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Coding Sequence and Growth Regulation of the Human Vimentin Gene

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We have established the complete coding sequence of the human vimentin gene. It had 91% homology to the coding sequence of the Syrian hamster vimentin gene (Quax et al., Cell 35:215–223, 1983) and partial homology to several other sequences coding for intermediate filament proteins. The most striking difference between the Syrian hamster and human vimentin genes was in the 3′ untranslated region, which was considerably longer in the Syrian hamster. Using RNA blots and a human vimentin cDNA clone from an Okayama-Berg library, we have established that expression of the vimentin gene was growth regulated. The steady-state levels of cytoplasmic vimentin mRNA in 3T3 cells were increased by serum and platelet-derived growth factor, but not by epidermal growth factor, insulin, or platelet-poor plasma. The increase in expression of the vimentin gene that occurred when G0-phase cells were stimulated to proliferate was detected in six different cell types from four different species. The expression of the vimentin gene was also increased when HL60 cells were induced to differentiate by phorbol esters; it decreased when differentiation was induced by retinoic acid.

Vimentin is a protein belonging to the class of intermediate filaments of the cell, which includes other proteins such as keratins, desmin, neurofilaments, and nuclear lamins (for a review, see Lazarides [28]). It is present in the majority of cells of mesenchymal and nonmesenchymal origin, and its filaments are associated with both the nuclear and plasma membranes. Little is known about the fate of vimentin filaments during the cell cycle (28) and even less about the regulation of its expression by growth factors. We therefore thought it worthwhile to report our observation that vimentin mRNA levels are growth regulated. The observation originates in previous work from this laboratory, in which we had isolated, by differential screening of a cDNA library, five cDNA clones representing sequences that were inducible by serum (21). The cDNA clones were originally isolated from a cDNA library of *ts*13 cells, a G1-specific temperature-sensitive mutant derived from Syrian hamster BHK cells (54). Because the inserts in this library were unusually short, we screened for longer inserts in a human cDNA library from simian virus 40 (SV40)-transformed fibroblasts (41). When one of the original cDNA clones, called p4F1, was used for screening, it yielded more than 100 clones from the Okayama-Berg and other human cDNA libraries. One of these human cDNA clones, pL3-A7A, was sequenced and shown to be a partial-length cDNA clone of human vimentin with 91% homology to the coding sequence of the Syrian hamster vimentin gene (42).

Using this cDNA clone, we isolated from a human genomic library a clone that contains the remainder of the coding sequence as well as ~1.5 kilobases (kb) of 5′ flanking sequences. The purpose of the present communication is to report the complete coding sequence of the human vimentin gene and the most proximate 5′-flanking region and to confirm and extend our observation that the steady-state levels of vimentin mRNA are growth regulated.

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MATERIALS AND METHODS

Cell cultures. BALB/c 3T3 fibroblasts were plated in Dulbecco modified minimal essential medium supplemented with 10% fetal calf serum (FCS), L-glutamine, and antibiotics. When cells were semiconfluent, high-serum medium was removed, the cells were washed, and medium containing 1% FCS was added to the cultures. Five days later the cells were quiescent (less than 1% of the cells incorporated [^{3}H]thymidine following a 24-h incubation period). The cells were then treated with either fresh 10% FCS, platelet-derived growth factor (PDGF; Collaborative Research), epidermal growth factor (EGF; Collaborative Research), insulin (Sigma Chemical Co.), or platelet-poor plasma (PPP) prepared from human blood as described by Ross et al. (46). Except for FCS, growth factors were added to the conditioned low-serum medium. Eight hours after addition of growth factors, the cells were washed with ice-cold phosphate-buffered saline and harvested with a rubber policeman, and total cytoplasmic RNA was extracted. HL-60 cells were grown as described by Rovera et al. (47).

