IUBMB *Life*, 61(8): 800–830, August 2009

Critical Review

Erythropoiesis: Model Systems, Molecular Regulators, and Developmental Programs

Asterios S. Tsiftsoglou[1], Ioannis S. Vizirianakis[1] and John Strouboulis[2]

[1]*Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

[2]*Institute of Molecular Oncology, Alexander Fleming Biomedical Sciences Research Center, Vari, Athens, Greece*

Summary

Human erythropoiesis is a complex multistep developmental process that begins at the level of pluripotent hematopoietic stem cells (HSCs) at bone marrow microenvironment (HSCs niche) and terminates with the production of erythrocytes (RBCs). This review covers the basic and contemporary aspects of erythropoiesis. These include the: (*a*) cell-lineage restricted pathways of differentiation originated from HSCs and going downward toward the blood cell development; (*b*) model systems employed to study erythropoiesis in culture (erythroleukemia cell lines and embryonic stem cells) and *in vivo* (knockout animals: avian, mice, zebrafish, and xenopus); (*c*) key regulators of erythropoiesis (iron, hypoxia, stress, and growth factors); (*d*) signaling pathways operating at hematopoietic stem cell niche for homeostatic regulation of self renewal (SCF/c-kit receptor, Wnt, Notch, and Hox) and for erythroid differentiation (HIF and EpoR). Furthermore, this review presents the mechanisms through which transcriptional factors (GATA-1, FOG-1, TAL-1/SCL/MO2/Ldb1/E2A, EKLF, Gfi-1b, and BCL11A) and miRNAs regulate gene pattern expression during erythroid differentiation. New insights regarding the transcriptional regulation of α- and β-globin gene clusters were also presented. Emphasis was also given on (i) the developmental program of erythropoiesis, which consists of commitment to terminal erythroid maturation and hemoglobin production, (two closely coordinated events of erythropoieis) and (ii) the capacity of human embryonic and umbilical cord blood (UCB) stem cells to differentiate and produce RBCs in culture with highly selective media. These most recent developments will eventually permit customized red blood cell production needed for transfusion. © 2009 IUBMB IUBMB *Life*, 61(8): 800–830, 2009

Keywords erythropoiesis; molecular mechanisms; hematopoietic stem cells; erythroid; transcription factors.

Received 4 December 2008; accepted 8 April 2009

Address correspondence to: Asterios S. Tsiftsoglou, Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki GR-54124, Greece. Tel: +30-2310-997631. Fax: +30-2310-997618. E-mail: tsif@pharm.auth.gr

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.226

INTRODUCTION INTO THE CURRENT STATE OF ERYTHROPOIESIS

Human erythropoiesis is a dynamic complex multistep process that involves differentiation of early erythroid progenitors to enucleated red blood cells (*1, 2*). Basically, pluripotent hematopoietic stem cells (HSCs) and early multipotent progenitors (MPP) generate committed erythroid precursors (erythroblasts), which then give birth to mature erythrocytes (red blood cells; RBCs) (*3*). HSCs residing within the bone marrow microenvironment (niche) (*4*) are stimulated by cell–cell interactions and extrinsic factors (soluble macromolecules) (*5*), which interact on the cell surface receptors and initiate signaling pathways. Such primed hematopoietic progenitors undergo several transition steps of differentiation and generate the common myeloid progenitor (CMP) and the common lymphoid progenitor (CLP). Although CLP supplies T and B lymphocytes, natural killer (NK) cells, and maybe dendritic cells (DCs), CMP is converted into megakaryocytic/erythroid (MEP) and granulocyte-myeloid (GMP) progenitors. MEP cells upon the action of growth factors are differentiated into erythropoietin (Epo)-responsive erythroid burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E). The CFU-Es are then differentiated into orthochromatic normoblasts and finally via enucleation yield reticulocytes first and then red blood cells (RBCs) (Fig. 1).

As the hematopoietic process progresses from the early stages into erythroid cell maturation, cells gradually lose their potential for cell proliferation and become mature enucleated cells. Mature erythrocytes are biconcave disks without mitochondria and other organelles but full of hemoglobin able to bind and deliver O_2. Several reviews have been published over the last years and covered most, if not all, of the aspects of erythropoiesis (*6–10*). This review describes fundamental principles as well as contemporary aspects of erythropoiesis (see Fig. 2) providing new insights that have emerged in the field of erythropoiesis in recent years. Because hemoglobin switching, a major issue in erythropoiesis, has been extensively covered by

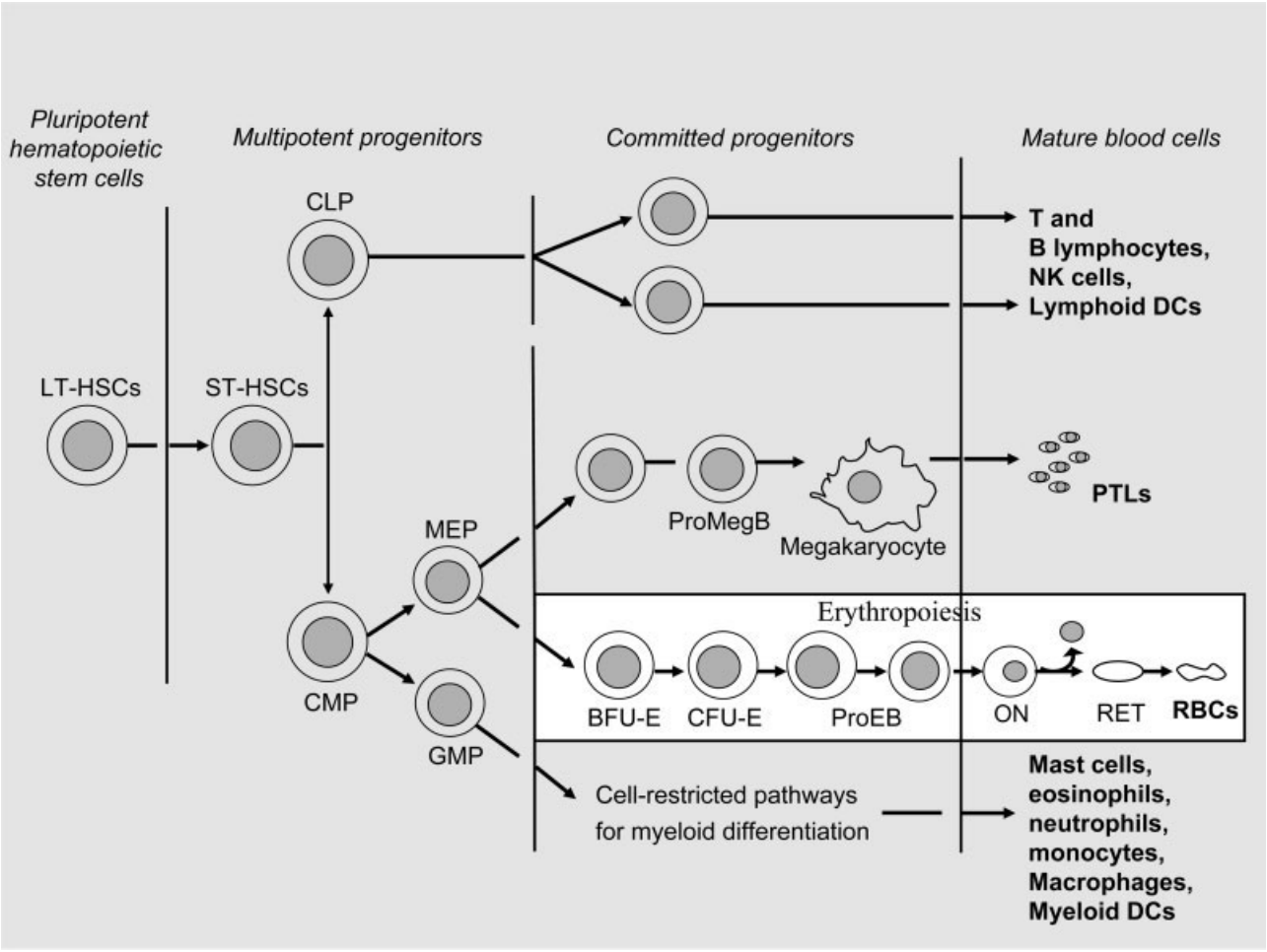


Figure 1. Schematic representation of normal hematopoiesis and illustration of the erythrocytic pathway of development. Hematopoiesis in adult life begins in bone marrow at the level of pluripotent long-term HSCs, which then give rise to short-term HSCs and multipotent hematopoietic progenitors. These cell types then yield committed progenitors and finally mature blood cells.Among various hematopoietic cell-restricted pathways illustrated above, erythropoiesis represents a unidirectional pathway of maturation. The erythrocytic pathway of differentiation (highlighted) begins from megakaryocytic/erythroid pluripotent progenitor (MEP) that gives rise to BFU-E, CFU-E, intermediate forms of proerythroblasts (ProEB), and then to orthochromatic normoblasts (ON). Enucleation of ONs leads to birth of reticulocytes (RETs). The latter cells finally yield mature enucleated red blood cells (RBCs). BFU-E, burst forming unit-erythroid; CMP, common myeloid progenitor; CFU-E, colony forming unit-erythroid; CLP, common lymphoid progenitor; DCs, dendritic cells; EB, erythroblast; GMP, granulocyte-myeloid progenitor; MEP, megakaryocytic/erythroid progenitor; MPP, multipotent progenitors; NK, natural killer; ON, orthochromatic normoblast; PLTs, platelets; RBCs, red blood cells; RET, reticulocyte.

excellent reviews (see refs. *8* and *9*), it will not be covered in detail in this review. Only structural aspects of human embryonic, fetal, and adult globin genes will be discussed.

EMBRYONIC DEVELOPMENT OF ERYTHROPOIESIS AND BONE MARROW MICROENVIRONMENT (NICHE)

Developmentally, at least in mouse, hematopoiesis begins in the yolk sac/blood islands, later at the aorta-gonad mesonephros (AGM) region, the chorio-allantoic placenta, the fetal liver, and finally in the bone marrow during adult life (*2, 11*). The direct precursors of hematopoietic cells are found to be hemogenic blasts and hemogenic endothelium of embryonic vasculature in early embryonic life. Developmentally, hematopoiesis in humans occurs in two waves: (*a*) primitive (embryonic) hematopoiesis occurs in blood islands in yolk sac and generate enucleated RBCs (*2, 10*) that express embryonic Hbs [Gower 1 ($\zeta 2\varepsilon 2$), Gower 2 ($\alpha 2\varepsilon 2$) and Portland Hb ($\zeta 2\gamma 2$)]; (*b*) definitive hematopoiesis occurs in fetal liver and gives rise to enucleated RBCs that express fetal hemoglobin [HbF ($\alpha 2\gamma 2$)]. Definitive (fetal/adult) hematopoiesis finally shifts from the fetal liver to the bone marrow where enucleated RBCs produce adult hemoglobin [HbA ($a2\beta 2$)] (*2, 3*).

