Effects of Platelet-Derived Growth Factor on Degradation, Nuclear Translocation of Nonhistone Proteins, and DNA Synthesis

(43375)

HERMAN POLET[1]

Department of Pathology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612

Abstract. **Stimulation of resting normal rat kidney fibroblasts, prelabeled with [^{3}H]leucine, by platelet-derived growth factor (PDGF) caused inhibition of cellular protein degradation and a parallel increased nuclear translocation of ^{3}H-labeled nonhistone proteins (^{3}H-NHP) and DNA synthesis. Nuclear translocation of these proteins was independent of protein synthesis. Fractionation of the nuclear ^{3}H-NHP in a pH gradient of 2.5–6.5 showed that the protein fractions with a high degree of proteolysis in resting cells corresponded to the protein fractions with a high extent of translocation in stimulated cells, suggesting that degradation and translocation of these proteins may be related. PDGF inhibited cellular uptake of [^{3}H]chloroquine, suggesting that PDGF inhibits NHP degradation via the lysosomal pathway. These observations support the hypothesis that PDGF induces NHP translocation to the nucleus by inhibiting lysosomal degradation of these proteins.** [P.S.E.B.M. 1992, Vol 199]

Previous investigations have suggested that lysosomal protein degradation and DNA synthesis may be related. Concanavalin A, fibroblast and epidermal growth factor, serum, conditioned medium, and lysosomal amines cause two parallel effects in cultured cells: inhibition of proteolysis of cellular proteins and increased translocation of nonhistone proteins (NHP) from the cytoplasm to the nucleus (1–5). This parallel effect suggests that these two effects may be related. The results of these investigations further suggested that the increased translocation of the NHP to the nucleus may be the result of inhibition of lysosomal degradation of these proteins caused by the growth stimulants.

NHP are believed to play an important role in gene activation and cellular proliferation (6, 7). The nuclear level of these proteins varies with the stages of the cell cycle: it is low in resting cells and, upon stimulation to grow, gradually rises during Gap_1 to attain a maximum in the early S phase (8). The addition of nuclear NHP to nuclei of resting cells alters the structure of chromatin (9, 10) and stimulates RNA synthesis (11). In the present report, it is shown that platelet-derived growth factor (PDGF) has similar effects as the above growth factors.

Materials and Methods

Cells, Medium, and Chemicals. Normal rat kidney fibroblasts (NRK cells) were obtained from the American Type Culture Collection. The cells were grown continuously in plastic bottles (Corning) of 150-cm^2 surface in Dulbecco's modified Eagle's medium (DME) containing 20 m*M* Hepes and 10% newborn calf serum (NBCS); bicarbonate was added to adjust the pH to 7.2–7.3. Serum was obtained from Biologos, Naperville, IL. Platelet-derived growth factor, produced by recombinant DNA technology followed by purification to apparent homogeneity, was obtained from Amgen Biologicals, Thousand Oaks, CA. Most experiments were performed with the latter product; a few experiments were performed using PDGF purified from human platelets and obtained from Collaborative Research Inc., Bedford, MA. Powdered culture media, Hepes, cycloheximide, and crystalline bovine serum albumin were from Sigma Chemical Co., St. Louis, MO. [^{3}H]Leucine (40–50 Ci/mmol) was purchased

[1] To whom requests for reprints should be addressed at Department of Pathology, 446 CMW M/C 847, University of Illinois, 1853 West Polk Street, Chicago, IL 60612.

Received June 27, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted November 4, 1991.

0037-9727/92/1994-0417$3.00/0

from ICN, Irvine, CA. [^{3}H]Chloroquine (210 mCi/mmol) was from New England Nuclear Corp., Boston, MA. All other chemicals were of reagent grade.

Preparation and Labeling of Cultures. After reaching confluent monolayers in the 150-cm^2 bottles, the cells were trypsinized, divided into two halves and inoculated into 75-cm^2 bottles in medium containing 10% NBCS.

For optimal growth, it was important to expose the cells to trypsin for as short a time as possible, as follows. After removing the medium, the cells were washed with 10 ml of Hanks' basal salt solution without calcium or magnesium, followed by overlaying the cells with 10 ml of the same solution containing 0.2% trypsin and 0.2% EDTA for 1 min only. This solution was aspirated and, 1 min later, the cells were removed by gentle shaking with 10 ml of DME. The cells were used for experiments, when monolayers had reached confluency.

To study the effect of PDGF on protein degradation and nuclear translocation of proteins, cellular proteins were labeled by incubating the cells in minimal essential medium (15–20 ml/culture) containing 2 μCi/ml of [^{3}H]leucine for 48 hr. The normal leucine concentration of 0.8 m*M* was reduced to ⅓ by mixing minimal essential medium with leucine-deficient minimal essential medium. The medium contained 0.5–1.0% NBCS to induce the cells into stationary growth.

