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Growth-Regulated Expression of Vimentin in Hamster Fibroblasts Is a Result of Increased Transcription

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Abstract We have previously shown that vimentin is a growth-regulated gene whose mRNA levels increase after serum stimulation of quiescent hamster fibroblasts. In this study, the control of the growth-regulated expression of vimentin was determined in ts13 cells induced to proliferate by serum. Both transcriptional and post-transcriptional mechanisms of regulation were examined by determining transcriptional rates, cytoplasmic transcript abundance, transcript stability, and protein abundance. We observed a fourfold increase in vimentin transcripts in the cytoplasm of serum-stimulated ts13 cells. Since transcripts are stable in both quiescent and stimulated cells, this induction of vimentin expression is a result of a fivefold increase in vimentin-specific transcriptional activity. As a result of this increased transcript availability, the abundance of polymerized vimentin protein increased following serum stimulation of quiescent fibroblasts. Overall, the induction of vimentin expression in fibroblasts by serum is a consequence of increased vimentin-specific transcriptional activity. The significance of this with regard to cytoskeletal organization and cell division is discussed.

Key words: hamster, fibroblasts, vimentin, transcription

Vimentin, a class III intermediate filament (IF), is a component of the cellular cytoskeleton. It is expressed in cells of mesenchymal origin and in most cultured cells in a developmental and tissue-specific fashion. The regulation of vimentin expression has been studied in a number of systems, including fibroblasts, lymphocytes, leukemic cell lines, and myeloma cells [Hirschhorn et al., 1984; Calabretta et al., 1985; Ferrari et al., 1986; Gibson et al., 1986; Geisse and Traub, 1986; Rittling et al., 1989]. We have demonstrated that the vimentin gene is a growth-regulated gene [Hirschhorn et al., 1984] as defined by an increase in transcript abundance in the cytoplasm when quiescent fibroblasts or lymphocytes are stimulated to proliferate with serum or purified mitogens [Ferrari et al., 1986; Gibson et al., 1986]. The expression of vimentin is also induced when HL-60 cells, mouse myeloma cells, and fibroblasts are exposed to phorbol esters [Ferrari et al., 1986; Geisse and Traub, 1986; Rittling et al., 1989]. From in vitro deletion analysis, it has been determined that the activation of the vimentin promoter by serum and growth factors is mediated by an intermediate filament consensus element [Rittling and Baserga, 1987; Quax et al., 1983, 1985]. In separate studies the synthetic rate of vimentin protein was shown to increase in fibroblasts and lymphocytes induced to proliferate [Low et al., 1985; Podolin and Prystowsky, 1991].

In normal cells, the cytoskeleton has been shown to extend from the nuclear envelope to the plasma membrane [Lazarides, 1980; Aubin et al., 1980; Goldman et al., 1985; Georgatos and Blobel, 1987]. Because of this physical link, there has been speculation that, in addition to a structural function, vimentin may provide a more interactive role in cellular events leading to growth and division. First, vimentin has been shown to bind synthetic and natural nucleic acids, telomeric sequences, core histones, and membrane phospholipids in vitro [Traub et al., 1983, 1987; Kuehn et al., 1987; Shoeman et al., 1988]. This information and immunofluorescent data demonstrating the IF array throughout the cytoplasm [Goldman et al., 1985] sug-

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gest that IFs may participate in transmitting extracellular signals from the plasma membrane to the nucleus. Secondly, vimentin exhibits structural similarity to the DNA binding-leucine zipper domain of known cellular oncogenes and transcription factors, such as c-*fos,* c-*jun,* c-*dbl,* CREB, and tpr [Ron et al., 1988; Capetanaki et al., 1990]. Thirdly, vimentin is phosphorylated by a number of protein kinases in a cell cycle fashion, as are other growth-regulated genes. In particular, it was shown that MPF/p34^{cdc2} kinase, the cell cycle regulator, phosphorylates vimentin prior to mitosis [Chou et al., 1990] at a time that correlates with the collapse of the IF network [Rosevear et al., 1990]. Together, this information suggests a role for vimentin during cell growth.

The studies presented here examine the in vivo regulation of vimentin transcript and protein accumulation in the same cell system. Specifically, we examined both transcriptional and post-transcriptional mechanisms of regulation of vimentin expression in hamster fibroblasts stimulated to proliferate by determining transcriptional rates, cytoplasmic transcript abundance, transcript stability, and protein abundance. We observe that the extent of the increase in vimentin protein expression following serum stimulation of quiescent fibroblasts was a direct consequence of increased vimentin-specific transcriptional activity.