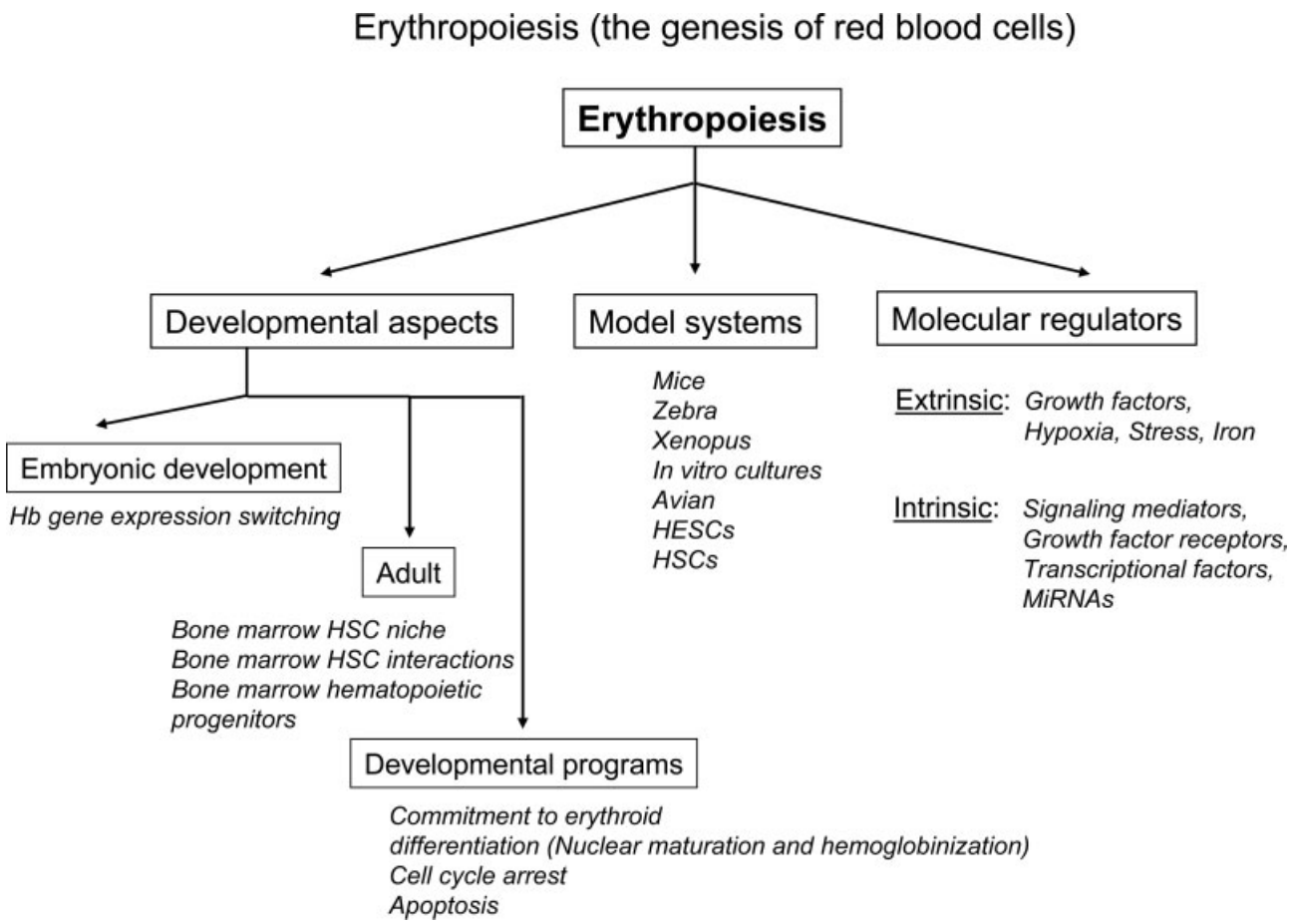


Figure 2. Schematic illustration of various developmental aspects, model systems, and molecular regulators of erythropoiesis (the genesis of red blood cells). Each aspect, model system, and key-regulators are presented in the text.

As Bessis (*12*) observed 50 years ago, erythropoiesis in bone marrow takes place in erythroblastic islands composed of erythroblasts surrounding a central macrophage that facilitates phagocytosis of nuclei derived from enucleated orthochromatic normoblasts as well as providing iron needed for erythropoiesis. Long term liquid cultures of bone marrow cells developed by Allen and Dexter in early 1980s (*13*), consisting of main stromal cells of mesenchymal type (MSCs) and extracellular matrix (ECM) allowed generation of erythroblastic islands *in vitro*. The erythroblasts interact with each other and most important with the central macrophage via cell adhesive macromolecules including integrins, like a4β1 (*14, 15*). The number of erythroblasts surrounding the central macrophage may be increasing during stress erythropoiesis.

Emerging evidence indicates that erythropoiesis is regulated by the balance of positive and negative feedback regulatory mechanisms operating within the erythroblastic islands niche (see ref. *14*). This occurs *via* cell–cell interactions and soluble factors like stem cell factor (SCF), the ligand for the SCF receptor (c-kit), erythropoietin (Epo), VEGF, and others produced by erythroblasts and macrophages. Obviously, SCF, Epo, and VEGF activate erythropoiesis via the positive feedback mechanisms. However, a negative network of feedback regulatory factors including IL-6, TGF-β, TNFα, and INFγ appears to function negatively by inhibiting erythropoiesis and promoting cell death of erythroblasts. IL-6 prevents iron release from central macrophage thus reducing erythropoiesis. Finally, extracellular matrix (EMC) components, such as fibronectin and laminin, affect erythropoiesis by influencing terminal differentiation and migration of erythroid cells via interactions with fibronectin receptors found in erythroblasts, integrins, and maybe other molecules (*15*).

MODEL SYSTEMS FOR STUDYING ERYTHROPOIESIS

Permanent Cell Lines

The discovery in early 1970s by Friend et al. that induction of differentiation of erythroleukemic cells (MEL) *in vitro* resembles normal erythroid differentiation (*16*), triggered several research groups to use malignant hematopoietic cell lines (HL-60, K-562, HEL, U937, TF-1, ML-1, KG-1, and others) as suitable model systems to study hematopoiesis (erythropoiesis, granulopoiesis, and

lymphopoiesis) in culture (*17, 18*). Permanent cell lines grown in culture maintain their neoplastic phenotype and behave like relatively homogeneous cell populations. Under certain growth conditions, such cells are induced to differentiate extensively into cells resembling normal counterparts (*e.g.* orthochromatic normoblasts, granulocytes). Each permanent cell line used as model system depending on its potential, unipotent, and/or multipotent, can give birth to one or more than one cell types. Several fundamental questions as shown below have been investigated using *in vitro* model systems: (*a*) how do hematopoietic progenitor cells commit to differentiate along one or another cell-restricted pathway? (*b*) What major events occur during cell differentiation in hematopoiesis? (*c*) Is apoptosis a regulatory homeostatic process integrated in hematopoiesis? MEL and human leukemic K562 cells have been most widely used for studying *in vitro* erythropoiesis and hemoglobin gene regulation (*17, 18*).

Cultured MEL (or Friend) cells permitted analysis of unipotent hematopoietic cells, like CFU-E, along the erythrocytic pathway. MEL cells growing in suspension culture are induced to terminally differentiate by several agents into cells resembling orthochromatic normoblasts. Such cells produce vast amounts of hemoglobin and exhibit a selective expression/repression program of several genes (Fig. 3) (*18*). Cells committed to differentiation undergo a programmed loss of proliferative capacity, restricted to only a few divisions because of their irreversible growth arrest in G1. Commitment to erythroid maturation activates the expression of several genes involved in the biosynthesis of globins, heme, and hemoproteins. Unfortunately, MEL cells are unable to respond to Epo and differentiate into reticulocytes, although enucleation of terminally differentiated MEL cells has been achieved in culture (*19*).

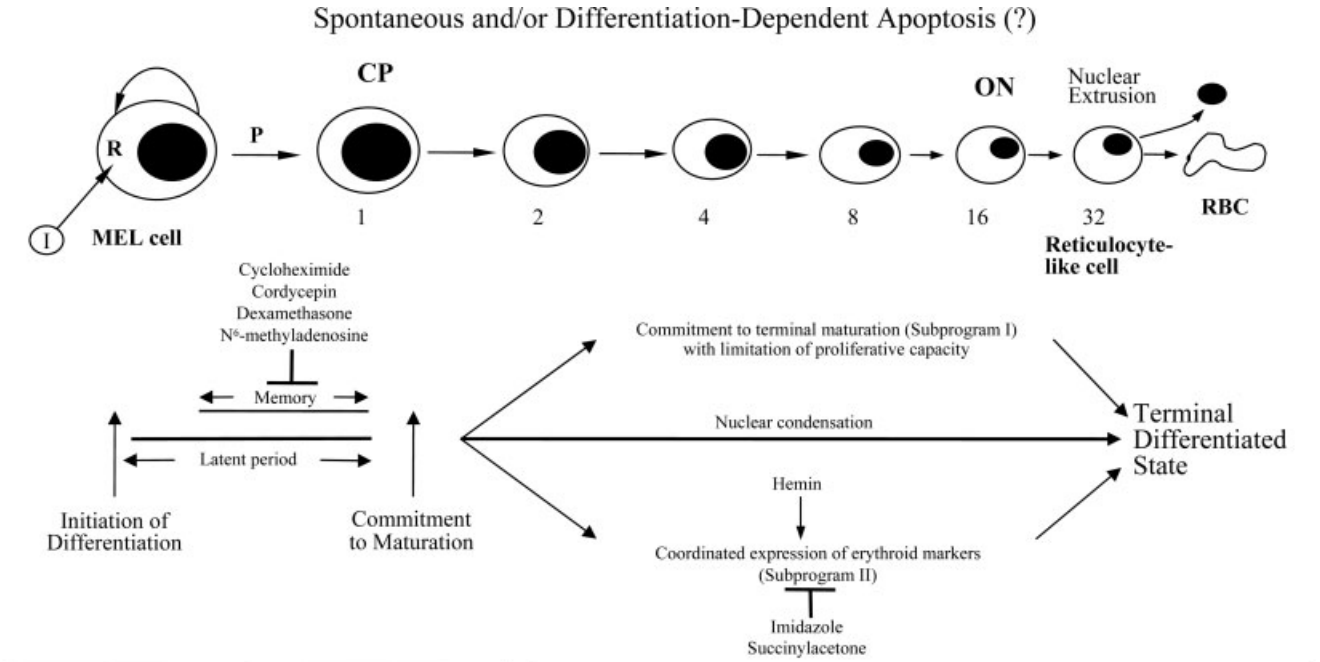
K562 cells are human leukemia cells derived from a patient with bcr-Abl(+) chronic myelogeneous leukemia (CML). K562 cells bear the Philadelphia chromosome (9:22 translocation) and grow well in suspension culture. On treatment with hemin and other inducing agents (like anthracyclines, sodium butyrate, and hydroxyurea), K562 cells are induced to differentiate into hemoglobin producing cells (*18*). Differentiated K562 cells synthesize embryonic and fetal but not adult hemoglobins despite the fact that these cells also bear intact the β-globin gene cluster. To a certain extent, K562 cells behave like thalassemia cells and serve as a suitable model for hemoglobin "switching" (see refs. *8* and *18* for details). Moreover, evidence exists to indicate that differentiated K562 cells can give birth to platelets and macrophage-monocyte-like cells on treatment with phorbol ester TPA. In most studies reported so far, K562 cells have been used as a model system for understanding the mechanism of action of heme/hemin, a natural key regulator of bone marrow hematopoiesis (*20*). Developmentally, K562 cells are more primitive in nature than MEL cells and behave more like undifferentiated early hematopoietic multipotent progenitors.

In addition to permanent cell lines, genetically-engineered hematopoietic cells lines, like G1E, were also established and used in delineating transcriptional regulation upon erythropoiesis. G1E cells are an immortalized GATA-1-null erythroblast line derived from gene-targeted embryonic stem cells (*21*). This system has been extensively used to uncover specific signaling pathways regulating erythropoiesis. These cells proliferate continuously in culture as developmentally arrested erythroid precursors and, on restoration of GATA-1 activity, undergo cell cycle arrest and terminal maturation in a fashion that largely recapitulates normal erythropoiesis (*22, 23*). In this regard, stem cell factor (SCF) (the ligand for c-Kit) and Epo are required for the growth and survival of these cells at distinct developmental stages. During GATA-1-induced maturation, c-Kit is downregulated, and Epo becomes an essential survival factor (*23–26*). Such a process shows that recapitulation of cytokine requirements during normal erythroid maturation may occur.

Embryonic Stem Cells and Production of Functional Human RBCs

Embryonic stem (ES) cells are derived from the inner cell mass of preimplantation blastocysts and maintain their capacity to self-renew and differentiate into several different tissues under controlled conditions *in vitro*. Upon withdrawal of leukemia inhibition factor (LIF) and stromal cell contact, ES cells differentiate to form embryoid bodies, that is, cell aggregates containing several differentiated cell types. Mouse ES cells have been extensively used for *in vitro* differentiation into several hematopoietic lineages and, in particular, into erythroid cells (recently reviewed in ref. *27*). In general, two experimental approaches have been applied in the differentiation of mouse ES cells into erythroid cells. In the first approach pioneered by Keller and coworkers (*28, 29*), disaggregated embryoid bodies are cultured in the presence of Epo and kit ligand (KL or SCF), which promote growth and differentiation of erythroid progenitors. By day 10 of culture, definitive erythroid colonies arise and can be harvested for further studies. Furthermore, treatment with Epo alone (without KL) at an earlier stage leads to the generation of primitive erythroid colonies (*29*), which are harvested within 2–3 days. Primitive erythroid cells are distinguishable by virtue of their large nucleated morphology and the expression of embryonic globin genes such as βh1, whereas definitive erythroid colonies are larger and contain smaller cells that express adult stage globin genes such as β^{major}. A related approach described by Beug and coworkers (*30*) relies on culturing disaggregated embryoid bodies under conditions that mimic erythropoietic stress. Definitive erythroid cells can be derived from disaggregated embryoid bodies by culturing in serum free media in the presence of dexamethasone (DEX), which activates the glucocorticoid receptor (GR) pathway, and of kit ligand and low amounts of Epo. Terminal differentiation can be induced by replacement of the proliferative factors with high Epo and insulin and is normally complete within 72 h (*30*).

