Nutrition and Metabolism

Second Edition

Edited on behalf of The Nutrition Society by

Susan A Lanham-New
Ian A Macdonald
Helen M Roche

WILEY-BLACKWELL
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Nutrition and Metabolism

The Nutrition Society Textbook Series

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Nutrition and Metabolism

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Series Foreword

The early decades of the twentieth century were a period of intense research on constituents of food essential for normal growth and development, and saw the discovery of most of the vitamins, minerals, amino acids and essential fatty acids. In 1941, a group of leading physiologists, biochemists and medical scientists recognised that the emerging discipline of nutrition needed its own learned society and the Nutrition Society was established. Our mission was, and remains, 'to advance the scientific study of nutrition and its application to the maintenance of human and animal health'. The Nutrition Society is the largest learned society for nutrition in Europe and we have over 2000 members worldwide. You can find out more about the Society and how to become a member by visiting our website at www.nutsoc.org.uk.

The revolution in biology initiated by large-scale genome mapping and facilitated by the development of reliable, simple-to-use molecular biological tools makes this a very exciting time to be working in nutrition. We now have the opportunity to get a much better understanding of how specific genes interact with nutrient intake and other lifestyle factors to influence gene expression in individual cells and tissues and, ultimately, affect health. Knowledge of the polymorphisms in key genes carried by an individual will allow the prescription of more effective, and safe, dietary treatments. At the population level, molecular epidemiology is opening up much more incisive approaches to understanding the role of particular dietary patterns in disease causation. This excitement is reflected in the several scientific meetings which the Nutrition Society, often in collaboration with sister learned societies in Europe, Africa, Asia and the USA, organise each year. We provide travel grants and other assistance to encourage students and young researchers to attend and to participate in these meetings.

Throughout its history a primary objective of the Society has been to encourage nutrition research and to disseminate the results of such research. Our first journal, *The Proceedings of the Nutrition Society*, recorded, as it still does, the scientific presentations made to the Society. Shortly afterwards, *The British Journal of Nutrition* was established to provide a medium for the publication of primary research on all aspects of human and animal nutrition by scientists from around the world. Recognising the needs of students and their teachers for authoritative reviews on topical issues in nutrition, the Society began publishing *Nutrition Research Reviews* in 1988. We subsequently launched *Public Health Nutrition*, the first international journal dedicated to this important and growing area. These journals are available in electronic and conventional paper form, and we are exploring new opportunities to exploit the web to make the outcomes of nutritional research more quickly and readily accessible.

Just as in research, having the best possible tools is an enormous advantage in teaching and learning. This is the reasoning behind the initiative to launch this series of human nutrition textbooks designed for use worldwide. The Society is deeply indebted to the founding Editor-in-Chief, Professor Michael J Gibney (University College Dublin), for his foresight and hard work in bringing the first editions of this major publishing exercise to successful fruition and for overseeing the production of the second edition of the *Introduction to Nutrition* textbook. We are particularly grateful to Dr Susan A Lanham-New (University of Surrey) for agreeing to take on the challenge of being Editor-in-Chief for the second editions of the other three textbooks (*Nutrition and Metabolism*, *Public Health Nutrition* and *Clinical Nutrition*) and for also having the vision to add a fourth textbook, *Sports and Exercise Nutrition*. Read, learn and enjoy.

Ian A Macdonald
President of the Nutrition Society

Preface

More than a decade has passed since the idea of a Nutrition Society Textbook Series was first raised and it has proved to be an enormously successful venture. It is a great honour for me to be the new editor-in-chief of the series and credit should go to the first ever Editor-in-Chief, Professor Michael J Gibney (University College Dublin) for his tremendous vision and hard work in the early days of the Series' development.

Nutrition and Metabolism 2e is the second of this series of four textbooks: *Introduction to Human Nutrition 2e* was published last year and launched at the 2009 Nutrition Society Conference, held at the University of Surrey, Guildford. It was seen very much as an 'introductory' textbook and, as such, was designed not only for students of nutritional sciences but also for the many undergraduate and postgraduate students who have aspects of nutrition in their courses (e.g. medicine, pharmacy, nursing and food science). *Nutrition and Metabolism 2e* is aimed at the student (undergraduate and postgraduate) opting to pursue nutrition as a main academic subject. This textbook, as the title implies, has as its focus the physiological and biochemical basis for the role of nutrients in metabolism. The first seven chapters cover some core areas, some traditional areas, such as the integration of metabolic nutrition or areas related to stages of growth, and also focuses on molecular nutrition. This is an area of considerable growth and development. Following on from this, the chapters are organised in a slightly different manner, taking the view that the role of individual nutrients should be integrated into chapters on a 'systems' level rather than a specific nutrient one.

Plans are well underway for the second edition of *Public Health Nutrition*, and hence this topic is avoided in *Nutrition and Metabolism 2e*. The second edition of *Clinical Nutrition Textbook* will address the diet–disease links on a system-by-system basis.

The first edition of *Nutrition and Metabolism* was published in 2003 with Professor Ian A Macdonald (University of Nottingham) and Professor Helen M Roche (University College Dublin) as the specific N&M Textbook Editors, doing a splendid job. It has been a great pleasure to have had the opportunity to work with them again on the production of this new edition, and I thank them sincerely for all their hard work.

We have tried to minimise within-textbook overlap and have cross-referenced chapters where possible. However, some level of overlap across texts will undoubtedly occur, but from different perspectives. For example, *Nutrition and Metabolism 2e* introduces an analysis of how nutrients influence risk factors for coronary heart disease with a perspective on the metabolic dimension. Much of this will again arise in both *Public Health Nutrition 2e* and *Clinical Nutrition 2e*, from a population and preventive approach and from a patient and therapeutic approach, respectively.

Nutrition and Metabolism 2e is dedicated to Professor Vernon Young, who contributed greatly to the first edition and who sadly died in 2004. We acknowledge the tremendous contribution that he has made to our field of Nutritional Sciences.

There are plans for further titles in the Nutrition Society Textbook Series, which is certainly a fast-moving product, and it is a pleasure for me, as the new Editor-in-Chief, to be driving them.

The Nutrition Society Textbook Series is hugely indebted to Wiley-Blackwell, who have proved to be extremely supportive publishers. Special mention should go to Nigel Balmforth and Laura Price for their commitment to this Series. The Society is also indebted to Jennifer Norton, who is the new assistant editor of the textbooks. Her hard work, focus and organisation are first rate and we would certainly not be pressing ahead with such pace and efficiency without her input.

I hope that you will find the book of great use. Please enjoy!