METHODS

Cells and Culture Conditions

The ts13 cell line was derived from Syrian *hamster* kidney cells [Talavera and Basilico, 1977]. The cells were cultured in Dulbecco's modified Eagle's medium (1,000 mg/L glucose (DMEM); GIBCO, Gaithersburg, MD) supplemented with 10% (v/v) calf serum (10% CS; GIBCO, Gaithersburg, MD), 200 mg/L penicillin, 80 mg/L streptomycin, and 2 mM glutamine (Sigma Chemical Co., St. Louis, MO) in a humidified incubator at 34°C and 10% CO_2. Cell cultures were passaged when the cell density approached 75% confluence in a 100 mm tissue culture plate.

Synchronized quiescent cell populations were obtained by culturing cells to approximately 80% confluence, and replacing the growth media with DMEM containing 0.5% (v/v) calf serum (0.5% CS) for 48 h in a humidified incubator at 34°C and 10% CO_2. Quiescent cells were stimulated to re-enter the cell cycle by replacing the medium with DMEM (4,500 mg/L glucose; GIBCO, Gaithersburg, MD) containing 15% (v/v) fetal bovine serum (15% FBS; GIBCO, Gaithersburg, MD) followed by incubation at 34°C in 10% CO_2.

To score the percentage of the cell population undergoing DNA synthesis, cells were incubated either continuously in the presence of 1.5 μCi/ml of [^{3}H]-thymidine (20 Ci/mmole; ICN Biomedical, Inc., Irvine, CA) or pulsed for 1 h in the presence of 2.5 μCi/ml of [^{3}H]-thymidine. After the appropriate labeling period, the cells were rinsed three times with 1× PBS and fixed to the dish with −20°C methanol/acetone (1:1) for 10 min. After the cells were air dried overnight, they were processed for autoradiography. Nuclei were counterstained with Giemsa before scoring for the percentage of nuclei that incorporated [^{3}H]-thymidine [Hirschhorn et al., 1984].

RNA Isolation and Northern Blot Hybridization

Cytoplasmic RNA was isolated from cells following lysis with 150 mM NaCl, 10 mM Tris-HCl, pH 7.9 at 4°C, 1.5 mM $MgCl_2$, 0.65% Nonidet P-40, 10 mM vanadyl-ribonucleoside complex (New England Biolabs, MA) [Hirschhorn et al., 1984]. RNA (15 μg) was dried under vacuum and resuspended in 20 μl of loading buffer. RNA samples were size-fractionated by formaldehyde-agarose (1%) gel electrophoresis at constant current (20–60 mA) and were transferred by capillary action to reinforced nitrocellulose (Nitroplus, 0.45 μm; Micron Separations Inc., Westboro, MA). To monitor loading equivalence and efficiency of RNA transfer to nitrocellulose, either 40 μg/ml ethidium bromide was incorporated into the loading buffer [Rosen and Vila-Komaroff, 1989] or the gel was placed on a thin layer chromatography plate. The RNAs were detected by ultraviolet light. Northern blots were prehybridized at 42°C, hybridized at 42°C to nick-translated cDNAs, and washed three times at room temperature in 2× SSC, pH 7.0, 0.1% SDS and then washed twice at 52°C in 0.1× SSC, 0.1% SDS [Maniatis et al., 1982]. Blots were exposed to Kodak XAR5 x-ray film at −80°C with intensifier screens and processed for autoradiography. Signal intensities were assessed by densitometric scanning of each signal. The following cDNAs were used for northern analysis or in nuclear transcriptional assays: the plasmid hp4F1 is a 1.5 kb human vimentin cDNA in an Okyama/Berg vector [Ferrari et al., 1986] and was provided by Dr. Renato Baserga (Temple University, Philadelphia, PA). rDNA is a mouse

18s ribosomal RNA inserted in a pBR322 vector and was provided by Dr. Dylan Edwards [Edwards et al., 1985]. pSVc-*myc* 1 contains the full length murine c-*myc* locus in the pSV2 vector [Land et al., 1983] and was obtained from ATCC (Rockville, MD) (ATCC #41029). pRSVneo contains a Hind III–BamH I fragment from pSV2neo with neo coding and SV40 mRNA processing signals [Gorman et al., 1983].

