Expression of Elongation Factor-1α and S1 in Young and Old Human Skeletal Muscle

Stephen Welle,¹² Charles Thornton,¹ Kirti Bhatt,¹² and Matthew Krym¹

¹University of Rochester and ²Monroe Community Hospital, Rochester, New York.

Previous research has indicated that reduced expression of elongation factor-1α (EF-1α) may be an important determinant of the reduced rate of protein synthesis in senescent animals and cultured cells. The present study examined whether expression of EF-1α or S1, a homologous protein found exclusively in postmitotic tissues, is reduced in senescent human skeletal muscle. Muscle biopsies were obtained from the vastus lateralis muscles of healthy young (22–31 yr old) and old (61–74 yr old) subjects. As reported previously, myofibrillar protein synthesis was ~40% slower in the older muscle (p < .001) as determined by incorporation of a stable isotope. Immunoblotting revealed no difference in the concentration of EF-1α + S1 between younger and older muscle. RT-PCR assays indicated that S1 mRNA was much more abundant than EF-1α mRNA in muscles of both age groups, with no reduction in either EF-1α or S1 mRNA abundance in older muscles. We conclude that expression of EF-1α and S1 is not diminished in older muscles and does not explain the age-related slowing of protein synthesis in human skeletal muscle. However, we cannot exclude the possibility that the activity of these proteins declines during senescence due to post-translational modifications.

There is a reduction in the rate of protein synthesis during senescence in many organisms (Makrides, 1983; Webster, 1985; Richardson and Sensei, 1987). This slowing of protein synthesis could have significant functional consequences for two reasons. First, the mass of proteins is directly proportional to the rate of protein synthesis when protein half life is constant. Second, even if protein half life is prolonged enough to maintain protein mass when the protein synthesis is slower, the quality of the proteins could be decreased because chemical modifications in aged proteins can impair their functions (Stadtman, 1988). Recently it has been reported that the rate of synthesis of myofibrillar proteins (Welle et al., 1993, 1994, 1995) and total tissue proteins (Yarasheski et al., 1993) is slower in skeletal muscle of healthy older humans than it is in muscle of young adults. There was no difference between young and old muscle in the abundance of total mRNA or mRNAs encoding the most abundant myofibrillar proteins (Welle et al., 1996), suggesting that the slower protein synthesis results from slower mobilization or translation of the mRNAs.

Several lines of evidence suggest that elongation of peptide chains slows during aging. Blazewiowski and Webster (1984) reported that there was only a slight decrease in the protein synthesis initiation rate in various tissues of old mice, whereas the elongation rate was reduced 67–85%. Reduced GTP-dependent binding of t-RNAs to ribosomes, which is catalyzed by elongation factor-1 (EF-1), has been observed in various tissues of senescent animals (Moldave et al., 1979; Webster and Webster, 1982; Gabius et al., 1983), whereas initiation, translocation, and termination are relatively unaffected by age. A decrease in the synthesis of EF-1 was reported to precede the overall decrease in protein synthesis observed in senescent Drosophila (Webster and Webster, 1983). Use of a probe specific for the mRNA encoding the α subunit of EF-1 (EF-1α) in Drosophila revealed an age-related decrease in expression (Webster, 1985). The amount of translatable EF-1 mRNA was reported to also decrease in livers of old mice (Webster, 1985). Cultured human fibroblasts near the end of their life span have reduced EF-1α activity (Cavallius et al., 1986). However, there are recent reports that the amount of mRNA encoding EF-1α does not decrease significantly with aging in the brains, hearts, or livers of old rats (Lee et al., 1992) or in old Drosophila (Shikama et al., 1994) or nematodes (Fabian and Johnson, 1995).

In the brain, heart, and skeletal muscles of rodents and humans, abundant expression of an EF-1α variant, termed S1 or EF-1α2, has been observed (Lee et al., 1992; Knudsen et al., 1993; Lee et al., 1994). It has 92% amino acid sequence homology with EF-1α, and is assumed to have a similar function in protein synthesis (Knudsen et al., 1993). In human muscle, S1 mRNA is much more abundant than EF-1α mRNA (Knudsen et al., 1993). Lee et al. (1992) did not observe a change in S1 mRNA during senescence in rats.

The relevance of the studies cited above to the aging of a human postmitotic tissue such as skeletal muscle is uncertain. Thus, we have examined whether skeletal muscle of healthy older human subjects, in whom reduced protein synthesis rates were demonstrated previously (Welle et al., 1993, 1994, 1995), expresses less EF-1α and S1 protein and mRNA than muscle from young adults.

