Review article

Regulatory signals in messenger RNA: determinants of nutrient–gene interaction and metabolic compartmentation

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Nutrition has marked influences on gene expression and an understanding of the interaction between nutrients and gene expression is important in order to provide a basis for determining the nutritional requirements on an individual basis. The effects of nutrition can be exerted at many stages between transcription of the genetic sequence and production of a functional protein. This review focuses on the role of post-transcriptional control, particularly mRNA stability, translation and localization, in the interactions of nutrients with gene expression. The effects of both macronutrients and micronutrients on regulation of gene expression by post-transcriptional mechanisms are presented and the post-transcriptional regulation of specific genes of nutritional relevance (glucose transporters, transferrin, selenoenzymes, metallothionein, lipoproteins) is described in detail. The function of the regulatory signals in the untranslated regions of the mRNA is highlighted in relation to control of mRNA stability, translation and localization and the importance of these mRNA regions to regulation by nutrients is illustrated by reference to specific examples. The localization of mRNA by signals in the untranslated regions and its function in the spatial organization of protein synthesis is described; the potential of such mechanisms to play a key part in nutrient channelling and metabolic compartmentation is discussed. It is concluded that nutrients can influence gene expression through control of the regulatory signals in these untranslated regions and that the post-transcriptional regulation of gene expression by these mechanisms may influence nutritional requirements. It is emphasized that in studies of nutritional control of gene expression it is important not to focus only on regulation through gene promoters but also to consider the possibility of post-transcriptional control.

Gene expression: Untranslated regions: Post-transcriptional regulation

Nutrition and gene expression

Although nutritional science has defined many of the necessary constituents of an adequate diet in terms of both macro- and micronutrients, the feeding of such a diet to two different individuals or to the same individual under different circumstances can give rise to different metabolic or even clinical effects. For example, on the same energy intake one individual becomes obese but another does not; equally, different individuals have varying requirements for micronutrients to maintain health. A major challenge to modern nutrition is to understand the basis for such differences in nutrient requirements and to relate this to the achievement of optimal health and performance. This requires an appreciation not only of the traditional links between nutrition and endocrine status but also of the less well-defined links between nutrition and gene expression. With the on-going expansion in molecular biology and genetics we are now beginning to be able to address the latter aspects. An ability to define the interactions between nutrition and gene expression will enable nutritionists to identify individuals with particular dietary needs.

In a broad sense this entails understanding how the role of nutrients in health and metabolism is linked to biochemical events which lead to gene expression. As shown schematically in Fig. 1, this can be reduced to two inter-linked

Abbreviations: apo, apolipoprotein; cGSH-Px, cytosolic glutathione peroxidase; GLUT, glucose transporter; G6PDH, glucose-6-phosphate dehydrogenase; GRP78, glucose-regulated protein 78; IDI, iodothyronine 5′ deiodinase; IRE, iron-regulatory elements; IRP, iron-regulatory proteins; MT, metallothionein; PHGSH-Px, phospholipid hydroperoxide glutathione peroxidase; poly(A), polyadenylate; UTR, untranslated regions.

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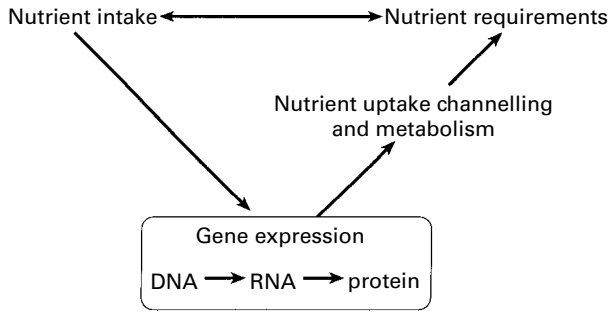

Fig. 1. Inter-relationships of nutrition and gene expression.

processes: the influence of nutrient intake on gene expression and protein synthesis, and the influence of gene expression on nutrient requirements. In specific terms it involves addressing important fundamental questions: which genes, particularly those involved in the control of metabolism, growth and differentiation are regulated by nutrition; how do nutrients and diet regulate the expression of specific genes; and how is the expression of specific gene products involved in metabolism and channelling of nutrients?

The genetic information in the DNA is transcribed into an RNA copy of the gene (the primary transcript) which is processed to produce a mature mRNA molecule. The mRNA is then transported from the nucleus to the cytoplasm where it is translated to produce a protein according to the amino acid sequence encoded in the mRNA and the gene. Potentially, the expression of any gene can be regulated at different steps between its transcription and synthesis of the final active protein product. There is increasing evidence that in addition to regulation of transcription itself, many genes are regulated post-transcriptionally during the processing, transport and translation of the mRNA. It is important to emphasize that nutrition may alter the amount of functional protein expressed by a specific gene through a range of transcriptional, post-transcriptional and post-translational mechanisms. However, this review focuses on post-transcriptional regulation, illustrates its relevance to nutrition and develops an integrated picture of how post-transcriptional regulation is involved in the interaction between nutrition and gene expression. To achieve this we briefly describe the principal features of post-transcriptional regulation and then describe the increasing number of genes whose post-transcriptional regulation has been shown to be affected by nutrients. The mechanisms of regulation by specific nutrients and their role in controlling protein synthesis, protein distribution and nutrient channelling within cells are highlighted. The approach is selective rather than comprehensive, and our overall aim is to highlight the possibilities of nutrient control of gene expression through post-transcriptional mechanisms, without diminishing the roles of other regulatory events.

The regulatory mechanisms and signals

Critical control points

The amount of protein produced from any gene can be regulated at many points between conversion of the gene sequence to the mRNA to the protein. The activity of the gene itself in producing RNA transcripts is regulated by the promoter region to which specific transcription factors can bind. Transcription produces a copy of the whole gene sequence, including both the exons (regions which code for protein) and introns (sections of non-coding DNA). Following gene transcription to produce a primary RNA transcript, the RNA is converted to a mature mRNA within the nucleus; this involves splicing and polyadenylation where introns are removed and adenine residues (polyadenylate (poly(A)) tails) added at the 3′ end of the mRNA. During processing certain mRNA, apparently only a few, can be edited. The mRNA is then transported from the nucleus to the cytoplasm. Theoretically, there are three possible fates for fully processed mRNA in the cytoplasm: they may be actively translated to produce protein, they may be sequestered in an untranslated form, or they may be degraded. Translation can be controlled either by regulating the availability of the mRNA for translation or regulating the initiation or elongation steps of the translation process. Certain mRNA are localized at specific subcellular sites. Post-transcriptional regulation of gene expression refers to the modulation of these processes (Wolfe, 1993) to determine how much, where and when specific proteins are produced (see Fig. 2).