Nuclear Transcriptional Assays

Nuclei for nuclear transcriptional assays were isolated from 5×10^7 cells and stored in liquid nitrogen in 50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, and 100 mM EDTA [Greenberg and Ziff, 1984]. Transcripts that were in the process of being synthesized at the time of isolation were elongated in transcription buffer (25 mM Tris-HCl, pH 8.0, 12.5 mM $MgCl_2$, 750 mM KCl, 1.25 mM each ATP, GTP, and CTP) containing 250 μCi [alpha-^{32}P]UTP (3,000 Ci/mmole; ICN Biomedicals, Inc., Irvine, CA). Purified radiolabeled transcripts (1×10^7 dpm nuclear transcripts) were isolated and hybridized to 10 μg denatured cDNAs that were immobilized on nitrocellulose. After hybridization at 65°C for 36 h, blots were washed twice for 10 min at room temperature in 2× SSC, 0.1% SDS and once for 30 min at 60°C in 0.1× SSC, 0.1% SDS. Blots were air dried and exposed to Kodak XAR5 X-ray film for up to 10 days. Plasmid DNA prepared in the absence of RNase A treatment [Ish-Horwicz and Burke, 1981] was used for generating slot blots for nuclear transcriptional assays. For each slot, 10 μg of plasmid DNA were diluted into 100 μl of TE buffer, pH 7.5, and incubated with 10 μl of 3 M NaOH at 65°C for 1 h. The samples were placed on ice and mixed with 110 μl of 2 M ammonium acetate, pH 7.0. After wetting a piece of nitrocellulose in 10× SSC and assembling the slot blot apparatus by BRL, 220 μl of a sample were loaded per slot. Each slot was rinsed with 500 μl of 10× SSC. The slot blot was air dried and baked under vacuum for 2 h at 80°C.

Protein Isolation and Immunoblot Analysis

Proteins enriched for intermediate filaments were isolated from cells (2.5×10^6) which were rinsed with ice-cold PBS and lysed directly on the plate for 3–5 min with lysis buffer (140 mM NaCl, 1% Triton X-100, 5 mM EDTA, 10 mM Tris-HCl, pH 7.6, 1 mM phenylmethysulfonyl fluoride) [Franke et al., 1981]. Lysis buffer was removed and cell residues incubated for 30 min in 1.5 M KCl, 0.5% Triton X-100, 5 mM EDTA, 10 mM Tris-HCL, pH 7.6, 1 mM phenylmethysulfonyl fluoride. The residual cell material was scraped from the tissue culture plate and collected by centrifugation for 20 minutes at 3,500*g*. The pellet was washed twice by resuspension in PBS and centrifugation, and the final pellet was stored at −80°C. Prior to electrophoresis, samples were solubilized by sonication in 100 μl sample buffer (10 mM $NaPO_4$, pH 7.4, 5% SDS, 0.1 mM phenylmethylsulfonyl fluoride, 10% beta-mercaptoethanol, 5% glycerol).

Protein samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [Towbin et al., 1979]. Briefly, 5 μl of each protein sample were mixed with 1 μl of bromophenol blue tracking dye and 1 μl of beta-mercaptoethanol. Samples were then boiled and separated by 10% SDS-PAGE [Laemmli, 1970]. The proteins were transferred electrophoretically from the gel to Immobilon-P membrane (Millipore Corp., Bedford, MA). Blots were blocked in 5% non-fat dry milk in PBS for 2.5 h at room temperature. Blots were immunostained by incubation for 2 h at room temperature with a 1:100 dilution of polyclonal goat anti-vimentin antibody (Sigma Chemical Co., St. Louis, MO), washed twice in PBS and once in 5% milk/PBS, followed by a second incubation for 1 h at room temperature with a 1:1,000 dilution of an anti-goat IgG alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO). Blots were washed and protein detected by the alkaline phosphatase chromogenic detection system (BCIP and NBT from BRL, Gaithersburg, MD).

RESULTS

The expression of vimentin transcripts has been previously defined as growth regulated in fibroblasts and lymphocytes [Hirschhorn et al., 1984; Calabretta et al., 1985]. Vimentin cytoplasmic transcript abundance was shown to increase in the G_1 phase of the cell cycle following a mitogenic stimulation of fibroblasts [Hirschhorn et al., 1984]. Although the transient increase of vimentin transcripts following mitogenic stimulation has been amply established, the cellular mechanisms controlling this event have not yet been determined. Transcriptional and post-transcriptional mechanisms affecting the expression of vimentin were examined by determining transcriptional activities of the endogenous pro-

