Proc. Natl. Acad. Sci. USA
Vol. 77, No. 8, pp. 4746–4750, August 1980
Cell Biology

Regulation of the production of secretory proteins: Intracellular degradation of newly synthesized "defective" collagen*

(hydroxyproline/microtubules/microfilaments/proteases/lysosomes)

RICHARD A. BERG, MARTIN L. SCHWARTZ, AND RONALD G. CRYSTAL

Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

Communicated by Donald S. Fredrickson, April 22, 1980

ABSTRACT **Confluent cultures of human fetal lung fibroblasts degrade approximately 10% of their newly synthesized collagen within the cell prior to secretion. This basal level of intracellular degradation could not be inhibited by colchicine or cytochalasin B, inhibitors of microtubular and microfilament function, respectively, or by N^{α}-*p*-tosyl-L-lysine chloromethyl ketone, chloroquine, or NH_4Cl, inhibitors of lysosomal enzymes. In contrast, cells in early logarithmic growth degrade approximately 30% of their newly synthesized collagen. This enhanced degradation of collagen in rapidly growing cells could be suppressed by inhibitors of lysosomal proteases and partially inhibited by disrupters of microtubular and microfilament function. A significant proportion of the collagen synthesized by these cultures contained prolyl residues that were incompletely hydroxylated. Because such collagen is "defective" (i.e., not capable of assuming a triple helical conformation), the results suggest that enhanced intracellular degradation may be a mechanism by which cells control the quality of collagen they produce. To test this hypothesis, confluent cells were incubated with the proline analog *cis*-4-hydroxyproline; such cells demonstrated enhanced collagen degradation that could be inhibited by agents that interfere with lysosomal, microtubular, or microfilament function. Because collagen containing *cis*-4-hydroxyproline cannot form a perfect triple helix, the data are consistent with the concept that defective collagen is recognized by cells and degraded prior to secretion. Thus, the proportion of newly synthesized collagen that undergoes intracellular degradation seems to be modulated, in part, by the conformation of the collagen molecule. Intracellular proteolysis may represent a means by which collagen-producing cells regulate the quality and quantity of collagen available for extracellular function. Although the exact mechanism of intracellular collagen degradation is unknown, the data presented here are consistent with a role for lysosomal proteases in this process.**

The normal collagen molecule is a triple helical, rod-like structure. Inherent in this structure is the property to polymerize into extracellular fibers that play a major role in defining organ structure and function (1–4). The ability of collagen to form fibers is dependent on its triple helical conformation. To form this helix, each collagen polypeptide chain must have a specific primary sequence, the most important aspect of which is a repetitive Gly-X-Y triplet; approximately 30% of the X residues are proline and 30% of the Y residues are *trans*-4-hydroxyproline (1–5). Several studies have demonstrated that, if a sufficient number of Y position residues are not filled with hydroxyproline, the resulting collagen molecule is "defective"—i.e., it cannot form a perfect triple helical structure at body temperature (5). Such defective collagen molecules will not polymerize into normal collagen fibers and thus cannot serve their function in the extracellular matrix.

It is reasonable to hypothesize, therefore, that collagen-producing cells might have mechanisms to control the quality of the collagen molecules they produce. In this regard, it has recently been demonstrated that such cells have the capacity to degrade to small peptides significant quantities of newly synthesized collagen within the cell prior to secretion (6, 7). To test this hypothesis, we have evaluated the ability of cultured human lung fibroblasts to degrade newly synthesized defective collagen and have tested compounds that interfere with microtubular, microfilament, or lysosomal systems to determine the role of these systems in degradation of this collagen.

MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's medium and phosphate-buffered saline (pH 7.4) were prepared by the Media Unit, National Institutes of Health. Chloroquine, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK), *N*-ethylmaleimide, ascorbate, glutamine, and β-aminopropionitrile fumarate (BAPN) were obtained from Sigma. Colchicine and *cis*-4-hydroxyproline were from Calbiochem. Cytochalasin B was from Aldrich and leupeptin was from the Protein Research Foundation (Osaka, Japan). [^{14}C]Proline was obtained from either Schwarz/Mann or Amersham/Searle, Centriflo(R) membrane cones (CF25) were obtained from Amicon (Lexington, MA), and Dowex 50W-X8 cation exchange resin, 100–200 mesh, hydrogen form, was from Bio-Rad.