Dr Susan A Lanham-New
University of Surrey and Editor-in-Chief,
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First Edition Acknowledgements

The Nutrition Society would like to express its appreciation and thanks to all our Authors and Editors of the first edition of Nutrition and Metabolism

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To Vernon Young

1
Core Concepts of Nutrition

Ian A Macdonald and Michael J Gibney

Key messages

- The change in body reserves or stores of a nutrient is the difference between the intake of that nutrient and the body's utilisation of that nutrient. The time-frame necessary to assess the body's balance of a particular nutrient varies from one nutrient to another.
- The concept of turnover can be applied at various levels within the body (molecular, cellular, tissue/organs, whole body).
- The flux of a nutrient through a metabolic pathway is a measure of the rate of activity of the pathway. Flux is not necessarily related to the size of the pool or pathway through which the nutrient or metabolite flows.
- Nutrients and metabolites are present in several pools in the body. The size of these metabolic pools varies substantially for different nutrients/metabolites, and a knowledge of how these pools are interconnected greatly helps us to understand nutrition and metabolism.
- Darwinian theory of evolution implies a capacity to adapt to adverse conditions, including adverse dietary conditions. Many such examples can be cited. Some allow for long-term adaptation and others buy time until better conditions arrive.

1.1 Introduction

This textbook on nutrition and metabolism covers macronutrient aspects of nutrition in an integrated fashion. Thus, rather than considering the macronutrients separately, this book brings together information on macronutrients and energy in relation to specific states or topics (e.g. undernutrition, overnutrition, cardiovascular disease). Before considering these topics in detail it is necessary to outline the core concepts that underlie nutritional metabolism. The core concepts to be covered in this chapter are nutrient balance, turnover and flux, metabolic pools, and adaptation to altered nutrient supply.

1.2 Balance

As discussed in Chapter 3, nutrient balance must be considered separately from the concepts of metabolic equilibrium or steady state. In this chapter, the concept of balance is considered in the context of the classical meaning of that term, the long-term sum of all the forces of metabolic equilibrium for a given nutrient.

The concept of nutrient balance essentially restates the law of conservation of mass in terms of nutrient exchange in the body. It has become common practice to refer to the content of the nutrient within the body as a 'store' but in many cases this is not appropriate and the term 'reserve' is better. Thus, the idea of nutrient balance is summarised by the equation:

$$\begin{bmatrix}\text{nutrient}\\ \text{intake}\end{bmatrix} - \begin{bmatrix}\text{nutrient}\\ \text{utilisation}\end{bmatrix} = \begin{bmatrix}\text{change in body}\\ \text{nutrient reserves}\end{bmatrix}$$

The above equation can have three outcomes:

- zero balance (or nutrient balance): intake matches utilisation and reserves remain constant
- positive balance (or positive imbalance): intake exceeds utilisation and reserves expand
- negative balance (or negative imbalance): utilisation exceeds intake and reserves become depleted.

In relation to macronutrient metabolism, the concept of balance is most often applied to protein (nitrogen) and to energy. However, many research

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studies now subdivide energy into the three macronutrients and consider fat, carbohydrate and protein balance separately. This separation of the macronutrients is valuable in conditions of altered dietary composition (e.g. low-carbohydrate diets) where a state of energy balance might exist over a few days but be the result of negative carbohydrate balance (using the body's glycogen reserves to satisfy the brain's requirement for glucose) matched in energy terms by positive fat balance.

Balance is a function not only of nutrient intake but also of metabolically induced losses. Fat balance is generally driven by periods where energy intake exceeds energy expenditure (positive energy balance) and by periods when intakes are deliberately maintained below energy expenditure, such as in dieting (negative energy balance). However, nutrient balance can also be driven by metabolic regulators through hormones or cytokines. For example, the dominance of growth hormone during childhood ensures positive energy and nutrient balance. In pregnancy, a wide range of hormones lead to a positive balance of all nutrients in the overall placental, foetal and maternal tissues, although this may be associated with a redistribution of some nutrient reserves from the mother to the foetus (Chapter 6). By contrast, severe trauma or illness will dramatically increase energy and protein losses, an event unrelated to eating patterns.

Balance is not something to be thought of in the short term. Following each meal, there is either storage of absorbed nutrients [triacylglycerol (TAG) in adipose tissue or glucose in glycogen] or a cessation of nutrient losses (breakdown of stored TAG to non-esterified fatty acids or amino acid conversion to glucose via gluconeogenesis). As the period of post-prandial metabolism is extended, the recently stored nutrients are drawn upon and the catabolic state commences again. This is best reflected in the high glucagon to insulin ratio in the fasted state before the meal and the opposite high insulin to glucagon ratio during the meal and immediate post-prandial period. However, when balance is measured over a sufficient period, which varies from nutrient to nutrient, a stable pattern can be seen: zero, positive or negative (Figure 1.1). It is critically important with respect to obesity that the concept of balance is correctly considered. While at some stage energy balance must have been positive to reach an overweight or obese stage, once attained most people sustain a stable weight over quite long periods.

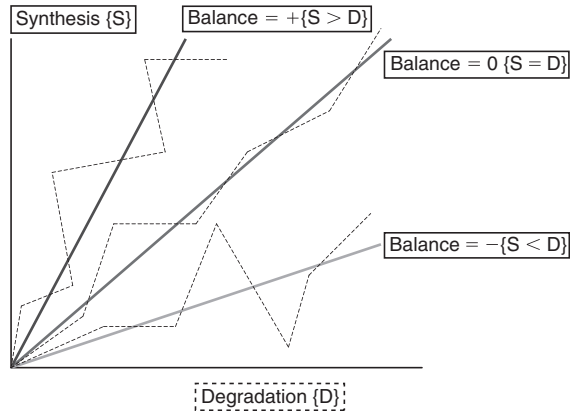

Figure 1.1 Positive, zero and negative nutrient balance over time with fluctuations upwards and downwards within that time.

In the context of the present chapter, it is worth reflecting on the reasons why the period to assess energy balance correctly varies for different nutrients.

Fat and adipose tissue (Chapter 5)

- There is a very large capacity to vary the body's pool of adipose tissue. One can double or halve the level of the fat reserves in the body.
- The capacity to vary the level of TAG in blood en route to and from adipose tissue can vary considerably.
- Almost all of the TAG reserves in adipose tissue are exchangeable.

Calcium and bone (Chapter 12)

- The human being must maintain a large skeleton as the scaffold on which the musculature and organs are held.
- There is a very strict limit to the level of calcium that can be transported in blood. Excess or insufficient plasma calcium levels influence neural function and muscle function, since calcium is also centrally associated with both.

- Only a small fraction (the miscible pool) of bone is available for movement into plasma.

Because of these differences, calcium balance will require months of equilibrium while fat balance could be equilibrated in days or at most a few weeks.

