Regulation of Protein Degradation in Normal and Transformed Human Cells

EFFECTS OF GROWTH STATE, MEDIUM COMPOSITION, AND VIRAL TRANSFORMATION*

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In WI-38, a normal human fibroblast, the rates of degradation of short lived and long lived proteins are identical whether the cultures are growing exponentially or at a density-inhibited. Replacement of the growth medium with fresh medium does not alter these rates. In VA-13, an SV-40 transformed derivative of WI-38, the rates of protein degradation are also independent of growth rate and fresh medium. However, in both WI-38 and VA-13 the rate of long lived protein degradation increases as the serum concentration is reduced below 5%. After complete serum withdrawal, the rate increases by 60 to 100% in both cell types. Withdrawal of arginine and phenylalanine triples the rate of long lived protein degradation, while addition of 10% dialyzed serum to this amino acid-deficient medium reduces the effect to twice that of the controls. Incubation of both types of cells in phosphate-buffered saline also increases protein degradation. This effect is reduced by glucose, albumin, and dialyzed serum. Therefore, the rate of protein degradation is independent of growth rate in normal and transformed human cells. However, the rate of degradation is closely coupled to certain medium alterations.

Mammalian cells continuously synthesize, degrade, and resynthesize most of their proteins. The balance between the rates of protein synthesis and degradation determines the steady state amount and rate of accumulation or loss of protein in a cell. For growing cells the overall rate of protein synthesis must be greater than the overall rate of protein degradation, while for non-growing cells the rates should be equal. Regulation could occur at the level of protein synthesis, of protein degradation, or at both levels simultaneously.

Many changes occur in cellular metabolism during growth inhibition in crowded cultures or after serum deprivation. Among these changes are declines in DNA, RNA, and protein synthesis (1, 2) and also in nutrient uptake (3, 4). Different cultured cell lines appear to reduce protein synthesis to different extents during growth limitation. For instance, rat hepatoma (5), Vero (6), and mouse 3T6 cells (7) reduce protein synthesis rates by almost 90%, while mouse 3T3 cells (8), hamster embryo fibroblasts (9), and certain human cells (1) reduce protein synthesis rates by only 50%. On the other hand, some cell lines such as BHK 21/13 (10) do not appear to reduce protein synthesis rates at all during growth inhibition but presumably regulate the rate of protein degradation or excretion in order to maintain a stationary state. In support of this presumption are studies in Yoshida ascites (11) and mouse L cells (12) showing higher rates of protein degradation in cells during stationary phase than in exponential growth. In contrast, Eagle's early work (13) as well as a more recent study (14) suggest that protein degradation rates are essentially independent of growth rate.

Serum deprivation halts cell growth and stimulates protein degradation rates in rat hepatoma (15, 16) and fibroblast cultures (17). In addition, serum starvation is reported to decrease protein, RNA, and DNA precursor incorporation to a greater extent in normal 3T3 cells than in their simian virus 40-transformed counterparts, SV3T3 (8). In these cells, serum starvation increases protein degradation in the normal 3T3 as expected, but not in the transformed SV3T3 (8). These results suggest that normal and transformed cells regulate macromolecular synthesis and degradation differently.

These various responses of different cell lines to growth inhibition raises a number of questions. Are protein degradation rates regulated during growth transitions as protein synthesis rates appear to be? Does the regulation of protein degradation differ between cultured normal and transformed human cells as it does for mouse cells? What effects do variations in medium composition have on degradative rates? This study examines these questions using the cultured normal human embryonic fibroblast WI-38 (18) and its SV40-transformed counterpart VA-13 (19). The rates of protein degradation have been measured during logarithmic growth, density inhibition, serum starvation, serum stimulation, conditioned medium treatment, and amino acid deficiency. A double isotope method that minimizes precursor reutilization was used to measure degradation in both the slowly and rapidly turning over proteins (17, 20, 21).