Exposure of Cells to Growth Conditions. The labeled cultures were washed three times with 10 ml of Hanks' solution mixed ⅓ with DME followed by incubation in 10 ml of DME containing various concentrations of PDGF for 24 hr. Control cultures were without growth factor. The medium contained 2 mg/ml of bovine serum albumin and 2 m*M* leucine, the latter to minimize reincorporation of ^{3}H-labeled degradation products into newly synthesized proteins (12). At the end of incubation, the medium was collected and the cells, after removal by trypsinization, were sedimented and frozen as a pellet at −20°C. Acid-soluble ^{3}H in the medium was determined as described, and represented the degradation products (1).

The cellular pool of acid-soluble ^{3}H-labeled degradation products was determined in three cultures each of control cells and PDGF-stimulated cells after 24 hr of incubation. The medium was removed and the acid-soluble ^{3}H was determined. The cultures were submerged in ice water and washed four times with 10 ml of 10% trichloroacetic acid. Aliquots of the latter extract, assayed for radioactivity, served to measure the cellular pool of ^{3}H.

Isolation of Nuclei and Determination of NHP and DNA. The procedures have been described in detail previously (1). Briefly, the frozen cell pellet was dispersed in 4 ml of fluid composed of 10 m*M* Tris-HCl (pH 8) in water, 0.32 *M* sucrose, 30 m*M* $CaCl_2$, and 0.5% Triton X-100. The cells were homogenized 20 strokes in 7-ml Dounce homogenizers. The nuclei were spun down at 500*g* for 5 min and the supernatant was discarded.

This procedure was repeated once, and then the nuclei were washed twice in the same fluid. Previous investigators (13) have compared the total protein content of nuclei by the present aqueous and a nonaqueous method and could find no significant difference. The nuclei were extracted twice for 10 min with 1.5 ml 0.25 *M* HCl to extract the histones. The remaining pellet was dissolved in 3 ml of 0.2 *M* NaOH. This procedure was carried out at 4°C. Cell pellets of one experiment were processed in parallel using one set of homogenizers (Deltaware; Kimble) for which tight-fitting pestles had been selected. Aliquots of the above samples were used to determine protein, DNA, and radioactivity in duplicate cultures, as described previously (1). Measurements of total DNA per culture served to determine growth. During homogenization, a small fraction of the cytoplasmic ^{3}H-labeled proteins adsorbed to the nuclei. This was determined by adding the cytoplasmic fraction of labeled cells to an equal number of unstimulated unlabeled cells, followed by homogenization. The results showed that the fraction of labeled material bound to the nuclei amounted to 5–15% of the nuclear ^{3}H of labeled nuclei. Cells stimulated with PDGF adsorbed similar amounts of ^{3}H as unstimulated cells. Similar results were obtained in previous studies with lymphocytes (14).

Determination of Cellular [^{3}H]Chloroquine Uptake. The determination was performed as described (1). Culture tubes of 16 × 125 mm were inoculated with 2.5 × 10^5 NRK cells/2 ml of medium containing 0.5% NBCS and slanted for 48 hr. The cells were then washed twice with DME, followed by incubation in 3 ml of medium containing various concentrations of PDGF. The medium of the controls was without PDGF. [^{3}H]Chloroquine (5 μg/ml) was added to each culture at Time 0 and incubation lasted for 4 hr. The media in all of the tubes were of the same pH before and after incubation. The cells were washed three times with ice-cold Hanks' solution, followed by dissolving the cells in 1 ml of 0.2 *M* NaOH. To retain the accumulated [^{3}H]chloroquine in the cells, it is essential that, during the washing procedure, the cells are kept in an ice bath.

Isoelectric Focusing in Gels of Nuclear Proteins. This procedure was carried out as described in detail previously (1), with minor modifications. After isolation and washing as described above, the nuclei were suspended in 1 ml of wash fluid and transferred to 5-ml, thick-walled centrifuge tubes. The pellets were sedimented, the tubes were inverted and drained on absorbant paper, and the pellets were suspended in 0.6 ml of 8 *M* urea. After 2 hr of continuous stirring, using small magnetic stirring bars, the DNA was spun down at 30,000 rpm for 16 hr in a Beckman centrifuge using

a SW-55 Ti rotor. The total supernatants of one experiment were used to cast and focus gels in parallel. After focusing at 200 V for 16 hr at 4°C, the gels were frozen and cut in 0.5-cm segments using a gel slicer. The pH of each slice was determined to 0.05 pH accuracy, after placing the slices in 0.5 ml of 0.01 *M* KCl for 1 hr. Among gels cast and focused in parallel, gel slices of the same number had the same pH ± 0.05 with very few exceptions. Gel slices were dissolved by the addition of 1.5 ml of 30% H_2O_2 and incubation at 75°C overnight, followed by assay for radioactivity.

