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Intracellular trafficking of micronutrients: from gene regulation to nutrient requirements

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The intracellular distribution of micronutrients, as well as their uptake, is important for cell function. In some cases the distribution of micronutrients or their related proteins is determined by gene expression mechanisms. The 3′ untranslated region (3′UTR) of metallothionein–1 mRNA determines localisation of the mRNA, and in turn intracellular trafficking of the protein product. Using transfected cells we have evidence for the trafficking of metallothionein–1 into the nucleus and for its involvement in protection from oxidative stress and DNA damage. When nutritional supply of Se is limited, selenoprotein expression is altered, but not all selenoproteins are affected equally; the available Se is prioritised for synthesis of particular selenoproteins. The prioritisation involves differences in mRNA translation and stability due to 3′UTR sequences. Potentially, genetic variation in these regulatory mechanisms may affect nutrient requirements. Genetic polymorphisms in the 3′UTR from two selenoprotein genes have been observed; one polymorphism affects selenoprotein synthesis. These examples illustrate how molecular approaches can contribute at several levels to an increased understanding of nutrient metabolism and requirements. First, they provide the tools to investigate regulatory features in genes and their products. Second, understanding these processes can provide model systems to investigate nutrient metabolism at the cellular level. Third, once key features have been identified, the availability of human genome sequence information and single nucleotide polymorphism databases present possibilities to define the extent of genetic variation in genes of nutritional relevance. Ultimately, the functionality of any variations can be defined and subgroups of the population with subtly different nutrient requirements identified.

Résumé

La distribution intracellulaire des micronutriments ainsi que leur absorption sont importantes pour les fonctions cellulaires. Dans certains cas la distribution des micronutriments ou des protéines associées est déterminée par des mécanismes liés à l'expression des gènes. La région 3′ non traduite (3′UTR) de l'ARNm de la métallothioneine–1 détermine la localisation de ce message et, par conséquent, la localisation intracellulaire de la protéine qu'il code. En utilisant des cellules transfectées nous avons montré que la métallothioneine–1 est transportée vers le noyau ou elle exerce un rôle dans la protection contre le stress oxydant et les dommages causés à l'ADN. Quand l'apport nutritionnel en Se est limité, l'expression des sélénoproteines est altérée. Toutefois celle-ci n'est pas affectée de façon identique pour toutes les sélénoproteines; le Se disponible étant utilisé de façon prioritaire pour la synthèse de certaines d'entre elles. Cet ordre de priorité met en jeu des différences dans la traduction et la stabilité de leur ARNm qui sont sous le contrôle de séquences dans la région 3′ non traduite. Potentiellement, des variations génétiques affectant ces mécanismes régulateurs peuvent moduler les besoins en nutriments. Des polymorphismes génétiques ont été décrits dans le 3′UTR des ARNm de deux sélénoproteines; l'un d'entre eux affectant la synthèse de la sélénoproteine correspondante. Ces exemples illustrent comment des

approches moléculaires peuvent contribuer à accroître notre compréhension du métabolisme et des besoins en nutriments à différents niveaux. Premièrement, elles permettent d'étudier les effets régulateurs des gènes et de leurs produits. Ensuite, la compréhension de ces effets peut fournir un modèle pour étudier le métabolisme des nutriments au niveau cellulaire. Ainsi, lorsque des effets essentiels sont identifiés, la connaissance du génome humain et les bases de données sur les polymorphismes génétiques constituent des outils complémentaires pour définir l'étendue de la variation génétique des gènes revêtant une importance nutritionnelle. Enfin, la fonctionnalité de ces variations peut être définie et des sous-groupes de la population, possédant des besoins nutritionnels différents, peuvent être identifiés.

Nutrient–gene interactions: Polymorphism: Selenium: Metallothionein: 3′ Untranslated region

Nutrition at the cellular level

Cells transport micronutrients across the plasma membrane, and then these micronutrients are utilised in a variety of proteins and organelles. As illustrated schematically in Fig. 1, this process presents cells with a series of different logistic and regulatory problems. First, how to get enough micronutrients into the cell. Second, how to coordinate their intracellular distribution so that the appropriate amounts of specific nutrients are delivered to different subcellular compartments. Third, if supply of a micronutrient is limiting, how to determine which metabolic process or enzyme is supplied with the nutrient in question; in other words, is there a prioritisation of the use of specific nutrients? Much attention has been given to the first step in the process of micronutrient delivery to the cell, and an extensive variety of nutrient transporters has been identified and characterised. Much less is known about the subsequent biochemical processes that determine what happens to the nutrients once inside the cell; what determines their subcellular distribution and their use in specific proteins or metabolic pathways.

It is probable that such intracellular trafficking and prioritisation of micronutrients are important for efficient use of nutrients, and that individual differences in these processes may potentially influence metabolic responses to nutrition; in turn, this situation suggests that, ultimately, such individual differences may affect an individual's micronutrient requirements. Any such individual differences in intracellular distribution mechanisms will be based on genotype. Thus, it is important to identify the cell components that play the key roles in determining intracellular nutrient distribution and prioritisation, to understand the regulation of their genes and to assess the extent to which individual variation in these genes has nutritionally-important effects on metabolism and requirements. The advent of molecular and genomic techniques presents opportunities to study the regulation of the key genes, to determine their variation within populations and to assess the impact of such variation on nutrition.

In the present article we will discuss how molecular approaches using novel gene constructs and transfected cell lines provide opportunities for studying two aspects of micronutrient distribution. First, micronutrients such as Zn and retinoic acid have important functions in the nucleus, but it is not known how their nuclear–cytoplasmic distribution is regulated; proteins such as metallothionein (MT) and cellular retinoic acid-binding protein (CRABP)-I may potentially play key roles in this intracellular trafficking. Second, selenoprotein gene expression is regulated by dietary Se supply such that when Se supply is low not all selenoproteins are affected to the same extent and there is effectively a prioritisation in the use of the available Se for selenoprotein synthesis.
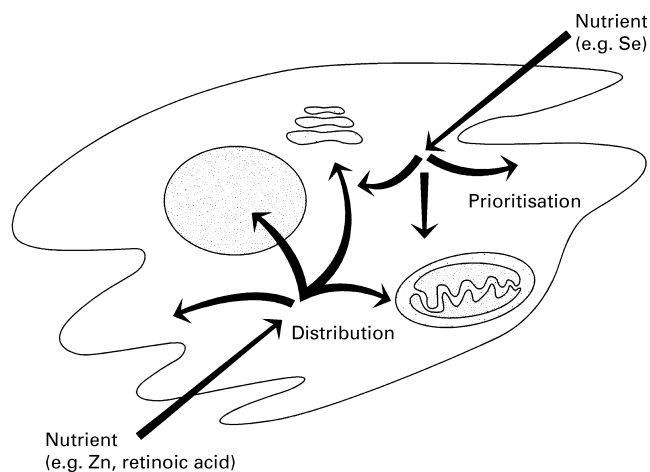

Fig. 1. Nutrition at the cellular level: intracellular trafficking and channelling of micronutrients. The schematic diagram illustrates the requirements of cells to control the intracellular distribution of nutrients. For example, in the case of zinc or retinoic acid these micronutrients are distributed to different subcellular compartments, and we propose that mechanisms exist for intracellular trafficking of these micronutrients and their associated proteins. Alternatively, as in the case of selenium, the available micronutrient is used in a hierarchical way so that it is channelled for use in one enzyme or metabolic function rather than another, i.e. there is a prioritisation in the metabolic use of the micronutrient.

