Selenite and Selenomethionine Promote HL-60 Cell Cycle Progression[1,2]

Huawei Zeng[3]

U.S. Department of Agriculture, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

ABSTRACT The essential role of selenium (Se) in nutrition is well established. The elucidation of the mechanisms by which selenium regulates the cell cycle can lead to a better understanding of the nature of selenium's essentiality and its role in disease prevention. In this study, the effects of selenium deficiency or adequacy (0.25 μmol/L selenite or selenomethionine) on HL-60 cell cycle progression were examined in serum-free media. Selenium was critical for promotion of HL-60 cell growth. Cell-cycle analysis revealed that selenium deficiency caused a decrease in G1 phase cells that corresponded to an increase in G2 and sub-G1 phase cells. Gene array analysis suggested that c-Myc, cyclin C, proliferating cell nuclear antigen, cyclin-dependent kinase (cdk)1, cdk2, cdk4, cyclin B and cyclin D2 mRNA levels were lower in selenium-deficient cells than in the cells supplemented with 0.25 μmol/L selenomethionine. The decrease in the c-Myc mRNA level in selenium-deficient cells was confirmed by reverse transcription-polymerase chain reaction analysis. Furthermore, the phosphorylation state of total cellular protein was higher (57%) in selenium-supplemented cells than in selenium-deficient cells. Collectively, these results suggest a novel role for selenium at 0.25 μmol/L in up-regulation of the expression of numerous cell cycle–related genes and total cellular phosphorylated proteins in HL-60 cells in serum-free culture media. This leads to the promotion of cell cycle progression, particularly G2/M transition and/or the reduction of apoptosis, primarily in G1 cells. These observations may have additional implications for understanding the nature of selenium's essentiality. J. Nutr. 132: 674–679, 2002.

KEY WORDS: • *selenium* • *cyclin C* • *c-Myc* • *cell cycle* • *HL-60 cell*

Selenium is an essential trace element for humans and many other forms of life (1); the current recommended daily allowance is 55 μg for a healthy adult (2). Selenium has been shown to regulate the functions of many intracellular proteins (3) by being a chemical component of selenoproteins as either selenocysteine or selenomethionine. Most of the currently known selenoproteins are selenium-dependent enzymes such as glutathione peroxidases and thioredoxin reductase, which are antioxidant enzymes (4). Interestingly, even without being incorporated into proteins, selenium can modulate the activities of certain transcription factors and kinases (5). For example, selenium is directly involved in the regulation of functions of a variety of proteins through the oxidation of reactive cysteine residues in proteins including Jun N-kinase activities (6) and caspase-3 (7). In recent years, selenium has been shown to mediate numerous insulin-like actions both in vivo and in vitro, although the molecular mechanism by which selenium mimics insulin is not fully understood (8). Selenium also acts as an antioxidant, a potent stimulator of tyrosyl phosphorylation and activator of mitogen-activated protein (MAP)[4] kinase (9,10). Although the essential role of selenium in growth of certain animal cell lines has been reported (11–13), delineation of the functions of selenium at low concentrations on cell cycle progression is lacking. Selenium can either stimulate or inhibit cell growth, depending on the concentration and chemical form of selenium (14). At concentrations higher than nutritional requirements, selenium has anticancer effects. These may be mediated through changes in the proliferation of certain cells (such as promotion of immune cells), cell apoptosis and/or a toxic effect on cancer cells (5,14,15). In most cases, ~3–5 μmol/L selenite is the lowest concentration to inhibit cell growth (14). In contrast, the effects of selenium at low concentrations (nmol/L) on cell cycle/growth are not obvious because most cell culture media and serum already contain trace amounts (nmol/L) of selenium. It has been shown that selenite at 50 nmol/L, but not at >1 μmol/L, is an essential trace nutrient for growth of WI-38 diploid human fibroblasts and Chinese hamster cells and enhances growth of other human cell lines (11,16). In fact, the finding that selenium enhances growth of cells in culture (11,16) is well recognized; up to 0.1 μmol/L selenite is added in some commercial cell culture media such as IMDM media (Gibco, Rockville, MD) for optimal growth of certain mam-

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[3] To whom correspondence should be addressed.
E-mail: hzeng@gfhnrc.ars.usda.gov.