METHODS

Subjects. — Subjects were healthy men and women who participated in studies of myofibrillar protein synthesis, as previously reported (Welle et al., 1993, 1994, 1995). None had any unusual dietary or exercise habits. Young subjects ranged in age from 21 to 31 yr, and older subjects ranged in age from 61 to 74 yr.
**Procedures.** — Muscle samples were obtained by needle biopsy from the vastus lateralis after the skin and muscle were anesthetized with lidocaine. Subjects followed a standard weight-maintaining diet provided by the Clinical Research Center and avoided strenuous activities for 3 days before biopsies were taken. They slept at the Clinical Research Center the night before the biopsy, and did not engage in any activities on the morning of the biopsy. Muscle samples were frozen immediately in liquid nitrogen, then stored at -70 °C until assayed.

**EF-1α + S1 protein abundance.** — Proteins were solubilized by mincing the muscle tissue with a razor and warming the fragments at 50 °C for 30 min in 20 volumes of 6 M urea, 1% SDS, 1% 2-mercaptoethanol. Insoluble remnants were removed by centrifugation, and the total protein concentration of the solution was determined with a commercial kit (BioRad, Hercules, CA). Equal amounts of protein (45 μg) from each sample were subjected to SDS-PAGE, then were transferred by electrophoresis to a nylon membrane (Immobilon, Millipore Corp., Bedford, MA). The membrane was blocked with 4% nonfat dry milk in PBS, then exposed overnight at 4 °C to a mouse monoclonal anti-EF-1α antibody (Upstate Biotechnology, Lake Placid, NY) at a concentration of 1 μg/ml in the blocking solution with 0.03% Triton X-100 and 1% normal goat serum. Although the reactivity of this anti-EF-1α antibody with S1 has not been determined, the very high amino acid sequence homology between EF-1α and S1 makes it likely that antibody binding reflects the combined abundance of EF-1α and S1. The amount of bound primary antibody was detected by reaction with a rabbit anti-mouse affinity purified IgG antibody (Cappel/Organon Teknika, West Chester, PA), then with 125I-labeled goat anti-rabbit IgG antibody (New England Nuclear, Boston, MA). The amount of 125I bound to the membrane was determined with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). Preliminary assays indicated that signal intensity was proportional to the amount of total protein applied to the gel, over the range of 15 to 90 μg. A positive control sample provided by the supplier of the primary antibody gave a band at the same molecular weight as the strong band observed at 50 kD in the blots of the muscle samples. Omission of the primary antibody from the above procedure eliminated the strong band at 50 kD.

**EF-1α and S1 mRNA abundance.** — Total RNA was extracted from the biopsy specimens by the phenol/guanidine thiocyanate method as described previously (Welle et al., 1996). We previously determined that there was no age-related difference in the muscle tissue abundances of total RNA or polyadenylated RNA relative to DNA (Welle et al., 1996). Thus, to compare young and old groups, equal amounts of total RNA were analyzed by RT-PCR.

Primers were identified to amplify segments of the 3' untranslated regions of human S1 (Knudsen et al., 1993) and EF-1α (Madsen et al., 1990) cDNAs (Figure 1). PCR amplification with the S1 primers (A and C in Figure 1) produced a 134 base pair product, and amplification with the EF-1α primers (D and E in Figure 1) produced a 220 base pair product (all primers were prepared by DNA International, Lake Oswego, OR). Automated sequencing by the University of Rochester core nucleic acid laboratory verified that the PCR products corresponded to the published mRNA sequences.

An internal standard RNA, which was the same as the amplified segment of the S1 mRNA except for a 16 base deletion 3' to the primer A sequence, was prepared using the general strategy described by Riedy et al. (1995). Dou-

---

**Figure 1.** Primers used to amplify S1 and EF-1α cDNAs. Numbers refer to nucleotide numbers from published sequences for S1 mRNA (Knudsen et al., 1993; GenBank Accession Number X70940) and EF-1α mRNA (Madsen et al., 1990; GenBank Accession Number X16869).
ble stranded DNA corresponding to S1 mRNA was prepared by PCR with human muscle first-strand cDNA as the template, using primers A and C (Figure 1). The product of this first step was then amplified by PCR with primers B and C (Figure 1) to produce the internal deletion. The DNA template for standard RNA synthesis was then made by amplifying the product of the second step with primer F (the T7 promoter sequence, ATGCACCACGAGGCTCTGAG, followed by the primer A sequence). This DNA was then transcribed with T7 polymerase to produce the standard RNA, which could then be amplified by RT-PCR using the same primers used for the S1 mRNA.

