tumors examined. It is possible that A-MuLV could have initiated an early step in ABPL tumorigenesis but it was not required for the maintenance of the neoplastic state, as was shown for several pre-B lymphosarcomas (*14*). Elimination of A-MuLV genome may have favored survival of that cell type because the gene product of v-*abl* has been shown to be toxic to certain cell types (*15*). Whether ABPL's can be induced by M-MuLV alone or by a concerted effort between the two viral elements in Abelson virus remains to be tested.

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Expression of the c-*fos* Gene and of an *fos*-Related Gene Is Stimulated by Platelet-Derived Growth Factor

Abstract. *Complementary DNA clones of genes induced by platelet-derived growth factor (PDGF) in BALB/c-3T3 cells were isolated; one such clone contains a domain having nucleotide sequence homology with the third exon of* c-fos. *This nucleotide sequence homology is reflected in the predicted amino acid sequences of the gene products. Under low stringency conditions, the mouse* v-fos *gene cross-hybridizes with the PDGF-inducible complementary DNA clone. However, the messenger RNA transcripts of mouse* c-fos *and the new* fos*-related gene can be distinguished by gel electrophoresis and by S1 nuclease analysis. Expression of the authentic* c-fos *gene is induced by PDGF and superinduced by the combination of PDGF and cycloheximide.*

We have described the isolation of a platelet-derived growth factor (PDGF)–inducible complementary DNA (cDNA) clone termed "pBC-JB" (*1*). Subfragments of pBC-JB were cloned into M13 mp8 and sequenced by the dideoxy method (*2*). The nucleotide sequence thus derived was screened for relationships to other genes recorded in the Genbank data base with a rapid similarity search algorithm of Wilbur and Lipman (*3*). This screen indicated that the pBC-JB cDNA insert (hereafter abbreviated as "r-*fos*") contained a domain with nucleotide sequence homology to the third exon of the mouse c-*fos* gene (*4*). The nucleotide sequence of this homologous region and the flanking sequences of r-*fos* which show little or no homology to the second and fourth exons of c-*fos*, are shown in Fig. 1.

The third exon of mouse c-*fos* contains an open reading frame encoding 36 amino acids. This sequence of 36 amino acids is strongly conserved between mouse c-*fos*, its viral homolog, and human c-*fos* (*5*). The domain of r-*fos*, which is homologous to the c-*fos* third exon, is contained within the longest open reading frame of the cDNA clone. This reading frame dictates synthesis of a peptide similar to that directed by the corresponding region of c-*fos* messenger RNA (mRNA) (Fig. 1). The r-*fos* cDNA clone represents only about 50 percent of the mRNA. We cannot detect an ATG (A, adenine; T, thymine; G, guanine) start signal for the longest open reading frame; however, this frame is contiguous through the 5′ end of the clone. If we assume that the longest reading frame depicted within r-*fos* is the authentic one, then the nucleotide sequence homology between r-*fos* and c-*fos* is reflected at the amino acid level.

The sequence homology between r-*fos* and c-*fos* is pervasive enough to suggest that these two genes would cross-hybridize with each other under relaxed stringency conditions. Southern gel electrophoresis (Fig. 2) (*6*) confirms this prediction.

Although there is sufficient sequence homology to allow cross hybridization of r-*fos* and c-*fos* DNA's at relaxed stringency, the mRNA's corresponding to these two genes can be readily distinguished by gel electrophoresis (Fig. 3). By sequentially probing a Northern blot (*7*) filter with nick-translated v-*fos* and then with r-*fos*, we were able to demonstrate differences in the molecular weight of their cognate mRNA's. A single band of mRNA that reacted with the v-*fos* probe (*8*) migrated with an apparent molecular weight of 2.2 kb (*5*). Another band reacting with the r-*fos* probe has an apparent molecular weight of about 2.0 kb. The r-*fos* mRNA appears to be more abundant than c-*fos* in these Northern blots (Fig. 3).

The Northern gel assay shown in Fig. 3 required isolation of poly(A)$^+$ (polyadenylated) RNA from large quantities of cell cultures. For this reason we were forced to use partially purified PDGF preparations in the experiment. To establish that PDGF itself, rather than some other agent within platelet extracts, regulates c-*fos* expression, we developed a c-*fos* probe suitable for S1 nuclease analysis of total cellular RNA in solution. With mouse v-*fos* provirus as a source of starting material, a Pst I restriction fragment homologous to authentic c-*fos* mRNA within a region spanned by the second and third c-*fos* exons was cloned into M13 mp8 bacteriophage. The region spanned by this M13 probe is indicated by the bracketed arrows in Fig. 4. The nucleotide sequence of the M13 probe dictates that c-*fos* mRNA will protect a 234-nucleotide fragment from S1 nuclease digestion. Under identical conditions, r-*fos* mRNA could protect no fragment of the probe greater than 24 nucleotides in length as indicated by the sequence data (Fig. 1). S1 nuclease analysis indicates that

expression of the c-*fos* gene is indeed stimulated within 3 hours after exposure to electrophoretically homogeneous PDGF (Fig. 4A). Other growth factors contained within platelet-poor plasma do not stimulate expression of c-*fos*. Serum-free culture medium conditioned by NRK-*sis* transformed fibroblasts also induces expression of c-*fos* (data not shown).