Fig. 1. Abundance of vimentin cytoplasmic transcripts in fibroblasts during serum deprivation and following serum stimulation. For serum deprivation experiments, exponentially growing ts13 cell were rinsed twice with Hank's balanced salt solution and incubated for 24, 48, and 72 h in DMEM supplemented with 0.5% CS. For serum stimulation experiments, quiescent (48 h serum-deprived cells) ts13 cells were stimulated with DMEM supplemented with 15% FBS for 6, 16, and 24 h. Cytoplasmic RNA was isolated from different cell populations at the indicated times in the presence of vanadyl-ribonucleoside complex. RNA (15 μg) was size-fractionated by formaldehyde-agarose gel electrophoresis and transferred to nitrocellullose. Loading equivalence was monitored as described in Methods. Autoradiographs shown were obtained after hybridizing the filters with nick-translated vimentin cDNA insert. **Lane A:** RNA isolated from exponentially growing cells. **Lanes B,C,D:** RNA isolated from cells incubated in low serum media for 24, 48, and 72 h, respectively. **Lanes E,F,G:** RNA isolated from cells 6, 16, and 24 h following serum stimulation. These data are representative of 3 similar experiments.

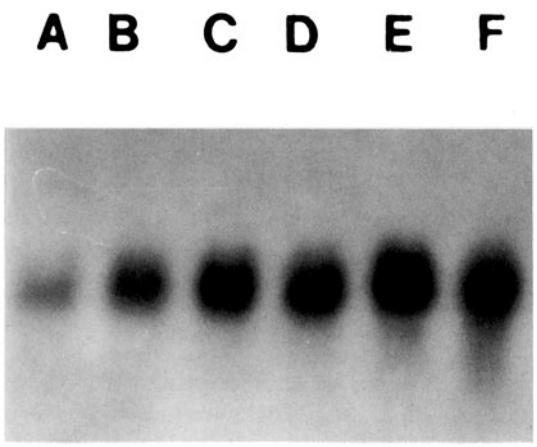

Fig. 2. Vimentin cytoplasmic transcript abundance increases following serum stimulation of quiescent fibroblasts. Quiescent ts13 cells were stimulated with DMEM supplemented with 15% FBS for 3, 6, 9, 16, and 24 h. Cytoplasmic RNA was isolated from different cell populations at the indicated times in the presence of vanadyl-ribonucleoside complex. RNA (15 μg) was size-fractionated by formaldehyde-agarose gel electrophoresis and transferred to nitrocellulose. Loading equivalence was monitored as described in Methods. Autoradiographs shown were obtained after hybridizing the filters with nick-translated vimentin cDNA insert. **Lane A:** RNA isolated from quiescent cells. **Lanes B–F:** RNA isolated from cells 3, 6, 9, 16, and 24 h following serum stimulation.

moter, cytoplasmic transcript abundance, transcript stability, and protein abundance following serum stimulation of quiescent hamster fibroblasts.

Vimentin Cytoplasmic Transcript Abundance Declines During Serum Deprivation and Increases Following Serum Stimulation of Fibroblasts

To determine the kinetics of expression of vimentin transcripts as ts13 cells entered quiescence, cytoplasmic RNA was extracted from exponentially growing cells and from cells at 24 h intervals following incubation in low serum at 34°C. Northern blot analysis showed that vimentin cytoplasmic transcripts (1.9 kb) were abundant in exponentially growing cells and cells deprived of medium for only 24 h and then diminished to barely detectable levels over the remaining 48 h period (Fig. 1). Parallel cultures were labeled with [^{3}H]-thymidine for 1 h to monitor ongoing DNA synthesis. The percentage of labeled nuclei in exponentially growing cells and cells deprived of serum for 24, 48, or 72 h were approximately 35%, 15%, 4%, and 4%, respectively. Thus as cells exited the cell cycle and entered quiescence, the expression of vimentin declined.

As quiescent ts13 cells were stimulated to re-enter the cell cycle, cytoplasmic RNA was isolated from quiescent cells and from cells stimulated for 6, 16, and 24 h. As shown in Figure 1, vimentin cytoplasmic transcripts began to increase 6 h following serum addition to quiescent cells. Cytoplasmic abundance of vimentin transcripts continued to increase throughout G_1 in serum-stimulated ts13 cells. As determined by densitometric analysis, vimentin cytoplasmic transcript levels increased fourfold in ts13 cells over levels in quiescent cells. In Figure 2, vimentin cytoplasmic transcripts can be seen to increase in abundance as early as 3 h following serum addition to quiescent cells. Parallel cultures of ts13 cells were incubated in the presence of [^{3}H]-thymidine to monitor the entry of cells into S phase following serum stimulation. In unstimulated cell populations less than 10% of the cells incorporated [^{3}H]-thymidine, verifying that over 90% of the cell population was quiescent at the time of serum addition. Approximately 16 h after serum stimulation, ts13 cells approached the G_1/S phase border. Furthermore, [^{3}H]-thymidine incorporation indicates that over 85% of the cells entered S phase by 24

