus converted cell lines

P.M. Brickell & M. Patel
The Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, UK.

Summary The c-fgr protooncogene is a member of the c-src family of tyrosine kinases. Expression of c-fgr was studied in a series of Epstein-Barr virus (EBV) negative Burkitt's lymphoma cell lines and their EBV-converted derivatives. Two transcripts, of 2.9 kb and 3.5 kb, were present at dramatically elevated levels following EBV-conversion.

The structure of the c-fgr transcripts was studied by the isolation and nucleotide sequence analysis of cDNA clones. This indicated that the c-fgr protein encoded by the mature mRNA would contain 529 amino acids and have a molecular weight of approximately 58,000. The N-terminus of the predicted c-fgr protein has low amino acid homology with the N-termini of other members of this family of proteins, suggesting a cell specific function for the N-terminal domain. Analysis of the c-fgr cDNA clones also revealed the presence of alternative polyadenylation signals, although the use of these does not account for the size difference between the two major c-fgr transcripts.

A variety of agents, including antigens, lymphokines and mitogens, can activate small resting B-lymphocytes to proliferate and progress to a terminally differentiated state. The molecular mechanisms whereby signals at the B-lymphocyte cell surface generate changes in DNA and RNA synthesis are of considerable interest. B-lymphocyte activation is accompanied by the appearance of a range of cell surface molecules, such as CD23 and Blast-1 (Swendeman & Thorley-Lawson, 1987), and it is probable that some of these activation markers represent receptors for growth and differentiation factors. It has been shown, for example, that a fragment of CD23 shed from the surface of activated B-lymphocytes can act as an autocrine B cell growth factor for normal and transformed B-lymphocytes (Swendeman & Thorley-Lawson, 1987).

Within the cell, a number of changes which may be involved in signalling have been described. These include increases in phosphatidylinositol 4,5-bisphosphate hydrolysis, intracellular free Ca2+ and protein kinase C activity (Ransom et al., 1986). It is likely that a range of other intracellular molecules are also involved in signalling during B-lymphocyte activation. One candidate is the cellular proto-oncogene c-fgr, which is a member of a family of genes encoding intracellular proteins with tyrosine kinase activity (Nishizawa et al., 1986). Transcripts of c-fgr are induced in B-lymphocytes immortalised by Epstein-Barr virus (EBV) and in EBV-negative Burkitt's lymphoma (BL) cell lines converted with EBV in vitro (Cheah et al., 1986). Both of these events bear many features in common with B-lymphocyte activation (Thorley-Lawson & Mann, 1985), involving changes in growth properties (Zeuthen, 1983) and in the expression of B-lymphocyte cell surface activation markers (Rowe et al., 1986).

Cheah et al. (1986) showed that EBV converts of the EBV-negative BL cell lines Ramos and BJAB contained a 3 kb transcript homologous to the cellular proto-oncogene c-fgr, which was undetectable in Ramos and BJAB themselves. Ramos and BJAB and their converts are long-established cell lines, and have had the opportunity to accumulate phenotypic changes unrelated to their initial EBV conversion. We have therefore studied changes in c-fgr expression in a series of recently established EBV-negative BL cell lines (Calender et al., 1987), freshly converted with the B95-8 strain of EBV. These cell lines have been well characterised with respect to the pattern of EBV gene expression within them (Murray et al., 1988), to their growth properties and to the B-lymphocyte activation markers which they express (Rowe et al., 1986; Calender et al., 1987). We also report here the isolation and sequencing of c-fgr cDNA clones, a necessary first step in the characterisation of the structure of the c-fgr protein and of its function in B-lymphocyte activation, and in the mapping of the c-fgr gene for studies of its regulation during B-lymphocyte activation.

We describe features of the 5' untranslated region and the 5' coding region of c-fgr mRNA, and demonstrate that the 3' untranslated region contains alternative polyadenylation sites.