[4] Abbreviations used: CDK, cyclin-dependent kinases; DTT, dithiothreitol; FBS, fetal bovine serum; MAP, mitogen-activated protein; PCNA, proliferating cell nuclear antigen; PI, propidium iodide; RT-PCR, reverse transcription-polymerase chain reaction.

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malian cell lines. Given the above findings, 50 nmol/L to 0.25 μmol/L selenite likely represent the nutritional concentrations of selenium that are needed for the growth of some human cell lines in culture.

The control of cell cycle progression plays a key role in terminal differentiation, growth and development (17,18). The connections between cancer and the "check-points" at the G1-S and the G2-M transitions of the cell cycle have become apparent (17–19). Because of the conserved nature of cell cycle control mechanisms in yeast, invertebrates and vertebrates (17,18), the elucidation of the effect of selenium on cell cycle control in a cell culture model will provide a better understanding of the nutritional roles of selenium (20). To obtain further insights into the function of selenium at nutritional concentrations in the cell cycle progression, HL-60 cells were chosen and optimized in serum-free culture conditions to maximize the effects of selenium on cell growth. The data represent the first observation of the molecular basis of selenium in promotion of cell cycle progression. The implications of this finding for human nutrition are discussed.

MATERIALS AND METHODS

Chemicals. Na_2SeO_3, selenomethionine, insulin, dithiothreitol (DTT) and propidium iodide (PI) were purchased from Sigma Chemical (St. Louis, MO). ATP (γ-^{32}P) and cytidine 5′-triphosphate (α-^{32}P) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Apo-transferrin was purchased from Calbiochem (La Jolla, CA). Oligonucleotides were synthesized by Gibco BRL (Rockville, MD).

Serum free cell culture and cell growth curve. Human leukemia HL-60 cells were maintained in RPMI media (GIBCO-BRL, Grand Island, NY) with 5% (v/v) fetal bovine serum (FBS) in a humidified atmosphere 95% air/5% CO_2 at 37°C; cell passage was between 28 and 38. Great care was taken to avoid any selenium contamination in each step in the serum-free HL-60 cell culture. Unless otherwise indicated, two steps were always taken to generate serum-free (selenium-deficient) HL-60 cells in this study. Step 1: HL-60 cells from RPMI media with 5% (v/v) FBS were centrifuged at 750 *g* for 10 min and washed with RPMI media and then were cultured for 5 d in a T75 flask in serum-free RPMI media containing 1.4 μmol/L $FeSO_4$, 75 mg/L insulin and 75 mg/L transferrin. Step 2: Cells (4 mL) from step 1 were seeded at 2.0–2.5 × 10^8 cells/L into the well of a 6-well plate in the same culture medium as that in step 1. Simultaneously with the step 2 cell seeding, 0.25 μmol/L of selenite or selenomethionine (in medium) or an equal volume of medium was added to study the effect of selenium on cell cycle/growth. Cells were counted with a hemocytometer. Cells that excluded trypan blue after incubation with an equal volume of PBS containing 4 g/L trypan blue dye were considered viable.

Cell cycle analysis. The cell cycle was analyzed by using flow cytometry with PI staining. Cells were washed once with PBS and incubated in 70% (v/v) ethanol at −20°C. After the incubation, cells were washed with PBS, and stained with 50 mg PI/L and 6000 U RNase A/L. The hypodiploid DNA content was used as a hallmark of apoptosis. The DNA contents of cells were determined by flow cytometry. Data were stored as list mode files of at least 10,000 single cell events and analyzed by EPICS profile II and ModFit LT software (Coulter, Miami, FL and Topsham, ME).