Intracellular trafficking of micronutrient-related proteins

Metallothioneins

Metallothioneins (MT) are a family of low-molecular-weight (6–7 kDa) metal-binding proteins, which exist as a series of isoforms. All MT bind heavy metals such as Cd, Cu and Zn, and they have been proposed to play important roles in metal detoxification and metabolism. Although MT are considered to be found mainly in the cytoplasm, there are an increasing number of reports of their nuclear localisation, and it has been suggested that MT protect DNA from oxidative damage (Bremner & Beattie, 1990). MT are found in the nucleus in fetal and neonatal rat liver (Panemangelore *et al.* 1983; Templeton *et al.* 1985), but not in adult liver. In hepatocytes MT–1 was observed to have a nuclear localisation in early S phase of the cell cycle, but not in G0–G1 (Tsujikawa *et al.* 1991). A body of further evidence supports the hypothesis that the nuclear localisation of MT is cell-cycle dependent: in 3T3-L1 cells and in myoblasts nuclear localisation is promoted by conditions that induce differentiation (Schmidt & Beyersmann, 1999; Apostolova *et al.* 2000); in transfected chinese hamster ovary cells nuclear localisation of MT–1 was observed in synchronised cells at the G1–S transition (early S phase) in the cell cycle (Levadoux *et al.* 1999; Levadoux-Martin *et al.* 2001). This temporal nuclear localisation of MT suggests

that this protein can shuttle from cytoplasm to nucleus during the cell cycle.

In parallel with these studies we have found that MT–1 mRNA is localised in perinuclear cytoplasm. In hepatoma cells, after induction of MT gene transcription by the addition of Zn, MT–1 mRNA was found to be localised in the cytoplasm around the nucleus and to be enriched in polysomes associated with the cytoskeleton (Mahon *et al.* 1995). Similarly, in chinese hamster ovary cells transfected with the rat MT–1 gene, the MT–1 transcripts were found localised in the perinuclear cytoplasm (Mahon *et al.* 1997). The phenomenon of mRNA localisation has been demonstrated in mammalian cells relatively recently (Kislauskis *et al.* 1993; Hesketh *et al.* 1994), but several mRNA encoding nuclear proteins (c-myc, c-fos) have been found to be both localised in the perinuclear cytoplasm and associated with the cytoskeleton by a mechanism which requires specific RNA sequences in the 3′ untranslated region (3′UTR) of the mRNA (Veyrune *et al.* 1996; Dalgleish *et al.* 2001).

We have investigated MT mRNA and protein localisation in cells transfected with a range of novel gene constructs. Exchange of the full 3′UTR of MT–1 mRNA for the 3′UTR from the cytosolic glutathione peroxidase (GPX)1 causes loss of perinuclear mRNA localisation (Mahon *et al.* 1997); in addition, removal of the whole 3′UTR or a 41-nt region within the 3′UTR causes loss of localisation (Levadoux-
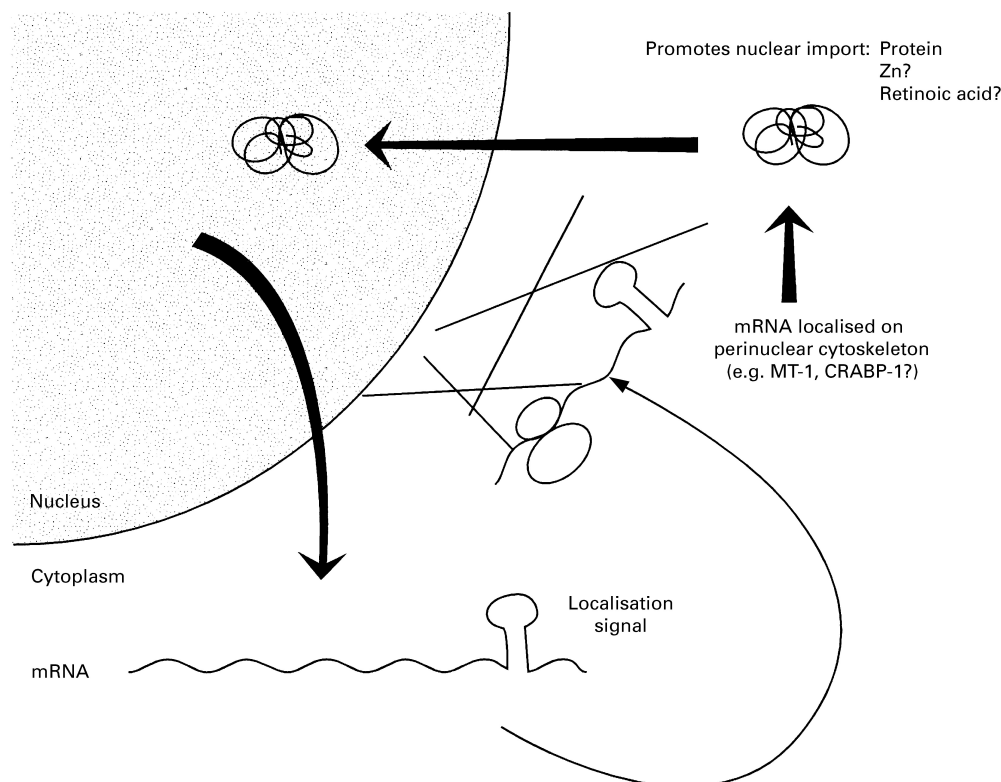

Fig. 2. mRNA localisation leads to localised synthesis of proteins: a mechanism to promote nucleo–cytoplasmic trafficking of proteins and associated micronutrients. Signals in the 3′ untranslated region of mRNA lead to their localisation. Our hypothesis is that such mRNA localisation in the perinuclear cytoplasm in association with the cytoskeleton promotes subsequent nuclear import of the synthesised protein. We propose that this nucleo–cytoplasmic shuttling of certain proteins provides a basis for intracellular trafficking of specific micronutrients. MT–1, metallothionein; CRABP–1, cellular retinoic acid-binding protein 1.

Martin & Hesketh, 2002). Furthermore, loss of mRNA localisation is accompanied by loss of MT–1 protein localisation; in unsynchronised cells the usual perinuclear localisation of MT–1 is not observed if the cells do not localise the MT–1 mRNA, and in synchronised cells entering the S phase the nuclear localisation of MT–1 is not observed if the cells cannot localise the mRNA (Levadoux et al. 1999; Levadoux-Martin et al. 2001). It thus appears that perinuclear localisation of MT1 mRNA is essential for subsequent nuclear import of the MT–1 protein as the cells enter the S phase. Since mRNA encoding other nuclear proteins are localised to the perinuclear cytoskeleton by 3′UTR signals (Veyrune et al. 1996; Dalgleish et al. 2001), it has been suggested that mRNA localisation promotes the nuclear import of a range of proteins, as illustrated schematically in Fig. 2.