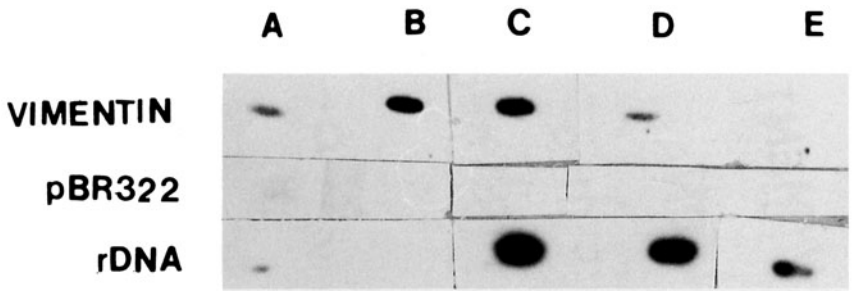

Fig. 3. Transcriptional activation of vimentin in fibroblasts following serum stimulation. Nuclei were isolated from quiescent cells (**lane A**) and from cells stimulated with DMEM supplemented with 15% FBS for 1, 2, 4, and 6 h (lanes B–E, respectively). Nuclear transcriptional assays were performed as described in Methods. Radiolabeled transcripts (1×10^7 dpm/sample) were hybridized to 10 μg plasmid DNAs (vimentin, rDNA, and pBR322) immobilized to nitrocellulose as described in Methods. These data are representative of 3 similar experiments.

h after serum addition. These results verify and extend results previously reported [Hirschhorn et al., 1984].

Vimentin Transcriptional Activity Increases in Serum-Stimulated Fibroblasts

The transcriptional activity of the endogenous vimentin promoter was examined in quiescent and serum-stimulated cells using nuclear transcriptional assays to determine if the increase in vimentin transcripts following serum stimulation of quiescent cells resulted from increased transcription. As shown in Figure 3, vimentin transcriptional activity increased by 1 h in ts13 cells following serum stimulation, remained elevated for 2 h, and then returned to quiescent levels by 4 h. In this experiment, the relative transcriptional activity increased approximately fivefold in ts13 cells following serum stimulation as determined by densitometric analysis. It has been previously shown that the transcriptional activity of the rDNA promoter is evident at low levels in quiescent fibroblasts and that the activity increases upon serum stimulation of fibroblasts [Edwards et al., 1985]. In Figure 3, it can be seen that the transcription of rDNA was apparent in quiescent ts13 cells and increased upon serum stimulation (positive control). There was no apparent hybridization to pBR322 sequences (negative control). Therefore, the increase in vimentin transcriptional activity of fivefold in serum-stimulated fibroblasts was reflected in a fourfold increase in cytoplasmic transcript abundance.

Nuclear transcriptional assays were performed in the absence or presence of 1 μg/ml α-amanitin to verify that the observed increases in transcriptional activities seen in Figure 3 result from RNA polymerase II–specific transcription. Only RNA polymerase II activity is sensitive to this concentration of α-amanitin while RNA polymerases I and III are insensitive [Lindell et al., 1970]. Nuclei were isolated from exponentially growing ts13 cells and were incubated in the absence or presence of 1 μg/ml α-amanitin (Fig. 4). Vimentin transcriptional activity was evident in the absence of α-amanitin and greatly diminished in the presence of α-amanitin. As a positive control for RNA polymerase I activity, the transcriptional activity of rDNA was examined and found to be insensitive to α-amanitin. As previously shown, c-*myc* transcriptional activity was found to be sensitive to α-amanitin, which indicated an effective inhibition of RNA polymerase II in our system [Greenberg and Ziff, 1984]. Hybridization of radiolabeled transcripts to pRSVneo sequences was undetectable (negative control). This suggests that the vimentin gene was transcribed by RNA polymerase II and that the observed increases in vimentin and rDNA transcriptional activity were not the result of spurious RNA polymerase I or III transcriptional activity.