In the second approach pioneered by Nakano and coworkers (*31, 32*), ES cells are cultured in the presence of the OP9 stromal cell line, which promotes hematopoietic differentiation by providing a supportive hematopoietic environment. OP9 cells



Gene expression patterns prior to commitment

Up-regulation	*Down-regulation*	*Biphasic regulation*
c-fos	EPOR	c-myc
jun B	H2a histones	c-myb
p21	Id	COX II
H1° histone	HSP70	GATA-1
MER5	ODC	Myn
COX IV	PU.1	p53
Notch-1	rRNAs	SCL
	Vimentin	

Gene expression patterns following commitment

No change	Down-regulation			Up-regulation	
β_2-microglobulin	CDK2	rRNAs	GAPDH	β^{major} globin	SCL
c-K-ras	CDK4	α-actin	H1° histone	β^{minor} globin	Bax
EKLF	c-myb	β-actin	H2a histones	α1-globin	bcl-X_L
H3.3 histone	c-myc	B22	H3.1 histone	e-ALAS	Prn-P
	Cyclin D3	COX II	H3.2 histone	GATA-1	pRb
	Id	COX IV	H3.3 histone	NF-E2	p21
	jun B	eEF-Tu	HSP70	EPOR	TfR
	Myn	ODC	MER5		
	P53	PU.1	RPS5		
	Notch-1	Vimentin	RPL35a		

Figure 3. Diagrammatic presentation of the erythroid developmental program of murine erythroleukemia (MEL) cell differentiation. *Upper panel*: MEL cell (virus-transformed CFU-E-like mouse hematopoietic spleen cell) treated with an inducing agent (I) acting upon a receptor (R) or otherwise commit into erythrocytic pathway of differentiation with probability (P). After 3–4 divisions, the committed progenitor (CP) of MEL gives birth to orthochromatic normoblast (ON), which via nuclear extrusion yields reticulocyte first and then matured red blood cell (RBC). Commitment to erythrocytic maturation occurs after a latent period. Inducer-treated cells "remember" the previous inducing stimulus. Memory is developed early and last for several hours. However, memory can be erased by metabolic inhibitors of protein (cycloheximide) and RNA (cordycepin) biosynthesis as well as by RNA methylation inhibitor (N^6-methyladenosine) and dexamethasone. Commitment to erythrocytic pathway is highlighted by progressive loss of cellular replication (subprogram I), nuclear condensation, and expression of erythroid markers (subprogram II). The latter subprogram II is activated by hemin and repressed by imidazole and succinylacetone. *Lower panel*: Gene expression patterns occurring before and after commitment to erythrocytic maturation of MEL cells have been initiated. Several classes of genes are indicated (for further details, see review ref. *18*). Note that the data included are referring only to changes observed in the mRNA steady-state levels of the genes examined. eEF-Tu, eukaryotic elongation factor Tu; ODC, ornithine decarboxylase; e-ALAS, erythroid form of 5-aminolevulinate synthase; NF-E2, erythroid-specific nuclear factor 2; COX, mitochondrial cytochrome c oxidase; pRb, retinoblastoma protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PU.1, *ets*-related transcription factor; MER5, gene preferentially expressed in murine erythroleukemia cells; RPS5, ribosomal protein S5; RPL35a, ribosomal protein L35a; Prn-P, prion protein; NF-E2, erythroid-specific nuclear factor 2; B22, B22 subunit of NADH-ubiquinone oxidoreductase complex of mitochondria; SCL (TAL1), stem cell leukemia; EpoR, erythropoietin receptor.

are derived from the calvariae of newborn op/op mutant mice, which lack functional macrophage colony-stimulating factor (M-CSF). Lack of M-CSF inhibits the survival of macrophages, which tend to outgrow all other lineages in differentiated ES cells (*32*). Mesodermal colonies arise by day 5 of ES cell cocultured with OP9 cells, trypsinized into single cell suspensions, and replated on OP9 cells. Addition of Epo at day 3 of culture leads to the production of almost exclusively primitive erythroid colonies around days 6–7, whereas Epo with KL leads to an additional wave of erythropoiesis around days 10–14 accompanied by the formation of definitive erythroid colonies (*31*). Definitive erythroid progenitors can be easily separated from primitive progenitors at day 6 by removing the nonadherent cell population from the culture, which represents the majority of primitive erythroid progenitors (*31*). Thus, both approaches for ES cell differentiation appear to recapitulate murine developmental erythropoiesis in that an early wave of primitive erythropoiesis is succeeded by a wave of definitive erythropoiesis with each wave expressing a developmentally appropriate complement of globin genes (and other markers).

There are important advantages to using mouse ES cell differentiation approaches in studying erythropoiesis. Firstly, erythroid differentiation proceeds in a synchronous manner, particularly in the OP9 and DEX-serum free systems, thus facilitating cellular and molecular studies. Secondly, genetically modified (*i.e.* targeted gene knockout) ES cells can be used to study the effects of the genetic modification(s) in erythroid differentiation. This is a particularly useful approach in testing gene knockouts that are early embryonic lethal (*i.e.* embryos that do not make it to the fetal liver stage). Probably, the most useful application of mouse ES cell differentiation has been in the study of transcription factor function in erythropoiesis, the best example being that of GATA-1 (for example, see ref. *33*; see also section Alpha-Globin Cluster). ES cell differentiation has also been used in studying early events in cell commitment to the erythroid lineage and the development of primitive and definitive erythropoiesis (for example, see refs. *34* and *35*).

Recent advances in the isolation, maintenance, and propagation of human ES cell lines have also allowed rapid progress in their application for studying hematopoiesis. Human ES cells (HuESCs) were first isolated in 1998 (*36*) and exhibit stem cell hallmarks such as indefinite propagation *in vitro*, embryoid body formation and pluripotentiality (recently reviewed in ref. *37*). As in mouse ES cells, hematopoietic progenitors can be generated by HuESCs either by treating embryoid bodies with growth factors or by coculturing with stromal cell lines that support and promote hematopoiesis (reviewed in ref. *38*). However, the precise pattern of developmental erythropoiesis in differentiated HuESCs remains somewhat unresolved. In one study using embryoid bodies and growth factor cocktails, the erythroid cells that were generated morphologically resembled definitive cells in the absence of a clear first wave of primitive erythropoiesis (*39*). It is of interest that practically all erythroid cells generated in this way expressed embryonic and fetal globin genes in the absence of any morphological evidence of a fetal-to-adult switch in β-globin expression at any stage under the culture conditions used. Another recent study using coculturing of human ES cells with mouse fetal liver derived stromal cells, showed an apparent maturation of erythroid cells with time from expressing predominantly embryonic ε-globin at day 12 of coculture, to predominant expression of adult β-globin by day 18 and the production of enucleated erythrocytes (*40*). Interestingly, expression of fetal γ-globin remained high throughout this maturation period. Importantly, enucleated erythrocytes generated using this protocol exhibited functional properties of mature red blood cells (*40*). Thus, it is presently unclear as to how well these studies recapitulate physiological developmental erythropoiesis in man, or to what extent differences in methods may influence outcomes. It is also of note that of five human ES cell lines tested only one was efficient in generating erythroid cells (*39, 41*).

After the successful isolation, culture and maintenance of human embryonic hematopoietic stem cells (HuESCs) in selected media in 1998 (*36*), several efforts were made to use these cells to replicate, reconstruct, and explore normal hematopoiesis *in vitro* from early stages up to the production of RBCs. The realization that hematopoietic differentiation of HuESCs, under selective growth conditions, progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development (*42*) promoted a number of studies directed toward the production of functional sound RBCs. Different methods and protocols were developed and applied. In most cases, investigators succeeded using HuESCs (*40, 43, 44*), to develop hemangioblasts, then erythroid progenitors and finally enucleated RBCs that are functional, because that produce embryonic, fetal, and adult hemoglobin produced during the entire course of erythropoiesis and to bind O_2 like their normal counterpart enucleated RBCs derived from humans. In other cases, cord blood $CD34^+$ HSCs were employed (*45*).

Although growth and differentiation of HuESCs or cord blood HSCs requires a mixture of soluble growth factors, the enucleation of differentiated erythroid cells produced requires coculture with either mouse fetal liver cells or use of BM and CD stromal mesenchymal stem cells (MSCs) containing macrophages/monocytes. Despite differences observed among the various protocols in methods used by various groups, it has been established that HuESCs and cord blood HSCs can be used not only as best possible model system to study hematopoiesis *in vitro* and/or ex vivo but most importantly to become able to produce donorless blood cells of ABO and O-Rhesus (-) type aimed for transfusion of people suffering of severe hematological disorders. Moreover, these systems can be used as models to uncover molecular and cellular events of hematopoiesis during the entire spectrum of hematopoiesis.

Mice

Production of red blood cells in the mouse occurs in two clearly defined and distinct waves during primitive and defini-

tive erythropoiesis (*46, 47*). In the primitive stage, erythropoiesis takes place in the blood islands of the yolk sac to produce large nucleated immature erythroblasts, which start circulating at E8.25. Primitive erythroblasts undergo a synchronous wave of maturation in the circulation with recent evidence showing that by E12.5 primitive erythroblasts undergo enucleation and continue circulating as erythrocytes (*48, 49*). Primitive erythrocytes express the embryonic complement of globin chains, which consist initially of ζ- and βh1-globin, followed by α1-, α2-, and εy-globin as primitive proerythroblasts at E7.5 mature and transition into reticulocytes by E15.5 (*50, 51*).

Definitive (fetal/adult) erythropoiesis first arises in the developing fetal liver of the mouse embryo. It is thought that the first erythroid progenitors that populate the fetal liver at around E9.5 originate from the yolk sac in a transient wave of definitive erythropoiesis before the emergence of adult hematopoietic stem cells (*52*). Around birth, the bone marrow becomes the major site of definitive erythropoiesis. In both cases, the fetal liver and bone marrow, definitive erythroid precursors mature while remaining attached to macrophage cells that form the erythroblastic islands (*14*). Macrophages in erythroblastic islands engulf and digest extruded nuclei during erythroid maturation and may also provide broader "nursing" functions to erythroid maturation. It is of interest that recent work demonstrated that circulating primitive erythroblasts can also interact with erythroblastic islands in the fetal liver in completing their maturation and enucleation steps (*53*). Definitive erythroid cells in the fetal liver and the bone marrow express the adult complement of globin chains consisting of α1- and α2-globin and of β^{major}- and β^{minor}-globin.

Zebrafish and Xenopus

Zebrafish (*Danio rerio)* has established itself as an extremely useful model for the study of hematopoiesis (recently reviewed in ref. *54*). Primitive erythropoiesis first occurs in the intermediate cell mass (ICM), a GATA-1 expressing axial hematopoietic tissue, which produces the first erythrocytes, akin to the blood islands in the mammalian yolk sac. Definitive erythropoiesis initiates by 48 h postfertilization in Runx-1 expressing cell clusters around the ventral wall of the dorsal aorta, similar to the aorta-gonad-mesonehpros (AGM) phase of erythropoiesis in mammals (*55, 56*). In the larval stage and in adult animals, the kidney becomes the primary site of hematopoiesis.

As a model organism, zebrafish offers many practical advantages such as the ease of husbandry, large numbers of progeny with short developmental times, and transparent embryos, which facilitate imaging and observation for example, using GFP or fluorescent *in situ* hybridization (FISH). It has also emerged as an excellent genetic model for large scale forward genetic screens by random chemical mutagenesis. This has resulted in the isolation of many mutants with hematopoietic phenotypes, the analyses of which have contributed significantly to our understanding of developmental, cellular, and molecular hematopoiesis. A good example is the *cloche* mutant, which presents with a total hematopoietic failure and an absence of anterior endothelial development (*36, 57*). The analysis of *cloche* mutants provided compelling evidence for the existence of the long sought hemangioblast, that is, the common progenitor for the development of the hematopoietic and vascular tissues. Furthermore, the use of antisense morpholinos RNAs allows single or multigene knockdowns in early embryos, whereas overexpression allows gain-of-function studies. Lastly, other classical hematopoietic approaches such as flow cytometry and transplantation studies are increasingly applied in zebrafish, thus extending the repertoire of tools and approaches for the study of hematopoiesis.

Xenopus laevis has also served as a useful model in the study of erythropoiesis (*58*). Primitive erythrocytes develop within the V-shaped ventral blood island, located on the ventral surface of the developing tadpole, at around 2 days postfertilization following a rostral to caudal "wave" of primitive erythrocyte differentiation. This stage of erythropoiesis in Xenopus is analogous to the yolk sac stage in mammals. Definitive erythrocytes first initiate in the lateral plate mesoderm located near the pronephros at a stage that is analogous to the aorta-gonad-mesonephros stage in mammals. Larval and adult erythropoiesis occurs in the liver in frogs.

The large numbers and size of eggs and of ensuing embryos have facilitated cell explant and transplantation studies carried out for the definition of the cellular origins and of the inductive signals of hematopoiesis in the early embryo. Cell fate maps tracing the origin of tissues to specific cells in the 32-cell stage embryo have been worked out in Xenopus. In addition, gene knockdown, overexpression, and dominant negative expression assays are possible through mincroinjection in Xenopus embryos (see for example, ref. *59*). Using such approaches, it has been shown for example that primitive erythropoiesis also contributes to the initiation of definitive erythropoiesis in the Xenopus embryo (reviewed by (*60*), or that BMP4 signaling is critical to primitive erythroid blood formation (*61*).

