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Conference on 'Over- and undernutrition: challenges and approaches'

Plenary Lecture 2
Transcription factors, regulatory elements and nutrient–gene communication

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Dramatic advances have been made in the understanding of the differing molecular mechanisms used by nutrients to regulate genes that are essential for their biological roles to carry out normal metabolism. Classical studies have focused on nutrients as ligands to activate specific transcription factors. New interest has focused on histone acetylation as a process for either global or limited gene activation and is the first mechanism to be discussed. Nuclear ATP-citrate lyase generates acetyl-CoA, which has been shown to have a role in the activation of specific genes via selective histone acetylation. Transcription factor acetylation may provide a second mode of control of nutrient-responsive gene transcription. The third mechanism relates to the availability of response elements within chromatin, which as well as the location of the elements in the gene may allow or prevent transcription. A fourth mechanism involves intracellular transport of Zn ions, which can orchestrate localized enzyme inhibition–activation. This process in turn influences signalling molecules that regulate gene expression. The examples provided in the present review point to a new level of complexity in understanding nutrient–gene communication.

Nutrient–gene communication: Transcription factor acetylation: Zn transport and cellular signalling: Zn status and response element placement

The understanding of how specific genes and groups of genes are regulated by nutrients has advanced dramatically in recent years. A historical perspective and some general concepts relating to nutritional regulation of gene expression have been presented earlier[1]. Since the nutrition–gene regulation field is now well developed, the latest methods for the study of gene regulation are rapidly being incorporated into projects designed specifically to examine how dietary components affect the genome to produce specific phenotypic effects. As a result, modes of nutrient action, once viewed as classical ligand–nuclear receptor interactions acting in a Newtonian fashion, now extend into aspects of genomic structure and intracellular trafficking of nutrients. The goal of the present brief discussion is to illustrate three newly-identified pathways in which nutrients use widely-divergent processes to influence gene expression.

Most attention in the area of nutrient control of gene expression has focused on the ligand-activated transcription factor-mediated activity. The ligands include metabolites of vitamins A or D, sterols, fatty acids and a plethora of organic compounds, such as flavinoids, present in foods as well as specific drugs[1–3]. These transcription factors, of which currently about fifty have been described[4], usually termed nuclear receptors, bind to response elements (such as the sterol regulatory element) as homo- or heterodimers and, unless modified, activate gene transcription. Such interactions have therapeutic potential[5]. The metal-responsive transcription factor 1 (MTF-1) has an approximately analogous role in Zn (and other transition metal)-activated gene

Abbreviations: ACL, ATP-citrate lyase; MTF-1, metal-responsive transcription factor 1; siRNA, small interfering RNA.

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transcription[6]. The ligand-activated mode of nutrient control is illustrated in Fig. 1(A).

Transcription factor acetylation

Much has been written about histones and regulation of gene expression and the roles that environmental factors, including diet and specific nutrients, play as modifiers of regulation[7–9] and which histone modifications are truly epigenetic[10]. While much attention has focused on methylation as a post-translational modifier of histones, recently major strides have been made in understanding how histone acetylation, also a post-translational modification, influences gene expression[9]. The concept of histone acetylation as a factor in gene regulation was first advanced 35 years ago[11]. Acetylation of the lysine residues of histone tails removes positive charges, thus decreasing histone–DNA affinity. This process yields easier access for RNA polymerase II and transcription factors to promoter regions. Targeted regions of chromatin are acted on by histone acetyltransferases and histone deacetylases to regulate transcription of specific genes.

Recently, an elegant example of histone acetylation has been shown to link acetyl-CoA production from glycolysis to the expression of genes that influence glucose metabolism[12]. Focusing specifically on ATP-citrate lyase (ACL), an enzyme that generates acetyl-CoA from citrate, it was found that, surprisingly, ACL is present in both cytoplasm and the nucleus. This finding suggests that citrate may diffuse into the nucleus, where it may facilitate acetyl-CoA production. Silencing of ACL with small interfering RNA (siRNA) decreases the acetylation of numerous histones. The effect on histone acetylation is prevented by the addition of acetate, which is an acetylation substrate used by histone acetyltransferases. ACL siRNA suppression reduces the expression of the glucose transporter GLUT4 as well as genes needed for glucose metabolism, i.e. hexokinase, phosphofructokinase-1 and lactate dehydrogenase A. Other genes not involved in glucose metabolism are not influenced by ACL siRNA. Acetate also reverses effects on those genes that are inhibited by ACL siRNA. Finally, immunoprecepitation studies with the GLUT4 promoter using antibodies for two histone acetylases (Ac-H3 and Ac-H4) have shown that histone acetylation at the GLUT4 promoter is reduced when ACL production is inhibited with siRNA. GLUT4 promoter activity is rescued with acetate. It was not possible to fully rule out that the observed effects on the *GLUT4* gene and those for the glucose-metabolizing enzymes (hexakinase 2, phosphofructokinase and lactate dehydrogenase A) are produced by global changes in acetylation. Nevertheless, histone acetylation by ACL at promoters relevant to a particular metabolic pathway opens a new avenue of inquiry.

