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NUTRIENT REGULATION OF CELL CYCLE PROGRESSION

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■ **Abstract** Cell replication is tightly controlled in normal tissues and aberrant during disease progression, such as in tumorigenesis. The replication of cells can be divided into four distinct phases: Gap 1 (G1), synthesis (S), gap 2 (G2), and mitosis (M). The progression from one phase to the next is intricately regulated and has many "checkpoints" that take into account cellular status and environmental cues. Among the modulators of cell cycle progression are specific nutrients, which function as energy sources or regulate the production and/or function of proteins needed to advance cells through a replicative cycle. In this review, we focus on the roles of specific nutrients (vitamin A, vitamin D, iron, folic acid, vitamin B12, zinc, and glucose) in the control of cell cycle progression and discuss how insights into the mechanisms by which these nutrients modulate this process can be and have been used to control aberrant cell growth in the treatment of prevalent pathologies.

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INTRODUCTION

In both plants and animals, growth is regulated with regard to overall size rather than cell number. However, regulation of cell number is critically important for achieving and maintaining the desired size of organs and organisms. When tissues are injured, the average cell size and/or cell number is increased to compensate for the loss of functional tissue mass. Provision of macronutrients (carbohydrates, proteins, and fatty acids), which supply cellular fuel, is essential for proper cell replication that is needed for tissue maintenance and growth. What is less well understood, and predominantly addressed herein, is the role of micronutrients (vitamins and minerals) in the control of cell cycle progression. To clearly dissect the role of such nutrients in the regulation of this process, it is necessary to overview the regulation of cell cycle control.

OVERVIEW OF CELL CYCLE PROGRESSION

The cell cycle is divided into four phases: gap 1 (G1), synthesis (S), gap 2 (G2), and mitosis (M) (Figure 1). Each phase is characterized by distinct cellular processes that are required for proper cell division and the formation of cyclin, and a cyclin-dependent kinase (CDK) complex regulates phase transitions. For the progression from G1 to S, D-type cyclins complex with CDK4 or CDK6 to form an active kinase that phosphorylates retinoblastoma (Rb) protein. In a hypophosphorylated state, Rb inhibits growth by sequestering the E2F transcription factor. Hyperphosphorylation of Rb by cyclin D-CDK4/6 results in the release of E2F, which activates transcription of cyclins for later phase transitions as well as proteins required for DNA synthesis such as DNA polymerase α, thymidine kinase, and dihydrofolate reductase (DHFR). External stimuli (i.e., nutrients) and internal signals (i.e., DNA damage) regulate the formation of cyclin-CDK complexes via cyclin-dependent kinase inhibitors (CKI), which include the cip/waf family (p21, p27, p57). The cip/waf family interacts with multiple cyclin-CDK complexes and the INK4 family (p15, p16, p18, p19), which specifically inhibits cyclin D-CDK complexes.

The regulation of cell cycle progression from one phase to the next is intricately controlled and has many "checkpoints" that take into account cellular status and environmental cues. The progression from G1 to S to G2 to M is regulated by cyclin-dependent kinase complexes, which are composed of two proteins—a cyclin (structural protein) and a kinase (enzyme) (37). For example, in G1 phase, growth factors or other stimuli induce the production of cyclin D1, which upon association with CDK4 or 6 forms an active kinase that targets the Rb protein. Phosphorylation of Rb blocks its growth-inhibitory activity and promotes the release of bound E2F transcription factor (88). E2F activates the transcription of specific genes, including the cyclin and kinase needed for the next phase transition as well as the

