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NEW INSIGHTS INTO ERYTHROPOIESIS: The Roles of Folate, Vitamin B_{12}, and Iron*[1]

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■ **Abstract** Erythropoiesis is the process in which new erythrocytes are produced. These new erythrocytes replace the oldest erythrocytes (normally about one percent) that are phagocytosed and destroyed each day. Folate, vitamin B_{12}, and iron have crucial roles in erythropoiesis. Erythroblasts require folate and vitamin B_{12} for proliferation during their differentiation. Deficiency of folate or vitamin B_{12} inhibits purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anemia from ineffective erythropoiesis. Erythroblasts require large amounts of iron for hemoglobin synthesis. Large amounts of iron are recycled daily with hemoglobin breakdown from destroyed old erythrocytes. Many recently identified proteins are involved in absorption, storage, and cellular export of nonheme iron and in erythroblast uptake and utilization of iron. Erythroblast heme levels regulate uptake of iron and globin synthesis such that iron deficiency causes anemia by retarded production rates with smaller, less hemoglobinized erythrocytes.

[1]ABBREVIATIONS: ALA-S2/eALA-S, erythroid-specific 5-aminolevulinic-acid synthase; BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; eIF-2, eukaryotic initiation factor 2; EPO, erythropoietin; FBP, folate-binding protein; GI, gastrointestinal; HO-1, heme oxygenase 1; HRI, heme-regulated inhibitor; IRE, iron-responsive element; Ireg1/MTP1, ferroportin 1; IRP, iron regulatory protein; LIP, labile iron pool; NTBI, nontransferrin-bound iron; RFC, reduced folate carrier; THF, tetrahydrofolate; UTR, untranslated region.

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INTRODUCTION

Erythropoiesis is the process by which the hematopoietic tissue of the bone marrow produces red blood cells (erythrocytes). The mean lifespan of a normal human erythrocyte is about 120 days. Erythrocytes are involved in transporting carbon dioxide and nitric oxide, but their principal function is to deliver oxygen from the lungs to the other tissues of the body. The amount of oxygen delivered to the tissues is a function of the number of circulating erythrocytes. In normal adults, approximately 200 billion of the oldest erythrocytes (about 1% of the total number) are replaced every day by an equal number of newly formed erythrocytes. In situations in which the erythrocytes are abnormally lost from the circulation by bleeding or by increased destruction (hemolysis), the rate of new erythrocyte production can exceed one trillion per day. Thus, erythropoiesis is a dynamic process that can respond promptly to the need for more oxygen delivery. Among the numerous requirements for active erythropoiesis are adequate supplies of three nutrients—folate, cobalamin (vitamin B_{12}), and iron. Deficiency of each of these three nutrients can lead to decreased erythrocyte production and subsequently to decreased numbers of circulating erythrocytes (anemia). Advances in erythropoiesis research have helped to explain the roles of these nutrients in the production of erythrocytes and how their respective deficiency states cause anemia. Recently reported findings related to the development of nutrition-related anemias that will be reviewed here include: (*a*) the uptake and intracellular effects of folate, vitamin B_{12}, and iron; (*b*) the induction of programmed death (apoptosis) of erythroid progenitor cells in folate or vitamin B_{12} deficiency; and (*c*) the cellular mechanisms in iron-deficient erythroblasts that avoid apoptosis, but nonetheless decrease erythrocyte production.

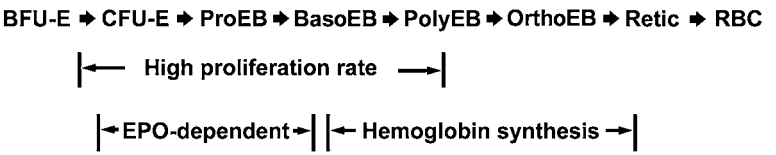

Figure 1 Stages of erythroid differentiation. Specific stages of erythroid differentiation beginning with the burst-forming unit-erythroid (BFU-E) and ending with the mature erythrocyte (RBC) are shown. Multiple cell divisions occur between stages prior to the polychromatophilic erythroblast (polyEB) stage after which the cells do not divide. Periods of high cellular proliferation, erythropoietin (EPO) dependence, and hemoglobin synthesis are demarcated. Other abbreviations: CFU-E, colony-forming unit-erythroid; ProEB, proerythroblast; BasoEB, basophilic erythroblast; OrthoEB, orthochromatic erythroblast; and Retic, reticulocyte.

STAGES AND REGULATION OF ERYTHROPOIESIS

All types of blood cells have a finite life span and must be constantly replaced by new cells formed in the hematopoietic tissue. Erythropoiesis is a continuous process of proliferation and differentiation beginning with the hematopoietic stem cell and ending with the erythrocyte (56, 60) (Figure 1). Hematopoietic stem cells are rare, less than one in ten thousand nucleated cells of the bone marrow, and they can self-renew or differentiate into all of the cells in the blood and the immune system. Their commitment to differentiation and the subsequent commitment of their progeny to the erythroid lineage appear to be stochastic events, but may be related to the prevalence and association of specific DNA transcription factors (18). The earliest stage of progenitor cell differentiation that is committed to the erythroid lineage is the burst-forming unit-erythroid (BFU-E; Figure 1). Human BFU-Es are defined by their ability to form large "bursts" of erythroblast colonies or one very large colony of erythroblasts, after two to three weeks in semisolid tissue culture. Erythroid bursts can contain more than one thousand erythroblasts and, thus, a single BFU-E and its progeny can have ten or more rounds of cell division before reaching the terminal postmitotic stages of differentiation. The next defined stage is the colony-forming unit-erythroid (CFU-E; Figure 1). Human CFU-Es require one week to form single colonies of up to 64 erythroblasts in tissue culture. Thus, CFU-Es and their progeny have six or fewer rounds of cell division. The erythropoietic stages subsequent to CFU-E are defined by their light microscopic appearance in stained preparations. Cellular proliferation is not shown in Figure 1, but the percentage of cells in active cell cycle is greatest in the CFU-E and proerythroblast stages, and cell division ceases at the polychromatophilic stage.

Erythropoietin (EPO) is the principal regulator of erythropoiesis (56, 63). EPO is a glycoprotein hormone produced by a subset of peritubular, interstitial cells in the renal cortex (61, 64). A few of these cells produce EPO under normal

circumstances. In response to decreased oxygen delivery as occurs with anemia, the number of these interstitial cells that produce EPO increases exponentially (62). In the bone marrow, EPO acts upon erythroid progenitors in the stages from CFU-E to the earliest of basophilic erythroblasts. This period of EPO dependence precedes and does not overlap the period of hemoglobin synthesis (Figure 1). As shown in Figure 1, progenitor cells in these stages are dependent upon EPO to prevent apoptosis (55, 119), but they display widely varying degrees of dependence (54). To survive this period of dependence, most erythroid progenitor cells require greater EPO concentrations than those concentrations normally found in the blood. Thus, the normal daily production rate of 200 billion erythrocytes requires the survival of only a minority of the maximal number of EPO-dependent erythroid progenitor cells.