Transfected cell lines expressing MT–1 with different localisation properties provide model systems in which to investigate functional aspects of the trafficking and the role of nuclear MT–1. For example, the cells that were unable to effectively redistribute MT–1 protein into the nucleus showed functional changes, including increased sensitivity to oxidative stress, increased DNA damage and reduced apoptosis (Levadoux-Martin et al. 2001). This finding supports the hypothesis that nuclear MT–1 has an important role in protecting cells from oxidative DNA damage. However, in cells transfected with MT–1 with an added nuclear localisation signal so that the MT–1 was exclusively nuclear (Woo & Lazo,1997) expression of this MT provided no protection from oxidative stress. Taken together these observations suggest that if MT–1 is exclusively nuclear it does not protect the cell from oxidative stress, but that the protective role of MT–1 requires a trafficking or shuttling of MT–1 between nucleus and cytoplasm (Levadoux-Martin et al. 2001). In view of the well-known metal-binding properties of MT–1, it seems likely that the redistribution of MT–1 is associated with a trafficking of Zn between cytoplasm and nucleus. This possiblity is supported by the observations that in myoblasts the redistribution of MT into the nucleus during differentiation is accompanied by a redistribution of Zn (Apostolova et al. 1999, 2000). We are now using our transfected cell model to study the relationship between Zn distribution and MT–1 localisation.

Retinoic acid and fatty acid-binding proteins

It is well known that retinoids bind to a series of nuclear receptors and have a major role in the regulation of gene expression during growth and development; in this way retinoic acid acts as a morphogen. Recently, a number of other ligand-activated nuclear receptors have been identified which are activated by other lipid molecules such as polyunsaturated fatty acids and prostaglandins: the peroxisome proliferator-activated receptors (PPAR; Desvergne & Wahli, 1999). Activation of retinoid receptors and PPAR therefore requires that retinoids and fatty acids are present in the nucleus. In addition, fatty acids are also required in other cellular compartments such as the mitochondria and internal membrane compartments and there is therefore an intracellular trafficking of lipids with cells such as the adipocyte (Bernlohr et al. 1999).

Cells not only have retinoid receptors and PPAR, but they also contain other proteins which have the capacity to bind either retinoic acid or fatty acids; cellular CRABPI and CRABPII and fatty acid-binding proteins (FABP) (Bernlohr et al. 1999; Noy, 2001). These are low-molecular-weight (approximately 15 kDa) proteins of related structure. The functions of both CRABPI and FABP have not been defined, but both families of proteins are prime candidates to function in the intracellular transport and distribution of retinoic acid and specific fatty acids. However, the evidence for such a role is minimal. In particular, the subcellular distribution of these proteins is still either contentious or unexplored in detail.

Using immunohistochemistry, CRABPI was found to be nuclear in some cell types but cytoplasmic in others (Gustafson et al. 1996; Venepally et al. 1996). It was later reported that detection of nuclear or cytoplasmic localisation of both CRABPI and CRABPII depends on the nature of the fixation used (Gaub et al. 1998). However, other researchers have found that there is an exclusion of CRABPI from the nucleus and an association with mitochondria (Ruff & Ong, 2000). Thus, the precise subcellular distribution of CRABPI remains unclear. However, it is a distinct possibility that it can be nuclear under certain circumstances, and that it may therefore shuttle from nucleus to cytoplasm and so play a role in retinoid trafficking. Further work is required to confirm that CRABPI has such a role. We have begun to use the transfected cell approach that was successful with MT–1 (see p. 407) to investigate the subcellular distribution of CRABP with a view to later examining its role in retinoid trafficking.

Data on the distribution of FABP are scarce, but results from a recent biochemical study suggest that liver FABP co-localises with PPARα in the nucleus of hepatocytes; furthermore, the two proteins co-immunoprecipitate, and a two-hybrid screen supports the contention that both PPARα and PPARα interact with liver FABP (Wolfrum et al. 2001). It thus appears that liver FABP may function to provide a mechanism for trafficking lipid ligands into the nucleus to the PPAR receptors. It is possible, therefore, that both CRABP and FABP may turn out to be key factors in determining the cytoplasmic–nuclear distribution of retinoids and other intracellular lipids.

Selenoprotein expression and the prioritisation of available selenium

Incorporation of selenium into the functional selenoproteins

Since the early descriptions of Se toxicity (Schwarz & Foltz, 1957), it has become apparent that Se is a micronutrient essential for optimal health in both man and animals (Yang et al. 1984). Indeed, severe nutritional deficiency of Se has been associated with a large number of clinical conditions (Kohrle et al. 2000; Rayman, 2000; Maehira et al. 2002). Recently, new potential roles for Se in human health have been recognised, particularly in relation to cancer (Clark et al. 1997), the immune system and viral diseases (Beck, 1996; Diamond et al. 2001). Furthermore, marginally-low Se intake may also have health implications. Low Se status may contribute to the aetiology of the disease process, but in

some cases it may be an outcome of the condition itself or may exacerbate disease progression (e.g. HIV infection).

Se is associated with several major metabolic functions in the body, due to its incorporation as the amino acid selenocysteine at the active site of a wide range of 'seleno-proteins'. In mammals, the selenoproteins have been estimated to be between thirty and fifty in number, but so far less than twenty have been fully characterised by sequence analysis and an enzymic function has been assigned to approximately ten of them. Among the identified and relatively well-characterised selenoproteins are four glutathione peroxidases (GPX1–4), at least three thioredoxin reductases, three deiodinases (including iodothyronine deiodinase types I (IDI) and III), selenophosphate synthetase-2, selenoprotein P present in plasma and a related variant in bovine brain, the selenoprotein W in muscle and a 15 kDa selenoprotein.

Selenocysteine is incorporated into nascent polypeptide chains of the proteins during the translation process; its site of incorporation in both prokaryotes and eukaryotes was found to be the UGA codon (Chambers & Harrison, 1987; Berry & Larsen, 1993), originally identified as one of the stop codons in the genetic code. This unique recoding of the UGA codon requires the presence of additional signals. In *Escherichia coli* UGA is read as a selenocysteine incorporation site only if it is immediately followed in the reading frame by a region that has been shown to form a stem–loop structure (Heider *et al*. 1992). An element with an apparently analogous function, referred to as the seleno-cysteine insertion sequence (SECIS; Berry *et al*. 1991*a*), exists in selenoprotein-encoding mRNA of eukaryotes (Berry *et al*. 1993; Berry & Larsen, 1993). However, unlike the prokaryotic signal, the eukaryotic element is found in the 3′UTR (Berry *et al*. 1991*a*; Shen *et al*. 1993), and must therefore be effectively acting at a distance. Initial studies showed that the 3′UTR from three mammalian seleno-proteins were functionally interchangeable (Berry *et al*. 1991*a*, 1993). While there is little primary sequence homology between these elements, the predicted secondary structures are similar. Sequence comparisons and structure–function experiments generated a consensus secondary structure model for the SECIS element in which a functional motif could be identified (Walczak *et al*. 1996, 1998). The rat and human selenoprotein P mRNA contain ten UGA and two SECIS elements (Berry *et al*. 1993; Hill *et al*. 1993), whereas multiple UGA and stem–loops have not been reported for prokaryotic selenoproteins.