Avian Systems

Erythropoiesis has also been extensively studied in avian models (chicken in particular), which offer some advantages when it comes to studying hematopoietic ontogeny and aspects of erythroid commitment and differentiation. For example, elegant grafting experiments of chick embryonic (blastoderm) cells onto the yolk sac of quail embryos provided early evidence that yolk sac hematopoiesis does not appear to contribute to definitive hematopoiesis (*62*). In addition, the avian model provided early evidence for an intraembryonic origin of definitive hematopoiesis (*63*), later found to also occur in mammals.

Hemoglobin in the chicken embryo can be detected in the blood islands of the developing yolk sac as early as 24 h after fertilization (2–4 somite stage). In contrast to mammals, avian embryos do not use the fetal liver but instead maintain the yolk sac as a site of erythropoiesis until the bone marrow takes over later in development. The α^{A} and the α^{D}-globin genes are expressed constitutively throughout development, whereas the embryonic α^{π} gene is expressed exclusively in primitive eryth-

ropoiesis. For the β-like globin genes, the embryonic ε and ρ-globin genes are expressed in the primitive stage, whereas the adult β^H and β^A genes are expressed in the definitive stage of erythropoiesis.

With regards to the study of erythroid differentiation, there are two aspects of the avian model that have proved to be useful. The first, as originally developed by Beug and coworkers (*64, 65*), is the ability to propagate *in vitro* mass cultures of primary bone marrow erythroid progenitors that can proliferate in the presence of specific growth factors and steroids while remaining at an immature progenitor stage. These progenitors can also be induced to undergo synchronous differentiation into mature erythrocytes upon stimulation with physiological signals such as Epo and insulin (*66*). This system has been effectively used by Beug and coworkers (*67, 68*) for example, to identify two distinct erythroid progenitors in chicken bone marrow, and investigate the signaling pathways governing proliferation versus terminal differentiation decisions in the avian system (reviewed in ref. *69*).

The second aspect of avian models in the study of erythropoiesis is the occurrence of avian leukemia viruses that have provided important clues as to the signaling and transcriptional regulation of erythroid differentiation. An important example is the avian erythroblastosis virus (AEV), which causes acute, lethal erythroleukemia through overproduction of two oncoproteins v-ErbB and v-ErbA. The study of these oncoproteins in erythroblastic transformation and of the physiological functions of their normal cellular counterparts c-ErbB (the chicken homolog of the Epidermal Growth Factor Receptor) and of the thyroid hormone receptor TRα provided invaluable insight as to the implication of receptor tyrosine kinase and nuclear hormone signaling pathways in the maintenance of an immature proliferative state of erythroid progenitors (reviewed in ref. *69*). It is also worth mentioning the E26 acute leukemia virus that expresses a transforming oncoprotein, which includes two mutated forms of the important hematopoietic transcription factors c-Myb and c-Ets-1 (reviewed in ref. *70*). *In vitro*, the E26 virus has the capacity to infect and transform multipotent hematopoietic progenitors and has been used in studying the role of specific transcription factors in the commitment and differentiation of distinct hematopoietic lineages (reviewed in ref. *71*). One important observation from such studies is the reprogramming function of key erythroid transcription factors such as GATA-1 and FOG-1 when ectopically expressed in myeloid or eosinophilic cells obtained from E26 transformed progenitors, which provided early evidence as to the differentiation plasticity of hematopoietic cells (*71*).

KEY REGULATORS OF ERYTHROPOIESIS: IRON HOMEOSTASIS-CELLULAR HEME, HYPOXIA, STRESS, GROWTH FACTORS, TRANSCRIPTION FACTORS, AND miRNAs

Erythropoiesis is a dynamic process regulated by oxygen tension in vertebrates and requires the orchestrated action of specific molecular mechanisms to strictly regulate cell proliferation, prevent apoptosis, and coordinate cell-cycle arrest with terminal maturation. Although many questions still remain unanswered regarding the underlying molecular mechanisms that govern lineage-determining decisions toward the path of terminal maturation, specific signaling pathways and molecular networks have been identified to control erythropoiesis. Iron homeostasis, hypoxia (low oxygen tension), and stress physiology affecting transcriptional factors and regulators represent key elements contributing to erythropoiesis (*72*).

Hypoxia, Stress, and HIF-Signaling Pathway

It is well established over the years that hypoxia promotes erythropoiesis via an increase of erythropoietin (Epo) production (Fig. 4). Epo, a member of the hematopoietic cytokine superfamily, is produced mainly in the fetal liver and adult kidney by transcriptional activation of Epo gene in response to low oxygen tension at high attitudes (*73*). This process is predominantly regulated by hypoxia-inducible factor (HIF) family as well as nuclear factor kappa B (NF-kB) (*74, 75*). HIF, a member of the basic helix-loop-helix (bHLH) PAS (Period-ARNT-Single minded) family, was discovered through its action as the transcriptional activator of erythropoietin, and has subsequently been found to control intracellular hypoxic responses throughout the body. The PAS domain, a homology region identified in the first three members of the PAS family: the drosophila period gene, the mammalian ARNT gene, and the drosophila single-minded gene (*76*). The importance of HIF functions in human physiology has been now well recognized and the data accumulated thus far indicate that in HIF-regulated gene products are included, besides Epo, vascular endothelial growth factor (VEGF), glucose uptake transporter-1, transferrin, transferrin receptor, plasminogen activator inhibitor-1, aldolase C, and endothelin-1 (*73*). The first member of the HIF family of transcription factors to be discovered, HIF-1, (a heterodimer consisting of an oxygen labile α-subunit and a constitutive β-subunit), was originally identified as a hypoxia-inducible nuclear factor that bound to the hypoxia-response element (HRE) enhancer region of the Epo gene (*77–79*) (Fig. 4). This was the first description of HIF-1α, whereas HIF-1β was found to be identical to the aryl hydrocarbon receptor nuclear transporter (ARNT), which is a dimerization partner for the aryl hydrocarbon receptor (*80*). In accordance with the effects of hypoxia on serum Epo, the DNA-binding activity of HIF-1 was found to be tightly regulated by cellular oxygen tension. As far as the HIF-1α nuclear transport is concerned, new information has been recently revealed. Interestingly, experimental data support the notion that nuclear transport of HIF-1α is mediated by MAPK-dependent regulatory mechanism via an identified on the molecule nuclear export signal (NES) (*81*). The function of HIF is primarily regulated by specific prolyl hydroxylase-domain (PHD) enzymes that initiate its degradation via the von Hippel-Lindau tumor suppressor protein (VHL) (Fig. 4). In particular, VHL binds to the HIF-a subunit and targets it for ubiquitin-

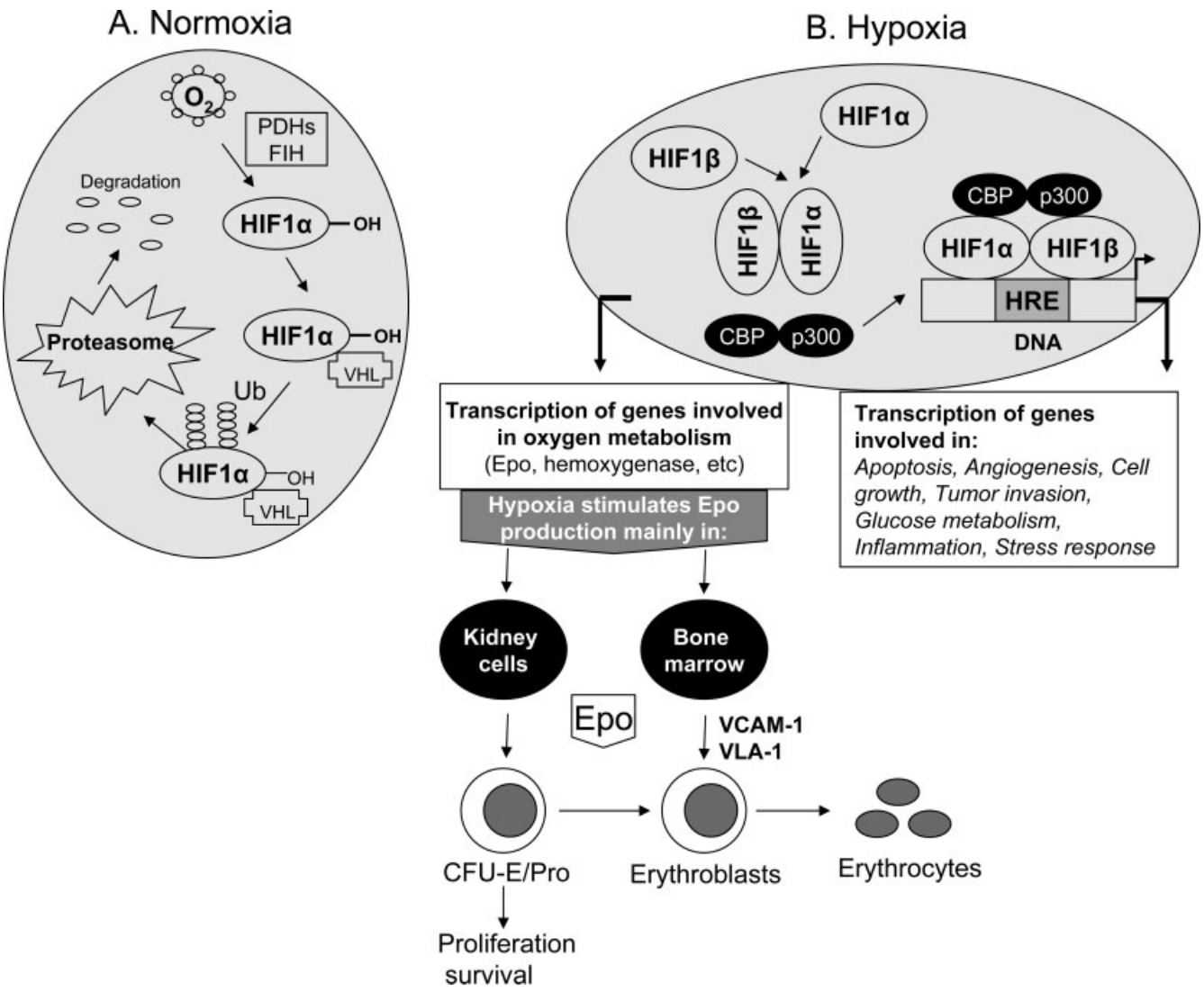


Figure 4. Schematic diagrams illustrating the major events occurring during erythropoiesis under normal oxygen consumption and hypoxia. (A) In normal oxygen (O_2) conditions (normoxia), the HIF-1α protein is subjected to hydroxylation by PDHs and FIH, both requiring 2-oxoglutarate and oxygen as cofactors. This allows recruitment of VHL, a component of ubiquitin ligase complex that ubiquitinates (Ub) HIF-1α and thereby targets it for proteasome-mediated degradation. (B) During hypoxia, when PDHs are not active, HIF-1α is stabilized, dimerizes with HIF-1β-CBP, a fact that results in transcription at HREs of hypoxia-inducible genes involved in angiogenesis, apoptosis, glucose transport and metabolism, erythropoiesis, inflammation, cellular invasion, and stress response. As far as erythropoiesis is concerned, hypoxia stimulates EPO production mainly in the adult kidneys and cell–cell contacts of erythroblasts and endothelial cells in the bone marrow through hypoxia-induced accumulation of HIF-2α. In the bone marrow, HIF-2α drives the expression of VCAM-1 and VLA-1 ($\alpha 1\beta 1$ integrin), molecules that facilitates cell–cell contacts between stromal and hematopoietic progenitors, thus modulating bone marrow microenvironment and finally inducing erythropoiesis. Epo, erythropoietin; FIH, factor inhibiting HIF-1; HREs, hypoxia-responsive elements (TACGTG); p300/CBP, CREB-binding protein (CREB, CCAAT enhancer-binding protein-ε); PHD, propyl hydroxylases; VCAM-1, vascular adhesion molecule-1; VHL: von Hippel-Lindau tumor-suppressor protein; VLA-1, very late antigen-1.

mediated proteasomal degradation by acting as the recognition component of a multiprotein ubiquitin ligase complex. This process is considered as the fundamental oxygen-sensitive step underlying the degradation of HIF-α and is mainly relied on the function of prolyl hydroxylase-domain (PHD) proteins (PHD1, PHD2, and PHD3). These catalyze oxygen-dependent hydroxylation of specific proline residues in HIF-a, which in turn directs the binding of VHL (reviewed in ref. *73*). The oxygen and iron

dependency of PHD activity accounts for regulation of the pathway by both cellular oxygen and iron status.

