Identification of a Transcript That Is Down-regulated in Senescent Human Fibroblasts

CLONING, SEQUENCE ANALYSIS, AND REGULATION OF THE HUMAN L7 RIBOSOMAL PROTEIN GENE*

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Normal eukaryotic cells divide only a limited number of times before proliferation ceases due to cellular senescence. We previously reported that a constitutively expressed, non-cell cycle-regulated transcript of unknown identity declines severalfold when human fibroblasts become senescent. We show here, from the sequence of cDNA and genomic clones, that this transcript encodes L7, a structural protein of the large ribosomal subunit. The human L7 protein shares >90% amino acid identity with the mouse and rat L7 proteins but is shorter than either rodent protein due to fewer basic repetitive motifs at the amino terminus. The position of the first intron is conserved between the mouse and human genes. The L7 mRNA was abundant, stable (t1/2 > 10 h), and polyadenylated in presenescent and senescent human fibroblasts; however, steady state mRNA levels were 5-10-fold lower in senescent cells, whether derived from fetal lung or neonatal foreskin. Quiescent and senescent cells synthesized protein at similar rates, yet only senescent cultures showed a decline in L7 mRNA. The mRNAs encoding five other ribosomal proteins (L5, P1, S3, S6, and S10) behaved similarly. The results suggest that the senescence-associated decline in L7 and other ribosomal protein mRNAs is unrelated to growth state or protein synthetic rate per se and support the view that senescence and quiescence are dissimilar states.

senescence may be a tumor suppressive mechanism. Whatever the case, the finite lifespan phenotype is under dominant genetic control and is most likely regulated by more than one locus (4-6).

Cellular senescence has been studied extensively in human fibroblasts (1-3). Fibroblasts from human tissue explants initially grow well in culture. However, the cultures (termed early passage or presenescent) inevitably show a progressive decline in proliferative capacity and eventually consist entirely of senescent cells. Senescent cells do not initiate DNA synthesis, despite high levels of growth factors and nutrients in the medium, but continue to synthesize RNA and protein, albeit at a reduced rate (1-13). Because senescent cells resemble early passage quiescent (reversibly growth-arrested) cells in several ways, it has been suggested that senescence entails a block to normal cell cycle progression (2, 8, 9). Alternatively, it has been proposed that senescence is a form of terminal differentiation, whereby cells irreversibly arrest growth with an attendant change in cell function (2, 10-12, 14).

Human fibroblasts express many genes throughout their proliferative lifespan, and many genes retain normal regulation in senescent cells, including growth factor responsiveness (9, 12). Nonetheless, senescence entails altered expression or regulation of specific genes (2, 3, 12-15). Some senescence-sensitive genes encode growth regulators, whereas others encode differentiated functions. Thus, the growth regulatory genes c-fos, cdc2, and cyclins A and B are repressed in senescent human fibroblasts (12, 13), while senescent cells underexpress tissue inhibitor of metalloproteinases (14) and overexpress collagenase (14), a1(I) procollagen (15), and cathepsin B (16), which contribute to the differentiated phenotype of fibroblasts.

We previously showed that a constitutively expressed (non-growth-regulated) RNA declined when human fibroblasts senesce (12). This RNA hybridized to a cloned cDNA fragment (pHE-7) from a human carcinoma (HeLa) cell library (17). We report here on the identity and characteristics of the pHE-7 RNA. From the sequence of cDNA and genomic clones, we show that this RNA encodes the human L7 ribosomal protein. The human L7 gene is very similar to the rat and mouse L7 genes, including the position of the first intron. However, the human L7 protein is smaller than either rodent protein due to fewer basic repetitive motifs at the amino terminus. We further show that there is a senescence-specific down-regulation of five other ribosomal protein mRNAs, in addition to L7 mRNA, and that this regulation is not related simply to the growth arrest or lower protein synthetic rate of senescent cells.