Gene array and reverse transcription-polymerase chain reaction (RT-PCR) assay. Total cellular RNA was isolated from HL-60 cells by using an RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, and the integrity of RNA samples was checked by electrophoresis. Each cDNA probe was prepared from 5 μg of total cellular RNA, and hybridized to Human Cell cycle-1 GFArray membrane (Superarray, Bethesda, MD). These membranes contain 23 sequence-verified known cell cycle–related genes. Each cell cycle–related gene was normalized by 10% of the β-actin signal in the same membrane, and only those gene signals (~10% of the β-actin signal) that were well above background were considered specific gene signals. These data were collected, stored and analyzed with Molecular Dynamics Image-Quant system (Sunnyvale, CA). To confirm the data generated by gene array analysis, a RT-PCR assay was performed (21). Briefly, independent total RNA samples were isolated from HL-60 cells and the abundance of the c-Myc and β-actin was analyzed by standard RT and PCR amplification (20 cycles) (Promega, Madison, WI). β-Actin primers 5′-ATG GGT CAG AAG GAT TCC TAT G-3′; 5′-CAG CTC GTA GCT CTT CTC CA-3; and c-myc primers 5′-ATG GTG AAC CAG AGT TTC ATC T-3′; 5′-AGG TGA TCC AGA CTC TGA CCT-3′.

Total cellular phosphorylated proteins. Briefly, HL-60 cells were washed twice with ice-cold PBS, and centrifuged for 5 min at 300 × *g*. The cell pellet was lysed for 25 min on ice in extraction buffer A, 20 mmol/L HEPES pH 7.6, 20% (v/v) glycerol, 10 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L EDTA, 1 mmol/L DTT, 0.1% (v/v) Nonidet P-40 containing protease inhibitor cocktail (1:100 dilution) (Sigma). The cell lysates were collected by centrifugation at 15,000 × *g* and 4°C for 15 min, and protein concentrations were determined by the BioRad protein assay (BioRad, Hercules, CA). Each cell lysate sample was diluted to the same concentration with lysis buffer and 1.85 × 10^5 Bq γ-^{32}P ATP was added and incubated for 30 min at 30°C. After electrophoresis in a 12% denaturing SDS polyacrylamide gel, the dried gel was exposed to the phosphorimaging screen for 16 h at room temperature and total cellular phosphorylated protein bands (signals) were visualized by the Molecular Dynamics Image-Quant system (Sunnyvale, CA).