Screening of human cDNA libraries. We obtained four different cDNA libraries as generous gifts. Two human cDNA libraries were obtained from Okayama and Berg, the first from SV40-transformed human fibroblasts (GM637) and the second from normal human fibroblasts. Both libraries are in the pcD vector (41). We obtained a third cDNA library (a generous gift from G. Rovera) established with polyadenylated [poly(A)$^+$] RNA from the Jurkat cell line (T cell lymphoma line, human) cloned in pUC9. The fourth cDNA library was obtained from the DNAX Research Institute of Molecular and Cellular Biology. It is a pcD cDNA library prepared from poly(A)$^+$ RNA from concanavalin A-stimulated human T lymphocytes (29). The plasmid DNA of the different cDNA libraries was transfected into *Escherichia coli* JM109 or HB101 by the high-efficiency transformation technique described by Hanahan (18). Approximately

800,000 transformants per library were grown directly on Millipore filters. The libraries were screened with the high-density colony-screening method of Hanahan and Meselson (19). The probe used for screening was the original hamster 4F1 insert (21). The insert was separated by electrophoresis through a 4% polyacrylamide gel and recovered by electroelution into a dialysis bag. The hamster 4F1 DNA fragment was labeled by nick translation with [^{32}P]dCTP to a specific activity of 2×10^8 to 4×10^8 cpm/μg under the conditions described by Rigby et al. (44) or by the oligonucleotide labeling method, also with [^{32}P]dCTP, to a specific activity of 2×10^9 cpm/μg as described by Feinberg and Vogelstein (11).

Screening of the human genomic library. The human genomic library (a kind gift from Tom Maniatis, Harvard University) was prepared from liver DNA cloned into phage Charon 4A. For screening, a human vimentin cDNA isolated from the Okayama-Berg library (see above) was used.

DNA sequencing. DNA sequencing was done by the method of Sanger et al. (50) after cloning in M13 (49), M13mp8, M13mp9 (37), and M13mp19 (40) with the use of a universal M13 sequence primer, TCCCAGTCACGACGT (New England Biolabs). The sequence gels were 6% acrylamide and 8 M urea in Tris-borate buffer. The genomic clone was sequenced both by the method of Sanger et al. (50) and by that of Maxam and Gilbert (33). The Genebank from Palo Alto, Calif., was searched for sequence homologies.

RNA blots. Total cytoplasmic RNA was extracted as previously described (21). Blotting was carried out as described by Thomas (55). Hybridization and autoradiography were done by standard procedures (55). The human vimentin probe (4F1) was nick translated (44) to 4×10^8 to 5×10^8 cpm/μg. The various lanes in Fig. 2, 3, and 4 were normalized by the use of a non-growth-regulated gene as previously described (23). The intensity of the bands was determined with a soft-laser densitometer within its linear range, as previously described (23).

RESULTS

Coding sequence of the human vimentin gene. In a previous paper (21), we described several cDNA clones whose cognate RNAs were markedly increased when G0 cells were stimulated to proliferate by serum. One of these clones was called 4F1. Using the original Syrian hamster 4F1 cDNA clone as the hybridizing probe, we isolated the corresponding human cDNA from an Okayama-Berg library (41). More than 100 clones were isolated from this and other libraries (see Materials and Methods), with inserts of different lengths, but none of them longer than ~1,000 bp. Since the RNA recognized by 4F1 had an estimated length of 1.8 to 1.9 kb on RNA blots, even the longer inserts represented only partial-length cDNA. The clone with the longest insert, pL3-A7A, was sequenced. Comparison with other sequences in the Genebank showed it to be a cDNA clone of human vimentin. Despite screening more than 100 clones, we could not obtain a full-length cDNA clone. Quax-Jeuken et al. (43) also could not obtain a full-length cDNA clone from a Syrian hamster library and had to complete the coding sequence by isolating the genomic clone. Perhaps vimentin RNA has some structural features that make it very difficult to obtain a full-length cDNA by the commonly used procedures. We emphasize that we screened four different human cDNA libraries without finding a single clone longer than 1,000 bp. One of the libraries (DNAX) yielded 75 vimentin clones, but here again, none of them was longer than 1,000 bp.

