

## Nutrition and Gene Expression

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Altering the expression of genes has become a rapidly evolving area of research in medicine. The realization that gene expression is important in a wide range of diseases, and not just in inherited disease, has resulted in recognition of the whole field of gene expression as one that may bring new therapeutic options. Although more recent attention has focused on the benefits of altering gene expression by inserting new genetic material into cells by a variety of vectors, the expression of genes can also be altered by other means, most notably by changing the molecular environment that cells inhabit. Utilizing the natural responses of a cell to changes in its surroundings offers a new and amenable way to alter the expression of its genes. Many ways of altering these surroundings can be proposed, but no single act alters the environment of the cells of the body more than the ingestion of food. Thus, the future of nutrition as a therapeutic tool may lie in its potential for influencing gene regulation. This chapter will examine this emerging field and will lay down some concepts that may prove useful in establishing the scientific basis from which future treatments may develop.

The survival of a child to reproductive age and beyond requires an ability to respond to external demands. Every organ in the body is attuned to this need. Many organ systems have two levels of response to external changes. There is a rapid response, often occurring within seconds of a new stimulus, for example, contraction of muscle fibers following a neuronal impulse or the breakdown of glycogen by the liver during hypoglycemia. Such responses do not involve changes in gene expression. The cells maintain themselves in a state of readiness, responding rapidly to external stimuli by altering protein/ion activity. Behind this immediate response, there lie other slower, but more lasting, responses that require genetic control. For example, exercise on a regular basis not only leads to muscle mass increase, but change in activity of the attendant enzymes that serve the increased metabolic need also occurs. Similarly, regular exposure of the liver to drugs induces the expression of enzymes that catalyze their breakdown.

There are few external stimuli on a child more important than his or her nutritional environment. The metabolic processes underlying the rapid response of cells to nutritional variations have long been documented in humans and other

mammals.<sup>1</sup> However, the mechanisms whereby gene expression changes in response to nutritional stimuli is still poorly understood in humans or indeed in any multiorgan animal. This is, at first, surprising because in bacteria, the study of nutritional changes led to our understanding of some of the most fundamental mechanisms of gene expression. The elucidation of the induction of proteins that transport and hydrolyze lactose (the *lac* operon) after adding lactose to bacterial culture media<sup>2</sup> was the first examination of any form of gene regulation. These observations spawned an explosion of research in other regulatory genes in bacteria and in unicellular, eukaryotic organisms such as yeast. The upregulation of the bacterial genes that handle tryptophan when this amino acid is scarce (the *trp* operon) has become another well-understood example of nutrient–gene interaction.<sup>3</sup>

Progress in the study of nutrient–gene interaction in eukaryotic cells has been slower for two main reasons. First, the molecular mechanisms controlling gene expression are more complex than in bacteria, and second, it is more difficult to identify the metabolites of nutrients that may be responsible for inducing such changes. This review will therefore cover some of the major advances in the study of nutrition and gene expression in the human and, where necessary, in other mammals. Nutritional changes ultimately impinge on most cells in the body; however, it is the epithelium of the gastrointestinal (GI) tract that first encounters any variation in nutrient intake. Much of this chapter will therefore concentrate on how nutritional factors can alter the expression of genes in intestinal epithelial cells. The relevance of nutrient–gene interactions to human physiology will also be stressed. Finally, because manipulating nutritional intake may be a way of treating disease in children, the review will discuss nutritional therapy in childhood in the light of its effects on gene expression.

### PHYSIOLOGIC IMPORTANCE OF NUTRITIONAL REGULATION IN GENE EXPRESSION

The effect of nutrients on gene expression may have different implications in different individual situations (Table 1). First, genes may be upregulated to better utilize the supply of a particular

**Table 1 Physiologic Importance of Nutritional Regulation of Gene Expression**

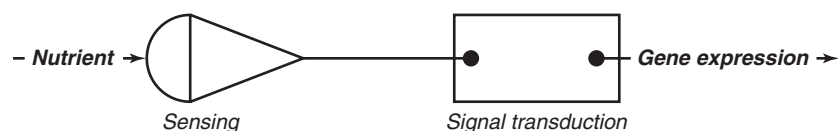
To satisfy the nutritional needs of an organ
To manage storage fuels required by other organs
Production of hormones essential to whole body metabolism
Direct interaction with the body's external environment along the GI tract

nutrient when it is scarce. Transporters of nutrients and the enzymes that metabolize them are examples of proteins that may be induced by nutrients. Second, the expression of genes required for the storage of a particular nutrient may be altered according to that nutrient's abundance. Third, nutrients regulate the secretion of hormones that control the homeostasis of metabolic processes. For example, insulin synthesis increases after increased carbohydrate intake to maintain glucose homeostasis. Finally, food is part of our external environment and, as such, represents a challenge to the cells that come into intimate contact with it. This challenge is met in the main body by the epithelial cell lining of the GI tract.<sup>4</sup> The ability of these cells to alter the expression of their genes with changes in food intake is one of the ways by which the intestinal epithelium senses and modulates the intestinal environment.

Certain fundamental characteristics are found in the mechanisms that underlie each of these different aspects of nutrient–gene interactions. They include (Figure 1) a specific interaction between the cell and a particular nutrient (sensing) and a pathway by which such an interaction may translate into alterations in gene expression (signal transduction). We have little understanding of these mechanisms at present. However, some aspects of the molecular biology of these two functions will be examined later in this chapter.

### Genes Controlling the Nutritional Requirements of Individual Organ Systems

Survival of cells depends on their ability to extract nutrients and other essential molecules from their surroundings, and they synthesize proteins specifically for this purpose. Broadly speaking, two categories of genes are involved: those genes that express proteins that transport nutrients across cell membranes and those genes that express enzymes that metabolize nutrients. In



**Figure 1** The regulation of gene expression by nutrients requires two generic mechanisms. First, molecules (either receptors or enzymes) within the cell must recognize the nutrient. Second, this interaction must initiate a sequence of events that ultimately alters the transcription or translation of genes. These functions of sensing and signal transduction have been depicted in this diagram using electronic symbols.

both cases, genes are regulated purely to satisfy the requirements of an organ system. This form of gene regulation is similar in concept to the regulatory genes of bacteria like the *trp* operon.<sup>3</sup> The cells are changing their phenotype to their own advantage, rather than to suit the needs of the body as a whole.

A good model of how cells may respond to their nutritional environment for their own needs is the utilization of glutamine by skeletal muscle.<sup>5</sup> Glutamine availability modulates muscle turnover, and glutamine is an important source of amino nitrogen for muscle cells.<sup>6</sup> Glutamine and glutamate are transported into cells by sodium-coupled transporters. For experimental purposes, rat skeletal muscle can be grown as primary cell cultures. These cells exhibit an upregulation in intracellular transport of both glutamine and glutamate when they are deprived of an exogenous glutamine supply.<sup>7-9</sup> In addition, the activity of glutamine synthetase (GS) simultaneously increases. This enzyme catalyzes the addition of an amino group to the carboxyl moiety of the glutamate molecule to form glutamine. Two different nutrients, glutamine and glutamate, can therefore ultimately satisfy the glutamine requirements of the muscle cell through separate pathways, each of which is under genetic control. This system exemplifies how different nutrients can cross-stimulate the expression of a related set of proteins: Removal of glutamine or glutamate from the cell medium results in an enhancement of the transport of both amino acids. Not only does lack of glutamine and glutamate enhance the expression of their own transport proteins, but deprivation of either substrate also upregulates the transporter of the other.<sup>8</sup> A study of the kinetics of transport<sup>9</sup> demonstrated that withdrawal of glutamine (or glutamate) enhanced the maximum rate at which the amino acid is transported into the cell ( $V_{\max}$ ), without altering the affinity of the transporter for its amino acid. This suggests that deprivation resulted in an increase in the production of the number of transporters available. The time required to double the  $V_{\max}$  was 4 hours. This fact, taken together with the observation that the induction of both glutamate and glutamine transporters was lost when glutamine was withdrawn in the presence of actinomycin D (inhibitor of RNA synthesis), indicates that their regulation is mediated through the initiation of transcription.

In recent years there has been an explosion in our understanding of the physiology of glutamine transporter systems.<sup>10</sup> At least four distinct

families have been identified to date, which include the  $\text{Na}^+$ -dependent glutamine transporter, system N (SN1), system A (ATA1 and ATA2), system ASC/B, and system y (+)L, the latter encoding for  $\text{Na}^+$ -independent glutamine transporter genes.<sup>11,12</sup> Molecular characterization of these systems suggest specific roles in glutamine efflux and uptake<sup>12</sup>; however, the exact mechanism(s) by which transport regulates gene expression remain unclear.

In vivo, glutamine homeostasis is to a large extent determined by the activities of GS and glutaminase.<sup>13</sup> Both enzymes exhibit regulation of gene expression at both transcriptional and post-transcriptional levels. During stress and sepsis, glutamine production is enhanced primarily due to greater GS activity in the skeletal muscle.<sup>14</sup> Beneficial effects of glutamine during sepsis include amelioration of endotoxin-induced chemokine production, a process that is associated with signal transducer of activator of transcription-4 (STAT-4) transcription activity.<sup>15</sup>

Although glutamine regulates the expression of health-promoting proteins in the gut epithelia,<sup>16</sup> overall the glutamate/glutamine circuit constitutes only a minor part of nutrient homeostasis in vivo. Glucose, on the other hand, is central to human metabolism. Glucose, galactose, and fructose are the major dietary saccharides and the derangement of glucose handling in diabetes mellitus constitutes the most common condition requiring nutritional advice. Intensive research in the last two decades has revealed the great complexity involved in glucose transport. Glucose is transported into enterocytes primarily by the sodium-glucose cotransporter (SGLT-1). Two  $\text{Na}^+$  ions must first bind to the transporter and then one molecule of glucose or galactose may be actively transported into the cell. To date, 11 members of the SGLT-1 family in humans have been identified on the basis of sequence homology within the human genome.<sup>17</sup> Efforts to curb the ever-burgeoning epidemics of diabetes and obesity have made dietary manipulation and therefore the SGLT family is an attractive therapeutic target.<sup>18</sup> One must also not forget that oral rehydration therapy (ORT) developed to treat diarrheal dehydration is intimately linked with carbohydrate digestion. The therapy relies on the ability of SGLT-1 to cotransport water without affecting  $\text{Na}^+$  or glucose uptake. In addition to SGLT proteins, facilitative diffusion glucose transporter (GLUT) proteins catalyze glucose transport into cells. Several mammalian GLUT proteins have been identified, each a separate gene product with unique properties.<sup>19</sup>