EXPERIMENTAL PROCEDURES

Materials — Materials were purchased as follows: Aquasol, [3H]toluene standard, and [14C]toluene standard from New England Nuclear; bovine serum albumin and cycloheximide from Sigma;
BME1 G-13 powdered media, trypsin, and sodium bicarbonate from Grand Island Biological; fetal calf serum from Flow; Gentamicin from Schering; L-[U-14C]leucine (312 mCi/mmol) and L-[4,5-3H]leucine from Schwarz/Mann, 1-5, 10-m1 plastic pipettes, 25 cm² and 70 cm² plastic tissue culture flasks from Falcon Plastics. All other chemicals were of reagent grade.

**Cell Culture** — The normal human embryonic lung fibroblast strain WI-38 (18) and its SV40-transformed counterpart VA-13 (19) were serially subcultured at 1.5 × 10⁵ cells per passage in Falcon Plastic Flasks (75 cm² growth area). The medium used was 40 ml of BME-10% containing 1 mM L-glutamine, 50 μg/ml of gentamycin, and 2.0 g/liter of sodium bicarbonate. pH was controlled by filling the flasks with 5% CO₂, 95% air before closing them. Cell sheets were harvested with 0.05% trypsin, 0.02% EDTA and aspirated to a single cell suspension with a 10-ml plastic pipette. Cell number was determined with a Coulter Counter model B. Cultures used in these experiments were free of mycoplasma contamination as determined by Flow Laboratories Inc. WI-38 cultures were used before their 28th population doubling.

**Protein Degradation** — Prelabeling of the long-lived, stable proteins was done by inoculating cultures with 2.5 × 10⁵ WI-38 cells/cm² or 5 × 10⁴ VA-13 cells/cm² and allowing the cells to grow to confluence during 5 days in 0.5 μCi/ml of [14C]leucine. For experiments with exponentially growing cells, 25 cm² flasks were inoculated with 2.5 × 10⁴ cells that had been prelabeled as above and harvested by trypsination. For experiments with density-inhibited cells, the cultures were not trypsinated but were rinsed three times with BME and reincubated for 2 days in fresh medium. The short lived proteins were labeled 2 days after seeding the exponential cells and changing the medium of the density-inhibited cells with 5 μCi/ml of [3H]leucine added to the culture medium for 30 min. This second isotope was removed by seven rapid washes with BME containing 2 mM excess leucine but without serum at approximately 37°. The cultures were incubated in 16 ml of the appropriate medium (containing added leucine) at zero time and the rate of protein degradation was measured by a method described previously that reduces leucine reutilization but that may not eliminate it completely (20, 21). At each time point 1.0 ml of medium was removed and added to 0.25 ml of 30% (w/v) trichloroacetic acid. After precipitation at 4° overnight, the samples were analyzed for acid-soluble and -insoluble radioactivity as described (21). The total radioactivity in the system was determined for each flask as described (21).

**Conditioned Media** — Conditioned medium was taken from WI-38 cultures at population-doubling level 25 that had been incubated 1 week previously in 75 cm² flasks with 1.5 × 10⁵ cells in 40 ml of BME-10%. During this time the cells grow to confluence. This conditioned medium was sterile filtered with a 0.2-μm Nalgene filter before use. The pH was adjusted to 7.2 with NaHCO₃.

VA-13 (B-l %) — This cell line was derived from VA-13 previously cultured in BME-10% for 1 month. Then these cells were cultured in BME-1%-1% for 1 day, and the rate of degradation determined during 8 h as described under "Experimental Procedures." O-O, final cell number 3 days after cycloheximide addition; △-△, per cent of protein degraded per h; --, the number of cells originally inoculated. Each point is the mean of two determinations.