The experiments on ACL acetylation have all been carried out with cells in culture (see Rathmell & Newgard[13]). However, experiments in animals are lending support for the role of acetylation in nutrient-responsive transcription factor activation. A notable example is the transcriptional activation of the fatty acid synthase gene on feeding[14]. In this model a transcription factor upstream, transcription

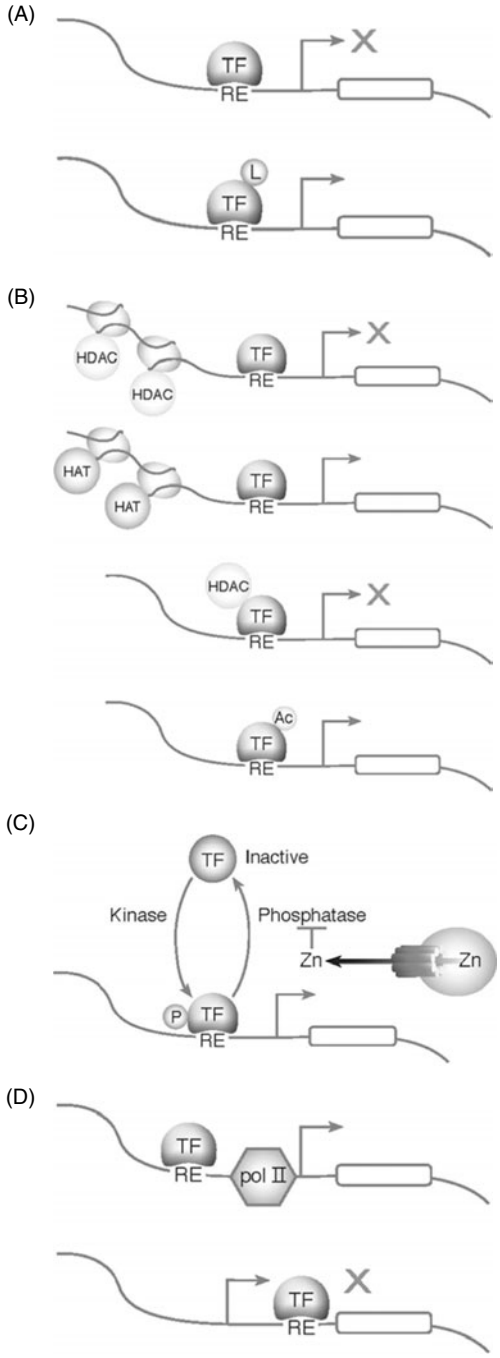

Fig. 1. Modes of nutrient–gene communication. (A) Nuclear receptor-mediated transcription. Ligand (L)-activated transcription factors (TF) as hetero- or homodimers interact with response elements (RE) and activate transcription. Without L they may act as repressors. (B) Acetylation-activated gene transcription. Histone acetylation can produce global and possibly limited activation of specific genes. Alternatively, acetylation of specific TF may provide a focused level of control of gene expression via production of acetyl-CoA (AC). HDAC, histone deacetylases; HAT, histone acetyltransferases. (C) Nutrient transport modifies cell signalling pathways and indirectly influences TF activity. P, phosphate. (D) Placement of the RE within the non-coding region can dictate whether a nutrient-binding TF acts as an activator or a repressor of transcription. Placement that prevents RNA polymerase (pol) II movement causes the TF to act as a gene repressor.

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factor 1, associates with histone deacetylase 9, which acts as a transcriptional repressor through upstream transcription factor 1 deacetylation. On feeding, upstream transcription factor 1 is acetylated and activated, thus allowing recruitment of accessory factors and increased fatty acid synthase expression. The results in relation to fatty acid synthase gene expression suggest that histone deacetylases may influence transcription factor access to specific promoters of other genes that respond to nutrients. In this context histone deacetylase 9 has been shown to associate with numerous transcription factors[15]. This mode of nutrient–transcription factor responsiveness is illustrated further in Fig. 1(B). It needs to be further explored through further research.

Nutrient transport-influenced transcription factors

Homeostatically-influenced transport of nutrients control cell function through provision of needed substrates. Recent evidence from experiments with various cell types and integrative systems suggest targeted nutrient transport has physiological consequences. Of particular interest for the authors' laboratory has been the linkage of Zn transport with cellular signalling. The idea that Zn could act as a specific second messenger was initially presented in 1984[16]. Cellular compartmentalization and transport are central to the signalling role of Zn. Zn transporters are from either the ZnT family (ten members) or ZIP family (fourteen members)[17]. The most-clearly-defined evidence for transporter-mediated signalling roles for Zn have been derived for the ZIP family. Specifically, ZIP6, ZIP8 and ZIP10 have each been identified with signalling processes[18–20]. Of the three transporters, nutrient–transcription factor communication has been shown for ZIP8 in transcriptional activation of the interferon-γ gene[19].