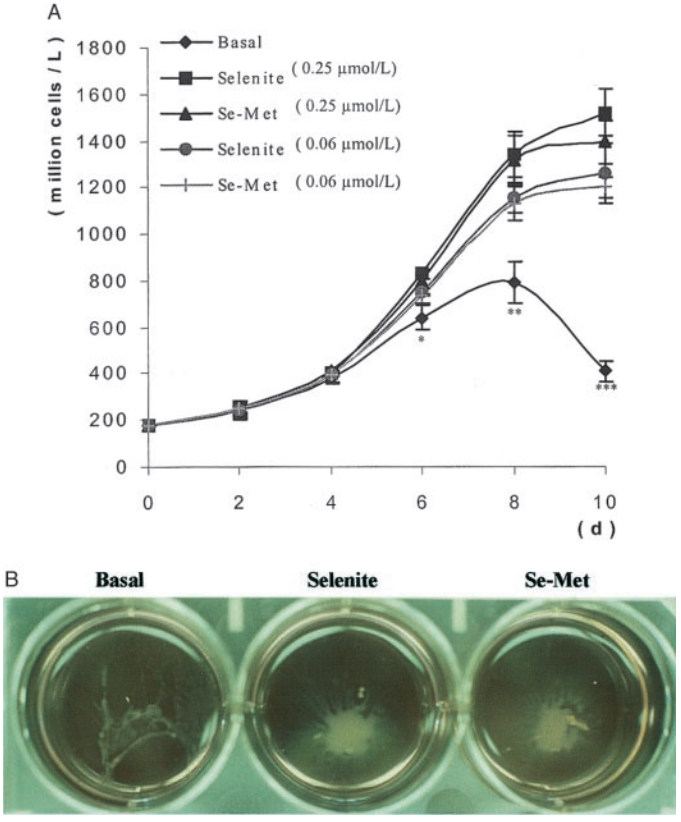

FIGURE 1 Effect of selenium on the growth of HL-60 cells in the absence of selenium (basal) or in the presence of selenite or selenomethionine (Se-Met), respectively. *Panel A*: HL-60 cell growth curve. Values are means ± SD, $n = 4$. Cell density was greater in HL-60 cells supplemented with selenite or Se-Met than in selenium-deficient cells (*$P < 0.03$ at 0.25 Se μmol/L); (**$P < 0.01$ at 0.25 Se μmol/L; $P < 0.05$ at 0.06 Se μmol/L); (***$P < 0.002$ at 0.25 Se μmol/L; $P < 0.01$ at 0.06 Se μmol/L). *Panel B*: HL-60 cell morphologic features on d 4: selenium-deficient cells (basal, *left well*), selenite at 0.25 μmol/L (*middle well*) and Se-Met at 0.25 μmol/L (*right well*).

Statistical analysis. Results are given as means ± SD. Data were analyzed by ANOVA and the Tukey post-hoc test. Differences with a P-value < 0.05 were considered significant.

RESULTS

Effect of selenium on HL-60 cell growth. To determine the effect of selenium on cell growth, HL-60 cells were cultured in serum-free RPMI media in 6-well plates and the effects of selenite or selenomethionine on the HL-60 growth curve were examined. There was a greater cell density ($P < 0.03$ at 0.25 Se μmol/L) in HL-60 cells supplemented with selenite or selenomethionine than in selenium-deficient (basal) cells on d 6, and this observed difference was even greater at d 8 ($P < 0.01$ at 0.25 Se μmol/L; $P < 0.05$ at 0.06 Se μmol/L) and d 10 ($P < 0.002$ at 0.25 Se μmol/L; $P < 0.01$ at 0.06 Se μmol/L) (**Fig. 1***A*). In addition to the difference in growth curves, as early as d 4, selenium-deficient HL-60 cells appeared to be a thin layer of "silk." The cells became difficult to resuspend for the cell counts on d 8 and 10, although HL-60 cells are suspension cells (Fig. 1*B*). In contrast, HL-60 cells supplemented with 0.25 μmol/L selenite or selenomethionine tended to condense at the center of the culture wells, and cells were easily resuspended for cell counts (Fig. 1*B*).

Effect of selenium on cell cycle phase-distribution of HL-60 cells. To examine the dynamics of cell cycle progression, G1, S and G2 phase distribution of the cells supplemented with 0.25 μmol/L of selenite or selenomethionine was compared with that of selenium-deficient (basal) cells over time. Intriguingly, G2 phase cell distribution gradually accumulated on d 4 and 6 and became significantly increased on d 8 ($P < 0.05$) and d 10 ($P < 0.01$) in selenium-deficient cells. In contrast, there was little change in G2 phase cell distribution in the cells supplemented with selenite or selenomethionine at 0.25 μmol/L (**Fig. 2**; **Table 1**). Interestingly, S phase cell distribution slightly but consistently increased in the cells supplemented with selenite or selenomethionine at 0.25 μmol/L on d 8 and 10 but not in the selenium-deficient cells (Fig. 2; Table 1). Although G1 phase cell distribution of both the selenium-deficient cells and the cells supplemented with 0.25 μmol/L selenite or selenomethionine slowly decreased during the 10-d incubation period, the decrement was more dramatic ($P < 0.05$) in the selenium-deficient cells than in the cells supplemented with 0.25 μmol/L selenite or selenomethionine (Fig. 2; Table1). Moreover, apoptosis of mainly sub-G1 selenium-deficient cells was slightly but significantly higher ($P < 0.05$) than that of the cells supplemented with 0.25 μmol/L selenite or selenomethionine on d 10 (Fig. 2; Table 1).