A salient feature of other PDGF-inducible genes is that induction is potentiated by agents that perturb cellular protein synthesis such as cycloheximide, puromycin, canavanine, and ouabain. The magnitude of superinduction observed when protein synthesis is arrested ranges from 4- to 20-fold or more (*1, 9, 10*). The c-*fos* oncogene shows strong superinduction response when cycloheximide is added together with PDGF (Fig. 4B).

Expression of c-*fos* has been observed in placenta and embryonic tissues (*11*) but not in cultured fibroblasts. That c-*fos* was not detected within fibroblasts in earlier studies may be due to the low abundance of c-*fos* mRNA and the periodic nature of PDGF-stimulated gene expression in mitogen-treated cells. From previous studies on the abundance of c-*myc* RNA, we estimate that quiescent BALB/c-3T3 cells contain less than a tenth of a molecule of *myc* mRNA gene per cell. After exposure to saturating doses of PDGF, the cells contain no more than five to ten molecules of *myc* mRNA per cell (*9, 10*). Thus even in the fully induced state, the abundance of c-*myc* mRNA is near the sensitivity threshold for detection by Northern gel electrophoresis with the use of poly(A)$^+$ mRNA. The abundance of c-*fos* in PDGF-treated cells does not exceed that of c-*myc* mRNA. In the Northern gel analysis (Fig. 3), c-*fos* mRNA was resolved only by superinducing the gene by treatment with PDGF in the presence of cycloheximide.

The biological significance of the sequence homology between r-*fos* and c-*fos* is unknown at present. The homologies are not as exact as those established for p28^{sis} and PDGF (*12*) or for the v-*erbB* protein and the EGF receptor (*13*) where virtual identity exists. However the r-*fos*:c-*fos* homology is extensive enough to allow cross hybridization of the two genes under low stringency conditions (Fig. 2). The homologies that we describe between r-*fos* and c-*fos* are at approximately the same level of significance as those described for c-*myc* and N-*myc* (*14*) and somewhat better than the relation between *myc* and *myb* (*15*). The exact match of eight amino acids between r-*fos* and c-*fos* is longer than the match of five to six amino acids which is considered to be the statistical minimum for significance (*16*). The additional fact that another exact match of four amino acids follows after a two-residue gap gives us additional confidence that this homology is not simply fortuitous. There is another smaller domain of homology within the nontranslated sequences of c-*fos* and r-*fos* (data not shown) and perhaps other homologies exist within areas of r-*fos* not represented in our cDNA clone.

The observation that both c-*fos* and r-*fos* are inducible by PDGF suggests a functional relation between these two

Second exon domain

```
GTGTTGCAGTGTTATAATAAGGCCAGCGCGGGATGGCGGGTTTGGTATTGGCGAGGTGTTTATTTCTAAGAAGGAATAAT
                 *    **   * *   * ***   * **     *       * ** * *  *** *
      GAGACAGATCAACTTGAAGATGAGAAGTCTGCGTTGCAGACTGAGATTGCCCAATCTGCTGAAAGAGAAGGAA
```

Third exon domain

```
CTATCTCCTGAAGAGGAAGAGAAAGAGAAACGGAGAATCCGAAAGGGAACGGAATATGAGACAGATCAACTTGAAGAT--
************************      ************* ************ **          *  *
CTATCTCCTGAAGAGGAAGAGAAA------CGGAGAATCCGAA-GGGAACGGAATAAGATGGCTGCAGCCAAGTGCCGGA

-------GAGAAGTCTGCGTTGCAGACTGAGATTGCCAA
       ***   ***      * ***   *  *
ATCGGAGGAGGGAGCTGACAGATACACTCCAAGCGTACG
```

Fourth exon domain

```
ATATATTCTAATTTAACCGCTTGGGTATCCCTTTCATGGCCCCCCTTTCCATTTATAGGAGGGGAAGGTTGGAACCAACC
  *   * *     * *** *** * *    * *      *       *    *** *  *  * *
GAACTCCCCACCCAGTCTGCTGGGGCTTACGCCAGAGCGGGAATGGTGAAGACCGTGTCAGGAGGCAGAGCGCAGAGCAT
```