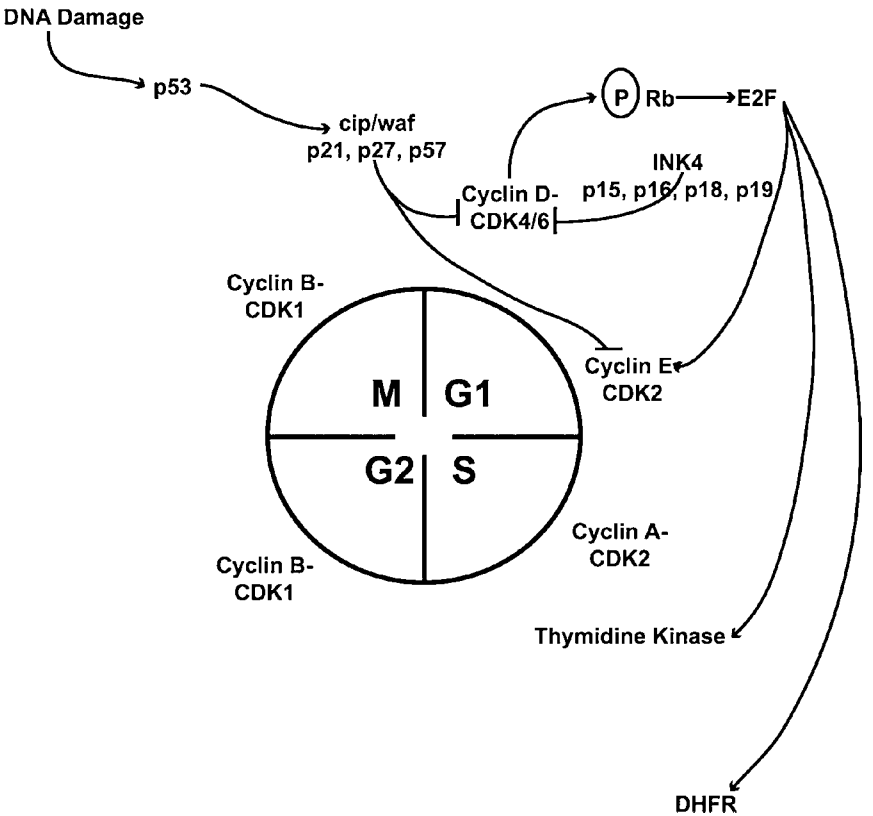

Figure 1 An overview of cell cycle progression.

enzymes required for DNA synthesis, such as thymidine kinase and dihydrofolate reductase.

The formation of cyclin and CDK complexes that are necessary for phase transitions is also regulated by the two families of CKIs, INK4 and cip/waf (88). The INK4 family specifically blocks G1 progression by inhibiting the association of CDK4/6 with cyclin D (46). The cip/waf family, on the other hand, interacts with most cyclin/CDK complexes as well as with other unrelated kinases. Both internal and external signals can control cell cycle progression via CKI production. For instance, p53, a tumor-suppressor protein that is activated by DNA damage, activates p21 transcription to inhibit cell cycle progression. Thus the availability of cyclins and kinases, and their ability to form active complexes, collectively determine whether a cell will transition from one phase to the next and complete a replicative cycle. Throughout this review we discuss how specific nutrients control the production and function of these cell cycle regulators and influence cell growth.

DIRECT REGULATION OF CELL CYCLE PROTEINS BY NUTRIENTS

The regulation of cell cycle proteins by different nutrients is important in both normal and pathological processes. The understanding of the basic function of nutrients is based within developmental schemes; however, this knowledge has aided in the prevention and treatment of various cancers. Two nutrients, vitamin A and vitamin D, are highlighted here for their multiple roles in the regulation of cell cycle proteins.

Vitamin A

Vitamin A, or retinol, is a naturally occurring retinoid that regulates cellular differentiation programs during embryogenesis and in adult tissues. Retinol can be derived from precursors such as β-carotene in the diet and is the primary form found in the blood (reviewed in 13, 82). Retinol-binding proteins transport retinol to target tissues and mediate their cellular uptake. Retinol, through a series of enzymatic steps, is converted into active all-trans retinoic acid (RA) as well as into different isomers, including 9-cis and 13-cis RA.

The actions of RA are mediated through retinoic acid receptors (RARs) and retinoid X receptors (RXRs), members of the nuclear hormone receptor family (reviewed in 13, 82). All-trans RA is the direct ligand for three classes of RARs, α, β, and γ, which are each distinct genes that produce multiple isoforms through alternative splicing and multiple transcription start sites. Similarly, there are three classes of RXRs: α, β, and γ. The 9-cis RA isomer, but not the all-trans RA, directly binds to RXR; however, it is undetermined whether 9-cis RA is a physiologically relevant ligand for RXRs. Activation of gene transcription by all-trans RA requires heterodimerization of RAR and RXR and the binding of this complex to RA response elements (RAREs). RAR and RXR isoforms have distinct expression patterns in both embryonic and adult tissues, resulting in numerous potential combinations of RXR-RAR heterodimers that could differentially regulate target genes.

RA during embryogenesis is first expressed during gastrulation and its essential role is highlighted by different animal models of vitamin A deficiency and targeted mutations in RA synthesis and regulatory proteins (72, 108). RA inhibits cellular proliferation and induces cellular differentiation programs in some types of embryonic cells including hepatocytes, vascular smooth muscle cells, and endothelial cells (4, 55). For example, recent studies from our laboratory demonstrated that during mouse embryogenesis, RA induces p21 and p27 expression in primordial endothelial cells, resulting in G1 arrest (Figure 2). Inhibition of proliferation is required for endothelial cell maturation and vascular development (55). In addition, studies with embryonic stem cells demonstrate that RA increases p53 levels and accelerates differentiation toward a neural fate.