Effect of selenium on mRNA level of cell cycle related genes and cellular phosphorylated proteins. Gene array analysis showed that the cyclin E mRNA level was not affected, but c-Myc (59%), cyclin C (45%), proliferating cell nuclear antigen (PCNA; 33%), cyclin-dependent kinase (cdk)1 (35%), cdk2 (31%), cdk4 (31%), cyclin B (30%), cyclin D2 (31%) and egr-1 (16%) mRNA levels were lower in selenium-deficient cells than in cells supplemented with 0.25 μmol/L selenomethionine on d 6 (**Table 2**). The c-myc mRNA signals were weaker in selenium-deficient cells than that in the cells supplemented with 0.25 μmol/L selenite or selenomethionine, whereas β-actin signals did not differ (**Fig. 3**). The phosphorylation state of total cellular protein in the cells supplemented with 0.25 μmol/L selenite or selenomethionine was 57% higher ($P < 0.02$) than that of selenium-deficient cells on d 6 (**Fig. 4***A*, *B*).

DISCUSSION

The control of cell cycle/growth has been directly related to numerous human diseases including growth arrest during development and tumorigenesis (17–19,22). HL-60 cells have been used extensively as a model with which to study mammalian cell cycle and apoptosis (15,23). In agreement with previous reports (11–13), 0.06 and 0.25 μmol/L selenium enhanced the growth of HL-60 cells. Because 0.25 μmol/L selenium had a stronger promotional effect on cell growth and yet was well below the known toxic concentration (3–5 μmol/L of selenite) (14), 0.25 μmol/L selenium was chosen for further study.

The eukaryotic cell cycle is divided into four major phases

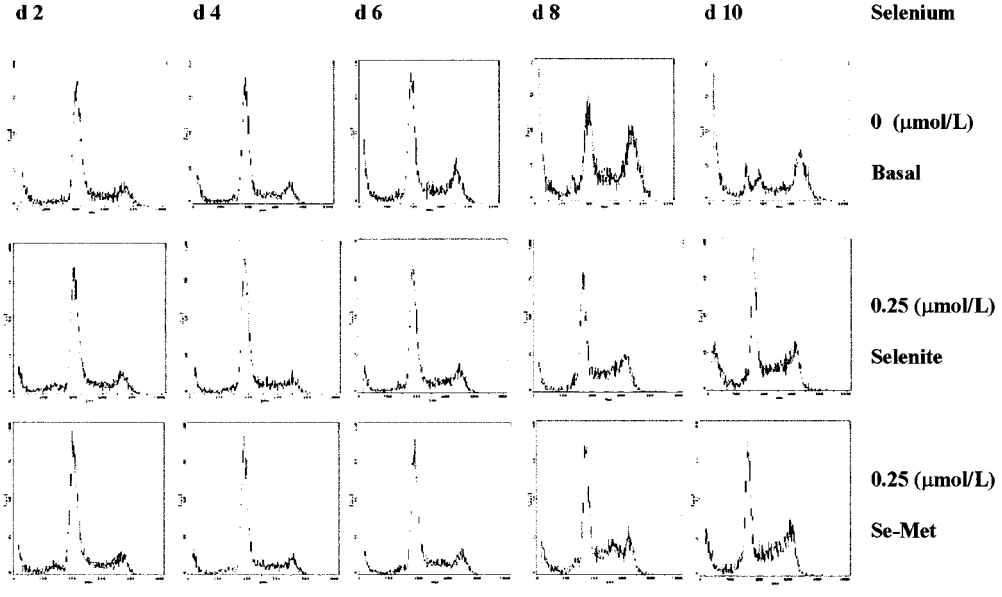

FIGURE 2 Flow cytometric profiles show cell cycle phase-distribution of the HL-60 cells in the absence of selenium (basal) or in the presence of 0.25 μmol/L selenite or selenomethionine (Se-Met). The data are from one experiment of three that yielded similar results.