Dietary Se is normally in the form of selenomethionine, selenocysteine or selenite. After absorption all these forms are metabolised to selenide, which is in turn converted to selenomonophosphate. Selenocysteine is incorporated into proteins by using selenocysteyl-tRNA^{Sec} formed from selenomonophosphate and seryl-tRNA. The homologue of the bacterial SelA gene product, selenocysteine synthase, catalysing the replacement of the side-chain O in the serine of seryl-tRNA^{Sec} by Se and yielding selenocysteyl-tRNA^{Sec}, has not been identified yet in archaea and eukaryotes. In contrast, homologues of the bacterial Sel D gene product, which delivers the Se donor monoselenophosphate, have been identified in mammals (Kim & Stadtman, 1995; Low *et al*. 1995).

Selenocysteyl-tRNA^{Sec} and the SECIS element in the 3′UTR are not sufficient for Se incorporation to occur; specific SECIS-binding proteins (SBP) and translation factors are also required. Direct binding studies using mammalian SECIS elements in gel retardation and u.v.-cross-linking studies have identified factors that are potentially involved in linking the 3′UTR to its potential targets such as ribosomes or tRNA. Protein complexes consisting of either 55 and 65 kDa proteins (DNA-binding protein B), nucleolin, or the 60–65 kDa SBP have been found to bind to SECIS elements such as a full-length stem–loop derived from GPX1 mRNA (Shen *et al*. 1995; Hubert *et al*. 1996; Wu *et al*. 2000). More recently, Lesoon *et al*. (1997) described a highly-specific 120 kDa protein (SBP2), the binding of which was highly correlated with seleno-protein synthesis. The precise roles of these proteins, if indeed all of them have roles, in Se incorporation remain to be clarified. However, it is possible that some of, or all, these proteins join some as yet unidentified other proteins to form a large complex that links the SECIS element in the 3′UTR to other elements in the coding region and the ribosome, so allowing the UGA 'stop' codon to be translated as selenocysteine.

There is strong evidence for the hypothesis that SBP2 is a key factor. Recent work by Driscoll and collaborators (Copeland *et al*. 2000) has established that SBP2 is essential for the co-translational insertion of selenocysteine into selenoproteins, and that it contains a putative RNA-binding domain similar to that found in the yeast SUP1 omnipotent suppressor of translation termination (Koonin *et al*. 1994). These findings led them to hypothesise that the binding activity of SBP2 may be involved in preventing termination at the UGA/Secys codon. A first eukaryotic selenocysteyl-tRNA-specific elongation factor, EFsec, has also been described. This factor forms a complex with mammalian SBP2, and these two components are thought to function together in selenocysteine incorporation in mammalian cells (Tujebajeva *et al*. 2000; Berry *et al*. 2001).

Prioritisation in the use of available selenium: role of the 3′ untranslated region

It is clear from many animal studies that dietary Se restriction causes decreases in the activity of the seleno-proteins. According to Se status, a hierarchy of Se retention occurs in different tissues. For example, testes retain Se stores approximately 20-fold better than liver or heart when dietary Se is limited (Behne *et al*. 1998). In addition, within a single tissue or cell line different selenoproteins are affected to varying extents (Bermano *et al*. 1995; Lei *et al*. 1995; Villette *et al*. 1998; Brigelius-Flohé, 1999). For example, in severely-Se-deficient rats liver GPX1 activity is decreased by approximately 99 % and GPX4 by 75 % (Bermano *et al*. 1995). Thus, the size of the decrease in selenoprotein activity varies from protein to protein and between tissues such that there is essentially, therefore, a 'prioritisation' of the available Se. The basis of this prioriti-sation is believed to partly lie in differences in 3′UTR sequences in the mRNA of the different selenoprotein gene products (Hesketh *et al*. 1998; Brigelius-Flohe, 1999).
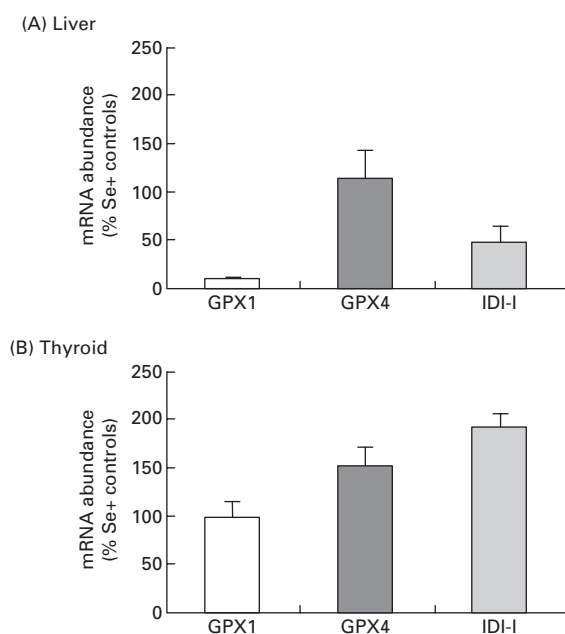
(A) Liver

(B) Thyroid

Fig. 3. The effects of dietary selenium deficiency on selenoprotein gene expression: evidence for the channelling or prioritisation of the available micronutrient. The differential effects of selenium deficiency on selenoprotein expression, assessed by mRNA abundance are shown. Values are means with their standard errors represented by vertical bars. Se+, selenium sufficient. In the rat liver (A)selenium deficiency causes a 90 % fall in glutathione peroxidase (GPX) 1 mRNA abundance and a 50 % fall in iodothyronine deiodinase (IDI) I mRNA abundance but no significant change in GPX4 mRNA abundance; in the thyroid (B) a hierarchy is also observed, but in this case IDI-I is preferentially maintained compared with GPX4 and GPX1. The relative effects of Se depletion are mirrored by enzyme activities. The data (taken from Bermano *et al*. 1995) show how there is a hierarchy of selenoprotein expression during dietary selenium depletion so that there is an effective prioritisation or channelling of the available selenium for synthesis of certain selenoproteins but not others.

The changes in activity or concentration of selenoproteins during limited Se supply are accompanied by alterations in mRNA abundances. Although the effects on selenoprotein activity and mRNA abundance do not always correlate exactly, the hierarchy of effects on protein expression are paralleled by that seen with the changes in mRNA abundance. For example, as illustrated in Fig. 3, in a comparison of GPX1, GPX4 and IDI-I it was found that in the rat liver GPX1 mRNA abundance is affected most by severe Se deficiency, IDI-I less so and GPX4 not at all (Bermano *et al*. 1995); a similar difference between the response of GPX1 and GPX4 was also found in hepatoma cells (Bermano *et al*. 1996) but a different pattern was seen in the thyroid (Fig. 3). In cultured colonic cells GPX2 mRNA abundance is not affected by Se supply (Wingler *et al*. 2000; Pagmantidis *et al*. 2002), and a similar observation has been made recently in the rat colon, whereas GPX1 mRNA abundance is decreased by 80 % and that of GPX4 by 20 % (Pagmantidis *et al*. 2002). Overall, these studies indicate that there is individual regulation of GPX1,

GPX2, GPX4 and IDI-I such that there are differences in the sensitivity of the synthesis of each enzyme and the abundance of its mRNA to Se supply, both within a given tissue and between tissues (Hesketh *et al*. 1998; Brigelius-Flohe, 1999; Wingler *et al*. 2000).

