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Selenoprotein W gene regulation by selenium in L8 cells

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Abstract

The effects of selenium on selenoprotein W gene expression were examined in cultured L8 rat skeletal muscle cells. Selenoprotein W contains selenium as selenocysteine in the primary protein structure and levels of this selenoprotein are affected by selenium. Northern blots indicated that there were no significant changes ($P < 0.05$) in selenoprotein W mRNA levels during cell proliferation and differentiation. Reduction of selenium concentration in the medium decreased the selenoprotein W mRNA levels. Nuclear run-on experiments with isolated L8 nuclei showed the same rate of selenoprotein W mRNA synthesis in cells cultured in either low selenium or selenium supplemented medium, suggesting that the transcription rate of the selenoprotein W gene is independent of selenium. Measurement of the selenoprotein W mRNA half-life in myoblasts treated with the transcription inhibitor, α-amanitin, showed that selenoprotein W mRNA levels decreased over time with an estimated half-life of 57 h for cells grown in low selenium medium. Selenium treatment increased the selenoprotein W mRNA half-life 2-fold. These data suggest that selenium stabilizes selenoprotein W mRNA but has no effect on transcription.

Introcution

Evidence for selenoprotein W was first reported as a missing component in muscle of lambs suffering from white muscle disease (Pedersen *et al.* 1972). Recent studies indicated that this protein was present in several tissues, but is highest in rat skeletal muscles (Yeh *et al.* 1995) and in both skeletal and cardiac muscles of sheep (Yeh *et al.* 1997) and monkeys (Gu *et al.* 2000). Tissue selenoprotein W and mRNA levels are influenced by selenium status in the rat (Yeh *et al.* 1995; Vendeland *et al.* 1995). Regulation of selenoprotein W gene expression in muscle cells is of interest because there is evidence that selenium is related to normal muscle function. Selenium deficiency is one factor contributing to white muscle disease (Schubert *et al.* 1961), characterized by degeneration of both skeletal and cardiac muscles in lambs and calves, and Keshan disease (Chen *et al.* 1980), an endemic juvenile cardiomyopathy in a human population in China.

Selenoproteins contain a unique amino acid, selenocysteine, in their primary protein structure. Selenocysteine is incorporated into selenoproteins at the translational step by a specific UGA decoding translational machinery (Burk & Hill 1993) and selenoprotein synthesis is dependent upon selenium. Regulation of glutathione peroxidase and selenoprotein P gene expression has been studied in selenium deficient or supplemented animals (Knight & Sunde 1988; Read *et al.* 1990; Burk *et al.* 1991) as well as in a human population (Hill *et al.* 1996). Even though mRNA levels directly affect the amount of template available for selenoprotein synthesis, the selenoprotein levels appear to be more sensitive to selenium status than their corresponding mRNAs .

The steady-state level of cytoplasmic mRNA depends upon its rate of formation as well as its degradation. Transcriptional control of mRNA formation has received the most attention, but post-transcriptional events such as processing of nuclear RNA, export from