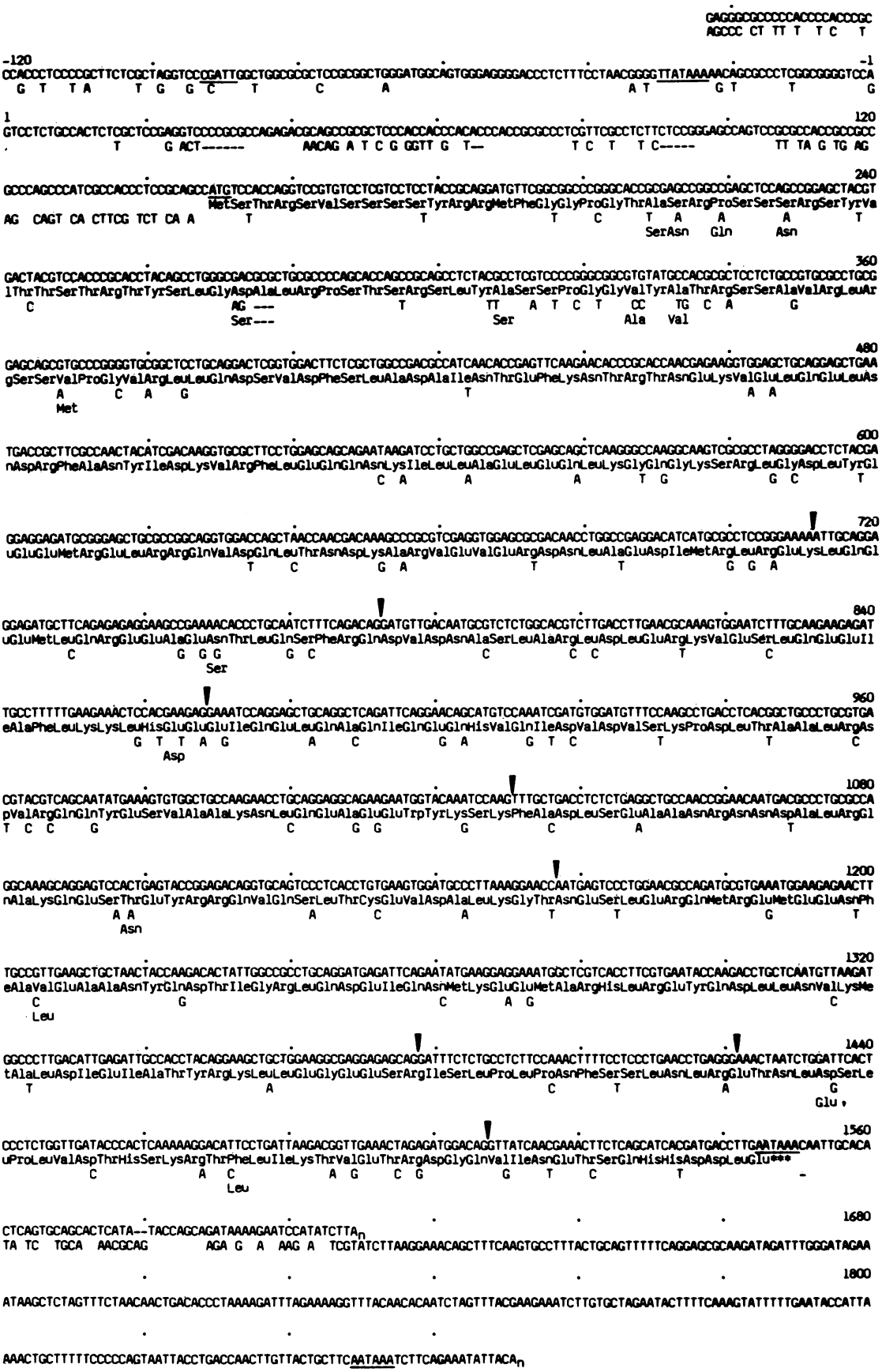
To complete the coding sequence of the human vimentin gene, we followed the example of Quax-Jeuken et al. (43) and isolated clones from a human genomic library. Several clones were isolated, but the one that is relevant to the present paper is clone λ4F1cl37, which allowed us to complete the coding sequence of human vimentin because it contained exons and introns from the 5′ half of the gene plus 1.5 kb of 5′-flanking sequences. Its coding sequence overlapped the sequence of pL3-A7A.

By combining the two sequences, we obtained the complete coding sequence of the human vimentin gene (Fig. 1) and compared it with the coding sequence of the Syrian hamster vimentin gene (42). The homology throughout the coding sequence was 91%. The homology was very high (~81%) in the 5′-flanking sequence, but some divergence was noted in the 5′ untranslated region (63% homology).

There was also a striking difference between the Syrian hamster cDNA sequence (43) and the human cDNA sequence in the length of the 3′ untranslated region, which was 296 bp in the Syrian hamster and only 57 bp in the human gene. As in the Syrian hamster (43), a canonic poly(A) signal coincided with the termination codon. We cannot say at this time whether this unusually located signal is the actual polyadenylation signal or whether the signal is contained in an imperfect sequence about 60 bp downstream from the termination codon. Figure 1 also shows the amino acid sequence of human vimentin deduced from the nucleotide sequence. The putative human sequence had 98% homology to the putative amino acid sequence of Syrian hamster vimentin.

