Role of the 3' untranslated region in the regulation of cytosolic glutathione peroxidase and phospholipid-hydroperoxide glutathione peroxidase gene expression by selenium supply

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Selenium is an essential nutrient and synthesis of selenoproteins is affected by limited selenium supply. During selenium deficiency there is a differential regulation of selenoprotein synthesis and gene expression; for example, there is a decrease in abundance of mRNA for cytosolic glutathione peroxidase (cGSH-Px) and a preservation of mRNA for phospholipid-hydroperoxide glutathione peroxidase (PHGSH-Px). This difference is not due to an alteration in the rate of transcription but might reflect differences in translation. The aim of the present work was to assess the role of cGSH-Px and PHGSH-Px 3' untranslated regions (UTRs) in the regulation of selenoprotein mRNA stability and translation by using H4-II-E-C3 cells transfected with different constructs containing a type I iodothyronine deiodinase-coding region

linked to different selenoprotein mRNA 3' UTRs. Translational efficiency results showed that the efficiency of the 3' UTRs in permitting selenocysteine incorporation is similar in selenium-replete conditions but, when selenium is limiting, the 3' UTR of cGSH-Px is less efficient than the 3' UTR of PHGSH-Px. The results suggest that the 3' UTR of these selenoprotein mRNA species influences their extent of translation when selenium levels are low. The different sensitivity of the 3' UTRs to selenium deficiency can explain the differential effect that selenium deficiency has on cGSH-Px and PHGSH-Px activity and mRNA levels, stability and translation. This might be partly responsible for channelling selenium for synthesis of PHGSH-Px rather than cGSH-Px.

INTRODUCTION

Cytosolic glutathione peroxidase (cGSH-Px) and phospholipid-hydroperoxide glutathione peroxidase (PHGSH-Px) are two of the many selenoproteins that mediate most of the biological functions of selenium in mammals [1]. The functions of the selenoproteins are essential for cell antioxidant systems (e.g. cGSH-Px and PHGSH-Px), thyroid hormone metabolism [e.g. iodothyronine deiodinase (IDI)] [2], maintenance of fertility and possibly some anti-cancer effects [3,4]. Several studies on the mechanisms of regulation of selenoprotein gene expression have shown that the activity and concentrations of these proteins are modified under conditions of limited selenium supply; however, the extent to which selenium deficiency affects the synthesis of the various selenoproteins differs between tissues and between the different enzymes. The changes in activity or concentration of selenoproteins during limited selenium supply are accompanied by alterations in mRNA abundances [5–7].

The different effects of selenium deficiency on the abundance of the mRNA for, and the activity of, hepatic cGSH-Px and PHGSH-Px are not due to alteration in the rate of transcription of the genes [7]. However, at least in hepatoma cells, selenium depletion does not change the half-life and therefore the stability of PHGSH-Px mRNA but decreases the half-life of cGSH-Px mRNA [8]. This selective action of selenium depletion on the stability of cGSH-Px mRNA can explain the observed differential effect of selenium deficiency on hepatic selenoprotein mRNA expression. The decrease in mRNA stability might be secondary to the effect of selenium depletion on mRNA translation [8].

Selenium is incorporated into selenoproteins by recognition of the stop codon UGA as a codon for selenocysteine (Se-Cys); in eukaryotes this requires stem-loop structures in the 3'

untranslated region (UTR) of cGSH-Px and IDI mRNA species [9,10]. In addition, a decreased availability of selenium leads to the termination of translation owing to recognition of the UGA codon as a normal stop codon [11]. The efficiency of translation of a particular selenoprotein can be affected by the 3' UTR, as shown by changes in the activity of the selenoenzymes type I and type III deiodinases when their native 3' UTRs were changed to those for other selenoproteins [12,13]. However, it is not known whether the effect of different 3' UTRs on translational efficiency is influenced by the amount of available selenium. The aim of the present work was to determine whether the 3' untranslated region of selenoprotein mRNA species is involved in the regulation of mRNA stability or translation during limited selenium supply. We therefore studied the effect of cGSH-Px and PHGSH-Px 3' UTRs on the expression and efficiency of translation of type I IDI in H4-II-E-C3 rat hepatoma cells under selenium-replete and selenium-deficient conditions. H4-II-E-C3 cells have been used as a model to study mechanisms of selenoprotein regulation in selenium-deficient conditions [8]; in addition, because they do not express IDI it is possible to use this as a reporter gene.