**RESULTS**

**Effect of Cycloheximide on Protein Degradation** — Even though leucine reutilization is unlikely to be occurring to a significant extent in these experiments (20, 21), I have tested the effect of cycloheximide on protein degradation in order to further test this hypothesis. Fig. 1 shows that at 0.1 μg/ml of cycloheximide cell growth continues at a rate equivalent to 50% of the control rate; protein synthesis as measured by [3H]leucine incorporation is also decreased by 48% (data not shown). At doses above 1.0 μg/ml growth is halted and [3H]leucine incorporation is reduced to 8% of the control rate (data not shown). In contrast, the apparent rate of protein degradation is inhibited 25% at concentrations of cycloheximide above 0.1 μg/ml (Fig. 1) even though growth and protein synthesis are only inhibited by 50% at the lowest cycloheximide concentration. These data suggest that the drug's effects on protein degradation are not closely correlated with its effects on protein synthesis or growth and that effects on degradation can be separated, at low drug concentrations, from effects on growth or synthesis. If reutilization for new synthesis of labeled leucine were occurring to a significant extent, then the apparent rate of protein degradation should have been increased by halting protein synthesis with cycloheximide. Since the drug slightly decreased the apparent rate of degradation independently of drug concentration, while growth and synthesis were still dependent upon drug concentration, leucine reutilization is unlikely to be significant. The slight inhibition of protein degradation by the drug remains unexplained.

**Protein Degradation in Growing and High Density Cultures of WI-38 and VA-13** — In BME-10% WI-38 cells seeded at 4 × 10⁵ cells/cm² grow logarithmically with a doubling time of 14 to 18 h for three generations. At approximately 4 × 10⁵ cells/cm², the growth rate declines until at 1 to 2 × 10⁵ cells/cm² detectable growth ceases. In this density-inhibited state, mitotic activity and DNA synthesis have almost stopped (22-24). Although the VA-13 cultures are not growth-regulated in the same way as WI-38, the fraction of VA-13 that are cycling is reduced at high density. Addition of either fresh medium or serum to high density-inhibited cul-

![Fig. 1. Effect of cycloheximide on growth and protein degradation. The WI-38 cells used in these experiments were at population-doubling level 21 and were inoculated at 10⁶ cells/75 cm² flask. For growth determinations, cycloheximide was added 1 day after seeding and the total cell number was determined 2 days after drug addition. For long lived protein degradation measurements, the cells were prelabeled for 3 days with 0.5 μCi/ml of [3H]leucine, rinsed, incubated in fresh BME-10% for 1 day, and the rate of degradation determined during 8 h as described under "Experimental Procedures." O-O, final cell number 3 days after cycloheximide addition; △-△, per cent of protein degraded per h; --, the number of cells originally inoculated. Each point is the mean of two determinations.](http://www.jbc.org/)
tures of VA-13 or WI-38 will stimulate a new wave of DNA synthesis followed by mitosis. By 48 h, the WI-38 cells are quiescent again except for a small cycling fraction (25) and VA-13 cultures have returned to the synthetic and mitotic patterns exhibited before medium change.

In the degradation experiments with exponentially growing cells, the stable long lived proteins were identified by labeling them with [14C]leucine for 4 days of growth and two population doublings. After labeling, the cells were trypsinized and reseeded at 10^6 cells/cm^2 in fresh unlabeled medium for 48 h. During this period, the unstable rapidly turning over proteins will have degraded, leaving behind a fraction of stable slowly turning over protein that is 14C-labeled. A subsequent incorporation with [3H]leucine for 30 min labels proteins having short half-lives with 3H. Density-inhibited cultures were labeled in the same manner except that they were not trypsinized and reseeded after 4 days of growth in [14C]leucine medium but were refed with fresh medium 48 h before the experiment.

Figs. 2 and 3 show the rates of protein degradation for growing and density-inhibited WI-38 cells; Figs. 4 and 5 show the same data for VA-13 cells. One sees that the rates of protein degradation are seemingly independent of the growth state of the cells. Long lived proteins are degraded with a half-life of approximately 69 h in either growing or density-inhibited WI-38 and with a half-life of approximately 80 h in either growing or density-inhibited VA-13 cells. These rates are determined as before (21) by the equation T = ln2/k, where T is the half-life and k is the rate constant for the protein fraction degraded per h.

The half-lives of the short lived proteins as a class cannot be determined exactly since their apparent rates of degradation vary as a function of time, no doubt because each short lived protein has a unique rate constant. Nevertheless, estimates can be made for the class as a whole if one extrapolates a rate from the initial slope of the degradation curve for short lived proteins (21): these estimates will tend to emphasize the half-lives of the least stable of the short lived proteins. Under these conditions for growing or density-inhibited VA-13 this half-life is approximately 8 h. For density-inhibited WI-38 it is also approximately 8 h. However, for growing WI-38 the apparent half-life of the short lived proteins is slightly less, approximately 5 h. This small but consistent difference may indicate that a different spectrum of less stable short lived proteins are synthesized in growing as compared to stationary WI-38 cells, or it may be due to limitations in this method of analysis.