Growth regulation of the vimentin gene. Using the human cDNA clone described above, we show here that the expression of the vimentin gene is regulated by growth factors. We use the term expression in one of its accepted usages, i.e., as steady-state levels of cytoplasmic mRNA. Previous experiments had shown that the RNA recognized by the 4F1 probe (Syrian hamster vimentin cDNA) increased markedly when *ts*13 cells (derived from Syrian hamster BHK cells) were stimulated by serum (21) or when human peripheral blood lymphocytes were stimulated by the mitogen phytohemagglutinin (PHA) (23). Levels of 4F1 RNA were also increased in serum-stimulated human diploid fibroblasts (44a), Swiss 3T3 cells (45), and rat fibroblasts (unpublished data). In those reports, however, 4F1 was not recognized as vimentin. We therefore thought that those reports should be confirmed and extended to identify the growth factors that regulate the expression of the vimentin gene.

Figure 2 shows an RNA blot with RNAs from BALB/c 3T3 cells, either quiescent (lane a) or 8 h after stimulation by different growth factors. Vimentin expression was increased by serum (lane b) and PDGF (lane c) but not by PPP, EGF, or insulin (lanes d, e, and f, respectively). In this respect, vimentin behaved like the KC-1 cDNA clone of 3T3 cells (7), which was also inducible by PDGF but not by EGF or insulin. Other growth-regulated genes respond to both PDGF and EGF, for instance, the proto-oncogenes c-*fos* and c-*myc* (3, 17, 24, 38), 2A9 (B. Calabretta, R. Battini, L. Kaczmarek, K. J. deRiel, and R. Baserga, J. Biol. Chem., in press), and 2F1 (unpublished data), which are two of the serum-inducible cDNA clones identified with 4F1 (21). Densitometric measurements showed a 10-fold increase in vimentin mRNA levels after serum stimulation and an 8-fold increase after PDGF treatment. PPP and insulin actually caused a small decrease in the signal.



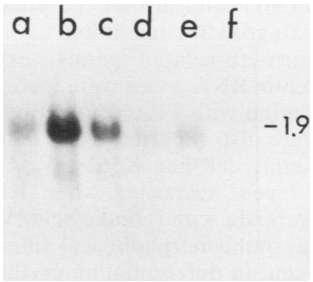


FIG. 2. Human cDNA clone of vimentin was hybridized to RNAs (20 μg/lane) obtained from BALB/c 3T3 mouse fibroblasts treated for 8 h with different growth factors (see Materials and Methods). Lanes: a, RNA from quiescent cells; b through f, RNA from cells treated with fresh medium containing 10% FCS (b), PDGF (2 U/ml) (c), PPP (5%) (d), EGF (20 ng/ml) (e), or insulin (10 μg/ml) (f). Fragment size is indicated (in kilobases).

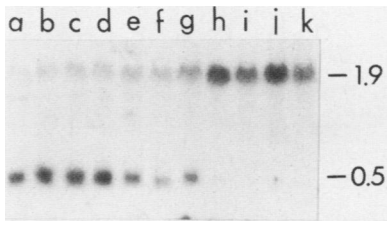


FIG. 3. Induction of vimentin in HL-60 cells by phorbol esters. Exponentially growing HL-60 cells were treated with TPA (20 ng/ml). At various intervals after induction with TPA, the cells were harvested, RNA was extracted, and RNA blots were prepared as described in Materials and Methods. The blots were hybridized with both the vimentin and the histone H3 probes. Lanes: Cells taken at 0 min (a), 15 min (b), 30 min (c), 1 h (d), 2 h (e), 4 h (f), 8 h (g), 24 h (h), 48 h (i), 72 h (j), and 190 h (k) after TPA treatment. The upper band (1.9 kb) is vimentin RNA, and the lower band (0.5 kb) is histone H3 mRNA.

It seems therefore that serum (or PDGF or PHA, but not EGF) can increase vimentin mRNA levels in six different cell types from four different species.

