INTRODUCTION

It has been well established that various tissue explants and cells synthesize and release matrix metalloproteinases (MMPs) (Birkedal-Hansen et al., 1993; Nagase 1996). These include the most recently found enamelysin (Bartlett et al., 1996) and MMP-19 (Cossins et al., 1996) as well as their intrinsic inhibitors, called tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2 and TIMP-3) (Denhardt et al., 1993; Hayakawa, 1994; Edwards et al., 1996), and the recently found TIMP-4 (Greene et al., 1996; Leco et al., 1997). The ability of these TIMPs to inhibit MMPs suggests that they play an important role in the regulation of MMP activity, effectively controlling the breakdown of extracellular matrix components in various kinds of important biological phenomena and pathological events such as inflammation and tumor invasion (Liotta et al., 1991; Docherty et al., 1992; Matrisian, 1992; Birkedal-Hansen et al., 1993; Nagase, 1996).

Although MMP inhibition is the primary action of the TIMPs, these inhibitors also have other properties, such as erythroid-potentiating activity (Docherty et al., 1985; Hayakawa et al., 1990; Stetler-Stevenson et al., 1992; Murate et al., 1993), cell growth-stimulating activity (Bertaux et al., 1991; Hayakawa et al., 1992; 1994; Yang and Hawkes, 1992; Nemeth and Goolsby, 1993; Nemeth et al., 1996; Kikuchi et al., 1997), transformation of chick embryo fibroblasts (Yang and Hawkes, 1992), inhibition of vascular endothelial cell proliferation (Murphy et al., 1993), as a survival factor (Matsumoto et al., 1993), and in embryogenesis-stimulating activity (embryogenin-1; Satoh et al., 1994), endometriosis (endometriosis protein-II; Sharpe-Timms et al., 1995) and steroidogenesis (steroidogenesis-stimulating protein; Boujrad et al., 1995).

A recent immunohistochemical study of ours suggested cell cycle-dependent accumulation of immunoreactive TIMP-1-like protein in the nuclei of human gingival fibroblasts (Gin-1 cells), reaching a maximum in the S phase of the cell cycle (Li, H., Nishio, K., Yamashita, K., Hayakawa, T. and Hoshino, T. (1995). Nagoya J. Med. Sci. 58, 133-142). Then we isolated this protein from a nuclear extract of Gin-1 cells and demonstrated it to be identical to human recombinant TIMP-1 by western blotting, by a sandwich enzyme immunoassay for TIMP-1 and by an assay for matrix metalloproteinase inhibition. The amount of TIMP-1 in the cytosolic fraction of quiescent Gin-1 cells after stimulation by fetal calf serum increased continuously for 48 hours, whereas that in the nuclear extract showed a maximum at 24 hours (S phase) and significantly decreased thereafter. Gin-1 cells expressed mRNAs for both TIMP-2 and TIMP-3 together with mRNA for TIMP-1. However, neither TIMP-2 nor TIMP-3 proteins seemed to accumulate in the nuclei of Gin-1 cells. These facts strongly suggest that TIMP-1 accumulates specifically in the nuclei of Gin-1 cells in a cell cycle-dependent manner.

SUMMARY

We first confirmed an earlier immunohistochemical study showing that immunoreactive TIMP-1-like protein accumulated in the nuclei of human gingival fibroblasts (Gin-1 cells), reaching a maximum in the S phase of the cell cycle (Li et al., 1995). Then we isolated this protein from a nuclear extract of Gin-1 cells and demonstrated it to be identical to human recombinant TIMP-1 by western blotting, by a sandwich enzyme immunoassay for TIMP-1 and by an assay for matrix metalloproteinase inhibition. The amount of TIMP-1 in the cytosolic fraction of quiescent Gin-1 cells after stimulation by fetal calf serum increased continuously for 48 hours, whereas that in the nuclear extract showed a maximum at 24 hours (S phase) and significantly decreased thereafter. Gin-1 cells expressed mRNAs for both TIMP-2 and TIMP-3 together with mRNA for TIMP-1. However, neither TIMP-2 nor TIMP-3 proteins seemed to accumulate in the nuclei of Gin-1 cells. These facts strongly suggest that TIMP-1 accumulates specifically in the nuclei of Gin-1 cells in a cell cycle-dependent manner.