Anemia occurs when the number of circulating erythrocytes is decreased. If the anemia is due to transient blood loss or hemolysis and the kidneys and bone marrow are normal, the erythropoietic system corrects the anemia. Specifically, the decreased erythrocytes in anemia reduce oxygen delivery, and the kidneys respond by increasing EPO production. The increased EPO results in the survival of more erythroid progenitor cells in the EPO-dependent stages and subsequently increased erythrocyte production. The increased erythrocytes in the circulation deliver more oxygen, lowering the elevated EPO levels, and ultimately returning the erythrocyte production rate and the number of circulating erythrocytes to their normal, steady-state levels prior to the onset of anemia. In many anemias, however, the kidneys are not normal, resulting in deficiency of EPO, or the hematopoietic tissue of the bone marrow is not normal, resulting in an inability to respond to the EPO. Among the numerous causes of an inability to respond to erythropoietic demand are deficiencies of folate, vitamin B_{12}, and iron. The majority of nutrition-related anemias can be attributed to deficiency of one of these nutrients (48). Folate and vitamin B_{12} are both required for the extensive DNA synthesis that accompanies the production of hundreds of billions of new erythrocytes each day. All proliferating cells require iron, but the iron requirements of erythroid cells in the late basophilic erythroblast through reticulocyte stages, when hemoglobin is synthesized and accumulates (Figure 1), are much greater than all other cell types.

FOLATE AND VITAMIN B_{12} AND THEIR DEFICIENCY STATES

Folate, an essential nutrient found in the tissues of plants and animals, consists of a pteridine (2-amino-4-hydroxy-pteridine) ring attached to para-aminobenzoate with a polyglutamyl tail. The reduced tetrahydrofolate (THF) form acts as a co-factor in multiple biochemical reactions by donating or accepting one-carbon units (4, 108). Folate is present in both plant and animal tissues, most commonly in the form of 5-methyl-THF. Reduced folates are absorbed in the jejunum after enzymatic cleavage to the monoglutamic form (40, 42). The absorbed folate

enters the blood and circulates in the body as 5-methyl-THF monoglutamate. Once folate is transported from the blood into a cell, it is retained there through the action of folylpolyglutamate synthetase that converts the folate to the polyglutamyl form (108). Folate is transported into cells by several mechanisms, but the endocytotic mechanism involving two specific glucosylphosphatidylinositol-anchored, cell-surface folate-binding proteins (FBPs) and the bidirectional membrane transporter termed the reduced folate carrier (RFC) are the best characterized (72). Both of these transport mechanisms have been examined in erythropoietic cells. In vitro studies with antibodies to the FBPs showed that FBPs are expressed on early stage hematopoietic cells, but they do not transport significant amounts of folate (98). Similar in vitro studies show some morphological changes in the progeny of BFU-E and CFU-E when antibodies to FBPs are added to the culture medium, but surprisingly the growth of these erythroid progenitors is enhanced by the antibodies (5, 6). Mice that are rendered null for one of the FBPs by homologous recombination have embryonic lethality due to neural defects, but no hematopoietic defect has been described (83). Mice rendered null for the RFC also have embryonic lethality, but the defect appears to involve the hematopoietic system (123). The prenatal mice can be rescued by loading the mothers with high doses of folic acid, but the pups die from hematopoietic failure a few weeks after birth (123), indicating that the RFC is necessary for folate transport in erythroid cells.

Clinical folate deficiency in the developed countries has been associated with those who have poor nutrition, such as the elderly or those with alcoholism. To reduce the incidence of neural tube defects that develop during the first trimester of pregnancy, grain products in the United States have been fortified with folic acid for the last seven years. Considering serum folate concentrations of less than 3 ng/ml as folate deficiency, one study found a reduction in folate-deficient individuals among a middle-aged/older population from 22% prior to fortification to 1.7% after fortification (52). Despite this dramatic decrease in the incidence of folate deficiency in the general population, others remain at increased risk for developing deficiency due to specific medical conditions. Among the individuals at increased risk are those with intestinal malabsorption; general malnutrition; high erythrocyte turnover rates such as in chronic hemolytic anemias; anticonvulsant medications that interfere with folate absorption or utilization such as phenytoin; and antibiotic medications with antifolate actions such as trimethoprim/sulfamethoxazole.

Vitamin B_{12} (cobalamin), an essential nutrient consisting of a tetrapyrrole (corrin) ring containing cobalt that is attached to 5,6-dimethylbenzimidazolyl ribonucleotide, is produced in microorganisms and is found in animal tissues. Vitamin B_{12} is a coenzyme in two biochemical reactions in humans. One of these reactions is the transfer of a methyl group from 5-methyl-THF to homocysteine via methylcobalamin, thereby regenerating methionine (4, 108) (Figure 2). This reaction represents the link between folate and vitamin B_{12} coenzymes and appears to account for the requirement of both vitamins in normal erythropoiesis (106, 114). The absorption of vitamin B_{12} is a relatively complex process (4, 106, 107). Protein-bound vitamin B_{12} in food is released by stomach acid and binds to

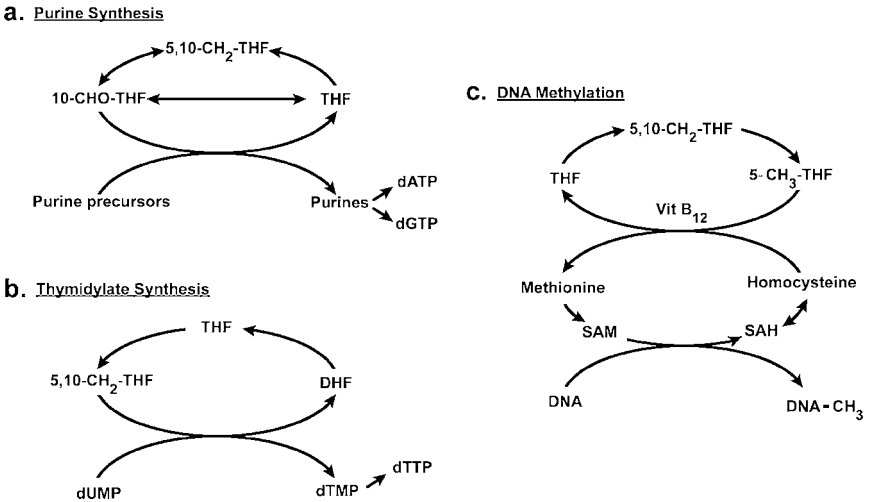

Figure 2 DNA synthesis pathways that require folate or vitamin B_{12} coenzymes. Abbreviations: THF, tetrahydrofolate; 5,10-CH_2-THF, methylenetetrahydrofolate; 10-CHO-THF, formyltetrahydrofolate; 5-CH_3-THF, methyltetrahydrofolate; DHF, dihydrofolate; DNA-CH_3, methylated DNA; dUMP, deoxyuridylate; dTMP, thymidylate; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; and dTTP, thymidine triphosphate.

specific vitamin B_{12}–binding glycoproteins termed haptocorrins that are present in the secretions of the salivary glands and stomach. In the duodenum, the haptocorrins are digested, and the vitamin B_{12} binds to intrinsic factor, another glycoprotein secreted by the stomach. The vitamin B_{12}–intrinsic factor complex subsequently binds to specific receptors in the terminal ileum. These receptors consist of cubilin, which binds the vitamin B_{12}–intrinsic factor complex, and megalin, an associated membrane transport protein (78). After endocytosis in the ileal epithelium, the vitamin B_{12} is freed from the intrinsic factor and binds to transcobalamin II (apotranscobalamin) that is produced by the microvascular endothelium of ileal villi (95). The vitamin B_{12}–transcobalamin II complex, termed holotranscobalamin II, enters the blood where it is the functional carrier of vitamin B_{12} to the other cells of the body. Holotranscobalamin II binds to specific homodimerized receptor proteins that are displayed on the surface of many different types of cells (107). Although this receptor has not been examined directly in hematopoietic tissue, its mechanism of transport in the other cells is via endocytosis with subsequent intracellular release of vitamin B_{12} from its complex with transcobalamin II. The incidence of vitamin B_{12} deficiency increases significantly with age such that up to 15% of older individuals are deficient in developed countries (8, 110). Most often this age-related deficiency appears to be due to atrophic gastritis and the resultant inability to dissociate vitamin B_{12} from the proteins to which it is bound

in food (19). Other causes include the autoimmune gastropathy termed pernicious anemia, in which both intrinsic factor and gastric acid are not produced; intestinal malabsorption involving the terminal ileum; previous surgery that removed the stomach or ileum; medications that interfere with gastric acid secretion such as H_2-histamine receptor blockers or proton pump inhibitors; and a strict vegan diet.