Purification of [^{14}C]Proline. Various commercial preparations of [^{14}C]proline were found to have up to 1% contaminants eluting from the amino acid analyzer in the region of HO[^{14}C]proline (Beckman resin PA28; 0.2 M Na citrate, pH 3.25). To determine accurately the amount of HO[^{14}C]proline synthesized by cells and degraded to small peptides, the contaminants had to be removed prior to utilizing the [^{14}C]proline for labeling cultured cells. To ensure that radiochemically pure proline was used, all [^{14}C]proline used was chromatographed on a cation exchange column (Dowex, 50W-X8; 0.9 × 30 cm) and eluted with 2 M HCl (8) to separate [^{14}C]proline from the contaminating material eluting in the region of HO[^{14}C]proline. The proline peak was pooled, lyophilized, and dissolved in 20 mM Tris·HCl (pH 7.4). This procedure reduced the level of contamination to <0.1% which was low enough not to interfere with subsequent determinations of HO[^{14}C]proline. The source of the residual contaminants is not known, but since they increased during storage in 2 M HCl, they may result from the oxidative decomposition of [^{14}C]proline.

Cell Cultures. Normal diploid human fetal lung fibroblasts,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone HCl; BAPN, β-aminopropionitrile.

* A preliminary account of this work was presented at the *63rd Annual Meeting of the Society of Biological Chemists*, Dallas, Texas, April 1–10, 1979.

HFL-1 (American Tissue Culture Collection CCL 153), between the 10th and 20th passages, were used for all studies. The cells were maintained and subcultivated at 37°C in growth medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units of penicillin and 100 μg of streptomycin per ml, and 0.06% glutamine) as described (6). Cells to be studied in the early logarithmic phase of growth were plated at 2.5×10^6 cells per 150-mm culture dish and incubated for 2 days in 20 ml of growth medium. Cells to be studied in the confluent phase of growth were plated at 10^6 cells per 100-mm culture dish and incubated for 5 days in 10 ml of growth medium.

Quantification of Intracellular Collagen Degradation. Twenty-four hours before labeling, the medium was changed to growth medium containing 50 μg of BAPN per ml; this change was repeated 1 hr prior to labeling. The cells were then rinsed three times with phosphate-buffered saline (pH 7.4) and incubated for 0.5–12 hr at 37°C with 4 ml (for 100-mm plates) or 6 ml (for 150-mm plates) of labeling medium (Dulbecco's modified Eagle's medium supplemented with 10% dialyzed fetal calf serum, 100 units of penicillin and 100 μg of streptomycin per ml, 0.06% glutamine, and 50 μg of ascorbic acid and 50 μg of BAPN per ml). The fetal calf serum was included for two reasons: (*i*) to avoid enhanced intracellular degradation resulting from so-called nutritional step-down (9); and (*ii*) to provide a source of antiprotease in the extracellular milieu and thus avoid confusion between intracellular and extracellular destruction of newly synthesized collagen. The fetal calf serum was dialyzed to remove amino acids, thus maximizing the specific activity of the [^{14}C]proline tracer in the labeling medium.

After labeling, the cell cultures were scraped with a rubber policeman and rinsed with 6 ml of 0.2 M NH_4HCO_3. The entire contents of the cultures were heated in plastic tubes at 100°C for 15 min to destroy proteolytic enzymes that might cause collagen degradation during the analytical procedures. The samples were then sonicated and divided into two portions. One portion was placed on an Amicon Centriflo filtration cone over a plastic centrifuge tube and centrifuged ($600 \times g$, 1 hr) to separate small peptides and free amino acids from protein. The filtrates were lyophilized and then hydrolyzed in 6 M HCl for 16 hr at 115°C and analyzed to obtain the amount of HO[^{14}C]proline in the cultures that was present in small peptides. The second portion was lyophilized and then hydrolyzed directly to obtain the total amount of HO[^{14}C]proline synthesized in the cultures. The hydrolysates were evaporated in a rotary evaporator, dissolved in 0.067 M sodium citrate buffer, and chromatographed on an amino acid analyzer ion exchange resin (Beckman PA-28) in a 0.9×50 cm glass column at 50°C in 0.067 M sodium citrate buffer at pH 3.25. The effluent from the column was collected in scintillation vials as 2-ml fractions and the amount of HO[^{14}C]proline was quantified as described (6, 7, 10). The percentage of collagen degraded in each culture was then quantified by dividing the amount of HO[^{14}C]proline appearing in the filtrate by the total HO[^{14}C]proline synthesized by the culture.