In eukaryotes all mRNA contain not only coding sequences, which are translated to produce the appropriate protein, but also untranslated sequences. These untranslated sequences are present at both the 5′ and 3′ ends of the coding region and are referred to as the 5′ and 3′ untranslated regions, 5′UTR and 3′UTR respectively. By using recombinant DNA techniques to produce modified genes in which 5′ and 3′UTR are altered, exchanged or removed, in combination with transfection techniques to introduce the modified genes into cells in culture, it has been demonstrated that regulatory elements are present in the UTR from a number of mRNA. From such studies it has become evident that UTR have a central and critical role in controlling gene expression by regulating the polyadenylation, translation, stability and localization of mRNA. This regulation is achieved by the interaction of the UTR with specific proteins. There is relatively little information available on the precise nature of many of the 5′ and 3′UTR signals, but in those cases where the regulatory elements have been closely mapped they have been found to consist of relatively short UTR sequences. The available data are compatible with some form of secondary RNA structure such as a bulge or a stem-loop being critical for regulation, and these structures in the RNA appear to exert their regulatory functions by binding specific proteins (Kozak, 1992; McCarthy & Kollmus, 1995). 5′UTR tend to be relatively short in length and to date their functions have been found to be restricted to regulation of translation (Kozak, 1992). In contrast, 3′UTR can be up to hundreds or thousands of bases in length, can contain conserved regions and have been implicated in a wide range of regulatory events such as control of mRNA stability, translation, polyadenylation and localization. For example, the regulation of the synthesis of 3-hydroxy-3-methyglutaryl-CoA reductase (*EC* 1.1.1.88) is at the post-transcriptional level and studies with constructs in which its 3′UTR is linked to β-globin show that

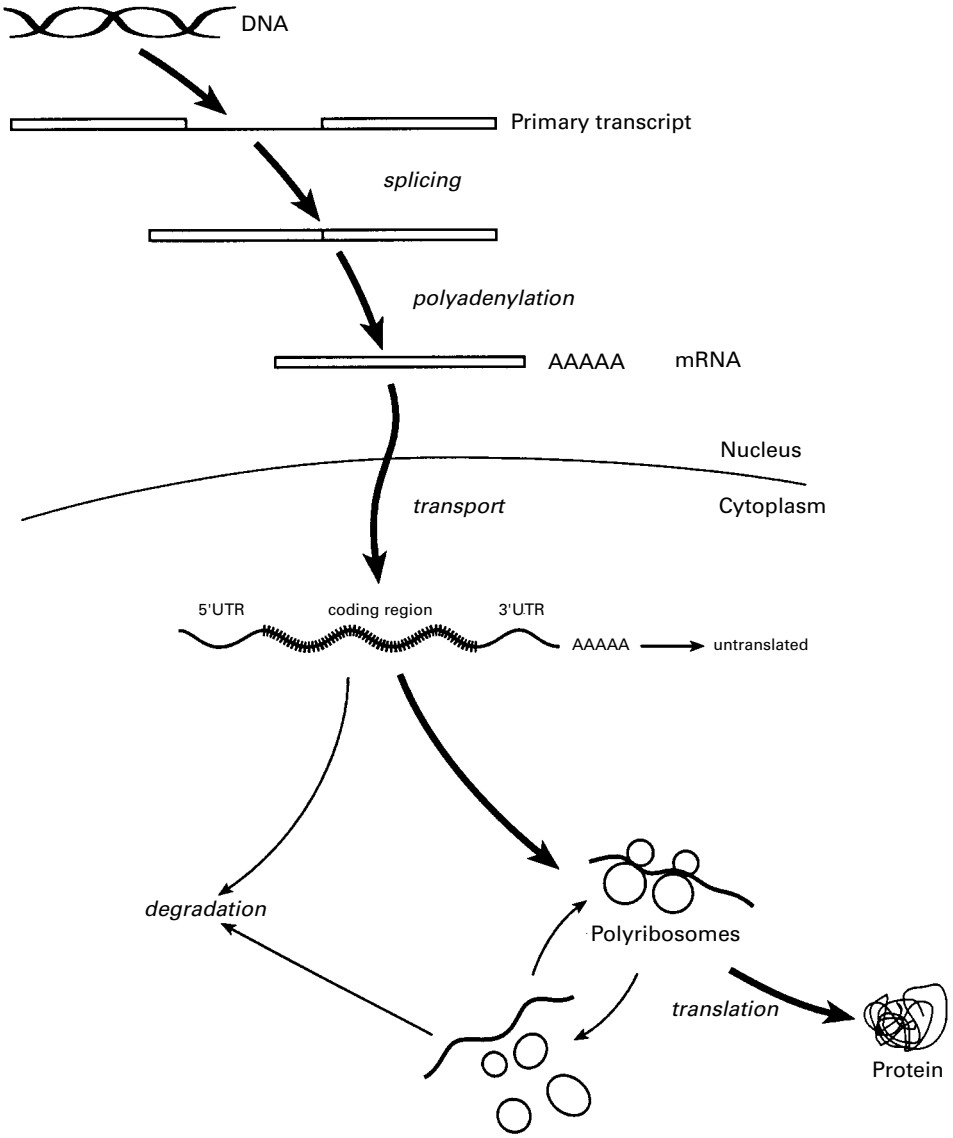

Fig. 2. Post-transcriptional control of gene expression. The diagram illustrates the critical stages and possible control points between transcription of the gene and synthesis of the corresponding protein. 5′UTR, 5′untranslated region; 3′UTR, 3′untranslated region.

regulation of synthesis by oxysterols depends on the 3′UTR (Choi & Peffley, 1995). As illustrated in Fig. 3, the picture emerging of post-transcriptional control is that translation, localization and stability of an mRNA are determined by the interactions between RNA structures in the 5′ and 3′UTR and proteins which bind to these structures.

Regulation of nuclear mRNA processing

A key step in RNA processing is the removal of intron sequences by splicing. In some cases this produces different mRNA from a single primary transcript, an event known as alternative splicing (Amara *et al*. 1982); this may generate mRNA with different coding sequences (and therefore producing subtly different proteins), inefficient or non-functional mRNA (Garrett *et al*. 1989) or UTR with different secondary structure and binding-sites for regulatory proteins (and therefore different responses to regulation). An example of the latter is the alternative splicing in the first three exons of the β-subunit of mouse thyrotropin gene which produces mRNA with different 5′UTR and different translational efficiencies (Januszeski & Gurr, 1991).

In a few cases the sequence of the primary transcript can be modified by a process called RNA editing. In mammals this event appears to be rare but it is more common in lower eukaryotes. Editing involves the binding of proteins or short RNA templates to specific regions of the primary transcript and subsequent alteration or ‘editing’ of the sequence, either insertion of one or more U nucleotides or base changes such as C to U or A to I (Scott, 1989). This mechanism allows production of different proteins from a single gene under different physiological conditions, as for example with apolipoprotein (apo) B (Baum *et al.* 1990).

