Constitutive expression of a gene encoding a polypeptide homologous to biologically active human platelet protein in Rous sarcoma virus-transformed fibroblasts

(gene expression/connective tissue-activating peptide III/pp60^{v-src}/growth-regulated message)

PIERRE-ANDRE BEDARD*, DAVID ALCORTA, DANIEL L. SIMMONS, KA-CHEUNG LUK†, AND R. L. ERIKSON

Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138

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ABSTRACT A molecular clone corresponding to a 1.2-kilobase mRNA enriched in Rous sarcoma virus-transformed chicken embryo fibroblasts (CEF) was identified by differential screening of a cDNA library. The induction of the cloned sequence (denoted pCEF-4) in CEF infected by the temperature-sensitive mutant NY72-4 Rous sarcoma virus is rapid and independent of protein synthesis. DNA sequencing of the 1.2-kilobase insert of CEF-4 revealed an open reading frame that predicts an 11-kDa protein. The predicted pCEF-4 gene product is homologous to human connective tissue-activating peptide III (CTAP-III) and platelet factor 4 (PF-4). Serum stimulation of quiescent normal CEF results in a rapid but transient expression of pCEF-4 mRNA. Hence, pCEF-4 mRNA is expressed at the G₀-G₁ transition and during the first G₁ phase of normal CEF reentering the cell cycle. The expression of pCEF-4 mRNA in Rous sarcoma virus-transformed CEF appears to be the result of transcriptional activation and stabilization of the transcript.

Much of our knowledge of the gene products responsible for cell transformation is derived from investigations of the virally encoded oncogenes and of the protooncogenes, their cellular ancestors. Functional and structural similarities often exist between oncogene products and growth factors, growth factor receptors, or the products of other genes expressed during the normal mitogenic response (1). In several instances, the alteration in cellular protooncogenes resulting in their oncogenic activation is understood (1-4). Hence, considerable progress has been accomplished regarding the biochemical characterization of some of the agents that cause cell transformation. In contrast, little is known about the biochemical pathways and cellular activities critically modified during the transformation process. The absence of functional assays and the lack of an appropriate model system amenable to genetic manipulation have clearly limited our progress in this respect. Various approaches have been utilized to circumvent these limitations. One involves the study of protein phosphorylation in normal and transformed cells, because a class of transforming proteins exhibits tyrosine-specific kinase activity. Some investigators have also compared the role of protooncogenes in normal and malignant growth because of the obvious links between this class of genes and the mitogenic response. We have followed a third approach by searching for genes repressed or expressed in response to transforming proteins or growth factors without regard to their possible relationship to known oncogenes.

In this report, we describe a cDNA clone (denoted pCEF-4) corresponding to a 1.2-kilobase (kb) mRNA species that is expressed transiently in response to serum and constitutively

in Rous sarcoma virus (RSV)-transformed chicken embryo fibroblasts (CEF) in the presence or absence of serum. Increased transcription and stabilization result in the accumulation of the pCEF-4 mRNA in RSV-transformed cells. A polypeptide of M_r 11,000 is predicted by the nucleic acid sequence.[‡] The predicted polypeptide shares significant homology with connective tissue-activating peptide III (CTAP-III) and other related platelet-associated factors. Pleiotropic effects including mitogenic stimulation have been attributed to these platelet factors and, hence, the constitutive expression of the pCEF-4 gene product may be important for the establishment of some aspects of the transformation phenotype. After this work was completed, Sugano *et al.* (5) published similar observations.

MATERIALS AND METHODS

Cells and Viruses. Cells and viruses studied in this report have been described elsewhere (6).

Construction and Screening of cDNA Libraries. A cDNA library was constructed from RNA purified from cycloheximide-treated CEF transformed by NY72-4 RSV according to published protocols (7, 8). The libraries were screened with radiolabeled cDNA prepared from uninfected and RSV-transformed CEF polyadenylated RNA by conventional filter hybridization techniques.