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A recent immunohistochemical study of ours suggested cell cycle-dependent accumulation of immunoreactive TIMP-1-like protein in the nuclei of human gingival fibroblasts (Gin-1 cells) (Li et al., 1995). In the present study, we isolated the TIMP-1-like protein from the nuclear fraction of Gin-1 cells and demonstrated for the first time that it was identical to TIMP-1 by western blotting, by a sandwich enzyme immunoassay for TIMP-1, and inhibitory activity against MMP.

MATERIALS AND METHODS

Materials

Materials used and their sources were as follows: Dulbecco’s modified Eagle’s minimal essential medium (D-MEM) and fetal calf serum (FCS) from Gibco Laboratories (Grand Island, NY); Histofine SAB-
PO(M) kit from Nichirei KK (Tokyo); PVDF membrane from Millipore Corporation (Bedford, MA); 0.45 μm cellulose nitrate membrane from Toyo Roshi Ltd (Tokyo); enhanced chemiluminescence (ECL)-western blotting detection reagents from Amersham Life Science (Buckinghamshire, England); fluorescein isothiocyanate (FITC)-labeled Dextran, Noniudet P-40 (NP-40), and phenylmethylsulfonyl fluoride (PMSF) from Sigma Chemical Co. (St Louis, MO); p-
aminophenyln mercuric acetate (APMA) from Aldrich Chemical Co. (Milwaukee, WI); ISOGEN from NPG Inc. (Toyama, Japan); DNase I, dNTP mixture, and recombinant T4 DNA polymerase from Takara (Shuzo Co., Ltd, Otsu, Shiga, Japan); oligo dT(12-18) primer from Pharmacia Biotech (Uppsala, Sweden); M-MLV reverse transcriptase from GibcoBRL. (Grand Island, NY); human gingival fibroblasts (Gin-1) from the American Type Culture Collection (Rockville, MD). Anti-
TIMP-1 monoclonal antibodies (mAbs) (clones 7-6C1, 7-3F1 and 7-
4F2), the horseradish-peroxidase (HRP)-labeled Fab' fragments of clone 7-6C1, and anti-TIMP-1-mAb-Sepharose affinity column were prepared as described previously (Kodama et al., 1987). Monoclonal antibody specific for TIMP-2, clone 67-4H11, was prepared against an oligopeptide R178-193 (Fujimoto et al., 1993), and that for TIMP-3, clone 136-13H4, was prepared against oligopeptide R170-188 (Fariss et al., 1997). Human recombinant (hr) TIMP-1 was prepared by a method described by Hayakawa et al. (1994).

Cell culture

Gin-1 cells were grown to 40-70% confluence in D-MEM with 10% FCS on 18 mm square glass coverslips at 37°C under an atmosphere of 95% air and 5% CO2. Cells growing at random under these conditions were designated as a 0 hour (h) control. Then, the cells were washed three times with phosphate-buffered saline (PBS) and maintained in serum-free D-MEM for an additional 48 hours without growth; these were designated as FCS(-)48 h. The FCS(-)48 h cells were then transferred into the medium containing 10% FCS and cultured for a further 24 hours (designated as FCS(+)/24 h. Cells of each stage were fixed in 4% paraformaldehyde (PFA) in PBS at 4°C for 1 hour and examined immunohistochemically. For biochemical studies, Gin-1 cells were cultured in 75 cm2 Falcon tissue culture flasks (Becton Dickinson and Co., Lincoln Park, NJ) by the same protocol as mentioned above.