Third exon domain reading frame

```
Leu Ser Pro Glu Glu Glu Glu Lys Glu Lys Arg Arg Ile Arg Lys Gly Thr Glu Tyr Glu
 *   *   *   *   *   *   *   *          *   *   *   *
                                        --------------
Thr Asp Gln Leu Glu Asp Glu Lys Ser Ala Leu Gln Thr Glu Ile Ala
```

Fig. 1. The r-*fos* cDNA contains a domain of strong nucleotide and amino acid sequence homology to the third exon of c-*fos*. For comparative purposes, flanking regions of r-*fos* that share little or no homology with the second and fourth exon of c-*fos* are also shown. The DNA sequences of r-*fos* (upper lines) and the c-*fos* coding regions (lower lines) were aligned with the use of the Nucaln program of Wilbur and Lipman (*3*). This program will introduce gaps in the sequence being fitted to achieve the best alignment. A "K-tuple" size of 3 window size of 20 and gap penalty of 7 were used to achieve the match. Exact matches are indicated by (*). Gaps within the c-*fos* sequences introduced for best alignment are shown by (−). The bottom panel shows the amino acid sequence of the r-*fos* open reading frame which is homologous to the c-*fos* third exon. Exact amino acid alignments are indicated by (*). The four amino acid residues indicated by (*) would align after a two-residue gap in the c-*fos* amino acid sequence. The positions of the r-*fos* second, third, and fourth exon domains relative to the c-*fos* gene can be visualized within Fig. 4 (inset). The c-*fos* data in Figs. 1 and 4 are adapted from Van Beveren *et al.* (*4*).

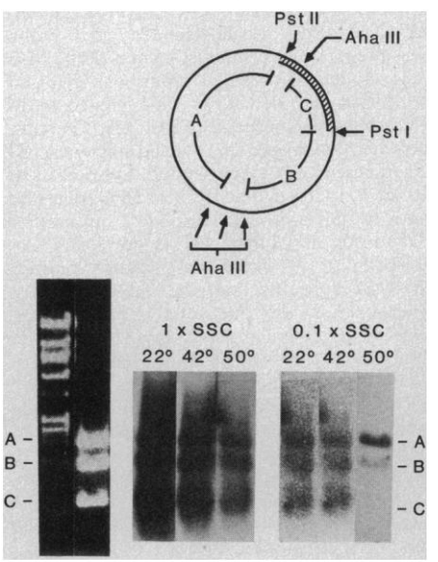

Fig. 2. Cross hybridization of r-*fos* and v-*fos* DNA's at low stringency. Top inset shows a restriction map of r-*fos* cDNA cloned into pUC-9. The hatched area is r-*fos*. A, B, and C are the three largest fragments of r-*fos* resolved on a 1 percent agarose gel after Pst I–Aha III digestion (1.9, 1.0, and 0.6 kb, respectively). The lower left panel shows the ethidium bromide staining of such a gel. The far left lane shows lambda–Hind III markers. The r-*fos* DNA fragments were then transferred to nitrocellulose and hybridized to a nick-translated v-*fos* probe (pFBJ-2) (*8*) at 37°C in 50 percent formamide, 5× SSPE (salt, sodium phosphate, and EDTA), 1× Denhardt's solution, 0.1 percent sodium dodecyl sulfate, and denatured salmon sperm DNA (50 μg/ml) for 20 hours. Filters were then washed successively at increasing temperatures and decreasing salt concentrations as indicated and exposed to x-ray film.

genes. It should also be noted that the strongest region of homology between these genes is in a region of the gene considered to be necessary for cellular transformation at least as scored by focus formation. The 3′ end of the c-*fos* gene downstream from the third exon must be disrupted to activate the transforming potential of the gene (*17*). Further structural and functional analysis of the r-*fos* gene will be necessary to fully assess the relationship of r-*fos* to c-*fos*.

There are now eight well-characterized members of the competence gene family. These include r-*fos*, the four other genes isolated as cDNA clones (*1*), c-*myc* (*1*), c-*myc* (*11*), c-*fos*, and a 29,000-dalton PDGF-inducible protein (*18*). Three of the eight competence gene products (c-*myc*, c-*fos*, and the 29,000-dalton protein) are localized within the nucleus (*19–22*). Perhaps this pattern of nuclear localization may also be found with r-*fos* and other competence gene products. These PDGF-inducible proteins may function within the nuclear matrix to coordinate transcription and replicative DNA synthesis (*20*).