ROLES OF FOLATE AND VITAMIN B_{12} IN ERYTHROPOIESIS

The importance of adequate folate and vitamin B_{12} in erythropoiesis is demonstrated by megaloblastic anemia, the clinical disease that can occur with deficiency of either vitamin. Megaloblastic anemia affects all hematopoietic lineages, but it is most prominent in the erythroid lineage. Megaloblastic anemia is characterized by pancytopenia with macrocytic erythrocytes, hypersegmented neutrophilic granulocytes, and reticulocytopenia. The bone marrow has increased numbers of large immature-appearing erythroblasts and myeloblasts (i.e., megaloblasts) that are undergoing increased rates of premature death as shown by elevated serum bilirubin, lactate dehydrogenase, and myeloperoxidase, and by increased iron turnover. This increased death of hematopoietic cells prior to their maturation is termed ineffective hematopoiesis. Studies in patients with anemia due to folate or vitamin B_{12} deficiency have shown that impaired DNA synthesis and its sequelae are key elements in the increased hematopoietic cell death that characterizes these anemias. The period of high proliferation rates during erythropoiesis (Figure 1) makes the erythroid progenitor cells more susceptible than other types of cells to the impaired DNA synthesis in folate or vitamin B_{12} deficiency. Erythroblasts from patients with folate or vitamin B_{12} deficiency anemia had no active incorporation of ^{3}H-thymidine into DNA despite total DNA content between 2N and 4N that characterizes cells in DNA synthesis (i.e., in S-phase of the cell cycle) (75, 115, 121). Flow cytometry of bone marrow cells from patients with folate or vitamin deficiency had increased percentages of cells in S-phase compared to controls (50). When rates of DNA synthesis were examined directly in mitogen-stimulated blood lymphocytes of patients with folate- or vitamin B_{12}–deficiency anemia, they were decreased (117), but a similar study using bone marrow cells did not show a decreased rate (13). Impaired DNA synthesis would be expected to result in chromosomal breakage and possibly nuclear damage. Previous studies have shown that chromosomal breakage is markedly increased in the bone marrow cells of patients with folate or vitamin B_{12} deficiency anemia (44, 75). Also, erythrocyte micronuclei (Howell-Jolly bodies), a marker of genetic damage when they are increased in splenectomized patients, are most increased in those patients who have folate or vitamin B_{12} deficiency (70).

One-carbon units are required in three biochemical pathways involved in the synthesis of DNA. These pathways are shown in Figure 2. They are (*a*) two steps in the de novo synthesis of purines in which 10-formyl-THF provides two carbons

of the purine ring structure; (*b*) the reaction catalyzed by thymidylate synthase in which 5,10-methylene-THF provides the methylene group and reducing equivalents for the methylation of deoxyuridylate to form thymidylate; and (*c*) the reaction catalyzed by DNA methyltransferase in which 5-methyl-THF provides the methyl group (indirectly through remethylation of homocysteine to form methionine and subsequently S-adenosylmethionine) for the methylation of cytosines in DNA. As mentioned above, the methylcobalamin form of vitamin B_{12} is the coenzyme involved in the transfer of the methyl group from 5-methyl-THF to homocysteine, thereby regenerating methionine and THF (Figure 2*c*). With vitamin B_{12} deficiency, not only does inhibited methionine regeneration lead to decreased S-adenosylmethionine and increased homocysteine and S-adenosylhomocysteine, but 5-methyl-THF accumulates intracellularly, while other forms of THF, specifically the 10-formyl-THF required for purine synthesis and the 5,10-methylene-THF required for thymidylate synthesis, decrease (108). This predicted "trapping" of intracellular folate in the 5-methyl-THF form (46) resulting in intracellular deficiencies of other forms of folate, including those required for de novo synthesis of deoxynucleotides, was demonstrated in the bone marrow cells of rats made functionally vitamin B_{12} deficient by nitrous oxide exposure (49). In folate or vitamin B_{12} deficiency, the de novo synthesis of deoxynucleotides is decreased, resulting in impaired synthesis and repair of DNA, and ultimately, in cell death. Erythropoiesis under these deficiency conditions is termed ineffective because the erythroid cells are present in the hematopoietic tissue, but most of them cannot mature to the late stages of differentiation before undergoing apoptosis. The decreased numbers of erythroid cells surviving until the postmitotic, terminal stages in ineffective erythropoiesis leads to anemia.

THE RELATIONSHIP BETWEEN IMPAIRED DNA SYNTHESIS AND ERYTHROID CELL APOPTOSIS

An in vivo murine model (10) and its in vitro extension (57) of folate-deficient erythropoiesis have provided some new insights into the cellular events that lead to erythroid cell apoptosis in folate deficiency. In this model, mice are fed an amino acid–based, folate-free diet that induces a pancytopenia with all of the characteristics of the human hematopoietic disease that results from folate or vitamin B_{12} deficiency (10). To study the cellular events of folate-deficient erythropoiesis in a purified population of developmentally synchronized cells, mice are fed the folate-free diet before and during the acute erythroblastosis phase of Friend virus disease. This virus induces a proliferation of erythroid cells that accumulate at the proerythroblast stage of differentiation, and when combined with the folate-free diet yield a population of folate-deficient proerythroblasts. When cultured with EPO under folate-sufficient conditions, almost all of these proerythroblasts differentiate into reticulocytes, but when cultured with EPO under folate-deficient conditions, most of these proerythroblasts undergo apoptosis before differentiating

into reticulocytes (57). Proerythroblasts freshly isolated from folate-deficient mice have decreases in all forms of folate (58). During their differentiation in vitro, the erythroblasts cultured under folate-deficient conditions accumulate in S-phase of the cell cycle. The majority of these cells undergo apoptosis in S-phase (59). The folate-deficient erythroid cells can be saved from their apoptotic fate if they are supplied in vitro with sufficient amounts of both thymidine and a purine that can be salvaged to provide the necessary deoxynucleotides that permit DNA synthesis (59). Hypoxanthine, inosine, adenosine, and deoxyadenosine are effective for this purine salvage, but guanosine and deoxyguanosine are not. The medium supplementation required in vitro for the survival and completion of erythroid differentiation is 60 μmol/L for the purine and 20 μmol/L for thymidine, indicating that the defects in DNA replication and repair that lead to apoptosis in folate-deficient erythroid cells are due to impaired de novo synthesis of primarily purines (Figure 2*a*) and secondarily thymidylate (Figure 2*b*). The methylation of cytosines in the DNA of folate-deficient murine erythroblasts is the same as in control erythroblasts (DJ Park and MJ Koury, unpublished data). Similarly, bone marrow cells of patients with vitamin B_{12} deficiency anemia and bone marrow cells of controls had similar percentages of methylated cytosines in their respective DNAs (97). These results in mice and humans suggest that inhibition of DNA methylation by folate or vitamin B_{12} deficiency (Figure 2*c*) does not play a role in the anemias resulting from deficiency of these vitamins.