Polyadenylation of mammalian mRNA at the 3′ end

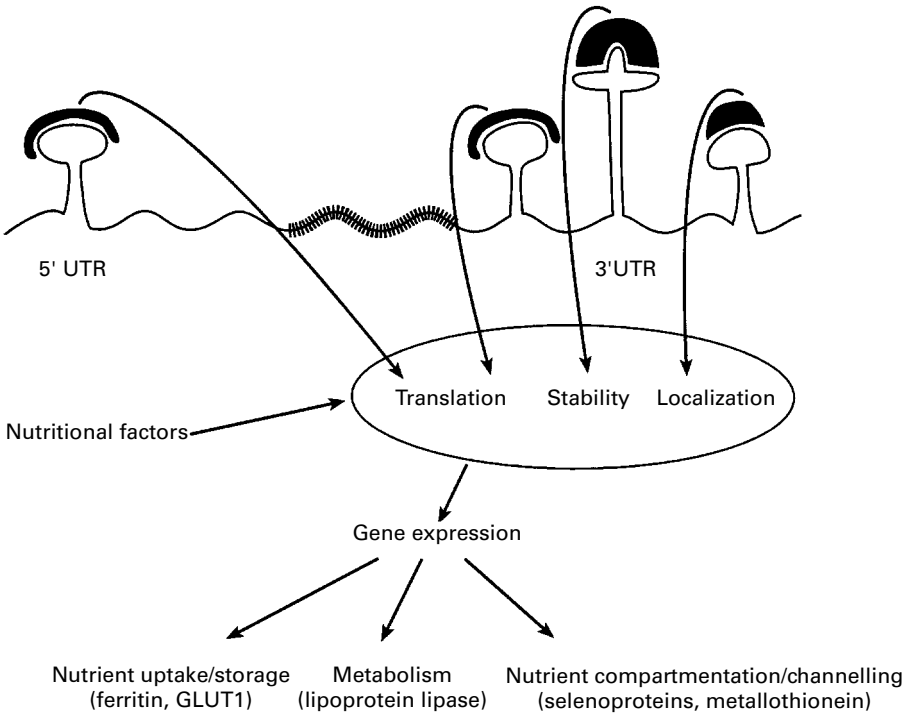

Fig. 3. Regulatory signals in the untranslated regions and their interactions with nutrition. The diagram illustrates schematically that untranslated regions (UTR) of mRNA contain regulatory signals which control the translation, stability and localization of mRNA, and thus gene expression. These regulatory signals in the mRNA bind specific proteins (■) and the protein–RNA interaction responds to nutritional factors to control the expression of gene products of nutritional significance, as shown schematically for certain specific examples. GLUT-1, glucose transporter-1.

occurs in the nucleus soon after transcription. Usually mRNA have 200–250 adenylate residues when transported into the cytoplasm and then these 'poly(A) tails' are usually shortened to 50–70 residues by cytoplasmic enzymes (Hershey, 1991). The polyadenylation status of many mRNA is important in determining the extent of both their translation and degradation (Jackson & Standart, 1990) in the cytoplasm. Once an mRNA has been spliced and polyadenylated it is transported to the cytoplasm. The mechanism of this RNA export from the nucleus is poorly understood (Izarrulde & Mattaj, 1995) but it is possible that regulation could also occur at this stage; indeed, recently it was suggested that synthesis of one of a family of cell-cycle regulators (cyclin D1) is regulated post-transcriptionally by the translation initiation factor eIF4E through its involvement in the transport of the cyclin D1 mRNA from the nucleus to the cytoplasm (Rosenwald *et al*. 1995).

mRNA translation

Regulation of the extent to which specific mRNA are translated is largely achieved by controlling the initiation of translation through formation of a complex between specific regulatory proteins, the mRNA and the small ribosomal subunit (Gray & Hentze, 1994; Morley, 1994). Several factors have been identified as particularly important in determining the extent to which an mRNA is translated. First, the structure of the 5′UTR, particularly specific stem-loops which bind proteins, is critical in determining whether an mRNA is translated or sequestered in an untranslated ribonucleoprotein complex (McCarthy & Kollmus, 1995). Some of these proteins are cell-specific and mRNA-specific, allowing precise post-transcriptional control over the synthesis of specific proteins (Richter, 1991; Hentze, 1995; McCarthy & Kollmus, 1995). Initiation of translation is dependent on the presence of the 5′ cap structure and the structure of the mRNA next to the initiation codon: indeed, secondary structures such as stem loops in the 5′ end of the mRNA seem to inhibit initiation of translation. Second, both the ability of the mRNA to compete for limited amounts of initiation factors and the phosphorylation of the elongation initiation factors eIF4E and eIF4G are important. Third, the poly(A) tails are also involved in the modulation of translation. Although they are not essential for translation to occur, mRNA lacking the poly(A) tail are less efficiently translated; for example, Xenopus β-globin mRNA without poly(A) tails formed smaller polyribosomes (Galili *et al*. 1988) indicating a lower translational efficiency. A protein which binds specifically to these poly(A) tails, the poly(A) binding protein, has a possible role in the formation of the translation initiation complex (Sachs & Davis, 1990). Some proteins, for example dihydrofolate reductase (*EC* 1.5.1.3) and thymidylate synthase (*EC* 2.1.1.45), are capable of regulating the translation of their own mRNA; thymidylate synthase regulates translation by binding to a part of the mRNA that includes the AUG codon (Chu *et al*. 1993).

mRNA stability

The amounts of some mRNA available for translation are regulated by controlling their stability and turnover, and thus their effective intracellular concentration. The regulation of mRNA stability is complex and can involve elements in both the coding and untranslated sequences, although in particular there is an increasing number of examples in which 3′UTR sequences are involved in determining stability of mRNA. The proto-oncogene *c-fos* codes for a highly unstable mRNA which has a half-life of approximately 30 min and its instability is due to sequences in the 3′UTR. Replacement of the endogenous 3′UTR in fos mRNA by that from *β*-globin, which has a half-life of approximately 24 h, produces a stable chimaeric transcript (Veyrune *et al*. 1995) whilst addition of the fos 3′UTR to *β*-globin confers instability. Within 3′UTR, certain AUUUA sequences, particularly multiple AUUUA sequences in close proximity or AU-rich regions, have been implicated in mRNA instability (Chen & Shyu, 1995). In contrast, isolated AUUUA sequences may have other regulatory functions, for example in translation and mRNA localization (Veyrune *et al*. 1996). Polyadenylation status also influences mRNA degradation; for example, *c-myc*, *c-fos* and metallothionein (MT) mRNA, deadenylation and degradation may be linked; when the poly(A) tail of MT mRNA is shortened to 30–40 residues the mRNA levels decrease (Mercer & Wake, 1985), indicating degradation. However, it is not known if poly(A) removal is a general mechanism of mRNA degradation (Surdej *et al*. 1994).

mRNA localization

Not only is the extent of translation of individual mRNA controlled but also the site of their translation in the cell. Secreted and membrane proteins are synthesized on the endoplasmic reticulum with the ribosome–mRNA complex being targeted there by a signal in the nascent polypeptide chain. In addition, it is now appreciated that there is a more complex spatial organization of the protein synthesis apparatus whereby mRNA for intracellular proteins are targeted to different parts of the cytoplasm (Hesketh, 1994, 1996*a*). Although mRNA localization was first observed in Drosophila embryos and Xenopus oocytes, it is now clear that the phenomenon occurs in somatic mammalian cells (Hesketh, 1996*a*); the perinuclear cytoplasm, peripheral cytoplasm, peri-mitochondrial cytoplasm and muscle costameres are amongst the various subcellular domains to which mRNA are directed. This targeting of mRNA coding for intracellular proteins is achieved by signals in the 3′UTR and it also involves the cytoskeleton (Hesketh, 1996*a*). Under growth conditions when actin synthesis is required in the cell periphery the 3′UTR from *β*-actin targets this transcript to the cell periphery (Kislauskis *et al.* 1993). In contrast, the 3′UTR of the c-myc mRNA is essential for its perinuclear localization and association with the cytoskeleton (Hesketh *et al*. 1994), and it has been suggested that this localization ensures efficient import of the protein into the nucleus (Hovland *et al*. 1995; Veyrune *et al*. 1996). In both these examples the localization signal has been at least partially defined and it was found that relatively short (40–90 nucleotide) sequences from these UTR are sufficient to produce localization (Kislauskis *et al*. 1993; Hesketh, 1996*a*). In Drosophila, localization has been demonstrated to provide a mechanism for translational control in which both localization and repression of translation are controlled by 3′UTR sequences (Gavis & Lehmann, 1994). The relationship between localization and translation in mammalian cells is less clear but correct localization appears not to be obligatory for translation. In general, the function of mRNA localization is to provide local synthesis of protein and thus contribute to the efficient delivery and compartmentation of specific proteins.