To evaluate the proportion of newly synthesized collagen that was secreted in an intact form, the media and cell layers of the cultures were evaluated separately for their content of HO[^{14}C]proline. These samples were then analyzed as described above for the relative proportions of the HO[^{14}C]proline that was in an intact or degraded form. The proportion of newly synthesized collagen that was secreted intact was quantified as the amount of nondialyzable HO[^{14}C]proline in the medium divided by the total HO[^{14}C]proline synthesized in the culture.

Modulation of Intracellular Collagen Degradation. To evaluate whether nonhelical, defective collagen molecules were degraded intracellularly to a greater extent than collagen molecules produced normally by confluent HFL-1 cells, 1.5 mM *cis*-4-hydroxyproline was included in the labeling medium. This concentration of *cis*-4-hydroxyproline is sufficient to prevent triple helix formation in collagen produced by cultured cells yet not interfere with collagen synthesis (11, 12).

To evaluate a possible role for microtubular, microfilament, and lysosomal processes in intracellular collagen degradation, early logarithmic and confluent cells were labeled for 4 or 6 hr in the presence of various compounds including: 1 mM colchicine, cytochalasin B at 10 μg/ml, 10 μM TLCK, 0.1 mM chloroquine, 25 mM NH_4Cl, or leupeptin at 50 μg/ml. Colchicine was used as a probe for microtubular processes, cytochalasin B for microfilament processes, and TLCK, chloroquine, NH_4Cl, and leupeptin as inhibitors of intracellular proteases, particularly those associated with the lysosomal system. The concentrations of colchicine, cytochalasin B, and TLCK were chosen, from preliminary studies, as the maximal concentration that could be added without inhibiting protein synthesis.

Extent of Prolyl Hydroxylation in Newly Synthesized Collagen. To determine the extent of hydroxylation of prolyl residues in collagen synthesized by HFL-1 fibroblasts under different conditions, the [^{14}C]proline-labeled cultures were heated to 100°C for 15 min and then dialyzed exhaustively against deionized water and lyophilized. Each sample corresponding to a single culture was dissolved in 2 ml of 5 mM $CaCl_2$/2.5 mM *N*-ethylmaleimide/10 mM Tris·HCl, pH 7.5. A 1-ml aliquot was incubated with 50 units of bacterial collagenase (Advance Biofactures, type III, Lynbrook, NY) for 12 hr at 20°C; as a control, the second 1-ml aliquot was incubated in the absence of collagenase (13, 14). Prior to use, the bacterial collagenase was tested for effectiveness and purity with [^{14}C]tryptophan-labeled cellular proteins (13). After collagenase digestion, the samples were dialyzed against 10 vol of H_2O and the dialyzed peptides were hydrolyzed and evaluated for the percentage of ^{14}C-labeled prolyl residues that were hydroxylated (14, 15).

Statistical Methods. All data are presented as mean ± SEM. Differences in intracellular collagen degradation in various experimental conditions were evaluated by using the Wilcoxon Mann–Whitney rank sum test for nonparametric analysis (16).

RESULTS

Confluent cultures of HFL-1 fibroblasts ($5.9 \pm 0.4 \times 10^4$/cm^2) degraded 9.1 ± 1.0% of their newly synthesized collagen within the cells prior to secretion (Fig. 1). This process represents the degradation of a constant fraction of newly synthesized collagen (6). For example, when the labeling period was varied from 0.5 to 12 hr, the degradation remained approximately 10% (data not shown). Under the conditions used in these studies, HFL-1 fibroblasts do not have any active collagenase in the culture medium (7). In addition, when ^{14}C-labeled type I and type III procollagens were added to the media of HFL-1 cultures, >98.5% of the added procollagen was recovered undegraded after 12 hr, suggesting that HFL-1 cells do not degrade procollagen molecules that have been secreted.