With the possible difference just noted, I conclude from these experiments that the rates of protein degradation for both short and long lived proteins are approximately independent of the growth and transformation states of these cultured human cells.

Effects of Different Media—The addition of fresh medium to arrested cultures of WI-38 and VA-13 stimulates the synthesis of RNA, protein, and DNA which subsequently leads to mitosis (25). In rat fibroblasts, fresh medium inhibits protein degradation (17). However, changing the medium from conditioned to fresh does not alter protein degradation in these human cells whether they are growing or density-inhibited (Figs. 2 to 5). Neither short nor long lived proteins have altered rates of turnover, nor do the transformed cells differ in their responses from the normal cells.

If medium without serum is added to either of the cells in both growth states, then the rates of protein degradation are increased as shown in Figs. 3 to 5. This stimulation of protein degradation occurs in the long lived proteins but does not occur to a significant extent in proteins with very rapid turnover.

After about 5 h, the rate of degradation for the pulse-labeled protein in serum-free medium appears to be increasing rela-

![Fig. 2 (left). Rates of short and long lived protein degradation in exponentially growing WI-38. Effects of different media. The long lived, slowly turning over proteins were prelabeled by inoculating cultures with 2.5 × 10^5 WI-38 cells/cm^2 into BME 10% containing 0.5 µCi/ml of L-[U-14C]leucine. The cultures grew to confluence during 4 days. After this labeling period, the cells were rinsed, trypsinized, and resuspended in fresh medium for 4 days of growth in [14C]leucine. The culture was then trypsinized and resuspended at 10^6 cells/cm^2 in fresh unlabeled medium for 48 h. During this period, the unstable rapidly turning over proteins will have degraded, leaving behind a fraction of stable slowly turning over protein that is 14C-labeled. A subsequent incorporation with [3H]leucine for 30 min labels protein having short half-lives with 3H. Density-inhibited cultures were labeled in the same manner except that they were not trypsinized and resuspended after 4 days of growth in [14C]leucine medium but were refed with fresh medium 48 h before the experiment.

![Fig. 3 (right). Rates of short and long lived protein degradation in confluent WI-38. Effects of different media. The experimental protocols are the same as in Fig. 2, except that the cells were initially seeded at 5 × 10^4 cells/cm^2. Symbols: same as in Fig. 2.

![Fig. 4 (left). Rates of short and long lived protein degradation in exponentially growing VA-13. Effects of different media. The experimental protocols are the same as in Fig. 2, except that the cells were initially seeded at 5 × 10^4 cells/cm^2. Symbols: same as in Fig. 2.

![Fig. 5 (right). Rates of short and long lived protein degradation in confluent VA-13. Effects of different media. The experimental protocols are the same as in Fig. 3, except that the cells were initially seeded at 5 × 10^4 cells/cm^2. Symbols: same as in Fig. 2.
to the fact that a pulse label for 30 min will also label some long lived proteins. Since the degradation curves are composites of all proteins labeled during the pulse, as time progresses the short lived proteins will all have been degraded leaving behind the long lived proteins which will degrade at their characteristic rates. Serum withdrawal accelerates the degradation rate of long lived proteins so that the degradation rate of the late part of the pulse-labeled curve appears to be faster. Therefore, these data suggest that the main effect of serum withdrawal is on the long lived proteins and that any effects on short lived proteins are slight and not clearly distinguishable by this method.

Serum withdrawal doubles the rate of degradation for the long lived proteins of both normal WI-38 and transformed VA-13 (Figs. 2 to 5). This finding with human cells is in contrast to previous work with mouse cells where serum withdrawal increases protein degradation in 3T3 but not in its SV40-transformed derivative SV3T3 (8). The effects of different serum concentrations on the rate of long lived protein degradation in exponentially growing cells are shown in Fig. 6. The degradation rate decreases the most between 0 and 5% serum, with the rate remaining approximately constant at higher serum concentrations. Fig. 6 emphasizes how coordinates the rates of protein degradation are regulated by serum concentration in both WI-38 and VA-13.