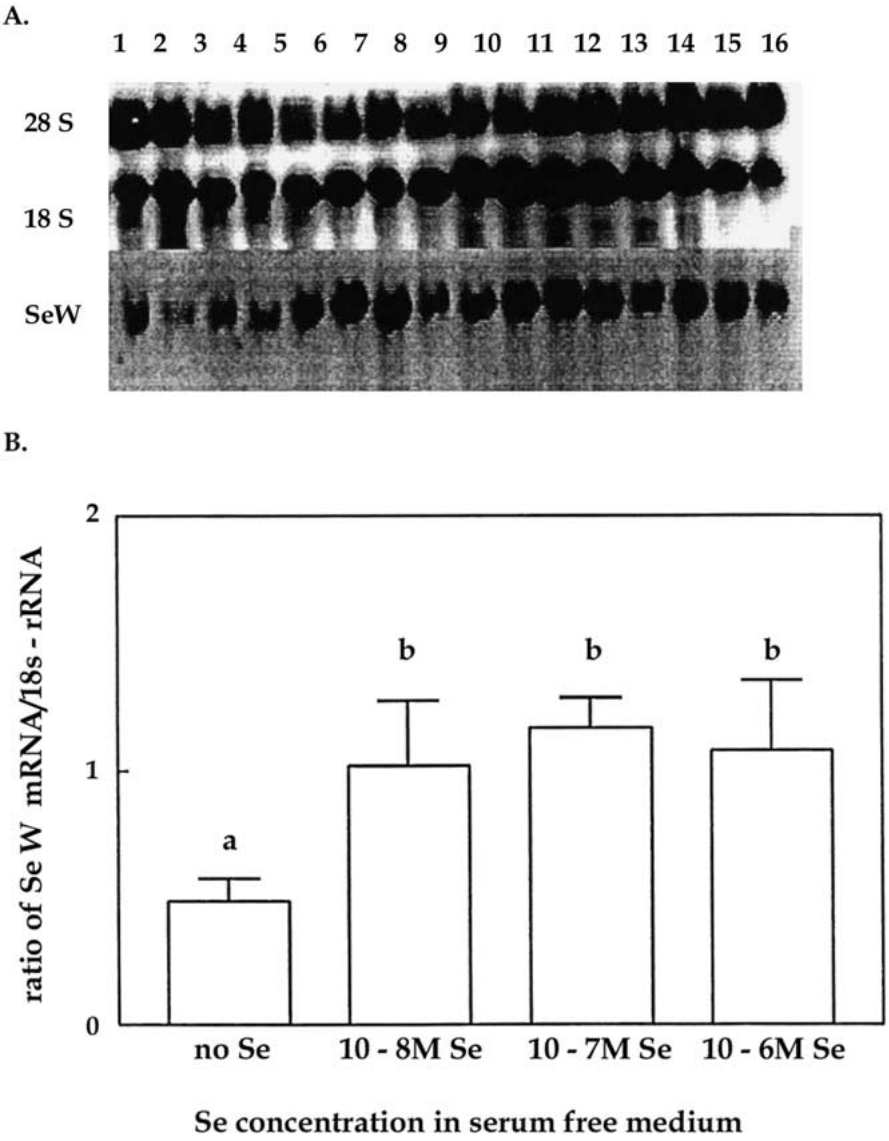

Fig. 1. Effects of different levels of selenium in the cell medium on selenoprotein W mRNA levels. Myotubules at 2 days of differentiation were incubated in serum-free medium with various concentrations of selenium for 3 days. A: Northern blot analysis with selenoprotein W cDNA and 18s-rRNA probes. Lanes 1–4: serum-free medium without selenium, lanes 5–8: serum-free medium with 10^{-8} M selenium, lanes 9–12: serum-free medium with 10^{-7} M selenium, lanes 13–16: serum-free medium with 10^{-6} M selenium. B: Bar graphs depicting the selenoprotein W mRNA levels normalized to that of 18s-rRNA to correct for loading variations. Error bars indicate standard error of the mean ($n = 4$). Treatments which do not share a common superscript differ significantly ($P < 0.05$).

the nucleus and mRNA degradation are increasingly recognized as processes affecting cytoplasmic mRNA levels. The 3′ untranslated region (3′-UTR) stem-loop mediated iron dependent regulation of transferrin receptor transcripts is an excellent example (Casey *et al.* 1988). Interestingly, mammalian cDNAs for selenoproteins also have a stem-loop structure in the 3′-UTR. This stem-loop structure (SECIS element) is considered to be an essential structure for selenocysteine incorporation at the UGA codon (Berry *et al.* 1994; Moriarty *et al.* 1998). The effects of selenium on glutathione peroxidase, deiodinase and selenoprotein P gene expression reside at post-transcriptional steps (Stadtman 1996; Christensen & Burgener 1992; Toyoda *et al.* 1989), which could be the same step for the regulation of selenoprotein W gene expression.

Table 1. Effects of long-term differentiation on selenoprotein W mRNA levels.

Differentiation (days)	DMEM+2% CS	Serum free medium + Se (3 10^{-8} M)	Serum free medium
Initial	2.51 ± 0.07	3.35 ± 0.43	3.28 ± 0.29^{a}
3	2.55 ± 0.28	3.17 ± 0.23	1.77 ± 0.27^{bc}
4	2.67 ± 0.18	3.19 ± 0.24	1.30 ± 0.29^{bc}
5	2.47 ± 0.06	3.20 ± 0.40	1.32 ± 0.22^{bc}
6	2.22 ± 0.24	2.93 ± 0.34	1.28 ± 0.62^{bc}
7	2.20 ± 0.23	2.85 ± 0.40	1.13 ± 0.27^{bc}
8	2.21 ± 0.16	3.08 ± 0.40	0.75 ± 0.09^{c}

Values are ratios of selenoprotein W mRNA/18s-rRNA expressed as the means of three individual determination $\pm$ standard errors. Different superscripts within column indicate significant differences ($P < 0.05$).

Thus in light of those studies, the present investigation was undertaken to determine whether selenium affects mRNA levels for selenoprotein W at the transcriptional or post-transcriptional levels. A preliminary report of this research has been presented (Ream *et al.* 2001).