Addition of colchicine to the confluent cultures caused the cells to lose their stellate, flattened appearance and become rounded. However, it did not affect the proportion of newly synthesized collagen that was degraded intracellularly (Fig. 1). Cytochalasin B had similar effects on the morphologic appearance of the cells and likewise had no effect on collagen

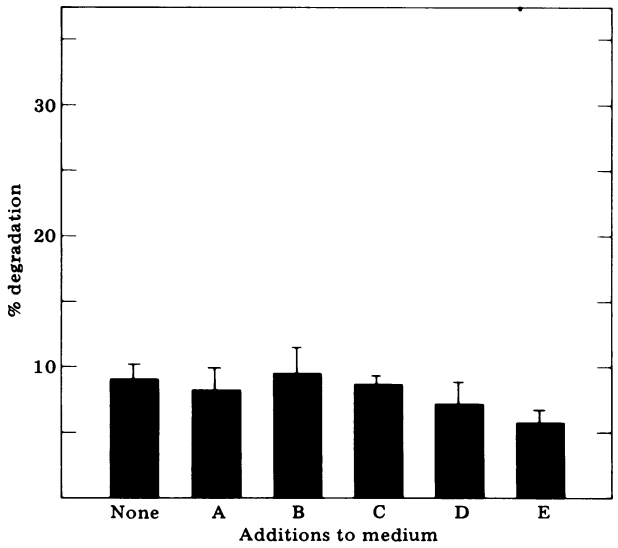

FIG. 1. Intracellular degradation of newly synthesized collagen by confluent cultures of HFL-1 fibroblasts. At the time of labeling with [^{14}C]proline, parallel cultures received colchicine (A), cytochalasin B (B), TLCK (C), chloroquine (D), or NH_4Cl (E). The proportion of newly synthesized collagen that was degraded (% degradation) was determined after 4 hr of incubation. Data are presented as mean ± SEM; each mean represents 4–10 cultures. Under the conditions utilized, the additions to the culture had no significant effect on degradation.

degradation. Under the conditions used, TLCK, chloroquine, and NH_4Cl also had no effect on intracellular collagen degradation by confluent cells, but both chloroquine and NH_4Cl caused the cells to appear vesiculated. Thus, confluent fibroblasts exhibit a small, but constant, level of collagen degradation that is not significantly influenced by compounds that affect microtubular, microfilament, or lysosomal processes within cells.

In contrast to the cells that had reached confluency, HFL-1 cells in the early logarithmic phase of growth ($1.8 \pm 0.1 \times 10^4/cm^2$) degraded large amounts of collagen intracellularly ($P < 0.05$ compared to confluent cells) (Fig. 2). In addition, a significant proportion of the collagen degradation by the early logarithmic cells could be inhibited by compounds that had no effect on the collagen degradation by confluent cells. Early logarithmic cells normally degraded 32 ± 4% of the newly synthesized collagen; in the presence of colchicine, cytochalasin B, TLCK, chloroquine, NH_4Cl, or leupeptin, the extent of this degradative process was significantly decreased ($P < 0.05$ for all comparisons). Thus, although the proportion of newly synthesized collagen degraded by early logarithmic fibroblasts was more than 3-fold that of confluent cells, at least some of this enhanced intracellular degradation was inhibited by compounds that interfere with cytoplasmic microtubular, microfilament, or lysosomal systems.

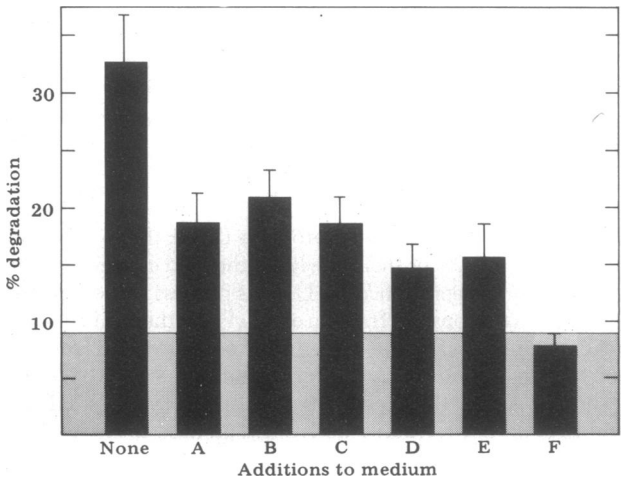

FIG. 2. Intracellular degradation of newly synthesized collagen by early logarithmic cultures of HFL-1 fibroblasts. At the time of labeling with [^{14}C]proline, parallel cultures received colchicine (A), cytochalasin B (B), TLCK (C), chloroquine (D), NH_4Cl (E), or leupeptin (F). The proportion of newly synthesized collagen that was degraded (% degradation) was determined after 6 hr of incubation. Data are presented as mean ± SEM; each mean represents three to eight cultures. In all cases, the additions to the medium significantly decreased the degradation compared to the untreated cultures ($P <$ 0.05 for all comparisons). The shaded area represents baseline degradation of collagen by untreated confluent cultures.