Materials and methods

Cell lines

EBV-negative BL cell lines IARC BL2, IARC BL31 and IARC BL41, and their EBV-converted sublines IARC BL2–B95/1, IARC BL31–B95/1 and IARC BL41–B95/1, were a gift from Prof. A.B. Rickinson, Cancer Research Campaign Laboratories, Department of Cancer Studies, The Medical School, Birmingham. They were maintained in RPMI 1640 medium supplemented with 15% (v/v) foetal calf serum (Sera-Lab) and 2 mM L-glutamine.

RNA preparation

Total RNA was purified from washed cell pellets using the guanidinium isothiocyanate method of Chirgwin et al. (1979). Polyadenylated RNA was isolated by affinity chromatography on oligo-dT cellulose (Collaborative Research Ltd.) according to Craig et al. (1976).

Northern blotting

Polyadenylated RNA (2 µg per track) was electrophoresed on a 1% (w/v) agarose MOPS-formaldehyde gel and blotted onto to Biodyne membrane (PALL) essentially as described by Taylor et al. (1984). Filters were probed overnight at 65°C with 32P-labelled antisense RNA, prepared as described below, in hybridisation buffer (60% (v/v) formamide, 5 x SSC, 5 x Denhardt’s solution, 20 mM sodium phosphate pH 6.8, 1% (w/v) SDS, 7% (w/v) dextran sulphate, 100 µg ml−1 single-stranded sonicated herring testis DNA, 100 µg ml−1 E. coli tRNA, 10 µg ml−1 poly A). Final washes were performed at 65°C in 0.1 x SCC/1% (w/v) SDS.

Correspondence: P.M. Brickell.
Received 10 June 1988.
Antisense RNA probes

Plasmid pFB52 is a subclone, in the plasmid vector pGEM1, of an 849 bp Bam HI-Sma I fragment of the v-fgr gene, containing only sequences from the tyrosine kinase encoding domain. Antisense RNA was synthesised by incubating 1 μg Sma I-linearised pFB52 DNA at 37°C for 2 h in 40 mM Tris-HCl (pH 7.5) containing 2 mM spermidine, 20 mM dithiothreitol, 0.43 mM UTP, 0.43 mM ATP, 0.43 mM GTP, 5 μM CTP, 1 unit μl-1 RNase inhibitor (BCL Ltd.), 70 μCi 32P CTP (NEN Ltd.), 0.2 units μl-1 SP6 RNA polymerase (BCL Ltd.). Template and 9 plasmid BluescriptSK III.Rsa I-linearised (Stratagene, San Diego). The cDNA was adenylated by incubation with 1 μg RNase-free DNase (Miles) at 37°C for 10 min, and unincorporated nucleotides were removed, following phenol extraction and other extraction, by ethanol precipitation.

Plasmid pF3.4 is a subclone, in the plasmid vector Bluescript SK M13+ (Stratagene, San Diego), of a 280 bp Rsa I-Eco RI fragment encompassing the 3' end of pF51 and 9 bp of sequence shared by pF51 and pFcl1 (see Figures 2 & 5). Antisense RNA was synthesised as above, using Bam HI-linearised pF3.4 DNA as a template, with 0.2 units μl-1 T3 RNA polymerase (Stratagene, San Diego) and including 50 mM sodium chloride and 8 mM magnesium chloride in the incubation buffer.

Construction of cDNA libraries

Blunt-ended, EcoRI-methylated, double-stranded cDNA was synthesised from 2 μg polyadenylated RNA extracted from BL2-B95/1 cells. It was then ligated to Eco RI linkers and cloned into the Eco RI site of the bacteriophage vector λgt 10, according to Watson & Jackson (1985). The ligated molecules were packaged in vitro and the resultant bacteriophage particles plated out according to Huyhn et al. (1985), yielding a cDNA library of 2 × 109 pfu.

The RPMI 4265 cDNA library was constructed in λgt 10 by Clontech Laboratories Inc. (California) from polyadenylated RNA isolated from the EBV-positive lymphoblastoid cell line RPMI 4265, and was a gift from Dr P. Beverley, ICRF Human Tumour Immunology Unit, University College, London.