Materials and methods

Materials

L8 rat myoblast cells were obtained from American Type Culture Collection (Rockville, MD). Fetal calf serum (CS) was purchased from Hyclone (Logan, UT). Dulbecco's modified Eagle's media (DMEM), penicillin/streptomycin solution, trypsin and DNA mass ladder were purchased from GIBCO (Gaithersburg, MD). Cell culture petri dishes were purchased from Corning (Corning, NY). GeneScreen Plus nylon membrane and α^{32}P-UTP (800Ci/mmole) were purchased from DuPont/NEN (Boston, MA). Plasmid Purification Kit was purchased from QIAGEN, Inc (Chatsworth, CA). PCR amplification Kit and RNase were purchased from Promega (Madison, WI). Chroma spin^{TM} columns were purchased from Clontech (Palo Alto, CA). All the restriction enzymes were obtained from New England Biolabs Inc (Beverly, MA). DIG Oligonucleotide Tailing Kit, digoxigenin-dUTP, anti-DIG-AP conjugate, blocking reagent, luminescent detection substrate CSPD and *E. coli* tRNA were purchased from Boehringer Mannheim Biochemical (Indianapolis, IN). DNase and RNase were purchased from Worthington Biochemical Co (Freehold, NJ). Non-radiolabeled nucleotides ATP, CTP and GTP were obtained from Pharmacia (Piscataway, NJ). Hyperfilm was purchased from Amersham (Arlington Heights, IL). All other chemicals were of molecular biology grade and purchased from Sigma Chemical Co. (St. Louis, MO).

Cell culture and treatment

Cells were grown in basal medium (DMEM, 100 units penicillin ml, 100 μg streptomycin ml and 44 mM sodium bicarbonate, pH 7.4) supplemented with 10% CS in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The medium was changed every two days. To induce differentiation, cells were grown to confluence and the medium was replaced with differentiation medium (basal medium plus 2% CS). Differentiating cells were sampled for 8 days to examine the long term effect of differentiation. Alternatively after inducing differentiation, the medium was replaced with serum-free medium (basal medium supplemented with 10^{-7} M insulin, 10^{-7} M dexamethasone, 5 μg/ml transferrin, 1 μg/ml linoleic acid, and 300 μg/ml fetuin) with various levels of selenium as selenite to examine the effect of selenium status on selenoprotein W mRNA levels. The effect of selenium on selenoprotein W mRNA stability was examined by blocking mRNA transcription with α-amanitin.

RNA extraction and Northern blot

Total cellular RNA was isolated by the single step guanidine isothiocyanate procedure (Chomczynski & Sacchi 1987). The final RNA pellet was dissolved in diethyl pyrocarbonate treated water and RNA concentration was determined spectrophotometrically at 260 nm. A sample of 20 μg of formaldehyde denatured RNA was separated by electrophoresis on a 1.5% agarose-2.2 M formaldehyde gel, and the RNA blotted onto a GeneScreen nylon membrane in 10× SSC (1.5 M NaCl and 0.15 M sodium citrate, pH 7.0) as transfer medium. The membrane