1.3 Turnover

Although the composition of the body and of the constituents of the blood may appear constant, this does not mean that the component parts are static. In fact, most metabolic substrates are continually being utilised and replaced (i.e. they turn over). This process of turnover is well illustrated by considering protein metabolism in the body. Daily adult dietary protein intakes are in the region of 50–100 g, and the rates of urinary excretion of nitrogen match the protein intake. However, isotopically derived rates of protein degradation indicate that approximately 350 g is broken down per day. This is matched by an equivalent amount of protein synthesis per day, with most of this synthesis representing turnover of material (i.e. degradation and resynthesis) rather than being derived *de novo* from dietary protein (Chapter 4).

Similar metabolic turnover occurs with other nutrients; glucose is a good example, with a relatively constant blood glucose concentration arising from a matching between production by the liver and utilisation by the tissues (Chapter 3).

The concept of turnover can be applied at various levels within the body (molecular, cellular, tissue/organs, whole body). Thus, within a cell the concentration of adenosine triphosphate (ATP) remains relatively constant, with utilisation being matched by synthesis. Within most tissues and organs there is a continuous turnover of cells, with death and degradation of some cells matched by the production of new ones. Some cells, such as red blood cells, have a long lifespan (c. 120 days), while others, such as platelets, turn over in a matter of 1–2 days. In the case of proteins, those with very short half-lives have amino acid sequences that favour rapid proteolysis by the range of enzymes designed to hydrolyse proteins. Equally, those with longer half-lives have a more proteolytic-resistant structure.

A major advantage of this process of turnover is that the body is able to respond rapidly to a change in metabolic state by altering both synthesis and degradation to achieve the necessary response. One consequence of this turnover is the high energy cost of continuing synthesis. There is also the potential for dysfunction if the rates of synthesis and degradation do not match.

The consequences of a reduction in substrate synthesis will vary between the nutrients, depending on the half-life of the nutrient. The half-life is the time taken for half of the material to be used up, and is dependent on the rate of utilisation of the nutrient. Thus, if synthesis of a nutrient with a short half-life is stopped, the level of that nutrient will fall quickly. By contrast, a nutrient with a long half-life will disappear more slowly. Since proteins have the most complex of structures undergoing very significant turnover, it is worth dwelling on the mechanism of this turnover. Synthesis is fairly straightforward. Each protein has its own gene and the extent to which that gene is expressed will vary according to metabolic needs. In contrast to synthesis, a reasonably small array of lysosomal enzymes is responsible for protein degradation.

1.4 Flux

The flux of a nutrient through a metabolic pathway is a measure of the role of activity of the pathway. If one considers the flux of glucose from the blood to the tissues, the rate of utilisation is approximately 2 mg/kg body weight per minute at rest. However, this does not normally lead to a fall in blood glucose because it is balanced by an equivalent rate of glucose production by the liver, so the net flux is zero. This concept of flux can be applied at the cellular, tissue/organ or whole body level, and can also relate to the conversion of one substrate/nutrient to another (i.e. the movement between metabolic pathways). Flux is not necessarily related to the size of the pool or pathway through which the nutrient or metabolite flows. For example, the membrane of a cell will have several phospholipids present and each will have some level of arachidonic acid. The rate at which arachidonic acid enters one of the phospholipid

pools and exits from that phospholipid pool is often higher in the smaller pools.

1.5 Metabolic pools

An important aspect of metabolism is that the nutrients and metabolites are present in several pools in the body (Figure 1.2). At the simplest level, for a given metabolite there are three pools, which will be illustrated using the role of dietary essential fatty acids in eicosanoid synthesis.

In the *functional pool*, the nutrient/metabolite has a direct involvement in one or more bodily functions. In the chosen example, intracellular free arachidonic acid, released from membrane-bound stores on stimulation with some extracellular signal, is the functional pool. It will be acted on by the key enzyme in eicosanoid synthesis, cyclo-oxygenase.

The *storage pool* provides a buffer of material that can be made available for the functional pool when required. Membrane phospholipids store arachidonic acid in the sn-2 position at quite high concentrations, simply to release this fatty acid when prostaglandin synthesis is needed. In the case of platelets, the eicosanoid thromboxane A_2 is synthesised from arachidonic acid released into the cytoplasm by stimuli such as collagen.

The *precursor pool* provides the substrate from which the nutrient/metabolite can be synthesised. Linoleic acid represents a good example of a precursor pool. It is elongated and desaturated in the liver to yield arachidonic acid. Thus, the hepatic pool of linoleic acid is the precursor pool in this regard. Not all nutrient pools should be thought of in the concept of the precursor, storage and functional pool model outlined above. The essential nutrients and the minerals and trace elements do not have a precursor pool. Nevertheless, no nutrient exists in a single homogeneous pool and an awareness of the existence of metabolic pools is essential to an understanding of human metabolism. For example, one might expect that a fasted individual would show a fall in all essential nutrient levels in the plasma pool. In many instances this is not the case initially because of the existence of storage pools, such as liver stores of iron or vitamin A. In the case of folic acid, fasting causes a rise in blood folic acid levels and this is explained by the concept of metabolic pools. A considerable amount of folic acid enters the gut via the bile duct and is reabsorbed further down the digestive tract. Thus there is an equilibrium between the blood folate pool and the gut folate pool. Fasting stops gallbladder contraction and thus the flow of folate to the gut, and hence folate is redistributed from one pool to another.

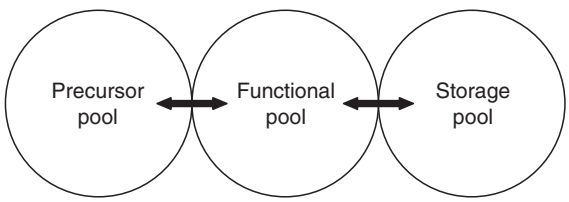

Figure 1.2 The pools in the body in which nutrients and metabolites may exist.

Another example of how an awareness of metabolic pools helps us to understand nutrition and metabolism is the intracellular free amino acid pool. This is the functional pool from which protein is synthesised. As this pool is depleted in the process of protein synthesis, it must be repleted, otherwise protein synthesis stops. Moreover, it is not just the intracellular pool of amino acids that matters but the intracellular pool of essential amino acids or, more precisely, the intracellular pool of the most limiting essential amino acid. Calculations show that if the pool of the most limiting amino acid in mammalian cells was not replenished, protein synthesis would cease in under 1 h. This highlights the need to transfer the limiting amino acid across the cell membrane, which raises the question of how that pool is repleted. Effectively, it can only be repleted if there is a comparable rate of protein degradation to provide the key amino acid, assuming the balance is zero. Thus there are links between the protein pool of amino acids and the extra- and intracellular pools of amino acids.