Isolation of c-fgr cDNA clones

The cDNA libraries were plated at high density and screened according to Benton & Davis (1977). The hybridisation probe was pFB52 DNA, radiolabelled with 32P-dCTP by the oligonucleotide method of Feinberg & Vogelstein (1984). Hybridisation was performed at 65°C in 6 x SSC containing 5 x Denhardt’s solution and 0.1% (w/v) SDS. Final washes were performed at 65°C in 2 x SSC containing 1% (w/v) SDS.

DNA sequencing

Single stranded templates were prepared from Bluescript SK M13+ sub-clones and dieoxy-sequencing was performed using modified T7 DNA polymerase (Sequenase; United States Biochemical Corporation, Ohio). Sequences were analysed with the aid of the Beckman MicroGenie sequence analysis programme (Queen & Korn, 1984). Oligonucleotides used as sequencing primers, whether homologous to vector or insert sequences, were kindly synthesised for us by Dr Len Hall, Department of Biochemistry, University of Bristol.

Results

Expression of c-fgr mRNA following EBV-conversion

Radiolabelled antisense RNA synthesised from the v-fgr tyrosine kinase domain probe, pFB52, hybridised to a single 2.9 kb transcript in Raji and Daudi cells (Figure 1, lanes a and b), as previously reported by Cheah et al. (1985). However the pattern of transcripts in the EBV-converted cell lines was more complicated (Figure 1, lanes d, h and k). All three EBVconverted lines contained transcripts at 2.9 kb and 3.5 kb. These hybrids were stable when blots were washed in 0.1 × SSC/1% SDS at 80°C and treated with RNase A (20 μg/ml; Sigma), as shown in Figure 1 (lane m), suggesting that they both derive from the c-fgr gene. A third transcript, of 3.9 kb, was visible in BL2-B95/1 cells only (Figure 1, lane h). This hybrid was not stable following RNase A treatment (Figure 1, lane h, m), suggesting that it derives from a related member of the tyrosine kinase gene family to which c-fgr belongs. It is clear from Figure 1 (lanes c, g and l), that low levels of the 2.9 and 3.5 kb c-fgr transcripts are present in BL2, BL31 and BL41 cells, but that there is significant induction upon EBV-conversion. Reprobing of blots with a 32P-labelled actin probe (Figure 1, lanes e, f, i and j) demonstrated that the polyadenylated RNA in each lane was intact and present in equal quantities, confirming that the induction of c-fgr transcripts upon EBV-conversion is a real phenomenon.

Isolation and nucleotide sequencing of c-fgr cDNA clones

In order to determine the relationship of the 2.9 kb and 3.5 kb transcripts to each other and to derive information about the 5’ end of the c-fgr coding region, we screened two cDNA libraries for c-fgr cDNA clones. Ten positively-hybridising recombinant phage were found amongst 120,000 colonies screened. These were plaque purified, and their inserts were excised with Eco RI and subcloned into the Eco RI site of the plasmid vector Bluescript SK M13+. Restriction maps of the inserts of the three longest clones (pF51, pFcl1 and pFcl2d) were shown in Figure 2. Together, the three clones span 2,347 bp and the restriction maps of their central regions are colinear with previously published partial restriction maps of the c-fgr transcript, predicted from the sequences of genomic clones (Parker et al., 1985; Nishizawa et al., 1986).