Furthermore, recent studies have shed more light on the mechanisms underlying the function of PHD-VHL-HIF axis pathway that acts as the central regulator of oxygen homeostasis in hypoxia-induced erythropoiesis (*73*). As it is shown, HIF-a subunit is synthesized continuously but is rapidly destroyed in the presence of oxygen and iron. In this way, oxygen- and iron-dependent PHD enzymes hydroxylate specific proline residues in HIF-α, increasing its affinity for the von Hippel-Lindau tumor suppressor protein (VHL). The binding of VHL to hydroxylated HIF-α then targets HIF-α for destruction by a multiprotein ubiquitin ligase that mediates proteasomal degradation of HIF-α subunits (Fig. 4). Another oxygen- and iron-dependent enzyme, -factor inhibiting HIF (FIH)-, hydroxylates an asparagine residue in HIF-α, reducing its ability to activate transcription by inhibiting binding of the transcriptional coactivator complex p300/CBP. Thus, under physiological cellular oxygen tension (euoxic condition or normoxia), HIF-α is continuously expressed and rapidly degraded, whereas under hypoxic conditions, the hydroxylation of HIF-α by PHDs and FIH is inhibited and proteasomal degradation is minimized. HIF-α rapidly accumulates and dimerizes with HIF-β which is expressed constitutively, a fact that leads to the recruitment of p300/CBP coactivator and the DNA binding complex subsequently upregulates hypoxia-responsive genes. The importance of these molecular events in the function of HIF was also verified by studying patients diagnozed with congenital polycythemia (a rare monogenic disease) (*82*). The most frequent cause of congenital polycythemia is likely to be mutations in VHL gene. The more extensively case studied among them is Chuvash polycythemia, where individuals are homozygous for a specific 598C>T mutation in the VHL gene. This results in an amino acid change of arginine to tryptophan (*73*). Such amino acid substitution diminishes the binding affinity of VHL for hydroxylated HIF-α, and by this way, the degradation of HIF-α is inhibited and HIF- targeted genes are pathologically activated (*83*). Another form of familial polycythemia caused by deregulation of HIF signaling has been recently identified as a result of mutations in EGLN1 gene (which encodes for PHD2). This genetic defect appears to reduce the activity of PDH2, the main PHD isoenzyme regulating the low steady-state level of HIF-α under physiologic oxygen tension conditions (*84*). Overall, these data confirm the importance the HIF-signaling pathway in regulating red cell production in the body.

Although HIF-1α was initially considered as the major regulator of Epo gene expression, recent studies indicate a dominant role for HIF-2α in regulating Epo synthesis in mice and in humans as well (*85–87*). Recently, it has been shown in HIF-2α knockdown gene expression in mice that the microenvironment required for erythropoiesis is dynamically regulated by oxygen through the regulation of vascular adhesion molecule-1 (VCAM-1) (*88*). Moreover, HIF-2α knockdown mice exhibited normocytic anemia, while their Epo levels were unaffected. As it is known, VCAM-1 supports the interaction of hematopoietic and endothelial cells in the bone marrow microenvironment and is required for maturation of erythroid cells. Hypoxia-inducible VCAM expression is selectively regulated by HIF-2α, and defective erythropoiesis can be rescued in HIF-2α knockdown mice with selectively restored HIF-2α expression in endothelial cells. Such data underscore the importance of HIF-2α in the oxygen-dependent regulation of erythropoiesis and add new insights on how low oxygen tension in the bone marrow promotes hematopoiesis by facilitating the interaction of stromal and hematopoietic cells (*89*) (Fig. 4). Because erythropoiesis is particularly sensitive to modulation of the function of HIF-α subunits and HIF-2α driven expression of VCAM-1, potential therapeutic targets can be developed. Overall, these functions are affected by cell–cell contacts between stromal and hematopoietic progenitors including erythroid committed progenitors like BFU-E and CFU-E, whereupon Epo acts in bone marrow microenvironment (niche) (*88, 89*).

Red blood cell homeostasis is an excellent example of redox balance: erythroid progenitors accumulate hemoglobin during development, and erythrocytes continuously transport large amounts of oxygen over the course of their ~120-day lifespan, a process that results in a high level of oxidative stress (*90, 91*). Thus, regulation of oxidative stress is particularly important to erythropoiesis and the production of mature erythrocytes (*92*). As far as the role of oxidative stress in erythropoiesis is concerned, it is interesting that forkhead box O3 (Foxo-3)-deficient erythrocytes exhibited decreased expression of reactive oxygen species (ROS) scavenging enzymes and had a ROS-mediated shortened lifespan as evidence of oxidative damage. Loss of Foxo-3 transcription factor induced mitotic arrest in erythroid precursor cells, leading to a significant decrease in the rate of *in vivo* erythroid maturation (*92*). To this respect, such data propose a potential role of Foxo-3 in the regulation of ROS in erythropoiesis implying that Foxo-3 may regulate the lifespan of red blood cells. Furthermore, it has been proposed that differential regulation of Foxo-3 target genes represents a novel mechanism controlling erythroid expansion and differentiation (*93, 94*). Therefore, understanding the direct targets of this transcription factor and its regulation is important for the regulation of erythropoiesis.

Iron Homeostasis/Cellular Heme (Iron Protoporphyrin IX)

Iron metabolism (*95, 96*) and cellular heme (see for review ref. *20*) are two of the most key regulators of erythropoiesis. Iron is entirely taken up from plasma via the iron-transferrin complex-transferrin receptor interaction. Heme derives from plasma as well as *de novo* biosynthesis inside the mitochondria as protoporphyrin IX first and then as iron-protoporphyrin IX (heme) after incorporation of iron with ferrochelatase. There is no free iron and cellular heme intercellularly. Both are bound to protein ferritins (for iron storage) and hemoproteins (proteins carrying heme as prosthetic groups).

Iron metabolism, cellular heme and erythropoiesis are inextricably linked. The major source of iron for erythrocyte precursors is plasma iron-transferrin (Fe-Tf). When iron supply to the bone marrow is inadequate because of systemic iron deficiency or iron sequestration, iron-restricted erythropoiesis or anemia results (*95, 96*). Iron homeostasis in mammals is regulated at the level of intestinal absorption, whereas the peptide hormone hepcidin was found to be the master regulator of systemic iron homeostasis by coordinating the use and the storage of iron with iron acquisition (*97*). Hepcidin is primarily produced by hepatocytes and is a negative regulator of iron entry into plasma by binding to ferroportin, an iron transporter present on cells of the intestinal duodenum, macrophages, hepatocytes, and cells of the placenta (*95, 97*). The magnitude of the iron flux is proportional to the concentration of ferroportin on the cell surface, and its binding with hepcidin induces ferroportin internalization and degradation (*98*). The loss of ferroportin from the cell surface prevents iron entry into plasma, a fact resulting in low transferrin saturation, and less iron delivery to the developing erythroblasts. Conversely, decreased expression of hepcidin leads to increased cell surface ferroportin and increased iron absorption. The expression of the gene encoding hepcidin (hepcidin antimicrobial peptide; HAMP) appears to be regulated at the level of transcription, whereas plasma levels of hepcidin are modulated by different stimuli, including cytokines (predominantly IL-6), plasma iron, erythropoiesis/anemia, and hypoxia (*97*). The induction of HAMP transcription in hepatocytes by IL-6 is mediated through the activation of Stat3 (*99*). Several studies identified the bone morphogenetic protein (BMP) pathway as a critical regulator of hepcidin expression. BMPs are members of the transforming growth factor-β (TGF-β) superfamily, and it has been shown that hemojuvelin (HJV), which was identified as an iron-specific BMP coreceptor, increased hepcidin expression by enhancing BMP signaling (*100*). In addition, manipulation of the HJV/BMP pathway *in vivo* altered hepcidin and iron levels. Administration of BMP2 in mice increased hepcidin expression and administration of soluble HJV decreased hepcidin expression, with consequent changes in iron plasma levels and tissue distribution (*101*). Hepcidin synthesis is stimulated by increased plasma iron and tissue iron stores, and hepcidin in turn decreases the release of iron into plasma, both from macrophages and from absorptive enterocytes in the duodenum (*95*). Although the molecular mechanisms underlying this effect are still elusive, it has been recently shown that Fe-Tf is implicated to hepcidin regulation by iron (*102*). To this regard, hepatocytes seem to sense Fe-Tf concentrations and increase hepcidin expression proportionally through the HJV/ BMP2,4 iron-regulatory pathway. Hepcidin regulation, however, during erythropoietic activity induced by hypoxia or increased Epo is still elusive (*95*).

The level of cellular heme (derived in part from plasma by uptake via hemopexin and in part from de novo biosynthesis in mitochondria) is very critical in the regulation of erythropoiesis. Heme biosynthesis is increased dramatically during Epo-moderated erythropoiesis to meet extra demand for red blood cell production under hypoxic conditions or stress erythropoiesis. Heme synthesized is needed for the production of large number of hemoproteins involved in cell respiration, O_2 tension sensing, and metabolism. Moreover, heme is needed to regulate the transcription of globin and nonglobin genes, because heme has been found to regulate the action of transcription factors at nuclear level (*20*).

Hepcidin-Mediated Iron Homeostasis and EpoR Signaling. HIF promotes iron transport by upregulating transferrin and the transferrin receptor (Tf-R) (*73, 95*). Furthermore, HIF negatively regulates hepcidin, the key hormonal regulator of iron homeostasis (*103*). Downregulation of hepcidin liberates ferroportin, thereby increasing intestinal iron absorption and mobilizing recycled iron from macrophages (*97*). Through the stabilization of HIF-a, hypoxia and iron-deficiency both suppress hepcidin and thereby increase iron availability, at least in mice (*103*). Similar involvement of HIF in humans seems likely, as hepcidin is likewise critical to human iron homeostasis. Indeed, hepcidin deficiency underlies most forms of the iron-overload disorder hereditary hemochromatosis (*97*). Thus, it appears that HIF simultaneously stimulates the polycythemic response to hypoxia and boosts the availability of iron so as to meet the resultant erythropoietic demands.

Iron and HIF are further coupled through the action of iron regulatory proteins IRP1 and IRP2. The importance of the cellular IRP-IRE regulatory network in systemic iron homeostasis has been recently reviewed by Muckenthaler et al. (*104*). IRPs posttranscriptionally regulate the expression of several proteins of iron metabolism by binding to their mRNA iron-responsive elements (IREs) (*105*). When iron is scarce, IRP-IRE binding promotes cellular iron uptake and prevents iron sequestration by stabilizing transferrin receptor (Tf-R) mRNA and inhibiting the translation of ferritin mRNA. In contrast, in iron-replete cells, IRP1 is biochemically inactivated, and IRP2 undergoes iron-dependent proteasomal degradation, resulting in reduced IRE-binding activity and opposite homeostatic effects. It is interesting to note that the IRP2 degradation pathway may involve an unidentified 2-oxoglutarate-dependent dioxygenase from the same family as the HIF hydroxylases (*106*), although VHL is not a necessary component of this pathway (*107*). Furthermore, it has been reported that HIF-2α (also called endothelial PAS domain protein-1, EPAS-1) is posttranslationally regulated by IRP-IRE binding (*108*). In particular, a regulatory link that permits feedback control between iron availability and the expression of key transcription factor promoting iron utilization was uncovered. This fact is mediated through a conserved, functional IRE that exists in the 5′-untranslated region (5′-UTR) of the mRNA of HIF-2α that permits IRP binding to control HIF-2α mRNA translation in response to iron availability. Because HIF-2a appears to be the most important HIF isoform in regulating Epo (*86*), this introduces a negative-feedback control mechanism that may operate during iron-deficiency to limit Epo

stimulation of erythropoiesis until iron availability is sufficient to allow effective red blood cell production.

Erythropoiesis requires Epo and stem cell factor (SCF) signaling via their receptors EpoR and c-Kit. As far as the EpoR function is concerned in erythropoiesis, signal transduction is mediated through the EpoR/Jak2/Stat5 signaling axis, regulating proliferation, differentiation, and survival. Interestingly enough, an unexpected mechanistic link between EpoR/Jak/Stat signaling and iron metabolism exist, that is absolutely essential for erythropoiesis and life. Mice completely lacking Stat5 suffered a reduction in the levels of transferrin receptor-1 (TfR-1) on erythroid cells. This fact was attributed to a reduced transcription of TfR-1 mRNA and iron regulatory protein 2 (IRP-2), the major translational regulator of TfR-1 mRNA stability in erythroid cells. Both genes were demonstrated to be direct transcriptional targets of Stat5 (*109*). Although EpoR acts via the kinase Jak2, the contribution of distinct EpoR/Jak2-induced signaling pathways (MAPK, PI-3, and Stat5) to functional erythropoiesis is still under investigation. Cytokine- and c-Kit pathways do not function independently of each other in hematopoiesis, but cooperate to attain full Jak2/Stat5 activation. Activated Stat5 has been shown to act as a critical downstream effector of Jak2 in erythropoiesis/myelopoiesis, whereas Jak2 functionally links cytokine- with c-Kit-receptor tyrosine kinase signaling (*110*). This is an intriguing situation where different signaling pathways are regulated by common molecules, thus allowing better regulation of erythropoiesis and red blood cell production.