The extent of intracellular collagen degradation exhibited by early logarithmic cells was inversely related to the extent of hydroxylation of the prolyl residues in the newly synthesized collagen produced by these cells. Whereas early logarithmic cells ($1.8 \pm 0.1 \times 10^4$ cells per cm^2) hydroxylated only 25 ± 1% of the prolyl residues within their newly synthesized collagen, they degraded 32 ± 4% of this collagen within the cell prior to secretion. In contrast, confluent cells ($5.9 \pm 0.4 \times 10^4$ cells per cm^2) hydroxylated 47 ± 1% of the prolyl residues within their newly synthesized collagen but degraded only 9.1 ± 1.0% (P

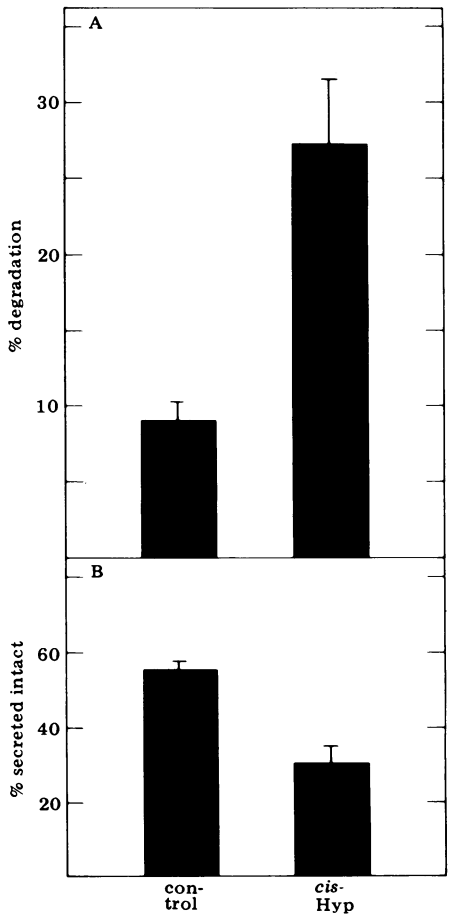

FIG. 3. Processing of defective collagen by HFL-1 fibroblasts. Confluent cells were labeled with [^{14}C]proline in the absence (control) or presence (*cis*-Hyp) of *cis*-4-hydroxyproline; under the conditions used, the collagen synthesized in the presence of *cis*-4-hydroxyproline is nonhelical. (*A*) Proportion of newly synthesized collagen degraded intracellularly (% degraded) during 4 hr of incubation. (*B*) Proportion of all newly synthesized collagen secreted into the culture medium in an intact form (% secreted intact). Data are presented as mean ± SEM.