GLUT-1, -3, and -4 can transport glucose and galactose but not fructose, GLUT-2 can transport all three, whereas GLUT-5 and -7 recognize fructose.<sup>19-21</sup> The GLUT family can be divided into three groups: class I (the previously known glucose transporters GLUT1-4), class II (GLUT-5, -7, -9, and -11), and class III (GLUT-6, -8, -10, and -12 and the myo-inositol transporter HM1T1). The various members exhibit both tissue and subcellular specificity, which most likely represents another level of regulation. (See reference 22 for a recent review.) Analysis of GLUT protein levels in patients with non-insulin-dependent diabetes mellitus revealed a four- to fivefold induction in SGLT-1, GLUT-2, and GLUT-5 protein levels, highlighting dysregulation in monosaccharide transport.<sup>23</sup> Identity of mechanism(s) by which these essential dietary carbohydrates directly affect their own uptake (via availability of GLUT and SGLT proteins) is most urgent if we are to understand the marked increase in the incidence of childhood obesity and diabetes facing the developed world.<sup>22,24</sup>

### Genes Regulating the Storage of Nutrients

The storage of nutrients is essential to provide energy in times of fasting, and the mechanisms that control this function must be regulated by nutrient intake to be effective. The two most important organs involved in nutrient storage are the liver and adipose tissue. Both cell types transport glucose and other nutrients and convert them into fat; in the case of the liver, glucose is also converted to glycogen. Insulin is the major hormone that promotes glucose utilization and induces storage. Its sensitive response to dietary intake complicates the study of the direct effects of nutrients on genes that regulate storage. The direct effects of glucose, in particular, on the expression of transporters and metabolic enzymes have to be dissected from the effects of insulin on these same genes. (The action of hormones, such as insulin, on target organs is beyond the scope of this review, but it is well covered by a number of excellent articles within the field of endocrinology.)<sup>25,26</sup> Experiments performed in vivo make the distinction between the direct effects of nutrients and those mediated through hormonal changes difficult to achieve.

The molecular mechanism(s) by which glucose directly modulates the transcriptional regulation of lipogenic enzymes was until recently poorly understood. In 2001 Kawaguchi and coworkers identified a hepatic transcription factor carbohydrate response element-binding protein (ChREBP), which is regulated at two different levels: nuclear entry and DNA-binding via phosphorylation events mediated by glucose and cyclic adenosine monophosphate (cAMP).<sup>27,28</sup> Earlier studies investigating the direct contribution of glucose toward increased nutrient storage in the liver have focused on the three main control points of glucose metabolism before it enters the mitochondrion as pyruvate. These are the enzymes glucokinase (GK),

6-phosphofructokinase (PFK), and L-type pyruvate kinase (PK). Fructose is also metabolized to pyruvate, bypassing the first two enzymes. PK is therefore a regulatory enzyme in the metabolism of both monosaccharides. The transcription factor ChREBP was purified by its ability to bind to the glucose-carbohydrate response elements present in the PK promoter sequence.<sup>27</sup>

Both in vitro and in vivo studies have identified a second critical transcription factor, sterol regulatory element-binding protein (SREBP) in insulin-mediated transcriptional control of genes involved in glucose, fatty acid, and triglyceride metabolism.<sup>29</sup> The involvement of at least two transcription factors (ChREBP and SREBP) for optimum lipogenesis may reflect the degree of molecular complexity required for coordinated integration of hormonal and nutritional signals in regulating energy requirements of a cell.<sup>30</sup> More recent studies have identified TFE3/Foxo1 and SREBP-1c to reciprocally regulate insulin sensitivity in the liver.<sup>31</sup>

Insulin has a major effect on the activity of the three glycolytic enzymes via transcriptional and/or posttranslational modifications. GK mRNA expression is induced by insulin via an insulin receptor substrate (IRS)-1-phosphatidylinositol-3(P13)-kinase-dependent pathway.<sup>32</sup> P13-kinase and p44/p42 mitogen-activated protein kinase (MAPK) activities play a role in the transient activation of PK by insulin.<sup>33</sup> However, glucose itself independently alters the expression of PK by increasing the transcription of its mRNA.<sup>34</sup> Glucose similarly enhances the expression of fatty acid synthetase (FAS), a key enzyme in lipogenesis. GK regulates the flow of substrate to PK. Because GK requires insulin for its activity, it has been difficult to study the independent effects of glucose and its metabolites on PK expression. Nevertheless, two separate approaches directly demonstrate the effects of glucose on PK expression. The first approach was to demonstrate that the effects of insulin on transcription of PK mRNA was glucose dependent, thus identifying glucose as a separate variable.<sup>35</sup> The second approach examined a particular hepatoma cell clone (mhAT3F), whose GK (which is insulin dependent) had been replaced through spontaneous mutation by hexokinase (which is not insulin dependent). This allowed the effects of PK to be examined in the absence of insulin.<sup>36</sup> Several signaling pathways that may play a key role in glucose-dependent *L-PK* gene transcription are now known (for reviews, see references 31 and 37). The glucose response elements (GREs) in the promoter region of the *L-PK* gene interact directly or indirectly with various families of transcription factors, including the Sp1 family and the upstream stimulatory factors (USFs).<sup>38</sup> The complex interactions between the various factors (USFs, chicken ovalbumin upstream promoter transcription factor II [COUP-TF II], and ChREBP) is likely to act as a “glucose-sensing” mechanism, allowing the cell to

respond appropriately to continuous variation in its nutritional status.<sup>31,37</sup>

### Nutrient Stimulation of Hormone Secretion

**Insulin.** In children, hormones are secreted into the circulation in response to nutritional stimulation for two main reasons: (a) to effectively store nutrients in appropriate storage compartments, and (b) to signal to tissues that substrate for growth is available, thus coordinating cellular manifestations of growth (cell proliferation, differentiation, and controlled cell death) with the influx of nutrients. These two actions are mutually compatible, and a number of hormones and growth factors serve both functions to varying degrees. Insulin, for example, is mainly a storage hormone, but it is also able to stimulate growth; the increased weight of babies born to diabetic mothers is due to increased fetal insulin acting as a growth hormone (GH). Similarly, factors primarily responsible for growth, such as insulin-like growth factor 1 (IGF-1), which mediates many of the actions of GH, are able to vary the rate of glucose uptake into cells.

The secretion of insulin by the pancreatic islets is tightly controlled by glucose concentrations in the blood. Alterations in gene expression play no part in these rapid responses to glucose. Regulatory proteins within islet cells are ready to respond to increases in glucose. Release of insulin from storage granules occur following calcium influx, which in turn is probably induced by minor increases in ATP from the metabolism of glucose and other nutrients. Circulating hormones also affect insulin release. The quick-acting mechanisms that do not involve control lie beyond the scope of this review. Again, the reader is referred to several excellent reviews on the subject.<sup>39-41</sup>

In addition to these rapid responses, glucose also exerts a more lasting effect on insulin production through increased translation and transcription of the insulin gene. This allows a child to adapt to longer periods of starvation or carbohydrate repletion. Glucose enhances the rate of translation of proinsulin mRNA to protein by three methods.<sup>42</sup> It increases the transfer of initiated insulin mRNA from free to membrane-bound ribosome. It also decreases the rate of pausing of the proinsulin mRNA as it passes along the ribosome. Lastly, glucose directly stimulates the cellular machinery involved in the elongation of proteins in pancreatic islet cells.<sup>43,44</sup> Long-term changes in insulin production are also mediated by transcription. Rats fasted over 4 days have low insulin mRNA concentrations, which return to normal on refeeding.<sup>45,46</sup> These changes control the ability of the pancreas to secrete insulin during dietary manipulations, and they enable the islet cell to adapt to long-term dietary changes.

In addition to whole animal studies, pancreatic islet cell preparations have also been used to study how glucose alters insulin expression. Interesting information regarding signal transduction from nutrients to the initiation of transcription has been gleaned from this system (to

be discussed). The studies on insulin expression complement those on the role of glucose in PK and FAS expression.

**IGF-1 and IGF-Binding Proteins.** Although we do not fully understand the mechanisms whereby children whose nutritional intake is reduced beyond a certain point stop growing, it is certainly true that nutrient intake has a significant effect on final adult height. This is common knowledge in developed countries with large immigrant populations from the developing world. The children in the immigrant communities grow up to be taller on average than their parents, whose childhoods may have been spent in lands where food was less abundant. Poor linear growth also occurs in childhood diseases such as cystic fibrosis (CF). In inflammatory diseases, such as Crohn’s disease (CD) or juvenile rheumatoid arthritis, the characteristic rate of growth may, in part, be related to poor nutritional intake,<sup>47,48</sup> as well as to the direct effects of intestinal inflammation.<sup>49,50</sup>

IGF-1 and -2 are a family of polypeptide growth factors whose functions include mediation of GH action, stimulation of insulin activity, and autocrine regulators of cellular proliferation.<sup>51,52</sup> IGF-1 has been shown to be a key player in regulating organ and body growth during postnatal development both in rodents and in humans.<sup>53</sup> There is now compelling evidence that IGF-1 secretion may be the point at which regulation of growth is controlled both in health and in disease.<sup>54,55</sup> Although IGF is an important modulator of GH action, GH itself does not correlate with depressed linear growth under nutritional restriction<sup>56</sup> or in children with active inflammation.<sup>57</sup> Alterations in tissue responses to GH may therefore mediate the effects of nutrition on growth. This has resulted in two separate lines of investigation:

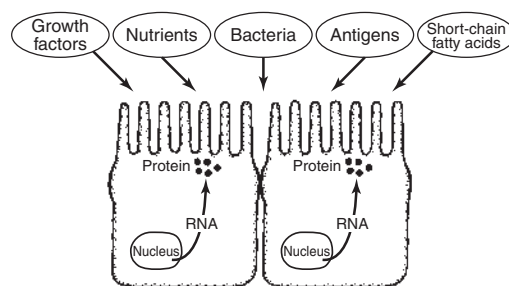
1. The effect of nutritional alterations on expression of the GH receptor.<sup>58,59</sup>
2. The study of nutritional factors on IGF-1 and -2 secretion in association with secretion of IGF-binding proteins (IGFBPs).