The size of these various pools varies substantially for different nutrients and metabolites. When studying the activities of metabolic processes within the body, it is often necessary to measure or estimate the size of the various pools in order to derive quantitative information about the overall rates of the processes. In addition, the actual situation may be more complex than the simple three-pool model described

above. Nutritional assessment often involves some biochemical assessment of nutritional status. Blood is frequently the pool that is sampled and even there, blood can be separated into:

- erythrocytes, which have a long lifespan and are frequently used to assess folic acid status
- cells of the immune system, which can be used to measure zinc or ascorbic acid status
- plasma, which is used to ascertain the levels of many biomarkers
- fractions of plasma, such as cholesteryl esters used to ascertain long-term intake of polyunsaturated fatty acids.

In addition to sampling blood, nutritionists may take muscle or adipose tissue biopsies, or samples of saliva, buccal cells, hair and even toenails. A knowledge of how a nutrient behaves in different metabolic pools is critically important in assessing nutritional status. For example, the level of folic acid in plasma is determined by the most recent intake pattern and thus is subject to considerable fluctuation. However, since erythrocytes remain in the circulation for about 120 days, a sample of erythrocytes will represent very recently synthesised cells right through to erythrocytes ready for recycling through the turnover mechanism previously described. As erythrocytes do not have a nucleus, they cannot switch on genes that might influence folate levels, and so the cell retains the level of folate that prevailed at the time of synthesis. Thus, erythrocyte folate is a good marker of long-term intake. The free form of many minerals and trace elements is potentially toxic, and for this reason their level in the plasma is strictly regulated. Hence, blood levels are not used to assess long-term intake of selenium, but toenail clippings can be used.

1.6 Adaptation to altered nutrient supply

In many circumstances, the body is able to respond to altered metabolic and nutritional states in order to minimise the consequences of such alterations. For example, the brain has an obligatory requirement for glucose as a substrate for energy and it accounts for a significant part of resting energy expenditure. During undernutrition, where glucose input does not match glucose needs, the first adaptation to the altered metabolic environment is to increase the process of gluconeogenesis, which involves the diversion of amino acids into glucose synthesis. That means less amino acid entering the protein synthesis cycle of protein turnover. Inevitably, protein reserves begin to fall. Thus, two further adaptations are made. The first is that the brain begins to use less glucose for energy (replacing it by ketones as an alternative metabolic fuel). The second is that overall, resting energy expenditure falls to help sustain a new balance if possible (Chapter 8). Stunting in infants and children, reflected in a low height for age, can be regarded as an example of successful adaptation to chronic low energy intake. If the period of energy deprivation is not too long, the child will subsequently exhibit a period of accelerated or catch-up growth (Chapter 7). If it is protracted, the stunting will lead to a permanent reprogramming of genetic balance. In many instances, the rate of absorption of nutrients may be enhanced as an adaptive mechanism to low intakes. Some adaptations appear to be unsuccessful but work for a period, effectively buying time in the hope that normal intakes will be resumed. In essential fatty acid deficiency the normal processes of elongation and desaturation of fatty acids take place but the emphasis is on the wrong fatty acid, that is, the non-essential 18-carbon monounsaturated fatty acid (oleic acid, C18:1 n-9) rather than the deficient dietary essential 18-carbon polyunsaturated fatty acid (linoleic acid, C18:2 n-6). The resultant 20-carbon fatty acid does not produce a functional eicosanoid. However, the body has significant reserves of linoleic acid which are also used for eicosanoid synthesis and so the machinery of this synthesis operates at a lower efficiency than normal. Eventually, if the dietary deficiency continues then pathological consequences ensue. In effect, adaptation to adverse metabolic and nutritional circumstances is a feature of survival until the crisis abates. The greater the capacity to mount adaptations to adverse nutritional circumstances the greater the capacity to survive.

1.7 Perspectives on the future

These basic concepts of nutrition will remain forever but they will be refined in detail by the emerging

subject of nutrigenomics (Chapter 2). We will develop a greater understanding of how changes in the nutrient content of one pool will alter gene expression to influence events in another pool and how this influences the flux of nutrients between pools. We will better understand how common single nucleotide polymorphisms will determine the level of nutrient intake to achieve nutrient balance in different individuals.

Further reading

Frayn KN. Metabolic Regulation: a Human Perspective, 2nd edn. Oxford: Blackwell Publishing, 2003.

Websites

health.nih.gov/search.asp?category_id=29
http://themedicalbiochemistrypage.org/
www.nlm.nih.gov/medlineplus/foodnutritionandmetabolism.html

2
Molecular Aspects of Nutrition

Helen M Roche, Herman E Popeijus and Ronald P Mensink

Key messages

- The genome forms the information or blueprint to build up an organism and contains the full complement of genes (genotype) that when expressed determines the phenotype. The genome determines nutritional requirements and metabolic responses. Nutrients can modulate gene expression. These interactions between nutrition and the genome are referred to as *molecular nutrition* or *nutrigenomics*.
- The specific order of nucleotides within DNA forms the basis of genetic information. It is organised into chromosomes and every cell contains the full complement of chromosomes.
- Genetic variation can be the result of DNA alterations or damage that lead to genetic mutations. Genetic polymorphisms are common forms of genetic heterogeneity whereby there are several different forms of the sample allele in a population.
- Gene expression refers to the process whereby information encoded in the genes is converted into an observable phenotype.
- There are several tools to investigate molecular aspects of nutrition: animal models, cell/tissue-culture models, molecular cloning, gene expression analysis [polymerase chain reaction (PCR) and DNA microarrays], protein analysis, stable isotopes and metabolomics.
- Genetic background or common polymorphisms can determine nutrient requirements, the metabolic response to nutrients and/or susceptibility to diet-related diseases.
- Nutrients can interact with the genome and modulate gene expression. Hence, it is possible that nutrients could be used to manipulate an individual's metabolic response or to reduce their predisposition to diet-related diseases.

2.1 Introduction

Our genes determine every characteristic of life: gender, physical characteristics, metabolic functions, life stage and responses to external or environmental factors, which include nutrition. Nutrients have the ability to interact with the human genome to alter gene, protein and metabolite expression, which in turn can affect normal growth, health and disease. The human genome project has provided an enormous amount of genetic information and thus a greater understanding of our genetic background. It is true that we are only beginning to understand how nutrients interact with the genome. This aspect of nutritional science is known as *molecular nutrition or nutrigenomics*.

Molecular nutrition looks at the relationship between the human genome and nutrition from two perspectives. First, the genome determines every individual's genotype (or genetic background), which in turn can determine their nutrient state, metabolic response and/or genetic predisposition to diet-related disease. Secondly, nutrients have the ability to interact with the genome and alter gene, protein or metabolite expression. Gene expression is only the first stage of the whole-body or metabolic response to a nutrient and a number of post-translational events (e.g. enzyme activity, protein half-life, co-activators, co-repressors), but metabolomic events can also modify the ability of nutrients to alter an individual's phenotype. This chapter will review the core concepts in molecular biology, introduce the genome and discuss how we can characterise the effect of nutrition on gene, protein and metabolite expression using state-of-the-art transcriptomic, proteomic and metabolomic technologies, identifying some important research tools used to investigate these molecular aspects of nutrition, such as characterising how genetic background can determine nutrition and health. Some examples of how nutrients regulate gene, protein and metabolite expression will also be explored.

