UNIT 19 NUTRITIONAL REGULATION OF GENE EXPRESSION

Structure

19.1 INTRODUCTION

This unit introduces you to the recent developments in the area of nutrient-gene interactions. We will first review the concepts related to gene expression followed by a description of the basic processes involved in gene expression. Next we will discuss the role of different nutrients in gene expression and finally touch upon the health implications of nutrient-gene interactions. Before you begin the study of this unit, we suggest you look up the structure and functions of the DNA which has been described in the Nutritional Biochemistry Course (MFN-002) in Unit 2,

Objectives

After studying this unit, you will be able to:

describe the different levels of gene regulation,

discuss the sole of specific nutrients in regulating gene function, and

critically analyze how specific nutrients at the cellular/gene level can influence health and disease.

19.2 GENE EXPRESSION – AN OVERVIEW

The last two decades have witnessed tremendous development in our understanding of the cellular processes at the molecular level including the mechanism of action of certain nutrients. This has been feasible largely by the application of modern molecular and cellular biological techniques within the discipline of nutrition.

DNA (deoxyribonucleic acid) in all cells of a species, we already know, carries all of the genes for all the body's characteristics. However, not all genes are expressed in all cells at all times. Controls of gene expression exist, that determine which genes are transcribed and translated into gene products. Besides metabolic control mechanisms, which involve hormones, metabolites, ions, second messenger systems and others modify the phenotypic expression of genes.

Dietary factors, which include both nutrients and non-nutritive components, can influence gene expression at various levels. Specific nutrients can turn on or turn off specific genes. Nutrient-gene interactions have the potential to influence the life process from conception through growth and development to adulthood. These interactions are also likely to determine a healthy life span by influencing both infectious and chronic degenerative diseases.

Although the Human Genome Project has unravelled the genetic code, gene expression is a process that is still under investigation. An understanding of the molecular mechanisms underlying human health and disease is fundamental to both prevention and treatment of disease. Ultimately, as knowledge about genetic identity expands and gene-nutrient interactions are well understood, nutritionists may be able to recommend nutrient intakes that enhance the expression of genes associated with good health and suppress the expression ,of genes associated with disease.

So then what is meant by gene expression? Let us find out.

What is Meant by Gene Expression?

The synthesis of protein under the influence of gene is called *Gene Expression.* All human cells are derived from a single cell, fertilized egg or zygote. Therefore, all cells contain the same genetic information. However, a cell from the gastrointestinal epithelium is different from a cell of the central nervous system or from the liver, both by structure and function. In a cell about 90% of DNA is permanently inactive. Histones and specific proteins help in this inactivation process and consequent differentiation.

The fundamental dogma of molecular biology is that DNA produces RNA (ribonncleic acid), which in turn produces proteins. Thus, the genetic information in the DNA specifying particular functions is converted into an RNA copy by the process of *transcription,* which is then translated into protein. The action of the protein then produces the phenotype. Current scientific evidence indicates that only 10% of the human DNA contains genes, i.e., those sequences that are transcribed and translated.

In simple terms, gene expression is *the process by which a gene's DNA sequence is converted into the structure and functions of a cell.* Gene expression is **a** multi-step process that begins with transcription of DNA into messenger RNA (mRNA). It is then followed by post transcriptional modifications and translation into a gene product, followed by folding, post-translational modification and targeting. For those of you who would like to know more about the process, a brief description of the process is given in Box 19.1.

Box 19.1 | Gene Expression Process

The process of converting genetic information, contained in a gene, into RNA, and, ultimately, to protein is described herewith.