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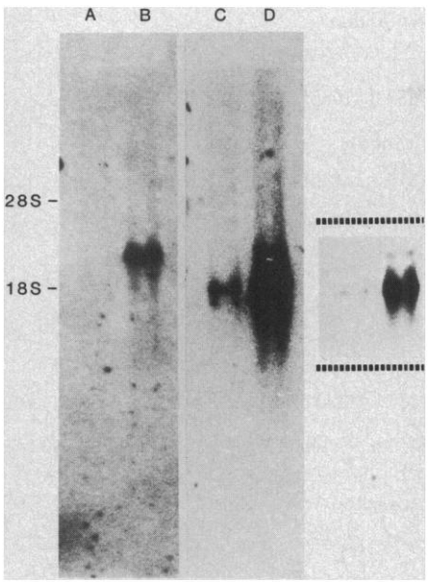
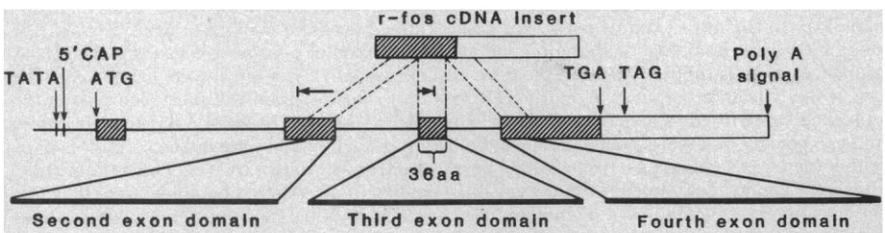


Fig. 3. r-*fos* and c-*fos* mRNA's resolved by Northern gel (*7*) electrophoresis. Poly(A)$^+$ RNA was isolated from 3T3 cells in which c-*fos* and r-*fos* were maximally superinduced by exposure to partially purified PDGF (200 unit/ml) in the presence of cycloheximide (10 μg/ml). A sample (3 μg) of poly(A)$^+$ RNA from control cells (A and C) or treated with PDGF and cycloheximide from cells (B and D) was subjected to electrophoresis through a 1.5 percent agarose gel containing 10 m*M* methyl mercury hydroxide and transferred to a nitrocellulose filter. (Lanes A and B) Hybridization to a nick-translated v-*fos* probe. This same filter was then hybridized (without eluting the previous band) to an r-*fos* probe of approximately one-fourth the specific activity of the v-*fos* probe (lanes C and D). The inset on the far right shows a lighter exposure of lanes C and D.



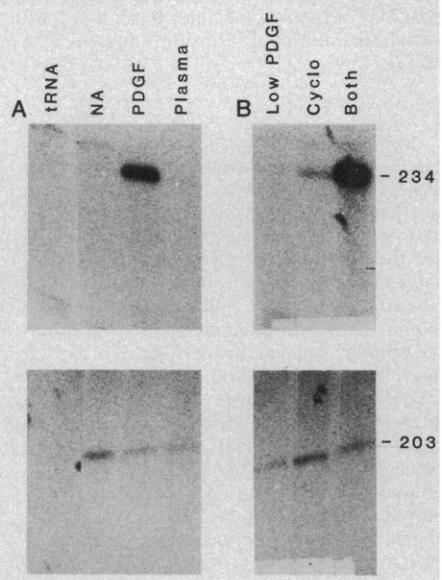


Fig. 4. Induction of c-*fos* by PDGF displayed by S1 nuclease assay. A Pst I restriction fragment of v-*fos*, which spans the second and third exons of the c-*fos* gene (the bracketed arrows), was cloned into M13 mp8. A uniformly labeled, single-stranded c-*fos* probe was prepared by primer extension of the M13 mp8 clone. The labeled probe was digested with Eco RI, denatured, and subjected to electrophoresis through an 8*M* urea, 5 percent polyacrylamide gel. The labeled single-stranded fragment was electroeluted, hybridized to 10 μg of total cellular RNA at 56°C, digested with S1 nuclease, and displayed on another denaturing acrylamide gel as previously described (*10*). As a control for slight variations in RNA concentration, the amounts of β_2-microglobulin were measured in parallel samples with a similar S1 assay (*10*). (A) S1 analysis for c-*fos* (upper lanes) and β_2-microglobulin (lower lanes) from quiescent BALB/c 3T3 cells treated for 3 hours with electrophoretically homogeneous PDGF (60 ng/ml), 5 percent platelet-poor plasma, or neither. As a control for self-annealing of the probe, 10 μg of transfer RNA was hybridized in each assay. (B) Similar S1 analysis of c-*fos* (upper panels) and β_2-microglobin (lower panels) from quiescent BALB/c-3T3 cells 3 hours after treatment with partially purified PDGF (200 unit/ml), cycloheximide (10 μg/ml), or both.

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