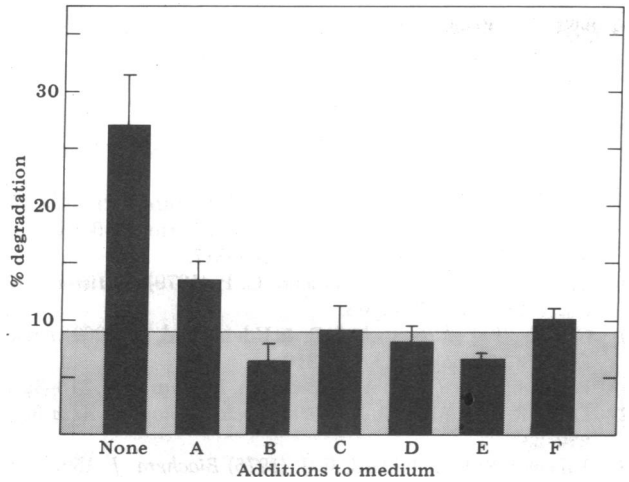

FIG. 4. Intracellular degradation of newly synthesized collagen by confluent cultures of HFL-1 fibroblasts induced to synthesize defective (nonhelical) collagen by incubation in the presence of *cis*-4-hydroxyproline. At the time of labeling with [^{14}C]proline, parallel cultures received nothing in addition to the *cis*-hydroxyproline or they received colchicine (A), cytochalasin B (B), TLCK (C), chloroquine (D), NH_4Cl (E), or leupeptin (F). The proportion of newly synthesized collagen that was degraded intracellularly (% degradation) was determined after 4 hr of incubation. Data are presented as mean ± SEM; each mean represents 4–10 cultures. In all cases, the additions to the medium significantly reduced the percent degradation of the defective collagen below that of the confluent cultures incubated only with *cis*-4-hydroxyproline ($P < 0.05$, all comparisons). The shaded area represents baseline degradation of collagen by untreated confluent cultures.

< 0.05). Because collagen with underhydroxylated prolyl residues is defective in that it cannot form a normal triple helix at body temperature (5), the inverse relationship between the extent of hydroxylation and the extent of degradation suggest that defective newly synthesized collagen may be more susceptible to intracellular degradation than is normal triple helical collagen.

To test this hypothesis, confluent cells were incubated with *cis*-4-hydroxyproline, a proline analog that is incorporated as prolyl residues into the collagen molecule and prevents the molecule from assuming a normal triple helical conformation. Under these conditions, confluent cells increased the fraction of newly synthesized collagen that was degraded from 9.1 ± 1.0% to 27 ± 4% ($P < 0.01$) (Fig. 3*A*). Increasing the concentration of *cis*-4-hydroxyproline in the labeling medium from 1.5 to 5 mM had no effect on increasing the fraction of collagen degraded in confluent cultures. At least one result of this enhanced degradation was a marked decrease in the proportion of the newly synthesized collagen secreted by the cells in an intact form (Fig. 3*B*). Whereas confluent fibroblasts normally secreted 55 ± 2% of newly synthesized collagen in an intact form within 4 hr, the confluent fibroblasts exposed to *cis*-4-hydroxyproline secreted only 30 ± 6% of the newly synthesized collagen in an intact form within the same time ($P < 0.05$). The fraction of intact collagen in the cells at the end of 4 hr was increased slightly, from 36% to 43%. To demonstrate that the newly synthesized procollagen containing *cis*-4-hydroxyproline was degraded intracellularly prior to secretion, ^{14}C-labeled type I and type III procollagens containing *cis*-4-hydroxyproline were added to cultures of HFL-1 cells. Under the conditions used, <2% of this material was degraded in 12 hr, suggesting that once the collagen containing *cis*-4-hydroxyproline was secreted, these cells had no mechanisms to degrade it.

As seen with the increased degradation observed in early logarithmic cells, the increased degradation induced by *cis*-4-hydroxyproline in confluent cells could be significantly inhibited by colchicine, cytochalasin B, TLCK, chloroquine, NH_4Cl, and leupeptin ($P < 0.05$ for all comparisons) (Fig. 4). In all cases, the extent of inhibition induced by these compounds brought the enhanced degradation of *cis*-4-hydroxyproline treated confluent cells close to the baseline degradation of approximately 10% observed in untreated confluent cells. Thus, newly synthesized defective collagen is apparently degraded intracellularly to a greater extent than is normal collagen and this enhanced degradation appears to require microtubular, microfilament, or lysosomal processes to operate.

DISCUSSION

Collagen is a well-characterized extracellular protein that traverses a defined intracellular pathway from its site of synthesis to its secretion (1–5, 17–19). Interestingly, a significant fraction of newly synthesized collagen is degraded to small peptides within the cell prior to secretion (6, 7). The proportion of newly synthesized collagen that undergoes intracellular degradation seems to be modulated, in part, by the conformation of the collagen molecule. In the early logarithmic phase of growth, diploid human fibroblasts synthesize large amounts of collagen that has fewer-than-normal hydroxylated prolyl residues, even though the cells are incubated under conditions optimal for the hydroxylation reactions to occur (20, 21). At 37°C, such underhydroxylated collagen is incompletely helical (5). The cells seem to recognize this and destroy approximately one-third of all such collagen they synthesize. In contrast, when the cells are confluent, the prolyl residues in the newly synthesized collagen are hydroxylated to a normal extent and intracellular collagen degradation is reduced to a low "basal" level.