Nutrition and Metabolism, Second Edition, edited by S. A. Lanham-New, I. A. Macdonald and H. M. Roche.

Overall the principal aim of nutrigenomics/molecular nutrition is to understand how the genome interacts with food, nutrients and non-nutrient food components, within the context of nutrition-related diseases. It attempts to determine nutrients that enhance the expression of gene, protein and metabolic pathways/networks that are associated with health and suppress those that predispose to disease. While it is unrealistic to assume that food intake and good nutrition can overcome our genetic fate, good nutrition can improve health and quality of life. Therefore, it is essential that we extend our understanding of the molecular interplay between the genome, food and nutrients; and therefore have a greater understanding of the molecular relationship between diet, health and disease.

2.2 Core concepts in molecular biology

The genome, DNA and the genetic code

The *genome* refers to the total genetic information carried by a cell or an organism. In very simple terms the genome (or DNA sequence) is the full complement of genes. The expression of each gene leads to the formation of a protein, which, together with many other proteins that are coded by other genes form tissues, organs and systems, constitute the whole organism. In complex multicellular organisms the information carried within the genome gives rise to multiple tissues (muscle, bone, adipose tissue, etc.). The characteristics of each cell type and tissue are dependent on differential gene expression by the genome, whereby only those genes are expressed that code for specific proteins to confer the individual characteristics of the cells that constitute each organ. For example, gene expression in muscle cells may result in the formation of muscle-specific proteins that are critical for the differentiation, development and maintenance of muscle tissue, and these genes are completely different from those expressed in osteoblasts, osteoclasts and osteocytes, which form bone. These differentially expressed proteins can have a wide variety of functions: as structural components of the cell or as regulatory proteins, including enzymes, hormones, receptors and intracellular signalling proteins that confer tissue specificity.

It is very important to understand the molecular basis of cellular metabolism because incorrect expression of genes at the cellular level can disrupt whole-body metabolism and lead to disease. Aberrant gene expression can lead to cellular disease when proteins are produced in the wrong place, at the wrong time, at abnormal levels or as a malfunctioning isoform that can compromise whole-body health. Furthermore, different nutritional states and intervention therapies can modulate the expression of cellular genes and thereby the formation of proteins. Therefore, the ultimate goal of molecular nutrition is to understand how nutrients interact with the genome and alter the expression of genes, and the formation and function of proteins that play a role in health and disease.

Deoxyribonucleic acid (DNA) is the most basic unit of genetic information, as the DNA sequence codes for the amino acids that form cellular proteins. Two individual DNA molecules are packaged as the chromosomes within the nucleus of animal and plant cells. The basic structure and composition of DNA are illustrated in Figure 2.1. DNA is composed of large polymers, with a linear backbone composed of residues of the five-carbon sugar residue deoxyribose, which are successively linked by covalent phosphodiester bonds. A nitrogenous base, either a *purine* [*adenine* (A) or *guanine* (G)] or a *pyrimidine* [*cytosine* (C) or *thymine* (T)], is attached to each deoxyribose. DNA forms *a double-stranded helical structure*, in which the two separate DNA polymers wind around each other. The two strands of DNA run *antiparallel*, such that the deoxyribose linkages of one strand runs in the 5′–3′ direction and the other strand in the opposite 3′–5′ direction. The double helix is mainly maintained by hydrogen bonds between nucleotide pairs. According to the *base-pair rules*, adenine always binds to thymine via two hydrogen bonds and guanine binds to cytosine via three hydrogen bonds. This complementary base-pair rule ensures that the sequence of one DNA strand specifies the sequence of the other.

The *nucleotide* is the basic repeat unit of the DNA strand and is composed of deoxyribose, a phosphate group and a base. The 5′–3′ sequential arrangement of the nucleotides in the polymeric chain of DNA contains the *genetic code* for the arrangement of amino acids in proteins. The genetic code is the universal language that translates the information stored within the DNA of genes into proteins. It is universal between species. The genetic code is read in groups of three nucleotides. These three nucleotides, called a

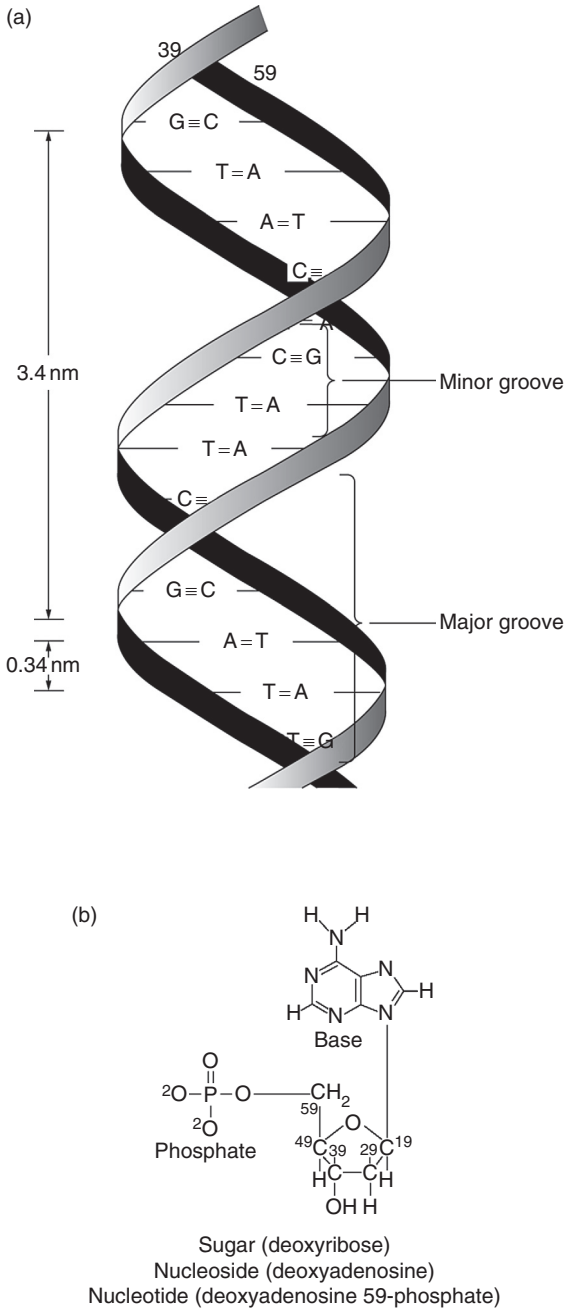

Figure 2.1 Structure and composition of DNA. DNA contains deoxynucleotides consisting of a specific heterocyclic nitrogenous base (adenine, guanine, cytosine or thymine) joined to a deoxyribose phosphate moiety. Adjacent deoxynucleotides are linked through their phosphate groups to form long polynucleotide chains. (a) The DNA double helix; (b) a nucleotide; (c) the purine and pyrimidine bases. (Cox TM and Sinclair J, Molecular Biology in Medicine, 1997. Copyright © Wiley-Blackwell.)