Vimentin Cytoplasmic Transcripts Are Stable in Quiescent and Serum-Stimulated ts13 Cells

The increase in vimentin cytoplasmic transcript abundance could result from a stabilization of vimentin transcripts after serum stimulation of quiescent cells. To investigate this possibility, cytoplasmic RNA was isolated from quiescent cells and cells stimulated for 16 h that

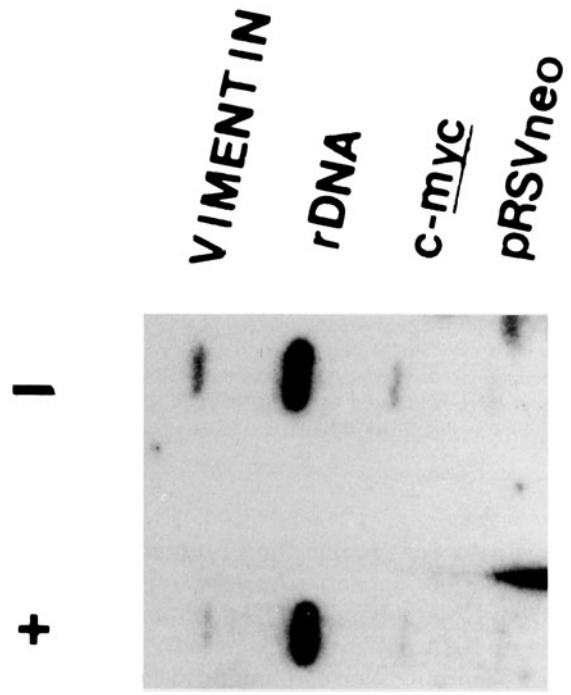

Fig. 4. Vimentin transcription is mediated by RNA polymerase II. Cell nuclei were isolated from exponentially growing ts13 cells and nuclear transcription assays were performed in the absence (−) or presence (+) of α-amanitin (1 μg/ml). Radiolabeled transcripts (1×10^7 dpm) were hybridized to 10 μg of plasmid DNA (vimentin, rDNA, c-*myc*, and pRSVneo) immobilized on nitrocellulose as described in Methods. These data are representative of duplicate experiments.

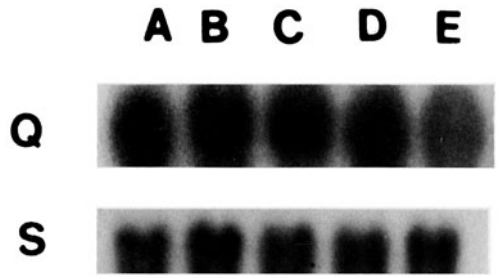

Fig. 5. The stability of vimentin cytoplasmic transcripts in quiescent and serum-stimulated ts13 cells. At time zero, actinomycin D (5 μg/ml) was added to quiescent cells and cells that were serum-stimulated for 16 h. Cytoplasmic RNA was isolated from the cells after 45, 90, 180, and 360 min of incubation in actinomycin D. RNA (15 μg) was size-fractionated by formaldehyde-agarose gel electrophoresis and transferred to nitrocellulose. Loading equivalence was monitored as described in Methods. The autoradiographs shown were obtained after hybridizing the filter with a nick-translated vimentin cDNA insert. The upper panel shows vimentin transcripts isolated from quiescent cell populations (**Q**). The lower panel shows vimentin transcripts isolated from cell populations serum-stimulated for 16 h (**S**), then treated with actinomycin D and samples collected at various times. **Lanes A–E:** RNA isolated from cells after 0, 45, 90, 180, and 360 min of actinomycin D treatment.

were treated with 5 μg/ml actinomycin D to inhibit all RNA synthesis. Northern blot analysis showed a gradual decrease in vimentin cytoplasmic transcript abundance during the 6 h chase period in both cell populations (Fig. 5), suggesting that the stability of vimentin transcripts was similar in quiescent and serum-stimulated ts13 cells. Parallel cultures of control cells and actinomycin D treated cells were labeled with 1 μCi/ml [^{3}H]-uridine (16 Ci/mmole; ICN Biomedicals, Inc., Irvine, CA) for 1 h. Radiolabeled nucleic acids were precipitated with trichloroacetic acid and quantitated using a scintillation counter. RNA synthesis was inhibited at least 97% in the presence of actinomycin D in quiescent and stimulated cell populations, verifying that ongoing RNA synthesis was not contributing to the mRNA levels detected. Therefore, since vimentin transcripts were relatively stable in quiescent cells, the increase in vimentin cytoplasmic transcript abundance (Fig. 2) reflects the increased transcriptional activity.