Immunohistochemistry

The direct HRP-labeled antibody method was applied for human TIMP-1 staining. The fixed cells were treated with methanol containing 0.01% H2O2 for 20 minutes to inhibit endogenous peroxidase activity. For blockage of nonspecific immunoreactivity, cells were treated with 100 μg/ml Fab' of normal mouse IgG in PBS for 30 minutes at room temperature. For immunostaining, the specimens were incubated with 30-50 μg/ml HRP-labeled Fab' of clone 7-6C1 overnight at 4°C. As a negative control, HRP-labeled normal mouse IgG Fab' (30-50 μg/ml) was used instead of the specific mAB. The specimens were dipped in 0.025% diaminobenzidine solution containing 10 mM H2O2 for exactly 5 minutes. They were then rinsed in PBS, dehydrated in a graded series of ethanol without counter staining, cleared in xylene, and mounted on a glass slide.

The indirect HRP-labeled antibody method was used for staining bovine TIMP-1. Normal rabbit serum (10%) was applied for 10 minutes, followed by incubation with 30-50 μg/ml anti-TIMP-1 mAbs (clones 7-3F1 and 7-4F2), which react exclusively with bovine TIMP-1, at 37°C for 60 minutes. The specimens were then successively treated with biotinylated rabbit anti-mouse IgG for 10 minutes and with HRP-labeled streptavidin for 10 minutes at room temperature following the instructions included in the SAB-PO(M) kit.

Preparation of cytosolic fraction and nuclear extract of Gin-1 cells

After harvesting, Gin-1 cells were washed three times in Ca2+- and Mg2+-free PBS, pelleted by centrifugation (12,000 g, 15 seconds), and resuspended in 50-100 volumes of 20 mM Tris-EDTA buffer, pH 7.4, containing 5 mM PMSF and 20% sucrose. The cells kept in ice with continuous swirling were lysed by slowly adding 5% NP-40 (Sambrook et al., 1989; Stubbs et al., 1996) to the suspension to make the final concentration below 0.4%. Cell lysis was ascertained under a light microscope. After thorough mixing, the cell lysate was kept at 0°C for 5 minutes. The nuclear fraction was pelleted by centrifugation at 8,000 g for 3 minutes, and the supernatant was pooled as the cytosolic fraction. The nuclear pellet was washed 3 times in PBS, centrifuged as mentioned above, and all washings were added to the cytosolic fraction. The isolated nuclei were free from glucose-6-
phosphate dehydrogenase activity (a marker enzyme for endoplasmic reticulum and Golgi apparatus). Transmission light microscopic analysis also demonstrated the absence of cytoplasmic membranes and organelles in the nuclear fraction. To ensure that the nuclear membranes were intact size-dependent nuclear transport of FITC-
labeled Dextran into the nuclei was examined. Only M1, 4,400 but not 19,600 or 50,700 Dextran was able to be transported into the nuclei, suggesting the nuclear membranes were intact (Duverger et al., 1995). The nuclei were solubilized by homogenization in 0.1% NaCl containing 0.1% Tween-20, 2 mM EDTA, and 5 mM PMSF, and then sucrose was added to make a final concentration of 15%. The supernatant collected after centrifugation (200,000 g, 2 hours) was dialyzed against deionized water, which was changed 3 times at 6 hour intervals, and then lyophilized. The sample obtained after dissolving the lyophilized material in 0.01 M sodium phosphate buffer, pH 7.4, containing 0.81% NaCl was designated as the nuclear extract.

Determination of TIMP-1 content and assay for the inhibition of gelatinolytic activity

TIMP-1 content was determined by a sandwich enzyme immunoassay (Kodama et al., 1990).