A line of VA-13 (VA-13 (B-1%)) has been adapted to grow in 1% serum (see "Experimental Procedures"). The doubling time is 43 h compared to 20 h for cells in BME-10%. The effect of serum concentration on protein degradation rates in these cells was determined. As seen in Table I, the cells adapted to grow in BME-1% have reduced their basal rate of protein degradation to 0.78%/h, a value characteristic of cells normally grown in 10% serum (0.8%/h). However, the low serum cells are less responsive to changes in serum concentration than are cells normally grown in BME-10%. Serum withdrawal increases the degradation rate in the low serum cells by only 28%, whereas it is increased between 60% and 100% in cells normally cultured in 10% serum.

These experiments show that transformed human cells, over a prolonged time, can adapt to growth in low serum concentrations. One component of that adaptation is to reduce the rate of protein degradation from an accelerated rate characteristic of serum withdrawal to the same rate as that of cells grown at normal 10% serum concentrations. This observation implies that an absolute rate of long lived protein degradation of 0.6 to 1.0%/h is associated with long term cell survival, and furthermore that cells can regulate the overall rate of degradation irrespective of certain medium alterations.

There are other components in medium besides serum that affect cell physiology. For instance, the deprivation of required amino acids markedly reduces the rate of ribosomal synthesis in Landschütz ascites cells (26). Amino acid deprivation also reduces leucine, uridine, and thymidine uptake and incorporation into macromolecules in 3T3/SV3T3 cells (8). In the latter systems deprivation of valine and tryptophan is reported to not affect protein degradation (27).

Since human and mouse cells seem to differ in their responses to serum withdrawal, I have investigated the effects of amino acid and glucose withdrawal on long lived protein degradation in the human system. Table II shows that the substitution of 10% dialyzed serum for 10% whole serum increases by 30 and 36%, respectively, the rate of degradation in both WI-38 and VA-13. This result suggests that there are dialyzable components in serum that play some role in regulating protein degradation.

Fig. 6. Effect of different serum concentrations on the rate of long lived protein degradation. Exponentially growing WI-38 and VA-13 cells were incubated at 2.5 µCi/ml of L-[4,5-3H]leucine for 24 h. The radioactive medium was removed, the cultures were rinsed twice with medium without serum and reincubated in fresh medium for 24 h. After this period, during which the rapidly labeled proteins degraded, the cultures were rinsed twice more and reincubated in medium containing different serum concentrations. The percentage of the total radioactivity released to the medium in acid-soluble form was determined every 2 h for 8 h. The rate of release was constant during this period. The figure shows the percentage of H released after 8 h for WI-38 and VA-13 at each serum concentration. • = WI-38; ○ = VA-13.

**Table I**

**Effects of serum concentration on long lived protein degradation in VA-13 cells adapted to 1% serum**

Exponentially growing cultures of VA-13 (B-1%) in BME-1% were grown in 0.25 µCi/ml of [3H]leucine for 5 days. After this period the medium was removed, the cultures rinsed three times in BME, and reincubated in BME-1% for 24 h. Then the cultures were rinsed once again with BME, incubated in the appropriate medium, and the rate of protein degradation determined during 8 h. The values represent the mean ± the standard deviation for three determinations.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Percentage of protein degraded per h VA-13 (B-1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BME</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td>BME-1%</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>BME-10%</td>
<td>0.68 ± 0.02</td>
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**Table II**