Nutritional status is a major determinant of IGF-1 mRNA expression in liver and nonhepatic tissues. Fasting, caloric restriction animal model studies all show a decrease in circulatory IGF-1 levels,<sup>60</sup> although circulating levels of GH remain elevated. Although fasting reduces the binding of GH to its receptor,<sup>61</sup> downregulation of the GH receptor does not occur with dietary restriction,<sup>62</sup> and it is believed that neither of the above are principal causes of reduced IGF-1. Further, administration of IGF-1, but not GH, stimulates mucosal hyperplasia in surgically stressed rats with intestinal atrophy induced by hypocaloric total parenteral nutrition.<sup>63</sup> Low protein intake studies in a liver-specific IGF-1-deficient mouse model led to a decrease in nonhepatic IGF-1 secretion into the circulation, with an increase in GH levels. Increased IGFBP-3 mRNA was also observed, which supports the hypothesis that the binding protein may contribute to greater sequestration of locally synthesized IGF-1.<sup>64</sup> These

studies support the view that the splenic GH/IGF-1 axis responds to nutritional stress to maintain tissue homeostasis.

Regulation of IGF mRNA expression is complex. The rat *IGF-1* gene contains six exons spanning 100 kb of DNA. Exons 1 and 2 encode alternative amino terminal sequences of the IGF-1 protein. The gene has two distinct promoter regions upstream of exons 1 and 2, which can be separately activated.<sup>65</sup> After transcription, alternative splicing results in further heterogeneity of the IGF-1 mRNA. Furthermore, there are two different terminal poly-A addition sequences in exon 6, adding to the general complexity. However, these myriad possibilities result in only three different-sized mRNA transcripts: a 3.8 kb, a 4.2 kb, and an 8 kb transcript. The importance of these different mRNAs to IGF-1 production and to growth awaits further investigation, but they are of interest because several studies have demonstrated that different forms of malnutrition affect the transcripts differently.<sup>51,52,66</sup> Total energy restriction induces a coordinate decrease in all three forms of mRNA. In contrast, protein restriction results in a profound decrease in the 8 kb fragment with less effect on the two smaller fragments.<sup>51,66</sup> These observations open up two questions of further interest: (1) How do different nutrients differentially affect gene expression in a particular family of genes? (2) How do these differences in gene expression translate into differences in a child's final phenotype? We will be returning to similar questions when we consider the small intestinal epithelium, where nutritional variations are greater than those seen in the circulation. Corresponding changes in IGF-1 have been seen in childhood illness. IGF-1 concentration correlates with increased intake and subsequent growth of children with CD, whereas growth has no correlation with GH production.<sup>52,57</sup> Children with CF frequently manifest hormonal abnormalities that may contribute to malnutrition. Interestingly in a study by Bucuvalas et al no effect of exogenous IGF-1 on linear growth in prepubertal children with CF was found, although an increase in glucose/insulin ratio was observed.<sup>67</sup>

In extracellular fluids, IGF-1 is bound to IGF-BPs, of which six have been cloned and characterized.<sup>51,52</sup> IGF-BP-3 is the main carrier of IGF-1 and in the presence of an acid-labile subunit, a bio-inactive ternary complex is maintained during circulation. The complex allows greater control on IGF-1 bioavailability.<sup>68</sup> IGF-BP-3 may also modulate IGF-1-stimulated DNA synthesis.<sup>69</sup> The biological function of each binding protein is under intense investigation.<sup>70</sup> Protection from the hypoglycemic effects of IGF-1 has also been suggested as a function. Nutritional factors alter the circulating levels of IGF-BPs. There is an inverse correlation of the expression of binding proteins with that of IGF-1. Protein restriction results in an increase of IGF-BP-1, which falls on refeeding. However, IGF-BP-1 is sensitive to insulin, and it is not clear how nutrients in the liver alter their expression directly.



**Figure 2** The luminal factors that influence gene expression in the enterocyte. Factors can affect individual genes or they may influence enterocyte differentiation and new cell lineages.

Interestingly, IGF-BPs are predominantly secreted by Kupffer cells,<sup>71</sup> unlike IGF-1, which is synthesized by hepatocytes. The difference between these two cell types in terms of their location and their expression of surface receptors could allow for subtle regulation of IGF action in the face of nutrients and other factors in the circulation, such as cytokines.

### Effect of Intestinal Contents on Genes in the Epithelium

The GI tract is the only part of the body that normally comes into contact with nutrients before they are absorbed. The GI tract is therefore exposed to a wider variety of nutrient molecules than any other organ of the body. The picture is further complicated because the lumen of the intestine is not a direct reflection of the food ingested. It also contains bacteria and their by-products and factors secreted into lumen in response to the ingestion of food (Figure 2). The study of nutrient effects on the enterocyte should therefore consider how changes in diet may affect the area around the apical aspect of a particular epithelial cell. The dissociation between nutrients ingested and the changes observed in the bowel lumen becomes greater the further one proceeds down the GI tract. The contents of the distal colon are completely different from food, although even here they are affected to some extent by dietary intake. This relationship between ingested nutrients and the local environment of the lumen is a separate issue from the interaction of that local environment with genes in the enterocyte.

It has been traditional to assume that the expression of genes in the small intestinal epithelium is preprogrammed and that their expression is not influenced by events in the lumen of the intestine. However, this view may be incomplete. An alteration in epithelial cell phenotype secondary to nutritional factors would have three possible advantages (Table 2). First, the intestine could adapt to absorb nutrients more effectively if specific digestive enzymes and transporters of the epithelium were upregulated by the repeated intake of a particular nutrient. Second, as all mammals are fed from mother's milk, the opportunity exists for breast milk to influence the development of the epithelium through actions of

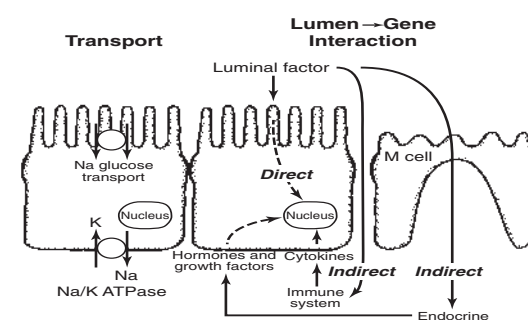
**Table 2** Relevance of Dietary Regulation of Enterocyte Gene Expression

Intestinal adaptation
Influence of maternal breast milk
Signaling from lumen to mucosal immune system

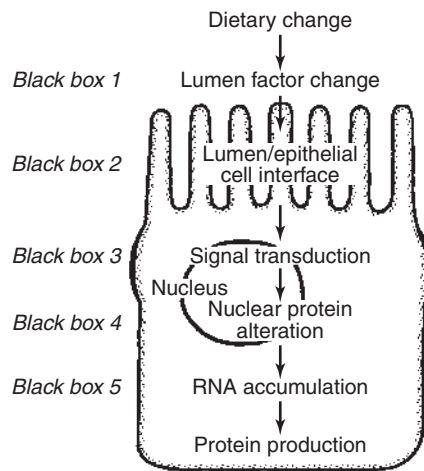
its own constituents. Third, if the genes affected in the epithelium were immunologically important, the intestinal epithelium could influence mucosal immune responses by signaling information to the mucosal immune system and beyond through changes in the expression of epithelial cell genes. Each of these areas is likely to represent important physiologic mechanisms that have implications for child health.

### Polarity of Epithelia

The cell types that we have discussed earlier in this chapter do not depend on a separation of functions to different cellular poles. For example, the pancreatic islet receives signals from the entry of glucose at any point on the plasma membrane, and as far as we can judge, the insulin response is not affected by the site of glucose entry. Insulin can also be released in any direction, eventually diffusing into the circulation. Cells that form epithelia are different in that they exhibit polarity. This separation between the apical side (bordering the lumen in the case of the intestinal epithelium) and the basolateral side is central to epithelial activity. This property is well recognized in the field of intestinal transport. Ions,<sup>72</sup> small molecules,<sup>22,73</sup> and macromolecules<sup>74</sup> are all transported differently across the apical membrane than across the basolateral membrane (Figure 3). It is the polarity of the epithelium that gives direction to the movement of these substances across the epithelium into or out of the body. But this property must also be considered when one is examining all aspects of intestinal epithelial cell function. Polarity is also of fundamental relevance in the study of nutrient-gene interactions in the intestinal epithelium. The polarity of the epithelial cell distinguishes the two major mechanisms by which nutrients (and other luminal factors) affect genes: (1) a direct effect on enterocytes and (2) an indirect effect mediated through hormones, growth factors, and cytokines (Figure 3). As with the effects



**Figure 3** Dietary changes may affect gene expression directly (being mediated through the apical membrane) or indirectly (through the basolateral membrane).



**Figure 4** The pathway connecting dietary alterations to changes in gene expression is not well understood. It is likely that the pathway is mediated by a series of steps that, at present, we can regard only as black boxes. Different luminal events will influence gene expression through different series of black boxes.

of insulin on target tissues, the mechanism(s) of action of cytokines and growth factors on the epithelium is beyond the scope of this chapter. The reader is again referred to excellent reviews on how epithelial cell expression can be regulated by a multitude of agents.<sup>22,75,76</sup>

The apical membrane mediates the direct effects of luminal factors on the epithelium. The functions of nutrient recognition (sensing) and signal transduction (see Figure 1) will therefore lie between the apical membrane and the apparatus of gene expression. The exact molecular mechanisms involved require further investigation. For purposes of investigation they can be considered as a series of black boxes connecting the lumen and gene expression (Figure 4). These black boxes contain the mechanisms that regulate gene expression and constitute the way by which molecules in the lumen “cross-talk” with the nucleus of the enterocyte. Figure 4 depicts the pathway linking single factors in the lumen to the expression of a single cell in a single gene. This is, of course, a gross oversimplification. As we have mentioned, an alteration in diet may alter different factors at the cell surface, and any one of these factors may have an effect on several different genes through different pathways (Figure 5). In addition, method(s) by which a particular protein may be expressed is not necessarily always due to the induction of a single gene in cells along the crypt–villus axis.<sup>22,77</sup> It can also be due to changes in the state of differentiation of the cells in the epithelium, the expression of a particular gene being just one component of that change. Lastly, alterations in luminal contents may not necessarily affect epithelial cells that are already formed along the epithelium, but instead result in the generation of new lineages of cells that express the gene arising from the crypt.<sup>77</sup> In this case, the mechanisms linking the lumen to gene expression would lie in the stem cell at the base of the crypt, rather than in the individual enterocyte itself.