Vimentin Protein Abundance Increases in ts13 Cells Following Serum Stimulation

Since in vivo vimentin exists predominantly in intermediate filaments [Bilkstad and Lazarides, 1983; Moon and Lazarides, 1983], the abundance of polymerized vimentin was examined in serum-stimulated fibroblasts. Intermediate filament-enriched samples were isolated from quiescent cell populations and serum-stimulated cells. Immunoblot analysis showed an increase in polymerized vimentin by 4 h following serum addition which continued to increase throughout G_1 (Fig. 6). There was little [^{3}H]-thymidine incorporation in quiescent cells, whereas following serum stimulation, DNA synthesis increased as a function of time. Therefore, the accumulation of vimentin protein closely paralleled that of vimentin transcript abundance.

DISCUSSION

The gene encoding the intermediate filament protein, vimentin, was previously identified as a growth-regulated gene as a result of a differential hybridization screen of a cDNA library constructed from mRNA isolated 6 h following serum stimulation of quiescent ts13 cells [Hirschhorn et al., 1984; Ferrari et al., 1986]. Vimen-

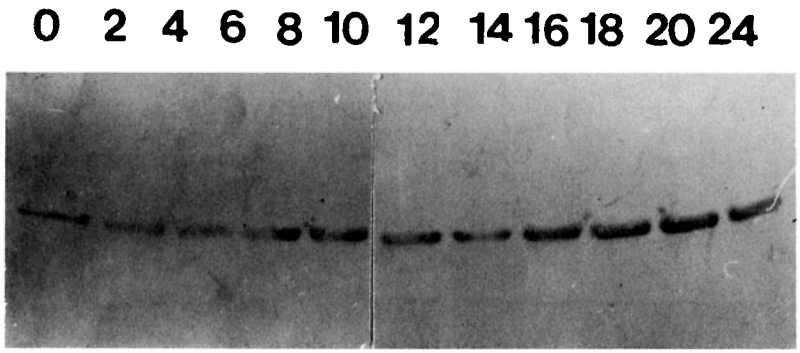

Fig. 6. Abundance of polymerized vimentin in quiescent and serum-stimulated cells. Samples enriched for intermediate filaments were isolated from quiescent and serum-stimulated cells. Protein was isolated from cells at 2 h intervals following serum addition. Protein samples were separated by 10% SDS-PAGE, transferred to Immobilon-P membrane, and immunoblotted. Lanes are labeled according to the time of protein isolation following serum stimulation. These data are representative of 3 similar experiments.

tin expression at the level of mRNA abundance has been shown to be growth regulated in human, hamster, and mouse fibroblasts and lymphocytes when induced by several mitogens [Ferrari et al., 1986; Rittling and Baserga, 1987; Calabretta et al., 1985; Kaczmarek et al., 1985] and in tumor cells when treated with phorbol esters [Rius and Aller, 1989; Paulin-Levasseur et al., 1989; Hass et al., 1990]. In the vimentin 5′ promoter region, three regulatory elements were identified by deletion studies using a heterologous reporter gene (CAT) [Rittling and Baserga, 1987]. Although the promoter regions involved in the induction of this exogenous promoter have been delineated, very little is known about the mechanisms regulating the expression of the endogenous vimentin gene in fibroblasts in response to mitogens. In this study, the transcriptional and post-transcriptional events leading to the increase of vimentin in intermediate filaments in hamster fibroblasts were examined by investigating transcriptional rates, cytoplasmic transcript abundance, transcript stability, and protein abundance in serum-stimulated ts13 cells.

The vimentin cytoplasmic transcript abundance in ts13 cells decreases gradually in response to serum deprivation and increases in abundance in response to serum stimulation (Figs. 1, 2). The increase in transcript abundance occurs in mid–late G_1 (Fig. 2) which is in agreement with previous studies [Hirschhorn et al., 1984; Ferrari et al., 1986]. Investigation of the mechanisms that could account for the increase in abundance of vimentin cytoplasmic transcripts when quiescent cells were serum-stimulated indicated that this was predominantly a result of transcriptional activation (Fig. 3). Vimentin-specific transcription increased fivefold by 1 h, remained elevated for 2 h, and then returned to basal levels by 4 h after serum stimulation. This increase is sufficient to account for the increase in vimentin cytoplasmic transcripts detected following serum stimulation. The stability of vimentin transcripts in quiescent and serum-stimulated ts13 cells was also examined to determine if an increase in transcript stability contributed to the observed increase in vimentin cytoplasmic transcript levels in response to serum (Fig. 5). The approximate half-life of vimentin transcripts in quiescent and serum-stimulated cells was estimated to be greater than 4 h, as determined by actinomycin D chase experiments. Therefore, since vimentin transcripts are stable in quiescent and serum-stimulated cells, the increase in vimentin cytoplasmic transcript abundance in response to serum was a result of increased vimentin-specific transcription.