RNA Gel Blot Analyses. RNA was extracted by high-salt precipitation (9). In RNA gel blot analyses, total cytoplasmic RNA was separated on 1% agarose gels containing formaldehyde. After transfer to Nytran membranes (Schleicher & Schuell), the RNA was probed with ³²P-labeled antisense RNA in the conditions described by Melton *et al.* (10). RNA loading was monitored by melting off the pCEF-4 probe in 50% (vol/vol) formamide, 10 mM sodium phosphate (pH 7), 5 mM EDTA at 85°C for 30 min and rehybridizing the blot to a chicken glyceraldehyde-3-phosphate dehydrogenase RNA probe (GAPD). Antisense myc transcripts were prepared from pMYC 4.1, a pGEM-2 plasmid (Promega Biotec, Madison, WI) containing the *Sph* I-*Sal* I fragment of the MC29 myc gene. The GAPD gene was isolated in our laboratory (Steve Jones and K.-C.L., unpublished data) and subcloned into pGEM-2. The chicken c-fos clone was isolated from the same cDNA library and will be described elsewhere (D.A.,

Abbreviations: CEF, chicken embryo fibroblast; RSV, Rous sarcoma virus; SR-A RSV, Schmidt-Ruppin strain (subgroup A) of RSV; CTAP-III, connective tissue-activating peptide III; wt, wild type; ts, temperature sensitive.

*To whom reprint requests should be addressed.

†Present address: McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02975).

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FIG. 1. Expression of pCEF-4 in RSV-transformed CEF. (A) The level of the pCEF-4 transcript in CEF infected by wt SR-A RSV (lane 1) and uninfected CEF (lane U) by RNA gel blot analysis. Total RNA was extracted from subconfluent cells actively growing in Dulbecco's modified Eagle's medium containing 10% (vol/vol) calf serum. The size of the pCEF-4 transcript (1.2 kb) was determined from the mobility of RNA species of known molecular weight (14) electrophoresed in parallel. RNA loading was monitored by melting off the pCEF-4 probe and rehybridizing the blot to a chicken GAPD probe. (B) The expression of pCEF-4 is analyzed in CEF infected with the ts mutant NY72-4 RSV. Total RNA was extracted from subconfluent CEF actively growing in presence of serum and maintained at the nonpermissive temperature of 41°C or transferred to the permissive temperature of 35°C for increasing periods of time. RNA was purified at the indicated time points and analyzed by RNA gel blots. (C) The effects of pp60^{v-src} inactivation on pCEF-4 expression is examined by RNA gel blot analysis. Actively growing CEF transformed by ts NY72-4 RSV were maintained at the permissive temperature of 35°C or transferred to the nonpermissive temperature of 41°C for increasing periods of time. Total RNA was extracted at each of the indicated time points and analyzed on RNA gel blots as described above. The RNA blot was hybridized sequentially with the pCEF-4 and GAPD(H) RNA probes.

unpublished data). The plasmids pA1 and pT2 containing a chicken α -actin and β -tubulin sequence, respectively, were provided by M. W. Kirschner (University of California, San Francisco, CA).

Sequencing and Computer Analysis. Both strands of the pCEF-4 subclone containing a 1.2-kb cDNA insert were sequenced by the method of Maxam and Gilbert (11). The protein sequence database⁸ maintained by the National Biomedical Research Foundation was searched for sequence homology using the FASTP program of Lipman and Pearson (12).

Transcription and Stability Analyses. Transcription analyses in isolated nuclei were performed as described by Linial *et al.* (13). To determine the half-life of mRNA, transcription was inhibited by the addition of actinomycin D at 2 μ g/ml. This concentration of actinomycin D is sufficient to inhibit 80–90% of RNA transcription within 15 min of addition (data not shown).