Table 2.1 The genetic code

First base	Second base T	C	A	G	Third base
T	TTT (Phe)	TCT (Ser)	TAT (Tyr)	TGT (Cys)	T
	TTC (Phe)	TCC (Ser)	TAC (Tyr)	TGC (Cys)	C
	TTA (Leu)	TCA (Ser)	TAA (Stop)	TGA (Stop)	A
	TTG (Leu)	TCG (Ser)	TAG (Stop)	TGG (Trp)	G
C	CTT (Leu)	CCT (Pro)	CAT (His)	CGT (Arg)	T

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

codon, are specific for one particular amino acid. Table 2.1 shows the 64 possible codons, of which 61 specify for 22 different amino acids, while three sequences (TAA, TAG, TGA) are stop codons (i.e. do not code for an amino acid). Some amino acids are coded for by more than one codon; this is referred to as *redundancy*. For example, the amino acid isoleucine may be coded by the DNA sequence ATT, ATC or ATA. Each amino acid sequence of a protein always begins with a methionine residue because the start codon (ATG) codes for methionine. The three stop codons signal the end of the coding region of a gene and the resultant polypeptide sequence.

Chromosome (karyotype)

In eukaryotic cells, DNA is packaged as *chromosomes* and every cell contains a set of chromosomes (Figure 2.2). Each chromosome has a narrow waist known as the centromere, which divides each chromosome into a short and a long arm, labelled p and q, respectively. The tip of each chromosomal arm is known as the telomere. DNA is packaged in a very compact structure within the nucleus. Condensing of DNA is essential because the human cell contains approximately 4×10^9 nucleotide pairs, termed *base pairs* (*bp*) of DNA, whose extended length would approach more than 1 m. The most basic unit of the chromosome is the *nucleosome*, which is composed of a 145 bp linear strand of double-stranded DNA wound around a complex of *histone proteins* (H2a, H2b, H3 and H4). Nucleosomes are linked together by the histone protein H1 to form *chromatin*. During

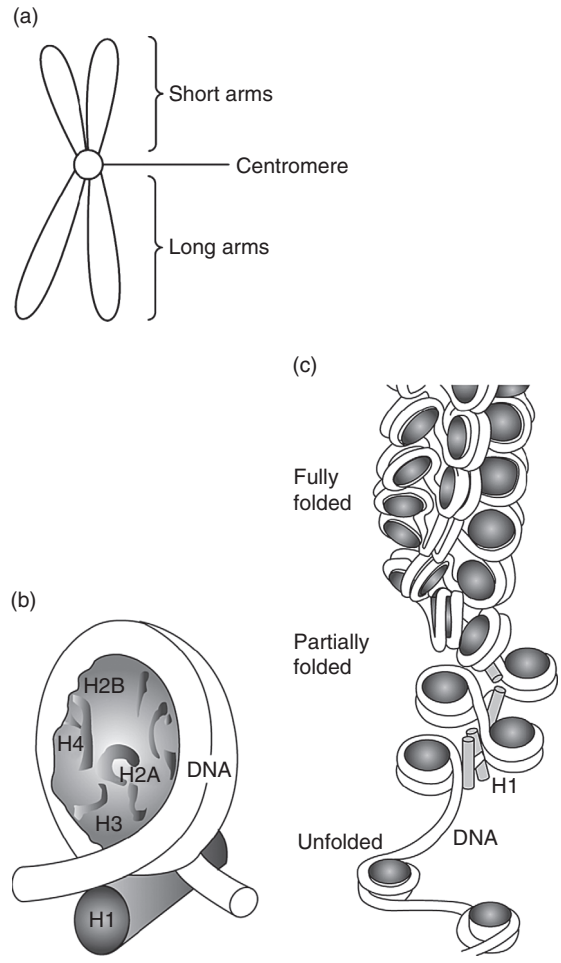

Figure 2.2 Structure of (a) a chromosome, (b) the nucleosome and (c) chromatin. (Cox TM and Sinclair J, Molecular Biology in Medicine, 1997. Copyright © Wiley–Blackwell.)

cell division this is then further compacted with the aid of non-histone chromosomal proteins to generate a chromosome. The structure of DNA in chromatin is important because it has profound effects on the ability of DNA to be transcribed.

The chromosomal complement or karyotype refers to the number, size and shape of the chromosomes. The human karyotype is composed of 22 pairs of autosomes and a pair of sex chromosomes: XX in the female and XY in the male. Most human cells contain 46 chromosomes, the *diploid* number. Chromosomal disorders are characterised by abnormalities of chromosomal number or structure. They may involve the autosomes or the sex chromosomes and may be the

result of a *germ-cell mutation* in the parent (or a more distant ancestor) or a *somatic mutation* in which only a proportion of cells will be affected (mosaicism). The normal chromosome number is an exact multiple of the *haploid* number (23) and is referred to as the diploid number. A chromosomal number that exceeds the diploid number (46) is called *polyploidy*, and one that is not an exact multiple number is *aneuploidy*. Aneuploidy usually occurs when the pair of chromosomes fails to segregate (non-disjunction) during meiosis, which results in an extra copy of a chromosome (*trisomy*) or a missing copy of a chromosome (*monosomy*). Down's syndrome is a common example of trisomy, and is due to the presence of three copies of chromosome 21 (trisomy 21).

Structural abnormalities of chromosomes also occur. A *translocation* is the transfer of chromosomal material between chromosomes. Chronic myeloid leukaemia results from the translocation of genetic material between chromosome 8 and chromosome 22. This results in an abnormal chromosome, known as the Philadelphia chromosome, the expression of which results in leukaemia. *Chromosomal deletions* arise from the loss of a portion of the chromosome between two break points. *Inversions* arise from two chromosomal breaks with inversion through 180° of the chromosomal segment between the breaks.

Genotype, phenotype and allelic expression

The *genotype* of an organism is the total number of genes that make up a cell or organism. The term, however, is also used to refer to alleles present at one locus. Each diploid cell contains two copies of each gene; the individual copies of the gene are called *alleles*. The definition of an allele is one of two (or more) alternative forms of a gene located at the corresponding site (*locus*) on homologous chromosomes. One allele is inherited from the maternal gamete and the other from the paternal gamete, therefore the cell can contain the same or different alleles of every gene. *Homozygous* individuals carry two identical alleles of a particular gene. *Heterozygotes* have two different alleles of a particular gene. The term *haplotype* describes a cluster of alleles that occur together on a DNA segment and/or are inherited together. *Genetic linkage* is the tendency for alleles close together to be transmitted together through meiosis and hence be inherited together.