Under the PCR conditions described below, amplification of all of the relevant cDNAs was exponential and highly efficient for at least 22 cycles. Thus, we examined the RT-PCR products in all samples after 20 cycles of PCR amplification. Even if there were slight differences in the efficiency of amplification of the different cDNAs, the relative effect of age on their ratios would not be invalidated.

Sample RNA (0.25 μg), plus 40 pg of internal standard, was reverse transcribed with M-MLV RT (Life Technologies, Grand Island, NY) using random hexamer priming. The first strand cDNAs were amplified by PCR with Taq polymerase (Promega, Madison, WI). Primers A and D were labeled with 32P using T4 polynucleotide kinase and γ-32P-ATP (New England Nuclear), then separated from free ATP on a Nensorb (NEN-20) cartridge (New England Nuclear). Specific activity of primer D was made fivefold more than that of primer A because preliminary trials indicated that the EF-1α cDNA was much less abundant than the S1 cDNA. PCR conditions were as follows: 20 cycles with 30 sec denaturing at 94 °C, 30 sec annealing at 47 °C, and 60 sec elongation at 72 °C. The first cycle in each assay included an additional 60 sec at 94 °C, and the final cycle had an additional 6 min at 72 °C. Taq polymerase (Promega, Madison, WI) was used at a concentration of 2.5 U/100 μl. Buffer concentrations were 10 mM Tris HCl, pH 9.0, 50 mM KC1, 1.5 mM MgCl2, 0.1% Triton X-100, 160 μM dNTPs, and 0.27 μM primers. The PCR products were separated on a polyacrylamide gel, and yield was determined by exposing X-ray film to the gel. Radiographs were quantified with a densitometer, with software obtained from the manufacturer (Microcomputer Imaging Device, Imaging Research, St. Catherines, Ontario, Canada). Duration of film exposure was adjusted to ensure that density was proportional to 32P activity.

Statistical analysis. — Data are presented as the mean ± one standard error. Statistical significance of differences between means of young and old groups was evaluated with t-tests. Analysis of covariance (NCSS 6.0, NCSS, Kaysville, UT) was used to determine whether the effect of age on myofibrillar synthesis was significant after adjustment for mRNA levels. Stepwise regression (NCSS 6.0) was used to determine whether there was a significant partial correlation between mRNA levels and myofibrillar protein synthesis after adjustment for age.

RESULTS

Muscle protein samples from 6 older subjects and 6 young adults were analyzed for S1 and EF-1α mRNA by immunoblotting. The average rate of myofibrillar protein synthesis in the older group was 42% (p < .001) slower than the synthesis rate in the young group. There was no difference between age groups in the immunoreactive EF-1α + S1 mass (Figure 2). Quantitative analysis of the results shown in Figure 2 is presented in Table 1. When the 50 kD band intensities from the immunoblot were divided by the respective intensities of the myosin heavy chain bands in the Coomassie stained gel (Figure 2B), the mean values were the same in younger and older groups (Table 1).

Muscle RNA samples from 10 young and 10 older subjects were analyzed with RT-PCR assays of EF-1α and S1 mRNA (Figure 3). Myofibrillar protein synthesis was 40% (p < .001) slower in the older muscles. In both age groups, the abundance of the RT-PCR product of EF-1α mRNA amplification was less than 10% of the abundance of the product of S1 mRNA amplification (Table 1). Note that the specific activity of the primer used to label the EF-1α cDNA was fivefold higher than the primer used to label S1 and standard cDNAs, so that the band intensities in Figure 3 do not represent the actual amounts of the RT-PCR products. Quantitative densitometry of the radiographs showed a higher EF-1α mRNA abundance in the older muscles, relative to the standard RNA (Table 1). There was no significant difference between age groups in the ratio of S1 mRNA to standard RNA or in the ratio of EF-1α mRNA to S1 mRNA (Table 1).
rates of myofibrillar protein synthesis did not correlate significantly with the EF-1α:S1 mRNA ratios \( (p = .49) \), the EF-1α mRNA:standard ratios \( (p = .79) \), or the S1 mRNA:standard ratios \( (p = .91) \) after adjustment for age. The myofibrillar synthesis rate was significantly less in the older group \( (p < .001) \) after adjustment for each of these ratios.