The mechanism by which the DNA damage in folate or vitamin B_{12} deficiency leads to apoptosis in hematopoietic cells has not been established. The inhibited conversion of deoxyuridylate to thymidylate has been associated with increased uracil misincorporation into DNA due to the utilization by DNA polymerase of deoxyuridine triphosphate in lieu of thymidine triphosphate (11, 12, 116). However, one study in vitamin B_{12}–deficient patients (97), and another in folate-deficient patients (99), did not find this increased incorporation of uracil in DNA of blood cells. Uracils misincorporated close to one another on opposite DNA strands have been proposed as a source of double-stranded DNA breakage in eukaryotic cells (38) and lead to double-stranded DNA cleavage in an experimental prokaryotic system (28). The DNA of the folate-deficient erythroblasts in the in vitro murine model have only a two- to threefold increased proportion of misincorporated uracil compared to controls (58), suggesting that uracil misincorporation may not be a significant source of DNA strand breakage leading to apoptosis. These two- to threefold increases in uracil misincorporation in folate-deficient erythroblasts are similar to the changes seen in lymphocytes of folate-deficient rats that have evidence of DNA damage (31), but less than found in patients with megaloblastic anemia (116). The rescue of folate-deficient erythroblasts by exogenous purines and thymidine suggests that insufficient deoxynucleotide triphosphates may be the cause of DNA damage and apoptosis. Murine granulocyte progenitors treated with the antifolate methotrexate are similarly rescued by exogenous purines and thymidine (84). Although one study of bone marrow from patients with megaloblastic anemia found increases in all deoxynucleotides (51), others have shown

specific deoxynucleotide depletions in splenic cells of folate-deficient rats (53), human lymphocytes cultured under folate-deficient conditions (111), and cell lines treated with antifolates (9, 122). Murine erythroblasts accumulate increased p53 protein when they are cultured under folate-deficient conditions (58), suggesting that *p53* expression is an indicator of DNA damage in folate-deficient erythroblasts. However, when erythroblasts from *p53*-null mice are cultured under folate-deficient conditions, the mice have similar rates of apoptosis as do their *p53* wild-type littermates, which indicates that p53 is not necessary for the apoptosis that results from folate deficiency–induced DNA damage (59).

The murine in vivo and in vitro systems have also provided insights into the morphological changes of ineffective erythropoiesis that occur in folate or vitamin B_{12} deficiency. When mice are made folate-deficient by being fed the folate-free diet, they develop a macrocytic anemia with decreased reticulocytes (10, 58). As the folate-deficiency anemia progresses, the bone marrow hematopoietic cells of the mice, including the erythroid cells, have decreased numbers of total nucleated cells, increased size of the individual cells, and increased numbers of cells undergoing apoptosis. While the absolute numbers of reticulocytes are decreased in folate-deficient mice, the absolute numbers of CFU-Es are increased in their bone marrow and spleen compared to controls (10). This result indicates that the folate-deficient mice, like their human counterparts, have increased EPO levels in response to the anemia, with a resultant increased survival of erythroid cells in the CFU-E and other early stages of the EPO-dependent period. However, most of these increased CFU-Es do not survive during the subsequent stages of erythropoiesis, but rather they succumb to apoptosis, most often while in S-phase of the cell cycle. Erythroid cells that are in the CFU-E stage or in S-phase during the post-CFU-E stages of differentiation are larger and more immature appearing than the normal erythroid cells which accumulate in the G_0/G_1 phase during the terminal stages of erythroid differentiation. Together, the shift to earlier stages of erythroid differentiation and the accumulation of cells in S-phase contribute to the increased size and immature appearance of erythroid cells in the bone marrow that characterize megaloblastic anemias (58).

IRON METABOLISM AND THE IRON-DEFICIENCY STATE

Iron is an essential element that is a component of heme-containing proteins (i.e., hemoglobin, myoglobin, and cytochromes) and innumerable nonheme iron-containing proteins with vital functions in many metabolic processes of all cells. However, at pH 7.4 and physiological oxygen tension, the relatively soluble ferrous ion is readily oxidized to the ferric ion, which forms virtually insoluble ferric hydroxides. Moreover, unless bound to specific ligands, iron plays a key role in the formation of harmful oxygen radicals, which ultimately cause peroxidative damage to vital cell structures. Because of this virtual insolubility and potential toxicity, specialized mechanisms and molecules for the acquisition, transport, and

storage of iron in a soluble, nontoxic form have evolved to meet cellular and organismal iron requirements. Moreover, organisms are equipped with sophisticated mechanisms that prevent the expansion of a catalytically active intracellular iron pool, while maintaining sufficient concentrations for metabolic use (2, 90, 100, 104).

Cellular iron acquisition and its proper intracellular targeting into functional iron proteins depend on an array of other proteins. "Traditional" proteins involved in iron metabolism include transferrin, transferrin receptor, and ferritin, but recent research has identified a number of novel genes whose products emerge as important players in iron metabolism (Table 1).

Iron represents 55 and 45 mg per kilogram of body weight in adult men and women, respectively. Normally, about 60% to 70% of total body iron is present in hemoglobin in circulating erythrocytes. In vertebrates, iron is transported within the body between sites of absorption, storage, and utilization by the plasma glycoprotein, transferrin, which binds ferric iron very tightly but reversibly. The daily turnover of transferrin iron is roughly 30 mg and, normally, about 80% of this iron is transported to the bone marrow for hemoglobin synthesis in developing erythroid cells. Senescent erythrocytes are phagocytosed by macrophages of the reticuloendothelial system where the heme moiety is split from hemoglobin and catabolized enzymatically via heme oxygenase-1 (HO-1) (71). Iron, which is liberated from its confinement within the protoporphyrin ring inside macrophages, is returned almost quantitatively to the circulation. The remaining 5 mg of the daily plasma iron turnover is exchanged with nonerythroid tissues, namely, the liver. About 1 mg of dietary iron is absorbed daily, and the total organismal iron balance is maintained by a daily loss of 1 mg via nonspecific mechanisms (mostly cell desquamation) (100).

Several important features of organismal iron metabolism must be mentioned. First, iron turnover is virtually an internal event in the body, and most of the iron turning over is used for the synthesis of hemoglobin in erythroid cells. Second, at least some nonerythroid cells can acquire nontransferrin-bound iron (NTBI), and this process likely operates in vivo only in severely iron-overloaded patients who have NTBI in their plasma. However, hemoglobin synthesis is stringently dependent on transferrin as the source of iron for erythroid cells. Third, although iron absorption is required for efficient erythrocyte formation on a long-term basis, quantitatively the most important source of iron for day-to-day erythropoiesis is macrophages that recycle hemoglobin iron. Fourth, erythrocytes contain about 45,000-fold more heme iron (20 mmol/L) than nonheme iron (440 nmol/L) (100). The fact that all iron for hemoglobin synthesis comes from transferrin and that this delivery system operates so efficiently, leaving mature erythrocytes with negligible amounts of nonheme iron, suggests that the iron transport machinery in erythroid cells is an integral part of the heme biosynthesis pathway. It seems reasonable to propose that the evolutionary forces that led to the development of highly hemoglobinized erythrocytes also dramatically affected numerous aspects of iron metabolism in developing erythroid cells, making them unique in this regard.