Results

Effects of PDGF on Protein Degradation, Translocation of NHP to the Nucleus, and DNA Synthesis. When monolayers reached confluence, the cells were incubated in minimal essential medium containing 0.5–1% NBCS for 48 hr in order to induce stationary growth. During this period, the cellular proteins were labeled with [^{3}H]leucine. At the end of labeling, the cells were washed and incubated for 24 hr in unlabeled DME containing various concentrations of PDGF, parallel to controls without growth factor. The results (Fig. 1) showed that PDGF inhibited proteolysis; in parallel, it increased the translocation of ^{3}H-NHP from the cytoplasm to the nucleus and stimulated DNA synthesis. These effects were dependent upon the concentration of PDGF. The increased nuclear translocation of NHP was slightly higher for the nonlabeled than for the ^{3}H-labeled variety in the presence of 20 and 40 ng/ml of PDGF and appeared significant only at the latter concentration of growth factor.

The decreased release of acid-soluble ^{3}H in the medium by the cells induced by PDGF indicated inhibition of proteolysis and was not due to retention of degradation products by the cells. This was shown by the following experiment, described in Materials and Methods. Prelabeled NRK cells, prepared as described above, incubated with 40 ng/ml of PDGF for 24 hr released a total of $1.543.10^6$ cpm of acid-soluble ^{3}H in the medium, while controls released $1.236.10^6$ cpm, i.e., PDGF reduced the cellular output of ^{3}H by 307.10^3 cpm or 20%. The ^{3}H-labeled, acid-soluble pool of the cells was $17.4.10^3$ cpm in PDGF-treated cells and $11.15.10^3$ cpm in controls, i.e., PDGF-stimulated cells contained 6250 cpm more than the control cells, accounting for 2% of the difference in the released ^{3}H between the two kinds of cells.

Influence of Blocking Protein Synthesis. The question can be raised of whether the growth factor-induced increase of the nuclear level of ^{3}H-NHP was due to increased protein synthesis, which takes place in cells stimulated to grow, despite the fact that reincorporation of degradation products from ^{3}H-labeled protein had been minimized by the addition of 2 m*M* leucine to the medium (12). This question was investigated by blocking protein synthesis with cycloheximide in prelabeled cells during incubation with PDGF, as follows: NRK cells were prelabeled with [^{3}H]leucine for 48 hr, followed by incubation for 24 hr in label-free medium with 40 ng/ml of PDGF or without PDGF and parallel to similar cultures in the presence of 4 μg/ml of cycloheximide. Initial experiments had shown that 2, 4, and 5 μg/ml of the drug inhibited the incorporation of [^{3}H]leucine in the nuclear NHP by 93.5, 95, and 96%, respectively. The results of four experiments showed that the nuclear level of ^{3}H-labeled nonhistone proteins was increased on the average by 28% (22–32%) in the absence of cycloheximide and by 45% (15–67%) in the presence of cycloheximide. Proteolysis was inhibited by 25% (18–38%) in the former and by 13% (13–17%) in the latter. The SE of the results of these experiments were 6% or less for the data on ^{3}H-labeled proteins and 3% or less for the results on proteolysis. The results indicated that presynthesized ^{3}H-NHP had accumulated in the cytoplasm during stationary growth and that these proteins were induced to translocate to the nucleus upon stimulation with PDGF. Also PDGF-induced proteolytic inhibition was observed in both untreated and cycloheximide-treated cells, although the inhibition was less in the latter. This indicated that the reduced release of degradation products from growth factor-stimulated cells was essentially not due to the use of degradation products for protein synthesis. However, the slightly higher nuclear level of nonlabeled NHP as compared with the ^{3}H-labeled variety (Fig. 1) could have been contributed by newly synthesized proteins.

Correlation between Protein Degradation and NHP Translocation. The association of inhibition of cellular proteolysis and increased NHP translocation suggested that these two events may be related. However, the data reflected degradation of total cellular proteins without proving participation by the NHP in proteolysis. To investigate this relationship directly, the extents of proteolysis of the ^{3}H-NHP were determined selectively in resting cells and were compared with their degree of translocation in PDGF-stimulated cells. This was accomplished by fractionating the nuclear proteins by isoelectric focusing in a pH gradient of 2.5–6.5, as follows. Three parallel cultures of NRK cells were induced into stationary growth for 48 hr while being labeled with [^{3}H]leucine. At the end of labeling, one culture was stopped and the second was incubated in unlabeled medium without PDGF for 24 hr. These two cultures served to determine the extents of degradation of the nuclear ^{3}H-NHP in resting cells. The nuclear proteins of these cells were fractionated in a pH gradient of 2.5–6.5. The radioactivity of the nuclear proteins of the second culture, determined as a percentage of that of the first culture, in fractions of similar pH, indicated the extents of degradation of ^{3}H-NHP in resting cells. The third culture was incubated parallel to the second culture, but contained unlabeled medium supple-