Single stranded templates were prepared from pF51, pF54 (insert of pF51 cloned in the opposite orientation), pFcl1 and pFcl2d and dieoxy-sequencing was performed. Sequences from regions of overlap with previously published genomic sequences (Parker et al., 1985; Nishizawa et al., 1986) exhibited 100% homology (data not shown). The sequence of the 5’ end of the c-fgr mRNA, derived from clones pFd97 and pF4a, is shown in Figure 3. Clone pFd97 contains 153 bp of 5’ untranslated region. The ATG codon at nucleotides 154 to 165 is likely to be the correct initiation codon since all three reading frames contain upstream termination codons, the remaining two reading frames
Figure 1. Nucleotide sequence of the 5′ end of the c-fgr cdNA clones pdf97, pFc11 and pFa1 and of the subclone pF3.4. The deduced sequence of the c-fgr mRNA is indicated by distances above which are given in base pairs. Restriction sites are: B = BamHI, Bs = BstEII, E = EcoRI, P = PstI, Pv = PvuII, R = RsaI, S = SmaI.

acid 76 in the predicted c-fgr protein to the carboxyl terminus.

Differential polyadenylation of c-fgr mRNA

As shown in Figure 5, the cdNA clone pFc11 is 271bp shorter than pFa1 at the 3′ end, and contains there a stretch of 60 adenosine residues. Polyadenylation at this site could be directed by the sequence UAUAAGA encoded by nucleotides 2054–2059. Polyadenylation at this site could also be directed downstream by the sequence AGUAAA encoded at nucleotides 2335–2340 in pFa1. It is possible that the use of alternative polyadenylation sites accounts for the difference in size between the 2.9 kb and the 3.5 kb c-fgr transcripts. In order to determine whether this is so, radiolabelled antisense RNA synthesised using the plasmid pF3.4 as a template was used to probe a Northern blot of polyadenylated RNA from Raji and BL2–B95/1 cells. The filter was washed at 80°C in 0.1 x SSC/1% SDS. Hybrids between the 9bp sequence shared by the probe and the RNA species corresponding to pFc11 would not be stable under these conditions. As shown in Figure 6, the probe hybridised to the 2.9 kb transcript of Raji cells (lane a) and to both the 2.9 kb and 3.5 kb transcripts of BL2-B95/1 cells (lane b). Thus, the downstream polyadenylation site is used in transcripts of both sizes, and use of the upstream site presumably contributes to the apparent heterogeneity of the 2.9 kb RNA (Figure 1, lanes d, h and k).

Discussion

We have shown here that in vitro conversion of three
recently-established EBV-negative BL cell lines with the B95.8 strain of EBV results in a dramatic elevation of levels of two c-fgr transcripts, of 2.9 kb and 3.5 kb. A similar pattern of transcripts has been described in the human B-lymphocyte cell line IM-9, derived by in vitro infection of normal peripheral blood B-lymphocytes with EBV (Inoue et al., 1987). Cheah et al. (1986) were unable to find c-fgr transcripts in the EBV-negative BL cell lines which they studied, but we find that low levels are detectable in the EBV-negative parent cell lines BL2, BL31 and BL41. Many of the changes in cellular phenotype which accompany EBV-conversion resemble changes seen during normal B-lymphocyte activation, most strikingly the acquisition of cell surface markers such as CD23 and Blast-1 (Swedeman & Thorley-Lawson, 1987). These results raise the possibility, therefore, that the c-fgr protein is involved in the normal B-lymphocyte activation, perhaps as part of a protein phosphorylation cascade which transduces signals leading to B-lymphocyte differentiation and proliferation. Transcripts of the c-fgr gene are not limited to B-lymphocytes, though, having also been detected in well-differentiated monocytic cells (Willman et al., 1987) in lung (Tronick et al., 1985) and in placenta (Nishizawa et al., 1986). It is not known which cell type(s) express c-fgr in the latter two tissues, but expression may be a reflection of their haematopoietic component.

The c-fgr gene is a member of a family which also includes c-src (Tanaka et al., 1987), lyn (Semba et al., 1987), hck (Quintrell et al., 1987), lck (Voronova & Selton, 1986), lyn (Yamashita et al., 1987), c-inkl (Strebhardt et al., 1987) and c-yes (Sukeyawa et al., 1987). Unlike other tyrosine kinases such as c-erb-B and c-fms these proteins do not appear to be cell surface receptors, since they are thought to be located at the cytoplasmic surface of the plasma membrane (Pellman et al., 1985). The c-fgr gene resembleslyn, hck, lck, lyn and c-inkl in having a restricted expression pattern, in contrast to c-src and c-yes whose expression is more widespread.