However, confluent cells can be induced to synthesize defective collagen by exposure to *cis*-4-hydroxyproline, an analog of proline that is incorporated into collagen in place of prolyl residues (12). The configuration of *cis*-4-hydroxyproline is such that, for steric reasons, it will not allow the collagen to form a triple helix (11). Under such conditions, intracellular degradation of collagen is enhanced severalfold.

That cells have mechanisms to degrade defective proteins is well known (22–25). Previously, this mechanism has been considered only in the context of proteins that function within the cell itself, not as a mechanism by which cells control the quality of proteins during secretion. However, it is apparent that intracellular proteolysis is a more general phenomenon and also may be used by cells for the conformation of proteins that function extracellularly. It is important to recognize, however, that such a mechanism is not foolproof. It is known that, under certain conditions, fibroblasts can secrete nonhelical collagen (26). In addition, when early logarithmic cells were incubated with *cis*-4-hydroxyproline or when confluent cells were grown with 5 mM instead of 1.5 mM *cis*-4-hydroxyproline, intracellular collagen degradation did not increase beyond approximately 30%. Thus, even though the conformation of collagen may be a major determinant of whether or not it is degraded intracellularly, fibroblasts clearly have a limited capacity to destroy defective collagen by this mechanism.

The enhanced intracellular degradation of defective collagen likely involves a lysosomal function because it is inhibited by TLCK, chloroquine, NH_4Cl, and leupeptin, all of which are thought to inhibit lysosomal proteases. TLCK, an alkylating agent that can inhibit intracellular proteases within living cells (27, 28), will irreversibly inhibit cathepsin B (29) and cathepsin L (30), major lysosomal endopeptidases. Chloroquine, a quaternary amine which is taken up by fibroblasts and concentrated in lysosomes, also inhibits cathepsin B (31). NH_4Cl increases

intralysosomal pH (32) and, as a result, probably inhibits lysosomal proteolytic enzymes that have acidic pH optima (33). Leupeptin also rapidly enters living cells and seems to inhibit intracellular proteases, particularly those in lysosomes, without disrupting cellular function (34). It inhibits thiol proteases such as cathepsin B (35) and cathepsin L (30) and also inhibits trypsin-like enzymes (36). The data presented here strongly suggest that intracellular degradation of defective collagen is not only mediated by lysosomal proteases but occurs within lysosomes. In support of this concept is the finding that antibodies directed against procollagen stain lysosomes in odontoblasts (19). In addition, it is known that lysosomes play a major role in the degradation of cellular proteins, particularly during enhanced degradation caused by altering cellular nutrition (9, 24, 25, 37–39) or inducing cells to synthesize defective proteins (22–25).

Enhanced intracellular collagen degradation also seems to require cellular microtubular and microfilament function, as does the degradation of cytoplasmic proteins (39). Colchicine retards translocation of secretory proteins such as collagen (40), probably by the association of colchicine with the microtubular apparatus (41). Cytochalasin B also inhibits translocation of collagen (40) but does so by disorganizing microfilaments (42). However, although microtubular and microfilament integrity seems to be required for enhanced intracellular collagen degradation, it is not clear whether the action of inhibitors on these processes is preventing collagen from reaching the site of degradation (e.g., lysosomes) or is preventing proteases from reaching the site of normal collagen translocation.