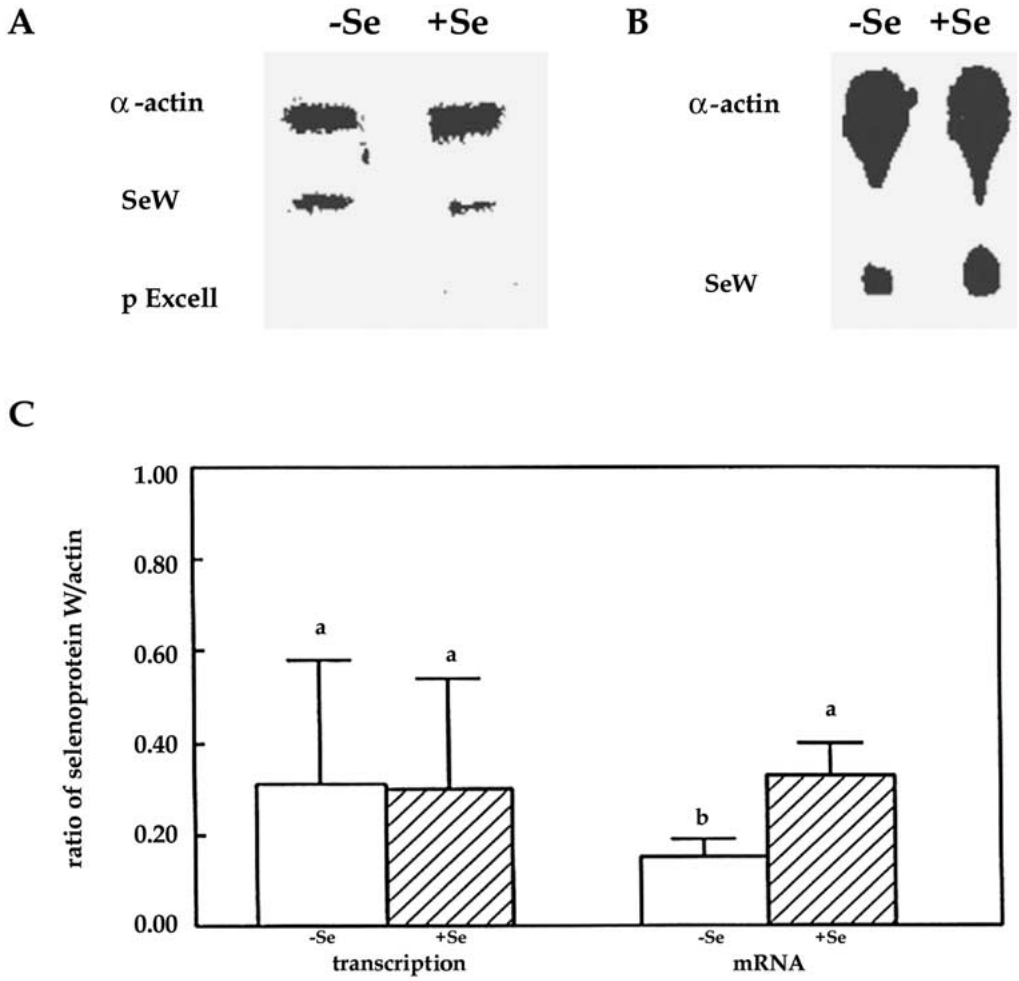

Fig. 2. Effects of selenium on transcription and on stability of mRNA for selenoprotein W in L8 muscle cells. A: Representative autoradiography from one set of experiments showing the amount of RNA transcribed *in vitro* by nuclei from cells grown in serum-free medium with 10^{-7} M selenium or without added selenium for 3 days following 2 days differentiation. The labels on the left indicate the cDNA probes used to identify the newly synthesized mRNA. Selenoprotein W probe represents the full length rat selenoprotein W cDNA to detect specific selenoprotein W gene transcripts, the α-actin probe representing the rat α-actin cDNA coding region was used as a positive control and pExcell plasmid without any insert as a negative control. B: Northern blot of cytoplasmic RNA hybridized with selenoprotein W cDNA and α-actin cDNA probes. C: Bar graph comparison of the steady-state selenoprotein W mRNA levels with newly synthesized selenoprotein W mRNA. Selenoprotein W mRNA levels were normalized to α-actin mRNA levels. Error bars indicate standard error of the mean ($n = 3$). Treatments which do not share a common superscript differ significantly ($P < 0.05$).

was prehybridized at 65 °C for 4 h in the prehybrization solution (16.3% SDS, 0.25 M $NaPO_4$, pH 7.2, 1 mM EDTA and 0.5% blocking reagent purchased from Boehringer Mannheim Biochemical, Indianapolis, IN). Hybridization was performed at 65 °C overnight in the hybridization solution with 30 ng/ml of dig-dUTP labeled rat selenoprotein W cDNA or α-actin cDNA probes. After being extensively washed with different concentrations of salt as described (Yeh *et al.* 1997) to remove excess probe and non-specific binding, hybridized RNA signals were detected by a chemiluminescence detection system based on the procedure described by Krueger & Williams (1995).

For reprobing, the membrane was boiled for 7 min in 0.1% SDS, then incubated at room temperature for 15 min in 0.1% SDS, 0.05 N NaOH solution and finally equilibrated in 2× SSC. The same conditions were used but the temperature for hybridization and post hybridization washing was decreased to 45 °C for dig-dUTP labeled oligonucleotide probe (1406R) (Lane *et al.* 1985). The relative intensities of the resulting products were determined by scanning den-

sitometry and analyzed by the Image-QuaNT program (Molecular Dynamics, Sunnyvale, CA). The level of selenoprotein W mRNA was expressed as the ratio of selenoprotein W mRNA/18s rRNA.

Preparation of plasmid filters for nuclear run-on assays

Cloned selenoprotein W cDNA in pExcell plasmid was used as the basic structure for constructing others. First, pExcell plasmid containing selenoprotein W cDNA was digested with restriction endonuclease *Eco*R1 and then size separated by 1% agarose gel. The vector fragment was purified using the Geneclean kit (BIO 101 Inc., La Jolla, CA) and re-ligated with T4 ligase. The circular pExcell plasmid without insert was recovered by transformation into NM522 competent cells.

Forward and reverse primers with additional restriction enzyme sites *Xho*1 in 5′ end and *Hin*dIII in 3′ end) were designed based on the rat α-actin cDNA coding region. A single product consisting of the 1.1 kb rat α-actin coding region was obtained with PCR amplification using a rat skeletal muscle cDNA library as the DNA template. The pExcell vector and PCR product were digested with *Xho*1 and *Hin*dIII. Vector and insert fragments were purified from the gel using the Geneclean kit and ligated with T4 DNA ligase. The recombinant pExcell plasmid containing α-actin cDNA was recovered by transformation into NM522 competent cells.

Plasmids were purified using QIAEX plasmid purification kit and linearized by the restriction enzyme *Eco*RI for pExcell, *Nco*I for selenoprotein W cDNA in pExcell and *Hin*dIII for α-actin cDNA in pExcell. The linear plasmid DNA was denatured by NaOH and neutralized in 6× SSC. A 125 μl aliquot of sample (5 μg plasmid) was applied on a GeneScreen nylon membrane slot under low vacuum provided by a water aspirator. After air-drying, the filter was UV-cross linked and stored at 4 °C.