Genetic polymorphisms are different forms of the same allele in the population. The 'normal' allele is known as the *wild-type allele*, whereas the variant is known as the polymorphic or mutant allele. A polymorphism differs from a mutation because it occurs in a population at a frequency greater than a recurrent mutation. By convention, a polymorphic locus is one at which there are at least two alleles, each of which occurs with frequencies greater than 1%. Alleles with frequencies less than 1% are considered as a recurrent mutation. The alleles of the ABO blood group system are examples of genetic polymorphisms. The acronym *single nucleotide polymorphism* (*SNP*) is a common pattern of inherited genetic variation (or common mutation) that involves a single base change in the DNA. More recently *copy number variation* (*CNV*) has been identified as another common form of genetic variation, it is estimated that about 0.4% of the human genome differ with respect to CVN. As yet CNV has not been associated with susceptibility or resistance diet-related diseases but it is possible that this type of genetic variation may also be linked to nutrition and health. The traditional way of identifying genetic variants was as *restriction fragment length polymorphism* (*RFLP*). RFLPs result in different lengths of DNA fragments when restriction enzymes cleave – or do not cleave – DNA at specific target sites because of nucleotide changes in the DNA sequence at the site where the restriction enzyme would usually cleave DNA.

Epigenetics is a relatively new field of research which refers to changes in gene expression due to mechanisms other than changes in the underlying DNA sequence. The molecular basis of epigenetics is complex; put simply it refers to altered DNA structure. It involves modifications of the activation of certain genes, but not the basic DNA sequence. For example, DNA methylation refers to the addition of methyl groups to the DNA, which in turn affects transcriptional activity. Folate status can affect DNA methylation, which in turn can affect gene expression through mechanisms that are being actively researched.

There is a considerable amount of research investigating the relationships between common genetic polymorphisms and epigenetics with disease because

certain genetic variations may predispose an individual to a greater risk of developing a disease. The effect of genetic variation in response to dietary change is also of great interest because some polymorphisms/epigenetic states may determine an individual's response to dietary change. Hence, genetic variation can determine the therapeutic efficacy of nutritional therapy, which may in turn determine the outcome of certain disease states. The interrelationship between diet, disease and genetic variation will be discussed in more detail in Section 2.5.

The *phenotype* is the observable biochemical, physiological or morphological characteristics of a cell or individual resulting from the expression of the cell's genotype, within the environment in which it is expressed. Allelic variation and expression can affect the phenotype of an organism. A *dominant allele* is the allele of a gene that contributes to the phenotype of a heterozygote. The non-expressing allele that makes no contribution to the phenotype is known as the *recessive allele*. The phenotype of the recessive allele is only demonstrated in homozygotes who carry both recessive alleles. *Codominant alleles* contribute equally to the phenotype. The ABO blood groups are an example of codominant alleles, where both alleles are expressed in an individual. In the case of *partial dominance* a combination of alleles is expressed simultaneously and the phenotype of the heterozygote is intermediate between that of the two homozygotes. For example, in the case of the snapdragon, a cross between red and white alleles will generate heterozygotes with pink flowers. *Genetic heterogeneity* refers to the phenomenon whereby a single phenotype can be caused by different allelic variants.

DNA damage, genetic mutations and heritability (monogenic and polygenic disorders)

Many agents can cause DNA damage, including ionising radiation, ultraviolet light, chemical mutagens and viruses. DNA can also change spontaneously under normal physiological conditions. For example, adenine and cytosine can spontaneously undergo deamination to produce hypoxanthine and uracil residues. A change in the nucleotide sequence is known as a *mutation*. A mutation may be defined as a permanent transmissible change in the nucleotide sequence of a chromosome, usually in a single gene, which may lead to loss or change of the normal function of the gene. A mutation can have a significant effect on protein production or function because it can alter the amino acid sequence of the protein that is coded by the DNA sequence in a gene. *Point mutations* include *insertions*, *deletions, transitions* and *transversions*. Two types of events can cause a point mutation: chemical modification of DNA, which directly changes one base into another, or a mistake during DNA replication that causes, for instance, the insertion of the wrong base into the polynucleotide during DNA synthesis. Transitions are the most common type of point mutations and result in the substitution of one pyrimidine (C–G) or one purine (A–T) by the other. Transversions are less common, where a purine is replaced by a pyrimidine or vice versa.

The functional outcome of mutations can vary very significantly. For example, a single-point mutation can change the third nucleotide in a codon and not change the amino acid that is translated, or it may cause the incorporation of another amino acid into the protein – this is known as a *missense mutation*. The functional effect of a missense mutation varies greatly depending on the site of the mutation and the importance of the protein in relation to health. A missense mutation can have no apparent effect on health or it can result in a serious medical condition. For example, sickle cell anaemia is due to a missense mutation of the β-globin gene: a glutamine is changed to valine in the amino acid sequence of the protein. This has drastic effects on the structure and function of the β-globin protein, which causes aggregation of deoxygenated haemoglobin and deformation of the red blood cell. A nucleotide change can also result in the generation of a stop codon (*nonsense mutation*) and no functional protein will be produced. *Frameshift mutations* refer to small deletions or insertions of bases that alter the reading frame of the nucleotide sequence; hence, the different codon sequence will affect the expression of amino acids in the peptide sequence.

Heritability refers to how much a disease can be ascribed to genetic rather than environmental factors. It is expressed as a percentage, a high value indicating that the genetic component is important in the aetiology of the disease. Genetic disorders can simply be classified as *monogenic* (or single-gene disorders) or *polygenic diseases* (multifactorial

diseases). In general, we have a far greater understanding of the *single-gene disorders* because they are due to one or more mutant alleles at a single locus and most follow simple *Mendelian inheritance*. Examples of such disorders are:

- autosomal dominant: familial hypercholestrolaemia, von Willebrand's disease, achondroplasia
- autosomal recessive: cystic fibrosis, phenylketonuria, haemochromatosis, α-thalassaemia, β-thalassaemia
- X-linked dominant: vitamin D-resistant rickets
- X-linked recessive: Duchenne muscular dystrophy, haemophilia A, haemophilia B, glucose-6-phosphate dehydrogenase deficiency.