### Adaptation of Enterocytes to the Nutritional Environment

The efficient utilization of food requires digestion, followed by absorption of digestion products and their release into the circulation. Adaptation is, by definition, the upregulation of one or all of these functions in the presence of a relevant nutrient. Adaptive mechanisms are more likely to be associated with foods whose intake varies between different individuals. The similarity here with prokaryotic cells is clear. Bacterial gene regulation was discovered in the study of enzymes that metabolized nutrients whose abundance varied widely in the environment, like lactose or tryptophan. For children, of course, there are many foods whose abundance can vary, but to illustrate this section we have chosen two: sucrose and dietary fat. These two examples demonstrate different aspects of adaptation: Digestion and absorption of sucrose is controlled entirely by the enterocyte itself; in contrast, fat digestion is primarily controlled by enzymes secreted from the pancreas and gastric glands.

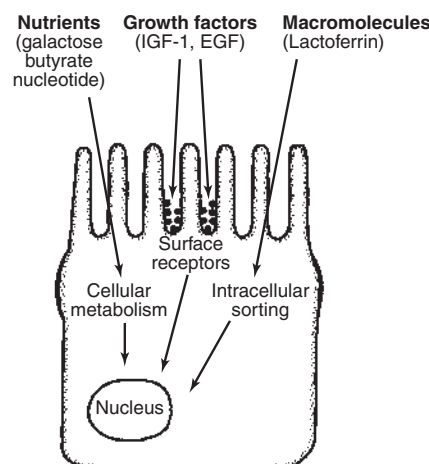
**Sucrose Intake.** The intake of sucrose can vary greatly between individual human beings. This is especially true when one compares individuals from different times in history. Until the industrial revolution, sucrose was present in the diet only when brought from the East as part of the spice trade, and its availability was limited. The introduction of plantations in the Caribbean in the eighteenth century, coupled with the industrial process of refining, resulted in an explosion of sucrose intake. In England in 1700, the estimated per capita daily consumption in adults was 4.5 g, rising to 37 g in 1800. By 1968 it had reached 140 g daily, which is around 15% of the total energy derived from food.<sup>78</sup> Sucrose is digested by the epithelial cell brush border, where it is hydrolyzed to fructose and glucose by sucrase isomaltase (SI). Fructose is absorbed by the carrier GLUT family members<sup>22</sup> and glucose by the specific sodium-dependent glucose transporter. Both sugars exit the cell through the basolateral membrane by facilitated diffusion via GLUT-2. The upregulation of any of these proteins by

their substrate would constitute a nutrient–gene interaction.<sup>22,79</sup>

The brush-border disaccharidases represent the final step in the digestion of carbohydrates.<sup>80</sup> Their activity can affect the glycemic response to oral carbohydrate.<sup>81</sup> More importantly, low levels of hydrolase activity in the face of carbohydrate in the intestine result in osmotic diarrhea.<sup>82</sup> A mechanism whereby expression of these enzymes could be linked to sugar intake would therefore have clear benefits. SI is one of three  $\beta$ -glucosidases (together with maltase and trehalase). The remaining disaccharidase, lactase, is a  $\beta$ -galactosidase. Sucrase is anchored to the microvillus membrane of the enterocyte by the isomaltase subunit of the SI protein.<sup>22,83</sup> Both subunits are synthesized as a single protein that is later cleaved.

The regulation of the induction of sucrase is not simple. The timing of its appearance during ontogeny in rodents was elegantly shown to be independent of luminal contents.<sup>84</sup> When the investigators transplanted isografts into animals 5 days younger than the donors, sucrase appeared earlier in the transplanted intestine than in the host. Therefore, luminal factors in this case did not induce sucrase nor did circulating factors in the host (whose intestine would have been susceptible to them). This evidence suggests that sucrase is controlled by an internal biologic clock within the intestine itself. However, luminal contents can have a marked effect on the activity of sucrase, which is separate from this internal mechanism: (1) early feeding with sucrose will induce precocious sucrase induction in rodents<sup>82</sup>; (2) reduction of carbohydrate intake results in a reduction of sucrase activity even when caloric intake is unchanged<sup>85</sup>; (3) feeding with glucose instead of sucrose reduces brush-border hydrolase activity<sup>86</sup>; and (4) refeeding sucrose after a period of abstinence leads to rapid recrudescence of the hydrolase activity.<sup>87</sup> Increase in sucrose from 2 to 55% of total energy intake has differing effects on the enterocyte depending on its location in the small intestine; that is, sucrase activity increases in the duodenum and upper jejunum, but not in more distal segments.<sup>88</sup> Further, cells from the midvillus of the proximal intestine show the greatest alterations, with lesser increases in the villus tip and crypt–villus junction.

The control of sucrase activity is complex and depends on events within the enterocyte in addition to external influences. Sucrase activity depends mainly on events in the sucrase promoter since expression of GH mirrors that of sucrase in transgenic animals containing a sucrase promoter–GH construct.<sup>89</sup> The expression of sucrase may therefore reflect nuclear protein–promoter interactions.<sup>90</sup> Evolutionarily conserved *cis*-acting elements in the promoter region have been found to interact specifically with transcription factors such as GATA-4 and caudal-related homeodomain (Cdx) proteins.<sup>91–93</sup> Further combinatorial interactions between hepatocyte nuclear factor 1 $\alpha$  (HNF-1 $\alpha$ ) protein, GATA-4, and Cdx-2 may play a critical role in the temporal and spatial



**Figure 5** Cross-talk through the apical membrane will depend on the particular signal involved. EGF = epidermal growth factor; IGF = insulin-like growth factor.

regulation of the SI gene expression during postnatal development.<sup>92,93</sup> Posttranscriptional events may also likely to influence enzymatic activity; for example, reduction in lactase activity following weaning does not necessarily correlate with lactase mRNA, but may be regulated during translation.<sup>94</sup>

**Glucose Transporter Expression.** Glucose enters the epithelial cell by active transport via a sodium-coupled glucose transporter, SGLT-1.<sup>22,95</sup> SGLTs represent part of a family of proteins that carry nutrients into the cell across concentration gradients. SGLT-1 is located in the brush border or apical surface of the enterocyte and along with Na<sup>+</sup> transports glucose and galactose into the cytosol. GLUT-2 is basolateral and transports glucose, galactose, and fructose (transported in from the lumen via GLUT-5) from the cytosol into circulation.

Direct transport studies undertaken before the identification of sodium-dependent transporters showed that glucose uptake increased with glucose feeding.<sup>96</sup> This increase was not due to changes in the affinity of glucose to the enterocyte ( $K_m$ ), but due to an increase in the uptake ( $V_{max}$ ), indicating an increase in the production of proteins that transport glucose. It is now well established that dietary sugars regulate the activity and the expression of intestinal SGLT-1. Structurally, an intact mucosa is necessary for rapid upregulation of glucose transporters by luminal glucose.<sup>97</sup> Modulation of SGLT-I expression by dietary carbohydrate has also been observed in the human intestine.<sup>17,22,95</sup> Mechanisms involved in the regulation of glucose transport are complex and have been difficult to characterize, as two distinct timescales of dietary carbohydrate exposure are now known to play a role.<sup>98</sup> Glucose transporters can respond within an hour to glucose<sup>97</sup> and also exhibit distinct changes in gene expression over a period of 1 to 3 days to a high-carbohydrate diet.<sup>99</sup> Intestinal glucose transport is also subject to diurnal changes, adding further complexity to the system. The increase in glucose transporters in response to dietary changes is analogous to the acquisition in bacteria of enzymes and transporters that are induced by lactose (*lac* operon). There are, however, notable differences between induction of enzymes and transporters in bacteria and those seen in the mammalian intestine. First, the activity of transporters in bacteria increases by 100- to 1000-fold, whereas the nutrient transporters in mammals show smaller variation with diet. For example, feeding mice with diets containing high levels of either D-glucose or nonmetabolizable analogs of D-glucose led to only a two- to threefold increase in SGLT-1 activity.<sup>100,101</sup> Second, in bacteria, the cell that receives the stimulus is the same cell that exhibits alterations in gene expression. In mammalian intestine, this appears not to be the case with the glucose transporter.<sup>102,103</sup> Sequential elution of different fractions of epithelial cells was used to distinguish different cells along the crypt-villus axis of

mice.<sup>104</sup> Increase in carbohydrate intake resulted in enhanced expression of the glucose transporter (as measured by phlorhizin binding). Importantly, changes were observed in the crypts before they were seen in the villi. Similarly, when carbohydrate was stopped, it was in the crypts that the reduction in transporter was first observed. Thus crypt cells respond to changes in diet before villus cells, indicating that the changes in the expression of the glucose transporter were effected by new cell lineages from stem cells. It is the stem cell producing the new cell lineage that therefore must contain the machinery for detection of carbohydrate. The resulting cell population that ascends along the crypt-villus axis expresses this change. This separates carbohydrate detection temporally and spatially from glucose transporter expression. It is not known whether other genes in the intestine follow a similar pattern. Identification of molecular mechanisms underlying carbohydrate detection and signal transduction are currently under active investigation in several laboratories<sup>105,106</sup> (see Figure 1).

Sucrose induces an increase in the enzyme (SI) that hydrolyzes it and in SGLT-1 that allows a greater influx into the enterocyte across a concentration gradient. This absorptive system is central to the efficacy of oral rehydration solution in the treatment of gastroenteritis and cholera.<sup>22</sup> The greater the number of transporters, the more sodium and fluid that can be transported into a child during dehydration. Upregulation of SGLT-1 has therefore a potential therapeutic role in alleviating the loss of sodium and fluid with glucose solutions. An understanding of the mechanisms regulating this process would define to what extent and over what time period sodium and fluid transport could be maximized in children with gastroenteritis.