Post-transcriptional mechanisms play an important role in this regulation of selenoprotein synthesis in response to altered Se supply. Se deficiency does not affect the rate of transcription of the GPX1, GPX4 or IDI-I genes in liver nuclei (Bermano *et al*. 1995), even though there are changes in mRNA abundance and enzyme activity, suggesting that control of the three selenoproteins involves regulation of mRNA stability. Indeed, in cell-culture systems Se supply has been shown to affect differentially the stability of GPX1, GPX2 and GPX4 mRNA (Bermano *et al*. 1996; Wingler *et al*. 1999). mRNA containing premature nonsense codons are eliminated from most cells via a pathway known as mRNA surveillance, or nonsense-mediated decay (Nagy & Maquat, 1998; Hentze & Kulozik, 1999). A critical feature in discrimination between physiological and premature termination codons in mammalian cells appears to be the last intron of the pre-mRNA relative to the termination codon. An intron more than approximately fifty nucleotides downstream from a termination codon will define this stop codon as premature (Nagy & Maquat, 1998). Thus, selenoprotein mRNA whose pre-mRNA contain introns downstream of the selenocysteine codon should be targeted for nonsense-mediated decay when selenocysteine incorporation is inefficient. This is the case for GPX1 mRNA (Weiss & Sunde, 1998; Maquat, 2001), whereas GPX4 mRNA is much less sensitive to nonsense-mediated decay, despite the presence of appropriately-spaced introns in both pre-mRNA (Lei *et al*. 1995).

Differences in the 3′UTR of the selenoprotein mRNA appear to be critical in determining the relative extent to which the different mRNA are translated. First, even when Se supply is adequate, the efficiency of translation of selenoprotein mRNA is affected by the 3′UTR, as indicated by the observations that the activities of the IDI-I and IDI-III are altered by exchanging their native 3′UTR for those of other selenoproteins (Berry *et al*. 1991*b*; Salvatore *et al*. 1995). Second, there is further influence of the 3′UTR when Se supply is low; translation of chimaeric constructs containing the IDI-I coding region linked to GPX4 3′UTR is affected less by low-Se conditions than translation of IDI-I coding region linked to GPX1 3′UTR (Bermano *et al*. 1996). This finding suggests that when Se supply is limiting, the 3′UTR of GPX1 is less efficient at maintaining translation than that of GPX4. Similarly, using a read-through assay and mRNA measurements the relative translation and stability of GPX1 and GPX2 mRNA under low-Se conditions has been shown to be determined by 3′UTR sequences (Wingler *et al*. 1999, 2000). These differences probably reflect different abilities to form a complex with seleno-cysteine-tRNA and the proteins forming the SECIS-binding complex, as illustrated in Fig. 4. Our hypothesis is that the differential expression of selenoproteins during limited Se supply reflects differential utilisation of selenocysteyl-tRNA dictated by the affinity of 3′UTR-binding proteins for different SECIS elements. Initially, some support for such a hypothesis came from studies of SBP which suggested that
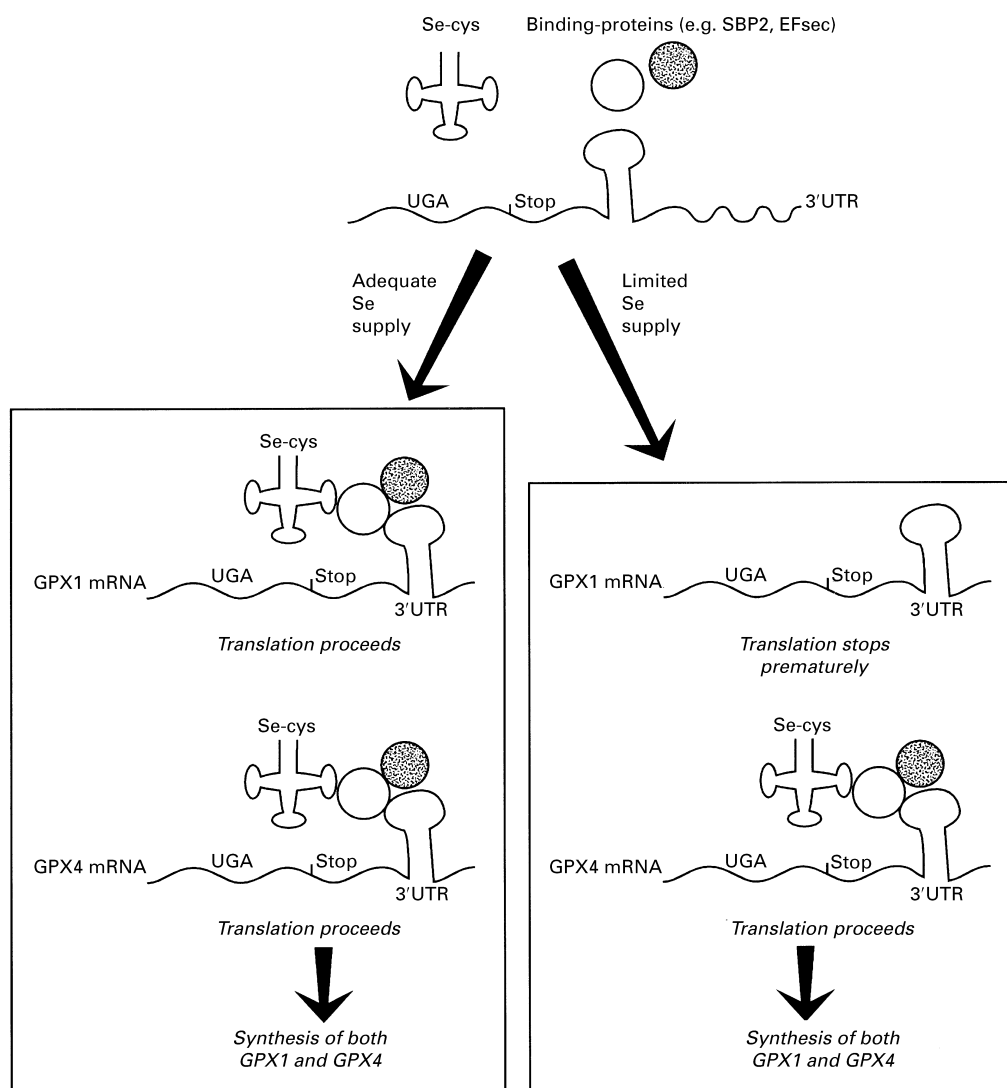
Fig. 4. A proposed mechanism to explain the prioritisation of selenium for synthesis of one selenoprotein rather than another when dietary selenium supply is limited. Our hypothesis is that prioritisation depends on small differences in the 3′ untranslated region (3′UTR) sequences and 3′UTR-binding proteins. We propose that subtle differences in the selenoprotein 3′UTR lead to differences in the complexes formed by 3′UTR-binding proteins, the selenocysteine (Se-cys) insertion sequence (SECIS) elements and other components of the selenium incorporation machinery; in this way the complex formation and the ability of the incorporation components to read UGA for Se-cys insertion varies when the supply of selenium, and thus Se-cys, is limited. The diagram illustrates this process in a schematic way, taking glutathione peroxidase (GPX) 1 and GPX4 as examples. When selenium is abundant both GPX1 and GPX4 mRNA are translated and both proteins synthesised, but when Se, and thus Se-cys-tRNA, is limiting translation of GPX1 mRNA is reduced while that of GPX4 mRNA is maintained, because the 3′UTR of the GPX4 mRNA is more effective at competing for components of the incorporation machinery. SBP, SECIS-binding protein; EFsec, an Se-cys-tRNA-specific elongation factor.