- *Transcription:* Transcription is the *enzymic synthesis of RNA on a DNA template.* This is the first stage in the overall process of gene expression and eventually leads to the synthesis of ***a*** protein encoded by the respective gene, It is catalyzed by an *RNA polymerase.* RNA polymerase binds to a specific DNA sequence called *promoter* to initiate RNA synthesis. These sequences are upstream end of the region that codes for protein and they contain short conserved DNA sequences, which are common to different promoters. The binding of RNA polymerase to the promoter sequence results in local DNA unwinding. Very few RNAs are transcribed directly into the final mature RNA product. Most newly transcribed molecules undergo various alterations to yield the mature product. The term RNA *processing* is used to describe these alterations to the primary transcript.

  Translation: The DNA which is transcribed into mRNA, is translated into protein with the help of ribosomes, tRNA, amino acids, a number of protein factors and enzymes. The sequence of nucleotides in the mRNA, which constitutes the genetic code, specifies the amino acid sequence in the proteins.

The actual mechanism of protein synthesis can be divided into three stages.

1) *Initiation:* The assembly of a ribosome on an mRNA molecule at the correct start point, the initiation codon. Three initiation factors IF_1, IF_2 and IF_3 and GTP (guanosine triphosphate) are involved in the process.

2) ***Elongation:*** RNA polymerase moves along the DNA and sequentially synthesizes the RNA chain. There is the repeated cycle of amino acid addition. This process involves 3 steps.

   i) Aminoacyl-tRNA delivery

   ii) Peptide bond formation, and

   iii) Translocation.

Three elongation factors along with GTP are also involved.

3) *Termination*: RNA polymerase recognizes the terminator, which results in no further nucleotides being incorporated and the release of the new protein chain. This involves the terminating codons and 3 release factors.

Having understood the basics process of gene expression, let us now study about the role of specific nutrients in gene expression.

19.3 ROLE OF SPECIFIC NUTRIENTS IN CONTROLLING GENE EXPRESSION

The role of several nutrients in controlling gene expression, as mentioned earlier, is in infancy, but with advancing bio-molecular techniques, newer roles will be evident. The information available of date is discussed herewith. We begin our study with the role of proteins in gene expression.

19.3.1 Proteins

Under proteins, we shall review the sterol regulatory element binding proteins (SREBPS) and leptin.

Sterol Regulatory Element Binding Proteins (SREBPS)

These belong to a general family of transcription factors. They are synthesized as membrane embedded proteins in response to depletion of cellular cholesterol.

These proteins lead to the transcription of genes encoding the low density lipoprotein (LDL) receptor and multiple enzymes in the cholesterol and fatty acid biosynthetic pathways. Mammalian cells use a very sensitive mechanism to control the amount of cholesterol and fatty acids in their membranes. Recent genetic. evidence reveals that the steroid sensor in this system is another membrane embedded protein called *SREBP cleavage activating* ***protein*** *(SCAP).*

Leptin

Leptin *is* **a** protein product (containing 167 amino acids) of the *ob* gene expressed exclusively in the adipose tissue. This protein molecule functions as an afferent signal in a negative feedback loop, centred in the hypothalamus, regulating the size of the adipose tissue mass.

Leptin has been shown to modulate the synthesis of neuropeptides, particularly Neuropeptide Y (NPY) that regulates food intake.

In human subjects, a highly significant correlation between body fat content and

increases energy expenditure as indicated by animal experiments. Leptin induced weight loss is completely specific for adipose tissue. While feeding does not appear to modulate leptin expression, fasting acutely decreases plasma leptin concentration.

In general, obese humans have high leptin levels and the available data suggest that human obesity is likely to be associated with insensitivity to leptin.

Next, let us look at the role of lipids.

19.3.2 Lipids

In the lipogenic pathway, the genes that encode the enzymes of the fatty acid synthase complex are co-ordinately expressed. Whether increased or decreased, all enzymes are synthesized to the same extent so that the pathway can function efficiently.