**Intake of Dietary Fat.** The intake of fat as a component of total energy varies considerably between different children. Fat consumption has been increasing in children from developed countries and has accelerated over the last 100 years. Contemporary comparison of fat intake between nations now shows large variations. For example, Perissé et al reviewed countries for the Food and Agriculture Organization of the United Nations (FAO), and showed that between wealthy and nonwealthy countries, fat intake varies from about 12% of total energy intake to over 40%.<sup>107</sup> It is not only healthy children who have seen a change in fat intake; children with CF have been given much larger amounts of fats. This is because increased calorie intake, particularly that of fat, results in improvements in respiratory function.<sup>108</sup> Much of this fat is digested in children with CF by the use of ingested enzyme preparations given with meals. However, lipases secreted in the GI tract proximal to the duodenum (gastric lipase) may also play a prominent role. It is important, therefore, to document whether secretion of this enzyme varies with fat. Knowledge of mechanism(s) underlying this possible adaptive effect may lead to therapeutic

optimization of enzyme production and better utilization of fat in children who are continually under threat of malnutrition.

### Gastric Lipase

Gastric lipase accelerates the digestion of triglycerides in human milk,<sup>109</sup> by releasing free fatty acids which enhance pancreatic lipase and colipase activities. The actions of gastric lipase are magnified in children with little pancreatic lipase activity, as in CF. Newborn babies also have relatively low pancreatic lipase activity<sup>110,111</sup> and the relative importance of gastric lipase is exaggerated in the premature neonate.<sup>112,113</sup> There are significant biochemical differences between pancreatic and gastric lipase, most significant of which is the difference in pH required for maximal activity.<sup>114,115</sup> In addition, gastric lipase activity requires no bile salt or colipase. In the human, the gastric mucosa secretes all the preduodenal lipase.<sup>116,117</sup> In the rodent, the tongue is the most significant source, and here the equivalent enzyme is termed lingual lipase. There may also be some lingual production in the preterm human infant because lipase activity has been detected in esophageal pouches of infants with esophageal atresia with no communication to the stomach.<sup>113</sup> The rabbit predominantly secretes its preduodenal lipase from the gastric mucosa, making the rabbit a more appropriate model than the rodent in which to study the effect of dietary influence on this enzyme.

Gastric lipase activity is more susceptible than pancreatic lipase to dietary changes. Increasing the dietary fat intake in rabbits from 2.7 to 6.0% for 2 weeks was sufficient to induce a 100% increase in gastric lipase activity.<sup>118</sup> This increase had no effect on pancreatic lipase production. Indeed, pancreatic lipase activity was enhanced only when the fat intake was increased to 12% of diet and then the increase was only modest, a 10% increase in pancreatic lipase activity. Studies in adult human volunteers have shown similar data with a doubling (5.7 to 9.95 U/mL) of gastric lipase after changing from a low-fat to a high-fat diet.<sup>119</sup> The mechanisms that detect the amount of fat intake (see Figure 1) need further examination, but triglyceride composition had no effect on the adaptive response in the rabbit.<sup>118</sup> This implicates a metabolite of fatty acid breakdown, which is not specific for either saturated or unsaturated fatty acids, as the cellular agent that initiates changes in gene expression. The signal may not have to come directly from the lumen of the GI tract, because there is preliminary evidence, in neonates at least, indicating that increasing lipid intake parenterally results in enhanced gastric lipase activity. This suggests that signals from the serosal side of the epithelial cell may be possible in this case.

Gastric lipase is therefore under the control of dietary signals. Although we do not at present understand the mechanisms that underlie this phenomenon, their elucidation may eventually allow us to manipulate gastric lipase expression when it

is clinically important, as in the nutrition of CF patients and in the oral intake of the newborn.

**Fatty Acid Uptake.** Fatty acid uptake in the GI tract is facilitated by the presence of fatty acid transporter proteins (FATPs) with intestinal FATP-4 attracting considerable attention.<sup>120</sup> FATP-4 is expressed in significant amounts in enterocytes and recent studies highlight FATP-4 localization to the endoplasmic reticulum (ER). Further, FATP-4 exhibits catalytic function with the ability to esterify fatty acids.<sup>121</sup> Whether FATP-4 can function both as a transporter and as an enzyme requires further clarification. Despite extensive research our understanding of how various lipids migrate from the site of absorption to the ER where complex lipid biosynthesis takes place remains unclear. Fatty acid-binding proteins (FABPs) have been implicated in this action. Ex vivo model studies of fetal jejunum explants implicate FABP-2 polymorphism to specifically influence small intestinal lipid absorption with minimal effect on glucose uptake or metabolism.<sup>122</sup> Epidemiological analyses of polymorphisms at codon 54(A/T) in *FABP-2* gene suggest that this variant may play a role in cardiovascular pathogenesis in the presence of insulin-resistance syndrome or hypertriglyceridemia.<sup>123</sup>

Further studies are required to clarify the potential role of these transporters in health and disease. A third fatty acid transporter (FAT; CD36) located mainly at the apical surface of the villus epithelia has been implicated in fatty acid uptake and in the pathogenesis of insulin resistance.<sup>124</sup> However, in a separate study CD36 knockout mice were shown to absorb triacylglycerol and fatty acid, as well as wild-type litter mates.<sup>125</sup> Although evidence suggests that CD36 plays a role in fatty acid uptake in adipose tissue,<sup>126</sup> muscle, and heart,<sup>127</sup> its role in the small intestine remains unclear.

### Breast Milk and Gene Expression

Nutrition and protection of the infant are the primary roles of breast-feeding, and the nutritional and immunologic properties of breast milk are reviewed in other chapters of this book. Nevertheless, the cells of the GI tract are the first cells of the child to encounter breast milk, and it is not fanciful to suppose that breast milk may influence the development of these cells. A number of factors in milk can affect the expression of genes in the intestinal epithelium, particularly those genes that are associated with enterocyte differentiation. Growth factors vascular endothelial growth factor (VEGF),<sup>128</sup> epidermal growth factor (EGF),<sup>129</sup> and hepatocyte growth factor (HGF) are found in breast milk<sup>130</sup> and enhance brush-border enzyme activity and cell proliferation. Although the exact effect of EGF in breast milk on the development of the intestinal epithelium depends on the age of the subject, its effect on enterocyte growth and development is not surprising. Less expected, perhaps, is the demonstration that other breast milk components such as nucleotides also affect the same cellular

processes.<sup>131,132</sup> Although the list of constituents in breast milk that may affect expression of genes in the intestinal epithelium is ever increasing (references 133 and 134 are recommended for recent update), due to space constraint we describe below our current understanding of the role of one critical milk component, that is, the role of lactoferrin in modulating intestinal homeostasis.

**Lactoferrin.** Lactoferrin, an 80 kDa iron-binding glycoprotein of the transferrin family, is a major component of mammalian colostrum and milk.<sup>135</sup> Lactoferrin is also found in mucosal secretions, neutrophil granules, and plasma, concentrations in the latter being relatively low.<sup>136,137</sup> Lactoferrin has bactericidal properties and is a source of iron in breast-fed infants. The protein has been found to have diverse biological functions, including facilitating iron absorption,<sup>138</sup> host defense,<sup>139</sup> regulation of cell proliferation and differentiation, modulating the immune system, and embryonic development.<sup>140,141</sup>

Specific binding sites for lactoferrin have been observed in activated human blood lymphocytes,<sup>142</sup> liver cells,<sup>143</sup> and cells lining the GI tract.<sup>144</sup> Lactoferrin receptor has been detected in intestinal brush-border membrane vesicles prepared from the human fetus,<sup>145</sup> rhesus monkey,<sup>146</sup> rabbit,<sup>147</sup> and mice.<sup>148</sup> Lactoferrin is relatively resistant to proteolytic degradation.<sup>149</sup> Intact protein with iron-binding capacity has been detected in murine fecal samples<sup>150</sup> and in urine samples from human infants.<sup>151</sup> The stability of the intact protein through transit in the gut suggests that it has the potential of interacting directly with the intestinal epithelium. The lactoferrin protein consists of a bactericidal domain (lactoferricin), which can be generated in vitro by pepsin digestion.<sup>152</sup> An understanding of the actions of lactoferrin at the molecular level is particularly relevant as some artificial milk formulas now contain added bovine lactoferrin. Its major role is thought to be delivery of iron to the intestinal epithelium.<sup>153</sup> The number of lactoferrin-binding sites increases in cultured epithelial cells when depleted of iron,<sup>154</sup> resulting in a specific increase of iron transport into enterocytes. The actions of lactoferrin are therefore interconnected with the actions of iron. Iron has well-characterized effects on the translation of ferritin and transferrin mRNA,<sup>155</sup> but its effect on gene expression in the enterocyte is less well understood and is currently an active area of investigation.<sup>156,157</sup>

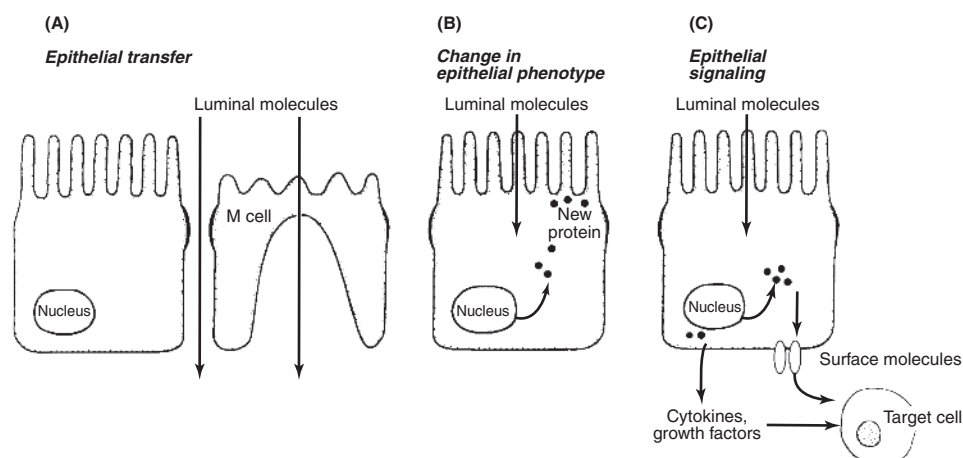
Although lactoferrin increases the bioavailability of iron in the neonatal intestine, studies suggest that lactoferrin may also act as a proliferative factor in a variety of cell types, including human lymphocytes,<sup>158</sup> murine osteoblasts,<sup>159</sup> and Caco-2 cells, a colonic adenocarcinoma cell line.<sup>160</sup> The effect of lactoferrin on the expression of brush-border enzymes has also been examined. In tissue culture experiments, the effect of lactoferrin on sucrase, lactase, and alkaline phosphatase-specific activities was found to

be dependent upon protein concentration<sup>160</sup> and on the degree of iron saturation.<sup>161</sup>