The proteins encoded by this gene family all have a highly conserved carboxy-terminal domain which encodes tyrosine kinase activity by analogy with pp60cs. The amino-terminal domains are quite diverged, however, and nucleotide sequence data presented here shows that the amino-terminal domain of the c-fgr protein is also quite different from those of the other members of the family. This sequence divergence may indicate that the various family members, expressed in different cell types, have different substrate specificities. It is unlikely that it engenders differences in intracellular location since all members of the family, including the predicted c-fgr protein, as shown here, retain a glycine residue at position two. In pp60cs this residue is the target for post-translational myristylation, which is necessary for the localisation of the protein to the inner surface of cytoplasmic membranes (Pellman et al., 1985). It is likely, then, that the c-fgr protein shares the same intracellular location as pp60cs. It is also noteworthy that against the general background of diversity in amino-terminal sequences, the predicted lyn, hck, and lyn proteins (but not c-src or c-yes) contain peptides with homology to the sequence Tyr-Gly-Pro-Asp-Pro-Thr-Lys found at positions 34-40 in the predicted c-fgr protein (Figure 4). In addition, the lyn protein has homology to the sequence Ile-Pro-Asn-Tyr-Ser-Asn-Phe found at positions 50-57 in the c-fgr protein. The functional significance of these peptides, if any, is unclear.

Inoue et al. (1987) and Katamine et al. (1988) have recently reported nucleotide sequence derived from c-fgr cDNA clones. The nucleotide sequence of the 5' end of the c-fgr cDNA clone pFD97 (Figures 2 and 3), is identical to that reported by Inoue et al. (1987), starting 3 bp downstream of their sequence. However, both our sequence and that of Inoue et al. (1987) differ from the 5' untranslated region from that reported by Katamine et al. (1988). This latter sequence was derived from a cDNA clone representing an incompletely processed c-fgr transcript containing intron 2. It is therefore likely that the sequence of the 5' untranslated region reported by Katamine et al. (1988) is in fact derived from intron 1, and that the sequence shown in Figure 3 is that of the 5' untranslated region of the mature mRNA.
The 5′ untranslated regions of the mRNAs encoding different members of the tyrosine kinase family may have a role in regulating their differential expression. Marth et al. (1988) have recently shown that the 5′ untranslated region of lek mRNA contains AUG codons which reduce the efficiency of translation from the authentic initiation codon. As shown here, the 5′ untranslated region of the c-fgr mRNA contains two AUG codons, both of which are out-of-frame with respect to the initiating AUG. One of these, at nucleotides 101–103, obeys the rules of Kozak (1987) and so could be recognised by the 40S mammalian ribosomal subunit and be used to initiate translation, masking the authentic AUG. Kozak (1987) has noted that 65% of sequenced proto-oncogene mRNAs have AUGs in their 5′ untranslated regions, in contrast to 10% of other mammalian genes surveyed, suggesting that cells might regulate the use of translational start sites in these mRNAs.

