DEGRADATION OF ABNORMAL PROTEINS IN GROWING AND IN QUIESCENT FIBROBLASTS IN CULTURE

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1. Introduction

All proteins in living cells seem to be degraded and resynthesized continuously. The rate of this turnover depends upon structural features of the protein. Thus high turnover tends to correlate with high molecular mass, low isoelectric point, and pronounced hydrophobicity [1–3]. Cells can be induced to synthesize abnormal proteins by mutations and by addition of puromycin or amino acid analogues. Such abnormal proteins are generally rapidly degraded within the cell [1,2].

Protein degradation is enhanced during starvation and after transition from growth to quiescence. The relative enhancement is usually larger for the more stable proteins than for those with fast turnover [4–8]. Furthermore, a reduction in temperature or addition of many compounds inhibits the enhanced degradation of stable proteins more than that of unstable normal or abnormal proteins [5,9–11]. Such observations led to the hypothesis [5] that abnormal proteins are catabolized by a pathway that is distinct from that utilized for enhanced degradation. This suggestion was questioned in [12,13], where puromycinyl-peptides were degraded at a much slower rate in growing than in non-growing rat liver.

Here I report that results similar to those in [12,13] can be obtained with cell cultures. However, the difference between growing and quiescent cells in degradation rates of abnormal proteins resides in a difference in proteins rather than in the degradative machinery of the cells.

2. Materials and methods

L-[4,5-^{3}H] Leucine was obtained from the Radiochemical Centre, Amersham. D,L-*p*-Fluorophenylalanine and puromycin were purchased from Sigma.

Balb/3T3 cells were grown in Eagle's MEM with Earle's salts and 10% (v/v) newborn calf serum (Gibco-Biocult). Media used for experiments contained penicillin G (100 IU/ml) and streptomycin sulphate (100 μg/ml) and the bicarbonate buffer was substituted by 10 mM TES (*N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid) and 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). PBS, as used here, refers to a solution containing 137 mM NaCl, 2.7 mM KCl, 4.1 mM Na_2HPO_4, and 0.73 mM KH_2PO_4, pH 7.4.

All experiments were carried out at 37°C. Data relevant for each experiment are given in the figure legends. The methods are detailed in [8].

3. Results and discussion

The fibroblast cell line used here is subject to growth control by serum concentration and culture density [14]. Fig.1 shows, in accord with [8] that protein is degraded faster in quiescent cells than in growing ones, and that proteins containing puromycin or fluorophenylalanine [1,2] are more rapidly degraded than normal cell proteins. Fig.1 also shows that with this experimental protocol quiescent cells degrade both normal and abnormal proteins faster than do growing cells.

The difference in degradation rates of abnormal proteins between growing and quiescent cells may reflect differences in the degradative apparatus, or quantitative differences in the effects of puromycin and fluorophenylalanine on protein synthesis in the two growth states. Indeed, fig.2 shows that protein

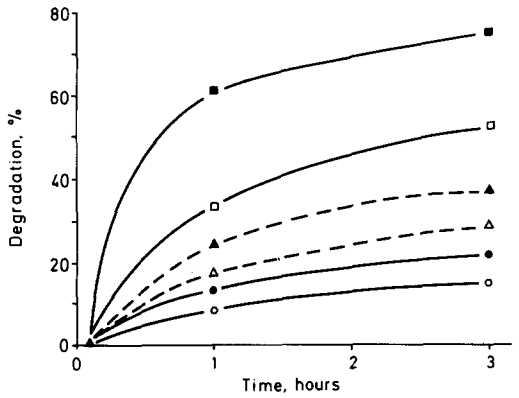

Fig.1. Degradation of normal cell proteins, abnormal proteins containing *p*-fluorophenylalanine and of puromycin-peptides in growing and in density-inhibited Balb/3T3 cells. Growing and density-inhibited Balb/3T3 cultures were prepared in 5 cm Petri dishes as in [8]. The cultures were incubated for 30 min in 3 ml of either normal growth medium (○,●) or in medium with 5 mM D,L-*p*-fluorophenylalanine instead of phenylalanine (△,▲) or in medium with puromycin, 20 μg/ml (□,■). The medium was then changed to the same medium, but with 0.2 mM [^{3}H]leucine (10 μCi/ml), whereupon the cultures were incubated for 1 h. The cultures were then washed in 3 × 6 ml PBS with 0.2 mM leucine and added 9 ml growth medium with 10% v/v calf serum and 4 mM unlabelled leucine. Protein degradation was assessed from the trichloroacetic acid-soluble radioactivity in samples of medium, taken of the indicated times [8]. Open and filled symbols denote growing and density-inhibited cultures, respectively.