Hemoglobin production during erythropoiesis is mechanistically coupled to the acquisition and metabolism of iron. Recently, a new mechanism through which hemoglobin may be modulated according to iron status has been proposed (*111*). The mRNA of α-hemoglobin-stabilizing protein (AHSP) contains a nucleotide sequence at the 3′-untranslated region (3′-UTR) that resembles the IREs (noncanonical IREs). Through this structure, iron regulates the expression of AHSP, which acts like a molecular chaperone that binds and stabilizes free α-globin during hemoglobin synthesis. Overall, these observations suggest that IRPs can regulate mRNA expression through noncanonical iron responsive elements (IREs) and extend the repertoire of known iron-regulated genes.

Another interesting line of investigation implicates involvement of UCP2, (an inner membrane mitochondrial protein implicated in bioenergetics) and ROS modulation in the regulation of erythropoiesis, because UCP2 has been postulated to function as a facilitator of both heme biosynthesis and iron metabolism by reducing ROS production. Expression of UCP2 gene occurs at early stages of erythroid maturation (when cells are not fully committed to heme biosynthesis), and high levels of UCP2 protein were found in erythroid cells. Detailed analysis of bone marrow and fetal liver progenitor cells, *in vitro* and *in vivo*, revealed that UCP2 deficiency led to a significant decrease in cell proliferation at the Epo-dependent phase of erythropoiesis, without affecting heme biosynthesis (*112*). This is an interesting finding indicating that UCP2 is another regulator of erythropoiesis and suggests that inhibition of UCP2 function may contribute to the development of anemia.

Growth Factors and Signaling Pathways

The hematopoietic system is hierarchically organized with a rare population of HSCs that give rise to progeny that progressively lose self-renewal potential and successively become more and more restricted in their differentiation capacity. Finally, this progeny generates functionally mature cells (*113*). During this process, self-renewal is critical for sustaining the HSCs compartment and thus is a prerequisite for lifelong hematopoiesis. In this direction, several developmentally conserved signaling pathways have emerged as important components of the fate of HSCs in the body by supporting expansion of the HSCs pool by a combination of survival and induced self-renewal. These include the SCF/c-kit receptor, Notch, wingless-type (Wnt), sonic hedgehog (Shh), and Smad [families of TGF-β, activins and bone morphogenetic proteins (BMPs)] signaling pathways (*113–115*). Although the intention of this review is not to comprehensively discuss each one of all the signaling pathways involved in hematopoiesis, the significance of SCF/c-kit receptor, Wnt, Notch, and EpoR pathways will be further considered in regulating erythropoiesis.

SCF receptor (CD117, the gene product of c-kit proto-oncogene) and its ligand SCF play a central role in gametogenesis, melanogenesis, and hematopoiesis. SCF receptor is a member of the type III subfamily of receptor tyrosine kinase (RTK) that also includes the receptor for M-CSF, Flt-3, and PDGF. It is expressed in hematopoietic progenitor cells, normal B- and T-cell progenitors, mast cells, germ cells, melanocytes, neurons, glial cells, placenta, kidney, lung, and gut cells (*116*). Sporadic mutations of c-kit and autocrine/paracrine activation mechanisms of the SCF/c-kit pathway have been implicated in a variety of malignancies. C-kit has been found to be mutated and activated in gastrointestinal tumors and lung carcinomas. C-kit receptor tyrosine kinase has been the target of drug Gleevec/Glivec (imatinib mesylate). Deficiency in SCF or c-kit product affects erythropoiesis. Gain of function mutations of c-kit is associated with malignancies such as AML, gastrointestinal stromal tumors (GISTs), and mastocytomas (*117, 118*). Binding of SCF to c-kit receptor leads to dimerization and activation of protein kinase that autophosphorylates the receptor. C-kit receptor tyrosine kinase controls the function of primitive hematopoietic cells, melanocytes, and germ cells (*119*). C-kit is an excellent target for new drug development, because inhibition of the underlined signaling pathway affects cell proliferation, survival, and differentiation (*120, 121*).

Recent studies indicate an important role for Wnt signaling in hematopoiesis during mammalian development by modulating the fate of HSCs through the regulation of bone marrow microenvironment, the so-called “niche” (*98, 115, 122*). Expression of Fz5, Wnt5a, and Wnt10b has been found in both

mouse embryonic yolk sac and fetal liver. Furthermore, Wnt10b was also found to be expressed by murine fetal liver hematopoietic progenitors (*122, 123*). The progenitor cell populations can be expanded greatly *in vitro* by addition of Wnt1-, Wnt5a-, or Wnt10b-conditioned media. In addition, human fetal bone marrow stromal cells have also been shown to express Wnt2b, Wnt5a, and Wnt10b, and human $CD34^+$ hematopoietic progenitor cell number was also increased when cocultured with stromal lines transfected with these Wnts (*124*). The role of Wnt signaling in the maintenance and proliferation of hematopoietic stem cells has also been more thoroughly characterized, because genes implicated in HSC self-renewal, such as HoxB4 and Notch1, were shown to be upregulated on activation of Wnt signaling in HSCs. (*125, 126*). Interestingly, blocking Wnt signaling with over-expression of Axin inhibits proliferation *in vitro* and the ability of HSC to reconstitute blood cells *in vivo* (*125*).

Following the importance of Wnt signaling in regulating hematopoiesis upon development, accumulating evidence suggests that also the Notch pathway affects survival, proliferation, and cell fate choices at various stages of hematopoietic cell development, including the decisions of HSC to self-renew and/or differentiate (*127*). Interestingly, it has been recently reported that the regulation of primitive erythroid development is controlled, at least in part, through the coordinated interaction of Wnt and Notch pathways (*128*). It has also been reported that deregulated Notch-1 gene expression in MEL cells inhibited apoptosis and abrogated erythroid differentiation induced by chemical inducers (*129, 130*). To this respect, Notch signaling has been recently shown to have definitive roles in the differentiation and maintenance of HSCs, mediated through Wnt signaling the self-renewal of HSC renewal, thus linking Notch and Wnt signaling in the process of HSC proliferation (*131*). The interaction between Notch and Wnt signaling pathways by acting in concert to maintain the balance between self-renewal and differentiation of hematopoietic stem cells has been also further confirmed in another study. In the latter case, the activation of Notch signaling in hemangioblasts dramatically reduced their survival and proliferative capacity, lowered the levels of hematopoietic stem cell markers CD34, and c-kit and the myeloid marker CD11b, as well as identified putative downstream targets of oncogenic forms of Notch, among others, the noncanonical Wnts Wnt4 and 5A (*132*). The inter-talk between Notch and Wnt signaling seems to be modulated through the association of Notch with Armadillo/β-catenin thus regulating β-catenin transcriptional activity in the nucleus (*133*).

Epo stimulates erythropoiesis via the promotion of proliferation, differentiation, and survival of erythroid precursors (BFU-E, CFU-E). Upon Epo binding to its receptor (EpoR), the receptor undergoes a conformational change that activates the prebound, cytoplasmic, tyrosine kinase, Janus kinase 2 (JAK2) (*134–136*). Subsequently, JAK2 phosphorylates several cytoplasmic tyrosine residues in the cytoplasmic tail of the EPoR that act as docking sites for proteins that contain Src-homology 2 (SH2) domains. Thus, the activation of multiple pathways begins including signal transducer and activator of transcription 5 (Stat5), phosphoinositide-3 kinase (PI-3K)/Akt, and p42/44 mitogen-activated protein kinase (MAPK) (*137, 138*). It is interesting, however, the fact that upon challenge of adult human hematopoietic progenitor cells *in vitro* on various oxygen tension (pO_2) conditions, hypoxia alters progression of erythropoiesis via modulation of expression of EpoR and erythroid transcription factors GATA-1, EKLF, and SCL/TAL-1, whereas this effect is well-correlated with the level of Epo in the culture (*75*). SH2 domains are crucial structures in regulating Epo-signaling pathway by other molecules as it has been shown in the case of Lnk protein. Lnk is an adaptor protein implicated in cytokine receptor signaling and through its SH2 domain negatively modulates EpoR signaling by attenuating JAK2 activation, and regulates Epo-mediated erythropoiesis (*138*).

Transcriptional Regulation of Erythropoiesis

Erythroid cell differentiation proceeds in response to specific signaling stimuli and according to a transcriptional program that is regulated by DNA binding, lineage-restricted transcription factors (*72, 139, 140*). Erythroid transcription factors were initially isolated through their binding to specific DNA binding motifs in the promoters of β-globin genes (for example, see Ref. *141, 142*). The use of gene knockouts in mice has provided unequivocal evidence for a number of transcription factors fulfilling essential functions in erythroid differentiation. Furthermore, the analysis of transcription factor null hematopoietic cells has provided invaluable insight into the cellular and molecular functions of these factors in erythropoiesis, whereas biochemical and proteomic analyses have begun uncovering protein interactions and signaling pathways regulating the activities of transcription factors in erythroid maturation. The application of genomic approaches such as expression profiling and chromatin immunoprecipitation (ChIP) assays is also having an impact in uncovering, on a global scale, the precise gene target networks, and *cis*-acting elements regulated *in vivo* by these factors.

Transcription factor function involves not only the activation of the erythroid specific genes but also the suppression of the multilineage transcriptional "promiscuity" associated with hematopoietic stem cells and multipotential hematopoietic progenitors as well as the suppression of cell division (*143, 144*). Thus, transcription factors are called upon to serve as activators as well as repressors in directing erythroid differentiation. The salient features of a number of key transcription factors and their cofactors in erythropoiesis are discussed below.

GATA-1. GATA-1 is a critical transcription factor for erythroid differentiation. It is the founding member of the GATA family of transcription factors, all of which execute essential developmental roles as transcriptional regulators (reviewed in refs. *145–147*). GATA factors are characterized by the presence of two conserved zinc finger domains and their binding to the (A/

T)GATA(A/G) consensus DNA binding motif. GATA-1 is expressed in erythroid cells, megakaryocytes, mast cells, eosinophils, and dendritic cells in hematopoiesis (*148, 149*). GATA-2 and GATA-3 are also hematopoietically expressed with GATA-2 predominantly expressed in hematopoietic stem and progenitor cells, whereas GATA-3 expression is T cell restricted.

GATA-1 was originally isolated as a factor binding to the β-globin promoter and has subsequently been recognized as a transcription factor that binds to most, if not all, known erythroid genes (reviewed in (*147, 150*). Three functional domains have been described for the GATA-1 protein: an N-terminal domain reported to act as a transcriptional activation domain in transient transcription assays, and two zinc finger domains located toward the C-terminus of the protein. The second, C-terminal-most, zinc finger is responsible for binding to DNA, whereas the first, N-terminal zinc finger modulates and stabilizes DNA binding, for example, to more complex palindromic GATA motifs (*151*). Both zinc fingers are involved in protein-protein interactions.

GATA-1 expression is essential for normal erythropoiesis. The GATA-1 gene knockout in mice results in embryonic lethality at around day E10.5–E11.5 of development due to severe anemia (*152*). Chimeric mice obtained from GATA-1 null ES cells fail to generate mature red blood cells whilst generating all other hematopoietic lineages (*153*). Detailed genetic analysis showed that GATA-1 null cells (primitive or definitive) are unable to differentiate beyond the proerythroblastic stage due to apoptotic cell death (*33, 154*). These studies highlight an essential anti-apoptotic function for GATA-1 in erythroid maturation. Importantly, genetic experiments have indicated that as little as 5% of physiological GATA-1 expression can prevent apoptosis, but is not sufficient for completing erythroid differentiation (*155*). Thus, GATA-1 fulfills additional essential functions in erythroid maturation beyond its anti-apoptotic role (see below).

In addition to genetic studies documenting an essential role for GATA-1 in erythroid differentiation, enforced ectopic GATA-1 expression studies have provided evidence for an instructive role for GATA-1 in erythroid differentiation. For example, ectopic GATA-1 expression in highly purified murine progenitor cells (myeloid or lymphoid) "instructed" their differentiation toward the erythroid and megakaryocytic lineages that GATA-1 normally regulates (*156, 157*). Thus, GATA-1 is capable of imposing a transcription program that promotes unilineage (*i.e.* erythroid) differentiation in cells that have the potential to give rise to different cell types (*158*).