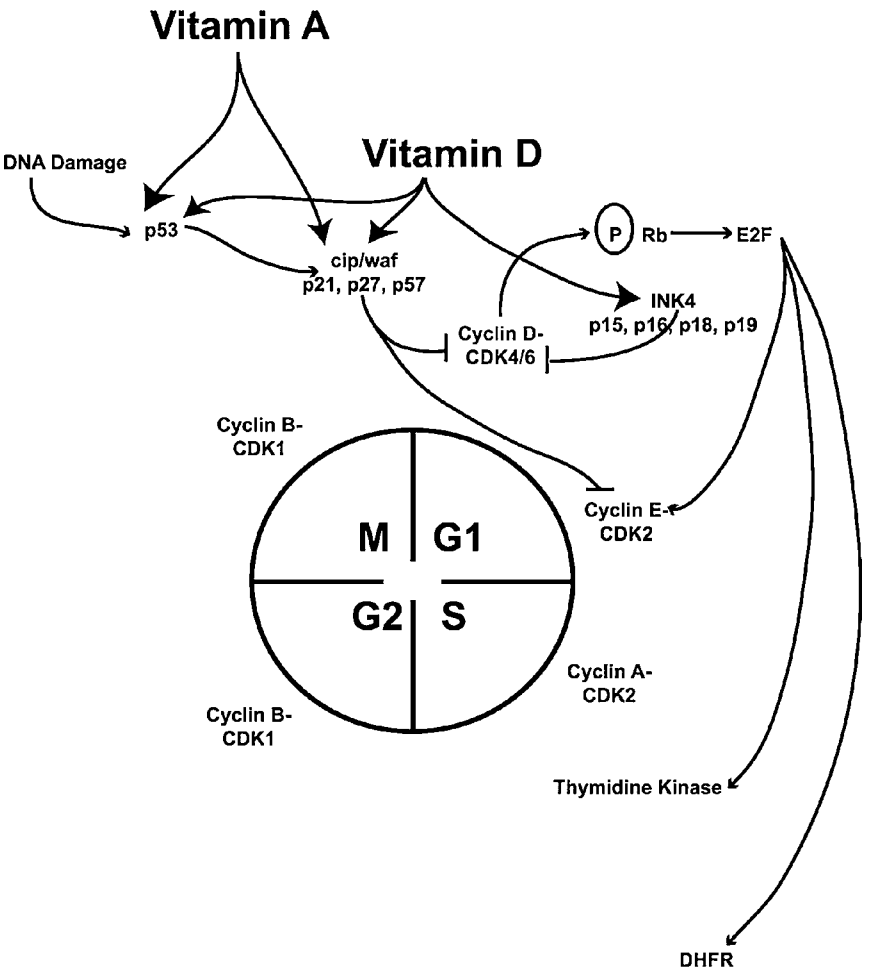

Figure 2 Nutrient regulation of cell cycle proteins. Nutrients regulate cellular growth through direct regulation of cell cycle proteins. Vitamin A–derived retinoic acid upregulates p21 and p27 as well as the tumor suppressor protein p53, thereby resulting in decreased cyclin-CDK complex formation. Vitamin D inhibits cellular proliferation by upregulating both families of CKIs (cip/waf and INK4), leading to G1 phase arrest.

In contrast, in other types of cells, RA maintains cellular proliferation for appropriate tissue patterning. In heart ventricular wall morphogenesis, epicardial production of retinoic acid induces trophic factors that stimulate cyclin D1 and cardiomyocyte proliferation (12, 92). Furthermore, RA stimulates proliferation and survival of neural crest–derived mesenchyme in the forebrain and frontonasal processes that are required for appropriate facial patterning (86). Thus during

embryonic development, RA has different effects in regulating cellular proliferation, depending on the cell type.

Vitamin A–derived RA is also required for normal physiological functions of different organs including the brain, testes, and liver as determined by various mutant mice models (42, 47, 52, 100). In adult tissues, RA also inhibits vascular smooth muscle cell proliferation and promotes differentiation through the downregulation of cyclins D3 and E, and CDK2, CDK4, and CDK6, resulting in hypophosphorylation of Rb and the inhibition of G1/S phase progression (8, 51, 76). Furthermore, RA promotes the differentiation of tracheal epithelial cells to form a mucus-secreting columnar epithelial layer (44, 79). The role of RA as an inducer of cellular differentiation in adult tissues, however, has been emphasized in the prevention and treatment of pathological conditions.

It has long been known that RA has a cancer-preventive effect because vitamin A–deficient rodents develop squamous metaplasia, specifically in the trachea (103). Epidemiological evidence, as well as clinical trials, corroborates these findings (31, 40). Studies by Langenfeld et al. (56, 57) demonstrated that treatment of immortalized human bronchial epithelial cells with RA in the presence of carcinogens, compared to cells only exposed to carcinogens, decreases cyclin D1 and cyclin E protein expression, resulting in G1 arrest. Interestingly, the change in protein levels occurred without any change in mRNA expression. Further experiments demonstrated that RA induces ubiquitination of cyclin D1, resulting in its proteolysis and cell cycle arrest (9, 91). In addition, microarray analyses of myeloid leukemic cell lines demonstrated that proteins involved the proteasomal degradation program are induced by RA treatment (97). Thus RA may act to prevent cancerous transitions by inhibiting cell cycle progression through the proteolysis of cyclins.