Nutrition and post-transcriptional control

Both macronutrients (carbohydrates, fatty acids, proteins and/or amino acids) and micronutrients (trace metals, Se, vitamins) participate, in concert with endocrine factors, in the regulation of gene expression in response to nutritional changes (Clarke & Abraham, 1992). There is increasing evidence that the response of gene expression to nutrients involves control of post-transcriptional events but it is often unclear whether the regulatory factors involved are the dietary components themselves, their metabolites or hormonal changes produced in response to the dietary changes.

Much of the evidence for post-transcriptional control comes from observed discrepancies between either mRNA abundance and transcription rates (altered mRNA abundance associated with unchanged gene transcription implies an altered mRNA stability) or in mRNA abundance and protein concentrations (altered protein concentration in the absence of any change in mRNA abundance implies either altered translation of the mRNA or changes in proteolytic breakdown of the protein). Many examples exist where alterations in nutritional status lead to such discrepancies. In the case of many mRNA–nutrient interactions, as for example in the post-transcriptional events playing a role in metabolic responses to dietary restriction and changes in macronutrient supply, this remains essentially the only evidence for post-transcriptional control. In some other cases, for example the glucose transporters and lipoprotein lipases, evidence for mechanisms and a role of the UTR is emerging whilst in a few instances, such as the incorporation of Se and the regulation of ferritin and transferrin receptor by intracellular Fe, details of the role of the UTR and associated binding proteins have been elucidated.

Starvation, macronutrients and energy restriction

In rats starvation decreases both the amount and activity of glycogen synthase (*EC* 2.4.1.11) but glycogen synthase mRNA abundance does not differ significantly between fed, starved and refed rats (Nur *et al*. 1995). In addition less glycogen synthase mRNA is associated with polyribosomes in starved rats than in fed rats, suggesting some form of translational control. There is evidence that this regulation involves an mRNA-binding protein but it is not clear with which part of the mRNA it associates or how it regulates translation (Nur *et al*. 1995).

Whereas starvation has an inhibitory effect on lipogenesis, a high-carbohydrate diet generally has a stimulatory

effect. Both dietary changes alter the activity of key lipogenic enzymes, in particular acetyl-CoA carboxylase (*EC* 6.4.1.2) and fatty acid synthase (*EC* 2.3.1.85) (Clarke *et al.* 1990; Katsurada *et al.* 1990*a*). Feeding a high-carbohydrate diet increases the acetyl-CoA carboxylase mRNA concentration, the rate of transcription and enzyme activity but the increase in acetyl-CoA carboxylase mRNA level is greater than that accounted for by the increase in transcription rate, suggesting that the high-carbohydrate diet both stimulates transcription of the acetyl-CoA carboxylase gene and stabilizes the mRNA (Katsurada *et al.* 1990*a*). Similarly, the dietary regulation of hepatic fatty acid synthase mRNA levels has been reported to occur at both transcriptional and post-transcriptional levels (Katsurada *et al.* 1990*b*). The mechanism by which feeding a high-carbohydrate diet leads to enhanced expression of these mRNA has not been identified. However, it has been proposed that products of glucose metabolism, possibly glucose-6-phosphate, stimulate the production of lipogenic enzymes (Foufelle *et al.* 1992).

Similarly, nutritional control of hepatic glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH) and malic enzyme (*EC* 1.1.1.40) activities involves regulation of mRNA stability. In rats fed on a high-carbohydrate diet after starvation, changes in G6PDH activity are accompanied by alterations in G6PDH mRNA abundance (Kletzien *et al.* 1985; Fritz *et al.* 1986) and measurement of rates of transcription and mRNA degradation (Prostko *et al.* 1989) indicated both a stimulation of transcription of the G6PDH gene and an increased stability of the mRNA. In the case of malic enzyme, mRNA levels are readily increased by dietary carbohydrate, but transcription rates, as determined *in vitro* using isolated nuclei (nuclear run-on assays) are unchanged; this suggests that changes in mRNA abundance are determined by mRNA stability and not gene transcription (Dozin *et al.* 1986).

Dietary energy restriction also affects the expression of various genes, in particular that encoding the glucose-regulated protein 78 (GRP78) (Tillman *et al.* 1996), a protein located in the endoplasmic reticulum which is essential for proper folding, assembly and glycosylation of proteins (Little *et al.* 1994). In mice fed on an energy-restricted diet, a reduction in GRP78 protein levels was observed to be closely paralleled by a reduction in hepatic GRP78 mRNA levels (Spindler *et al.* 1990), indicating that energy restriction does not influence the rate of translation or the stability of GRP78 protein. However, measurement of transcription rates *in vitro* using isolated nuclei failed to show any effect of energy restriction on the rate of transcription, suggesting that dietary restriction regulates synthesis of GRP78 protein by destabilization of its mRNA.

Restriction of dietary protein or amino acid intake reduces the rate of growth of young animals. There is some evidence that this is accompanied by post-transcriptional changes in the expression of certain growth-related genes such as c-myc (Yokota *et al.* 1995*a*). Furthermore, a similar effect has also been observed *in vitro*: in media deprived of amino acids cells show increased abundance of c-myc mRNA which is due to an approximately 6-fold increase in mRNA stability, as shown by the prolonged half-life (Yokota *et al.* 1995*b*). It was suggested that this was due to the suppression of the synthesis of a short-lived cytosolic protein which is involved in the degradation of the mRNA. The expression of genes associated with growth arrest has also been reported to be regulated in part by post-transcriptional mechanisms (Coccia *et al.* 1992; Fleming *et al.* 1998).

A protein-restricted diet also increases the transcription of the insulin-like growth factor binding protein-1 gene (Straus *et al.* 1993). However, because the observed increase in the primary transcripts was less than that of the mature insulin-like growth factor binding protein-1 mRNA, it is probable that there was also a stabilization of the mRNA when protein synthesis was reduced.

Lipoproteins: RNA editing, stability and translation

The two apolipoproteins apoB-100 and apoB-48 are both encoded by the same gene but apoB-48 is formed as a result of a post-transcriptional modification of the apoB-100 transcript (Scott, 1989; Baum *et al.* 1990) in which RNA editing converts the cytosine at position 2153 to uracil and so forms a premature stop codon (UAA). This editing produces two dramatically different proteins from the one gene, the apoB-48 produced from the truncated transcript being of much smaller molecular mass (34 kDa) than the apoB-100 produced from the full transcript; the larger apoB-100 contains the LDL-receptor binding site. The degree of editing appears to differ between tissues: in the intestine apoB-48 is produced and incorporated into chylomicrons for absorption of dietary lipids; in man the liver produces only the larger apoB-100 form (an important component of VLDL and LDL complexes) but in the rat the liver synthesizes both apoB-48 and apoB-100 proteins. Although high-sucrose diets have no effect on total hepatic apoB secretion (Strobl *et al.* 1989), the ratio apoB-100 : apoB-48 proteins is markedly altered (Baum *et al.* 1990). It has been suggested that the effect of carbohydrate feeding on apoB-100 : apoB-48 is due to regulation of the editing of the apoB RNA transcripts by dietary carbohydrate. Fasting has itself also been reported to reduce apoB RNA editing (Leighton *et al.* 1990). These data indicate that diet influences apoB-48 and/or apoB-100 synthesis by modulation of mRNA processing, specifically mRNA editing.