Since vimentin exists mainly in intermediate filaments in vivo [Bilkstad and Lazarides, 1983; Moon and Lazarides, 1983; Soellner et al., 1985; Vikstrom et al., 1989], the abundance of polymerized vimentin was examined in serum stimulated cells (Fig. 6). The accumulation of polymerized vimentin increased throughout G_1 following mitogenic stimulation, suggesting that as vimentin transcripts become available in the cytoplasm they are translated into protein which is readily incorporated into filaments (Fig. 7).

Overall, the increased vimentin-specific transcriptional activity mediated by serum stimula-

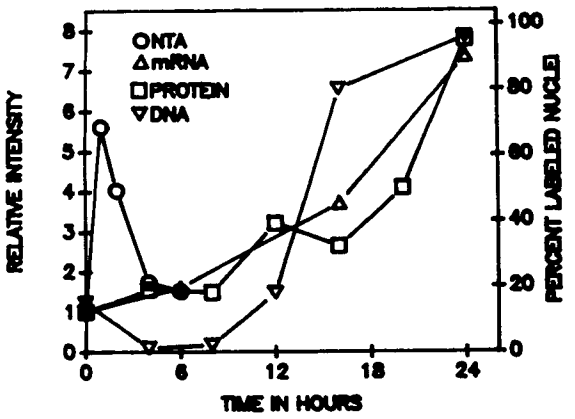

Fig. 7. Composite of vimentin transcriptional activation, mRNA abundance, and protein abundance levels in serum-stimulated ts13 cells at 34°C. The relative levels of vimentin transcripts (mRNA) in each sample were determined by scanning the previous autoradiographs with an LKB Ultroscan XL laser densitometer. Relative levels of vimentin transcripts and protein in serum-stimulated cells were normalized to vimentin transcript and protein levels in quiescent cells (t_n/t_o, where n = time of isolation). For nuclear transcriptional assays (NTA), the relative levels of the transcripts were corrected for background hybridization to pBR322 before normalizing to hybridization levels in quiescent cells. To score the percentage of the cell population undergoing DNA synthesis (DNA), quiescent and serum-stimulated cells were incubated continuously in the presence of 1.5 μCi/ml [^{3}H]-thymidine. Cells were stimulated for 0, 6, 16, and 24 h and then processed as described in Methods.

tion of quiescent ts13 cells results in the accumulation of stable cytoplasmic transcripts. These transcripts appear to be translated and readily incorporated into intermediate filaments. The delay observed between the fivefold induction of vimentin transcription by 1 h and the appearance of vimentin transcripts in the cytoplasm by 3 h suggests that the transcripts are not immediately transported to the cytoplasm after synthesis. This possibility is currently under investigation.

Although advances have been made in understanding the dynamics of intermediate filament assembly, the fundamental function of the cytoskeleton and its components remains obscure. While the conservative view presents an exclusively structural function, an alternative hypothesis suggests that the cytoskeleton not only responds to stimulation from the cell's external environment, but that it is actually part of the signal transduction mechanism that results in differential gene expression [Skalli and Goldman, 1991; Ben-Ze'ev, 1991]. In view of this possibility, it is interesting to note, that in addition to vimentin [this paper; Hirschhorn et al., 1984; Ferrari et al., 1986; Rittling and Baserga, 1987], the expression of other cytoskeletal components and extracellular matrix proteins has been characterized as growth regulated [reviewed in Hofbauer and Denhardt, 1991]. These proteins include actin [Elder et al., 1988], fibronectin [Blatti et al., 1988], and an actin-associated protein [Almendral et al., 1988]. Disruption of the normal cytoskeletal structure with colchicine or cytochalasin D selectively alters the expression of some growth-regulated genes, including c-*fos*, an immediate early gene, and actin [Zambetti et al., 1991; Miura et al., 1987]. The growth-regulated expression of cytoskeletal components, and their ability to modulate the growth-regulated expression of other genes when the cytoskeletal structure is disrupted, further supports a more active role in cell growth and division, possibly at the level of signal transduction. A more complete understanding of the regulation of the expression of these individual cytoskeletal components will provide information to allow a more extensive testing of this intriguing hypothesis.

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