MATERIALS AND METHODS

Cell culture

H4-II-E-C3 rat hepatoma cells (European Cell Culture Collection, Porton Down, U.K.) were grown in Dulbecco's minimal essential medium supplemented with 10% (v/v) foetal calf serum until confluence. To produce selenium-deficient and selenium-replete cells, the cells were then grown in the same medium without serum but supplemented with either insulin (5 µg/ml),

Abbreviations used: cGSH-Px, cytosolic glutathione peroxidase; IDI, iodothyronine deiodinase; PHGSH-Px, phospholipid-hydroperoxide glutathione peroxidase; Se-Cys, selenocysteine; UTR, untranslated region.

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Figure 1 Chimaeric constructs containing the coding region of IDI linked to the 3' UTRs from IDI, cGSH-Px or from PHGSH-Px mRNA

Restriction sites used to clone the chimaeric constructs and to insert them into the pcDNA3 plasmid for transfection of rat H4-II-E-C3 hepatoma cells are shown. The size of these constructs is shown. Key: open, IDI coding region; filled, IDI 3' UTR; hatched, cGSH-Px 3' UTR; cross-hatched, PHGSH-Px 3' UTR.

transferrin (5 µg/ml) and sodium selenite (7 ng/ml Na₂SeO₃) (selenium-replete medium) or with insulin and transferrin only (selenium-deficient medium) [8]. Medium was changed every 2 days.

Plasmid constructions and DNA transfections

All plasmid DNA manipulations were performed in accordance with standard protocols [14]. The IDI-IDI and IDI-cGSH-Px plasmids used for subsequent subcloning were generously provided by Dr. M. Berry and Dr. P. R. Larsen (Boston, MA, U.S.A.) [15]. The plasmid IDI-IDI resulted from insertion of the IDI cDNA (2.1 kb) into the *Bst*XI and *Not*I sites of CMD8 plasmid, whereas IDI-cGSH-Px resulted from insertion of the cGSH-Px 3' UTR (236 bp), obtained from the rat cGSH-Px gene and introduced into the *Sma*I/*Not*I sites of the IDI-IDI plasmid after removal of the 5'IDI 3' UTR (1208 bp). The plasmid IDI-PHGSH-Px was constructed in a similar fashion. The IDI-PHGSH-Px plasmid resulted from insertion of the PHGSH-Px 3' UTR (a 324 bp *Nco*I-to-*Not*I fragment) obtained from the PHGSH-Px cDNA [6] into the *Sma*I/*Not*I sites of the IDI-IDI plasmid after removal of the IDI 3' UTR. The IDI-IDI and IDI-cGSH-Px constructs were subsequently cloned into pcDNA3 at *Bst*XI and *Not*I sites, whereas IDI-PHGSH-Px construct was inserted at the *Xba*I site (Figure 1). pcDNA3 contains the neomycin resistance gene.

H4-II-E-C3 cells were stably transfected with the various constructs in the pcDNA3 vector by using lipofectamine. In a 35 mm dish, 2.5×10^5 cells were plated out and incubated overnight until the cells were 80% confluent. Cells were then incubated for 5 h with 2 µg of plasmid and 6 µl of lipofectamine (2 mg/ml). Thereafter cells were grown in normal Dulbecco's minimal essential medium/10% (v/v) foetal calf serum, and after 72 h they were moved into a selective medium that contained the antibiotic geneticin (400 µg/ml) to select cells expressing the pcDNA3 plasmid with both neomycin and the required reporter gene.