TABLE 1

Comparison of cell cycle phase-distributions of HL-60 cells in serum-free RPMI media without (basal) and with 0.25 μmol/L selenite or selenomethionine (Se-Met)

	d 2	d 4	d 6	d 8	d 10
	phase-specific cells/total detected cells, %				
G1 phase					
Basal	59.04 ± 4.60	62.17 ± 2.04	58.33 ± 5.76	35.34 ± 9.60	17.96 ± 5.22*
Selenite	58.84 ± 2.56	65.06 ± 0.61	59.32 ± 3.61	45.41 ± 7.11	34.02 ± 2.14
Se-Met	55.69 ± 5.41	64.83 ± 2.75	59.69 ± 3.46	43.85 ± 8.39	29.69 ± 3.08
S phase					
Basal	33.04 ± 4.74	28.65 ± 1.48	28.97 ± 1.99	34.60 ± 3.26	35.09 ± 10.98
Selenite	31.05 ± 3.36	28.16 ± 0.76	31.77 ± 2.29	46.58 ± 8.11	49.68 ± 1.74
Se-Met	32.09 ± 3.22	28.55 ± 0.91	31.57 ± 3.35	42.47 ± 10.61	54.67 ± 3.89
G2 phase					
Basal	8.59 ± 1.92	9.18 ± 0.76	12.70 ± 3.83	30.02 ± 8.85*	46.98 ± 10.08**
Selenite	10.12 ± 2.24	6.78 ± 0.50	8.91 ± 1.35	14.31 ± 1.95	16.30 ± 0.40
Se-Met	12.23 ± 2.19	6.61 ± 1.86	8.74 ± 1.15	13.67 ± 2.26	15.64 ± 2.36
Apoptosis					
Basal	1.97 ± 0.50	2.01 ± 0.51	3.19 ± 0.62	6.07 ± 3.27	9.98 ± 1.64*
Selenite	2.29 ± 1.26	1.45 ± 0.32	1.43 ± 0.42	2.03 ± 0.75	3.99 ± 0.55
Se-Met	2.50 ± 0.99	1.73 ± 0.29	2.06 ± 0.39	4.53 ± 1.04	5.28 ± 2.42

[1] Values are means ± SD, $n = 3$.

[2] * $P < 0.05$ and ** $P < 0.01$ indicate significant differences on a given day between basal and the cells supplemented with 0.25 μmol/L selenite or 0.25 μmol/L Se-Met. Cells supplemented with 0.25 μmol/L selenite or 0.25 μmol/L Se-Met did not differ.

as follows: the G1 phase before DNA replication, the periods of DNA synthesis (S phase), the G2 phase before cell division and cell division (M phase) (18). The check-points between phases are critical for accurate transmission of genetic information; they have important implications in growth, embryonic development and cancer development (19,22). In contrast, at concentrations higher than nutritional requirements, selenium has anticancer effects by causing cell growth arrest and apoptosis. For example, cells exposed to inorganic selenite (5 μmol/L) or organic methylselenocyanate (5 μmol/L) were arrested in the S/G2-M phases and G1 phase of the cell cycle by 24 h, respectively (24,25). The findings that 0.25 μmol/L selenium promoted G2/M transition and/or reduced apoptosis (sub-G1 peak) primarily in G1 cells explain the subcellular basis of the opposite effects of selenium on cell growth due to high and low selenium concentrations (5,8). Because HL-60 cells are human lymphocyte cells, it is conceivable that serious selenium deficiency may reduce the proliferation of certain immune cells, or cause embryogenetic and developmental retardation due to cell growth arrest in humans, particularly those with poor nutritional status.

Eukaryotic cell cycle progression is orchestrated by CDK (18). Because selenite and selenomethionine similarly affected cell cycle progression in this experiment, only selenium-deficient vs. selenomethionine–treated cells were compared to study the effects of selenium on cell cycle gene expression. We used a gene array, followed by a conventional RT-PCR analysis for the confirmation of the genes with differential expression > 2.0. The above technical strategy has been well accepted in gene expression research because it avoids repeatedly

TABLE 2

Human cell cycle-1 GEArray analysis of gene expression of HL-60 cells without (basal) and with 0.25 μmol/L selenomethionine (Se-Met) on d 6[1,2,3]