Diets rich in lipid, particularly saturated fatty acids and cholesterol, also influence lipoprotein metabolism; they increase LDL and HDL particles in plasma (Goldberg & Schonfeld, 1985; Sorci-Thomas *et al.* 1989; Srivastava *et al.* 1991) and increased levels of plasma LDL have been identified as a risk factor for CHD (Gofman *et al.* 1966). Thus, the influence of diet on lipoprotein metabolism is of major interest. Turnover of components of both LDL and HDL has been shown to involve post-transcriptional mechanisms. *In vivo*, oxidized LDL is taken up by scavenger receptors in macrophages. The expression of the scavenger receptor gene is inhibited by tumour necrosis factor-α and this regulation occurs through a decrease in mRNA stability (Hsu *et al.* 1996).

Since the synthesis and secretion of plasma lipoproteins are often correlated with the production of their corresponding apolipoproteins (Srivastava *et al.* 1992), the expression of

apoAI, the major protein component of HDL, has received considerable attention. The synthesis and secretion of apoAI is regulated by transcriptional, post-transcriptional and post-translational control mechanisms. In rats fed on diets of high lipid content plasma concentrations of apoAI and HDL were elevated but the level of apoAI mRNA was unaltered (Srivastava, 1994) and there was an increase of 20 % in the translation of apoAI mRNA as judged by the amounts of apoAI mRNA in isolated polysomes. These data suggest that there is some translational control of apoAI synthesis but since this was insufficient to account for the 50–60 % increase in plasma apoAI, additional mechanisms, such as reduced protein degradation, may also be important.

Glucose transporters and lipoprotein lipase: evidence for a role of the untranslated regions

Both glucose transporters and lipoprotein lipase play key roles in the metabolic response to nutrient intake. Not only is the regulation of the amounts of these proteins partly controlled by post-transcriptional mechanisms, but it is emerging that the regulatory elements are present within the UTR of the respective mRNA. Expression of glucose transporter 1 (GLUT1) is regulated at a post-transcriptional level under different pathophysiological conditions (Boado, 1995); for example, glucose deprivation increased GLUT1 mRNA stability without changes in gene transcription (Boado & Pardridge, 1993). Recent studies suggest that regulatory elements occur in both the 5′ and 3′UTR with those in the 5′UTR interacting with certain proteins in a sequence-specific manner to control translational efficiency (Boado *et al*. 1996) and those in the 3′UTR binding specific cytosolic proteins and mediating post-transcriptional regulation of GLUT1 gene expression (Dwyer *et al.* 1996). The fact that the sequence of the GLUT1 mRNA is highly conserved among five different mammalian species, particularly in the 5′ and 3′UTR (Boado & Pardridge, 1990) confirms the importance of regulatory elements in these regions. A report suggesting post-transcriptional control of GLUT4 expression (Munoz *et al*. 1996) leads one to speculate that such mechanisms may occur throughout the GLUT family of proteins.

Lipoprotein lipase mRNA exists in two forms, one of 3·2 kilobases (kb) and one of 3·6 kb; the difference lies in the 3′UTR with the two forms using different polyadenylation signals and the 3′UTR being involved in tissue-specific expression (Ranganathan *et al*. 1995). In adipose tissue both transcripts are expressed, but in muscle and heart only the longer transcript is present. Both transfection and *in vitro* translation studies indicate that the longer transcript is translated more efficiently resulting in greater synthesis of the protein. Furthermore, regulation of lipoprotein lipase expression by noradrenaline and thyroid hormone in adipocytes also depends on 3′UTR regulatory elements which control mRNA translation (Kern *et al*. 1996; Ranganathan *et al*. 1997); this involves a cytoplasmic repressor protein which binds to the 3′UTR. In conclusion, the 3′UTR of lipoprotein lipase both controls tissue-specific differences in expression and allows response to regulatory factors.

Iron and the iron-regulatory element: details of the untranslated region-dependent mechanisms

The expression of both transferrin receptor and ferritin is controlled by post-transcriptional regulation of their synthesis, and there is now very strong evidence showing clear roles for 3′UTR and 5′UTR regulatory elements.

Transferrin and its receptor are required for the uptake of Fe into cells. Transferrin receptor mRNA has five regulatory sequences, named Fe-regulatory elements (IRE) in the 3′UTR. These sequences are predicted to fold into a stem loop structure (Hershey, 1991) and this structure is required for regulation (Müllner & Kuhn, 1988). In the absence of Fe, *trans*-acting repressor proteins, the Fe-regulatory proteins (IRP), bind to these sequences protecting the mRNA from degradation by ribonucleases (Koeller *et al*. 1989). In the presence of Fe, these repressor proteins are removed from the transferrin receptor mRNA leading to mRNA destabilization, decreased translation of the mRNA, decreased transferrin receptor synthesis and therefore decreased Fe uptake (Madani & Linder, 1992; Theil, 1994) (Fig. 4). During liver cell proliferation *in vivo* following partial hepatectomy, this mechanism of post-transcriptional control is capable of fully accounting for the transferrin receptor mRNA induction (Cairo & Pietrangelo, 1994).

The expression of ferritin, required for storage and detoxification of Fe, is also controlled post-transcriptionally (Cairo *et al*. 1985) by IRE and IRP (Leibold & Munro, 1988). In contrast to the transferrin receptor mRNA, where the IRE are in the 3′UTR and act by regulating mRNA stability, ferritin mRNA has an IRE in the 5′UTR which regulates translation. When the cellular Fe levels are low the IRP binds to the IRE in the 5′UTR and represses translation of the mRNA (Fig. 4). In the presence of Fe IRP is removed from the mRNA and ferritin translation is increased to allow storage of Fe (Madani & Linder, 1992). Additionally, when Fe is abundant or the IRP is absent, the ferritin IRE region enhances the binding of initiation factors to the mRNA (Dix *et al*. 1992). The contrasting effects of Fe on translation of ferritin mRNA and degradation of transferrin receptor mRNA appears to be related to the location of the IRE in different UTR: 5′UTR in the case of ferritin and 3′UTR in the case of transferrin receptor (Theil, 1990).

The presence of similar IRE in both transferrin receptor and ferritin mRNA is important because it allows co-ordinated regulation of synthesis of the two proteins by Fe concentration. This was the first example of concerted regulation of eukaryotic mRNA (Klausner & Harford, 1989; Theil, 1990) and illustrates how post-transcriptional control can produce an integrated regulation of different proteins in response to change in intracellular concentrations of a nutrient. There are several known IRE sequences in various species but they are not all the same for ferritin and transferrin receptor, neither in primary nor secondary structure. Nevertheless, they all enable a co-ordinated regulation of Fe uptake, storage and detoxification (Theil, 1994). IRE have been identified in other RNA, such as erythroid 5-aminolevulinate synthase and mitochondrial aconitase, suggesting that Fe may regulate the expression of a range of genes by post-transcriptional mechanisms (Klausner *et al*. 1993).