Nuclear run-on assays

Five culture dishes at densities of 1×10^6 cells/dish were used for each nuclear run-on assay. Cells were harvested and resuspended in 4 ml sucrose buffer I (0.32 M sucrose, 3 mM $CaCl_2$, 2 mM MgOAc, 0.1 mM EDTA, 10 mM Tris-HCl pH 8.0, 1 mM DTT, 0.5% (v/v) Nonidet P-40) and broken in an ice-cold Dounce homogenizer with five to ten strokes with a pestle. The cell lysates were mixed with 4 ml sucrose buffer II (1.8 M sucrose, 5 mM MgOAc, 0.1 mM EDTA, 10 mM Tris-Cl pH 8.0, 1 mM DTT) and carefully loaded onto 4 ml of 1.8 M sucrose cushion with sucrose buffer I to top off the gradient. The gradient was centrifuged at 30,000 *g* for 1 h. Supernatants were removed by vacuum aspiration, and the nuclei were resuspended in ice-cold glycerol storage buffer and immediately frozen. A 10 μl portion of each sample was removed, adjusted to 50 μl with TES, boiled for 5 min, sonicated and the absorbance was measured at 260 nm to estimate the amount of nuclei in each sample. Each sample was adjusted to a concentration of 2.5 mg nucleic acid/ml (equal to 2.5×10^8 nuclei/ml) with glycerol storage buffer and frozen in liquid nitrogen.

Transcription of nuclei and subsequent RNA isolation were performed based on procedures described by Greenberg and Bender (1985); 5×10^7 nuclei per sample were used for transcription and equal counts per minute of the ^{32}P-labeled transcription products were diluted in 50% formamide, 50 mM Na_2PO_4 , 3× SSC, 10× Denhardt's solution, 250 μg/ml salmon sperm DNA at 2×10^7 cpm ml. Hybridization of de novo nuclear RNA transcripts to cDNAs immobilized on nylon membrane strips was performed at 42 °C with shaking for 72 h. After hybridization, non-specific binding was removed from membranes by washing with increasing stringencies. The membranes were exposed to X-ray film for 7 days at −70 °C with an intensifying screen. Autoradiograms were quantified by densitometry and the level of selenoprotein W transcript in each slot was expressed as a ratio of selenoprotein W/α-actin RNA.

mRNA half-life

The rate of mRNA degradation in L8 cells grown in either low selenium medium or selenium supplemented medium was determined by measuring the changes of mRNA levels in the presence of the transcription inhibitor, α-amanitin. L8 myoblast cells were routinely grown in DMEM medium with 10% CS. After the cells reached 50–60% confluence, the medium was replaced with serum-free medium, and 2.5 μg/ml α-amanitin was added at the same time selenium was either removed or added. The cells were harvested at 0, 3, 6, 12, 24, 48 and 72 h. Total cellular RNA was isolated by the guanidinium isothiocyanate procedure. Northern blots were performed with non-radiolabeled probes to measure the relative levels of specific selenoprotein W mRNA. The amount of selenoprotein W

mRNA at any time was expressed relative to the initial level.

Statistical analysis

ANOVA (analysis of variance) with Fisher's LSD (least-significant difference) was performed to determine statistical significance (Steel & Torrie 1980). Each selenoprotein W/18s-rRNA ratio or selenoprotein W/α-actin ratio was expressed as means $\pm$ SE of three replicates taken at each point. For selenoprotein W decay curves, selenoprotein W/18s-rRNA ratios were subjected to linear regression analysis followed by ANOVA. Regression equations were considered to be significantly different from zero or from one another if the probability was less than 5%, ($P < 0.05$).

Results

Northern blots revealed the presence of selenoprotein W mRNA in both proliferating and differentiating cells, but quantitative analysis showed no significant difference in selenoprotein W gene expression between undifferentiated and differentiated cells (data not shown). To examine the long term effect of cell differentiation on selenoprotein W gene expression, Northern blot analysis of total cellular RNA from L8 cells at various differentiation stages indicated that selenoprotein W was expressed continuously with time and the levels of mRNA remained constant during different stages of differentiation (Table 1). This indicates that the selenium (10^{-8} M) in 2% CS was adequate for selenoprotein W gene expression. To examine the influence of selenium on selenoprotein W expression, L8 cells were cultured in serum-free medium with or without 3×10^{-8} M selenium. After 2 days of differentiation, total RNA was isolated for Northern blot analysis. As shown in Table 1, selenium depletion caused a drop in selenoprotein W mRNA levels ($P < 0.05$) while selenium supplementation maintained the selenoprotein W mRNA at its original level during the various differentiation stages ($P > 0.05$).