**DISCUSSION**

The present results do not support the hypothesis that reduced expression of EF-1α or the related protein S1 is responsible for the slowing of protein synthesis associated with aging in human skeletal muscle. These data are consistent with the data of Lee et al. (1992), who found no age-related decrease in expression of EF-1α or S1 mRNAs in rat tissues. Failure to find a reduction in the amount of EF-1α:S1 in older muscle does not necessarily mean that activity of these proteins is not reduced. In a study of *Drosophila*, Shikama et al. (1994) noted that EF-1α activity decreased with age even though EF-1α mRNA and protein levels were not significantly affected by age. However, earlier research in *Drosophila* and mouse liver had demonstrated that aging reduced EF-1α mRNA levels and reduced synthesis and amounts of EF-1 (Webster, 1985). If the slowing of protein synthesis in old human muscle is related to reduced EF-1α activity (i.e., binding of amino acyl-tRNA to ribosomes), there must be a post-translational modification in the protein that is affected by age.

S1 mRNA has been detected only in postmitotic tissues (Lee et al., 1992; Knudsen et al., 1993; Lee et al., 1994). Because of its very high amino acid sequence homology with EF-1α, S1 is assumed to be functionally similar to EF-1α (Knudsen et al., 1993), although this assumption has not been verified experimentally. The high level of amino acid homology between these proteins has prevented determination of their relative abundances by immunoblotting or by two-dimensional gel electrophoresis. Northern blotting previously revealed abundant expression of S1 mRNA in adult human skeletal muscle, whereas EF-1α mRNA was barely detectable (Knudsen et al., 1993), which is consistent with the present finding that the RT-PCR product of S1 mRNA amplification was much more abundant than the product of EF-1α mRNA amplification. Thus, it seems likely that most of the immunoreactive protein measured in the present study was S1 rather than EF-1α in both age groups, unless there is preferential translation of EF-1α mRNA. If EF-1α has a higher specific activity than S1 with respect to binding of amino acyl-tRNA to ribosomes (or if S1 has no amino acyl-tRNA binding activity at all), then the increased expression of EF-1α mRNA in the older muscles would be inconsistent with the hypothesis that reduced EF-1α expression is responsible for the slower protein synthesis in older muscles. Further evidence that neither EF-1α nor S1 mRNA abundance was the limiting factor in determining myofibrillar synthesis was that neither correlated with the observed protein synthesis rates after adjustment for the age effect, and that the statistical significance of the age difference in myofibrillar synthesis was not diminished by adjustment for the abundance of these mRNAs or their ratio.

In recent years there has been much interest in EF-1α because it appears to have several roles other than binding of amino acyl-tRNA to ribosomes. In rapidly growing cells, EF-1α abundance appears to be much greater than what is necessary for its role in peptide chain elongation, being 7-35 times more abundant on a molar basis than other components of the elongation cycle (EF-2, EF-1β, ribosomes, tRNAs) (Condeelis, 1995). It is involved in microtubule severing (Shiina et al., 1994), it binds to calmodulin (Kaur and Ruben, 1994) and actin filaments (Yang et al., 1990), and is required for ubiquitin-dependent proteolysis of N-acetylated proteins (Gonen et al., 1994). Other potential

---

**Table 1. Quantitative Densitometry**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Old</th>
<th>Young</th>
<th>95% CI (Old minus Young)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoreactive protein (Arbitrary Units)</td>
<td>32.1 ± 1.9</td>
<td>33.0 ± 1.1</td>
<td>-5.9, +4.1</td>
<td>.68</td>
</tr>
<tr>
<td>Immunoreactive protein: myosin heavy chain</td>
<td>3.2 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>-0.6, +0.6</td>
<td>.95</td>
</tr>
<tr>
<td>S1 mRNA:standard ratio (Arbitrary Units)</td>
<td>5.2 ± 0.8</td>
<td>4.3 ± 0.6</td>
<td>-1.3, +2.9</td>
<td>.41</td>
</tr>
<tr>
<td>EF-1α mRNA:standard ratio (Arbitrary Units)</td>
<td>0.35 ± 0.08</td>
<td>0.17 ± 0.03</td>
<td>0.00, -0.36</td>
<td>.05</td>
</tr>
<tr>
<td>EF-1α:S1 mRNA ratio (%)</td>
<td>7.7 ± 1.5</td>
<td>4.5 ± 1.1</td>
<td>-0.8, +7.2</td>
<td>.11</td>
</tr>
</tbody>
</table>

*Notes:* Values are mean ± one standard error. Confidence intervals (CI) are for the mean difference between the old and young groups; with a positive value denoting a higher mean in the old group. *P*-values based on *t*-tests for comparisons between means of young and old groups.