RESULTS

Identification of a cDNA Corresponding to a mRNA Enriched in Transformed CEF. A cDNA library representative of RNA sequences expressed in RSV-transformed CEF was screened as described. From an initial pool of 3000 recombinants, we identified one sequence enriched in transformed cells. The expression of the sequence, denoted pCEF-4, was confirmed by RNA gel blot analysis (Fig. 1). In CEF transformed by the wild-type (wt) Schmidt-Ruppin strain (subgroup A) of RSV (SR-A RSV), pCEF-4 hybridized to a 1.2-kb RNA species that was virtually undetectable in normal cells (Fig. 1A). Moreover, the sequence was induced in cells infected by a temperature sensitive (ts) mutant, NY72-4 RSV, at the permissive temperature of 35°C (Fig. 1B). The 1.2-kb transcript accumulated within 30 min of temperature shift and reached a nearly maximal level 1 hr after transfer. RNA slot-blot hybridization showed that the pCEF-4 transcript was 20 times more prevalent in cells 24 hr after transfer to the permissive temperature, and fully transformed NY72-4 RSV-infected CEF maintained at 35°C for 3 days also expressed high levels of pCEF-4 mRNA (data not shown). The accumulation of pCEF-4 mRNA was independent of protein synthesis, since NY72-4 RSV-infected CEF treated with cycloheximide expressed the sequence upon transfer to the

permissive temperature. In all RNA analyses described in this report, we controlled for RNA loading by rehybridization to a glyceraldehyde-3-phosphate dehydrogenase probe (GAPD). This sequence is expressed constitutively in a variety of cell lines (13) and with little variation at different phases of the cell cycle (15).

To determine to what extent the expression of pCEF-4 mRNA is linked to pp60^{v-src} activity, the expression of the transcript was analyzed in CEF transformed by NY72-4 RSV after transfer to the nonpermissive temperature of 41°C. Under these conditions, the kinase activity of pp60^{v-src} diminishes by 75% within 1 hr of temperature shift (6). The level of pCEF-4 mRNA decreased rapidly upon temperature shift, suggesting that the increased expression of pCEF-4 requires a rather high level of kinase activity in RSV-infected cells (Fig. 1C). Quantitation by RNA slot-blot of the pCEF-4 decline in the analysis of Fig. 1C indicated that the transcript is less prevalent by roughly a factor of 8 in cells transferred to the nonpermissive temperature for 1 hr. Hence, the apparent half-life of the transcript is only 20 min, indicating that pCEF-4 is relatively unstable under these conditions.

The pCEF-4 Gene Product Is Homologous to CTAP-III. The complete nucleic acid sequence of the pCEF-4 cDNA insert is shown in Fig. 2. An open reading frame encoding an 11-kDa protein, 103 amino acids long, is found within the nucleic acid sequence. Sequence comparison of the predicted pCEF-4 gene product indicated striking homology with CTAP-III (18), also known as low-affinity platelet factor 4. Identical amino acid residues (37) shared by pCEF-4 and CTAP-III are indicated by asterisks in Fig. 2. The C termini of pCEF-4 and CTAP-III are 56% homologous when conserved residues are taken into account. Other related proteins such as β -thromboglobulin (19), platelet basic protein (a probable precursor of both CTAP-III and β -thromboglobulin, ref. 20), platelet factor 4 (16), and γ IP-10, an interferon-inducible gene (21), also have homology with pCEF-4. The predicted N terminus of pCEF-4 is highly hydrophobic and thus may function as signal sequence (22). The coding region of pCEF-4 is followed by $\approx$ 800 base pairs of nontranslated sequence containing a polyadenylation signal at the 3' end and two A+T-rich regions located 250 and 650 base pairs upstream from the poly(A) tail (underlined in Fig. 2). These regions consist of repeats of the octanucleotide TATTTATT or pentanucleotide ATTAA also found in sequences of lymphokines, cytokines, or protooncogenes (23).

Transient Expression of pCEF-4 Following Serum Stimulation. Thus far, the expression of pCEF-4 has been investigated in subconfluent cells growing in complete medium. The

⁸Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 48.