Studies of the transcription and translalion of all of the constituent components of this multifunctional complex have revealed that the fatty acid *synthase* is represented by a single gene that encodes seven gene products, the seven enzyme components of the fatty acid synthase complex. This enzyme complex is regulated by nutrients at the levels of both transcription and translation. *Arachidonic* acid, a fatly acid with 20 carbons and four double bonds, suppresses gene transcription. It is likely that the mechanism is one that involves a fatty acid binding protein that in turn binds to DNA as trans-acting element.

S14, a protein that seems to be important in the regulation of mRNA transcription of not only fatty acid synthase but also a number of carbohydrate metabolic enzymes, also, is suppressed by long-chain polyunsaturated fatty acids. The S14 gene has a carbohydrate-responsive element as well. When diets rich in sugar are consumed, S14 mRNA increases, as do the mRNAs for glucokinase, glucose-6-phosphate dehydrogenase; 6-phosphogluconate dehydrogenase, and malic enzyme. In each of these instances, nutrients (glucose, fatty acids) are actively involved as parts of transcription regulatory elements.

Next, a review on fuel molecules and lipogenesis follows.

19.3.3 Fuel Molecules and Lipogenesis

Lipogenesis, you may recall studying, is a collective *name* for *the complex* process *of producing lipids (fatty* acids) *from smaller* precursor molecules. The synthesis of long-chain fatty acids occurs primarily in the liver in many animals. The flux of carbon through this pathway is slow in starved animals and fast in fed ones, especially if the diet is high in carbohydrate and low in fat. The activities of several enzymes involved in lipogenesis, including L-type *pyruvate kinase* (L-PK) and *malic* enzyme (ME) (refer to Unit 7 in Nutritional Biochemistry MFN-002) have similar patterns. The regulation of these enzymes by starvation and refeeding and by altering the carbohydrate content of the diet is briefly described next.

L-PK catalyzes the conversion of phosphoenolpyruvate to pyruvate and plays an important role in directing pyruvate toward glucose synthesis in starved animals or towards oxidation of and incorporation into long-chain fatty acids in fed animals. The level of L-PK decreases greatly in starved rats and is increased when starved rats are refed, particularly if the diet contains a high level of carbohydrate. In diabetic animals, L-PK concentration is very low; treatment with insulin restores normal levels. Feeding a diet high with insulin restores normal levels. Feeding a diet high in fructose, but not glucose, partly restores the normal level of L-PK in a diabetic rat. These changes in concentration of L-PK are due to changes in the synthesis rate of the protein. Thyroid hormone also stimulates accumulation of L-PK protein in thyroidectomized rats.

The level of L-PK mRNA is increased in rats treated with insulin and decreased in diabetic animals or animals treated with glucagons or cyclic adenosine monophosphate (cAMP). Fructose and certain other simple sugars cause an increased accumulation of L-PK and its mRNA, even in diabetic animals.

Regulation of rate of synthesis of L-PK mRNA is not exclusively transcriptional, and different hormones may regulate expression of this gene by regulating different reactions in the gene expression pathway.

ME catalyzes the oxidative decarboxylation of malate to pyruvate and CO_2,(refer to Figure 7.6 in Unit 7 in the Nutritional Biochemistry Course MFN-002) simultaneously generating the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$, much of the NADPH generated by this reaction is utilized in the ***de novo*** synthesis of long-chain fatty acids. ME responds to dietary and hormonal manipulation in much the same way as L-PK does. The level of the hepatic enzyme is low in starved animals and high in fed animals, especially if the diet is high in carbohydrate. The concentration of ME is controlled by regulating its synthesis rate which, in turn correlates positively with abundance of ME mRNA, indicating pretranslational regulation.

A 50- to 100-fold increase in mRNA level is caused by refeeding starved chicks or ducklings; this is accompanied by about a 50-fold increase in transcription of the ME gene.

Hormones also play an important role in regulating ME. The concentration of ME is low in diabetic and hypothyroid animals and restored to normal or higher levels by treatment with insulin or T_3, respectively. Insulin has little effect by itself but amplifies the effect of T_3, such that the total increase is 100 to 150-fold.