There is a school of thought that proposes that the main function of lactoferrin in vivo is not to transport iron into the epithelium, but to scavenge iron (and possible other substances) in the intestinal lumen. This unbound iron might otherwise cause free radical-mediated damage to sensitive tissues.<sup>162</sup> Accordingly, lactoferrin would have no direct effect on the intestinal epithelium, and only indirectly interact with it through the sequestration of other substances in the lumen. These luminal factors, if left free in solution, may have an impact on enterocyte gene expression. An example of this is lactoferrin's binding to lipopolysaccharide,<sup>163</sup> a major component of bacterial cell walls, thereby modulating the effect of this moiety on the epithelium. However, a purely luminal role for lactoferrin is difficult to reconcile with the identification of specific receptors for lactoferrin on the surface of the small intestinal brush-border membrane.<sup>147</sup> Initially, the binding of lactoferrin to brush borders was explained on the assumption that lactoferrin was binding to transferrin receptors,<sup>164</sup> but the use of rhesus monkeys has made possible an examination of the specificity of lactoferrin binding to its receptor.<sup>146</sup> Binding was specific and time dependent and reached saturation; monkey and human lactoferrin effectively inhibited the binding of rhesus lactoferrin at 50-fold excess, while a similar excess of bovine lactoferrin or human transferrin had no effect on binding. These results taken together are good evidence for a specific lactoferrin receptor. Any direct effect of lactoferrin on gene expression should be transmitted through this receptor, and its analysis may therefore give clues as to how lactoferrin might do this. Human<sup>165</sup> and porcine<sup>166</sup> intestinal lactoferrin receptors have been cloned and further studies are likely to highlight molecular interactions critical for the activation of this ligand-receptor complex.

### Signaling from the Intestinal Lumen to the Mucosal Immune System

The function of the small intestinal epithelium is to absorb nutrients from the environment while providing a barrier to the external world.<sup>4</sup> The barrier is not complete, as the intestine allows macromolecules to be sampled and actively absorb nutrients. The success of the intestine in achieving the balance between these competing aims was the critical step in the evolution of multiorgan animals. The ability of the intestine to monitor the environment is a key element in this process, in which the mucosal immune system plays an important part. Curiously, however, the possibility that epithelium may respond to luminal factors (and signal their presence to the mucosal immune system) has been substantially ignored. The epithelium has mainly been regarded as a passive barrier with points of selective filtration (Figure 6A), surveillance of the luminal contents occurring after this filtration has taken place.



**Figure 6** Possible interactions of molecules from the intestinal lumen with the epithelium. Traditionally, the epithelium has been seen as a selective barrier to molecules, admitting those required for energy intake or immunosurveillance and excluding others (A). However, nutrients can alter the phenotype of the epithelium to adapt to changing nutritional needs (B). When changes in the intestinal lumen induce new proteins that interact with mucosal immune cells, the epithelium can act as a membrane signaling information from the lumen to the immune system (C).

Earlier in this chapter we examined genes whose activity is restricted to the confines of the epithelial cell monolayer of the intestine (Figure 6B). Nutrient transporters and disaccharidases all contribute to the actions of the epithelium. When genes within the enterocyte express proteins whose actions occur away from the epithelium, such as cytokines and growth factors, the relevance of luminal factors on enterocyte gene expression becomes different. In this new situation (Figure 6C), the epithelium acts as a mediator between the luminal contents of the intestine and target cells beyond the epithelial barrier. Such cells include immunocytes from the mucosa, with the potential for additional cells being recruited from the circulation. The small intestinal epithelium is now recognized to be a part of the mucosal immune system.<sup>4,167-169</sup> For example, it expresses innate host pattern recognition receptors, such as Toll-like receptors and nucleotide oligomerization domain proteins, which are involved in microbial detection.<sup>170</sup> Dysregulated activation of bacteria-driven epithelial inflammatory processes is kept under tight control by a spectrum of negative regulators who collectively ensure maintenance of intestinal homeostasis.<sup>171,172</sup> It is the breakdown of these cellular processes that is now known to contribute to disease pathogenesis in inflammatory bowel disease(s).<sup>76,173</sup> The epithelial cell also expresses proteins that may interact with immunocytes within the intestine. These include surface molecules such as class II major histocompatibility complex<sup>75</sup> and secretory chemokines<sup>174</sup> and cytokines<sup>167</sup> that may directly alter immune responses. The epithelium also expresses families of growth factors and their binding proteins, which may affect the proliferation of immune cells. It may also itself directly present antigen to T cells.<sup>175</sup> The molecular mechanisms governing the expression of these genes have features in common with other enterocyte genes that respond to nutrients and factors in the lumen. For example, the epithelial cell must recognize (or “sense”) the nutrient or other luminal factor(s), and mechanism(s) must then “transduce” this recognition into molecular

changes that can directly alter gene expression (see Figures 1 and 4).<sup>176</sup> The main difference in molecular terms from the adaptive response described earlier (Figure 6B) is that the resultant proteins escape from the compass of the epithelium and interact with receptors on target cells (Figure 6C).

Signaling, by transducing molecular interactions with the epithelium into secretion of messenger proteins, allows luminal factors to influence immune responses while maintaining full epithelial barrier integrity (Figure 6C). Although the absorption of luminal factors (Figure 6A) is an important method by which the mucosal immune system surveys the intestinal lumen,<sup>4</sup> it requires the entry of molecules through the epithelial barrier. This provides a potential source for invasion by pathogens. The poliovirus, for example, enters by this means, exploiting the normal uptake of macromolecules. The ability of the epithelium to signal information to the mucosal immune system without the need for a physical pathway from the lumen to the serosal surface offers obvious advantages in terms of epithelial protection.

The notion of epithelial signaling (Figure 6C) implies that the epithelium can modulate the level of immune activity in the mucosal immune system according to the environment of the intestinal lumen. Mucosal immune responses play a large part in GI inflammatory diseases, particularly CD, ulcerative colitis, and celiac disease. The milieu of the intestinal lumen could therefore be manipulated for therapeutic purposes, by alterations in diet.

**Genes of Immunologic Importance in the Intestinal Epithelium.** An increasing list of immunologically active proteins is now known to be synthesized by the intestinal epithelium (Table 3),<sup>177-180</sup> and these originally were thought to be produced only by other cell types. Human diseases are associated with the increased production of some of these proteins in the circulation and in the GI tract. Emerging data from our and other laboratories show that the expression of these proteins in the epithelium is influenced by dietary factors.

**Cytokines.** An interesting example of one of many cytokines secreted by the epithelium is interleukin 8 (IL-8). This protein is small (8 kDa) and has a long half-life in vivo relative to other cytokines, making it a feasible candidate for long-lasting control over mucosal immune responses. IL-8 is a member of a superfamily of cytokines, “the chemokines,” which have chemotactic properties, recruiting as well as activating a number of immunologic cell types. It is a potent chemotactic factor for neutrophils and T lymphocytes and may be important in recruiting them to the GI tract. Invasion by neutrophils is a hallmark of inflammatory bowel disease (greater influx in ulcerative colitis compared to CD), a condition in which IL-8 production is enhanced.<sup>181,182</sup> IL-8 also stimulates the release of superoxide radical in neutrophils as well as other potential mediators of damage in intestinal inflammation. Furthermore, it increases the permeability of vascular endothelium to albumin resulting in tissue edema. IL-8 production in intestinal epithelial cells has

**Table 3 Immunologically Relevant Genes Expressed by Intestinal Epithelial Cells**

Gene	Experimental System	Reference
<i>Chemokines</i>		
IL-8	Primary human ileal and colonic epithelia	177
ENA-78	Primary human colonic epithelia and cell lines	178
IP-10, MIG	Human epithelial cell lines	179
IL-1R antagonist	Primary human ileal and colonic epithelia	177
CCL28	Primary human colonic epithelium and cell lines	180
<i>Cytokines</i>		
GM-CSF, TNF- $\alpha$	Colon carcinoma cell lines	167
<i>Ant-microbial peptides</i>		
<i>Acute-phase respondents</i>		
<i>Surface molecules</i>		
Class II	Sections of murine small intestine	201
MHC Invariant chain	Primary mouse epithelial cells, IEC-6 cells	202
<i>Growth factors</i>		
IGF-I	Colon carcinoma cell lines	75, 184, 208
IGFBPs	Colon carcinoma cell lines	207

IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; IL = interleukin; MHC = major histocompatibility complex; MIP = macrophage inflammatory protein.

been documented in response to bacterial invasion<sup>167</sup> and by other agents, including phorbol-12-myristate-13-acetate (PMA) and IL-1. Studies from our laboratory have shown unequivocally that the surface epithelium and its products can orchestrate the mucosal immune system of the gut. Macrophage inflammatory protein 2 (MIP-2) (mouse chemokine similar to human IL-8) transgenic mice were generated where the chemokine expression was confined to the small intestinal and colonic epithelium by the use of fatty acid-binding protein promoter.<sup>174</sup> The epithelium from the first generation of the founder showed effects on both neutrophil and lymphocyte recruitment. In the small intestine where the FABPI promoter is active neutrophil, recruitment (myeloperoxidase activity as the indicator) was significantly greater. In the distal colon where the promoter is inactive there was no effect. These studies clearly show that the epithelium can, through the release of chemokines, alter the mucosal immune function in vivo.