Gelatinolytic activity was determined by the method of Harris and Krane (1972) with heat-denatured [14C]glycine-labelled collagen solution as substrate. One unit of gelatinolytic activity was defined as the amount of enzyme necessary to degrade 1 μg of [14C]glycine-labelled gelatin per minute at 37°C. Conditioned medium of TNF-α-treated HT 1080 cells, containing the desired number of units of gelatinolytic activity, was first mixed with the nuclear extract of Gin-1 cells, and subsequently incubated with [14C]glycine-labelled gelatin in the presence of 1 mM APMA at 37°C for 16-18 hours.

Measurement of diameters of cells and nuclei

Gin-1 cells at FCS(+)24 h were harvested by treatment with trypsin-EDTA solution and washed with PBS. The diameters of both cells and nuclei were measured by use of a microruler in a Zeiss microscope equipped with a ×40 objective. The average volumes of cells and nuclei calculated from their diameters were 38.77±4.56 and 4.19±0.50 μm3, respectively. The average volume of cytosol calculated from those values was 34.59±4.06 μl/107 cells.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli and Favre (1973) after reduction of samples with 10% 2-mercaptoethanol. Molecular mass markers (Bio-Rad Lab) were used as standards. After electrophoresis, we employed either of two methods for immunoblotting as follows. (1) The protein bands in the gel were transferred to a cellulose nitrate membrane. The membrane was then incubated with 3% skimmed milk solution overnight at 4°C for the blocking of non-specific reaction, and stained with monoclonal antibody. After the membrane had been washed, the bound first antibody was detected with an HRP-
conjugated second antibody, 3,3', diaminobenzidine, and H2O2. (2) The protein bands were transferred onto a PVDF membrane. After blockage with 5% skimmed milk solution, the membrane was stained
with monoclonal antibody together with ECL western blotting detection reagents.

**RNA isolation and RT-PCR**

Total RNA was isolated from Gin-1 cells with ISOGEN. Subsequent chloroform extraction, RNA precipitation and RNA wash were performed according to the manufacturer’s instructions. About 20 μg of isolated total RNA was treated with 10 units of DNase I at 37°C for 60 minutes. 2 μg of total RNA was reverse transcribed to cDNA by use of oligo dT12-18 primer with M-MLV reverse transcriptase for 60 minutes at 37°C in the presence of 0.5 mM dNTPs. cDNA was amplified with each primer set using 0.63 units of recombinant Taq DNA polymerase. The amplification conditions were as follows: 94°C (3 minutes) for 1 cycle, followed by 94°C (15 seconds), 55°C (30 seconds) and 72°C (30 seconds) for 25 to 40 cycles in GeneAmp PCR System 2400 (Perkin Elmer, Branchburg, NJ). The following primer pairs were used for RT-PCR amplification: TIMP-1 (Docherty et al., 1985): 5'-primer, 5'-TTCGTGGGGACACCAGAAGTCAAC-3' (198-221); 3'-primer, 5'-TGGACACTGTGCAGGCTTCAGTTC-3' (725-702); TIMP-2 (Stetler-Stevenson et al., 1990): 5'-primer, 5'-AAGCGGTCACTGAGAAGGTGG-3' (413-436); 3'-primer, 5'-CCTGGAGGCTTTTTTCAGTTG-3' (981-958); TIMP-3 (Wick et al., 1994): 5'-primer, 5'-GCA TGTAAGAGTGAGGGAGG-3' (1216-1236); 3'-primer, 5'-CCAAAGCGTTTTCCAGGAGG-3' (981-958); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Arcari et al., 1984): 5'-primer, 5'-ACCACAGTC-CA TGCCA TCAC-3' (586-605); 3'-primer, 5'-TCCACCCCTTGTGCTGTA-3' (1037-1018). The products of these reactions were analyzed by electrophoresis on a 2% agarose gel.

**RESULTS**

**Immunolocalization of TIMP-1 in Gin-1 cells**

We first confirmed the positive nuclear staining of Gin-1 cells with anti-TIMP-1 monoclonal antibodies (Li et al., 1995). In the 0 h control cells (Fig. 1A), the intensity of nuclear staining varied from cell to cell. The staining in the nuclei at FCS(-)48 h decreased and became fainter than that in the cytoplasm in most cells (Fig. 1B). In contrast, the nuclear staining increased remarkably in almost all the cells at FCS(+)24 h (Fig. 1C).