One fuel that affects ME, apparently independently of insulin, is fructose. As with other lipogenic enzymes, dietary fructose increases the levels of ME and its mRNA even in diabetic animals. Hormones control the synthesis of ME by regulating the abundance of its mRNA, indicating pretranslational control.

Insulin, glucagons, glucocorticoids and triiodothyronine (T_3) modulate the rate of fatty acid synthesis and the activities of the lipogenic enzymes. Evidence that these hormones are mediators of the effects of diet on hepatic enzyme activities is based on a variety of mainly correlative evidence. The blood of animals fed high-carbohydrate diets has an elevated level of insulin and a decreased level of glucagons. The opposite is observed in starved animals – lowered insulin and elevated glucagons. In diabetic animals, insulin levels are low and glucagons levels high; the rate of Iipogenesis and the activities of the lipogenic enzymes are low. In perfused liver, liver slices and isolated hepatocytes; insulin stimulates and glucagons inhibits the rate of fatty acid synthesis. Increases in lipogenic flux and activities of the lipogenic enzymes caused by refeeding starved animals are blocked by the simultaneous administration of glucagons. Finally, in hepatocytes in culture, insulin stimulates and glucagon inhibits accumulation of the lipogenic enzymes. These results suggest that insulin and glucagons play important roles in the metabolic transitions between the fed and starved states.

Although, the level of thyroxine is not affected by starvation or feeding, the concentration of the active form of the thyroid hormone T_3, is regulated in a manner similar to that for insulin – decreased in starved animals and increased in fed animals. These results are consistent with a role for T_3 in the regulation of lipogenic enzyme activity during the transitions between the fed and starved states.

Glucocorticoids are another class of hormones involved in the regulation of lipogenic enzymes, but their role in nutritional regulation of these enzymes is not well understood. The glucocorticoids may be permissive for the expression of a variety of hepatic enzymes, involved in intermediary metabolism.

Changes in the levels of fuels derived directly or indirectly from dietary nutrients also regulate the activities of lipogenic enzymes. Thus, dietary fructose stimulates an increase in the activities of the lipogenic enzymes in starved rats, whether they are diabetic or not. Glucose is effective only in non-diabetic animals.

Glucose-6-phosphatedehydrogenase (G6PD) is another enzyme involved in lipogenesis that is regulated by dietary factors. G6PD activity is needed in all cell types for the production of NADPH and for the control of carbon flow through the pentose phosphate pathway. G6PD catalyzes the first reaction of this pathway, oxidizing glucose-6-phosphate to 6-phosphogluconolactone (refer to Figure 6.16 in Unit 6 of the Nutritional Biochemistry Course MFN-002), and in the process reducing $NADP^+$ to NADPH + H^+. This reaction, you may recall studying, is the rate-determining step of the oxidative portion of the pentose phosphate pathway. Together with 6-phosphogluconolactone dehydrogenase, the enzyme provides NADPH for reductive biosynthetic reactions, such as fatty acids, cholesterol and amino acids and for the maintenance of reduced glutathione concentration. In addition, it regulates the rate of glucose conversion to ribose-5-phosphate, the precursor for nucleotide biosynthesis. G6PD expression is regulated by hormonal and nutritional factors in a few tissues such as the liver and mammary glands.

Long-term starvation decreases the activity of the enzyme, while re-feeding with a fat-free, high-carbohydrate increases the activity to an amount greater than in the "normal" fed state. Glucose and fructose increase activity to a larger extent than starch and the response is greater with fructose than with glucose.

In contrast, polyunsaturated fatty acids (PUFA) inhibit G6PD in both intact animals and in primary hepatocytes in culture. The inhibition occurs in rats switched from a high-carbohydrate fat-free diet to one with PUFA and when starved rats are re-fed a high-carbohydrate diet supplemented with PUFA. Addition of saturated fatty acids or monounsaturated fatty acids does not inhibit G6PD activity. The inhibitory effect of PUFA is unique to G6PD activity in the liver. PUFA in the diet do not affect the enzyme activity in the adipose tissue.