We have also demonstrated, by the nucleotide sequencing of c-fgr cDNA clones, that the mature c-fgr mRNA can be polyadenylated at either of two sites. The upstream polyadenylation signal, at nucleotides 2054–2059 does not encode the usual AAUAAA consensus sequence (Proudfoot & Brownlee, 1977), but UAAUAA, a sequence which has also been found to be used as a polyadenylation signal in the gene encoding hepatitis B virus surface antigen (McLauchlan et al., 1985). The c-fgr cDNA clones of Inoue et al. (1987) and Katamine et al. (1988) have the extended 3′ end of pF1, and the data of Katamine et al. (1988) show that the RNA species from which their cDNA clones derive are polyadenylated at the upstream of the 3′ end of pF1, presumably using the non-consensus polyadenylation signal AGAAUA encoded at nucleotides 2335–2340. This sequence is also used as a polyadenylation signal in the genomes of baboon erythroblastosis virus and mouse mammary tumour virus (McLauchlan et al., 1985). The sequence AAUAAAG encoded at nucleotides 2331–2336 is probably non-functional, since it has been shown not to direct polyadenylation of transcripts of a mutant α2-globin gene in a case of α-thalassaemia (Higgs et al., 1983). On the basis of the numbers of cDNA clones of each type isolated, the downstream polyadenylation site appears to be used most frequently. Alternative polyadenylation does not account for the size difference between the 2.9 kb and 3.5 kb c-fgr transcripts but probably contributes to heterogeneity in the lower molecular weight band on Northern blots. This result agrees with recent data from Katamine et al. (1988), which suggests that the 3.5 kb transcript is an incompletely processed precursor, containing intron 2 of the c-fgr gene. Several genes have been characterised which contain alternative polyadenylation sites. In some cases, such as the bovine and human kinogen genes (Kitamura et al., 1985) and the immunoglobulin μ heavy chain gene (Rogers et al., 1980), the use of alternative polyadenylation sites generates proteins with different carboxyl-termini. In the case of the immunoglobulin μ heavy chain gene, polyadenylation site selection is developmentally regulated. The c-fgr gene resembles the rat disulphide isomerase gene (Edman et al., 1985) and the hamster HMG CoA reductase gene (Reynolds et al., 1984), however, in which the choice of polyadenylation site does not affect the protein product. It is not clear whether the choice of polyadenylation sites in the case of c-fgr is regulated. If it were, it could influence the stability of c-fgr mRNA in different cell types and thus be a mechanism for regulation of c-fgr gene expression.

Finally, the availability to us of cDNA clones representing 5′ sequences of the c-fgr mRNA now allows us to map the limits of the c-fgr transcription unit in cosmid genomic clones (our unpublished data), in order to study the basis of the regulation of c-fgr gene expression during EBV conversion, and B-lymphocyte activation. In addition, the predicted amino-acid sequence of divergent portions of the c-fgr protein allows the synthesis of peptides for production of specific antisera with which to study the expression and function of this protein during the activation of B-lymphocytes.

This work was funded by the Cancer Research Campaign. We thank Professor R.K. Craig for helpful discussions and critical reading of this manuscript.

References

BENTON, W.D. & DAVIS, R.W. (1977). Screening Ig recombinant clones by hybridisation to single plaques in situ. Science, 196, 180.

CALENDER, A., BILLAUD, M., AUBRY, J.P., BANCHEREAU, J., VUILLEME, M. & LENOIR, G.M. (1987). Epstein-Barr virus (EBV) induces expression of B-cell activation markers on in vitro infection of EBV-negative B-lymphoma cells. Proc. Natl Acad. Sci. USA, 84, 8060.

CHEAH, M.S.C., LEY, T.J., TRONICK, S.R. & ROBBINS, K.C. (1986). fgr proto-oncogene mRNA induced in B-lymphocytes by Epstein-Barr virus infection. Nature, 319, 238.

CHIRGWIN, J.M., PRZYBYLA, A.G., MACDONALD, R.J. & RUTTER, W.J. (1979). Isolation of biologically active ribonuclease acid from sources enriched in ribonuclease. Biochemistry, 18, 5294.

CRAIG, R.K., BROWN, P.A., HARRISON, O.S., MCLREAVY, D. & CAMPBELL, P.N. (1976). Guinea pig milk protein synthesis: isolation and cDNA sequence characterization of the major and minor bands. Biochem. J., 160, 57.

EDMAN, J.C., ELLIS, L., BLACHER, R.W., ROTH, R.A. & RUTTER, W.J. (1985). Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin. Nature, 317, 267.

FEINBERG, A.P. & VOGELSTEIN, B. (1984). A technique for radio-labeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 137, 266.

HIGGS, D.R., GOODBOURN, S.E.Y., LAMB, J., CLEGG, J.B., WETHERALL, D.J. & PROUDFOOT, N.J. (1983). α-Thalassaemia caused by a polyadenylation signal mutation. Nature, 306, 398.