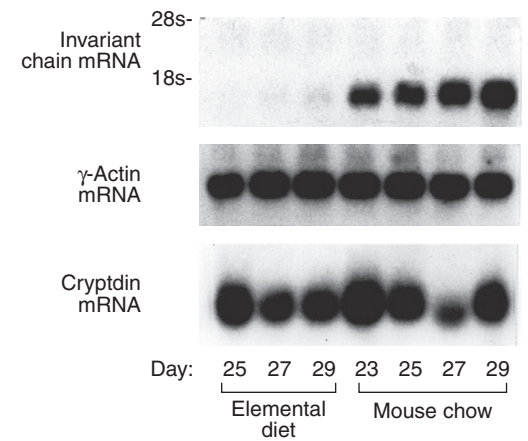
Further analysis of the immune system demonstrated that the small intestine has increased lymphocyte infiltration, in addition to the presence of increased neutrophils. Lymphocyte numbers in the lamina propria are significantly increased ( $P < .05$ ), and there was also a doubling of the number of intraepithelial (IEL) lymphocytes. The increase in IELs was due to an increase in both  $\alpha\beta$  and  $\gamma\delta$  lymphocyte populations. Interestingly, these cells express CXCR2, the receptor for MIP-2. These studies highlight the potential recruitment capacity of a single chemotactic activity; the in vivo situation, however, is likely to be more complex with simultaneous alteration of various immune regulators.

Increasing evidence suggests that expression of various epithelial-derived immune regulatory molecules is altered by dietary factors.<sup>4,75,176</sup> *n*-Butyrate is a short-chain fatty acid produced by the metabolism of normal intestinal bacteria whose levels in the intestine vary with diet.<sup>183</sup> Newborn babies have very low butyrate levels in either the small or large intestine. However butyrate levels increase with time, reaching adult levels by 2 years of age.<sup>184</sup> It has long been known that infants fed casein-based formulas<sup>185</sup> produce large amounts of butyric acid and propionic acid in the stool, whereas the predominant fatty acid in breast-fed infants is acetic acid. Furthermore, the bacterial flora (*Lactobacillus*) responsible for high acetate/butyrate production is inhibited by casein.<sup>185</sup> In addition, the amount of butyrate produced in the stool is altered by the type of fiber consumed in the diet.<sup>186</sup> Butyrate levels depend on the type of bacteria in the gut and how much substrate is available for butyrate production. Butyrate levels therefore reflect events in the intestinal lumen, and we hypothesized that their concentrations may alter cell signaling. We therefore examined its effects on IL-8 and *MCP-1* gene and protein expression.<sup>187</sup> We found that butyrate increased IL-8 secretion in enterocytes while simultaneously downregulated another chemotactic cytokine, macrophage chemotactic protein 1 (MCP-1).<sup>187</sup> More recent studies suggest that butyrate can also modulate

pathogen-driven epithelial inflammatory responses.<sup>188</sup> Studies from our laboratory have defined a role for butyrate-induced histone acetylation in the regulation of chemokine gene expression.<sup>189</sup> Unlike IL-8, which attracts neutrophils, MCP-1 attracts macrophages and monocytes. It is constitutively secreted by the intestinal epithelium,<sup>190</sup> whereas IL-8 is not. These observations are consistent with events in the whole intestine in vivo where, in healthy tissue, neutrophils are absent and macrophages abound. With increasing butyrate levels, IL-8 secretion is induced but MCP-1 production is depressed. Such “chemokine switching” in response to butyrate may well alter the population of immune cells in the lamina propria. Thus, butyrate may affect both the number and types of cells in inflamed tissue through changes in chemokine production of epithelium during inflammation.

**Antimicrobial Peptides.** In the last decade a new family of endogenous epithelial cell-derived antimicrobial peptides has been identified. (For detailed reviews, see references 168, 191, and 192.) To date, mRNA expression of members of two families, the  $\beta$ -defensin and cathelicidin (LL-37), has been observed in enterocytes. (The expression of  $\alpha$ -defensins is confined only to Paneth cells.) Several studies have shown dynamic changes in the expression of these peptides during infectious and inflammatory episodes implicating them in the front line of host defense in the GI tract.<sup>193–196</sup>

**Class II Major Histocompatibility Complex and Invariant Chain Expression.** The initiation of an immune response to protein antigen normally requires the help of T lymphocytes. Activation of T cells in turn depends on the processing and presentation of peptides by an antigen-presenting cell (APC).<sup>197</sup> Class II MHC heterodimers (Ia in the rodent) are the molecules that present the processed exogenous antigen to the T-cell receptor. In addition to classic APCs (dendritic cells, macrophages, and B lymphocytes), a number of cell types express class II MHC (Ia antigen) including intestinal epithelial cells and may function as APCs. In recent years, several studies have noted that both the small intestinal and colonic epithelium may function as an APC.<sup>198,199</sup> The requirement for cell viability and an intact cytoskeleton suggests pinocytosis as a major mechanism for active antigen uptake by intestinal epithelial cells; however, passive diffusion and adsorption may also contribute to the process. Processing and presentation of exogenous antigen requires an additional protein, the invariant chain (Ii).<sup>200</sup> Diet has a marked effect on the expression of class II MHC and its associated invariant (Ii) chain in the mouse intestinal epithelium. The expression of class II MHC and Ii mRNA is developmentally regulated<sup>201</sup> in the epithelium (unlike its expression in the lamina propria, where it is expressed from before birth). In addition, the timing of expression can be altered by delaying the age of weaning from mother’s milk to normal chow.<sup>202</sup> Expression of class II MHC and Ii chain is apparent 3 to



**Figure 7** Dietary factors alter the expression of class II MHC and invariant chain expression in intestinal epithelial cells. Figure shows Northern blot of ribonucleic from epithelial cells taken from individual mice of a single split litter weaned at day 17 onto elemental diet or Purina mouse chow. The blot was probed with invariance chain complementary deoxyribonucleic acid (cDNA) and autoradiographed for 48 hours. Mice were examined at days 23, 25, 27, and 29. Blots were also probed with  $\gamma$ -actin and cryptdin cDNA to verify uniformity of enterocyte extraction mRNA = messenger ribonucleic acid. (Reproduced from Sanderson et al<sup>201</sup>).

4 days after weaning on chow. However, weaning onto an elemental diet (which contains chemically synthesized amino acids, simple sugars, and fats) did not induce the expression of class II MHC or invariant chain (Figure 7). Thus the expression of these genes in the intestinal epithelium is influenced by dietary manipulation in vivo. The fact that elemental diets used in these experiments were the same as those administered therapeutically in CD lends support to the idea that alterations in gene expression may be a significant tool in the treatment of disease (see below).

**Insulin-Like Growth Factor-Binding Proteins.** Immune responses depend not only on the activation of T cells, but also on the ability of these T cells to proliferate. Earlier sections of this chapter described the importance of IGF-1 on growth. However, IGF-1 is also an agent that affects cells of the immune system. IGF-1 exerts a range of effects on T-cell physiology.<sup>203</sup> The growth factor induces a marked increase in the gene and protein expression of both CD25 and IL-2 and modulates T-cell proliferation via both autocrine and paracrine mechanisms.<sup>204</sup> Treatment of adult mice with recombinant human IGF-1 induces striking modifications in lymphocyte number and function.<sup>205</sup> Fourteen days of treatment with IGF resulted in increases in both CD4<sup>+</sup> T cells and splenic B cells. Mitogenic responses of T and B cells were also enhanced, demonstrating that IGF-1 increases lymphocyte numbers and activity. As discussed earlier, IGF-BPs modulate the actions of IGF-1. Because cultured intestinal epithelial cells<sup>206,207</sup> secrete IGF-BPs, it is possible that the epithelium can influence the proliferation of activated lymphocytes by this means. Furthermore, studies have shown that nutritional factors affect the production of IGF-BPs in cell lines in vitro.<sup>208</sup> Therefore, not only may nutritional

factors influence IGF/IGFBP secretion by the liver into the circulation, resulting in changes in growth of the whole individual, but nutrients may also affect the IGF/IGFBP system in the intestinal epithelium, leading to alterations in the proliferation of the local immune system.

### Nutrient Recognition and Signal Transduction

The mechanisms whereby a nutrient (or other factor in the intestinal lumen altered by dietary change) results in changes in RNA transcription are not well understood (see Figure 1). It is likely that increases in transcription are the consequence of alterations in the binding of nuclear transcription and accessory factors to the promoter of a particular gene, but the steps by which a cell recognizes a change in that nutrient will differ for every nutrient examined, so too will the mechanisms that transduce this signal into alterations of nuclear protein binding. In the enterocyte we have seen the variety of mechanisms that might occur simply by appreciating the many factors that may alter when the diet is changed (see Figure 2). Different theoretic mechanisms of interaction exist between the lumen and epithelial cells. These mechanisms differ, depending on the exact luminal factor involved (see Figure 5). Small nutrients such as butyrate or galactose are likely to enter the cell and affect gene expression through their respective metabolic pathways. Growth factors and lactoferrin involve specific receptors on the surface of the epithelium, and large proteins may enter the cell and alter gene expression as they are sorted. Some complex entities may affect more than one pathway. Bacteria for instance may interact through all three pathways: they could influence enterocyte metabolism; they may produce factors or components of their cell walls (e.g., endotoxin), which may interact with surface receptors; and the macromolecules from which they are composed may enter enterocyte compartments and be sorted inside the cell. Each pathway may influence aspects of nuclear function. In addition, indirect actions of cytokines produced from immune cells may also play a role.

Although sensing and signal transduction mechanisms (see Figure 1) in the epithelium require further investigation, there are some data on the mechanisms by which glucose regulates the expression of PK (in the liver) and insulin (in pancreatic islet cells). Three key questions relate to the effect of glucose on the expression of these two proteins: (1) What are the glucose metabolites that set in train the effects on transcription? (2) How is binding of nuclear proteins to gene promoter elements induced? (3) What is the sequence of DNA in the promoter that confers glucose sensitivity on the gene in question (the so-called nutrient response element)?