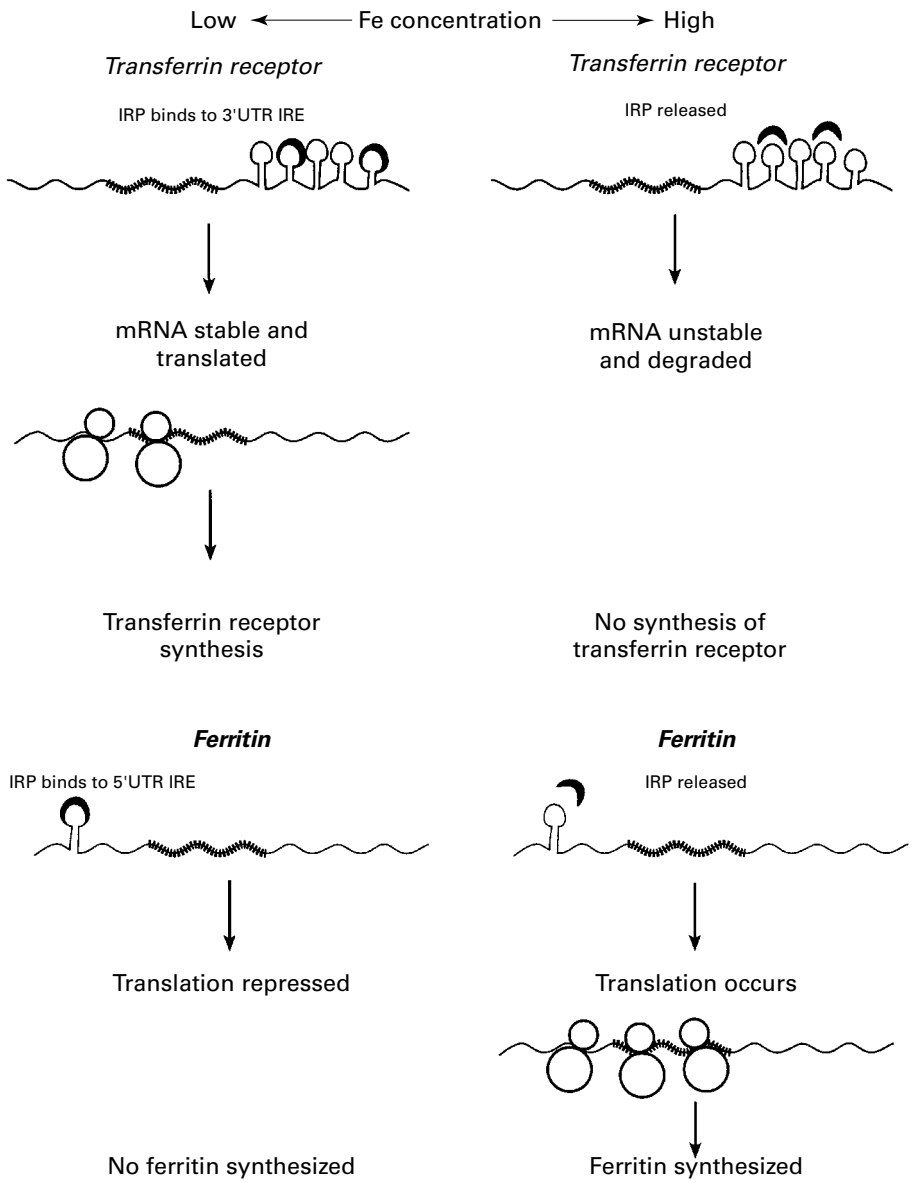

Fig. 4. Regulation of transferrin receptor and ferritin by iron. The diagram illustrates the mechanism by which iron concentrations, through specific iron-regulatory proteins (IRP) and iron-regulatory elements (IRE) in the mRNA, control transferrin receptor and ferritin synthesis. 5′UTR, 5′untranslated region; 3′UTR, 3′untranslated region.

The IRP are conserved in both structural and functional properties between species (Rothenberg *et al*. 1990) and exert different effects in different mRNA by the different context of the IRE in the mRNA. In the cases so far examined Fe appears to exert its effects by changing the activity, rather than the amount, of IRP (Hentze *et al*. 1989; Müllner *et al*. 1992). One IRP has now been identified as cytoplasmic aconitase (IRP-1) on the basis of amino acid sequence and enzyme activity (Kennedy *et al*. 1992; Beinert & Kennedy, 1993), but a second IRP seems distinct in that it has no aconitase activity (Henderson *et al*. 1993; Guo *et al*. 1994). Alterations in the Fe-S cluster structure within IRP-1 changed RNA-binding activity (Haile *et al*. 1992) suggesting that this structure acts as a 'regulatory switch' which alters the binding properties of the protein in response to Fe availability.

NO can mimic the effects of Fe on IRP-1; it activates binding to the IRE, represses ferritin translation and stabilizes transferrin receptor mRNA (Pantopoulos & Hentze, 1995; Domachowske *et al*. 1996). Thus it appears that IRP-1 responds not only to Fe but also to NO; therefore in inflammation the generation of NO in response to cytokines may change cell Fe metabolism by acting at the Fe-S cluster in the IRP-1 protein (Klausner *et al*. 1993; Mascotti *et al*. 1995). It has been suggested that other agents, such as superoxide radicals or molecular O_2, may also act on this IRP-1 'switch'.

Selenoproteins: role of 3′untranslated regions in incorporation and channelling of selenium

The essential micronutrient Se is incorporated into selenoproteins (e.g. glutathione peroxidases and iodothyronine

5′deiodinases) as selenocysteine. The incorporation of Se occurs during mRNA translation and specific UGA codons are recognized not as stop signals but for insertion of selenocysteine from selenocysteine-tRNA; this requires specific 3′UTR sequences which form stem-loop structures (Berry *et al.* 1991, 1993; Shen *et al.* 1993). Feeding diets of different Se content modifies the activity and amounts of selenoproteins (Burk & Hill, 1993), and more recent studies have shown that dietary Se supply regulates both the activity of the selenoproteins cytosolic glutathione peroxidase (*EC* 1.11.1.9; cGSH-Px), phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px) and type 1 iodothyronine 5′ deiodinase (*EC* 3.8.1.4; IDI) and the abundance of their respective mRNA to different extents (Bermano *et al.* 1995). There is individual regulation of cGSH-Px, PHGSH-PX and IDI 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.

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 cGSH-Px, PHGSH-PX or IDI 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. In hepatoma cells in culture, Se depletion had no effect on the rate of degradation of the PHGSH-Px mRNA, but decreased that of the cGSH-Px mRNA (Bermano *et al*. 1996*a*). This observation suggests that differences in the effect of Se deficiency on stability of the two mRNA can account for the effects of Se deficiency on cGSH-Px and PHGSH-Px in liver. Thus, under conditions of reduced Se availability there is termination of translation (Chambers *et al.* 1986; Berry *et al.* 1991), which leads to destabilization of the mRNA (Bermano *et al*. 1996*a*); the differential effects on cGSH-Px and PHGSH-Px mRNA produce a channelling of Se to allow preferential synthesis of a particular selenoprotein.

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 (see Fig. 5). 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 activity of the selenoproteins type I and type III deiodinases are altered by exchanging their native 3′UTR for those of other selenoproteins (Berry *et al.* 1991; 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 coding region linked to PHGSH-Px 3′UTR is affected less by low Se conditions than translation of IDI linked to the glutathione peroxidase 3′UTR (Bermano *et al*. 1996*b*). This suggests that when Se supply is limiting the 3′UTR of cGSH-Px is less efficient at maintaining translation than that of PHGSH-Px and this probably reflects different abilities to form a complex with selenocysteine-tRNA and appropriate protein factors. In turn, this could involve subtle differences in stem-loop structures and different strengths of complex formation when either selenocysteine tRNA or an mRNA-binding protein (elongation factor) is limiting so that the available selenocysteine is utilized for preferential synthesis of a particular selenoprotein. Recently an mRNA-binding protein that binds to the stem-loop structure of cGSH-Px mRNA has been isolated (Shen *et al.* 1995) but it is not known if the synthesis of this protein, or the affinity for the stem-loop, changes in Se-deficient conditions. Whatever the detailed mechanism, it is clear that the 3′UTR-based Se-insertion mechanism responds to Se supply and channels the available Se for synthesis of particular selenoproteins.