Enzyme activity

cGSH-Px enzyme activity was measured in cells that had been washed and scraped into PBS, pH 7.4, and then incubated with 0.3 mM NADPH, 5 mM GSH and 0.7 unit/ml glutathione reductase. Oxidation of NADPH was followed spectrophotometrically at 340 nm after addition of 2.2 mM H₂O₂ to the samples [16].

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IDI enzyme activity was measured on the same cell suspension after sonication. Cells were incubated with [¹²⁵I]3,3',5'-tri-iodo-thyronine (1 µM) and 1 mM dithiothreitol in a phosphate/EDTA buffer (0.125 M potassium phosphate/1 mM EDTA) in a final volume of 300 µl. Incubation was for 30 min at 30 °C. [¹²⁵I]⁻ was separated by precipitation by trichloroacetic acid after the addition of horse serum, and the release of [¹²⁵I]⁻ was then quantified as described by Arthur et al. [17].

cDNA probes and chemicals

The cGSH-Px probe [9], a gift from Dr. P. Harrison (Beatson Institute, Glasgow, Scotland, U.K.), and the 18 S rRNA cDNA [18] from Dr. R. Fulton (Beatson Institute) have been described previously [7]. The IDI probe, a 0.77 kb *Nco*I/*Sma*I fragment of the rat cDNA, was a gift from Dr. M. Berry and Dr. P. R. Larsen [11]. Multiprime labelling kits, Hyperfilm-MP and [³²P]dCTP were purchased from Amersham International (Little Chalfont, Bucks., U.K.). Other chemicals were either of analytical or molecular-biology grade.

RNA extraction and hybridization

Cells were scraped in 4 M guanidinium thiocyanate/25 mM sodium citrate (pH 7.0)/0.5% sarcosyl/0.1 M 2-mercapto-ethanol. Total RNA was then extracted by the acid/guanidinium/phenol/chloroform procedure of Chomczynski and Sacchi [19] and the purity of each preparation was assessed by the A_{260}/A_{280} ratio. RNA species were then separated by electrophoresis through a denaturing 2.2 M formaldehyde/1.2% (w/v) agarose gel [14] and transferred to nylon membrane (Genescreen from NEN-Dupont Ltd.) by capillary blotting. RNA was fixed to the membrane by exposure to UV and the membranes were stored dry until required.

Membranes were prehybridized for at least 6 h with 0.1 mg/ml denatured salmon sperm DNA in 50% (v/v) formamide/10% (w/v) dextran sulphate/0.2% BSA/0.2% polyvinylpyrrolidone/0.2% Ficoll/0.1% sodium pyrophosphate/1% SDS/50 mM Tris/HCl (pH 7.5). Portions (50–100 ng) of the DNA probes were labelled with [³²P]dCTP by random priming and hybridization was performed at 42 °C for 24 h as described previously [7]. Membranes were washed to remove non-specifically bound probe; two washes in $2 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl/0.015 M sodium citrate) at room temperature for 5 min, followed by two washes at 65 °C for 1 h in either $1 \times$ SSC/1% (w/v) SDS (cGSH-Px and IDI) or $0.2 \times$ SSC/1% (w/v) SDS (18 S rRNA), and a final wash in $0.1 \times$ SSC at room temperature. Specific hybridization was then detected both by direct imaging with a Packard Instantimager and by autoradiography with Hyperfilm-MP (Amersham International) at -70 °C. After analysis, membranes were washed in 0.1% SDS for 5–7 min at 95 °C before rehybridization with other probes.

Quantification of the bound probe was carried out with the Instantimager and results for each probe were expressed per unit of hybridization achieved with the 18 S rRNA probe; this permitted the correction for any variation between loading of RNA on the gel or transfer to the nylon membrane. RNA abundances were estimated in this manner from cells analysed in triplicate for each condition, and all hybridizations were performed on duplicate filters. Estimates of mRNA levels were calculated by combining the data from both filters.