Some single-gene disorders show non-Mendelian patterns of inheritance, which are explained by different degrees of penetrance and variable gene expression. *Penetrance* means that a genetic lesion is expressed in some individuals but not in others. For example, people carrying a gene with high penetrance have a high probability of developing any associated disease. A low-penetrance gene will result in only a slight increase in disease risk. *Variable expression* occurs when a genetic mutation produces a range of phenotypes. *Anticipation* refers to the situation when a Mendelian trait manifests as a phenotype with decreasing age of onset and often with greater severity as it is inherited through subsequent generations (e.g. Huntington's chorea, myotonic dystrophy). *Imprinting* refers to the differential expression of a chromosome or allele depending on whether the allele has been inherited from the male or female gamete. This is due to selective inactivation of genes according to the paternal or maternal origin of the chromosomes. Although there are only a few examples of diseases that arise as a result of imprinting (e.g. Prader–Willi and Angelman's syndromes), it is thought that this form of gene inactivation may be more important than previously realised.

Polygenic (or multifactorial) diseases are those due to a number of genes (e.g. cancer, coronary heart disease, diabetes and obesity). Even though polygenic disorders are more common than monogenic disorders, we still do not understand the full genetic basis of any of these conditions. This reflects the fact that there is interaction between many candidate genes, that is, those genes that are thought to play an aetiological role in multifactorial conditions. Furthermore, in polygenic inheritance, a trait is in general determined by a combination of both the gene and the environment.

The Human Genome Project

The Human Genome Project (HGP) is an important source of genetic information that will be a resource to molecular nutrition, especially in terms of understanding the interaction between nutrition and the human genome. The working draft sequence of the human genome was published in February 2001. The project showed that the size of the human genome is 30 times greater than that of the fruit fly and 250 times larger than yeast. Only 3% of the DNA in the human genome constitutes coding regions. Compared with the fruit fly, the human genome has much more non-coding or intronic regions. Although these intronic regions do not code for genes they may have a functional role, and these functional effects could, for example, include important promoter and/or repressor regions. The number of genes coded by the human genome was much less than expected. The human genome codes for approximately 25 000 genes. Indeed, a human has only two to three times as many genes as a fruit fly. The two- to three-fold difference between humans and fruit flies may largely be accounted for by the greater number of control genes (e.g. transcription factors) in the human genome.

In the future, we will have a greater understanding of the human genome and how it interacts with the environment. The HGP showed that humans are very alike: it estimated that humans are 99.8% genetically similar. Nevertheless, the implications of the HGP in relation to molecular nutrition are immense. The challenge is to identify the proportion of that genetic variation that is relevant to nutrition. The term 'gene mining' refers to the process that will identify the new genes involved in nutrition, health and disease. At the most basic level we already know that the human genome determines nutrient requirements, for example gender determines iron requirements – the iron requirement of menstruating women is greater than that of men of the same age. In the case of folate, research would suggest that the methylenetetrahydrofolate reductase (MTHFR) polymorphism could determine an individual's folate requirements. Since there are fewer genes than anticipated, it has been proposed that different isoforms of the same gene with different functionality are important. Already

there are examples of this (e.g. the three isoforms of the apolipoprotein E (Apo E) gene determine the magnitude of postprandial triacylglycerol metabolism). Furthermore, it has been proposed that the interaction between the human genome and the environment is an important determinant of within- and between-individual variation. Within the context of molecular nutrition we will have to determine how nutrients alter gene expression and determine the functional consequences of genetic polymorphisms. With the information generated from the HGP we will have a more complete understanding and information in relation to the relevance of genetic variation, and how alterations in nutrient intake or nutritional status affect gene expression in a way that is relevant to human health and disease processes. In essence, the challenge is to bridge the gap between the genome sequence and whole-organism biology, nutritional status and intervention.

2.3 Gene expression: transcription and translation

Gene expression

Gene expression refers to the process whereby the information encoded in the DNA of a gene is converted into a protein, which confers the observable phenotype upon the cell. A *gene* may be defined as the nucleic acid sequence that is necessary for the synthesis of a functional peptide or protein in a temporal and tissue-specific manner. However, a gene is not directly translated into a protein; it is expressed via a nucleic acid intermediary called *messenger RNA* (*mRNA*). The transcriptional unit of every gene is the sequence of DNA transcribed into a single mRNA molecule, starting at the promoter and ending at the terminator regions. The essential features of a gene and mRNA are presented in Figure 2.3. The DNA sequence of a gene comprises two non-coding (or untranslated) regions at the beginning and end of the gene coding region. The non-coding promoter and terminator regions of the DNA are partially transcribed, but not translated and therefore form the 5′ and 3′ *untranslated regions* (*UTR*) of mRNA. Although the non-coding regions of a gene and mRNA are not translated into the protein product of the gene, they contain critical parts of the genetic information involved in regulation of gene expression and the characteristics of the protein production. The *promoter region* is located immediately upstream of the gene coding region; it contains DNA sequences, known as the TATA and CAAT boxes, which define the DNA binding sites at which transcription starts and regulate the rate of gene expression. The *TATA box* is an AT-rich sequence that occurs about 30 bp (−30 bp) upstream from the transcriptional start site. The *CAAT box* contains this short DNA sequence about 80 bp upstream (−80 bp) of the start site. These sequences, together with binding sites for other *transcription factors*, regulate the rate of tissue-specific gene expression. Transcription starts at the *CAP site*, so-called because following transcription the 5′ end of the mRNA molecule is capped at this site by the attachment of a specialised nucleotide (7-methyl guanosine). The CAP site is followed by the *initiation*, or *start codon* (ATG), which specifies the start of translation; hence, according to the genetic code every polypeptide begins with methionine. The DNA coding sequence for a gene in eukaryotes is not contiguous or uninterrupted. Each gene contains DNA sequences that code for the amino acid sequence of the protein, which are called *exons*. These exons are interrupted by non-coding DNA sequences, which are called *introns*. The last exon ends with a *stop codon* (TAA, TAG or TGA), which represents the end of the gene-coding region and it is followed by the terminator sequence in the DNA sequence that defines the end of the gene-coding region. The *3 UTR* of the mRNA molecule includes a *poly*(*A*) *signal* (*AATAAA*) that is added to the mRNA molecule following transcription.

Ribonucleic acid

RNA, like DNA, carries genetic information. The composition of RNA is very similar to DNA, and it plays a key role in all stages of gene expression. RNA is also a linear polynucleotide, but it differs from DNA in that it is single stranded and composed of polymers of ribose rather than deoxyribose, the pyrimidine base *uracil* (*U*) replaces thymine (T), and it is relatively unstable when compared to DNA. There are at least five different types of RNA in eukaryotic cells and all are involved in gene expression:

- Messenger RNA (mRNA) molecules are long, linear, single-stranded polynucleotides that are direct copies of DNA. mRNA is formed by transcription of DNA.
- Small nuclear RNA (snRNA) is a short, ±150 nucleotide (nt) RNA molecule that forms, together with