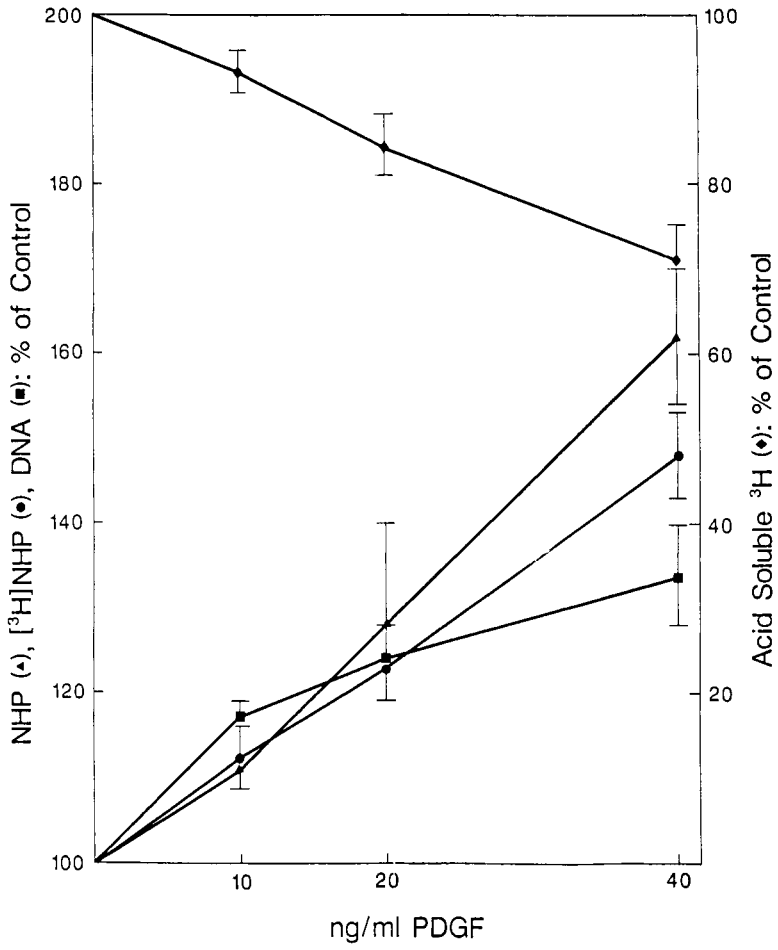

Figure 1. Dose response to PDGF of degradation of cellular proteins (♦), translocation of ^{3}H-labeled (●) and nonlabeled (▲) NHP to the nucleus, and DNA synthesis (■). Proteins of confluent monolayers of NRK cells were labeled in medium containing 0.5–10% NBCS and 2 μCi/ml of [^{3}H]leucine for 48 hr. After washing, the cells were further incubated for 24 hr with various concentrations of PDGF and 2 mg/ml of BSA. The medium of the controls contained bovine serum albumin only. The data represent averages of three experiments with the SE. The SE of the controls was within 5% for DNA and NHP and within 3% for the acid-soluble fraction of the medium. The control values of the three experiments were: acid-soluble ^{3}H in the medium, 129,070, 89,420, and 77,168 cmp/ml; ^{3}H-NHP, 215,877, 57,624, and 130,587 cpm/culture; nonlabeled proteins, 463, 273, 627 μg/culture; and DNA, 115, 122, and 203 μg/culture.

mented with PDGF. This culture served to determine the extents of translocation of ^{3}H-NHP. The nuclear proteins were fractionated in parallel to those of the other cultures. The radioactivity of the nuclear proteins of the third culture, determined as a percentage of the same of the second culture, in fractions of similar pH, showed extents of translocation of ^{3}H-NHP in PDGF-stimulated cells.

The results (Fig. 2) showed that the ^{3}H fractions with a high extent of degradation in resting cells corresponded to the protein fractions with a high extent of translocation in PDGF-stimulated cells. Repeated experiments yielded similar results, showing coincidence according to pH of high extents of degradation and translocation of ^{3}H-NHP fractions in resting and stimulated cells, respectively; however, the distribution of these high extents in the pH gradient varied slightly. Only the acidic nuclear proteins were investigated, because in lectin-activated lymphocytes, it was found that nuclear translocation involved mainly this class of proteins with no significant effect on the nuclear proteins with pI > 7 (1).