Statistical analysis

Both enzymic activity and mRNA abundance results are expressed as means $\pm$ S.E.M. for three experiments. Results from selenium-adequate cells were compared with those from selenium-deficient cells by using an unpaired Student *t* test.

RESULTS AND DISCUSSION

H4-II-E-C3 hepatoma cells cultured in medium without a supplement of sodium selenite become selenium-depleted [8]. After 3 days in such medium H4-II-E-C3 cells lose approx. 80 % of their cGSH-Px activity and there is a differential effect of selenium depletion on the enzyme activity and mRNA levels of cGSH-Px and PHGSH-Px. Furthermore H4-II-E-C3 cells do not seem to express the IDI gene because no enzyme activity (results not shown) or mRNA (Figure 2) is detectable in these cells. Therefore it is possible to use IDI coding region as a reporter gene to study the effect of cGSH-Px and PHGSH-Px 3' UTRs on the regulation of expression of type I IDI in selenium-replete and selenium-deficient conditions. cGSH-Px and PHGSH-Px 3' UTRs were linked downstream of the IDI coding region and the resulting chimaeric constructs were assayed for the production of deiodinase activity and the levels of the specific mRNA were determined by Northern hybridization.

H4-II-E-C3 cells were transfected successfully with the three chimaeric constructs, and stable transfected cell lines were obtained. Figure 2 shows results from hybridization, with the

Figure 2 Northern hybridization showing expression of IDI mRNA in the transfected cell lines

Total RNA was extracted from the three transfected H4-II-E-C3 cell lines and from the untransfected line under selenium-replete conditions. All lanes were loaded with 20 μ g of total RNA and the filter was hybridized with IDI cDNA probe. The three corresponding RNA species were detected by autoradiography and showed the expected differences in sizes. No IDI mRNA was detected in the untransfected cells.

IDI cDNA probe, of a Northern blot with total RNA from selenium-replete cells transfected with the three constructs and from untransfected H4-II-E-C3 cells. A single mRNA species was detected in each transfected cell line and no IDI mRNA was detected in untransfected H4-II-E-C3 cells. The chimaeric and native IDI mRNA species were of different sizes: IDI-IDI, IDI-cGSH-Px and IDI-PHGSH-Px mRNA species were respectively approx. 2.0, 1.1 and 1.1 kb (Figure 2), sizes that are consistent with the known lengths of the three 3' UTRs.

The extent of translation of the three mRNA species was assessed by measuring deiodinase activity in these cells, and the selenium status was monitored by measuring cGSH-Px activity. In all three cell lines, cGSH-Px enzyme activity was decreased by approx. 75 % after three days in a selenium-deficient medium, confirming the low-selenium status of the cells under these conditions (see Table 1). IDI enzyme activity was also lowered in selenium-deficient conditions (see Table 1) but the extent of the decrease was different in the three cell lines. IDI activity in cells transfected with the construct IDI-cGSH-Px was decreased by 85 %, whereas it fell by only 64 % in cells transfected with IDI-IDI or IDI-PHGSH-Px constructs. The lowering of IDI activity in IDI-cGSH-Px cells is statistically different from that in IDI-IDI and IDI-PHGSH-Px cells ($P < 0.05$). The much higher IDI activity in IDI-cGSH-Px-transfected cells cultured in selenium-replete medium, compared with the other transfected cell lines, can be explained by differences in transfection efficiency. This was confirmed by the observation that the expression of IDI-cGSH-Px mRNA was higher than that of IDI-PHGSH-Px or IDI-IDI in the corresponding transfected cells (see Figure 2).