**Western blotting of both cytosolic and nuclear TIMPs**

Immunoreactive TIMP-1-like protein in both the cytosolic fraction and nuclear extract of Gin-1 cells cultured in serum-containing medium was partially purified by use of an anti-TIMP-1 mAb-Sepharose affinity column. Each TIMP-1 preparation eluted from the affinity column showed a single band indicating a molecular mass around 30 kDa on a nitrocellulose membrane by immunoblotting analysis, which was indistinguishable from that of hrTIMP-1 (Fig. 2).

**TIMP-1 contents in both cytosol and nuclei of Gin-1 cells**

TIMP-1 concentrations were measured in both the cytosolic fraction and nuclear extract of Gin-1 cells at FCS(+)+24 h by the sandwich EIA. As shown in Fig. 3, the nucleus showed a significantly higher TIMP-1 concentration than the cytoplasm with respect to the original volumes of both cytosolic and nuclear compartments. The sandwich EIA for TIMP-1 that we employed in this study uses two mAbs that detect different epitopes on the TIMP-1 molecule simultaneously.

**MMP inhibitory activity of nuclear TIMP**

We next examined the inhibitory activity of nuclear extract at FCS(+)+24 h on the gelatinolytic activity in the conditioned medium of TNF-α-treated HT 1080 cells, in which the major gelatinolytic activity has been ascribed to gelatinase B (MMP-9) (Okada et al., 1992). The nuclear extract of Gin-1 cells displayed potent dose-dependent inhibitory activity toward MMP, as shown in Table 1. The inhibitory activity was completely abolished by the presence of anti-TIMP-1
monoclonal antibody. The extract itself had no gelatinolytic activity.

**Time course of TIMP-1 accumulation in both cytosol and nucleus of Gin-1 cells**

We next examined the dynamics of TIMP-1 through the cell-cycle from G₀ to G₁ and S phase. The expression of mitogen-inducible gene 5 (mig-5) was used as a marker for mid G₁ (Wick et al., 1994), positive reaction with 5-bromo-2′-deoxyuridine (BrdU) for S phase, and positive reaction with proliferating cell nuclear antigen (PCNA) for the period 24-48 hours after serum stimulation of quiescent Gin-1 cells (Li et al., 1995). As shown in Fig. 4, TIMP-1 accumulation in the cytosolic fraction increased continuously until 48 hours. TIMP-1 in the nuclear fraction, however, showed a maximum accumulation at 24 hours after serum stimulation and diminished thereafter.

**RT-PCR of mRNA for TIMPs in Gin-1 cells**

Fig. 5 shows the induction of TIMP-1 and TIMP-3 mRNAs after stimulation of quiescent Gin-1 cells as determined by RT-PCR. The expression of both TIMP-1 and TIMP-3 mRNA reached peak levels at 6-9 hours after FCS stimulation. Induction of both TIMP-1 and TIMP-3 genes seemed to be transient since their expressions decreased to basal levels by 12 - 24 hours after stimulation. The expression of TIMP-2 mRNA, however, was not affected at all by FCS stimulation, nor was that of MMP-2 (data not shown), which is known to be a housekeeping gene.

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**Table 1. Inhibition of gelatinolytic activity by nuclear extract of Gin-1 cells**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Activity (mU/tube)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM alone</td>
<td>59.7</td>
<td>0</td>
</tr>
<tr>
<td>+nuclear extract (1.3 pg*)</td>
<td>30.0</td>
<td>49.7</td>
</tr>
<tr>
<td>+nuclear extract (2.6 pg)</td>
<td>17.4</td>
<td>70.9</td>
</tr>
<tr>
<td>+nuclear extract (5.3 pg)</td>
<td>9.0</td>
<td>84.9</td>
</tr>
<tr>
<td>+nuclear extract (2.6 pg) + anti-TIMP-1 mAb† (10 ng)</td>
<td>59.6</td>
<td>0.2</td>
</tr>
<tr>
<td>nuclear extract (2.6 pg) alone</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

*TIMP-1 amount determined by the sandwich EIA.†clone 7-6C1.