In all of the above experiments, PDGF produced by recombinant DNA technology was used. In a few experiments, PDGF purified from human platelets was investigated for its effects on the parameters shown in Figure 1; results were obtained that were similar to those obtained with the synthesized growth factor.

Cellular Uptake of [^{3}H]Chloroquine. To investigate whether PDGF inhibits proteolysis via the lysosomal or extralysosomal pathway, PDGF was tested for an effect on the uptake of [^{3}H]chloroquine by NRK cells. This drug has been shown to be accumulated markedly and almost exclusively in lysosomes (15). Cultures of NRK cells were incubated for 4 hr with 4, 20, or 40 ng/ml of PDGF, parallel to controls without PDGF. The results (Fig. 3) showed that PDGF inhibited cellular [^{3}H]chloroquine accumulation significantly.

Discussion

Growth factors induce stationary cells to grow and multiply; however, the mechanism is not known. When

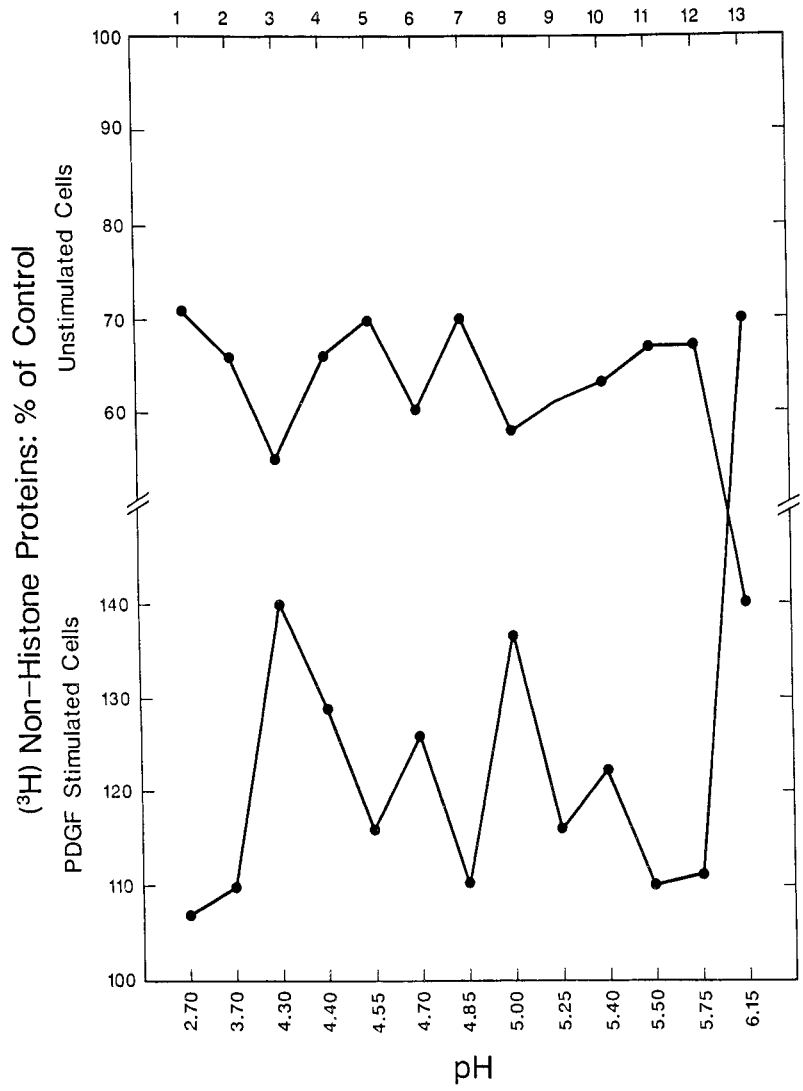

Figure 2. Comparison of rates of degradation of ^{3}H-NHP in unstimulated cells (upper curves) to rates of translocation of ^{3}H-NHP in NRK cells stimulated with PDGF (lower curves) after fractionation of the proteins in a pH gradient of 2.5–6.5. From three cultures with prelabeled proteins, one (Gel 1) was stopped at Time 0, and the two others were incubated for 24 hr, (Gel 2) without PDGF or (Gel 3) with 40 ng/ml of PDGF. Nuclear proteins were fractionated by isoelectric focusing in gels. The amount of ^{3}H in Gel 2, expressed as the percentage of ^{3}H in Gel 1, shows rates of degradation in resting cells. The amount of ^{3}H in Gel 3, expressed as the percentage of ^{3}H in Gel 2, shows rates of translocation in PDGF-stimulated cells. Proteolysis was inhibited by 24%. Total cpm in the gels after focusing were: Gel 1, 15,488; Gel 2, 8,910; and Gel 3, 13,179.