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EXPERIMENTAL PROCEDURES

Cell Culture—Human fetal lung fibroblasts strain WI-38 (AG06814) and SV40 immortalized WI-38 cells (AG07217) were obtained from the National Institute on Aging Cell Repository; neonatal foreskin fibroblasts (strain HCA-2) were obtained from Dr. O. M. Pereira-Smith (Baylor College of Medicine).

Cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with glutamine, antibiotics, and 10% fetal calf serum in a 10% CO₂ atmosphere, as described (12). Cultures were considered presenescent or early passage if greater than 70% of the cells synthesized DNA within 48 h. DNA synthesis was assessed by the incorporation of [³H]thymidine into nuclei, detectable by autoradiography (12). The doubling time of presenescent or early passage cultures was roughly 24 h, whereas senescent cultures failed to double after >2 weeks. WI-38 and HCA-2 cells reached senescence after approximately 45 and 70 population doublings, respectively. Presenescent cells were made quiescent by incubating barely confluent cultures in 0.2% serum for 48–72 h, after which less than 10% of the cells synthesized DNA over the ensuing 48 h.

RNA Isolation and Analysis—Total cellular RNA was isolated by lysing cells in guanidine isothiocyanate and pelleting the RNA through cesium chloride (18). Polyadenylated RNA was partially purified by oligo(dT) cellulose affinity chromatography (19). RNA (5–20 µg) was fractionated by electrophoresis through formaldehydeagarose gels, blotted onto nitrocellulose or nylon membranes, and fixed by baking. Blots were then hybridized to radiolabeled probes, washed, and subjected to autoradiography as described (20, 21). Signals were quantitated by laser densitometry.

Plasmids and Probes—Plasmid inserts were labeled with [³²P]dCTP by random priming or [³²P]UTP by in vitro transcription (12, 21). pHE-7 was obtained from Drs. C. Simon (Harvard University Medical School) and J. Nevins (Duke University Medical Center). pL5-6-4, pP1-13, pS3-15, pS6-4, and pS10-9, containing partial cDNAs for the L5, P1, S3, S6, and S10 ribosomal proteins, were obtained from Dr. Ira Wool (University of Chicago). The rabbit Gna, rat actin, v-Ha-ras, and human c-myc clones have been described (12, 21). pHE-7 was obtained from Drs. Simon (Harvard University Medical School) and I. Wool (University of Chicago). The rabbit v-Ha-ras, and human c-myc clones have been described (12, 20, 21).

Hybrid Selection and Analysis of Translation Products—Denatured pHE-7 plasmid (50 µg) was immobilized on nitrocellulose filters and hybridized to 1 mg of total RNA from WI-38 cells. The filters were hybridized for 3 h at 50 °C in 65% formamide, 20 mM PIPES (pH 6.4), 0.2% SDS, 0.4 M NaCl, and 100 µg/ml Escherichia coli tRNA and washed in 10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 mM EDTA, 0.5% SDS. The bound RNA was eluted at 100 °C with 400 µl of distilled water containing 30 µg tRNA and translated in vitro using a commercially available rabbit reticulocyte lysate and [³⁵S]methionine (Promega). The translation product was analyzed on a 7.5% denaturing polyacrylamide gel (22) or an 8% denaturing polyacrylamide gel (23). The signals were quantitated by laser densitometry.

Cloning and Sequence Determination.—The 280-bp insert from pHE-7 plasmid was cloned into Xgtll (Stratagene, Inc.). Phage DNA was isolated from two independent plaques showing positive hybridization. After screening, the cDNA inserts were excised with EcoRI, and double-stranded DNA sequencing was initiated from either an independent plaque showing positive hybridization, the inserts were excised with NotI and PstI, and a 2.7-kb fragment that hybridized to pHE-7 was subcloned into Bluescript KS⁺. The ends of the 2.7-kb fragment were sequenced using either T3, T7, or HE7-3 primers. Internal regions were sequenced on both strands by priming from previously determined sequence.