Metallothionein: translational control and mRNA localization

MT exists in several isoforms (MT-1, MT-2 etc.) which bind metals such as Zn, Cd and Cu. The precise function(s) of the MT isoforms remains unknown, although they are thought to transport metals and maintain metal homeostasis in the cell, as well as possibly having an antioxidant function in protecting DNA from damage (Bremner & Beattie, 1990). Following administration of metals there is an induction of MT isoforms in liver and kidney. This involves increased gene transcription but there is also evidence for post-transcriptional control of MT synthesis. For example, during development MT protein abundance does not parallel mRNA abundance (Andersen *et al*. 1983). More recently it has been shown that, after metal administration, there is induction of both MT-1 and MT-2 mRNA in rat liver but only the MT-1 isoform shows an increased amount of protein, indicating that there is differential control of translation of the two isoforms (Vasconcelos *et al*. 1996). Similarly, MT-2, but not MT-1, is post-transcriptionally regulated in fish (Scudiero *et al*. 1997). The reason for this differential regulation of the two isoforms is not known but it is possible that the two isoforms have different functions and/or different subcellular compartmentation.

Recent data suggest that indeed there may be some compartmentation of the isoforms and that, furthermore, this involves localization of the mRNA. In rat hepatoma cells the mRNA for MT-1 has a perinuclear location and is associated with the cytoskeleton, whereas the mRNA for MT-2 shows less association (Mahon *et al.* 1995). Thus, it appears that the mRNA for these two isoforms are compartmentalized, suggesting that the proteins are synthesized in different subcellular sites. Expression of hybrid gene constructs in transfected cells has shown that localization of MT-1 mRNA is lost on exchange of the 3′UTR for that of cGSH-Px, and the 3′UTR of MT-1 mRNA itself is able to target β-globin transcripts to the perinuclear cytoplasm and the cytoskeleton (Mahon *et al*. 1997). Therefore the 3′UTR of MT-1 mRNA determines the site of MT-1 synthesis. The role of this targeting is still unclear, but it may be important in compartmentation of MT within the cell. Under certain circumstances, such as during cell proliferation, MT is found in the nucleus (Tohyama *et al*. 1993). The mRNA for several nuclear proteins are associated with the cytoskeleton or localized in the perinuclear cytoplasm (Hesketh, 1996*a*), and so it is probable that the targeting of MT-1 mRNA to the cytoskeleton in the perinuclear cytoplasm is related to the compartmentation of the MT-1 protein.

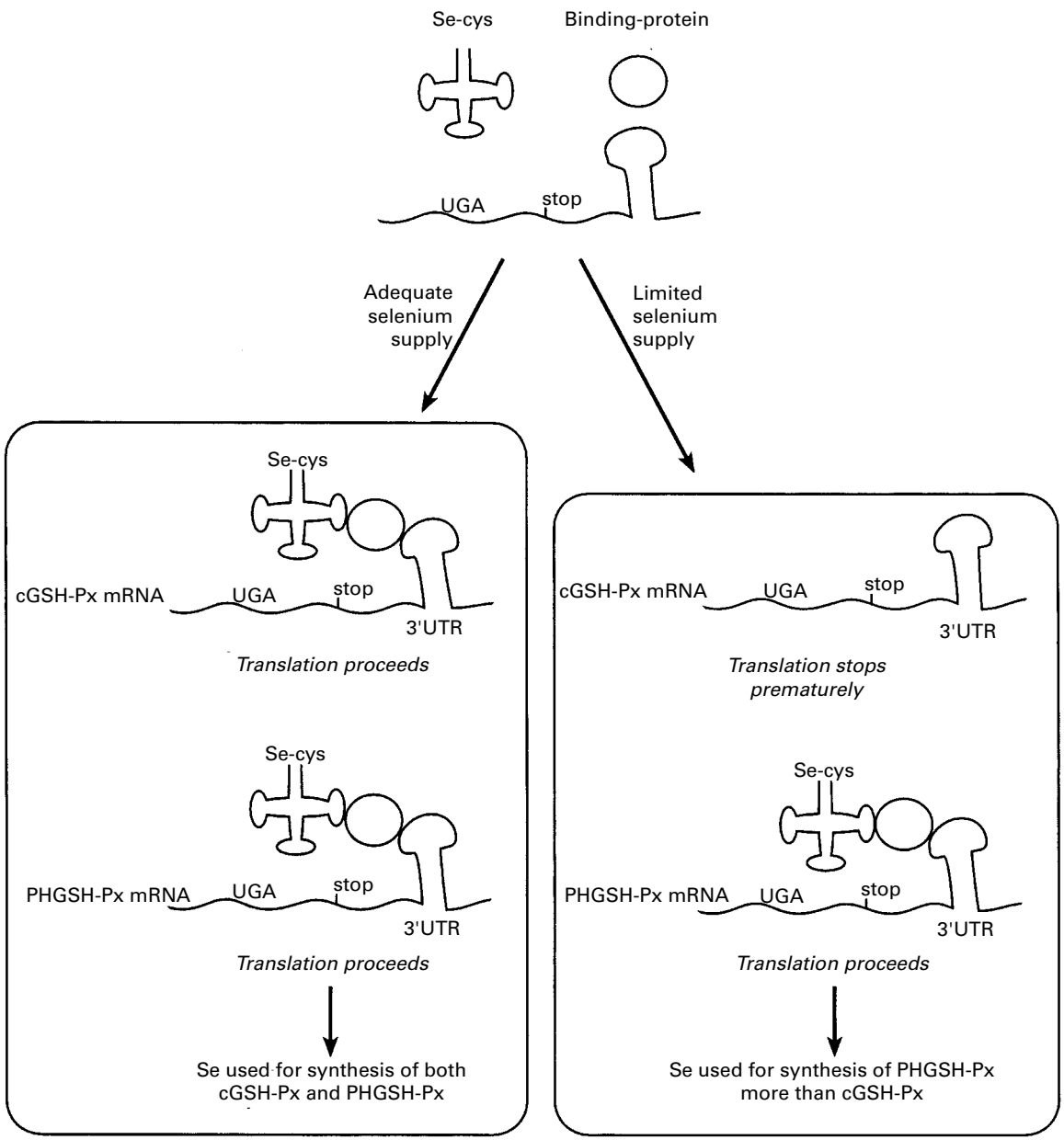

Fig. 5. Channelling of selenium for preferential synthesis of a particular selenoprotein. The diagram illustrates schematically how the post-transcriptional mechanism for incorporation of selenium into selenoproteins responds to altered selenium supply by allowing preferential translation of phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px) mRNA rather than cytosolic glutathione peroxidase (cGSH-Px) mRNA. This results in preferential synthesis of one selenoprotein rather than the other. 3′UTR, 3′untranslated region; Se-cys, selenocysteine.