To further establish the relationship between selenoprotein W mRNA in the cytoplasm and selenium concentration in the medium, concentrations of supplemented selenium were varied in serum-free medium. As shown in Figure 1A, additional selenium in the incubation medium caused an increase of selenoprotein W mRNA levels. Quantitative densitometry of Northern blots indicated that selenoprotein W mRNA levels were higher in cells cultured with added selenium than in those cultured in low selenium medium ($P < 0.05$; Figure 1B). Selenium concentration as low as 10^{-8} M increased selenoprotein W mRNA above basal levels. There were no differences among the various selenium (10^{-8} to 10^{-6} M) treated groups ($P > 0.05$).

Nuclear run-on assays were performed to examine the transcription rates of selenoprotein W mRNA in L8 cells grown in serum-free medium with or without addition of 10^{-7} M selenium. Figure 2A shows a typical autoradiogram from a transcriptional run-on analysis in which nascent RNA transcripts corresponding to the selenoprotein W mRNA were detected by the rat selenoprotein W cDNA probe. Hybridization of the RNA transcripts was specific because no activity was detected with non specific pExcell plasmid DNA. The transcripts were normalized to α-actin RNA to account for slight variations in total RNA transcript concentration in the hybridization solution. Parts of the cells were reserved for cytoplasmic RNA isolation and Northern hybridization. Figure 2B shows one set of Northern blots developed with selenoprotein W cDNA and α-actin cDNA probes. The levels of selenoprotein W mRNA were normalized to that of α-actin mRNA to account for variations in sample loading. As shown in Figure 2C, nuclear run-on analysis revealed no difference in transcription rate of selenoprotein W mRNA in cells grown in either selenium depleted or supplemented medium ($P > 0.05$), however, the level of selenoprotein W mRNA was higher in cells incubated in medium containing selenium than those in selenium depleted medium ($P < 0.05$). Thus, selenium depletion causes a decrease of selenoprotein W mRNA levels at the post-transcriptional stage.

L8 myoblast cells were cultured in DMEM medium with 10% CS for 2 days and then replaced with serum-free medium with or without added selenium. Further RNA synthesis was blocked with 2.5 μg/ml of α-amanitin and the decay rate of preexisting selenoprotein W mRNA was monitored by Northern blots. When cells were grown in low selenium medium, selenoprotein W mRNA levels decreased progressively over time (Figure 3A), while selenoprotein W mRNA levels in cells grown in selenium supplemented medium decreased at a significantly slower rate for the duration of the experiment (Figure 3B). Quantitation of Northern blots by densitometry and linear curve analysis indicated significant differences between the low and high selenium treatments. The estimated half-lives of selenoprotein