RA has also been shown to have a strong antiproliferative effect in numerous types of tumors, including breast cancer, prostate cancer, non-small-cell lung cancer, thyroid cancer, and myeloid leukemias. These antiproliferative effects of retinoids are utilized in chemotherapeutic regimens, commonly in the treatment of promyelocytic leukemia, head and neck carcinoma, and non-small-cell lung cancer. Numerous studies have illustrated the effects of RA on cell cycle proteins in tumor cells. In general, RA treatment results in hypophosphorylation of Rb and G1 arrest to inhibit cellular proliferation. RA-induced specific changes in cyclin, CDK, and CKIs differ in various tumors and in in vitro cell lines. For example, in neuroblastoma cells, cyclin D3 is downregulated (107); in human myeloid U-937 cells, cyclins A, B, D2, D3, and E are all downregulated (19); and in hepatoma cells, cyclin D1 is decreased (94). In numerous cell types, including cervical squamous carcinoma cells (5), hepatoma cells (4, 94), and myeloid cell lines (18, 63), RA induces p21 or p27 expression. Thus RA regulation of cyclins and CKIs inhibits cell cycle progression at G1 and mediates tumor cell differentiation. In summary, RA induces the cellular differentiation program and mediates the inhibition of cellular proliferation in multiple types of cells during development, in mature tissues, and in the progression of diseases such as tumorigenesis.

Vitamin D

Vitamin D, unlike most other nutrients, can be endogenously synthesized or obtained from the diet. Vitamin D_3, or cholecalciferol, is produced from 7-dehydrocholesterol present in the skin by UV light. In addition, in the diet, the vitamin D precursor ergosterol is naturally present in fish, plants, and grains. Active vitamin D is hydroxylated twice: first at carbon 25 in the liver and subsequently at carbon 1 in the kidney to produce active 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$]. Similarly to RA, the actions of $1,25(OH)_2D_3$ are mediated by nuclear hormone receptors (reviewed in 11, 33, 45). The vitamin D receptor (VDR) directly binds to DNA at vitamin D response elements (VDREs) as a homodimer or as a heterodimer with RXR to activate gene transcription. Ligand binding to the VDR recruits a complex of coactivators that modulates gene expression in different cell types.

$1,25(OH)_2D_3$ has multiple cellular roles; the most well known is the maintenance of calcium and phosphorus plasma levels. $1,25(OH)_2D_3$, however, also regulates proliferation and differentiation of different kinds of cells including keratinocytes, osteoblasts, and hematopoietic cells. $1,25(OH)_2D_3$ has varying effects on keratinocyte proliferation depending on conditions, $1,25(OH)_2D_3$ concentration, and physiological state. However, in general, $1,25(OH)_2D_3$ ($>10^{-8}$M) inhibits keratinocyte proliferation and induces differentiated function, as illustrated by cornified envelope formation (41, 43). The VDR is specifically expressed within cycling keratinocytes, which suggests that proliferating cells are the preferential target for $1,25(OH)_2D_3$ (87). In support of this idea, vitamin D analogs are effective treatments for hyperproliferative skin disorders such as psoriasis (54, 98, 99). In epidermal keratinocytes, $1,25(OH)_2D_3$ has been associated with increased synthesis of TGF-β and IL-8 (38, 58), which decrease proliferation, as well as with increased synthesis of TNFα, which is associated with increased differentiation (29). Similarly, in osteoblasts (33) and hematopoietic cells (1, 14, 30, 74), $1,25(OH)_2D_3$ is generally associated with inhibiting their proliferation and inducing differentiation.

Direct regulation of the cell cycle by $1,25(OH)_2D_3$ has been studied predominantly in in vitro systems. In the monomyelocytic cell line U937, $1,25(OH)_2D_3$-activated VDR directly binds to the promoter of p21 and induces its expression. Furthermore, $1,25(OH)_2D_3$ increases the expression of p27 as well as p15, p16, and p18, which effectively inhibits the G1-to-S phase transition (Figure 2) (25, 83, 84). Furthermore, in the MCF-7 breast cancer cell line, treatment with $1,25(OH)_2D_3$ inhibits cellular proliferation, and microarray analysis demonstrated in upregulation of a number of cell cycle regulatory genes, including p21-activated kinase 1 and p53 (95).

The strong antiproliferative effect of $1,25(OH)_2D_3$ has made it a target for chemotherapeutic drug development. The major drawback of $1,25(OH)_2D_3$, however, is its effects on calcium metabolism, which results in hypercalcemia and hypercalciuria. Thus synthetic analogs such as the 20-epi analogs, which maintain the antiproliferative effects but do not have strong calcemic activity, have been

developed and have shown promising results. Studies using the human osteosarcoma cell line MG-63 demonstrated that three of these analogs, KH1060, EB1089, and CB1093, have a greater antiproliferative effect than $1,25(OH)_2D_3$. Treatment with the analogs increases p27 protein levels by increasing expression and decreasing degradation. This results in decreased cyclin E, decreased CDK2 kinase activity and hypophosphorylation of Rb, and inhibition of the G1-to-S phase transition (85). Similar results have been seen in neuroblastoma cell lines treated with a 20-epi-$1,25(OH)_2D_3$ analog (32), suggesting that in the future, these $1,25(OH)_2D_3$ analogs may be useful in chemotherapeutic regimens.

Thus vitamin D is a potent regulator of cellular differentiation and proliferation through direct regulation of cell cycle proteins. Although it is not currently used as a chemotherapeutic agent, vitamin D is clearly a potential target for drug development in the treatment of certain cancers.