AGATCACAGC TCCACAAAC CTCAGCTCAG AAAACAAGCC AAACACTCCT AACC ATG AAC GGC AAG TTT GGA GCT GTC CTG GCC CTC CTC CTG GTT TCA GCT GCT CTG TCG CAA GGT AGG ACG 123
pCEF-4 MET ASN GLY LYS PHE GLY ALA VAL LEU ALA LEU LEU LEU VAL SER ALA ALA LEU SER GLN GLY ARG THR
CTAP-III ASN LEU ALA LYS GLY LYS GLU GLU SER LEU
*
CTG GTA AAG ATG GGG AAT GAG CTG CGG TGC CAG TGC ATT AGC ACT CAT TCT AAG TTC ATC CAC CCT AAA TCC ATT CAA GAT GTG AAG CTG ACG CCA AGC GGC CCC CAC 231
pCEF-4 LEU VAL LYS MET GLY ASN GLU LEU ARG CYS GLN CYS ILE SER THR HIS SER LYS PHE ILE HIS PRO LYS SER ILE GLN ASP VAL LYS LEU THR PRO SER GLY PRO HIS
CTAP-III ASP SER ASP LEU TYR ALA GLU LEU ARG CYS MET CYS ILE LYS THR THR SER GLY --- ILE HIS PRO LYS ASN ILE GLN SER LEU GLU VAL ILE GLY LYS GLY THR HIS
* * * * *
TGC AAG AAT GTT GAA ATC ATA GCT ACT CTA AAG GAT GGA AGA GAG GTG TGC TTG GAC CCC ACT GCT CCC TGG GTA CAG CTG ATC GTA AAG GCA CTT ATG GCC AAG GCT 339
pCEF-4 CYS LYS ASN VAL GLU ILE ILE ALA THR LEU LYS ASP GLY ARG GLU VAL CYS LEU ASP PRO THR ALA PRO TRP VAL GLN LEU ILE VAL LYS ALA LEU MET ALA LYS ALA
CTAP-III CYS ASN GLN VAL GLU VAL ILE ALA THR LEU LYS ASP GLY ARG LYS ILE CYS LEU ASP PRO ASP ALA PRO ARG ILE LYS LYS ILE VAL GLN LYS LYS LEU ALA GLY ASP
* * * * *
CAG CTC AAT TCT GAT GCA CCA CTG TGA GAAATTCG AGACAGGAAA AATCCTCAGA ACTGCTCTG ATTTCTACTG GGAGAAACAT CCGAAGAAGG CATCATGAAG CATTCCATCT TCCACCTTCC 466
pCEF-4 GLN LEU ASN SER ASP ALA PRO LEU OPAL
CTAP-III GLU SER ALA ASP
*
ACATCGGTGC CTCATGTTAA TTGACGATCC TTGTATCTAT TTATTTATTT ATTTAACTGC ATGTATTTAA AAAAGCTTTT CATATAGGTC AGTGCTGGG GATTCACTGT CCACTGAAAC TGAAGACACT GAATAGCAAA 466
AGGGCTGCT AGGGGAAATG AAGATCCCTT GGAAGCCACT TCAGTCAGAC ACAATCAGTT AAGTGCAATG CACTTACAGC ACAGCTGTT TGTATTAAGC CACTACTGTG TGCTATTACA GCAGCAAACT GGTAATTCCT 746
CCTGCTCCCC TGGAGTGCTC TAGTATGTTG TGTCACAAC AGTTTCCTAG TCAGAGTCAG CTCATGCCGA CTGCAGACTG TGTTTAAAAA CTCAGAAAT CTAACCTGCA GAATCTGTAA GACTGTGGGT TTGGTATTTA 886
TTATGATTC CATGGATTT ATAAATATAT TTATTACTA GTTCTATAC AAGATGGAAG GAGATGATAA CTGTGTAAAT TTCTACTGGA TTTCTGTTC TTAATGATGA ATACTTAAGA AACATTGACA TACCCATTAC 1026
TCTGCATAAG GACTTGTTTC TATGTCTAAT ACGTGAGTTA TTCAGCTAAT GGAAGAAAAA CTACAGCATG CACACAGCA ATTTGCTGTG GAGAATGTAA TTACCTCTTA CAATATATTA ATAAATATTT TATTTAAAAA 1166