1. Bornstein, P. & Traub, W. (1979) in *The Proteins*, eds. Neurath, H. & Hill, R. L. (Academic, New York), 3rd Ed., Vol. 4, pp. 163–273.
2. Prockop, D. J., Kivirikko, K. I., Tuderman, L. & Guzman, N. A. (1979) *N. Engl. J. Med.* **301**, 13–23, 77–85.
3. Fessler, J. H. & Fessler, L. I. (1978) *Annu. Rev. Biochem.* **47**, 129–162.
4. Rennard, S., Ferrans, V. J., Bradley, K. H. & Crystal, R. G. (1980) in *Toxicology of the Lung*, ed. Witschi, H. (CRC, Cleveland, OH), in press.
5. Prockop, D. J., Berg, R. A., Kivirikko, K. I. & Uitto, J. (1976) in *Biochemistry of Collagen*, eds. Ramachandron, G. N. & Reddi, A. H. (Academic, New York), pp. 163–273.
6. Bienkowski, R. S., Baum, B. J. & Crystal, R. G. (1978) *Nature (London)* **276**, 413–416.
7. Baum, B. J., Moss, J., Breul, S. D., Berg, R. A. & Crystal, R. G. (1980) *J. Biol. Chem.* **255**, 2843–2847.
8. Stein, W. H. & Moore, S. (1949) *Cold Spring Harbor Symp. Quant. Biol.* **14**, 179–190.
9. Hershko, A. & Tomkins, G. M. (1971) *J. Biol. Chem.* **246**, 710–714.
10. Bienkowski, R. S., Cowan, M. J., McDonald, J. & Crystal, R. G. (1977) *J. Biol. Chem.* **253**, 4356–4363.
11. Inouye, K., Sakakibara, S. & Prockop, D. J. (1976) *Biochim. Biophys. Acta* **420**, 133–141.
12. Uitto, J. & Prockop, D. J. (1977) *Arch. Biochem. Biophys.* **181**, 293–299.
13. Peterkofsky, B. & Dieglmann, R. (1971) *Biochemistry* **10**, 988–994.
14. Kao, W. W.-Y., Berg, R. A. & Prockop, D. J. (1977) *J. Biol. Chem.* **252**, 8391–8397.
15. Juva, K. & Prockop, D. J. (1966) *Anal. Biochem.* **15**, 77–83.
16. Kraft, C. H. & Von Eeden, C. (1968) *A Nonparametric Introduction to Statistics* (Macmillan, New York).
17. Weinstock, M. & Leblond, C. P. (1974) *J. Cell Biol.* **60**, 92–127.
18. Jamieson, J. D. & Palade, G. E. (1977) in *International Cell Biology 1976–1977*, eds. Brinkley, B. R. & Porter, K. R. (Rockefeller Univ. Press, New York), pp. 308–317.
19. Karim, A., Cournil, I. & Leblond, C. P. (1979) *J. Histochem. Cytochem.* **27**, 1070–1083.
20. Gribble, T. J., Comstock, J. P. & Udenfriend, S. (1969) *Arch. Biochem. Biophys.* **129**, 308–316.
21. Peterkofsky, B. (1972) *Arch. Biochem. Biophys.* **152**, 318–328.
22. Dean, R. T. & Riley, P. A. (1978) *Biochim. Biophys. Acta* **539**, 230–237.
23. Knowles, S. E. & Ballard, F. J. (1976) *Biochem. J.* **156**, 609–617.
24. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803.
25. Schimke, R. T. & Bradley, M. O. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 515–530.
26. Kao, W. W.-Y., Prockop, D. J. & Berg, R. A. (1979) *J. Biol. Chem.* **254**, 2234–2243.
27. Shaw, E. (1970) *Physiol. Rev.* **50**, 244–296.
28. Etlinger, J. D. & Goldberg, A. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 54–58.
29. Barrett, A. J. (1977) in *Proteases in Mammalian Cells and Tissues*, ed. Barret, A. J. (North-Holland, Amsterdam), pp. 181–208.
30. Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S. & Bokley, P. (1977) *Eur. J. Biochem.* **74**, 293–301.
31. Wibo, M. & Poole, B. (1974) *J. Cell Biol.* **63**, 430–440.
32. Ohkuma, S. & Poole, B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3327–3331.
33. Amenta, J. S., Hlivko, T. J., McBee, A. G., Shinozuka, H. & Brocher, S. (1978) *Exp. Cell Res.* **115**, 357–366.
34. Libby, P. & Goldberg, A. L. (1978) *Science* **199**, 534–536.
35. Huisman, W., Lanting, L., Doddema, H. J., Bouma, J. M. W. & Gruber, M. (1974) *Biochim. Biophys. Acta* **370**, 297–307.
36. Umezawa, H. & Aoyagi, T. (1977) in *Proteases in Mammalian Cells and Tissues*, ed. Barret, A. J. (North-Holland, Amsterdam), pp. 637–662.
37. Chandler, C. S. & Ballard, F. J. (1978) *Biochem. J.* **176**, 151–158.
38. Warburton, M. J. & Poole, B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2427–2431.
39. Amenta, J. S., Sargus, M. J. & Baccino, F. M. (1977) *Biochem. J.* **168**, 223–227.
40. Ehrlich, H. P., Ross, R. & Bornstein, P. (1974) *J. Cell Biol.* **62**, 390–405.
41. Dustin, P. (1978) *Microtubules* (Springer, New York), pp. 167–184.
42. Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. & Yamada, K. M. (1971) *Science* **171**, 135–143.