In practice, this last question is the easiest to answer because DNA sequences can be easily manipulated in the laboratory. Promoters linked to reporter genes allow researchers to directly measure promoter activity. This was first examined in

the *PK* gene in response to glucose. The actual sequence in the PK promoter was determined by mutational analysis of the promoter and its effects on reporter activity. The GREs in the *PK* gene and in the insulin 1 gene were discovered by this means. To determine the GRE in the *PK* gene promoter, promoter DNA sequences were transiently transfected into hepatocytes in culture<sup>209,210</sup> and were also examined in transgenic mice.<sup>211</sup> Mutations induced into the DNA demonstrated a GRE occurring twice in the PK promoter. This motif is known as an E box because of its resemblance to a similar sequence in a promoter of the adenovirus, which had been given this name. This same DNA sequence also occurs in other genes that regulate glucose metabolism, most notably the *FAS* gene, whose protein product catalyzes one of the later steps in the conversion of carbohydrate to fat, and one that is upregulated when glucose is abundant in the diet.

The rat insulin gene also contains an E box, which is termed the Far element. Paradoxically, this element does not confer glucose sensitivity to the insulin gene. This attribute is conferred by a sequence 10 bases away termed the FLAT element. We now understand some of the features of the signal transduction from nutrient to nuclear function in the insulin gene. MacFarlane et al<sup>212</sup> identified a nuclear factor, insulin upstream factor 1 (IUF-1), which binds to the insulin promoter. IUF-1 binding was examined in vitro by assessing the degree of binding of nuclear extracts from cultured islet cells to radiolabeled promoter DNA. There was a high degree of IUF-1 binding in cells incubated with 20 mmol/L glucose, but this was abolished in cells incubated in 3 mmol/L glucose. In addition, this protein–DNA binding depended on the phosphorylation of IUF-1. Phosphorylation therefore alters a factor in the initiation of transcription of at least one glucose-sensitive gene. It is particularly interesting that phosphorylation is the signal that transduces glucose intake to induce insulin transcription, because it is also phosphorylation that directly affects the activity of enzymes in the Embden–Meyerhoff pathway. Thus, in this system phosphorylation acts as a common transduction mechanism, responsible for both the quick and slow responses to glucose uptake.

The metabolites derived from nutrients responsible for the change in nutrient gene expression have not been elucidated in any system. Glucose-6-phosphate may possibly be the metabolite that activates the GRE in the *FAS*<sup>213</sup> and *PK* genes. This was demonstrated by comparing the effects of the two glucose analogs, 2-deoxyglucose (2dG) and 3-*O*-methyl glucose (3OMeG). Neither analog can be metabolized. However, 2dG can be phosphorylated, but not 3OMeG; 2dG stimulated transcription, but 3OMeG did not. Therefore, only the phosphorylated sugar was able to stimulate the transduction mechanisms that resulted in transcription, which it did without further metabolism. Whether this phosphate bond provides the energy to phosphorylate a nuclear factor to bind

to DNA is not known, but it is an interesting possibility.

### CLINICAL AND THERAPEUTIC IMPLICATIONS OF NUTRIENT–GENE INTERACTIONS

The role of diet on fetal<sup>214</sup> and adult<sup>215</sup> health is an area attracting considerable attention from medical scientists and the general public. However, the relationship between dietary factors and the health of most organs has been difficult to examine, other than by using epidemiology.<sup>215</sup> Occasionally, data have emerged suggesting that particular diets benefit certain disease states. For example, renal failure was shown to be less progressive in rats whose protein intake had been restricted,<sup>216</sup> but even this has been difficult to substantiate in children.<sup>217</sup> Furthermore, it is not easy to see how the protein load has its effect. We have no knowledge of which cell types in the kidney may be affected, let alone how their phenotype may be altered by protein intake. On the other hand, in the small intestine, the relation between diet and GI function is clearer.

In clinical practice, one of the most substantial changes in the content of the intestine occurs before and after parenteral nutrition. The effects of such changes have profound consequences for the small intestinal epithelium in human subjects (see Figure 6B). Intestinal brush-border hydrolase activity is diminished during parenteral feeding.<sup>218</sup> Moreover, the ability of the mucosa to form a barrier is compromised. Moore et al studied 74 patients who had suffered abdominal trauma randomized to receive either enteral or parenteral nutrition.<sup>219</sup> Those fed parenterally had more episodes of infective complications (pneumonia and abdominal abscesses) than those fed enterally. Calorie and nitrogen intake was equivalent in the two groups. As it is thought that the main source of sepsis following trauma is the GI tract, this study suggests that enteral feeding directly affects the ability of the injured patient to contain bacteria within the GI lumen.

The effect of parenteral nutrition on the morphology of the intestine in animals has long been known.<sup>220</sup> Considerable attention has been paid to the question of which enteral components are responsible for maintaining normal bowel morphology and function. No clear answer has yet emerged. For example, Spector et al observed that luminal infusions of both 30% dextrose and 5% amino acids were effective in restoring intestinal weight, protein content, and DNA content after a period of intravenous alimentation.<sup>221</sup> Amino acids were more potent on a weight-for-weight basis or on a molar basis than dextrose. Changes in the intestine seen in animals fed via parenteral nutrition have been compared with the changes seen during weaning.<sup>87</sup> Intestinal length, mucosal mass, DNA, protein, and disaccharidase activities were significantly lower in animals sustained by intravenous nutrition when compared to normally weaned controls. However, restoration of luminal feeding resulted in a return to

normal of the intestine in the total parenteral nutrition–fed group.

There are now many reports of the relationship of intestinal function to parenteral nutrition.<sup>222,223</sup> However, a study of the interaction between luminal content and the molecular control of intestinal cell function is just the beginning, and this relationship is likely to provide the key to our understanding of how enteral feeds maintain a healthy epithelium.

It is also likely that epithelial signaling (see Figure 6C) may have important therapeutic implications for diseased intestine. First, the contents of the intestinal lumen are directly amenable to therapeutic manipulation, whereas the environment that surrounds other, more internal, organs is maintained in strict homeostasis by a plethora of mechanisms. Second, although we do not know exactly how the local environment surrounding the epithelial cell is changed by diet (see Figure 2), dietary manipulations are an important aspect of management of GI disease states.

The activity of CD is greatly influenced by altering the contents of the intestinal lumen. Inflammation subsides in patients that are fed parenterally and receive no oral nutrients<sup>224</sup> and in those receiving elemental diets.<sup>225,226</sup> These chemically defined diets induce remission as effectively as high-dose oral corticosteroids in both adults<sup>226</sup> and children.<sup>227,228</sup> The return of inflammatory changes to normal in the intestine has been documented indirectly by the use of markers of intestinal permeability,<sup>229,230</sup> scanning of labeled neutrophils,<sup>231</sup> endoscopic examination,<sup>232</sup> and quantifying protein metabolism.<sup>233</sup>

It has been hypothesized that a reduction in antigen load in the intestine may underlie the efficacy of elemental diets in CD<sup>227</sup> in a fashion similar to that in cow's milk–sensitive enteropathy and celiac disease. However, the switch from normal diet to an elemental diet alters many constituents of the intestinal milieu apart from food antigens (see Figure 2). In particular, bacterial populations (and therefore bacterial products) are profoundly altered by dietary alteration. Moreover, giving whole protein in the form of liquid diets (polymeric diets) is also an effective treatment for CD.<sup>232</sup> Removal of protein antigen is therefore not essential for the efficacy of the formula diet. It is more likely that the diets are altering many factors in the local environment of the intestinal lumen (see Figure 2). The concept of genes in the epithelium signaling events to the mucosal immune system is a first step to understanding the basis of how such events may occur because it seeks to identify molecular pathways from the intestinal lumen, through enterocytes to the mucosal immune system and other target cells beyond the epithelium. Certainly, this would be in keeping with data presented earlier (see Figure 7), showing that class II MHC and invariant chain expression in the epithelium are related to dietary factors. There was no expression in epithelial cells of the mice weaned onto an elemental diet<sup>202</sup> and this diet was the same as that used in the treatment of CD. Genes other than class II

MHC, with common promoter elements, may also be altered by similar maneuvers, and may be important in regulating intestinal inflammation.

## CONCLUSIONS AND SPECULATION

Certain concepts have been laid down in this chapter that can be used as the basis for examining how dietary factors may affect the expression of genes in various cell types including the intestinal epithelium. The essential components for an effective transition between nutrient and gene expression in cells have been documented. These components (see Figure 1) comprise a system of recognizing changes in factors as they interact with a cell (sensing) and a mechanism of converting these external molecule–cellular molecule interactions into changes in gene expression (signal transduction). It is not yet understood how these mechanisms integrate to yield tissue homeostasis. This chapter has presented evidence that nutritional factors do indeed alter gene expression in cells, but it is likely that different molecular species are sensed by completely different mechanisms. In the case of the epithelial cell (see Figure 5) these sensing mechanisms can be at the cell surface or part of the internal mechanisms of the cell. It is also possible that special receptors exist on the surface of the cell and detect more complex external molecules, such as large proteins and oligosaccharides. The apical surface of mucosal epithelial cells expresses a number of molecules belonging to the immunoglobulin superfamily whose expression can also be modulated by nutrition.<sup>234</sup> The function of many members remains unclear; however, one family member ICAM-1 allows rhinovirus attachment.<sup>235</sup> As the primary function of these molecules on the mucosal surface cannot be to serve as a conduit for viral infection, it is tempting to suggest that they and other similar molecules might play a role in the immunosurveillance of macromolecules in the GI tract. Signaling through these molecules would require alterations in epithelial cell gene expression for this surveillance to be transmitted to the mucosal immune system. If antigens are recognized by epithelial cells of the GI tract, it is possible that this information is correlated with information garnered from the previous penetration of similar antigens across the epithelium.<sup>74</sup>

Finally, this field of nutrient–gene interaction may have important therapeutic implications; the ability of the epithelium to interact with other immune cells makes it an active member of normal human defense.<sup>179,199,236</sup> This physiologic role may allow it to influence inflammatory reactions in pathologic situations. The opportunity exists through dietary means to manipulate the constituents of the lumen of the intestine by opening up a new vista for treatment of human disease. Genetic expression is now regarded as a central feature of human disease states, particularly those in which certain genes are defective. However, genetic

influences also affect a much wider variety of diseases than those that are due to simple inherited gene mutations. The possibility that expression of genes can be modulated by means other than insertion of new DNA should be added to the armamentarium of the medical community. An understanding of how dietary factors may alter gene expression is an early step in this direction.

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