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**Fig. 2. Immunoblotting of cytosolic TIMP-1 and nuclear TIMP-1.** TIMP-1 was partially purified by subjecting cytosolic fraction and nuclear extract of 2.3×10⁶ cells to anti-TIMP-1 mAb-Sepharose affinity column chromatography (Kodama et al., 1987). Following the SDS-PAGE (12% polyacrylamide) of partially purified samples, the protein bands were transferred to a cellulose nitrate membrane and stained with anti-TIMP-1 mAb (clone 7-6C1), HRP-conjugated second antibody, 3, 3′-diaminobenzidine, and H₂O₂ as described in Materials and Methods. Lane 1, cytosolic TIMP-1; lane 2, nuclear TIMP-1; lane 3, hrTIMP-1.

**Fig. 3. TIMP-1 concentrations in cytosolic fraction (cyt) and nuclear extract (nuc) of Gin-1 cells at FCS(+)24 h.** TIMP-1 concentrations were determined with a sandwich EIA for TIMP-1. Results are the means of 5 experiments, with standard deviations. *Original volume of cytosolic and nuclear compartments calculated from average diameters of cells and nuclei measured by the method described in Materials and Methods. **P<0.05 from the value for the cytosolic fraction.

**Fig. 4. Time course of TIMP-1 accumulation in cytosolic fraction and nuclear extract.** Gin-1 cells (1.25×10⁶ cells) were quantitatively fractionated into cytosolic fraction and nuclear extract at the indicated times after serum stimulation of the quiescent cells starved in serum-free D-MEM for 72 hours. Following SDS-PAGE (10% polyacrylamide) of the entire amount of each cytosolic fraction (cys) and nuclear extract (nuc), protein bands were transferred to a PVDF membrane and stained with anti-TIMP-1 mAb (clone 7-6C1) together with ECL western blotting detection reagents as described in Materials and Methods. The expression of mig-5 is a marker for mid G₁; BrdU-positive cells, for the S phase; PCNA-positive cells, for the period 24-48 hours after serum stimulation.
Selective accumulation of TIMPs in the nuclei of Gin-1 cells

All three TIMP proteins, TIMP-1, TIMP-2 and TIMP-3, were detected in the cytosolic fraction of Gin-1 cells. These results are consistent with those obtained by RT-PCR of mRNA as described above (Fig. 5). Only TIMP-1 protein, however, was detected in the nuclear extract, as shown in Fig. 6.

DISCUSSION

The frequency of nucleus-located TIMP-1 in cells changed in line with that of PCNA (Li et al., 1995). These results indicate that the immunoreactive TIMP-1-like protein is localized in the nuclei of Gin-1 cells and that its content changes in accordance with the cell cycle.

The question arises as to whether nuclear TIMP-1 might be derived from the FCS added to the culture medium. As TIMP-1 is known to be a growth factor in serum and to act on a wide range of cells (Hayakawa et al., 1992), it might be possible that bovine TIMP-1 could enter the cells after internalization together with its receptor. We could not see, however, any positive staining with anti-TIMP-1 mAbs (7-3F1 and 7-4F2), which specifically react with bovine TIMP-1 but not with human TIMP-1. Therefore, it is unlikely that the TIMP-1 in the nuclei originated from the FCS.

One of the critical points in this work was how to fractionate the cells quantitatively into cytosolic and nuclear fractions and furthermore how to obtain pure nuclei having intact membranes. We carefully checked the purity by monitoring for activity of a marker enzyme for endoplasmic reticulum and Golgi apparatus, together with a morphological examination of the nuclear fraction under a transmission light microscope. The intactness of the nuclear membrane was also checked by size-dependent nuclear transport of FITC-labelled Dextran into the nuclei (Duverger et al., 1995).