The essential functions of GATA-1 in erythroid differentiation are achieved through the orchestrated regulation, which involves activation as well as repression, of several classes of target genes implicated in all facets of erythroid cell maturation (*148, 159*). Thus, apart from the upregulation of the erythroid transcription program, GATA-1 is also a critical suppressor of early hematopoietic progenitor multipotentiality and of alternative hematopoietic cell fates, for example, through the repression of GATA-2 expression (*160*) or repression of the critical myeloid PU.1 transcription factor [P. Rodriguez and J. Strouboulis, unpublished observations; (*149*)]. Interestingly, in erythroid cells, GATA-1 appears to repress alternative transcription programs that GATA-1 itself regulates in other hematopoietic lineages such as eosinophils (*161*). In addition, GATA-1 promotes survival of maturing erythroid cells through the upregulation of anti-apoptotic genes such as Bcl-x_L (*22*). GATA-1 also promotes G1 cell cycle arrest in terminal erythroid differentiation through the repression of mitogenic genes such as myc (*24, 161*). Many of the above features of GATA-1 gene regulatory functions have been elegantly demonstrated in the study of Welch et al. (*26*). Here, GATA-1 expression was restored in G1E cells (see section Permanent Cell Lines), leading to the synchronous terminal erythroid differentiation of these cells. By carrying out microarray expression analysis at specific timepoints during differentiation in this system, Welch et al. (*26*) showed a wave of repression early in the differentiation process, which included genes of early acting hematopoietic transcription factors and mitogenic genes. Genes upregulated early in differentiation include erythroid acting transcription factors such as FOG-1 and EKLF (see below), whereas genes implicated in red cell physiological functions, such as globins and heme biosynthesis enzymes, were upregulated later in differentiation (*26*).

How does GATA-1 carry out these functions in erythropoiesis? Protein interactions are an important factor in this respect. GATA-1 has been reported to interact with many transcription factors, such as FOG-1, EKLF, TAL-1/SCL, PU.1 and cofactors such as CBP/p300, Brg1, MeCP1/NuRD, and others (for reviews (*139, 148, 162*). Using an *in vivo* biotinylation tagging approach coupled with mass spectrometry, Rodriguez et al. (*161*) isolated and characterized nuclear GATA-1 protein complexes in differentiated MEL cells. This work showed that GATA-1 forms two independent complexes with FOG-1, with and without the repressive MeCP1/NuRD chromatin remodeling/histone deacetylase complex. In addition, GATA-1 forms distinct complexes with the hematopoietic transcription factors TAL-1/SCL (and associated partners) or Gfi-1b and with the chromatin remodeling complex ACF/WCRF (*161*). Preliminary analysis suggested a model whereby each GATA-1 protein subcomplex regulates a distinct subset of target genes. For example, the GATA-1/FOG-1/MeCP1 complex represses early hematopoietic and alternative lineage transcription programs, whereas the GATA-1/Gfi-1b complex suppresses cell division by repressing mitogenic genes such as myc. Additionally, the GATA-1/FOG-1 and the GATA-1/TAL-1/SCL complexes upregulate the erythroid transcription program. By using various assays, GATA-1 has also been reported to interact with many other proteins including transcription factors and cofactors with documented roles in erythropoiesis, such as TAL-1/SCL and FOG-1 (see below). The application of biotinylation tagging in the complementary isolation and characterization of GATA-1 protein partners such as TAL-1/SCL has led to the identification of novel GATA-1 protein interactions and the expansion of its protein interaction networks (see below).

Posttranslational modifications of GATA-1 protein have also been proposed to modulate GATA-1 functions. GATA-1 protein is subject to phosphorylation, acetylation, and sumoylation. GATA-1 phosphorylation has been mapped at seven serine residues, one of which (S310) becomes phosphorylated upon terminal erythroid differentiation (*163*). Although various models have been proposed for phosphorylation modulating GATA-1 functions (reviewed in (*148*), specific serine-to-alanine mutations did not have any effect in normal erythropoiesis in mouse (*164*). GATA-1 can be acetylated by the p300 and CBP protein acetyltransferases at two conserved lysine-rich motifs localized C terminally to each of the zinc fingers (*165, 166*). Although acetylated GATA-1 has been estimated to account for less than 5% of total GATA-1 protein (*141*), GATA-1 acetylation has been shown to be a requirement for terminal erythroid differentiation (*166*) and for binding to target genes in chromatin (*167*). It has been proposed that GATA-1 acetylation increases GATA-1 DNA binding activity at the same time marking the highly "active" acetylated GATA-1 protein fraction for degradation in the cell (*168*). SUMOylation of GATA-1 at lysine residue K137 has been reported (*169*); however, the functional significance of GATA-1 SUMOylation remains unclear.

GATA-1 protein levels have also been implicated in regulating GATA-1 functions in erythroid differentiation. GATA-1 protein levels decline in the very last stages of terminal erythroid differentiation and GATA-1 degradation by caspases has been reported (*170*). Interestingly, GATA-1 overexpression inhibits erythroid differentiation *in vivo* (*171*). Taken together with observations that wild type mature erythroid cells can rescue the defective differentiation phenotype of GATA-1 overexpressing cells, led to a model whereby mature erythroid cells signal to immature cells to undergo GATA-1 degradation in completing the final steps of maturation as a homeostatic mechanism for maintaining red cell production (*172*). Degradation of the acetylated fraction of GATA-1 protein has also been proposed as a safety mechanism for controlling levels of the highly transcriptionally active acetylated form of GATA-1 (*168*).

A critical question to be addressed in erythropoiesis is how proliferation and differentiation demands upon erythroid maturation can be met through the action of GATA-1, Epo, and SCF? Recent advances have provided evidence toward understanding the signaling mechanisms by which EpoR and c-Kit regulate cooperatively growth, survival, and differentiation of erythroid progenitors (*173*). To this respect, earlier studies have indicated that GATA-1-mediated proliferation arrest during erythroid maturation is mediated *via* c-Myc repression by directly binding to this gene (*24*). By extending these observations and using G1E cells, (an immortalized GATA-1-null erythroblast line derived from gene-targeted embryonic stem cells), it has been highlighted that a distinct antiproliferative program of GATA-1 related to gene repression exists and can be uncoupled from its ability to activate erythroid marker genes during terminal maturation (*173*). In particular, immature G1E cells lacking GATA-1 depend on SCF for cell cycle progression through effects mediated by induction of c-Myc via the Src kinase signaling pathway. Moreover, a regulated signaling cascade, in which GATA-1 inhibits the expression of c-Kit, Rac1, Akt, and c-Myc, to induce G1 arrest during terminal erythroid maturation has been uncovered (*173*). Such data suggest that GATA-1 induces cell cycle arrest in erythropoiesis by blocking expression of multiple components of a c-Kit signaling cascade that lead to c-Myc activation. Of particular importance in erythropoiesis are studies demonstrating reversal of tumorigenicity and differentiation in MEL cells by enforced expression of GATA-1 (*174*). Terminal erythroid differentiation and reduced proliferation in MEL cells were associated with repression of c-Kit and c-myc (*174, 175*). Therefore, this possible overexpression of GATA-1 in MEL cells that induces terminal erythroid differentiation *in vitro* and reverses tumorigenicity *in vivo* may be closely related with the modulation of c-Kit and c-Myc signaling pathways (*173*). This provides new insight into how c-Kit and GATA-1 interrelate to each other during normal hematopoiesis.

An interesting direction on how the GATA-1 regulatory axis controls erythroid regulation has emerged through the identification that GATA-1 regulates the expression of a miRNA locus essential for erythropoiesis (*176*). In an effort to identify miRNAs involved in erythropoiesis, researchers detected two conserved miRNAs, miR-144 and miR-451, as direct targets of GATA-1. In particular, it has been shown that GATA-1 binds a distal upstream regulatory element to activate RNA polymerase II-mediated transcription of a single common precursor RNA encoding both mature miRNAs (see the role of miRNAs in section MicroRNAs as Major Regulators of Erythropoiesis).

FOG-1. Friend of GATA (FOG-1), as the name implies, is a very close interacting partner of GATA-1 originally isolated through a yeast two-hybrid screen (*177*). It is a large nine zinc finger-containing protein, which does not bind to DNA directly. Four of the FOG-1 zinc fingers contribute to interactions with GATA-1 (*178*). FOG-1 is highly expressed in erythroid cells and megakaryocytes, mirroring expression of GATA-1 in these lineages. The phenotype of the FOG-1 gene knockout is very similar to that of the GATA-1 knockout as it results in midembryonic lethality due to severe anemia (*179*). An elegant study utilizing a GATA-1 point mutant that disrupts interaction with FOG-1 (but not DNA binding) provided conclusive evidence for the GATA-1/FOG-1 interaction being essential for erythroid differentiation, because proerythroblasts expressing the GATA-1 point mutant fail to undergo erythroid maturation (*180*). A similar point mutation in GATA-1 has been linked to severe congenital dyserythropoietic anemia and thrombocytopenia in man (*181*). The study by Crispino et al. (*180*) provided evidence for the GATA-1/FOG-1 interaction resulting in activation and repression of GATA-1 target genes. Subsequent studies identified the MeCP1/NuRD complex as a GATA-1/FOG-1 interacting partner in repressing gene expression (*161, 182*). However, it is unclear how the FOG-1/GATA-1 interaction mediates gene activation. One possibility is that FOG-1 facilitates GATA-1

mediated DNA looping, which juxtaposes distal enhancer elements onto gene promoters, as was shown for the β-globin locus (*183*).

Further evidence for the importance of the FOG-1/GATA-1 interaction in erythroid differentiation has been provided by the ectopic FOG-1 expression in nonerythroid hematopoietic lineages or in progenitors. Ectopic expression of FOG-1 in eosinophils or mast cells where GATA-1 (but not FOG-1) is physiologically expressed, resulted in the reprogramming of these cells toward the erythroid/megakaryocytic lineages (*184, 185*). Ectopic expression of FOG-1 by itself in multipotential hematopoietic progenitor cells (where GATA-1 expression is low or none) is not sufficient to divert these to an erythroid fate, thus further highlighting the necessity for interactions with GATA-1 (*157*). It is of interest that FOG-1 expression appears to be regulated by GATA-1 (*26*), thus suggesting a model whereby an early event in erythroid differentiation is FOG-1 upregulation by GATA-1 leading to stable FOG-1/GATA-1 protein interactions, which direct erythroid differentiation.

A defined balance of transcription factors GATA-1 and GATA-2 is a part of erythropoiesis. The "GATA switch" (*i.e.* raising GATA-1 levels displaces GATA-2 from its binding sites) facilitated by FOG-1 provides an important component of the GATA-related repression mechanism (*72*). Such conditions have been found recently to play major role in deregulation of erythropoiesis by tumor necrosis factor alpha (TNFα), because it has been shown that the inhibition of Epo-mediated erythroid differentiation by TNFα is mediated by activation of p38/MAPK pathway, GATA-1, and FOG-1 downregulation and GATA-2 upregulation (*186*). Interestingly, in a study of kit gene chromatin conformation, a GATA-switch was observed to alter the higher-order chromatin organization thus modulating the developmental regulation of kit gene expression during early erythropoiesis (*187*). Another interesting feature of erythropoiesis is the ability of specific transcription factors to regulate more than one hematopoietic cell lineages because they are able to interact each time with different target proteins, like Id2, a member of inhibitors of DNA binding (Id) family. These molecules act as negative regulators of E proteins, [the transcription factors of the basic helix-loop-helix (bHLH) family of class I are also known as E proteins; the basic region associates with a hexanucleotide (CANNTG) "E box" sequence on the DNA of target genes]. By analyzing Id2-knockout mice and retroviral-transduced hematopoietic progenitors, researchers were able to demonstrate that Id2 regulates B lymphopoiesis by acting as a physiologically relevant regulator of E2A, which contributes to erythroid development as well by interacting with transcription factor PU.1 and modulating PU.1 and GATA-1 activities (*187*).

TAL-1/SCL/LMO2/Ldb1/E2A Complex. TAL-1/SCL is a basic helix-loop-helix (bHLH) transcription factor that binds to a short consensus DNA motif (CANNTG) called the E-box. TAL-1/SCL expression essentially mirrors that of GATA-1 as it is expressed in erythroid cells, megakaryocytes, and mast cells (for review (*72, 139*). The TAL-1/SCL gene knockout results in total absence of hematopoiesis in the yolk sac, and TAL-1/SCL null ES cells fail to contribute to hematopoiesis in mouse chimeras (*188–190*). Significantly, conditional knockout of the TAL-1/SCL gene in adult hematopoiesis led to a failure in erythropoiesis (*191*), thus establishing a critical role for TAL-1/SCL in erythroid differentiation.