PDGF binds to its receptor, it stimulates a series of early events: among others, activation of tyrosine kinase, hydrolysis of phophatidylinositol, altered cellular pH, increased cellular calcium, increased expression of c-*myc* and c-*fos* protooncogenes, and internalization with degradation of the receptor (16). However, it is not understood how these events lead to DNA synthesis.

Role of Protein Degradation in Cell Proliferation. In earlier investigations, no relationship could be found between protein degradation and cell growth (17, 18). Subsequently, Warburton and Poole (19) showed a close correlation between inhibition of degradation of long-lived proteins and DNA synthesis in growth factor-stimulated fibroblasts, and concluded that these events are likely to be related.

The study of synthesis and degradation of proteins in liver regenerating after partial hepatectomy has produced one of the best documented examples of the role of degradative processes in the regulation of cell mass. The contribution of increased synthesis of long-lived proteins to liver cell regeneration is small, while the decreased rate of degradation of these proteins proves to be the most important factor (20). Extensive studies in liver tissue (21, 22) and liver cells in culture (23), have shown that lysosomes participate in both short-lived and long-lived protein degradation. The species of proteins causing regeneration has not yet been identified. The present studies suggest that the NHP could participate in such a role.

Initiation of cell growth in stationary cells is also associated with a rising nuclear level of NHP (1, 13).

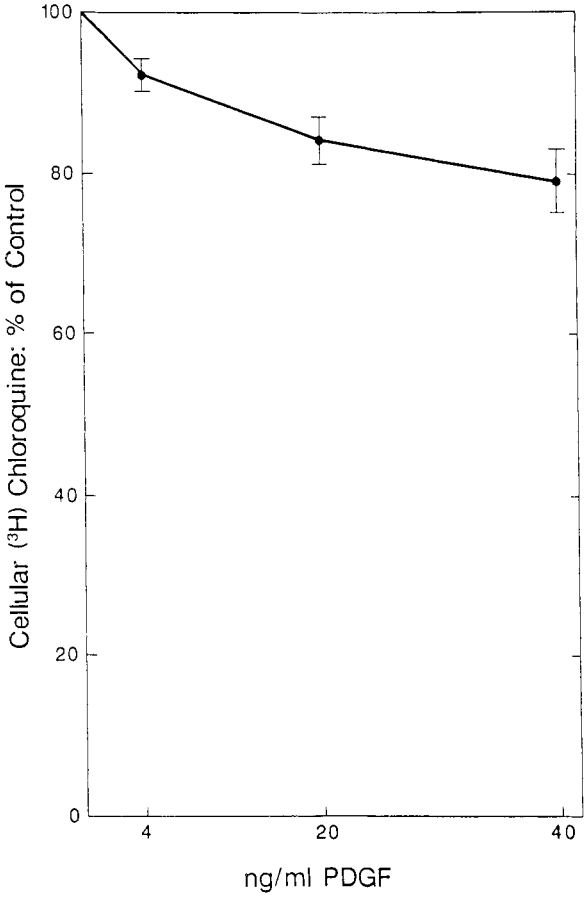

Figure 3. Effect of PDGF on the cellular uptake of [^{3}H]chloroquine. Confluent monolayers of NRK cells, prepared in 16 × 125-mm culture tubes, were incubated for 48 hr in medium with 0.5% NBCS. After washing, the cells were incubated with 2 ml of medium containing various concentrations of PDGF, 5 μg/ml of [^{3}H]chloroquine, and no bovine serum albumin for 4 hr. The results are averages of two experiments with SE, with triplicate cultures in each experiment. The controls of each experiment contained 126.10^3 and 143.10^3 cpm.

The present results (Fig. 1) showed that PDGF induced three parallel effects: it inhibited cellular proteolysis, increased the translocation of ^{3}H-NHP from the cytoplasm to the nucleus, and stimulated DNA synthesis. The association suggests that these events may be related, i.e., that the increased nuclear translocation of these proteins may be the result of their inhibited degradation. This is supported by comparing the extents of degradation of the ^{3}H-NHP of resting cells to the extents of translocation of these proteins in PDGF-stimulated cells after fractionation of these proteins in a pH gradient of 2.5–6.5. These results (Fig. 2) showed that the ^{3}H-NHP fractions with the highest extent of degradation in resting cells corresponded to the protein fractions of the same pI with the highest extent of nuclear translocation in PDGF-stimulated cells. Degradation rates of the various NHP species are heterogeneous (24). If the translocation of these proteins to the nucleus is the result of their inhibited degradation, it would be expected that the proteins with the highest turnover rate would have the highest translocation rate. This is exactly what the data show. The proteins migrating to the nucleus would then lead to stimulation of DNA synthesis, since NHP are known to increase gene activity (see Introduction).