---

**Figure 3. Representative radiograph of gel separating 32P-labeled cDNAs of human muscle EF-1α mRNA, human muscle S1 mRNA, and in vitro synthesized internal standard RNA.** Equal amounts of total RNA from each sample were analyzed in RT-PCR assay as described in text. Abundances of EF-1α and S1 cDNAs cannot be directly compared because of differences in primer specific activity. Three lanes have samples from older muscle (O) and three from younger (Y) muscle.
roles include involvement in centrosome formation and mitotic spindle nucleation (Marchesi and Ngo, 1993) association of ribosomal subunits (Herrera et al., 1991), and activation of phosphatidylinositol 4-kinase (Yang et al., 1993). Its expression is much higher in cultured cells than in tissues in vivo (Sanders et al., 1992), and is higher in tumors than in normal tissues (Grant et al., 1992). The possible role of EF-1α in longevity also has received attention. Drosophila transformed with a vector containing the EF-1α gene under control of hsp-70 regulatory sequences were found to have increased longevity (Sheperd et al., 1989). However, a later study showed that EF-1α was not overexpressed in the transformed flies (Shikama et al., 1994). Increased longevity was found in fungal strains with mutagenesis and increased longevity was found in fungal strains with mutagenesis to have increased longevity (Sheperd et al., 1989). However, a later study showed that EF-1α was not overexpressed in the transformed flies (Shikama et al., 1994). Increased longevity was found in fungal strains with muta-
tions of the EF-1α gene that increased fidelity of translation (Silar and Picard, 1994). It is not known whether EF-1α or S1 have any essential role in postmitotic human cells other than binding of amino acyl-tRNA to ribosomes.

In summary, there is no evidence that the slowing of protein synthesis associated with senescence in human skeletal muscle is caused by reduced expression of EF-1α or S1. Further research is needed to elucidate whether EF-1α or S1 activity changes with age, or whether other components of the translational apparatus are primarily responsible for the slower protein synthesis in old muscles.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health (AG-13070, AG-10463, RR-00044). Dr. Thornton is supported by a Clinical Investigator Development Award from the National Institute of Neurological Disorders and Stroke (NIH).

Address correspondence to Dr. Stephen Welle, Monroe Community Hospital, 435 East Henrietta Road, Rochester, NY 14620.

REFERENCES


Herrera, F.; Correia, H.; Triana, L.; Fraile, G. Association of ribosomal subunits. A new functional role for yeast EF-1α in protein biosyn-


Knudsen, S.M.; Frydenberg, J.; Clark, B.F.C.; Leffers, H. Tissue-dependent variation in the expression of elongation factor-1α isoforms: iso-


Lee, S.; Ann, D.K.; Wang. E. Cloning of human and mouse brain cDNAs for coding the S1, the second member of the mammalian elongation fac-

Madsen, H.O.; Poulsen, D.; Dahl, O.; Clark B.F.C.; Hjorth, J.P. Retro-


Marchesi, V.T.; Ngo, N. In vitro assembly of multigene complexes con-


Riedy, M.C.; Timm, E.A.; Jr.; Stewart, C.C. Quantitative RT-PCR for mea-

Sanders, J.; Maassen, J.A.; Moller, W. Elongation factor-1 messenger-
RNA levels in cultured cells are high compared to tissue and are not drastically affected further by oncogenic transformation. Nucl. Acids Res. 20:5907–5910; 1992.

Sheperd, J.C.W.; Walldorf, U.; Hug, P.; Gehring, W.J. Fruit flies with addi-

Shina, N.; Gotob, Y.; Kubomura, N.; Iwamatsu, A.; Nishida, E. Micro-


Webster, G.C. Protein synthesis in aging organisms. In: Sohal, R.S.; Birm-


Webster, G.C.; Webster, S.L. Decline in synthesis of elongation factor one (EF-1) precedes the decreased synthesis of total protein in aging.


Welle, S.; Thornton, C.; Jozefowicz, R.; Statt, M. Myofibrillar protein syn-


Yang, F.; Demma M.; Warren, V.; Dharmawardhane, S.; Condeelis, J. Identification of an actin-binding protein from dictyostelium as elonga-

Yatsu, W.; Burkart, W.; Cavalli, J.; Merrick, W.C.; Boss, W.F. Purifica-

Yarasheski, K.E.; Zachwieja, J.J.; Bier, D. Acute effects of resistance exer-

Received July 22, 1996
Accepted May 1, 1997

ELONGATION FACTOR-1α and S1

B239

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/52A/5/B235/617470 by guest on 30 December 2018