interactions of one of the SBP, SBP2, with the SECIS element dictate selenocysteine incorporation efficiency and selenoprotein hierarchy (Low *et al.* 2000). However, more recent data suggest that the affinity of SBP2 for the SECIS elements is not responsible for Se prioritisation and the hierarchy of selenoprotein synthesis (Fletcher *et al.* 2001).

Polymorphisms in human selenoprotein 3′ untranslated region sequences

Dietary intake of Se in the UK has fallen over the past 20 years, and it is now reported to be only half the UK reference nutrient intake of 1 µg Se/kg body weight (Molnar *et al.* 1995; Ministry of Agriculture, Fisheries and Food, 1997). The nutritional and health implications of the decline

in Se status in the UK are of concern (Shortt *et al.* 1997; Rayman, 2000), as it may result in suboptimal selenoprotein function, with deleterious long-term health implications. It has been reported that individuals in the UK population differ in their response to Se supplementation (Brown *et al.* 2000). One possibility is that subtle differences in genotype corresponding to the selenoprotein genes may underlie, at least in part, such variation between individuals.

As discussed earlier, there is now a body of evidence from cell-culture work with transfected cell lines that shows that relatively small changes in selenoprotein 3′UTR sequences influence incorporation into the selenoproteins. Thus, exchange of selenoprotein 3′UTR causes changes in the extent of mRNA translation, Se incorporation and mRNA stability. In particular, different 3′UTR influence the effect of Se depletion on Se incorporation into protein and mRNA stability (Bermano *et al.* 1996; Wingler *et al.* 1999, 2000). If such small changes in 3′UTR sequences could influence Se metabolism in man, then it is possible that subtle genetic changes in 3′UTR sequences might produce differences between individuals in their selenoprotein synthesis in response to Se supply.

Recently we embarked on sequence analysis of the GPX4 gene in a Scottish population. No sequence variation was found in the region corresponding to the SECIS, indicating the absolute requirement for this control element. However, a common single nucleotide polymorphism (T/C) was identified in a part of the 3′UTR close to the proposed SECIS region (Villette *et al.* 2002). The functional significance of this polymorphism is at present under investigation both in transfected cell lines and human studies. A further example of a polymorphism in a selenoprotein 3′UTR has been found in the 15 kDa selenoprotein 3′UTR (Hu *et al.* 2001). In this case there were statistically significant differences in allele frequencies by ethnicity ($P < 0.001$) and, moreover, in cell-culture studies the identity of the nucleotides at the polymorphic sites was found to influence SECIS function in a Se-dependant manner. It would appear, therefore, that there are indeed genetic variations in 3′UTR gene sequences, and we hypothesise that such genetic poymorphisms may lead to differences in the way in which individuals use available dietary Se and so lead to a gene–nutrient interaction that underlies differences in nutrient requirements or susceptibility to disease.

Concluding remarks: the potential of molecular and genomic approaches

Molecular approaches with gene-expression studies, gene constructs and transfected cell lines have allowed the description of gene regulatory mechanisms involved in mRNA translation, stability and localisation (Hesketh *et al.* 1998). For example, the role of 3′UTR sequences in mRNA localisation and Se incorporation has been demonstrated using such methods. The transfected cell lines provide model systems in which to carry out functional studies, both of mechanisms regulating selenoprotein synthesis and of protein trafficking and its role in micronutrient distribution. Such studies present a paradigm of how such

molecular studies and techniques can be used to address nutritional questions and to increase knowledge about specific aspects of nutrient metabolism. Furthermore, as illustrated by the ongoing analysis of the selenoprotein genes, once the cell components involved in a nutrient process of interest have been identified, it is possible to analyse the regulation of the genes for those components and search for genetic polymorphisms which may underlie phenotypic variation in individual responses to diet. The availability of human genome sequence data, single nucleotide polymorphism databases and the advent of genomic technologies will all contribute to such studies in the future; combined with both molecular cell biology approaches and human nutrition studies they will promote our understanding of the extent to which subtle genetic differences influence an individual's optimal nutrition, their nutrient requirements and susceptibility to disease.

References

Apostolova MD, Ivanova IA & Cherian MG (1999) Metallothionein and apoptosis during differentiation of myoblasts to myotubes: protection against free radical toxicity. *Toxicology and Applied Pharmacology* **159**, 175–184.

Apostolova MD, Ivanova IA & Cherian MG (2000) Signal transduction pathways and nuclear translocation of zinc and metallothionein during differentiation of myoblasts. *Biochemistry and Cell Biology* **78**, 27–37.

Beck MA (1996) The role of nutrition in viral disease. *Nutritional Biochemistry* **17**, 683–690.

Behne D, Hilmert H, Scheid S, Gessner H & Elger W (1998) Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochimica et Biophysica Acta* **966**, 12–21.

Bermano G, Arthur JR & Hesketh JE (1996) Role of the 3′ untranslated region in the regulation of cytosolic glutathione peroxidase and phospholipid-hydroperoxide glutathione peroxidase gene expression by selenium supply. *Biochemical Journal* **320**, 891–895.

Bermano G, Nicol F, Dyer A, Sunde RA, Beckett GJ, Arthur JR & Hesketh JE (1995) Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochemical Journal* **311**, 425–430.

Bernlohr DA, Coe NR & Licata VJ (1999) Fatty acid trafficking in the adipocyte. *Seminars in Cell and Developmental Biology* **10**, 43–49.

Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD, Harney JW & Larsen PR (1991*a*) Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′ untranslated region. *Nature* **353**, 273–276.

Berry MJ, Banu L, Harney JW & Larsen PR (1993) Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. *EMBO Journal* **12**, 3315–3322.

Berry MJ, Banu L & Larsen PR (1991*b*) Type-I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* **349**, 438–440.

Berry MJ & Larsen PR (1993) Recognition of UGA as a seleno-cysteine codon in eukaryotes: a review of recent progress. *Biochemical Society Transactions* **21**, 827–832.