The available evidence suggests that the regulation of G6PD gene expression by nutritional and hormonal factors occurs at the post-transcriptional step. The abundance of G6PD RNA appears to be regulated during the processing of the nascent transcript, steps that involve either splicing or polyadenylation of the pre-mRNA. Re-feeding enhances the efficiency of splicing and increase polyadenylation.

The discussion above focused on the role of macronutrients on gene expression. Next, let us review the role of minerals and vitamins in controlling gene expression.

19.3.4 Minerals

The role of iron and zinc in controlling gene expression are covered in this, sub-section. Let us begin our study with iron.

A) ***Iron***

Iron metabolism is regulated by a complex co-ordinate mechanism. Recent evidence supports specific past-transcriptional mechanisms in the cytoplasm of vertebrate cells that directly affect the stability and the translation of mRNAs coding for central proteins in iron metabolism.

Ferritin, the storage form of iron in the body, is expressed in all tissues and is abundant in the liver. The quantity of ferritin synthesized is directly proportional to the available cellular iron and reflects body iron stores. Its synthesis is controlled at the level of mRNA translation by a regulatory network described next.

The Iron Response Element (IRE) and the Iron Regulatory Protein (IRP) play key roles in co-ordinating regulation of iron uptake, storage and utilization. The IRE has specific sequences in the 5' untranslated region of L and H chain ferritin mRNAs. These sequences are conserved in evolution and form a specific RNA stein loop structure. The IRE interacts at high affinity with a cytoplasmic protein known as Iron Regulatory Protein. When the IRE is situated sufficiently close to the cap site and before the start codon, binding of IRP prevents ribosome attachment and thereby inhibits translation initiation. The IRE binding activity of IRP varies as a function of iron availability: it increases after iron chelation, but decreases when iron supply is plentiful. As a result of the IRE-IRP interaction and the inhibition of ferritin synthesis, iron storage diminishes in iron-deprived cells. In contrast, cells with a high iron supply synthesize and store iron normally.

In addition, transferrin receptor mRNA is regulated by IRP, but in the reverse. Here, iron deprivation increases the stability of transfemn receptor mRNA, whereas an abundance of iron has a destabilizing effect. Cells respond to natural fluctuations in iron metabolism by modulation of the IRPs.

The co-ordinate opposite control of iron storage and uptake has physiologically a cumulative effect. While iron deprivation is compensated by higher iron uptake and less storage, iron overload is re-equilibrated by opposite effects. An individual's iron status triggers a feedback control on ferritin and transferrin receptor levels.

Next, the role of zinc is highlighted.

B) ***Zinc***

Zinc atoms have specific structural roles in enzyme molecules, as well as, in many other proteins and in biomembranes. These structural roles of zinc are ubiquitous and of outstanding importance in cellular and sub-cellular metabolism. One outstanding example that has generated a great deal of is the *zinc finger motif,* the most common recurring motif in transcription proteins. The configuration of these "fingers," which determines their binding to DNA, is determined by the single zinc atoin at their base. The linking of these zinc fingers to corresponding sites on DNA initiates the transcription process and gene expression. Similar motifs have been identified in nuclear hormonal receptors, including those for oestrogen, testosterone and vitamin D.

Zinc, you might recall studying earlier in this course, plays an important role in the maintenance of the immune system. While the mechanisms of zinc ions interaction with immune cells are still poorly understood, a striking concurrent effect of zinc is the induction of the biosynthesis of *metallothioneins* (*MT*), a group of low molecular weight, cysteine-rich metal-binding proteins, believed to play a role in zinc homeostasis. *Dr. Cousins* was the first, in the late 1970s, to elucidate the presence of intestinal metallothionein, a protein involved in the regulation and kinetics of the intestinal absorption of dietary zinc, while also discovering its vital role in cellular zinc metabolism, its relationship to zinc deficiency and the consequences that can have on the body. In his studies, he was the first to demonstrate that a dietary trace mineral could actually influence the transcriptional regulation of gene expression.