TABLE 1 Some proteins involved in iron metabolism

Protein	Function	Result of deficiency	References
Transferrin (Tf)	Fe^{3+}-carrier in plasma	Severe Fe deficiency anemia; generalized Fe overload	(88, 90)
Tf receptor	Membrane receptor for diferric-Tf	Embryonic lethality	(67, 91)
Ferritin (H and L)	Cellular Fe storage	H: embryonic lethality	(7, 32, 90)
IRP (1 and 2)	Fe "sensors"; bind to IREs	IRP2: brain Fe overload	(2, 15, 65, 76, 104)
DMT1/DCT1/Nramp2	Membrane transporter for Fe^{2+}	Hypochromic microcytic anemia	(16,17, 34, 41)
Duodenal cytochrome b (Dcytb)	Ferric reductase (provides Fe^{2+} for DMT1 in duodenum)	Unknown	(73)
Ferroportin 1/Ireg1/MTP1	Fe export from cells	Hemochromatosis type 4	(1, 29, 74, 79, 88)
Ceruloplasmin (Cp)	Regulation of Fe export from cells	Hypochromic microcytic anemia	(45)
Hephaestin	Regulation of Fe export from enterocytes (membrane-bound Cp homolog)	Hypochromic microcytic anemia	(3, 112)
ALA-S2/eALA-S	First enzyme of heme synthesis; erythroid-specific 5-aminolevulinic-acid synthase	X-linked sideroblastic anemia	(33, 85)
Ferrochelatase	Last enzyme of heme synthesis; Fe^{2+} insertion into protoporphyrin IX	Erythropoietic protoporphyria	(24, 85)
Mitochondrial ferritin	Mitochondrial Fe storage (?)	Unknown; high expression in "ring" sideroblasts	(20, 30)
Heme oxygenase-1 (HO-1)	Recycling of hemoglobin Fe	Severe anemia and inflammation	(71, 88, 93, 94, 120)
Hepcidin	Plasma peptide which appears to inhibit Fe absorption	Fe overload; overexpression of hepcidin causes severe Fe deficiency anemia	(35, 80–82)

Abbreviations: ALA-S2/eALA-S, erythroid-specific 5-aminolevulinic-acid synthase; DCT, divalent cation transporter; DMT, divalent metal transporter; IRE, iron-responsive element; IRP, iron regulatory protein.

Iron deficiency is the most prevalent cause of anemia, affecting more than half a billion people worldwide. The anemia of iron deficiency is caused by a decreased supply of iron for heme synthesis and, consequently, hemoglobin formation in developing erythroid cells. Decreased hemoglobinization leads to the production of erythrocytes that are smaller than normal (microcytic) and contain reduced amounts of hemoglobin (hypochromic). Blood loss is the most common cause of iron deficiency. One milliliter of blood contains about 0.5 mg of iron and, hence, a steady blood loss of as little as 3 to 4 mL per day (1.5 to 2 mg of iron) can result in a negative iron balance. In men and postmenopausal women, unexplained iron deficiency is nearly always due to occult bleeding from the gastrointestinal (GI) tract. Sources of GI bleeding include hemorrhoids, hiatus hernia, peptic ulceration, diverticulosis, tumors of the stomach and colon, adenomatous polyps, colitis, esophageal varices, and ingestion of salicylates, steroids, and nonsteroidal anti-inflammatory agents. Worldwide, the leading cause of GI blood loss is hookworm infection (87). In premenopausal women, menstrual blood loss is the most common cause of iron deficiency. The average menstrual blood loss in normal healthy women is about 40 mL, and women who lose 80 mL or more become iron-deficient. Increased iron requirements during periods of rapid growth, diminished iron absorption, or both may also cause iron deficiency.

In the anemia of chronic disease, iron-deficient erythropoiesis results from a defect in the recycling of hemoglobin iron in the reticuloendothelial system (109). In patients with anemia of chronic inflammation, there appears to be a defect in the release of iron from macrophages that is probably caused by cytokine-induced ferritin synthesis. As a result, iron is plentiful in macrophages, but this iron is not available to erythroid precursors.

IRON EXPORT FROM CELLS TO TRANSFERRIN: A NECESSARY PREREQUISITE FOR ERYTHROPOIESIS

There are specialized mammalian cells that must export iron. Absorption of dietary iron for transfer to transferrin in plasma requires iron efflux across the basolateral surface of the intestinal epithelia. A second major site of iron release is from macrophages where senescent or damaged red cells are degraded to export the metal from hemoglobin and provide it for binding to transferrin. Iron release from these "donor cells" to plasma transferrin is poorly understood, but a number of recent studies have provided new clues in this important area of iron metabolism. A likely candidate for iron export from cells is ferroportin 1 (29), also known as Ireg1 (74) or MTP1 (1), with the ferroxidase activity of hephaestin (112) and ceruloplasmin (45) facilitating the movement of iron across the membranes of enterocytes and macrophages, respectively. Ceruloplasmin and hephaestin exhibit a high degree of homology; both proteins contain several copper atoms that are necessary for their ferroxidase (i.e., oxidation of Fe^{2+} to Fe^{3+}) activity.

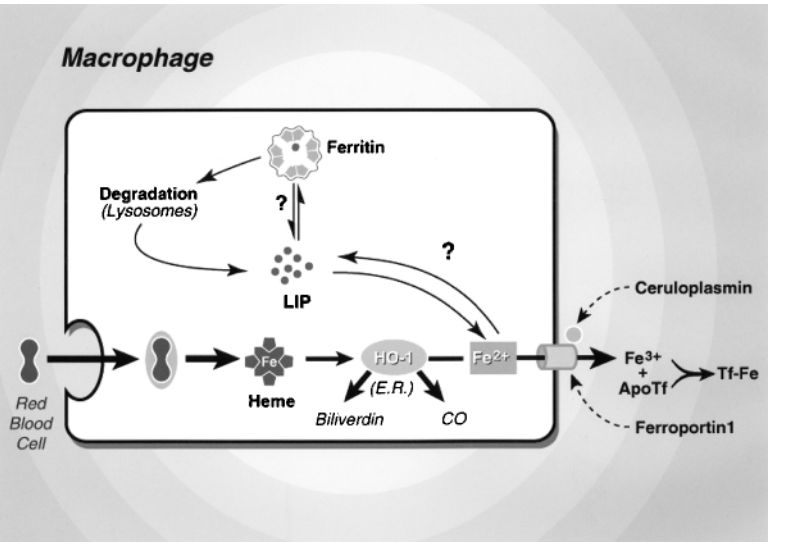

Figure 3 Scheme of possible iron (Fe) pathways in reticuloendothelial macrophages involved in the recycling of hemoglobin iron. Following phagocytosis of senescent red blood cells, the erythrocyte membrane is lysed and heme is transported to the endoplasmic reticulum (E.R.) to be degraded by heme oxygenase-1 (HO-1). Most of the iron derived from hemoglobin catabolism is promptly returned to the circulation, likely being transported across the plasma membrane by ferroportin 1. In Kupffer cells, ferroportin 1 (MTP1, Ireg1) is present not only at the plasma membrane but also in the cytoplasm (1). (Reprinted from Reference 89 and used with permission.)