Berry MJ, Tujebajeva RM, Copeland PR, Xu XM, Carlson BA, Martin GW III, Low SC, Mansell JB, Grundner-Culemann E, Harney JW, Driscoll DM & Hatfield DF (2001) Selenocysteine incorporation directed from the 3′UTR: Characterization of eukaryotic EFsec and mechanistic implications. *BioFactors* **14**, 17–24.

Bremner I & Beattie JH (1990) Metallothioneins and the trace minerals. *Annual Reviews of Nutrition* **10**, 63–83.

Brigelius-Flohé R (1999) Tissue-specific functions of individual glutathione peroxidases. *Free Radical Biology and Medicine* **27**, 951–965.

Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG & Arthur JR (2000) Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphoctyes, granulocytes, platelets and erythrocytes. *Clinical Science* **98**, 593–599.

Chambers I & Harrison PR (1987) A new puzzle in selenoprotein biosynthesis – Selenocysteine seems to be encoded by the Stop codon, UGA. *Trends In Biochemical Sciences* **12**, 255–256.

Clark LC, Combs GF, Turnbull BW, Slate E, Alberts D, Abele D, Allison R, Bradshaw J, Chalker D, Chow J, Curtis D, Dalen J, Davis L, Deal R & Dellasega M (1997) The nutritional prevention of cancer with selenium 1983–1993: a randomized clinical trial. *Journal of the American Medical Association* **276**, 1957–1963.

Copeland PR, Fletcher JR, Carlson BA, Hatfield DL & Driscoll DM (2000) A novel RNA binding protein, SBP2, is required for the translation of mammalian selenoprotein mRNAs. *EMBO Journal* **19**, 306–314.

Dalgleish GD, Veyrune J-L, Blanchard J-M & Hesketh JE (2001) mRNA localization by a 145- nucleotide region of the c-fos 3′ untranslated region. *Journal of Biological Chemistry* **276**, 13593–13599.

Desvergne B & Wahli W (1999) Peroxisome-proliferator activated receptors: nuclear control of metabolism. *Endocrine Review* **20**, 649–688.

Diamond AM, Hu YJ & Mansur DB (2001) Glutathione peroxidase and viral replication: Implications for viral evolution and chemoprevention. *BioFactors* **14**, 205–210.

Fletcher JE, Copeland PR, Driscoll DM & Krol A (2001) The selen-ocysteine incorporation machinery: Interactions between the SECIS RNA and the SECIS-binding protein SBP2. *RNA* **7**, 1442–1453.

Gaub M-P, Lutz Y, Ghyselinck NB, Scheuer I, Pfister V, Chambon P & Rochette-Egly C (1998) Nuclear detection of cellular retinoic acid binding proteins I and II with new antibodies. *Journal of Histochemistry and Cytochemistry* **46**, 1103–1111.

Gustafson A-L, Donovan M, Annerwall E, Dencker L & Eriksson U (1996) Nuclear import of cellular retinoic acid-binding protein type I in mouse embryonic cells. *Mechanisms in Development* **58**, 27–38.

Heider J, Baron C & Bock A (1992) Coding from a distance – Dissection of the messenger – RNA determinants required for the incorporation of selenocysteine into protein. *EMBO Journal* **11**, 3759–3766.

Hentze MW & Kulozik AE (1999) A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* **96**, 307–310.

Hesketh JE, Campbell GP, Piechaczyk M & Blanchard J-M (1994) Targeting of c-myc and ß-globin coding sequences to cytoskeletal-bound polysomes by c-myc 3′ untranslated region. *Biochemical Journal* **298**, 143–148.

Hesketh JE, Vasconcelos MH & Bermano G (1998) Regulatory signals in messenger RNA: determinants of nutrient–gene interaction and metabolic compartmentation. *British Journal of Nutrition* **80**, 307–321.

Hill KE, Lloyd RS & Burk RF (1993) Conserved nucleotide sequences in the open reading frame and 3′ untranslated region of selenoprotein P mRNA. *Proceedings of the National Academy of Sciences USA* **90**, 537–541.

Hu YJ, Korotkov KV, Mehta R, Hatfield DL, Rotimi CN, Luke A, Prewitt TE, Cooper RS, Stock W, Vokes EE, Dolan ME, Gladyshev VN & Diamond AM (2001) Distribution and functional consequences of nucleotide polymorphisms in the 3′-untranslated region of the human Sep15 gene. *Cancer Research* **61**, 2307–2310.

Hubert N, Walczak R, Carbon P & Krol A (1996) A protein binds the selenocysteine insertion element in the 3′UTR of mammalian selenoprotein mRNAs. *Nucleic Acids Research* **24**, 464–469.

Kim IY & Stadtman TC (1995) Selenophosphate synthetase – Detection in extracts of rat-tissues by immunoblot assay and partial-purification of the enzyme from the archean *Methanococcus-Vannielii*. *Proceedings of the National Academy of Sciences USA* **92**, 7710–7713.

Kislauskis EH, Li Z, Taneja KL & Singer RH (1993) Isoform-specific 3′untranslated sequences sort α-cardiac and ß-cytoplasmic actin messenger RNAs to different cytoplasmic compartments. *Journal of Cell Biology* **123**, 165–172.

Kohrle J, Brigelius-Flohé R, Bock A, Gartner R, Meyer O & Flohé L (2000) Selenium in biology: Facts and medical perspectives. *Biological Chemistry* **381**, 849–864.

Koonin EV, Bork P & Sander C (1994) A novel RNA-binding motif in omnipotent suppressor of translation termination, ribosomal-proteins and a ribosome modification enzyme. *Nucleic Acids Research* **22**, 2166–2167.

Lei XG, Evenson JK, Thompson KM & Sunde RA (1995) Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. *Journal of Nutrition* **125**, 1438–1446.

Lesoon A, Metha A, Singh R, Chisolm GM & Driscoll DM (1997) An RNA-binding protein recognizes a mammalian seleno-cysteine insertion sequence element required for cotranslational incorporation of selenocysteine. *Molecular and Cellular Biology* **17**, 1977–1985.

Levadoux M, Mahon C, Beattie JH, Wallace HM & Hesketh JE (1999) Nuclear import of metallothionein requires its mRNA to be associated with the perinuclear cytoskeleton. *Journal of Biological Chemistry* **274**, 34961–34966.

Levadoux-Martin M & Hesketh JE (2002) Localisation of metallothionein in CHO cells requires a 41-nucleotide sequence within the 3′ untranslated region. *Biochemical Society Transactions* (In the Press).

Levadoux-Martin M, Hesketh JE, Beattie JH & Wallace HM (2001) Influence of metallothionein–1 localisation on its function. *Biochemical Journal* **355**, 473–479.

Low SC, Grundner-Culemann E, Harney JW & Berry MJ (2000) SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. *EMBO Journal* **19**, 6882–6890.

Low SC, Harney JW & Berry MJ (1995) Cloning and functional characterization of human selenophosphate synthetase, an essential component of selenoprotein synthesis. *Journal of Biological Chemistry* **270**, 21659–21664.

Maehira F, Luyo GA, Miyagi I, Oshiro M, Yamane N, Kuba M & Nakazato Y (2002) Alterations of serum selenium concentrations in the acute phase of pathological conditions. *Clinica Chimica Acta* **316**, 137–146.