REGULATION OF CELLULAR GROWTH THROUGH BIOCHEMICAL METABOLIC PATHWAYS

The regulation of cellular growth can also be influenced by nutrients through their use as cofactors in enzymes required for DNA synthesis and the control of genomic structural integrity. Such nutrients include iron, folic acid, vitamin B12, and zinc; dietary deficiencies of any of these nutrients can result in general cellular dysfunction and lack of proliferative control. Recently some of these nutrient-dependent enzymes have become targets in chemotherapeutic treatments to inhibit DNA synthesis in tumor cells.

Iron

Iron deficiency is one of the most common nutritional disorders worldwide. Iron is an essential nutrient that is utilized as a cofactor by multiple biochemical pathways and that regulates both metabolism and cellular growth. Hence, the clinical manifestations of iron deficiency are often global and nonspecific, including general atrophy and anemia.

Iron derived from the diet is absorbed by the duodenum and bound to transferrin (Tf) in the serum (68). Tf regulates the cellular uptake of iron through receptor-mediated endocytosis (68, 78, 81). Once in the cell, iron is released from Tf and then either incorporated as a cofactor into both heme-containing and nonheme proteins or stored intracellularly by ferritin (36).

The role of iron in the regulation of cellular growth is most obviously connected to its function in oxygen transport and utilization. Oxygen as the ultimate electron acceptor in the respiratory chain and oxidative phosphorylation is required for aerobic adenosine triphosphate (ATP) generation by cells. Iron, in its lower oxidation state (Fe II), has a strong affinity for oxygen, and when complexed to protoporphyrin IX, a tetrapyrrole ring, it forms heme. Heme, when incorporated into hemoglobin, prevents the oxidation of iron and the protected transport of

oxygen to cells. Furthermore, iron-containing heme molecules as well as iron-sulfur centers are utilized in the majority of oxidative reactions in the mitochondrial respiratory chain.

In addition to the role of iron in oxygen metabolism, iron-containing enzymes are also utilized in DNA synthesis. Ribonucleotide reductase (RR) is an iron-containing enzyme that catalyzes the rate-limiting step in the conversion of ribonucleotides (NTPs) into deoxyribonucleotides (dNTPs) (Figure 3). RR is composed of two subunits: R1, the catalytic subunit that binds the NTP; and R2, the regulatory subunit that requires Fe III for the stabilization of a tyrosyl radical. Although the expression of R1 is constant throughout the cell cycle, R2 is expressed predominantly during S-phase to ensure the production of dNTPs for DNA replication.

It has long been known that RR, when compared to numerous key enzymes required for DNA synthesis, has the greatest increase in activity in tumor cells in order to sustain the highly proliferative state (59, 96, 102). Thus inhibition of RR activity has been targeted in chemotherapeutic regimens. Hydroxyurea (HU) scavenges the tyrosyl radical, thereby inhibiting RR activity; however, the effectiveness of HU is limited by its low affinity for RR and the development of drug resistance by the tumor (59, 67, 73). The use of iron chelators has been shown to have antiproliferative effects both in vitro and in vivo on numerous types of tumors, including rat mammary adenocarcinoma, human hepatocellular carcinoma, human neuroblastomas, and human acute leukemias (17, 20–24, 35, 80, 101).

In addition to the role of iron in the regulation of RR and DNA synthesis, there are other effects of iron on the cell cycle as demonstrated by iron chelation; however, the direct targets of iron are not well defined. In a variety of tumor tissues, including neuroepithelioma, breast adenocarcinoma, and leukemia, iron chelation causes decreased levels of cyclin D1, D2, and D3, and hypophosphorylation of Rb resulting in G1/S arrest (Figure 3) (3, 28, 53, 89). Furthermore, iron chelation results in decreased CDK2 expression; however, in neuroepithelioma cells there was a large increase in cyclin E (3). The discontinuity in expression patterns may be indicative of overall cell cycle dysregulation or, as suggested by Le & Richardson, may be simply due to inhibition of G1 at the time when cycle E levels are normally at their highest (59).

In addition to cyclin and CDK regulation, iron chelation also has an effect on CKIs. Iron chelation results in a large increase in p21 transcription; however, it concurrently inhibits p21 translation (60). Furthermore, iron depletion markedly increases p53 activity, resulting in the upregulation of known p53 target genes, including p21 and mdm-2 (93). The increase in p53 activity is not due to increased protein expression, but rather to increased activation from the latent form into the active DNA-binding form, as well as to increased stabilization of p53 resulting from serine-15 phosphorylation mediated by ATR (6, 10, 34).

Thus the role of iron as a cofactor for numerous enzymes involved in integral biochemical pathways places iron at the center for regulating normal as well as aberrant cellular proliferation.