FIG. 2. Sequence of the pCEF-4 cDNA insert. Both strands of the pCEF-4 cDNA insert were sequenced by the method of Maxam and Gilbert (11). The sequence of a 103-amino acid polypeptide is predicted from the nucleic acid sequence and is indicated underneath. Also included is the sequence of CTAP-III (16), a human platelet-associated factor showing extensive homology with the predicted pCEF-4 gene product. Identical amino acids shared by pCEF-4 and CTAP-III are marked by asterisks. The polyadenylation signal sequence AATAAA and two A+T-rich regions characteristic of lymphokine, cytokine, or protooncogene sequences (17) are underlined.

following experiments were developed to analyze the expression of pCEF-4 in cells entering the cell cycle from G₀. In the analysis of Fig. 3, normal uninfected CEF were grown to confluence and deprived of serum to establish quiescence. Serum was added to stimulate cell proliferation, and RNA was extracted at various times thereafter. Under these conditions the cells are capable of at least one more round of division after serum addition. RNA blot hybridization showed that pCEF-4 mRNA is 4-fold more abundant after 15 min (data not shown) and reached a fully induced level 20-fold greater than basal within 1–2 hr of serum addition (Fig. 3). By 8 hr the transcript began to decline and returned to the uninduced level within 16 hr of serum addition. Hence, the expression of pCEF-4 during normal cell proliferation is only transient. The 1.2-kb transcript accumulated upon serum stimulation of normal CEF pretreated with cycloheximide indicating that the expression of pCEF-4 under these conditions is also independent of protein synthesis. Additional rounds of cell division did not result in reexpression of the

sequence. Thus, the expression of pCEF-4 in normal cells may be restricted to reentry in the cell cycle, that is, to the G₀–G₁ transition and the first G₁ phase of the cycle. The expression of pCEF-4 was compared to that of other known cellular sequences induced by serum stimulation. In agreement with previous investigations (24, 25), c-myc mRNA was maximally induced within 1 hr of serum stimulation and decreased gradually thereafter (Fig. 3A). In the same RNA samples, c-fos mRNA also accumulated with the rapid and transient kinetics reported for other cell types (data not shown). Therefore, like other sequences activated following serum stimulation and, particularly, like the c-myc protooncogene, pCEF-4 mRNA is rapidly but only transiently expressed during reentry of quiescent CEF in the cell cycle.

Effect of pp60^{v-src} on Serum-Deprived Cells. The expression of pCEF-4 mRNA in serum-starved CEF infected with ts NY72-4 RSV showed that upon transfer to the permissive temperature, pCEF-4 was induced and, hence, did not require the presence of serum to accumulate upon pp60^{v-src} activation in transformed CEF (Fig. 3B). The accumulation was less rapid than shown in Fig. 1 (data not shown) and probably reflected differences in the metabolism of starved cells. We often observed elevated levels of c-myc transcripts at both permissive and nonpermissive temperatures (Fig. 3), but this level was variable in different cell batches.

Regulation of pCEF-4 Expression. The level of control of pCEF-4 expression was investigated by analysis of transcriptional activity in isolated nuclei (13) from normal actively growing CEF and SR-A RSV-transformed CEF. Fig. 4A shows a difference of 14-fold in labeling intensity between normal and transformed cells indicating that, under these conditions, an increased rate of transcription determines the accumulation of pCEF-4 mRNA in RSV-transformed CEF. The same type of analysis on cells infected with the ts mutant NY72-4 RSV (Fig. 4B) indicated that the relative rate of transcription increases upon temperature shift, but, in this case, the difference in labeling intensity was 2- to 4-fold. RNA gel blot analysis of the cytoplasmic RNA extracted from the same cells showed a difference of 12-fold in the steady-state level of pCEF-4 mRNA between cells transferred to the permissive temperature for 4 or 24 hr and cells maintained at the nonpermissive temperature. Thus, under these conditions, transcriptional activation may not be the only component of pCEF-4 expression. The transcriptional analysis of pCEF-4 in nuclei isolated from serum-stimulated cells also indicated an increased but insufficient level of

FIG. 3. Expression of pCEF-4 in uninfected CEF. (A) Uninfected CEF were grown to confluence, transferred to medium containing 10% (vol/vol) plasma (6) for 24 hr, and starved in plasma-free medium for an additional 24 hr. The quiescent cells were then stimulated with 10% (vol/vol) calf serum, and RNA was extracted at each of the indicated time points. After electrophoresis and transfer onto a nylon membrane, the RNA was hybridized sequentially to pCEF-4, v-myc, and GAPDH(H) RNA probes. (B) CEF infected with ts NY72-4 RSV were grown to confluence at the nonpermissive temperature of 41°C cultured in plasma-containing medium and starved in plasma-free medium as described above. Transformation and cell division were initiated by transferring the cells to the permissive temperature of 35°C. RNA was extracted at the indicated time points as well as at intermediary points not represented here. RNA samples in A and B were analyzed on the same blot.