DNA sequences were determined by the dideoxynucleotide chain termination method (24) using a commercially available kit (Sequenase, U. S. Biochemical Corp.) and 1 µCi [³²P]dATP (500 Ci/mmol). The sequencing reaction products were analyzed on 5% polyacrylamide 7 M urea gels and detected by autoradiography.

Protein Synthesis Measurements—Presenescent cells were plated in 10% serum and used at subconfluence (<1 x 10⁶/cm²) as growing cells. Confluent cells (2-3 x 10⁶/cm²) were shifted to 0.2% serum to generate quiescent cultures. Senescent cells (1 x 10⁶/cm²) were plated in 10% serum. Cells were labeled for 60 min with [³H]leucine (20 µCi/ml, 142 Ci/mmol) in 10 or 0.2% serum, washed with ice-cold phosphate-buffered saline, and precipitated and washed with cold 10% trichloroacetic acid (20). The precipitate was solubilized in 0.2% SDS in 0.2 N NaOH and neutralized with HCl. Acid-insoluble radioactivity was determined by scintillation counting; cell protein was determined with a commercial dye binding kit (Pierce Chemical Co.).

RESULTS

Down-regulation of the pHE-7 Transcript in Senescent Human Fibroblasts—The 280-bp insert of pHE-7 detects a 1.2-1.4-kb transcript expressed by a variety of cells, including HeLa (17) (a human cervical carcinoma cell line) and WI-38 (9, 12) (a human fetal lung fibroblast strain). The pHE-7 RNA is easily detectable on Northern blots of 10–20 µg total RNA, and its abundance does not change with cellular growth state or position in the cell cycle (9, 12).

In agreement with our earlier findings (12), presenescent WI-38 cells expressed the pHE-7 transcript at nearly identical levels, whether they were actively growing (in 10% serum) or quiescent (due to serum deprivation) (Fig. 1). By contrast, senescent WI-38 cells expressed approximately 5-fold lower levels of this transcript, whether they were maintained in serum (Fig. 1) or deprived of serum (12) (data not shown).

Down-regulation of the pHE-7 RNA in senescent, but not quiescent, cells was not limited to WI-38 cells. Very similar results were obtained with another human fibroblast strain, HCA-2, derived from neonatal foreskin (Fig. 1). In addition, the down-regulation was not due to an overall decline in mRNA in senescent cells. At least two transcripts, one encoding Gna, a GTP-binding protein (21) (Figs. 1 and 2), the other encoding the c-Ha-ras protooncogene (12) (see Fig. 4), were expressed at nearly identical levels in presenescent and senescent cultures.

The pHE-7 transcript did not decline gradually over the proliferative lifespan of WI-38 cells. There was little or no change in the level of this transcript, as the fraction of cells capable of DNA synthesis declined from >70 to 20%; a significant decline was detectable only after the culture had senesced to a point where 5% or less of the cells were capable of DNA synthesis (Fig. 2). As expected, there was no change

1 The abbreviations used are: PIPES, 1,4-piperazinediethanesulfonic acid; bp, base pair(s); kb, kilobase.

FIG. 1. Down-regulation of the pHE-7 RNA with senescence in two strains of normal human fibroblasts. Total cellular RNA was isolated from exponentially growing (E), quiescent (Q), and senescent (S) cultures of human fetal lung fibroblasts (WI-38) and human neonatal foreskin fibroblasts (HCA-2), and 20 µg were analyzed for the level of pHE-7 RNA, as described under "Experimental Procedures." The abundance of Gna mRNA does not change with growth state or proliferative capacity and therefore serves as a control for RNA quantitation, loading, and transfer.
plates by a 48-h exposure to \[3H\]thymidine. Cells at different population doubling levels was isolated and analyzed as described in the legend to Fig. 1. All cultures were maintained in 10% serum, and proliferative capacity was determined on parallel plates by a 48-h exposure to \([H]\)thymidine (10 \(\mu\text{Ci}/\text{ml} ; 60 \text{ Ci/mmol})\), followed by autoradiography to determine the percent cells with radiolabeled nuclei (Labeling Index) (12). The pHE-7 RNA level declined only after the labeling index reached 5. In several experiments, even cultures with a labeling index of 10 often showed only a marginal down-regulation of the pHE-7 RNA (data not shown).