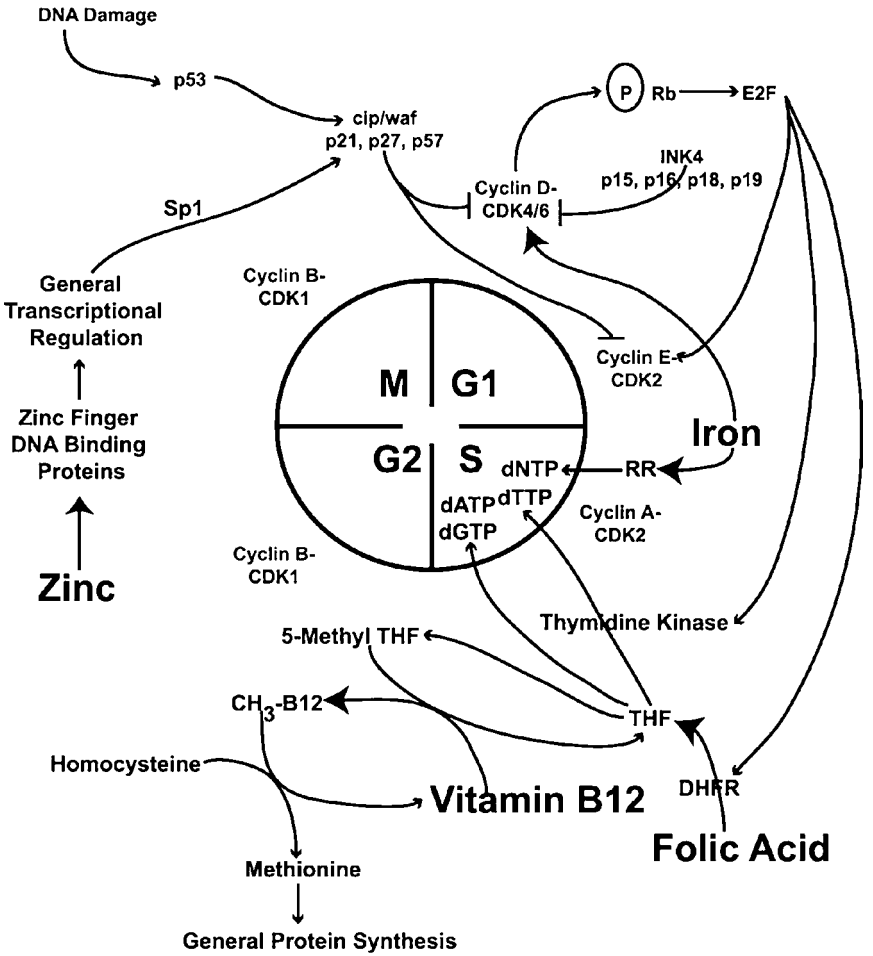

Figure 3 Nutrient regulation of cellular growth through biochemical pathways. Nutrients utilized as cofactors in enzymes required for DNA synthesis, general transcriptional regulation, and protein synthesis have indirect effects on cellular growth. Iron as a cofactor for ribonucleotide reductase (RR) is integral for deoxynucleotide (dNTP) synthesis. Iron also appears to have an effect on G1/S progression; however, its direct targets have not been characterized. Folic acid is converted to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR), and acts as a mediator of single-carbon transfers that are necessary for the de novo synthesis of purines (ATP, GTP), deoxythymidylate triphosphate (dTTP) synthesis, and methionine synthesis. Vitamin B12 accepts single carbons from folate derivatives, which facilitates the synthesis of methionine. Zinc is required for certain DNA-binding motifs (zinc fingers) that regulate general transcription as well as the transcription of specific cell cycle proteins, such as p21.

Folic Acid

Folic acid or pteroymonoglutamic acid is predominantly found in leafy green vegetables such as spinach, asparagus, and broccoli. Unlike iron, which is required for multiple processes, the primary role of folic acid is the generation of single-carbon groups for transfer to various compounds. The primary form of folic acid in the diet is folylpolyglutamate, which must be split into the monoglutamates for absorption by the proximal jejunum. DHFR converts folic acid into THF, which has the ability to bind single-carbon functional groups that can be subsequently transferred. The three most important metabolic processes that are dependent on THF are the de novo synthesis of purines, the synthesis of methionine from homocysteine, and the synthesis of deoxythymidylate monophosphate (dTMP) (Figure 3). The de novo synthesis of purine nucleotides from aspartate, glutamine, and glycine requires the addition of two carbons that are transferred from 10-formyl THF. The addition of a methyl group to methionine is directly catalyzed by methylcobalamin, a vitamin B12 derivative; however, 5-methyl THF is required for the regeneration of methyl-cobalamin. Most importantly, 5–10 methylene THF is required for the conversion of deoxyuridine monophosphate (dUMP) to dTMP, the precursor of deoxythymidylate triphosphate (dTTP) that is essential for all DNA synthesis.

The clinical manifestation of folic acid deficiency, megaloblastic anemia, reflects the primary function of THF in DNA synthesis and cell cycle regulation. Due to the decreased availability of dTTP, DNA synthesis is inhibited, despite normal RNA and protein synthesis, resulting in cell cycle arrest. Hematopoietic cells are enlarged, reflecting the asynchrony between the inability to divide and normal maturation of the cytoplasm.

Because folic acid deficiency results in cell cycle inhibition, folic acid and THF have been used as effective targets for chemotherapeutics. Methotrexate, 6-mercaptopurine, and cyclophosphamide bind to dihydrofolate reductase and inhibit the conversion of folic acid to THF. This effectively decreases THF levels and inhibits DNA synthesis. 5-Fluorouracil (5-FU) is a potent chemotherapeutic whose mechanism of action is directly on thymidylate synthase. 5-FU is converted into FdUMP, and in the presence of THF inhibits thymidylate synthase, resulting in decreased production of dTMP. Thus the role of folic acid in cell cycle regulation is well characterized and has been utilized effectively in the development of chemotherapeutic agents.