FIG. 4. Transcription analysis of pCEF-4 in isolated nuclei. (A) Nuclei were isolated from actively growing, uninfected CEF (U) or wt SR-A RSV-transformed CEF (I). Run-off transcription analysis in isolated nuclei was carried out according to Linial *et al.* (13); labeled RNA was hybridized to pCEF-4, actin, tubulin, GAPDH(H), pGEM2 (the control plasmid), and *v-src* DNA, which had been denatured and transferred onto nitrocellulose. (B) Run-off transcription is analyzed in CEF infected with ts NY72-4 RSV at 41°C (nonpermissive temperature) or at 35°C (permissive temperature) after transfer for 20 min, 60 min, 4 hr, and 24 hr. (C) Normal quiescent CEF were stimulated by the addition of 10% (vol/vol) serum, and nuclei were isolated after 5 min, 15 min, 60 min, 2 hr, 5 hr, and 8 hr of stimulation. Run-off transcription analysis was as described above except that samples were also hybridized to *c-fos* and *v-myc* DNA.

transcription to account for the 20-fold accumulation of pCEF-4 during serum stimulation (Fig. 4C). In the same nuclei, *c-fos* transcription was increased >30-fold 5 min after serum addition with transient kinetics consistent with the accumulation of the *c-fos* transcript. The kinetics of pCEF-4 transcriptional activation was also transient and in agreement with the results depicted in Fig. 3. We observed no increase of *c-myc* transcription following serum stimulation in agreement with a report (15), suggesting that *c-myc* is regulated at a different level of expression. Finally, several genes including actin, tubulin, and GAPD did not vary in transcriptional activity following transformation by RSV or serum stimulation (Fig. 4).

Transcriptional activation results in the accumulation of pCEF-4 mRNA in RSV-transformed cells. However, this activation may be insufficient to account for the 20-fold increase in accumulation of pCEF-4 mRNA in infected CEF, particularly, in CEF infected with ts NY72-4 RSV (Fig. 4B). Therefore, we examined the stability of the pCEF-4 transcript in CEF infected with the ts virus at the permissive and nonpermissive temperatures. In the following analysis, NY72-4 RSV-infected CEF were maintained at the permissive temperature of 35°C or transferred to the nonpermissive temperature of 41°C for 24 hr. Then, actinomycin D was added to a final concentration of 2 μ g/ml, and RNA was prepared at various times thereafter. The analysis showed that pCEF-4 declines with a half-life of 15 min at the nonpermissive temperature and 45 min at the permissive temperature (Fig. 5). Therefore, the accumulation of pCEF-4

FIG. 5. Stability of the pCEF-4 transcript. NY72-4 RSV-infected CEF grown at 35°C were transferred to the nonpermissive temperature of 41°C (NP) for 24 hr or maintained at the permissive temperature of 35°C (P). Actinomycin D was added to a final concentration of 2 μ g/ml, and RNA was extracted at each of the indicated time points after addition. The level of pCEF-4 was probed by RNA gel blot hybridization, and RNA loading was monitored by rehybridization to a GAPDH(H) probe as described above. To obtain a signal of comparable intensity with pCEF-4 probe at the nonpermissive temperature, a 10-fold excess of RNA was loaded in each lane, and the RNA blot was exposed for twice as long.

at the permissive temperature is accounted for, partly, by stabilization of the mRNA.