FIG. 2. Down-regulation of the pHE-7 RNA with proliferative capacity of normal human fibroblasts. RNA from WI-38 cells at different population doubling levels was isolated and analyzed as described in the legend to Fig. 1. All cultures were maintained in 10% serum, and proliferative capacity was determined on parallel plates by a 48-h exposure to \([H]\)thymidine (10 \(\mu\text{Ci}/\text{ml} ; 60 \text{ Ci/mmol})\), followed by autoradiography to determine the percent cells with radiolabeled nuclei (Labeling Index) (12). The pHE-7 RNA level declined only after the labeling index reached 5. In several experiments, even cultures with a labeling index of 10 often showed only a marginal down-regulation of the pHE-7 RNA (data not shown).

FIG. 3. The pHE-7 RNA is polyadenylated in both presenescent and senescent cells. Total cellular RNA from early passage (Presenescent) and senescent WI-38 cells was separated into a polyadenylated enriched (Poly(A)+) and a polyadenylated depleted (Poly(A)-) fraction, and each fraction was analyzed for the presence of pHE-7 and actin RNA, as described under "Experimental Procedures."

in \(G_{1}\) mRNA, regardless of the proliferative capacity of the culture.

Taken together, these results indicate that a decline in expression of the pHE-7 transcript is not coupled to cell proliferation per se but rather occurs only as human fibroblast cultures near or reach the end of their proliferative lifespan.

pHE-7 RNA Is Polyadenylated and Relatively Stable in Presenescent and Senescent Cells—To learn more about the nature of the pHE-7 transcript, RNA from presenescent and senescent WI-38 cells was separated into polyadenylated (poly(A)+) and nonpolyadenylated (poly(A)-) fractions and analyzed on Northern blots for pHE-7-hybridizing species (Fig. 3). For both presenescent and senescent cells, all the RNA detected by the probe migrated as a single 1.2-1.4-kb transcript that was present exclusively in the poly(A)+ fraction. No pHE-7 hybridizing species were detectable in the poly(A)- fraction. As expected (26), actin mRNA was present predominantly in the poly(A)+ fraction, but a small amount was detectable in the poly(A)- fraction.

To estimate the stability of the pHE-7 transcript, presenescent and senescent cultures were incubated with actinomycin D (5 \(\mu\text{g/ml})\), an inhibitor of transcription. After 6 or 10 h, steady state levels of the pHE-7, c-Ha-ras, and c-myc transcripts were assessed by Northern analysis (Fig. 4). As expected, presenescent cells expressed higher levels of pHE-7 RNA than senescent cells. In both cases, however, there was virtually no decline in the abundance of the pHE-7 transcript in the presence of actinomycin D, even after 10 h. The level of c-Ha-ras mRNA, a relatively stable transcript (21) that is expressed at a similar level in presenescent and senescent cells (12), was also unaffected by a 10-h exposure to actinomycin D. By contrast, the labile c-myc mRNA (25) decayed to an undetectable level within 6 h of actinomycin D addition in both presenescent and senescent cells.