A more recently discovered role for folic acid has revolved around the effects of DNA methylation on cellular growth and function. DNA methylation involves the transfer of methyl groups by DNA methyltransferases onto cytosine-guanine (CpG) dinucleotide-rich regions, most often found in gene promoters or initial exons. S-adenosyl-L-methionine (SAM) serves as the DNA methyl donor; however, THF is required for the generation of methionine, the precursor of SAM. Studies by Friso et al. demonstrated that folate levels directly correlate with genomic DNA methylation (26, 27).

DNA methylation establishes general patterns of gene expression. Hypermethylation tends to decrease gene expression and transcription, whereas hypomethylation can result in increased transcript expression. Furthermore, within coding regions of genes, methylation may increase genomic stability as it protects the DNA from nucleases. Thus decreased DNA methylation can result in aberrant gene expression and increased mutability of certain genes that may give rise to uncontrolled cellular growth.

Animal studies have illustrated a connection between folate/methyl availability, DNA methylation status, and tumor formation. In folate/methyl-deficient rats, hepatocellular carcinoma formation is associated with different levels of methylation on the p53 gene, which is dependent on tumor stage. In preneoplastic areas the coding region of p53 is hypomethylated and the p53 mRNA is upregulated. Conversely, in tumor tissues, p53 is hypermethylated and the mRNA expression is downregulated (77). Hypomethylation of the coding region of the p53 gene (exons 5–8) in a folate-deficient rat model resulted in increased DNA breaks (49). In addition, in an animal model of chemical carcinogenesis, the p53 gene was shown to be hypomethylated in exon 8, and interestingly, the hypomethylation was reversed when dietary folate levels were increased (50). These studies suggest a close relationship between dietary folate and DNA methylation, and furthermore, the role of folate in maintaining normal cellular function and growth through suppression of aberrant gene expression and preservation of genomic integrity.

Vitamin B12

Unlike the majority of other nutrients, vitamin B12 or cobalamin is almost solely derived from animal products. Vitamin B12 absorption is complex, requiring multiple proteins to transport through the esophagus, stomach, and ileum. Most importantly, vitamin B12 absorption requires intrinsic factor (IF), which is produced by the parietal cells of the gastric mucosa. The IF–vitamin B12 complex binds to ileal cells through IF-specific receptors that allow vitamin B12 to transverse the cellular membrane and bind to the plasma protein, transcobalamin II.

The functions of vitamin B12 are closely related to folic acid because cobalamin serves as a cofactor for methionine synthase, the enzyme directly responsible for the conversion of homocysteine to methionine. The methylated form of cobalamin is replenished by 5-methyl THF (Figure 3). Vitamin B12 deficiency also inhibits DNA synthesis as THF becomes trapped in the 5-methyl form, thereby diminishing the supply of 5–10-methylene THF, which is required for dTTP synthesis. Hence the clinical manifestations of vitamin B12 deficiency are very similar to folic acid deficiency and are a result of decreased DNA synthesis and subsequent cell cycle arrest.

Zinc

Zinc, found in meat, fish, whole-grain cereals, and legumes, is a key cofactor for numerous enzymes including alcohol dehydrogenase, carbonic anhydrase, carboxypeptidase, and superoxide dismutase, as well as for DNA binding. The

clinical features of zinc deficiency, including growth retardation, impaired wound healing, impaired reproductive function, and a distinctive rash (acrodermatitis enteropathica), mirror the importance of zinc in general cellular function, including the regulation of proliferation.

The most direct connection between zinc and the control of cellular growth is the necessity of zinc finger proteins for gene regulation. The zinc finger, a common eukaryotic motif that conjugates a zinc ion to cysteine and histidine residues to form a loop structure that binds to the major groove of DNA, is involved in the general transcription machinery as well as in gene activators and repressors. For example, transcription factor IIIA (TFIIIA) contains nine consecutive zinc finger motifs that bind to the 5S RNA gene and is a component of the general transcription machinery for RNA polyermase III, which transcribes the tRNA genes necessary for translation. Another zinc finger protein, Sp1, is an important transcription factor that has been linked to the regulation of cellular proliferation in multiple systems. For instance, in non-small-cell lung cancer cells, the antiestrogen tamoxifen inhibits cellular proliferation through the upregulation of p27 via Sp1 (Figure 3) (61). Similarly, in vascular smooth muscle cells, Sp1 enhances p21 expression, resulting in inhibition of proliferation. However, Sp1 also binds to the cyclin D1 promoter in response to growth-promoting factors, and enhances cellular proliferation (62, 70). TFIIIA and Sp1 are only two examples of zinc finger proteins that affect cellular proliferation. Many other zinc finger proteins regulate gene expression. Dietary zinc is integral to the regulation of overall cellular function and hence is required for cellular proliferative control.