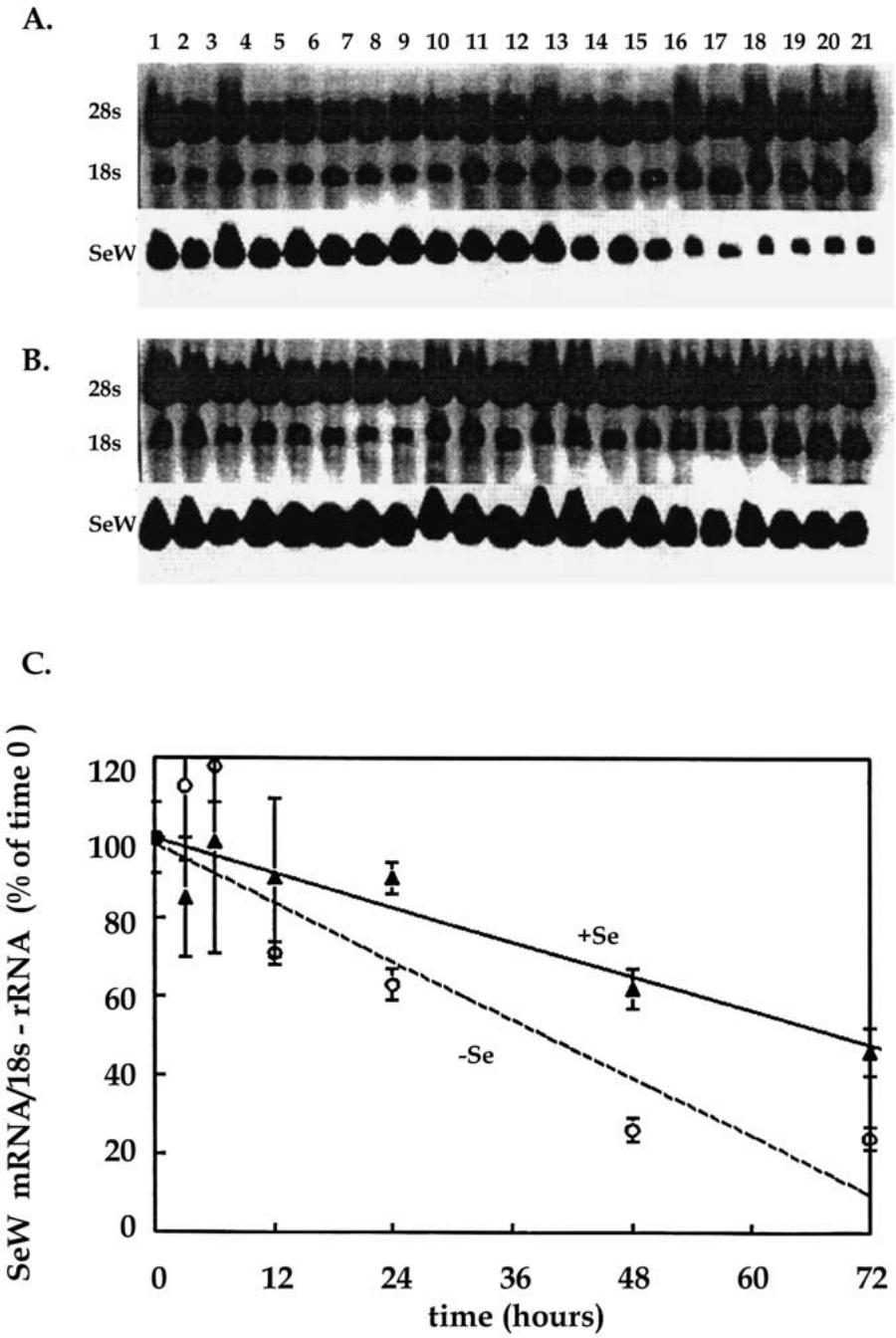

Fig. 3. Effects of selenium on selenoprotein W mRNA stability. L8 myoblasts were cultured in DMEM medium with 10% CS for 2 days and replaced with serum- free medium with either 10^{-7} M selenium or no selenium. At the same time, 2.5 μg/ml α-amanitin was added to block transcription. A: Northern blot of total RNA isolated from cells grown in selenium depleted medium. B: Northern blot of total RNA isolated from cells grown in selenium supplemented medium. Lanes 1–3: initial, lanes 4–6: 3 h, lanes 7–9: 6 h, lanes 10–12: 12 h, lanes 13–15: 24 h, lanes 16–18: 48 h, lanes 19–21: 72 h. C: Linear curve analysis depicts the selenoprotein W mRNA decay over 72 h following α-amanitin addition to block transcription. Selenoprotein W levels were normalized to levels of 18s-rRNA to correct for loading variations and expressed as a percent at time zero. Error bars indicate standard error of the mean ($n = 3$).

W mRNA were 56 h in the absence of selenium but 120 h in the presence of this element (Figure 3B).

Discussion

In the present study, the expression and regulation of selenoprotein W genes were investigated *in vitro* with an L8 cell line. Selenoprotein W is expressed both in proliferating myoblasts and in differentiating myotubules. There was no difference in selenoprotein W mRNA levels between growing cells and cells undergoing differentiation. Selenoprotein W mRNA levels remained constant at the various stages of differentiation when cells were cultured in standard differentiation medium (DMEM+2%CS) (Table 1). Even though mRNA levels remained constant there is a decrease in selenoprotein W content during differentiation (Yeh *et al.* 1997). Apparently the selenoprotein W protein levels are more sensitive to selenium status change than are selenoprotein W mRNA levels. Selenoprotein W mRNA levels decreased simultaneously with time of differentiation when selenium was reduced in medium, while selenoprotein W mRNA levels remained constant during differentiation in the presence of selenium in the medium (Table 1). Thus, the maintenance of selenoprotein W mRNA levels is dependent upon the presence of selenium instead of the cell stage.

Effects of selenium concentration in the medium indicated that a selenium concentration of 10^{-8} M is adequate to maintain selenoprotein W expression (Figure 1). Although the increase in selenoprotein W mRNA was slightly higher at 10^{-7} M compared to 10^{-8} M selenium, the difference was not statistically significant. The increase of selenoprotein W mRNA with selenium exposure is consistent with a previous in vivo study which demonstrated that selenoprotein W mRNA levels increased from deficient to 0.1 μg dietary selenium per gram diet for the rat, but there was no further increase with dietary selenium between 0.1 and 4 μg per gm (Vendeland *et al.* 1995). The selenoprotein W mRNA level does not parallel the selenoprotein W content as shown with the L8 cells (Yeh *et al.* 1997). In the *in vivo* system, selenoprotein W protein synthesis also showed a dose-response pattern. Selenoprotein W mRNA level was reduced to 25% of control but the selenoprotein W protein level was reduced to 13% of control when dietary selenium was decreased from 0.1 μg per gm to no added selenium. Selenoprotein W mRNA remained constant whereas selenoprotein W content increased when the dietary selenium was increased from 0.1 to 4 μg per gm (Vendeland *et al.* 1995). With respect to other selenoproteins, the regulating system is very similar. Levels of mRNAs for cellular glutathione peroxidase and selenoprotein P fell respectively to 3% and 11% of controls whereas cellular glutathione peroxidase activity was only 0.8% of control (Chada *et al.* 1989), and selenoprotein P concentration was only 4.3% of control (Hill *et al.* 1992) with deficient levels of selenium. Thus, selenium greatly affects selenoprotein synthesis but does not always affect the levels of selenoprotein mRNA to the same extent (Moriarty *et al.* 1998).