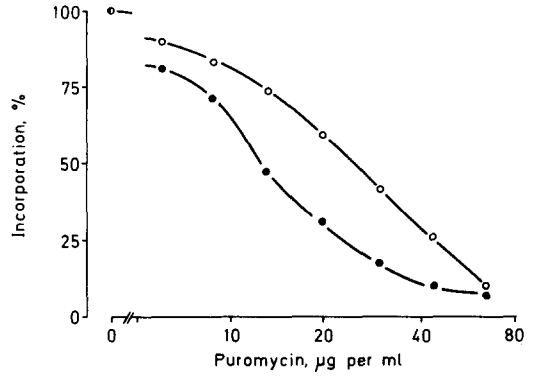

Fig.2. Effect of puromycin on [^{3}H]leucine incorporation into growing and quiescent Balb/3T3 cells. Growing and density-inhibited Balb/3T3 cultures in 5 cm Petri dishes were prepared as in [8]. The cultures were incubated for 30 min in 3 ml medium with the indicated concentration of puromycin. [^{3}H]Leucine was then added to final conc. 0.2 mM (10 μCi/ml), and the incubation continued for a further 30 min. The cultures were then washed in 3 × 6 ml PBS with 0.2 mM leucine and dissolved in 2 ml 0.1 M NaOH, 0.4% sodium deoxycholate. Incorporation was assessed from the trichloroacetic acid-insoluble radioactivity in this cell extract and calculated as % incorporation in the absence of puromycin. Open and filled symbols denote growing and density-inhibited cultures, respectively.

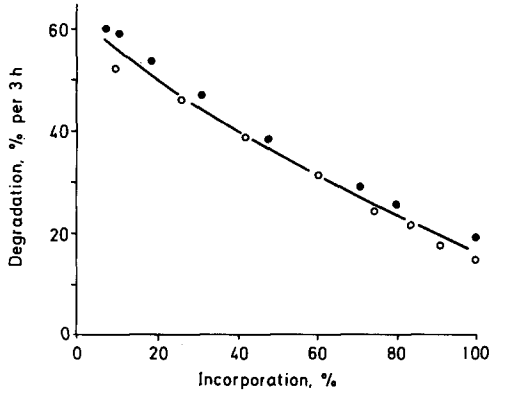

Fig.3. Correlation between inhibition of [^{3}H]leucine incorporation by puromycin and subsequent release of [^{3}H]leucine (protein degradation). Labelled Balb/3T3 cell cultures were prepared as in fig.2, whereupon they were washed by 3 × 6 ml PBS with 0.2 mM leucine and incubated in 5 ml growth medium with 10% (v/v) calf serum and 4 mM leucine. Protein degradation was assessed from the difference in trichloroacetic acid-soluble radioactivity between samples of medium taken 10 min and 1 h after the end of the labelling period. Incorporation was calculated as the total recovered activity less the acid-soluble activity in the medium at the first sampling. Incorporation is expressed as % of the incorporation found in cultures with the same cell density, but without puromycin. Open and filled symbols denote growing and density-inhibited cultures, respectively.

synthesis is more sensitive to puromycin in quiescent cells than it is in growing cells. Similar results were obtained with fluorophenylalanine (not shown).

Puromycin is a peptide-chain terminator, and the size of the puromycinyl-peptides decreases with increasing concentration of puromycin and, hence with increasing inhibition of amino acid incorporation [15]. Among puromycinyl-peptides, those with low molecular mass are the least stable [16]. The metabolic stability of puromycinyl-peptides is therefore also correlated to the inhibition of amino acid incorporation, as shown in fig.3. This correlation does not differ for growing and for density-inhibited cells (fig.3). It seems reasonable to suppose that the size of puromycinyl-peptides is comparable in cultures, that have had their [^{3}H]leucine incorporation inhibited to the same extent. Accordingly, fig.3 shows that comparable, abnormal proteins are degraded equally fast in growing and in quiescent cells.

The difference in degradation rates between puromycinyl-peptides in growing and in density-inhibited

cells is therefore due to differences in the molecular weight of the puromycinyl-peptides, and there is no need to suggest a difference in the degradative apparatus for abnormal proteins.

A similar explanation may also hold for the observations in [12,13] on differences in degradation rates between puromycinyl-peptides in growing and non-growing rat livers. The results therefore support the suggestion [5] that the majority of abnormal proteins follow a degradative pathway that is distinct from the pathway utilized for enhanced protein degradation.

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