Mahon P, Beattie JH, Glover LA & Hesketh JE (1995) Localisation of metallothionein isoform mRNAs in rat hepatoma (H4) cells. *FEBS Letters* **373**, 76–80.

Mahon PC, Partridge K, Beattie JM, Glover LA & Hesketh JE (1997) The 3′untranslated region plays a rôle in the targeting of metallothionein–1 mRNA to the perinuclear cytoplasm and cytoskeletal-bound polysomes. *Biochimica et Biophysica Acta* **1358**, 153–162.

Maquat LE (2001) Evidence that selenium deficiency results in the cytoplasmic decay of GPX1 mRNA dependent on pre-mRNA splicing proteins bound to the mRNA exon-exon junction. *Bio Factors* **14**, 37–42.

Ministry of Agriculture, Fisheries and Food (1997) *Dietary Intake of Selenium. Food Surveillance Information Sheet* No. 127. London: H.M. Stationery Office.

Molnar J, MacPherson A, Barclay I & Molnar P (1995) Selenium content of convenience and fast foods in Ayrshire, Scotland. *International Journal of Food Sciences and Nutrition* **46**, 343–352.

Nagy E & Maquat LE (1998) A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends In Biochemical Sciences* **23**, 198–199.

Noy N (2001) Retinoid-binding proteins: mediators of retinoid action. *Biochemical Journal* **348**, 481–495.

Pagmantidis V, Bermano G, Broom I, Arthur JR & Hesketh JE (2002) Selenoprotein expression in the rat colon during Se deficiency. *Proceedings of Nutrition Society* **61**, 51A.

Panemangalore M, Banerjee D, Onosaka S & Cherian MG (1983) Changes in the intracellular accumulation and distribution of metallothionein in rat liver and kidney during postnatal development. *Developmental Biology* **97**, 95–102.

Rayman MP (2000) The importance of selenium to human health. *Lancet* **356**, 233–241.

Ruff SJ & Ong DE (2000) Cellular retinoic acid binding protein is associated with mitochondria. *FEBS Letters* **487**, 282–286.

Salvatore D, Low SC, Berry MJ, Maia AL, Harney JW, Croteau W, St Germain DL & Larsen PR (1995) Type-3 iodothyronine deiodinase – Cloning, in-vitro expression, and functional-analysis of the placental selenoenzyme. *Journal of Clinical Investigation* **96**, 2421–2430.

Schmidt C & Beyersmann D (1999) Transient peaks in zinc and metallothionein levels during differentiation of 3T3-L1 cells. *Archives of Biochemistry and Biophysics* **364**, 91–98.

Schwarz K & Foltz CM (1957) Selenium as an integral part of factor 3 against necrotic dietary liver degeneration. *Journal of the American Chemical Society* **79**, 3292–3293.

Shen Q, Chu FF & Newburger PE (1993) Sequences in the 3′-untranslated region of the human cellular glutathione peroxidase gene are necessary and sufficient for selenocysteine incorporation at the UGA codon. *Journal of Biological Chemistry* **268**, 11463–11469.

Shen Q, McQuilkin PA & Newburger PE (1995) RNA-binding proteins that specifically recognize the selenocysteine insertion sequence of human cellular glutathione peroxidase mRNA. *Journal of Biological Chemistry* **270**, 30448–30452.

Shortt CT, Duthie GG, Robertson JD, Morrice PC, Nicol F & Arthur JR (1997) Selenium status in a group of scottish adults. *European Journal of Clinical Nutrition* **51**, 400–404.

Templeton DM, Banerjee D, & Cherian MG (1985) Metallo-thionein synthesis and localisation in relation to metal storage in rat liver during gestation. *Biochemistry and Cell Biology* **63**, 16–22.

Tsujikawa K, Imai T, Kakutani M, Kayamori Y, Mimura Y, Otaki N, Kimura M, Fukuyama R & Shimizu N (1991) Localization of metallothionein in nuclei of growing primary adult rat hepatocytes. *FEBS Letters* **283**, 239–242.

Tujebajeva RM, Copeland PR, Xu XM, Carlson BA, Harney JW, Driscoll DM, Hatfield DL & Berry MJ (2000) Decoding apparatus for eukaryotic selenocysteine insertion. *EMBO Reports* **1**, 158–163.

Venepally P, Reddy LG & Sani BC (1996) Analysis of the effects of CRABPI expression on the RA-induced transcription mediated by retinoid receptors. *Biochemistry* **35**, 9974–9982.

Veyrune J-L, Campbell GP, Wiseman J, Blanchard J-M & Hesketh JE (1996) A localisation signal in the 3′untranslated region of c-myc mRNA targets c-myc mRNA and ß-globin reporter sequences to the perinuclear cytoplasm and cytoskeletal-bound polysomes. *Journal of Cell Science* **109**, 1185–1194.

Villette S, Arthur JR, Bermano G & Hesketh JE (1998) Thyroid stimulating hormone and selenium supply interact to regulate selenoenzyme gene expression in thyroid cells (FRTL-5) in culture. *FEBS Letters* **438**, 81–84.

Villette S, Kyle JAM, Brown KM, Pickard K, Milne JS, Nicol F, Arthur JR & Hesketh JE (2002) A novel single nucleotide polymorphism in the 3′ untranslated region of human glutathione peroxidase 4 influences lipoxygenase metabolism. *Blood cells, Molecules and Disease* (In the Press).

Walczak R, Carbon P & Krol A (1998) An essential non-Watson-Crick base pair motif in the 3′UTR to mediate selenoprotein translation. *RNA* **4**, 74–84.

Walczak R, Westhof E, Carbon P & Krol A (1996) A novel RNA structural motif in the selenocysteine insertion element of eukaryotic selenoprotein mRNAs. *RNA* **2**, 367–369.

Weiss SL & Sunde RA (1998) Cis-acting elements are required for selenium regulation of glutathione peroxidase-1 mRNA levels. *RNA* **4**, 816–827.

Wingler K, Bocher M, Flohé L, Kollmus H & Brigelius-Flohé R (1999) mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. *European Journal of Biochemistry* **259**, 149–157.

Wingler K, Muller C & Brigelius-Flohé R (2000) Stability of gastrointestinal glutathione peroxidase mRNA in selenium deficiency depends on its 3′UTR. *Biofactors* **12**, 1–8.

Wolfrum C, Borrmann CM, Borchers T & Spener F (2001) Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α and γ-mediated gene expression via liver fatty acid binding protein: A signalling pathway to the nucleus. *Proceedings of National Academy of Sciences USA* **98**, 2323–2328.

Woo ES & Lazo JS (1997) Nucleocytoplasmic functionality of metallothionein. *Cancer Research* **57**, 4236–4241.

Wu R, Shen Q & Newburger PE (2000) Recognition and binding of the human selenocysteine insertion sequence by nucleolin. *Journal of Cellular Biochemistry* **77**, 507–516.

Yang G, Chen J, Wen Z, Ge K & Zhu L (1984) The role of selenium in Keshan disease. *Advances in Nutrition Research* **6**, 203–231.