Peripheral blood mononuclear cells provide a resource to evaluate the effects of Zn supplementation on expression of specific genes in human subjects. Using T-cells purified by negative magnetic selection and a procedure that simulates antigen presentation, *Zip8* mRNA has been identified as the most responsive Zn transporter transcript on cell activation[19]. The concurrent activation-induced increase in interferon-γ expression is further stimulated by supplemental Zn. Suppression of ZIP8 by siRNA silencing markedly reduces interferon-γ. ZIP8 has been found by confocal microscopy to be localized to the lysosome. On activation Zn is transported from the acidic environment of the lysosome, as shown using a Zn-responsive fluorophore (FluoZin 3). Analysis of the T-cell activation pathway involving calcineurin reveals that activation of the transcription factor cAMP-response element-binding protein, i.e. phosphorylated cAMP-response element-binding protein, is responsive to Zn. In addition, activation of cAMP-response element-binding protein is inversely correlated with calcineurin phosphatase activity. This activity is very sensitive to inhibition by Zn. It is concluded that ZIP8 produces a release of lysosomal Zn into the cytoplasm thereby inhibiting dephosphorylation by calcineurin and maintaining the cAMP-response element-binding protein transcription factor in its active phosphorylated form[19].

It is proposed that this experimental T-cell model is but one example of how, through mediated transport, Zn may communicate with a transcription factor. In this particular example, the influence could be through a classical enzyme inhibition mechanism involving a transcription factor that is active in its phosphorylated form. Similar roles for Zn and Zn transport influencing receptor-initiated events has been proposed[21] as shown in Fig. 1(C).

Nutrient-influenced gene repression via response element placement

Homeostatic balance in nutrient utilization implies that positive and negative control points are needed. Tight control of cellular Zn flux is of paramount importance because, unlike some other nutrients, Zn is not stored. The identification of the first mammalian Zn transporter, ZnT1, was through its ability to rescue cells in conditions of high extracellular Zn[22]. The positive response to Zn was traced to MTF-1. Indeed, nutritional transitions to an elevated Zn supply increase ZnT1 expression in the intestine and pancreas[23,24]. Most of the twenty-four genes in the ZnT and Zip families are refractory to changes in dietary Zn intake in these same tissues[24].

An expanding base of information has led to an analysis of most of these transporter genes in many tissues and their response to conditions of both dietary Zn restriction and Zn supplementation. Of particular note is the up-regulation of Zip10 expression with Zn restriction in mice[25,26].The experiments have shown that an increase in Zip10 expression occurs in liver and brain and is also detected in the erythrocyte membrane at the protein level on Zn restriction. Those observations merge well with those made with the Zip10 orthologue in zebrafish (*Danio rerio*) gill[27]. Sequence analysis of the Zip10 promoter has revealed no KLF4 binding site, which has been implicated in the similar up-regulation of Zip4[28]. However, there is a conserved metal response element for mouse, human and zebrafish Zip10. In each species this response element is placed downstream from the transcription start site of Zip10.

It has been found that MTF-1 is associated with the Zip10 promoter in a manner that is proportional to cellular Zn status[25]. In this context MTF-1 is responding to Zn occupancy in the classical fashion, i.e. the transcription factor translocates to the nucleus on binding Zn in the cytoplasm. Silencing of MTF-1 with siRNA increases Zip10 expression. The most likely explanation for this outcome is that nuclear MTF-1 acts as a repressor of Zip10 under conditions of normal Zn status. Chromatin immunoprecipitation analysis of RNA polymerase II binding to the *Zip10* gene has been conducted with antibodies for Ser2 and Ser5 phosphorylated polymerase II forms[25]. The results show that Zn-restricted conditions allow active transcription with clear elongation activity of RNA polymerase II. In Zn-supplemented conditions elongation does not occur. Placement of the metal response element at +17 of the *Zip10* gene allows MTF-1 binding, in response to Zn-stimulated nuclear translocation, but then prevents RNA polymerase II movement from the transcription start

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sites of the *Zip10* gene. In this fashion MTF-1 can act as an activator, e.g. for metallothionein and ZnT1, but because of metal response element placement also acts as a repressor of Zip10. A similar conclusion has been reached to explain the inverse relationship between Zn and zebrafish Zip10[27]. This mode of nutrient regulation is shown in Fig. 1(D).

Summary

The examples provided here have been derived from recent literature and point to a new level of complexity in nutrient–gene communication. Nutrition and gene regulation in this context has been commented on previously[29]. However, the application of new technologies such as siRNA silencing, quantitative PCR, global and targeted microarrays, cell transfection and reporter assays, plus the emergence of the understanding of histone acetyltransferases and deacetylases in transcriptional activation and repression, have given a far greater breadth to this area of nutritional science research than was possible a decade ago. Clearly, gene-regulation studies have a place in understanding the challenges and approaches directed at over-and undernutrition, the theme of the Nutrition Society meeting.

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