Expression of vimentin in HL-60 cells. The human promyelocytic leukemia line HL60, established by Collins et al. (8), consists predominantly of promyelocytes (85%) and a small fraction of more mature myeloid elements that can be markedly increased by treatment with dimethyl sulfoxide, retinoic acid, vitamin D, and several other compounds (for a review, see Koeffler [25]). 12-*O*-Tetradecanoyl-phorbol 13-acetate (TPA) induces terminal differentiation in HL-60 cells along the monocyte-macrophage pathway (47), while retinoic acid induces differentiation along the granulocyte pathway (for a review, see Koeffler [25]). Exponentially growing HL-60 cells were treated with TPA (20 ng/ml), and cytoplasmic RNA was extracted at various times (Fig. 3). The RNA blots were hybridized simultaneously to the vimentin and histone H3 probes (6, 30). As the cells differentiated (and therefore stopped proliferating), the amount of histone H3 mRNA rapidly decreased (Fig. 3). On the contrary, the amount of vimentin RNA increased to a maximum at 72 h after TPA addition. By densitometric measurement, the increase in vimentin mRNA levels reached a peak of eightfold the amount at zero time. A similar experiment was carried out with retinoic acid as the inducer of differentiation. Retinoic acid had the opposite effect of TPA, causing a rapid decrease in the levels of vimentin RNA which, at 70 h, was about 1/6 the level at zero time (Fig. 4).

The data thus far marshalled on the regulation of vimentin expression by growth factors and growth-regulated conditions can be summarized as follows. Vimentin RNA levels are increased by PDGF (this paper), serum in several cell types (21, 44a, 45; this paper), PHA in human peripheral blood mononuclear cells (23), TPA in HL-60 cells (this paper), serum in the presence of cycloheximide (45), and serum in G1-specific temperature-sensitive mutants at the restrictive temperature (21). Vimentin RNA levels are not increased by EGF, insulin, or PPP (this paper), adenovirus infection (30), or PHA in purified T lymphocytes (23). Vimentin RNA is overexpressed in human leukemia cells (5) and is decreased by retinoic acid in HL-60 cells (this paper).

DISCUSSION

We report here the complete coding sequence of the human vimentin gene and the confirmation and extension of previous results indicating that the steady-state mRNA levels of vimentin are regulated by factors that regulate cell growth.

When the human sequence was compared with the coding sequence of Syrian hamster vimentin, the following features were noted: (i) the homology in the coding sequences was 91%; (ii) the promoter region was also strongly homologous, but there was much less homology in the 5′ untranslated region; and (iii) the 3′ untranslated region was shorter in the human cDNA clone (57 nucleotides versus 296 nucleotides in the Syrian hamster cDNA [42]). A canonical polyadenylation signal coincided in the human sequence with the termination codon. Although this location was highly unusual, it is not without precedent. It has been reported that the mouse thymidylate synthase gene has no 3′ untranslated region and that the polyadenylation signal is embedded in the coding sequence (L. F. Johnson, personal communication). An imperfect polyadenylation signal was found downstream from the termination codon, about the same distance from the termination codon as in the Syrian hamster sequence (42). The chicken vimentin mRNA has at least three polyadenylation signals. The human vimentin sequence, like the Syrian hamster sequence (42), shows considerable homology to the sequences of other intermediate filament genes (nucleotide sequence or deduced amino acid sequence), such as desmin (12), keratins (22, 32, 52), and the lamins of the nuclear envelope (35). Our probe, however, gave a single RNA band in RNA blots at high stringency (Fig. 2 through 4 and references listed at the end of Results). This is at variance with chicken vimentin, in which two

FIG. 1. Nucleotide sequence of the coding regions and 5′-flanking sequences of the human vimentin gene. The sequence of the human vimentin gene is compared with the sequence of the Syrian hamster vimentin gene (42) (lower row; only differing nucleotides are indicated). The CAAT box, TATA box, cap site, AUG codon, and stop codon are underlined. The numbers refer to the human gene sequence. Note also the homologies and differences in the 5′-flanking and untranslated regions. Arrowheads indicate introns. The figure also shows a comparison of the amino acid sequences of human and Syrian hamster vimentin deduced from the nucleotide sequences. Only the divergent amino acids are given for the Syrian hamster sequence.

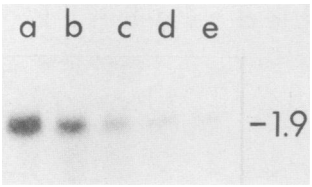


FIG. 4. Induction of vimentin in HL-60 cells by retinoic acid. Same conditions as for Fig. 3, except that retinoic acid (10^{-6}M) was used to induce differentiation. Lanes a, b, c, d, and e, cells harvested 0, 4, 8, 30, and 70 h after retinoic acid treatment, respectively. The 1.9-kb vimentin RNA band is indicated.

mRNA species have been found (58), but in agreement with Syrian hamster vimentin, which is a single gene whose product is a single RNA species (42). Incidentally, Southern blot analysis of human genomic DNA also indicated a single gene for human vimentin (data not shown). Also intriguing, but at the moment highly speculative, are the homologies reported to exist between cytoskeletal proteins and growth-related genes such as c-*myc*, Rous sarcoma virus, and adenovirus proteins (10).