3′Untranslated region signals, mRNA localization and metabolic compartmentation

The localization and targeting of MT-1 mRNA illustrates how, in a nutritional context, mRNA targeting by the 3′UTR may provide a mechanism for the compartmentation of proteins which bind or metabolize nutrients. Nutrients must not only be delivered to the cell but also to the correct compartment within the cell, whether this be a cytoplasmic domain or an organelle: post-transcriptional control of the site of synthesis of specific proteins which influence nutrient metabolism, compartmentation and function may provide a basis of such nutrient channelling. As discussed earlier, targeting of MT isoform mRNA may allow compartmentation of the protein isoforms, as with actin and creatine kinase isoforms (Kislauskis *et al.* 1993; Wilson *et al.* 1995) and possibly targeting of the protein to the nucleus.

The expression of retargeted mRNA in transfected cells does not prevent translation of the mRNA to synthesize the proteins. Thus, the modification of targeting signals to alter the site of synthesis of proteins provides opportunities to study the effects of mRNA relocalization on protein distribution. The production of such stably transfected cell lines will allow studies of the roles of specific proteins in

Table 1. Nutrition, post-transcriptional control and regulatory elements in untranslated regions (UTR)

Gene	Nutritional factor	Control point	Regulatory element
Ferritin	Fe	Translation	5′UTR
Transferrin receptor	Fe	Stability	3′UTR
Cytoplasmic glutathione peroxidase	Se	Translation	3′UTR
Phospholipid hydroperoxide glutathione peroxidase	Se	Translation	3′UTR
Metallothionein-1	–	Localization	3′UTR
Metallothionein-2	Zn	Translation(?)	?
Glucose transporter-1	–	Translation	5′UTR
Lipoprotein lipase	?	Translation	3′UTR
c-myc	Amino acid supply	Stability	?
c-myc	–	Localization	3′UTR
Argininosuccinate lyase	–	Localization	?
Creatine kinase	–	Localization	3′UTR
Retinol-binding protein	Vitamin A	Stability	?
Apolipoprotein CIII	Hyperlipidaemia	?	3′UTR

nutrient compartmentation; for example, the function of MT in the nucleus and whether the site of synthesis of the retinoic acid binding protein CRABP-1, which has been found in the nucleus and has been suggested to transport retinoic acid (Gustafason *et al*. 1996), is critical for its function. Such mechanisms may provide novel interactions between nutrition and gene expression. For example, differences in a fundamental mechanism of nutrient compartmentation could lead to altered nutritional requirements.

The spatial organization of protein synthesis within the cell and local synthesis of certain proteins has major implications for the study of protein synthesis in all tissues. For example, it is relevant to muscle because of its possible involvement in myofibrillar protein turnover (Hesketh, 1996*b*) and it concerns tissues with obvious polarity such as epithelia and secretory tissues such as the mammary gland where there is nutrient-driven regulation of export proteins. Compartmentation may influence the interpretation of protein synthetic measurements and indeed some kinetic data are compatible with certain proteins being synthesized from different amino acid pools (Connell *et al*. 1997). mRNA localization also has the potential to contribute to the compartmentation of enzymes and metabolic pathways. This has been elegantly demonstrated in the case of urea cycle enzymes such as argininosuccinate lyase (Cohen, 1995, 1996); both the enzymes and their mRNA are localized in the cytoplasm surrounding the mitochondria. This compartmentation allows reutilization of the ‘catalytic’ ornithine so that the cycle is not dependent on changes in cellular concentrations and thus insensitive to nutrient supply.

Conclusions and perspectives: untranslated regions as sites of nutritional regulation

It is now clear that post-transcriptional control is important in determining the synthesis of specific proteins. This is also exemplified in a number of nutrient-related systems, either by regulation of synthesis of specific proteins by nutrients or, alternatively, by the regulation of proteins which have important roles in nutrient metabolism. This reveals that the interactions between nutrition and gene expression are not restricted to the study of how nutrition interacts with the genome and gene transcription but also how nutrition affects mRNA.

This has two implications for studies of gene expression–nutrition interaction. First, the possibility that post-transcriptional interactions occur should always be considered. Second, since nutrition can regulate mRNA translation and stability, mRNA abundances may not reflect amounts of protein or rates of synthesis; therefore, observed changes in the abundance of a certain mRNA may not be followed by changes in amounts of the corresponding protein, or a lack of alteration in mRNA abundance may not mean there is no change in its protein. Thus, in order to relate, confidently, nutritional status to gene expression mRNA measurements should be accompanied by measurements of the protein product.

In certain specific cases, for example Fe and Se, post-transcriptional regulation plays a central role. However, in general, more information is required to assess the relative importance of post-transcriptional control relative to transcriptional and post-translational control in nutrient–gene interactions. Our view is that the role of different control mechanisms will vary from gene to gene. Post-transcriptional control of mRNA stability, translation and localization results in the regulation of expression of those genes becoming, at least partly, a cytoplasmic event. Regulation of gene expression in the cytoplasm offers certain advantages to the cell or organism. First, such control could respond directly and rapidly to cytoplasmic metabolite or nutrient concentrations rather than indirectly through nuclear transcription factors. Second, it allows regulatory mechanisms to use proteins with dual functions as both cytoplasmic enzymes and mRNA-binding proteins. Third, it allows development of mechanisms to localize mRNA and their translation, and thus regulate the sites of protein synthesis and delivery of newly-synthesized proteins; this has the potential to provide mechanisms to channel nutrients.

In those cases where details are known about the mechanism of nutritional effects on post-transcriptional control, the regulatory sequences involved have usually been found in the 5′ or 3′UTR (Table 1). This reflects the growing realization that UTR-based regulatory elements and RNA-binding proteins play a major role in post-transcriptional

control (Fig. 3, Table 1). In particular, it is now evident that 3′UTR are not lengths of redundant nucleotides but, on the contrary, are highly important parts of mRNA which contain a variety of regulatory elements that play a crucial role in determining the fate of individual mRNA within the cytoplasm. The number of examples in the literature where gene expression is controlled via such 3′UTR elements is increasing rapidly and it should be considered that this type of control exerts a major influence over the expression of many genes.

Furthermore, these UTR-based control mechanisms are of considerable relevance to nutrition: in several cases nutritional factors regulate gene expression through UTR elements and in other cases nutritionally-relevant proteins are regulated in this way. For example, the spatial organization of protein synthesis, the channelling of Se for preferential synthesis of one selenoprotein rather than another, and the regulation of the synthesis of the transferrin receptor and ferritin by the cellular Fe concentration, all involve UTR-based mechanisms. The regulation of other nutritionally-related mRNA (e.g. lipoprotein lipase and MT-1 mRNA) by 3′UTR elements, the considerable length of 3′UTR sequences in general, their conserved regions and the identification of regulatory elements within these regions shows that further studies on 3′UTR and RNA-binding proteins have the potential to produce many new insights into the interaction between nutrition and gene expression.

At present, nutritional regulation of post-transcriptional events is primarily a subject of fundamental research but in the long term it has the potential for considerable clinical relevance, as shown by the observation that the polymorphisms found in the 3′UTR of the apoCIII mRNA influence resistance and susceptibility to hyperlipidaemia (Dammerman *et al*. 1993). This illustrates the potential of future research to define the extent to which nutrition influences gene expression through post-transcriptional control and how individual variation in these regulatory mechanisms may alter nutritional requirements. These questions are currently being addressed using a combination of recombinant DNA technology and transfection into cells in culture, but the increasing availability of transgenic animals (Hall, 1997) should allow us to build on these approaches to define the interactions between UTR and nutrition.

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