Studies have demonstrated that various selenium levels affect selenoprotein synthesis at multiple levels (Burk & Hill 1993; Sunde 1997). Selenium is used in *de novo* synthesis of selenoproteins from pre-existing mRNA by co-translational insertion of selenocysteine into its primary peptide sequence and selenium also affects the steady-state levels of selenoprotein mRNA. In the *in vitro* transcriptional run-on analysis, RNA synthesis consists of elongation and completion of the synthesis of previously initiated RNA molecules. Incorporation of ^{32}P-UTP into initiated RNA indicated that these isolated nuclei were capable of transcript elongation. Results of the transcription assay using nuclei isolated from selenium deficient L8 cells showed that they have the ability to synthesize the same amount of selenoprotein W pre-mRNA as nuclei from selenium treated cells even though the steady-state level of selenoprotein W mRNA is lower in cells cultured in low selenium medium compared to selenium supplemented medium (Figure 2). These results are consistent with those of gene regulation for glutathione peroxidase (Toyoda *et al.* 1989). Nuclear run-on analysis with selenium deficient cells demonstrated that the decreases in mRNA levels of glutathione peroxidase are due to post-transcriptional regulation (Bermano *et al.* 1995; Hill *et al.* 1992). Similarly, regulation of selenoprotein W mRNA by selenium in the L8 cell line also resides at a post-transcriptional step. Therefore, selenium appears to affect the stability of selenoprotein W mRNA, which is similar to other selenoproteins (Christensen & Burgener 1992; Toyoda *et al.* 1989; Moriarty *et al.* 1998; Wingler *et al.* 1999).

The steady-state levels of any transcript represents a balance between pre-mRNA splicing and RNA export, its rate of nuclear synthesis, and its rate of cytoplasmic degradation. When transcription was blocked by α-amanitin, the difference of decay curves

of selenoprotein mRNA represents the different rates of mRNA degradation. In the absence of selenium, selenoprotein W mRNA levels decreased with an estimated half-life of 57 h, whereas in the presence of selenium selenoprotein W mRNA was much more stable with a half-life of 120 h (Figure 3). This greater stability of selenoprotein W mRNA in the presence of selenium is consistent with data on the glutathione peroxidases (Christensen & Burgener 1992; Toyoda *et al.* 1989; Moriarty *et al.* 1998; Wingler *et al.* 1999).

The stability of mRNA is probably regulated through the interaction of cis-elements with RNA-binding proteins that can affect the susceptibility of RNA to nucleolytic attack. A well-characterized example is the iron-responsive element (IRE) present in the 3′-UTR of transferrin receptor mRNA (Casey *et al.* 1988). The IRE forms a stem-loop structure that is recognized by an IRE-binding protein. This association modulates the stability of transferrin receptor mRNA in response to iron availability or deprivation (Mullner & Kuhn 1988). Selenoprotein W mRNAs also contain a stem-loop structure in the 3′-UTR (Gu *et al.* 1997) analogous to the conserved SECIS element in other mammalian selenoprotein RNAs (Berry *et al.* 1993; Walczak *et al.* 1996). The SECIS element is considered to be an essential structure for selenocysteine incorporation at the UGA codon (Berry *et al.* 1993, 1994; Walczak *et al.* 1996). It is possible that the same stem-loop structure has a dual function involved in selenocysteine insertion as well as selenoprotein mRNA stabilization. Selenium deficiency reduced the abundance of mRNA for glutathione petoxidase (Moriarty *et al.* 1998) and this is a method of regulation. Using Chinese hamster ovary cells, the 3′ UTR was shown to be necessary for selenium regulation of glutathione petoxidase mRNA levels (Weiss & Sunde 1997), providing another possible mechanism for regulation of selenoproteins. RNA binding proteins with the ability to bind the SECIS have been identified (Hubert *et al.* 1996; Shen *et al.* 1995). Alternatively, other critical cis-elements exist in some as yet unidentified region of selenoprotein mRNA. Functional studies of these RNA binding proteins may finally elucidate the relationship between the SECIS element and UGA read through and selenoprotein mRNA stabilization. The selenoprotein W gene for the rat contains five introns and six exons (Whanger *et al.* 1997). The coding sequence and SE-CIS element are much more highly conserved than other regions of the gene. Additional work will be required to determine the importance of these observations in relation to the future identification of the metabolic function of this selenoprotein.

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