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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.
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-
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 (Levadow 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 possibility 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 cytoplasmatic 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; Machira 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 ‘selenoproteins’. 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 (ID1) 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 selenocysteine insertion sequence (SECIS; Berry et al. 1991a), 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 of the mRNA and a related variant in bovine brain, the selenoprotein W in muscle and a 15 kDa selenoprotein.

In mammalian systems, two SECIS elements (Berry et al. 1993; Hill et al. 1993), 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 and collaborators (Lesoon et al. 1997) described a highly-specific 120 kDa protein (SBP2), the binding of which was highly correlated with selenoprotein 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 selenoproteins. 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-Flohe, 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 prioritisation 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).
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. 1991b; 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 selenocysteine-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...
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; Wingerter 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-dependent manner. It would appear, therefore, that there are indeed genetic variations in 3’UTR gene sequences, and we hypothesise that such genetic polymorphisms 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.

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