DISCUSSION

Gene Expression in Transformed Cells. We describe the expression of a cellular mRNA sequence, pCEF-4, constitutively expressed to high levels in RSV-transformed CEF. The same cDNA clone and its corresponding mRNA have also been described by Sugano *et al.* (5). Thus, pCEF-4 represents a member of a group of genes whose expression is modified in transformed cells. The expression of pCEF-4 mRNA is not limited to RSV-infected CEF since quiescent CEF express the sequence following serum stimulation and reentry into the cell cycle (Fig. 3). The abundance of the transcript is comparable in serum-stimulated and in RSV-transformed CEF, but the expression of pCEF-4 is only transient in serum-stimulated cells. Continuously growing, uninfected cells do not express pCEF-4 to significant levels suggesting that its expression is restricted to the G_0 - G_1 transition and the first G_1 phase of the cell cycle and, hence, that it is tightly controlled in noninfected cells. The constitutive expression of pCEF-4 is consistent with the idea that an early mitogenic signal is continuously present in RSV-transformed CEF and/or that the normal mechanisms of down-regulation are bypassed or ignored upon expression of pp60^{v-src}.

Control of pCEF-4 Expression. A 20-fold difference in the abundance of the pCEF-4 transcript exists between normal and RSV-transformed CEF. In cells infected by wt SR-A RSV, most of the accumulation of the transcript is accounted for by an increase in the rate of transcription, as indicated by the results of run-off experiments (Fig. 4). The transfer of ts NY72-4 RSV-infected CEF to the permissive temperature also resulted in the transcriptional activation of pCEF-4. In this case, however, the activation did not appear to be sufficient to account for the increase in steady-state levels of the pCEF-4 transcript. Measurements of mRNA decay in actinomycin D-treated cells indicated that the pCEF-4 transcript is three times more stable at the permissive temperature of 35°C than at the nonpermissive temperature of 41°C (half-life of 45 min vs. 15 min respectively). While this difference in pCEF-4 half-life may be attributed to differences in the metabolism of cells grown at 35°C and 41°C, we found that pCEF-4 decays with a half-life of 30 min in wt SR-A RSV-transformed CEF at 41°C. These results suggest that the accumulation of pCEF-4 mRNA in RSV-transformed CEF is the result of transcriptional activation and mRNA stabiliza-

tion. The 3' end of the pCEF-4 transcript includes two A+U-rich regions consisting in repeats of the consensus UAUUUUAUU motif. This repeat sequence has been found in a variety of short-lived transcripts belonging to the lymphokine, cytokine, or protooncogene classes of proteins (23). Shaw and Kamen (17) demonstrated that the addition of an A+U-rich region to globin mRNA is sufficient to confer instability to this chimeric transcript. It is possible that stabilization of the pCEF-4 transcript during transformation by RSV is mediated through modification of factors (or modifications of their expression) interacting with the A+U-rich region. Meijlink *et al.* (26) reported that the deletion of the A+U-rich region of the murine *c-fos* is sufficient to activate the oncogenic potential of this protooncogene. The mechanism of regulation of this truncated *c-fos* gene is of obvious interest.

The difference in transcriptional ability observed in wt SR-A RSV and ts NY72-4 RSV-infected CEF suggests that the full transcriptional activation of pCEF-4 is a gradual process.

The Nature of the pCEF-4 Gene Product. The sequence of the pCEF-4 insert predicts a protein sequence homologous to CTAP-III and other biologically active peptides found in human platelets (16, 18–20). Although pleiotropic effects on cellular metabolism have been ascribed to this family of related polypeptides, including the stimulation of synthesis of DNA, glycosaminoglycan, hyaluronic acid as well as glycolysis, the significance of constitutive expression of pCEF-4 in fibroblasts remains to be determined. Fundamental differences also exist between the predicted structure of pCEF-4 and CTAP-III thus raising the possibility that they have distinct biochemical properties. A phenotypic analysis of the constitutive pCEF-4 expression is clearly necessary to assess the role(s) of this gene product during normal growth and transformation. The transient and tightly controlled expression of pCEF-4 following serum stimulation implies a regulatory function for this gene product.

Note Added in Proof. After this manuscript was completed, we learned of a cDNA clone homologous to pCEF-4 that was obtained from transformed hamster and human cells (27).

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