The sandwich EIA, which we used as a quantitative measurement of TIMP-1 in the nuclear extract, is only able to detect antigens having two different epitopes, each of which is recognized by its corresponding monoclonal antibody (Kodama et al., 1989). Thus the fact that a significant amount of TIMP-1 was detected in the nuclear extract (Fig. 3) seems to be a convincing proof for the accumulation of TIMP-1 in the nuclei of Gin-1 cells. The TIMP-1 concentration calculated from the original volume of the nuclear compartment was significantly higher than for that of cytoplasmic compartment (Fig. 3). The result strongly suggests the presence of an active transport mechanism for the passage of cytosolic TIMP-1 into the nucleus against a concentration gradient (Melchior and Gerace, 1995). Our preliminary experiments suggested the temperature- and energy-dependent import of rhodamine-labelled TIMP-1 into nuclei (data not shown).

It has been well recognized that TIMPs are a family of intrinsic inhibitors specific for the MMP family, so the presence of the inhibitory activity in the nuclear extract (Table 1) provides further strong evidence to support our contention that TIMP-1 exists in the nuclei of Gin-1 cells.

Unlike that in the cytoplasm, TIMP-1 accumulated in the nuclei with a unique profile along the cell-cycle progression, showing a maximum at 24 hours after serum stimulation (Fig. 4). It has been demonstrated by pulse-labelling with BrdU that the period 24 hours after serum stimulation corresponds to the S phase of the cell cycle (Li et al., 1995). TIMP-3 (mig-5) mRNA expression has been found to be subject to cell-cycle regulation, showing a clear peak around mid G1 (Wick et al., 1994). We found that TIMP-3 mRNA, together with TIMP-1 mRNA was induced at 6-9 hours after FCS stimulation of quiescent Gin-1 cells (Fig. 5).

TIMP-1, TIMP-2 and TIMP-3, were expressed at both mRNA (Fig. 5) and protein levels, however, only TIMP-1 protein was detected in the nuclei (Fig. 6), suggesting the presence of a selection system specific for the nuclear accumulation of TIMP-1.

It is tempting to speculate that TIMP-1 participates in regulating the cell cycle by inhibiting some supposed nuclear MMP, as it has been realized that proteolysis plays a critical role in mitosis of the eukaryotic cell cycle (Tanaka et al., 1990; Glotzer et al., 1991). We recently detected the presence of stromelysin-like MMP activity in the nuclear fraction of cultured HeLa cells (W.-Q. Zhao et al., unpublished result), and are now investigating its function.

Another major question is how general is the trafficking of

Fig. 5. Induction of mRNA for TIMPs by FCS in Gin-1 cells. Quiescent Gin-1 cells were stimulated with 10% FCS for the indicated times. Each mRNA was detected by RT-PCR as described in Materials and Methods. GAPDH was used as the loading control.

Fig. 6. Selective accumulation of TIMPs in the nuclei of Gin-1 cells. Gin-1 cells (6.25x10⁵ cells) were quantitatively fractionated into cytosolic fraction (cyt) and nuclear extract (nuc) at FCS(+24 h). Other experimental conditions were the same as those described in Fig. 4.
TIMP-1 to the nucleus of cells with respect to different cell types. We recently observed that immunoreactive TIMP-1 protein was also localized in some of the nuclei of human fetal lung fibroblasts and human diploid fibroblast cell line WI-38 cells growing nonsynchronously (data not shown). These observations suggest that the accumulation of TIMP-1 in the nucleus seems to be widespread, at least among fibroblastic cell types. Further studies on other cell types besides fibroblasts together with studies on cell-cycle dependency and western blotting of nuclear extracts of such cells are under way in our laboratory.

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