HUMPHRIES, S., WHITALL, R., MINTY, A., BUCKINGHAM, M. & WHILLIAMS, R. (1981). There are approximately 20 actin genes in the human genome. Nucl. Acids Res., 9, 4895.

HUYNH, T.V., YOUNG, R.A. & DAVIS, R.W. (1985). Constructing and screening cDNA libraries in λgt 10 and λgt 11. In DNA Cloning: A Practical Approach, Vol. 1, Glover, D.M. (ed) p. 49. IRL Press: Oxford.

INOUE, K., IKAWA, S., SEMBA, K., SUKEGAWA, J., YAMAMOTO, T. & TOYOSHIMA, K. (1987). Isolation and sequencing of cDNA clones homologous to the ψ-fgr oncoprotein from the mouse B lymphocyte cell line, IM-9. Oncogene, 1, 301.

KATAMINE, S., NOTARIO, V., RAO, C.D. & others (1988). Primary structure of the human fgr proto-oncogene product p55/ψ Whereas. Mol. Cell. Biol., 8, 259.

KITAMURA, N., KITAGAWA, H., FUKUSHIMA, D., TAKAGAKI, Y., MIYATA, T. & NAKANISHI, S. (1987). Structural organisation of the human kinogen gene and a model for its evolution. J. Biol. Chem., 260, 8610.

KOZAK, M. (1987). An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucl. Acids Res., 15, 8125.

MARTH, J.D., OVERELL, R.W., MEIER, K.E., KREBS, E.G. & PELLMUTTER, R.M. (1988). Translational activation of the 1k proto-oncogene. Nature, 332, 171.

MC LAUCHLIA, J., GAPPENY, D., WHITTON, J.L. & CLEMENTS, J.B. (1985). The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3′ termini. Nucl. Acids Res., 13, 1347.

MURRAY, R.J., YOUNG, L.S., CALENDER, A. & others (1988). Different patterns of Epstein-Barr virus gene expression and of cytotoxic T-cell recognition in B-cell lines infected with transforming (B95.8) or non-transforming (P3HR1) virus strains. J. Virol., 62 (in press).
NISHIZAWA, M., SEMBA, K., YOSHIDA, M.C., YAMAMOTO, T., SASAKI, M. & TOYOSHIMA, K. (1986). Structure, expression and chromosomal location of the human c-fgr gene. Mol. Cell. Biol., 6, 511.

PARKE, R.C., MARDON, G., LEBO, R.V., VARMUS, H.E. & BISHOP, J.M. (1985). Isolation of duplicated human c-src genes located on chromosomes 1 and 20. Mol. Cell. Biol., 5, 831.

PELLMAN, D., GARRBER, E.A., CROSS, F.R. & HANAFUSA, H. (1985). An N-terminal peptide from p69src can direct myristylation and plasma membrane localization when fused to heterologous proteins. Nature, 314, 374.

PROUDFOOT, N.J. & BROWNLEE, G.G. (1976). 3' non-coding region sequences in eukaryotic messenger RNA. Nature, 263, 211.

QUEEN, C. & KORN, L.J. (1984). A comprehensive sequence analysis program for the IBM personal computer. Nucl. Acids Res., 12, 591.

QUINTRELL, N., LEBO, R., VARMUS, H. & 5 others (1987). Identification of a human gene (HCK) that encodes a protein-tyrosine kinase and is expressed in hematopoietic cells. Mol. Cell. Biol., 7, 2267.

RANSOM, J.T., HARRIS, I.K. & CAMBIER, J.C. (1986). Anti-Ig induces release of inositol 1,4,5-triphosphate, which mediates mobilization of intracellular Ca ++ stores in B lymphocytes. J. Immunol., 137, 708.

REYNOLDS, G.A., BASU, S.K., OSBORNE, T.F. & 5 others (1984). HMG CoA reductase: a negatively regulated gene with unusual promoter and 5' untranslated regions. Cell, 38, 275.