REGULATION OF CELLULAR FUNCTION AND PROLIFERATION BY GLUCOSE

The effects of glucose on overall cellular function are widespread and multifaceted due to its function in biochemical pathways, intracellular signaling, and osmolarity regulation. Glucose is the main energy source utilized by cells. The breakdown of glucose into pyruvate by glycolysis yields anaerobic production of two moles of ATP per mole of glucose. The subsequent conversion of pyruvate into acetyl-CoA and its utilization by the Krebs cycle generates an additional two moles of ATP as well as reduced electron carriers that, through the respiratory chain, transfer electrons to molecular oxygen and generate an additional 34 moles of ATP. Together these biochemical pathways are the mainstay for metabolic energy production; thus cellular functions including proliferation intrinsically depend on adequate levels of glucose. Studies in yeast have demonstrated that glucose is required for cellular proliferation because lack of glucose in the media induces cell cycle arrest. Furthermore, inhibition of glycolysis by an inhibitor of glyceraldehyde 3-phosphate dehydrogenase or mutations in phosphofructokinase stops cell cycle progression and links the necessity of glucose to proliferative control (71). In humans, physiological levels of glucose (100 mg/dl) are also required for cellular proliferation.

Singh et al. (90) demonstrated that the rapidly growing prostate cancer cell line DU145 is growth arrested when glucose levels in the media are decreased to half of normal physiological levels. Decreased glucose levels inhibited DNA synthesis as well as decreased cyclin D and E levels and CDK4 resulting in G1 arrest, which suggests that glucose is integral to maintaining cellular proliferation, whether it be normal or pathologic (90). Furthermore, high glucose levels (450 mg/dl) stimulate proliferation of different tumor cell types. The MCF-7 breast cancer cell line increases DNA synthesis as well as cyclin D1 and CDK2 levels, resulting in increased proliferation. In contrast, the multidrug-resistant breast cancer cell line NCI/ADR-RES is not affected by changes in glucose levels, which suggests that highly mutated tumors that are resistant to chemotherapeutics also become resistant to changes in their environment and utilize alternate fuel sources (75).

The majority of studies analyzing the effect of glucose on cellular proliferation revolve around the complications of diabetes mellitus. Increases in blood glucose levels stimulate insulin secretion from pancreatic beta cells, which results in the transport and metabolism of glucose in hepatocytes, skeletal myocytes, and adipocytes. Decreased production of insulin, as found in type I diabetes, or increased resistance to insulin, as is the case in type II diabetes, results in elevated glucose levels. Chronic hyperglycemia has profound physiological effects on renal function and the vasculature, which relate on the cellular level to cell cycle dysregulation. In the kidney, high glucose levels induce the formation of glomerular lesions, resulting in increased permeability of capillaries and massive proteinuria. Central to this pathogenesis is the effect of glucose on mesangial cells in renal glomeruli. Hyperglycemia induces biphasic growth in mesangial cells, an early stage of self-limited proliferation followed by cell cycle arrest and cellular hypertrophy (104–106). During the proliferative phase, high glucose decreases p21 expression and increases CDK2 and CDK4 levels, resulting in cellular growth (16). In contrast, during the hypertrophic phase, mesangial cell cycle arrest in G0/G1, which is mediated by p27 as well as by factors including TGF-β and angiotensin II, is concomitant with an increase in protein synthesis resulting in cellular hypertrophy (105). In the vasculature, high levels of glucose inhibit endothelial cell proliferation by increasing p21 and p27 expression (2). Thus regulation of the cell cycle by hyperglycemia directly relates to the pathogenesis of nephropathy as well as to vascular disease associated with diabetes.

In addition, glucose regulates intracellular signaling through the activation of insulin pathways as well as through other signal transduction pathways. Upon binding to its heterotetrameric receptor tyrosine kinase, insulin activates multiple downstream signaling pathways, including MAPK and PI3 kinase, which can result in diverse effects, depending on cell type. For example, in rat lactotrophs from the anterior pituitary, insulin increases cyclin D1 with a concomitant increase in cellular proliferation (48). However, in differentiated-arrested cycling C2C12 myoblasts, insulin activation of p38-MAPK and AKT results in p21-mediated cell cycle arrest and the induction of myotube formation (15). In addition, hyperglycemia upregulates TGF-β expression and activity in mesangial cells and endothelial cells (7, 39, 64). TGF-β has been shown to have antiproliferative

effects on multiple types of cells including endothelial cells (66, 69), and recent studies by McGinn et al. demonstrate that the effects of hyperglycemia-induced TGF-β production are specifically mediated by p38-MAPK (65). Thus the effects of glucose also relate to the activation of different signal transduction pathways, including insulin and TGF-β, which regulate cellular growth.

SUMMARY

In summary, it is apparent that nutrients are important regulators of cellular proliferation, not just as fuel substrates but also as specific modulators of the production and function of cell cycle–associated proteins. Clinical investigators continue to take advantage of accumulating knowledge of the mechanisms by which specific nutrients regulate cell cycle progression to manipulate this process in pathological conditions where aberrant cell growth is central to the progression of diseases, such as in tumorigenesis. Gaining further insight into the role of specific nutrients in the control of cell growth will improve our ability to manipulate their function for the development and optimization of clinical therapies.

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