At the end of an erythrocyte's life, it is phagocytosed by cells of the reticuloendothelial system and iron is liberated from its confinement within the protoporphyrin ring by HO-1. These cells have an enormous capacity to purge themselves of iron, which is likely exported via ferroportin 1 (Figure 3). However, the mechanism involved in the regulation of macrophage iron output is unknown (47). It has recently been proposed (35, 37) that the plasma peptide hepcidin may be involved in the regulation of iron release from macrophages, but direct evidence to support this hypothesis is missing.

Normally, the body iron content in humans is maintained within narrow limits by the regulation of intestinal iron absorption (77). Both heme and elemental iron are absorbed through the brush border of the upper small intestine. Heme iron is more readily available for absorption but usually constitutes only a small fraction of dietary iron. Heme (derived from hemoglobin or myoglobin) is taken up intact, probably via specific high-affinity heme-binding sites in the mucosal brush border (39, 118) (Figure 4). After entering the intestinal epithelial cells, iron is enzymatically released from heme by HO-1.

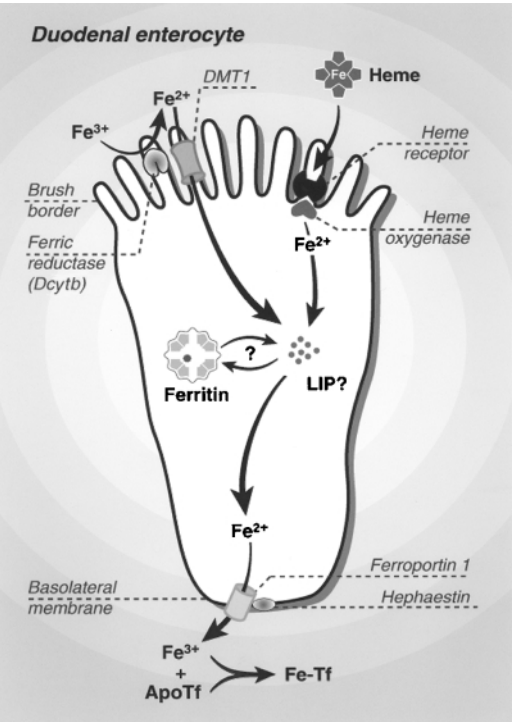

Figure 4 Iron transport across the intestinal epithelium. Iron (Fe) must cross two membranes to be transferred across the absorptive epithelium. The apical transporter has been identified as Nramp2/divalent metal transporter 1 (DMT1). It acts in concert with duodenal cytochrome b (Dcytb), which reduces ferric iron. The basolateral transporter, ferroportin 1, requires ferroxidase activity of hephaestin (a ceruloplasmin-like molecule) for the transfer of iron to the plasma. Hephaestin is depicted here at the basolateral surface of the cell, but it is not known whether it functions at this location. Heme iron is taken up by a separate process that is not well characterized. Excess iron within enterocytes is stored as ferritin. (Reprinted from Reference 89 and used with permission.)

Elemental Fe^{3+} is virtually insoluble at neutral pH and, therefore, the availability of dietary iron for intestinal absorption depends on the composition of intestinal secretions as well as ligands and reducing agents present in the diet. Ascorbic acid is the most powerful promoter of nonheme iron absorption, which is also enhanced by the organic acids (e.g., citric acid and amino acids). On the other hand, compounds that form insoluble complexes with iron (e.g., phosphates, phytates, and tannin) prevent absorption. Similarly, conditions in which there is a failure of gastric acid secretion (e.g., atrophic gastritis) may significantly reduce the availability of iron for absorption.

The process of inorganic iron absorption is not fully understood, but a compelling candidate for an iron transporter has recently been identified. Nramp2/divalent metal transporter 1 (DMT1), which is involved in iron transport across the endosomal membrane (see below), is also a principal transporter of iron in the intestine (41, 101). Nramp2/DMT1 transports only the ferrous (reduced) form of iron, and this explains why reducing agents enhance iron absorption. Moreover, the duodenal brush border contains a ferric reductase, duodenal cytochrome b (Dcytb) (73), which plays a role in the formation of Fe^{2+} prior to its transport into the enterocyte. The chemical nature of iron in the labile intermediate pool in enterocytes is unknown, but recently a novel protein necessary for iron egress from enterocytes was identified. This protein, ferroportin 1 (1, 29, 74), is identical to the Fe^{2+} exporter involved in iron egress from macrophages (Figure 3). The ferroxidase activity of hephaestin (3, 112), a membrane-bound ceruloplasmin (45) homologue, also plays an important role in iron export from intestinal epithelial cells to the circulation. Hephaestin is not an iron transporter itself but likely interacts with the ferroportin 1 to facilitate the movement of iron across the membrane (Figure 4). Hephaestin is mutated in sex-linked anemia (*sla*/*sla*) mice that take up iron from the intestinal lumen into the epithelial cells normally, but the subsequent exit of iron into the circulation is diminished (112). It is of interest that during the process of absorption, iron undergoes at least two changes in its oxidation status: reduction at the brush border and oxidation at the basolateral membrane.

Physiologically, the major factors affecting iron absorption are the amount of body iron stores and the rate of erythropoiesis (77). The uptake of iron by mucosal cells is inversely proportional to total body iron content but seems to be independent of changes in plasma iron or transferrin concentration. The 3′ UTR of mRNA for Nramp2/DMT1 expressed in intestinal cells contains the iron-responsive element (IRE) (16, 101); hence, based on the IRE/iron regulatory protein (IRP) paradigm (see below), diminished Fe levels would be expected to increase Nramp2/DMT1 expression and vice versa. It is unclear how increased erythropoietic activity (increased plasma iron turnover?) enhances iron absorption. Hypoxia can directly stimulate iron absorption, independent of changes in erythroid activity. Interestingly, the gene for Nramp2/DMT1 seems to contain regulatory elements that can be responsible for its increased transcription under hypoxic conditions (66).

Recent research, based on genetic studies, revealed that hepcidin probably plays, either directly or indirectly, an important role in iron metabolism. In its presumed active form, hepcidin is a 22- or 25-amino acid peptide that has intrinsic antimicrobial activity (82). Mice that are unable to express hepcidin develop iron overload associated with decreased iron levels in macrophages (80), whereas animals that overexpress hepcidin develop lethal iron-deficiency anemia (81). Hence, it has been suggested (35, 37) that hepcidin may be a putative signaling molecule mediating communication between the sites of iron storage (hepatocytes and macrophages) and iron release from duodenal enterocytes or macrophages. However, thus far no study has demonstrated that circulating hepcidin itself plays a direct role in iron metabolism.