Differences in transfection efficiency between cell lines can be circumvented by calculating the IDI activity per mRNA [15]; the translational efficiency of the three mRNA species was estimated in this way (Table 2). The two cell lines expressing chimaeric mRNA species containing the IDI-coding region and either cGSH-Px or PHGSH-Px 3' UTRs both exhibited IDI activity, showing that the stem-loop structure present in both 3' UTRs could direct the incorporation of Se-Cys on the UGA codon of the heterologous deiodinase coding region. Comparison of the ratio of enzyme activity to mRNA showed that, under selenium-replete conditions, cGSH-Px and PHGSH-Px 3' UTRs produced a similar translational efficiency, but were less efficient compared with the IDI stem loop (Table 2), as previously observed for the cGSH-Px 3' UTR [15]. The present result, and those of Berry et al. [15] contrast with those of Kollmus et al. [20] who found that the PHGSH-Px 3' UTR was more efficient at promoting translation compared with either IDI or selenoprotein P 3' UTRs. The reason for this discrepancy might be that Kollmus et al. assessed

Table 1 Effect of selenium deficiency on cGSH-Px and IDI enzyme activities in transfected H4-II-E-C3 cells

Values shown are means $\pm$ S.E.M. for three experiments. Groups were compared with an unpaired Student *t* test; statistical significances are shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; enzyme activities were compared with the selenium-replete groups, and the percentage decrease in IDI activity in selenium deficiency for IDI-cGSH-Px was compared with the decrease in IDI-IDI and IDI-PHGSH-Px.

Cell line	cGSH-Px enzyme activity (m-units/mg of protein)		Decrease (%) in cGSH-Px activity in selenium deficiency	5' IDI enzyme activity (pmol of I ⁻ released/min per mg of protein)		Decrease (%) in IDI activity in selenium deficiency
	Selenium-replete	Selenium-deficient		Selenium-replete	Selenium-deficient	
IDI-IDI	24.5 $\pm$ 2.8	6.5 $\pm$ 0.7**	73	0.093 $\pm$ 0.008	0.033 $\pm$ 0.004**	64
IDI-cGSH-Px	26.9 $\pm$ 2.1	5.6 $\pm$ 0.5***	79	0.622 $\pm$ 0.098	0.092 $\pm$ 0.014**	85*
IDI-PHGSH-Px	28.8 $\pm$ 5.5	6.1 $\pm$ 0.8*	79	0.076 $\pm$ 0.004	0.027 $\pm$ 0.005**	64

Table 2 Translational efficiency of native IDI mRNA and chimaeric IDI-cGSH-Px and IDI-PHGSH-Px mRNA species in transfected H4-II-E-C3 hepatoma cells cultured under selenium-replete and selenium-deficient conditions

The translational efficiencies of the three mRNA species were estimated by measuring IDI activity and IDI mRNA levels in the three cell lines and calculating the ratio between IDI activity and mRNA. In selenium-replete and selenium-deficient conditions the results are expressed as percentages of the translational efficiency of the IDI-IDI mRNA, which was considered as 100% in both cases. Results are shown as means $\pm$ S.E.M. for three experiments.

Cell line	Translational efficiency (IDI activity/IDI mRNA) (%)	
	Selenium-replete	Selenium-deficient
IDI-IDI	100 $\pm$ 6	100 $\pm$ 7
IDI-cGSH-Px	66 $\pm$ 15	34 $\pm$ 6
IDI-PHGSH-Px	49 $\pm$ 10	51 $\pm$ 4

Table 3 Effect of selenium deficiency on native and chimaeric IDI mRNAs

Values shown are means $\pm$ S.E.M. for three experiments. Results are expressed as a percentage of the mean selenium-replete values and the absolute values are respectively: IDI-IDI, 2.4 $\pm$ 0.5; IDI-cGSH-Px, 19.7 $\pm$ 3.6; IDI-PHGSH-Px, 3.8 $\pm$ 1.0. The groups were compared by using an unpaired Student *t* test; ****P* < 0.001 compared with the selenium-replete group.

Cell line	mRNA abundance (%)	
	Selenium-replete	Selenium-deficient
IDI-IDI	101.1 $\pm$ 1.3	97.2 $\pm$ 4.0
IDI-cGSH-Px	99.7 $\pm$ 0.1	78.3 $\pm$ 3.4***
IDI-PHGSH-Px	99.9 $\pm$ 0.3	91.8 $\pm$ 7.8

translational read-through, but not specifically Se-Cys incorporation, from the *lacZ* gene into the luciferase gene, whereas we and Berry et al. [15] have assessed the production of active IDI enzyme; this latter method depends on both translational read-through and Se-Cys incorporation. The reasons for differences in translation efficiency due to the different 3' UTRs are still not clear but Berry et al. [21] have postulated the existence of factors, such as the stem loop impeding progress of the advancing ribosomes or melting of base-pairing in the stem by ribosomes, contributing to the low efficiency.