Genebank	Gene name	Se-Met	Basal	Se-Met/ Basal
		signals 1000 × (intensity units/dot)		
(1) Y00272	cdk1	2.543 ± 0.068	1.637 ± 0.338	1.55
(2) X61622	cdk2	0.979 ± 0.051	0.677 ± 0.201	1.45
(3) M14505	cdk4	1.798 ± 0.002	1.252 ± 0.021	1.44
(4) J00120	c-myc	1.646 ± 0.034	0.671 ± 0.135	2.45
(5) M25753	cyclin B	1.014 ± 0.142	0.707 ± 0.069	1.43
(6) M74091	cyclin C	1.305 ± 0.112	0.715 ± 0.003	1.83
(7) X68452	cyclin D2	1.241 ± 0.078	0.860 ± 0.123	1.44
(8) M73812	cyclin E	1.348 ± 0.006	1.290 ± 0.120	1.05
(9) X52541	egr-1	0.969 ± 0.143	0.813 ± 0.119	1.19
(10) J04718	PCNA	2.647 ± 0.070	1.762 ± 0.175	1.50

[1] Values are means ± SD, $n = 2$.

[2] Signals were normalized with 10% of β-actin signals in the same membrane.

[3] cdk, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen.

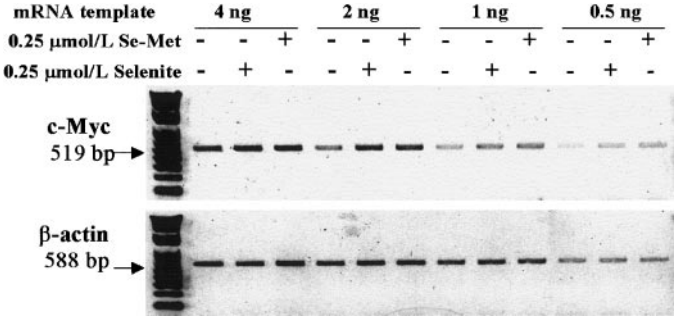

FIGURE 3 Effect of 0.25 μmol/L selenite or selenomethionine (Se-Met) on c-Myc and β-actin gene mRNA abundance in HL-60 cells. The c-Myc reverse transcription-polymerase chain reaction (RT-PCR) products from diluted mRNA templates from HL-60 cells (on d 6); β-actin RT-PCR products from diluted mRNA templates from HL-60 cells (on d 6). Weaker signals of c-Myc in selenium-deficient cells were consistently seen in three experiments.