The subsequent discovery and understanding of the zinc-binding properties of another protein – *cysteine-rich intestinal protein* or *CRIP* – has led to a more complete appreciation of how zinc behaves in intestinal and immune cells. CRIP is now understood to have a role in immune defense against infection. You may recall studying about this aspect earlier in Unit 10, section 10.4.

Most recently, the discovery by *Dr.* Cousins that zinc deficiencies can actually induce the expression of an intestinal honnone called *uroguanylin* is a breakthrough toward realizing the probable role that zinc deficiencies may play in zinc-responsive diarrhoea1 diseases common in young children, particularly in the developing world.

Finally, let us study about the role played by vitamins in controlling gene expression.

19.3.5 Vitamins

The role of vitamin A, vitamin K and vitamin D in controlling gene expression is highlighted in this sub-section.

A) Vitamin A

Vitamin A as *retinoic acid* has widespread effects on the synthesis of a variety of important proteins. These diverse effects are due largely to its combination with one or more nuclear receptors that serve as trans-acting factors that promote transcription. Several different receptors have been identified and each has a specific mode of action in growth and development. For example, should **a** mutation occur in the **a** receptor, which would result in decreased retinoic acid binding, embryonic cell differentiation is affected, with the result of embryonic wastage or foetal malformation. Congenital heart malformation has been attributed to such a mechanism.

B) Vitamin K

Prithrombin, a protein required for blood clotting is assembled with a large number of glutamic acid residues. In the presence of vitamin K, these residues are carboxylated, and this posttranslational change results in a dramatic increase in the calcium binding capacity of the resultant protein. Unless prothrombin can bind calcium, it cannot function in the clotting process, as you may recall studying earlier in Unit 7 in this course. This is another example of how a nutrient can affect gene expression; in this instance, it is the expression of functional prothrombin. The site of the nutritional effect is that of posttranslational protein modification.

C) ***Vitamin D***

Vitamin D is a secosteroid that is metabolically activated and degraded through the actions of three cytochrome P450 hydroxylase enzymes. Bioactivation occurs through the sequential actions of cytochromes (P450C25 and P450C1) resulting in synthesis of the pleiotropic hormone 1,25-dihydroxyvitamin D (1,25VD), which regulates over 60 genes whose actions include those associated with calcium homeostasis and immune responses, as well as, cellular growth, differentiation and apoptosis, as you may recall studying earlier in Unit 7. Inactivation of 1,25VD occurs by C23/C24 oxidation pathways that are catalyzed by the multifunctional cytochrome P450C24 enzyme. Both P450C1 and P450C24 are highly regulated enzymes whose differential expression is controlled in response to numerous cellular modulatory agents such as parathyroid hormone (PTH), calcitonin, interferon gamma, calcium, phosphorus and pituitary hormones, as well as, the secosteroid hormone 1,25VD.

With this, we end our study on nutritional regulation of gene expression.

19.4 LET US SUM *UP*

In this unit, we first reviewed the concepts related to gene expression followed by a description of the basic processes involved in gene expression. Next, we focused on the role of different nutrients, namely lipids, proteins, vitamins and minerals in gene expression and also touched upon the health implications of nutrient-gene interactions.

19.5 GLOSSARY

Genome : the total genetic material.

Promoters : these are present at the 5' end of all genes. They control the start position of the mRNA and govern the general level of transcription.

Reverse transcription : means making a DNA chain on a RNA template. This is part of the lifecycle of many RNA viruses (retroviruses) but is believed not to be part of normal cell metabolism. Reverse transcriptase activity is used as a marker of the presence of retroviruses.

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