The results suggest that the pHE-7 transcript is a relatively stable, polyadenylated mRNA that remains stable and polyadenylated in senescent cells.

pHE-7 mRNA Encodes a Basic, 30-kDa Protein—To confirm that the pHE-7 transcript is an mRNA and characterize the translation product, the transcript was hybrid-selected, using the pHE-7 insert, and translated in vitro (Fig. 5). A single, major translation product migrated with an apparent molecular mass of slightly greater than 30 kDa on a one-dimensional polyacrylamide gel. On a two-dimensional gel, the 30-kDa translation product was detectable at the extreme basic edge of the isoelectric focusing dimension. These results suggest that pHE-7 detects an mRNA that directs the synthesis of a basic, 30-kDa protein.

pHE-7 mRNA Codes for the L7 Ribosomal Protein—To determine the identity of the pHE-7 protein, we screened a WI-38 cDNA library with the 280-bp pHE-7 insert. Two phage inserts (roughly 800 and 1000 bp) hybridized to the pHE-7 insert and detected a 1.2-1.4-kb mRNA in Northern blots of total RNA. Double-stranded sequencing of overlapping fragments showed that the smaller phage insert was 780 bp and contained a polyadenylation signal (AATAAA) and poly(A) tract at the presumptive 3' end. The larger insert was identical to the smaller one but contained 200 bp beyond the poly(A) tract, probably the result of a recombination event. Both phage cDNAs contained a 5' segment that was identical

FIG. 4. The pHE-7 RNA is relatively stable in presenescent and senescent cells. Early passage (Presenescent) and senescent WI-38 cells maintained in 10% serum were given actinomycin D (5 \(\mu\text{g/ml})\) for 6 or 10 h. RNA was isolate before and after administration of the drug and was analyzed for the relative abundance of pHE-7, c-myc, and c-Ha-ras mRNA. c-myc and c-Ha-ras are expressed similarly by presenescent and senescent cells (12) but differ in stability (21, 25).
Fig. 5. The pHE-7 mRNA encodes basic protein of about 30 kDa. The pHE-7 mRNA was hybrid-selected from total RNA and translated in vitro with a rabbit reticulocyte lysate and [35S]methionine, and the translation products were analyzed on one- and two-dimensional gels, as described under "Experimental Procedures." Top panel, the in vitro translation reaction was run with no added RNA (-RNA) or with 5 μg of hybrid-selected (pHE-7), total (Total RNA), or Brome mosaic virus (BMV RNA) RNA, and the products were analyzed on a one-dimensional gel. Molecular mass markers are shown on left and are indicated by thin lines. The hybrid-selected RNA directed the translation of a protein that migrated with an apparent molecular mass slightly larger than 30 kDa, indicated by a thick line. Middle panel, the products of the in vitro translation reaction run with hybrid-selected RNA were analyzed on a two-dimensional gel. The acid and basic ends of the isoelectric focusing dimension are indicated at the bottom of the figure. The position of the 30-kDa molecular mass marker is indicated on the right by a thin line. A major translation product of about 30 kDa is visible at the extreme basic end of the gel and is indicated by a thick line. Bottom panel, for comparison, the products of the in vitro translation reaction run with total RNA were analyzed on a two-dimensional gel.

in sequence to the pHE-7 insert.

The 780-bp cDNA was analyzed for an open reading frame. One frame generated a continuous sequence of 238 amino acids (Fig. 6), whereas the other two generated multiple termination codons. A comparison of this cDNA with entries in the GenBank data base revealed extensive nucleotide and amino acid homology to the rat and mouse L7, a structural protein of the large subunit of the ribosome (27, 28).

From the alignment of the mouse, rat, and human L7 sequences, our human cDNA (designated pL7-900) appeared to lack sequences encoding 10-20 amino acids at the amino terminus; the open reading frame in pL7-900 did not contain a methionine start codon. The size of the L7 mRNA estimated from Northern blots also suggested that pL7-900 was missing 5'-coding sequences.

To obtain the 5' sequence of the L7 mRNA, we screened a human genomic library with an oligonucleotide corresponding to 30 nucleotides at the 5' end of pL7-900. We cloned a 2.7-kb PstI fragment (pKS6-1) from a positive hybridizing phage insert, and about 1809 bp of the 3' end of this clone were sequenced. This sequence is shown by Fig. 7, with the 5'-most nucleotide of the determined sequence designated as position 1.