IRON ACQUISITION FROM TRANSFERRIN BY DEVELOPING ERYTHROID CELLS

With some notable exceptions (e.g., enterocytes), physiologically, virtually all the cells in the organism, including erythroid precursors, take up iron from transferrin. Delivery of iron to cells occurs following the binding of transferrin to transferrin receptors on the cell membrane (91, 100). The transferrin receptor complexes are then internalized by endocytosis, and iron is released from transferrin by a process involving endosomal acidification. Identifying the mechanism of iron transport across the endosomal membrane was elusive, but a compelling candidate for an endosomal iron transporter has been identified (34, 41). The transporter, Nramp2 (also known as DMT1 or DCT1, divalent cation transporter 1), is encoded by a gene that belongs to the *Nramp* ("*natural resistance-associated macrophage protein*") family of genes (21). Interestingly, *Nramp2* generates two alternatively spliced mRNAs that differ at their 3′ untranslated regions (UTRs) by the presence or absence of the IRE and that encode two proteins with distinct carboxy termini (16, 17). Isoform II (derived from non-IRE-containing mRNA; for the definition of IRE see below) has been identified as the major Nramp2 protein isoform that is expressed in the developing erythroid cells (17). Also, Nramp2 was not found to be a limiting factor in erythroid cell iron acquisition via the physiological, transferrin-dependent, pathway. Because the substrate for Nramp2/DMT1 is Fe^{2+}, reduction of Fe^{3+} must occur in endosomes, but little is known about this process. A cDNA encoding a plasma membrane di-heme protein present in mouse duodenal cells was found to exhibit ferric reductase activity (73). This protein (Dcytb) belongs to the cytochrome b561 family of plasma membrane reductases, and it would seem important to examine whether this or a similar b-type cytochrome is involved in Fe^{3+} reduction within endosomes. Following its escape from endosomes, iron is transported to intracellular sites of use and/or storage in ferritin, but this aspect of iron metabolism, including the nature of the elusive intermediary pool of iron and its cellular trafficking, remains enigmatic. Only in erythroid cells does some evidence exist for specific targeting of iron toward mitochondria, the sites of heme production by ferrochelatase, the enzyme that inserts Fe^{2+} into protoporphyrin IX. This targeting is demonstrated in hemoglobin-synthesizing cells, where iron acquired from transferrin continues to flow into mitochondria, even when the synthesis of protoporphyrin IX is markedly suppressed (85). Moreover, inhibition of endosome motility decreases the rate of ^{59}Fe incorporation into heme from ^{59}Fe-labeled endosomes, suggesting that in erythroid cells a transient mitochondrion-endosome interaction may be involved in iron translocation to ferrochelatase (92).

DISTINCT CONTROL OF IRON METABOLISM IN ERYTHROID CELLS

In general, cells are equipped with a remarkable regulatory system that tightly controls iron levels in the labile iron pool (LIP), that is, iron in transit among various intracellular compartments. Sensitive control mechanisms exist that monitor iron

levels in the LIP and prevent its expansion, while still making the metal available for iron-dependent proteins and enzymes. In general, enlargement of the LIP leads to a stimulation of ferritin synthesis and to a decrease in the expression of transferrin receptors; the opposite scenario develops when this pool is depleted of iron. Pivotal players in this regulation are IRP1 and IRP2, which "sense" iron levels in the LIP.

Iron-dependent regulation of both ferritin and transferrin receptors occurs post-transcriptionally and is mediated by virtually identical IREs. IREs present in the 5′ UTRs of mRNAs, as in ferritin and erythroid-specific 5-aminolevulinic-acid synthase (ALA-S2, the first enzyme of heme biosynthesis), mediate inhibition of translation of the respective mRNAs in iron-deprived cells. Similar IREs are also present in the 3′ UTR of the transferrin receptor mRNA. These IREs confer differential stability to transferrin receptor mRNAs as a function of cellular iron levels. The IREs are nucleotide sequences that are recognized by specific cytosolic RNA-binding proteins known as IRP1 and IRP2. An IRE-binding form of each IRP accumulates in iron-depleted cells, but the mechanism of accumulation differs. When cellular iron is low, IRP1 is in a form that can bind to IREs, and IRP2 (which has constitutive RNA-binding activity) is stable. Binding of IRPs to IREs found in the 5′ end of mRNA (ferritin, erythroid-specific ALA-S2) inhibits translation of these transcripts, whereas binding to IREs in the 3′ UTR of the transferrin receptor mRNA (and probably also in the intestinal form of mRNA for Nramp2/DMT1) stabilizes the transcripts. Hence, iron deficiency promotes cellular iron acquisition and possibly intestinal iron absorption while it decreases levels of the cellular iron-storing protein, ferritin. On the other hand, the expansion of the LIP inactivates IRP1 and leads to a degradation of IRP2, resulting in efficient translation of ferritin mRNA (and ALA-S2 mRNA in erythroid cells) and rapid degradation of transferrin receptor mRNA (2, 15, 76, 91, 100, 104).

Some cells and tissues with specific requirements for iron evolved mechanisms that can override the IRE/IRP-dependent control of transferrin receptor formation. Erythroid cells, which are the most avid consumers of iron in organisms, use primarily a transcriptional mechanism to maintain very high transferrin receptor levels (68, 85). Moreover, erythroid cells are equipped with an important regulatory mechanism that coordinates protoporphyrin IX formation with iron supply (85). Because the 5′ UTR of mRNA for erythroid-specific ALA-S2 contains the IRE, the formation of ALA-S2 (the rate-limiting enzyme of porphyrin biosynthesis) and, consequently, protoporphyrin depends on the availability of iron.

THE AVAILABILITY OF IRON CONTROLS HEMOGLOBIN SYNTHESIS

Although three different and totally distinct pathways are involved in hemoglobin synthesis, virtually no intermediates, i.e., globin chains, porphyrin intermediates, or iron, accumulate in the developing erythroblasts and reticulocytes. This

regulation is achieved, at least in part, by a series of negative and positive feedback mechanisms in which both iron and heme play important roles. First and foremost, the supply of iron via the transferrin-receptor pathway limits, and thus controls, heme synthesis rate in erythroid precursors. Moreover, in erythroid cells "uncommitted" heme inhibits cellular iron acquisition and, consequently, heme synthesis. Furthermore, availability of heme is essential for the synthesis of globin at both the transcriptional and, more importantly, the translational levels (85, 86). Numerous reports indicate that heme stimulates globin gene transcription and is probably involved in promoting some other aspects of erythroid differentiation (105). Hemin treatment of erythroid precursors leads to rapid accumulation of globin mRNA, whereas heme deficiency leads to a decrease in globin mRNA levels (27, 36, 102, 103, 105). These effects can probably be explained by heme-mediated upregulation of the erythroid transcription factor NF-E2 binding activity (105).

It has long been known that the translation of globin in intact reticulocytes and their lysates is dependent on the availability of heme (14, 23, 69, 124). Heme deficiency inhibits protein synthesis through activation of heme-regulated inhibitor (HRI). HRI is a cyclic adenosine monophosphate (AMP)-independent protein kinase that specifically phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF-2). Recent research has revealed that autophosphorylation of threonine 485 is essential for the phosphorylation and activation of HRI and is required for the acquisition of the eIF-2α kinase activity (96). During translation initiation, eIF-2-GTP, associated with Met-tRNA^{Met}, binds to 40 S subunit and participates in the recognition of the initiation codon. After translation initiation, eIF-2-GTP is hydrolyzed to eIF-2-GDP. Because eIF-2 has a much greater affinity for GDP, a guanine nucleotide exchange factor, eIF-2B, is required to recycle eIF-2 to the GTP-bound form. Phosphorylation of eIF-α at serine 51 blocks the activity of eIF-2B, reducing the level of eIF-2-GTP. Heme binding to HRI inhibits the phosphorylation of eIF-2α by HRI, resulting in an efficient translation of globin and probably other proteins in erythroid cells (23, 113). Association of heme with HRI inhibits the enzyme by promoting the formation of disulfide bonds, perhaps between two HRI subunits (23). Disulfide bond formation reverses the inhibition of protein synthesis seen during heme deficiency. Interestingly, HRI contains two sequences that are similar to the heme regulatory motif found in numerous other proteins whose functions are regulated by heme. Importantly, the HRI homodimer has two distinct types of heme-binding sites (22). Binding of heme to the first site is stable (i.e., HRI is a hemoprotein), whereas binding of heme to the second site is responsible for the rapid down-regulation of HRI activity (22). The mRNA for HRI is present in uninduced murine erythroleukemia cells and is increased after the induction of erythroid differentiation. This accumulation of HRI mRNA in differentiating murine erythroleukemia cells is dependent upon the presence of heme because an inhibitor of heme synthesis markedly reduces HRI mRNA accumulation (26); hence, HRI plays an important physiological role in the translation of globin and probably other proteins synthesized in erythroid cells. This conclusion is further supported by the finding that expression of dominant-negative mutants

of HRI in murine erythroleukemia cells increased hemoglobin production in these cells upon DMSO induction of erythroid differentiation (25).