Role of Lysosomes in NHP Degradation. The finding that PDGF inhibited cellular uptake of [^{3}H]chloroquine indicates that it is lysosomotropic and suggests, but does not prove, that PDGF inhibits proteolysis via the lysosomes. The mechanism of action is not clear, it is most likely that PDGF increases the lysosomal pH. One could argue that PDGF inhibited [^{3}H]chloroquine uptake at the level of the cell membrane instead of by a lysosomal mechanism. While this possibility cannot be excluded, it is highly unlikely, since chloroquine, after entry into the cell by diffusion, is accumulated very rapidly and massively into lysosomes (15, 25, 26). There is no evidence that PDGF or any growth factor inhibits the diffusion of molecules through the cell membrane. However, it is known that certain growth factors can alter the cytoplasmic (27) or lysosomal pH (28). That lysosomes play a role in the degradation and nuclear translocation of ^{3}H-NHP has been shown by the effects of eserine and other lysosomotropic amines. These agents are known to increase the lysosomal pH (28), to inhibit cellular [^{3}H]chloroquine uptake and cathepsin D activity, and to inhibit strongly cellular proteolysis parallel to a markedly increased nuclear translocation of the ^{3}H-NHP. With these agents, it has further been observed that the ^{3}H-NHP fractions with the highest proteolytic rates in resting cells correspond to the protein fractions with the highest translocation rates in drug-treated cells (2–4).

The similarity between the effects of PDGF and the lysosomotropic amines on proteolysis and translocation of the ^{3}H-NHP supports the contention that the translocation of these proteins into the nucleus is linked to and is the result of their inhibited lysosomal breakdown. The mechanism of this linkage is not clear, because the process of lysosomal segregation of cellular proteins is poorly understood.

The NHP represent a large group of nuclear proteins; two-dimensional gel electrophoresis has identified 300 protein spots in the nucleus (29). Very few of these proteins have been purified and characterized. It is hoped that future investigations will reveal their specific role in cellular growth control and their turnover rate. In this respect, it is of interest that several oncoproteins, i.e., N-*myc*, C-*myc*, C-*fos*, p53, and E1A proteins, have been shown to have a short half-life (30). Short-lived proteins are believed to be degraded mainly by the ubiquitin pathway (31) and recent investigations have shown this to be the case, using an *in vitro* ubiquitin system (32). However, our knowledge of the various proteolytic pathways in the cell is only beginning to be understood (31). Although the lysosomal pathway degrades mainly long-lived proteins, it has been shown that this pathway is also involved in the degradation of

short-lived proteins (1–5, 21). Perhaps the cell may switch from one degradation pathway to another for certain or many proteins, depending on physiologic conditions (31). However, proteolysis via the ubiquitin pathway could play a role in cellular growth control as well.

The above results are similar to the ones obtained with concanavalin A in lymphocytes (1), fibroblast growth factor and serum in diploid cells (2), epidermal growth factor in diploid and transformed cells (51), with growth factors produced by autostimulating transformed cells (4), and with certain amino acids (33).

This research was supported by Grant R01-CA37197 from the National Cancer Institute.