**Effects of variations in BME composition on degradation of long lived intracellular proteins**

Exponentially growing cultures of WI-38 (PDL 22) and VA-13 were incubated in complete medium containing 2.5 µCi/ml of [3H]leucine for 24 h followed by three rinses with BME and reincubated in fresh complete medium. Twenty-four hours later the cultures were rinsed twice with BME and incubated for 6 h in the appropriate experimental media. The data are expressed as the percentage of the total radioactivity released to the medium in acid-soluble form per h during 6 h. Each value is the mean of from two to four experiments ± the standard deviation.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Percentage of protein degraded per h</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI-38</td>
<td></td>
</tr>
<tr>
<td>BME + 10% whole serum</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>BME + 10% dialyzed serum</td>
<td>1.10 ± 0.06</td>
</tr>
<tr>
<td>BME</td>
<td>1.50 ± 0.11</td>
</tr>
<tr>
<td>BME without Phe and Arg</td>
<td>3.12 ± 0.34</td>
</tr>
<tr>
<td>BME without Phe and Arg + 10% dialyzed serum</td>
<td>1.65 ± 0.13</td>
</tr>
<tr>
<td>VA-13</td>
<td></td>
</tr>
<tr>
<td>BME + 10% whole serum</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>BME + 10% dialyzed serum</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td>BME</td>
<td>1.32 ± 0.04</td>
</tr>
<tr>
<td>BME without Phe and Arg</td>
<td>2.97 ± 0.45</td>
</tr>
<tr>
<td>BME without Phe and Arg + 10% dialyzed serum</td>
<td>1.33 ± 0.09</td>
</tr>
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degradation. At the same time, there must be macromolecular components of serum that effect protein degradation, since in BME alone the rate is faster than with 10% dialyzed serum.

Removal of phenylalanine and arginine from BME caused the most dramatic effect, increasing the rate of protein degradation almost 4-fold. Re-addition of dialyzed serum to the amino acid-deficient medium reduces the effect. But protein degradation still remains 20 to 50% higher than in the dialyzed serum control. This experiment implies that the availability of amino acids alone can regulate protein degradation.

Phosphate-buffered saline contains none of the nutrients needed for normal cell growth, it simply provides the proper pH and osmolarity for mammalian cells. Table III shows that saline doubles the protein degradation rate relative to a complete medium control. The addition of an energy source, glucose, reduces the rate of degradation relative to saline alone; the further addition of 1.5 g/liter of bovine serum albumin reduces the rate even more.

DISCUSSION

In studies of protein degradation, the reutilization for protein synthesis of isotopic leucine derived from protein breakdown can confound the interpretation of results. Our previous work showed that leucine reutilization occurs in WI-38 cell cultures unless the leucine concentration in the medium is high enough (20, 21). Because leucine flux occurs rapidly in WI-38 cultures (20, 21), only a small fraction of the cell's leucine will be isotopic. Therefore the amount of reutilization occurring under these conditions should be small. Work by Van Venrooij et al. (28) with monolayers of HeLa cells demonstrates that reutilization does not occur in their system when the nutritional conditions are adequate. They concluded that transfer RNA was aminoacylated with extracellular leucine with little dilution from the intracellular leucine pool. Other work by Airhart et al. (29) tends to confirm this conclusion.

Utilisation of inhibitors of protein synthesis is another way to approach the problem of isotope reutilization. This approach can be dangerous since inhibitors of protein synthesis can also inhibit protein degradation, especially when cells are incubated in nutritionally deficient medium (see Ref. 30 for a review). However, in rat fibroblast (17) and hepatoma (15) cells growing in nutritionally complete medium, protein synthesis inhibitors do not appear to reduce protein degradation. The experiments with cycloheximide reported in Fig. 1 appear to contrast with some of these conclusions. First, in what should be nutritionally adequate conditions, the rate of protein degradation in WI-38 is decreased 25% by cycloheximide. Second, this inhibition is independent of cycloheximide concentration and occurs at low concentrations of drug when protein synthesis and growth rates are decreased by only 50%. These results suggest that the inhibition of degradation is not simply correlated with the inhibition of protein synthesis and that other mechanisms of action may be important. An alternative hypothesis is that a specific protein(s) is responsible for the altered degradation and that the synthesis of this protein(s) is more sensitive to cycloheximide than are the total cellular proteins. Since the apparent rate of degradation is independent of protein synthesis and cell growth rates, I conclude that leucine reutilization is unlikely to be significant in these experiments. If reutilization were occurring, the rate of degradation should have increased as the rate of synthesis, and thus reutilization, decreased at higher cycloheximide concentrations.