Mice rendered null for the HRI gene ($HRI^{-/-}$) appear to be normal, fertile, and without gross abnormalities of hematologic parameters (43). However, in erythroid cells from iron-deficient $HRI^{-/-}$ mice, a marked increase in both α- and β-globin synthesis led to accumulated globins that were devoid of heme and aggregated within the erythrocytes and their precursors. This resulted in a hyperchromic normocytic anemia with decreased erythrocyte counts, compensatory erythroid hyperplasia, and accelerated apoptosis in bone marrow and spleen (i.e., ineffective erythropoiesis). These important results established the physiological role of HRI in balancing the synthesis of α- and β-globins with the availability of heme in developing erythroid cells. Moreover, these results have demonstrated that the translational regulation of HRI in iron deficiency is essential for the survival of erythroid precursors (43).

In conclusion, in erythroid cells iron is not only the substrate for the synthesis of hemoglobin but also participates in its regulation. Moreover, the iron protoporphyrin complex appears to enhance globin gene transcription, is essential for globin translation, and supplies the prosthetic group for hemoglobin assembly.

CONCLUSION: ERYTHROPOIESIS UNDER NORMAL, FOLATE-DEFICIENT, AND IRON-DEFICIENT CONDITIONS

Erythropoiesis during normal conditions, folate deficiency, and iron deficiency is shown in Figure 5. Erythropoiesis during vitamin B_{12} deficiency is similar to that shown for folate deficiency. In normal erythropoiesis, a minority of the EPO-dependent cells survives the EPO-dependent period, giving rise to basophilic erythroblasts that divide and mature into orthochromatic erythroblasts and reticulocytes. Because the periods of EPO dependence and hemoglobin synthesis do not overlap, apoptosis of progenitors during normal erythropoiesis does not increase serum bilirubin. During folate-deficient erythropoiesis, increased apoptosis due to DNA damage extends into the post-EPO-dependent stages, where hemoglobin synthesis has begun but has not yet reached high levels. This apoptosis of cells that have begun hemoglobin synthesis causes slightly increased serum bilirubin. Those folate-deficient erythroblasts surviving to the late stages produce fewer but larger reticulocytes, leading to macrocytic anemia. The anemia induces EPO production, which in turn increases the survival of cells in the EPO-dependent stages compared to normal erythropoiesis. However, the expansion of these EPO-dependent populations is relatively incomplete due to increased apoptosis from the folate deficiency. In iron deficiency, decreased synthesis of heme results in the decreased protein translation, especially of globins, through the enhanced action of HRI. This decreased protein translation in the iron-deficient erythroid cells results in retarded reticulocyte production and smaller, less hemoglobinized

reticulocytes, leading to microcytic anemia. The resultant anemia induces EPO production, which decreases the apoptosis in the EPO-dependent cells relative to normal erythropoiesis. This increased survival in the EPO-dependent stages, however, does not result in increased reticulocyte production due to the inhibitory effect of HRI during the subsequent hemoglobin synthesis stages.

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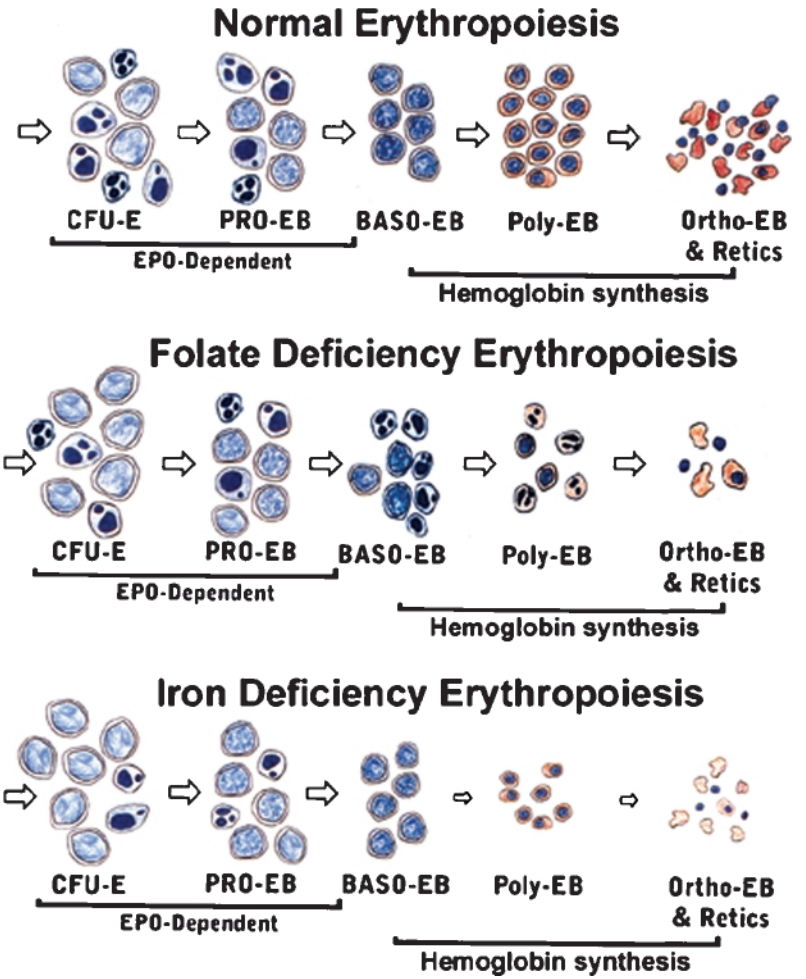

Figure 5 Models of erythropoiesis. Period of erythroid differentiation shown extends from the actively dividing CFU-E through the postmitotic Ortho-EB and Retics, which are shown with their enucleated nuclei. Irregular nuclear fragments in the Poly-EB and earlier stages represent apoptotic cells. The arrows between the stages represent relative rates of progression between stages. Colors are as stained with 3,3'-dimethoxybenzidine and hematoxylin. Orange represents accumulated hemoglobin. Abbreviations: Baso-EB, basophilic erythroblast; CFU-E, colony-forming unit-erythroid; Ortho-EB, Orthochromatic erythroblast; Poly-EB, polychromatophilic erythroblast; Pro-EB, proerythroblast; Retic, reticulocyte. Reprinted with modifications from Koury MJ, Horne DW, Brown ZA, Pietenpol JA, Blount BC, Ames BN, Hard R, and Koury ST. 1997. Apoptosis of late-stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production. *Blood* 89:4617–23. Copyright American Society of Hematology, used with permission.

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