Table 3 shows the abundance of IDI mRNA carrying the different 3' UTRs in selenium-replete and selenium-deficient cells. The abundance of IDI mRNA carrying cGSH-Px 3' UTR was decreased in selenium-deficient cells by approx. 20%, whereas that of IDI mRNA carrying its own or the PHGSH-Px 3' UTR was not affected by selenium deficiency. This differential effect is comparable with the effect of selenium deficiency on endogenous cGSH-Px and PHGSH-Px mRNA species [8] and is compatible with the stability and translation of IDI-cGSH-Px mRNA being decreased in selenium deficiency. The translational efficiency of IDI-cGSH-Px was 66% of that of IDI-IDI under selenium-replete conditions but only 34% under selenium-deficient conditions (Table 2). In contrast, IDI-PHGSH-Px translational efficiency was approx. 50% of that for IDI-IDI under both conditions. The efficiency of translation of all three chimaeric mRNA species was decreased in selenium-deficient conditions, that of IDI-cGSH-Px mRNA being decreased by 79%, whereas that of IDI-PHGSH-Px and IDI-IDI mRNA was decreased by only 60% (*P* = 0.05). The much higher

expression of the chimaeric IDI-cGSH-Px transcript (Table 1) raises the possibility that the lower translational efficiency of this mRNA might be due to limiting amounts of specific translation factors. However, the similar effect of selenium depletion on the endogenous cGSH-Px mRNA (which competes with the exogenous transcript for translation factors) in all three cell lines (Table 1) suggests that such factors are not in fact limiting. Thus the results indicate that cGSH-Px 3' UTR was as efficient as PHGSH-Px 3' UTR in the translation of IDI under selenium-replete conditions but it was less effective under selenium-deficient conditions. This suggests that the IDI, cGSH-Px and PHGSH-Px 3' UTRs differ in the efficiency with which they can regulate the insertion of the Se-Cys on the UGA codon.

The results confirm that the 3' UTRs of different selenoproteins can act to regulate the incorporation of Se-Cys by a UGA codon in a heterologous coding region. However, the efficiencies of the 3' UTRs in permitting Se-Cys incorporation are different; as shown by the present results, this is also affected by conditions of limiting selenium supply. Thus when selenium supply is limiting, cGSH-Px 3' UTR is less efficient than PHGSH-Px 3' UTR and this must reflect different abilities to form a stem-loop/elongation factor/selenocysteinyl-tRNA complex. The reason for this is not clear, but could involve subtle differences in stem-loop structures and different strengths of complex formation when either Se-Cys tRNA or a mRNA-binding protein (elongation factor) is limiting so that the available Se-Cys is utilized for preferential synthesis of one selenoprotein. Recently Shen et al. [22] have isolated an RNA-binding protein that binds to the stem-loop structure of the cGSH-Px mRNA and could be involved in the mechanisms of recognition of the UGA as the Se-Cys codon. It is possible that the synthesis of this protein or the affinity for the stem loop could change in selenium-deficient conditions, causing a decrease in the translational efficiency of specific selenoproteins. An additional factor that can play a role in the incorporation of Se-Cys in selenoproteins is the effect of the UGA codon context in the mRNA. Low and Berry [23] have reported that the bases following the UGA codon could be terminator factors that favour termination of translation over Se-Cys incorporation, especially when Se-Cys tRNA is limiting. This could be the case in cGSH-Px, which contains a strong terminator context after the UGA codon [23]: its translation is decreased more during selenium deficiency than is that of other selenoproteins. However, the present results show that, in addition to the codon context, the 3' UTR also plays a key role in determining the extent of selenoprotein mRNA translation when selenium supply is limiting. In conclusion, these responses of the 3' UTRs to selenium deficiency can partly explain the channelling of selenium for preferential synthesis of PHGSH-Px rather than cGSH-Px during selenium deficiency.