1. Polet H. The effects of concanavalin A and other agents on protein degradation and migration of non-histone proteins (NHP) to the nucleus in lymphocytes. Exp Cell Res **148**:345–362, 1983.
2. Polet H. The effects of lysosomotropic amines on protein degradation, migration of nonhistone proteins to the nucleus, and cathepsin D in lymphocytes. J Cell Physiol **122**:415–423, 1985.
3. Polet H. Effects of fibroblastic growth factor on protein degradation, the migration of non-histone proteins to the nucleus and DNA synthesis in diploid fibroblasts. Exp Cell Res **169**:178–190, 1987.
4. Polet H, Swager JS. The effects of serum and conditioned medium on protein degradation, migration of non-histone proteins to the nucleus, and DNA synthesis in transformed cells. J Cell Physiol **130**:436–443, 1987.
5. Polet H, Fryxell D. Effects of epidermal growth factor on protein degradation, the translocation of non-histone proteins to the nucleus and DNA synthesis. Biochim Biophys Acta **1013**:279–286, 1989.
6. Allfrey VG. DNA binding proteins and transcriptional control in prokaryotic and eukaryotic systems. In: Cameron IL, Jeter JR Jr, Eds. Acidic Proteins of the Nucleus. New York: Academic Press, pp1–20, 1974.
7. LeStourgeon WM, Totten R, Forer A. The nuclear acidic proteins in cell proliferation and differentiation. In: Cameron IL, Jeter JR Jr, Eds. Acidic Proteins of the Nucleus. New York: Academic Press, pp159–190, 1974.
8. Allfrey VG, Inoue A, Karn J, Johnson EM, Vidali G. Phosphorylation of DNA-binding nuclear acidic proteins and gene activation in the Hela cell cycle. Cold Spring Harbor Symp Quant Biol **38**:785–801, 1973.
9. Nicolini C, Ng S, Baserga R. Effect of chromosomal proteins extractable with low concentrations of NaCl on chromatin structure of resting and proliferating cells. Proc Natl Acad Sci USA **72**:2361–2365, 1975.
10. Polet H, Spieker-Polet H. Role of nuclear proteins on [^{3}H] actinomycin D binding during lymphocyte mitogenesis. Exp Cell Res **128**:419–429, 1980.
11. Stein G, Chanduri S, Baserga R. Gene activation in WI-38 fibroblasts stimulated to proliferate. J Biol Chem **247**:3918–3922, 1972.
12. Bradley MO, Hayflick H, Schimke RT. Protein degradation in human fibroblasts (WI-38). Effects of aging, viral transformation, and amino acid analogs. J Biol Chem **251**:3521–3529, 1976.
13. Johnson EM, Karn J, Allfrey VG. Early nuclear events in the induction of lymphocyte proliferation by mitogens. J Biol Chem **249**:4990–4999, 1974.
14. Polet H. Endocytosed non-histone proteins translocate in part to the nucleus in lymphocytes. Exp Cell Res **166**:455–464, 1986.
15. Wibo M, Poole B. Protein degradation in cultured cells. II: The uptake of chloroquine by rat fibroblasts and the inhibition of cellular protein degradation and cathepsin B. J Cell Biol **63**:430–440, 1974.
16. Williams LT. Signal transduction by the platelet derived growth factor receptor. Science **243**:1564–1570, 1989.
17. Goldberg AL, St John AC. Intracellular protein degradation in mammalian and bacterial cells: Part 2. Annu Rev Biochem **45**:747–803, 1976.
18. Bradley MO. Regulation of protein degradation in normal and transformed cells. J Biol Chem **252**:5310–5315, 1977.
19. Warburton WJ, Poole B. Effect of medium composition on protein degradation and DNA synthesis in rat embryo fibroblasts. Proc Natl Acad Sci USA **74**:2427–2431, 1977.
20. Scornik O, Botbol V. Protein metabolism and liver growth. In: Glaumann H, Ballard JF, Eds. Lysosomes: Their Role in Protein Breakdown. New York: Academic Press, pp445–484, 1987.
21. Marzella L, Glaumann H. Autophagy, microautophagy and crinophagy as mechanisms for protein degradation. In: Glaumann, H, Ballard JF, Eds. Lysosomes: Their Role in Protein Breakdown. New York: Academic Press, pp319–367, 1987.
22. Mortimore GE, Poso AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. Annu Rev Nutr 7:539–564, 1987.
23. Seglen PO, Gordon PB. Amino acid control of autophagic sequestration and protein degradation in isolated rat hepatocytes. J Cell Biol **99**:435–444, 1984.
24. Dice FJ, Schimke RT. Turnover of chromosomal proteins from rat liver. Arch Biochem Biophys **158**:97–105, 1973.
25. de Duve D, de Barsy T, Poole B, Trouet A, Tulkens P, Van Hoof F. Commentary. Lysosomotropic agents. Biochem Pharmacol **23**:2495–2531, 1974.
26. Polet H. Chloroquine-^{3}H: Mechanism of uptake by Chang liver cells in vitro. J Pharmacol Exp Ther **199**:687–694, 1976.
27. Molenaar WH. Effects of growth factors on intracellular pH regulation. Annu Rev Physiol **48**:363–376, 1986.
28. Poole B, Ohkuma O. Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages. J Cell Biol **90**:665–669, 1981.
29. Peterson JL, McConkey H. Non-histone chromosomal proteins from Hela cells. A survey by high resolution, two-dimensional electrophoresis. J Biol Chem **251**:548–554, 1976.
30. Rogers S, Wells R, Rechsteiner M. Amino acid sequences common to rapidly degraded proteins: The PEST hypothesis. Science **234**:364–368, 1986.
31. Hershko A, Ciechanover A. Mechanism of intracellular protein breakdown. Annu Rev Biochem **51**:335–364, 1982.
32. Ciechanover A, DiGiuseppe JA, Bercovich B, Orian A, Richter JD, Schwatz AL, Brodeur GM. Degradation of nuclear oncoproteins by the ubiquitin system in vitro. Proc Natl Acad Sci USA **88**:139–143, 1991.
33. Polet H, Molnar J, Goral J. The effects of amino acids on protein degradation and translocation of non-histone proteins to the nucleus in lymphocytes. Biochim Biophys Acta **886**:33–39, 1986.