Vimentin mRNA levels were increased by serum and PDGF, but not by EGF, insulin or PPP. In agreement with previous and unpublished data, the vimentin gene was inducible by mitogens in at least six different cell types from four different species. Most of these cell lines were fibroblasts or fibroblastlike cells, but vimentin was also inducible by PHA in peripheral blood mononuclear cells (23). Indeed, in the latter cells, it was induced very early after stimulation, the appearance of its RNA preceding even the appearance of c-*myc* RNA. Although the time course of induction is somewhat more sluggish in fibroblasts, vimentin mRNA is induced by serum in Swiss 3T3 cells in the presence of concentrations of cycloheximide that completely inhibit protein synthesis (45). This indicates that its induction, like that of c-*myc* (24), KC-1 and JE-3 (7), p53, 2F1, and 2A9 (45), and several other growth-regulated genes (27) does not require previous new protein synthesis, i.e., the products of other growth factor-inducible genes. Why so many genes are induced as primary responders to growth factors is a puzzle for which there is presently no clue. By runoff transcription assay, serum regulates the expression of the vimentin gene in Swiss 3T3 cells at the transcriptional level (unpublished data).

A very interesting feature of vimentin RNA is that it is increased in PHA-stimulated peripheral blood mononuclear cells but is not expressed in purified human T lymphocytes exposed to PHA (23). Under these conditions (i.e., without macrophages or interleukin-2), T lymphocytes grow in size, accumulate RNA, and develop nucleoli but do not enter S phase (31, 36). This suggests that the increase in the expression of vimentin is related more to the induction of cellular DNA synthesis (or mitosis) than to the growth in size of the cell that almost invariably accompanies the mitogenic process (for a review, see Baserga [1]). In this respect, it should be noted that vimentin has been associated with the mitotic apparatus (15). The amount of vimentin synthesis was also found to be high in sparse monolayer cultures and low in dense suspension cultures (2) and to increase in rapidly growing mesothelial cells (9). The findings that vimentin expression is increased in serum-stimulated G1-specific temperature-sensitive mutants of the cell cycle at the nonpermissive temperature (21), but not after adenovirus stimulation of DNA synthesis (30), confirm that the vimentin gene is an early responder to growth stimulation.

Like other growth-related genes, especially proto-oncogenes, vimentin RNA levels were increased after induction of differentiation with TPA. Vimentin biosynthesis and accumulation were also reported to be induced by TPA in the human leukemic cell line K562 (51). On the contrary, vimentin RNA levels decreased when HL-60 cells were induced to differentiate with retinoic acid. Vimentin synthesis also decreases in the morphological differentiation of 3T3 adipocytes (53) and in differentiating erythroleukemia cells (39). This ambivalence of vimentin expression, by which its RNA levels increase both after stimulation of cellular proliferation and after induction of differentiation, should not be surprising. There are very high levels of $pp60^{c\text{-}src}$ in postmitotic neurons (4) and in blood platelets (14), and nerve growth factor (or EGF) can induce c-*fos* and differentiation in PC12 cells (16, 26). Calcium, which is required for cell proliferation in fibroblasts (20, 34, 48) induces differentiation in keratinocytes (57). This ambivalence of growth factors and growth factor-related genes is perhaps best illustrated by TGF-β, which is at the same time a stimulator and an inhibitor of cell proliferation (56). Our previous finding (5) that vimentin 4F1 was overexpressed in human myeloid leukemias is consistent with another observation, that, at least by immunofluorescence, vimentin is particularly abundant in human non-Hodgkin's lymphomas (13).

In summary, our data provide the full coding sequence of human vimentin and clearly indicate that the vimentin gene is growth regulated in a variety of cell types. Especially interesting is the demonstration that the vimentin mRNA levels are increased by PDGF but not by EGF or insulin, indicating a specificity of response to certain growth factors. The changes occurring when HL-60 cells were induced to differentiate are also important, suggesting that the gene is subjected to both positive and negative regulation by factors that modify the proliferative behavior of cells.

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