Figs. 2 to 5 show that protein degradation rates are the same for human WI-38 and VA-13 cells whether they are growing exponentially or are density inhibited. Therefore the regulation of growth rate and protein degradation rate must be independent of each other. By the same reasoning the net rate at which protein mass per culture increases during growth must then be primarily a function of the rate of protein synthesis and not degradation.

Addition of fresh medium to density-inhibited cultures of human fibroblasts accelerates leucine incorporation rates within 1 h (34). However, Figs. 2 to 5 show that fresh medium has no effect on protein degradation rates up to 23 h. This finding is in contrast to density-inhibited rat fibroblasts where fresh medium inhibits protein degradation within 1 to 2 h (17).

In yeast (32, 33) and bacteria (30) the rates of protein degradation are closely coupled to the medium composition and growth rate; in nongrowing starving cultures the degradation rates are much faster than in exponential cultures. In contrast, previous work by Eagle et al. (31) showed that the malignant human lines HeLa and KB degraded protein at the same rates in exponential growth as they did in a growth-deficient medium lacking an essential amino acid. This experiment, however, is difficult to interpret. First, because growth limitation by amino acid deficiency is unphysiological and also because, as shown in Table II, some amino acid deficiencies may alter protein degradation. In the WI-38/VA-13 system, growth limitation is by density inhibition and may be more analogous to an in vivo tissue. Growth stimulation, by either subculture or medium renewal, has no effect on protein degradation.

As seen in Tables II and III and Figs. 2 to 5, variations in medium composition can greatly alter the rates of long-lived but not short-lived protein degradation. The human cells, studied here, contrast in their responses to the mouse and rat cells studied by others. For instance, Poole and Wibo (17) found that fresh medium added to confluent monolayers of rat fibroblasts inhibited long-lived protein degradation. This finding suggests that protein degradation may be decreased in growing rat fibroblasts, since a medium change stimulates growth. However, in both growing and confluent WI-38 and VA-13 fresh medium addition had no effect on protein degradation when compared to conditioned medium.

Protein degradation was accelerated by serum withdrawal in both WI-38 and VA-13 grown in 10% serum whether the cells were growing or confluent. This finding is in contrast to the mouse 3T3/SV3T3 system where serum withdrawal stimu-
lates protein degradation in normal cells but not in transformed ones (8). Fig. 6 emphasizes how similarly normal and transformed human cells respond to changes in serum concentration.

In the VA-13 (B-1%) line that had adapted to growth in BME-1%, the rate of long lived protein degradation had also adapted to a basal rate characteristic of the cells grown in 10% serum (Table I). In contrast to the high serum cells, the low serum cells alter their rates of degradation only slightly after complete serum withdrawal (Table I). These data suggest that cultured human cells adapt to transient nutritional deprivations by increasing their rate of protein degradation, but that there is an optimum rate of degradation consistent with long term cell survival.

Other authors have reported that amino acids can regulate protein degradation (see Ref. 30 for a review). Medium lacking amino acids accelerates degradation in perfused heart (34), liver (35), incubated skeletal muscle (36), and cultured rat fibroblasts (37). The effective amino acids differ for each system studied (30). However, Morhenn et al. (27) have reported that starvation for the amino acids valine and tryptophan has no effect on protein degradation in either mouse 3T3 or SV3T3. The results reported in Table II support the notion that starvation for at least arginine and phenylalanine accelerates protein degradation in cultured human WI-38 and VA-13 cells. This effect is more dramatic in medium without serum than in medium containing 10% dialyzed serum. Whether starvation for other amino acids is as effective as starvation for arginine and phenylalanine, both essential amino acids, remains to be determined.

Phosphate-buffered saline also accelerates protein degradation in human cells. That acceleration can be reduced by adding glucose, an energy source, to the saline. The addition of bovine serum albumin or dialyzed serum reduces the rate of degradation still further, although it still remains higher than in the complete medium controls.

From these data, it appears as if the rate of protein degradation in human cells is closely coupled to medium alterations, but not to growth rate. This coupling may represent a coordinated cellular regulatory mechanism which does not seem to vary greatly between normal and transformed human cells.

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M O Bradley


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