ROGERS, J., EARLY, P., CARTER, C. & 4 others (1980). Two mRNAs with different 3' ends encode membrane bound and secreted forms of immunoglobulin μ chain. Cell, 20, 303.

ROWE, M., ROONEY, C.M., EDWARDS, C.F., LENOIR, G.M. & RICKINSON, A.B. (1986). Epstein-Barr virus status and tumour cell phenotype in sporadic Burkitt’s lymphoma. Int. J. Cancer, 37, 367.

SEBMA, K., NISHIZAWA, M., MIYAJIMA, N. & 6 others (1986). yes-related proto-oncogene, syn, belongs to the protein-tyrosine kinase family. Proc. Natl Acad. Sci. USA, 83, 5459.

STREBHARDT, K., MULLINS, J.J., BRUCK, C. & RÜBSAMEN-WAIHGEMANN, H. (1987). Additional member of the protein-tyrosine kinase family: The src- and lck-related protooncogene c-akt. Proc. Natl Acad. Sci. USA, 84, 8778.

SUKEGAWA, J., SEMBA, K., YAMANASHI, Y. & 4 others (1987). Characterization of cDNA clones for the human c-yes gene. Mol. Cell. Biol., 7, 41.

SWENDEMANN, S. & THORLEY-LAWSON, D.A. (1987). The activation antigen BLAST-2, when shed, is an autocrine BCGF for normal and transformed B cells. EMBO. J., 6, 1637.

TAKEYA, T. & HANAFUSA, H. (1983). Structure and sequence of the cellular gene homologous to the RSV src gene and the mechanism for generating the transforming virus. Cell, 32, 881.

TAYLOR, J.B., CRAIG, R.K., BEALE, D. & KETTERER, B. (1984). Construction and characterisation of a plasmid containing complementary DNA to mRNA encoding the N-terminal amino acid sequence of the rat glutathione transferase Ya subunit. Biochem. J., 219, 223.

TANAKA, A., GIBBS, C.P., ARTHUR, R.R., ANDERSON, S.K., KUNG, H-J. & FUJITA, D.J. (1987). DNA sequence encoding the amino-terminal region of the human c-src protein: implications of sequence divergence among src-type kinase oncogenes. Mol. Cell. Biol., 7, 1978.

THORLEY-LAWSON, D.A. & MANN, K.P. (1985). Early events in Epstein-Barr virus infection provide a model for B-cell activation. J. Exp. Med., 162, 45.

TRONICK, S.R., POPESCU, N.-C., CHEAH, M.S.C. & 5 others (1985). Isolation and chromosomal localization of the human fgr proto-oncogene, a distinct member of the tyrosine kinase gene family. Proc. Natl Acad. Sci. USA, 82, 6595.

VORONOVA, A.F. & SEFTON, B.M. (1986). Expression of a new tyrosine kinase protein is stimulated by retrovirus promoter insertion. Nature, 319, 682.

WATSON, C.J. & JACKSON, J.F. (1985). An alternative procedure for the synthesis of double-stranded cDNA for cloning in phage and plasmid vectors. In DNA Cloning: A Practical Approach, Vol. I. Glover, D.M. (ed) p. 79. IRL Press: Oxford.

WILLMAN, C.L., STEWART, C.C., GRIFFITH, J.K., STEWART, S.J. & TOMASI, T.B. (1987). Differential expression and regulation of the c-src and c-fgr protooncogenes in myelomonocytic cells. Proc. Natl Acad. Sci. USA, 84, 4480.

YAMANASHI, Y., FUKUSHIGE, S.-H., SEMBA, K. & 5 others (1987). The yes-related cellular gene lyn encodes a possible tyrosine kinase similar to p56Lck. Mol. Cell. Biol., 7, 237.

ZEUTHEN, J. (1983). Epstein-Barr virus transformation: biological and functional aspects. Adv. Viral. Oncol., 3, 183.