A methionine codon at position 512 initiated an open reading frame that was similar to the rat and mouse L7. A 14-bp polypyrimidine stretch was about 30 bp upstream of this codon. Polypyrimidine elements are found in the 5'-noncoding regions of most eukaryotic ribosomal protein mRNAs and are thought to be translation control elements (29). The human (CTTCCTCTTTTCCCC), mouse (CTCTCTCTTCTTCCCC), and rat (CTCTCTCTTCTTCCCC) L7 polypyrimidines were very similar. Downstream of the initiation codon, a 5' splice junction in the fifth codon (position 526) indicated that the location of the first intron was conserved between the mouse (28) and human L7 genes.

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A 3' splice
junction at position 1319 reestablished a reading frame that was nearly identical to the rat and mouse L7. A sequence identical to the rat and mouse L7 was identical to the pL7-900 sequence (Fig. 7).

The coding sequence from both the genomic and cDNA clones were identical and are shown only in the genomic clone sequence. The stop codon (TAA) and polyadenylation signal clones were identical and are shown only in the genomic clone.

The human L7 contains four basic amino acid repetitions at position 1429, and a 3' splice junction at 1712 again established a reading frame that was nearly identical to the rat and mouse L7. A sequence identical to the rat and mouse L7 and was identical to the pL7-900 sequence (Fig. 7).

Regulation of Other Ribosomal Protein mRNAs and Protein Synthesis with Senescence—Ribosomal protein genes are often coordinately regulated (30-32). Therefore, we measured the levels of five other ribosomal protein mRNAs in proliferating, quiescent, and senescent WI-38 cells (Fig. 8). The L5, P1, S3, S6, and S10 ribosomal protein mRNAs were all detectable on Northern blots of 20 μg total RNA. At early passage, each mRNA was present at a nearly identical level in quiescent and proliferating cells. Moreover, early passage and SV40-immortalized WI-38 cells expressed similar levels of L7 mRNA. In senescent cells, by contrast, each ribosomal protein mRNA was present at a 3- to 8-fold lower level relative to presenescent cells. As expected, there was no change with growth state or passage number in G6PD mRNA. Although quiescent and senescent cells differed in the level at which six ribosomal protein mRNAs were expressed, they did not differ appreciably in their overall rate of protein synthesis (Table I). Relative to proliferating cells, the rate of protein synthesis in quiescent and senescent cells was 14 and 11%, respectively.

We conclude that senescence entails a down-regulation of at least six ribosomal protein mRNAs and that this regulation may not be due simply to the diminished rate at which senescent cells synthesize protein.

DISCUSSION

Down-regulation of Ribosomal Protein mRNAs with Senescence—Cellular senescence results in an essentially irreversible cessation of cell division. In human fibroblasts, the expression and regulation of many genes are similar throughout the proliferative lifespan (8, 9, 11, 12). However, several genes have been identified whose expression level or pattern of regulation show changes uniquely associated with senescence. Some senescence-regulated genes encode proteins that are important in controlling or facilitating cell proliferation (e.g. the c-fos or replication-dependent histone genes) (9, 12, 13).
TABLE I

| Growth state  | Cell protein | Average | Growing cell rate |
|---------------|--------------|---------|-------------------|
| Exponentially growing | 8703 | 7696 | 100 |
| Early passage, quiescent | 1252 | 1076 | 14 |
| Senescent | 930 | 811 | 11 |

13. Others encode proteins that are differentiated cell products (e.g. the collagenase or fibronectin genes) (14–16). Here, we identify the L7 ribosomal protein gene as a senescence-regulated gene and show that at least five other ribosomal protein genes are down-regulated when human fibroblasts senesce.