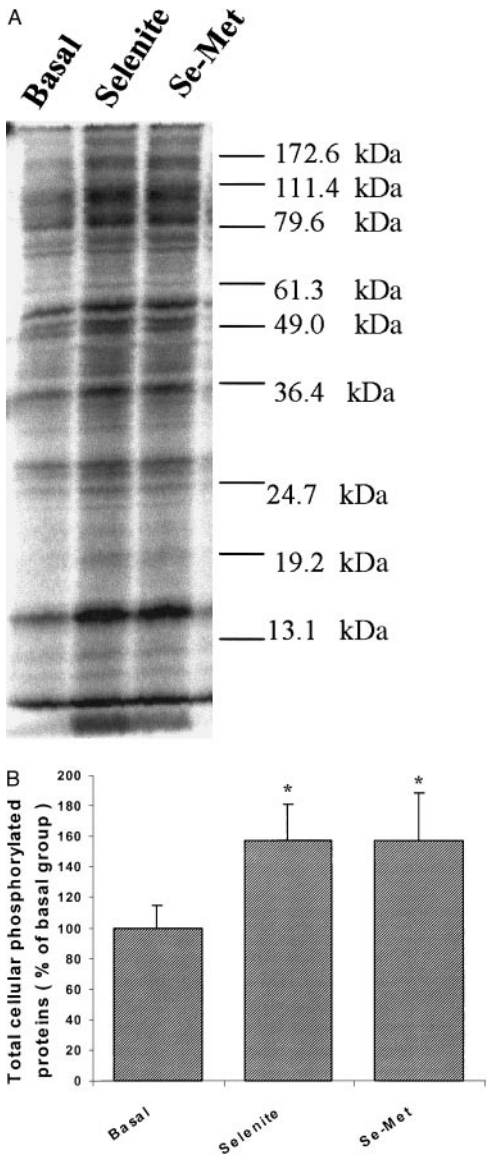

FIGURE 4 Effect of selenium on the phosphorylation state of total cellular protein in the HL-60 cells (on d 6) in the absence (basal) or in the presence of 0.25 μmol/L selenite or selenomethionine (Se-Met). *Panel A*: representative phosphorylation assay shows total cellular phosphorylated proteins. *Panel B*: total cellular phosphorylated proteins were quantified by the Molecular Dynamics Image-Quant system. Values are means ± SD, $n = 4$. *Different from basal, *$P < 0.05$.

using expensive cDNA arrays but validates the specificity of cDNA sequences by an alternate method (26). The changes observed in key cell cycle regulatory gene expression suggest several important molecular roles of selenium at low concentration in cell cycle progression. First, the up-regulation of c-Myc and cyclin C mRNA levels in the cells supplemented with selenomethionine (0.25 μmol/L) is consistent with the observation that cyclin C can cooperate with c-Myc in the promotion of cell proliferation (27). Second, PCNA is likely to be involved at several steps in HL-60 cell cycle progression. The transcriptional coactivator p300 interacts with many transcription factors that participate in a broad spectrum of biological activities such as cell cycle/growth. It has been shown that endogenous p300 binds PCNA and forms a complex that stimulates DNA synthesis and chromatin formation and is critical for DNA repair synthesis (28,29). The role for PCNA in the cell cycle control is related to its interaction with cyclin and CDK (30,31). Therefore, the up-regulation of PCNA mRNA in cells supplemented with selenomethionine (0.25 μmol/L) suggests that selenium directly or indirectly affects the function of basic transcriptional machinery in the cell. Third, the cell cycle progression is governed by the activities of the CDK, which are modulated through association with their regulatory subunits, the cyclins (17,18,22). Cyclin/cdk1 is thought to be the major kinase that initiates the onset of mitosis, and cdk2 and cyclin B are essential for the transition of S/G2 phases, initiation of DNA synthesis, activation of cdk1 and entry into mitosis in higher eukaryotes (32,33). In response to mitogenic stimuli in G1 phase, it was reported that cdk4 associated with the D-type cyclins that control cell cycle progression by phosphorylation of the tumor suppressor protein, pRb (34). Similarly, the up-regulation of cdk1, cdk2, cdk4, cyclin B and cyclin D2 mRNA levels in the cells supplemented with selenomethionine (0.25 μmol/L) is likely responsible in part for the promotion in the S/G2-M phase transition.

Key amino acid residue phosphorylation of total cellular proteins, particularly the CDK, plays a critical role in the regulation of cell cycle progression at the post-translation modification level (17,35). The finding that relatively high total cellular phosphorylated proteins existed in the cells supplemented with 0.25 μmol/L selenite or selenomethionine suggests a critical role for selenium in the up-regulation of total cellular kinase activity. In the future, the effect of selenium on the dynamics of key cell cycle–related gene expression will be studied, so that early and late differential gene expression in cell growth curves can be detected. Because of the limitation of conventional RT-PCR in the sensitivity of quantification, real-time (kinetic) RT-PCR or other more sensitive methods (36) will be developed to confirm the genes with differential expression < 2.0 in the current gene array data. Ultimately, the study of the specific biological function, such as the kinase activity of these cell cycle genes, is required to understand the effect of selenium on gene expression.

In summary, in contrast to the cell growth inhibition and apoptosis induced by selenium at high concentrations (22,37), the data in this study suggest for the first time that selenium at low concentration up-regulates multiple key cell cycle–related gene mRNA levels. Selenium also enhances total cellular phosphorylated proteins in HL-60 cells in serum-free culture media, which leads to the promotion of cell cycle progression, particularly the G2/M transition, and/or a reduction of apoptosis primarily in G1 cells.

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