This work was supported by the Scottish Office Agriculture, Environment and Fisheries Departement (SOAEFD). G.B. was supported by the University of Milan.

REFERENCES

- Weitzel, F. and Wendel, A. (1993) *J. Biol. Chem.* **268**, 6288–6292
- Arthur, J. R. and Beckett, G. J. (1994) In *Selenium in Biology and Human Health* (Burk, R. F., ed.), pp. 93–115. Springer-Verlag, New York
- Levander, O. A. (1987) *Ann. Rev. Nutr.* **7**, 227–250
- Arthur, J. R., Bermano, G., Mitchell, J. H. and Hesketh, J. E. (1996) *Biochem. Soc. Trans.* **24**, 384–388
- Saedi, M. S., Smith, C. G., Frampton, J., Chambers, I., Harrison, P. R. and Sunde, R. A. (1988) *Biochem. Biophys. Res. Commun.* **153**, 855–861
- Sunde, R. A., Dyer, J. A., Moran, T. V., Evenson, J. K. and Sugimoto, M. (1993) *Biochem. Biophys. Res. Commun.* **193**, 905–911
- Bermano, G., Nicol, F., Dyer, J. A., Sunde, R. A., Beckett, G. J., Arthur, J. R. and Hesketh, J. E. (1995) *Biochem. J.* **311**, 425–430

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- 8 Bermano, G., Arthur, J. R. and Hesketh, J. E. (1996) *FEBS Lett.* **387**, 157–160
 - 9 Chambers, I., Frampton, J., Goldforb, P., Affara, N., McBain, W. and Harrison, P. R. (1986) *EMBO J.* **5**, 1221–1227
 - 10 Larsen, P. R. and Berry, M. J. (1995) *Annu. Rev. Nutr.* **15**, 323–352
 - 11 Burk, R. F. and Hill, K. E. (1993) *Annu. Rev. Nutr.* **13**, 65–81
 - 12 Berry, M. J., Banu, L. and Larsen, P. R. (1991) *Nature (London)* **349**, 438–440
 - 13 Salvatore, D., Low, S. C., Berry, M., Maia, A. L., Harney, J. W., Croteau, W., St. Germain, D. L. and Larsen, P. R. (1995) *J. Clin. Invest.* **96**, 2421–2437
 - 14 Maniatis, J., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
 - 15 Berry, M. J., Banu, L., Chen, Y., Mandel, S. J., Kieffer, J. O., Harney, J. W. and Larsen, P. R. (1991) *Nature (London)* **353**, 273–276
 - 16 Paglia, D. E. and Valentine, V. W. (1967) *J. Clin. Lab. Med.* **70**, 158–178
 - 17 Arthur, J. R., Nicol, F. and Beckett, G. J. (1990) *Biochem. J.* **272**, 537–540
 - 18 Erickson, J. M., Rushford, C. L., Dorney, D. J., Wilson, G. N. and Schminckel, R. D. (1981) *Gene* **16**, 1–9
 - 19 Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
 - 20 Kollmus, H., Flohe, L. and McCarthy, J. E. G. (1996) *Nucleic Acids Res.* **24**, 1195–1201
 - 21 Berry, M. J., Maia, A. L., Kieffer, J. D., Harney, J. W. and Larsen, P. R. (1991) *J. Biol. Chem.* **266**, 14156–14158
 - 22 Shen, Q., McQuilkin, P. A. and Newburger, P. E. (1995) *J. Biol. Chem.* **270**, 30448–30452
 - 23 Low, S. C. and Berry, M. J. (1996) *Trends Biochem. Sci.* **21**, 203–208
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Received 11 July 1996/28 August 1996; accepted 4 September 1996