Even in early passage human fibroblast cultures, there is variability in the division potential of the cells that comprise the culture (33). Martin and colleagues (10) suggested that cell senescence, whether in vivo or in culture, is a process of clonal selection and that fully senescent cell populations are in fact comprised of clones that had the longest proliferative lifespan. The L7 mRNA level did not decline gradually as WI-38 cell senesced but rather declined only after the labeling index reached 5. In some experiments, cultures with a labeling index of 10 showed only a marginal down-regulation. This finding suggests that down-regulation of ribosomal protein mRNAs may not occur in cell clones that have a relatively short proliferative lifespan. Thus, by the time the cultures consist primarily of senescent cells, there has been selection for cell clones having the longest proliferative lifespan. This may be one of the clones that down-regulate the ribosomal protein mRNAs once they exhaust their proliferative capacity. Alternatively, down-regulation of ribosomal protein mRNAs may be a late event in the program of gene expression that defines the senescent state, occurring well after the cessation of cell proliferation.

Characteristics of the Human L7 Gene—The human L7 coding region shared extensive nucleotide and amino acid homology to that of the mouse and rat L7 genes. This was not surprising, given what is known about the extreme conservation among eukaryotic species in ribosome structure and function (34). Even within noncoding regions, some features were conserved between the human and rodent genes. These include the polypyrimidine element in the 5′-untranslated region of the mRNA and the position of the first intron. Because ribosomal protein genes are generally regulated coordinately (29–31), both of these characteristics may be important for appropriate regulation of L7 gene expression.

The human L7 differs most from the mouse and rat L7 in the amino-terminal portion of the protein. Lin et al. (1987) (27) identified five tandem repetitive arrays of 12 predominately basic amino acids near the amino terminus of the rat L7 protein. The mouse L7 protein contains an additional array between the first and second array present in the rat protein (28). Hence, the mouse protein contains six tandem repetitive arrays, whereas the rat protein contains five. Our analysis indicates that the human L7 protein contains four tandem arrays of 12 predominately basic amino acids. If the mouse sequence is the most representative of the ancestral L7 protein of mammals (or vertebrates), then it appears that cassettes of these arrays have been lost in at least a couple of mammalian lineages over the course of evolution.

Significance for the Senescent Phenotype—Several lines of evidence suggest that senescent fibroblasts express an altered phenotype and that senescence may entail processes analogous to terminal differentiation (10–12, 14–16). This view predicts that senescent and quiescent fibroblasts should differ in their pattern of gene expression, despite similarities in growth status and rate of protein synthesis. Our previous results on the expression of a histone variant mRNA (12) and the results presented here on the expression of ribosomal protein mRNAs support this view.

Proliferating cells have higher levels of ribosomal proteins than quiescent cells, and increased ribosome biogenesis often accompanies growth stimulation or malignancy (30–32, 35, 36). In these cases, altered protein synthetic capacity does not generally result in altered ribosomal protein mRNA levels. In contrast to its inactivation, pH1 was used to identify a “control” mRNA, which remained constant after growth stimulation by serum factors or adenovirus (9, 12, 17), although it declined after terminal differentiation of keratinocytes (37). Thus, when there is a change in growth state that is not coupled to differentiation, altered protein synthetic capacity may result from changes in ribosomal protein mRNA translation, rather than from changes in mRNA abundance. We suggest that the altered levels of ribosomal protein mRNAs in senescent fibroblasts reflect an altered differentiated state.

Increased ribosomal protein mRNA levels have been found in colorectal and hepatocellular tumors and adenomatous polyps (38–40). This increase did not correlate with increased cell proliferation per se, particularly in colorectal tumors where it is known that tumor tissue and normal colonic mucosa have similar proliferative indices (40). Anaplasia is a very common feature of tumor cells (41). Thus, the change in ribosomal protein mRNAs in these neoplastic cells may also reflect an altered state of differentiation. Our finding that human and SV40-immortalized human fibroblasts expressed similar levels of ribosomal protein mRNA is consistent with the idea that immortalization by T antigen prevents the change in differentiated phenotype that accompanies cell senescence, in addition to its well documented ability to prevent the growth arrest.

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