In erythroid cells, TAL-1/SCL forms a complex with the ubiquitous E47/E2A bHLH partner and also with the hematopoietic LMO2 and ubiquitous Ldb1 LIM domain containing cofactors (*192–195*). This complex interacts with GATA-1 to form a so-called pentameric complex that binds to composite E-box/GATA-1 DNA motifs that are spaced 9–11 nucleotides apart (*195, 196*). Such motifs are present in many erythroid genes and in the regulatory elements of key transcription factor genes including the TAL-1/SCL and GATA-1 genes themselves and the EKLF gene (*195–198*). Several lines of evidence support a critical role for the pentameric complex in erythropoiesis. For example, the LMO2 gene knockout phenotype in mice is identical to that of the TAL-1/SCL knockout (*199*). This is consistent with the suggestion that LMO2 acts as the bridge between TAL-1/SCL and GATA-1 in the pentameric complex (*195*). Furthermore, ectopic coexpression of TAL-1/SCL, LMO2, and GATA-1 in Xenopus embryos promoted erythropoiesis (*200, 201*), whereas antisense morpholino RNAs mediated knockdowns in zebrafish embryos directed against Ldb1 resulted in defects in definitive hematopoiesis (*202*).

The recent characterization of TAL-1/SCL and Ldb1 protein complexes from mouse erythroleukemic cells led to the identification of novel protein interacting partners, including the ETO-2 repressor protein (*192, 193, 202*). Knockdown experiments in zebrafish embryos showed ETO-2 to be essential for definitive erythropoiesis (*202*). Importantly, the stoichiometry of interaction between the ETO-2 repressor and the TAL-1/SCL complex declines with terminal erythroid differentiation (*192, 193, 202*). This is consistent with the activating functions associated with the TAL-1/SCL/GATA-1 pentameric complex in terminal erythroid differentiation; however, the precise function of ETO-2 containing TAL-1/SCL complex in early erythropoiesis is presently unclear.

EKLF. The erythroid Krüppel-like factor (EKLF) is an erythroid-specific zinc finger transcription factor that is critical for globin gene regulation and erythropoiesis in general (for review see refs. *203* and *204*). It binds to CACC box motifs (a subset of the broader 5′-NCNCNCCCN-3′ EKLF consensus sequence) present in the β-globin promoter and in the promoters of many erythroid genes (*205–207*). The significance of the CACC box in the human β-globin promoter is underscored by the fact that naturally occurring mutations in this element are associated with thalassemia (*208, 209*). The EKLF gene knockout results in embryonic lethality at around stage E14-15 due to lethal anemia (*210, 211*). Analysis of the EKLF knockout mice showed a failure in β-globin gene activation in the fetal liver stage,

accompanied by a "closed" chromatin structure at the β-globin gene promoter and loss of DNase I hypersensitivity at HS3 of the locus control region (LCR), thus suggesting important roles for EKLF in the organization of an active chromain domain in the β-globin gene locus (*212*). This is further supported by recent evidence suggesting a role for EKLF in the spatial nuclear organization of a transcriptionally active chromatin domain in the β-globin locus (*213, 214*). Expression profiling of EKLF null erythroid progenitors showed that impaired expression of genes involved in hemoglobin biosynthesis pathways and in the stability of the erythroid cell membrane may also contribute to the EKLF knockout phenotype (*205, 214*), whereas a recent study has also uncovered defects in cell cycle regulation in EKLF null cells (*215*). Recent evidence has also suggested important functions for EKLF in promoting erythroid differentiation of early progenitor cells at the expense of megakaryopoiesis (*216, 217*), a function that appears to be mediated through EKLF sumoylation and gene repression (*218*).

Gfi-1b. Growth factor-independent 1b (Gfi-1b) is a transcription factor isolated through its homology with the Gfi1 protein identified as an integration site for the Moloney leukemia virus following infection and transformation (*219*). Gfi1-1b is a six zinc-finger protein with a N-terminal SNAG domain associated with transcriptional repression (*220*). Overexpression of Gfi-1b in hematopoietic progenitors results in a dramatic expansion of erythroblasts, which cannot however complete erythroid maturation and undergo apoptosis (*221*). The Gfi-1b gene knockout results in embryonic lethality at around stage E15 due to a failure of definitive erythropoiesis, thus establishing a critical role for Gfi-1b in erythroid differentiation (*222*). The overexpression and loss-of-function studies suggest that Gfi-1b functions in erythroid differentiation must be closely linked to Gfi-1b expression level regulation. Indeed, there is evidence that Gfi-1b expression is upregulated by GATA-1 in early erythroid maturation with expression levels declining in later stages, possibly mediated through Gfi-1b repressing its own promoter in a negative feedback loop (*223*). Clues as to the possible Gfi-1b functions in regulating cell proliferation in erythropoiesis have been obtained through the identification of genes involved in cell cycle regulation and cytokine signaling suppression as being Gfi-1b targets (*161, 224, 225*).

BCL11A. The B-cell lymphoma/leukemia 11A (BCL11A) transcription factor is a C2H2 type zinc-finger protein that has been previously shown to be critical in lymphopoiesis for the development of B cell precursors (*226*). However, the role of BCL11A in erythropoiesis has only recently been assessed. In genome-wide association studies aiming to identify genes whose function is correlated with the "fetal hemoglobin switch" after birth, that is, the replacement of γ-globin by adult β-globin, BCL11A has emerged as a candidate factor involved in the control of γ-globin gene expression (*227–230*). Experimental data strongly suggested an association of SNPs within the BCL11A locus with HbF levels found in various ethnic populations. Consistent with a direct role of BCL11A in globin gene regulation, Orkin and coworkers (*231*) found that BCL11A is itself developmentally regulated and act as a stage-specific repressor involved in the silencing of γ-globin gene expression by occupying several discrete sites within the β-globin gene cluster. Interestingly, BCL11A functions in concert with the NuRD-repressor complex [that contain as core components histone deacetylase (HDAC) 1 and 2], GATA-1 and FOG-1. In agreement with these data, another study revealed that BCL11A binds to GGCCGG motif in nucleotide -56 to -51 on the HbF gene proximal promoter (*232*). The binding of BCL11A on the core motif of the promoter may recruit and interact with other partners to form a repression complex, leading to deacetylation of histones and downregulation of the HbF gene transcription. The possibility of unraveling the molecular mechanisms responsible for the gene expression switch in the β-globin cluster is of great therapeutic importance, because increased fetal hemoglobin (HbF; $\alpha 2\gamma 2$) levels or F-cell (HbF containing erythrocyte) numbers can ameliorate the clinical severity of diseases like β-thalassemia and sickle cell anemia (*233*). Therefore, on the basis of findings implicating BCL11A downregulation with increased HbF levels, BCL11A emerges as a valuable molecular target for reactivation of HbF in patients suffering of sickle cell disease and β-thalassemias (*231*). Furthermore, it has also been proposed that BCL11A cooperates with Nf1 in leukemogenesis. Within this frame, a therapeutic intervention targeting the BCL11A pathway may prove fruitful for leukemia therapy (*234*).

Transcriptional Cofactors in Erythropoiesis

The hematopoietic-restricted transcription factors are aided in their functions by interactions with a range of widely expressed cofactors. These include other ubiquitously expressed transcription factors, such as the E2A transcription factors interacting with TAL-1/SCL, catalytic activities mediating posttranslational modifications and chromatin remodeling and histone modification complexes (for review see ref. *72*). For example, specific acetylation by the p300 and CBP acetyltransferases has been reported for many of the key transcription factors, including GATA-1, TAL-1/SCL, and EKLF (*165, 166, 235, 236*). In all cases, transcription factor acetylation augments their transcriptional activation properties and in some cases modulates their DNA binding properties (*165, 235*), whereas in the case of GATA-1 acetylation has been shown to be required for GATA-1 binding to chromatin and erythroid differentiation (*166, 167*).

A number of hematopoietic transcription factors have also been shown to interact with chromatin remodeling enzymatic protein complexes that hydrolyze ATP to mobilize nucleosomes in transcriptional activation. Brg1, the catalytic subunit of the SWI/SNF chromatin remodeling complex, has been shown to interact with GATA-1, EKLF, and TAL-1/SCL in augmenting their transcriptional activation functions through changes in the chromatin structure of target genes (*237–239*). Mutant mouse

embryos expressing lower levels of Brg1 suffer from anemia due to a block in erythroid maturation, thus providing further evidence for a role for Brg1 in erythropoiesis (*240*). GATA-1 has also been reported to interact with the mediator complex, which serves as a coactivator by bridging transcription factors to RNA polymerase II (RNA Pol II) in transcriptional activation (*241*). A role for the mediator complex in erythropoiesis is further supported by the observation that the gene knockout of TRAP220, a component of the mediator complex, leads to defects in erythropoiesis (*241*).

Erythroid transcription factors also interact with repressive complexes and cofactors. The characterization of GATA-1 and FOG-1 protein complexes and interactions in MEL cells have shown that both of them interact with the repressive NuRD complex, which includes histone deacetylases (HDACs), the Mi2β nucleosome remodeling enzyme and methyl DNA binding proteins (*161, 182*). Significantly, the GATA-1/FOG-1/NuRD complex was shown to be responsible for the repression of the GATA-2 gene, a key step in terminal erythroid differentiation (*161*). FOG-1 also interacts with the CtBP corepressor (*178, 242*) though the significance of this interaction in erythropoiesis is presently unclear (*243*). EKLF and TAL-1/SCL have been reported to interact with the Sin3a corepressor and HDAC1 (*244, 245*). The EKLF repressor function mediated by Sin3A and HDAC1 appears to be stage specific in that it is more prominent in primitive erythroid cells (*246*). Remarkably, post-translational modification of EKLF by sumoylation appears to regulate its interaction with components of the NuRD repressive complex and EKLF's ability to suppress megakaryocytic differentiation (*218*). The interactions of TAL-1/SCL with Sin3A and HDAC1 appear to play a role in actively repressing erythroid genes in the early stages of erythroid differentiation (*239*). The characterization of TAL-1/SCL protein complexes from mouse erythroleukemic cells also identified ETO-2 as a TAL-1/SCL corepressor with an essential role in erythropoiesis (*202*), potentially through the active repression of erythroid genes in early differentiation stages and/or through regulating proliferation of early erythroid progenitors (*188, 192*). Lastly, characterization of Gfi-1b complexes from MEL cells identified the CoREST corepressor, the Lsd1 histone demethylase and HDACs 1 and 2 as Gfi-1b interacting partners (*247*). Importantly, knocking down LSD1 impaired terminal differentiation of MEL cells, which was accompanied by a derepression of a subset of Gfi-1b target genes (*247*).

MicroRNAs as Major Regulators of Erythropoiesis

MicroRNAs (miRNAs) are small (21–25 nt) noncoding RNAs that repress gene expression at the posttranscriptional level by binding to the 3′-UTRs of mRNAs and, depending on the extent of sequence complementarity, leading to mRNA degradation or translational inhibition (for recent review see ref. *248*). Much recent evidence has shown miRNAs to play important, mostly conserved, regulatory roles in practically all pathways including control of hematopoiesis, developmental timing, cell differentiation, apoptosis, proliferation, and others. The target range of a single miRNA can be very broad as it can bind to several mRNAs through imperfect complementarity. Hence, a relatively small number of miRNA genes can regulate a significant proportion of the cellular genes at any one instance.

MiRNAs are transcribed by RNA Pol II as long ($\sim$1 kb) primary miRNA transcripts (pri-miRNAs), which may encode several mature miRNAs. Pri-miRNAs are processed into $\sim$60 nt pre-miRNA stem-looped intermediates in the nucleus by Drosha, an RNase III enzyme. Pre-miRNAs are then actively transported into the cytoplasm and further processed into $\sim$20–24 nt duplexes by Dicer, an RNase III-like nuclease. In the last step of miRNA maturation, one strand of the dsRNA is loaded into the RNA-induced silencing complex (RISC) together with the Argonaut (Ago) proteins, thus forming the functional repressive complex. Interestingly, the hematopoietic specific conditional knockout of the Ago2 gene in mice resulted in severely defective erythropoiesis (*249*). Thus, in Ago2 -/- mice, erythropoiesis was blocked at around the basophilic stage accompanied by a large accumulation of early erythroblasts in the bone marrow and spleen and a sharp decline in the number of mature red blood cells thus leading to anemia. The erythropoietic block was accompanied by a reduction of mature miRNAs in the erythroblasts of Ago2-/- mice, thus suggesting critical roles for Ago2 and miRNA biogenesis in the differentiation of erythroid cells (*249, 250*).

A number of studies have investigated miRNA expression and function in hematopoiesis (reviewed in refs. *251–254*). For example, Chen et al. (*255*) cloned $\sim$100 miRNAs from mouse bone marrow and showed that three miRNAs expressed exclusively in hematopoietic cells. There have also been several studies examining differential miRNA expression in erythroid cells, which led to the identification of several miRNA species as being up or downregulated during erythroid differentiation. Felli et al. (*256*) identified miR-221 and miR-222 as miRNAs that are downregulated upon erythroid differentiation of human $CD34^+$ progenitors. Significantly, forced expression of miR-221 and miR-222 in $CD34^+$ progenitors blocked erythroid differentiation, most probably through the repression of the c-kit mRNA which may be a target of the two miRNAs (*256*). Similarly, a recent study by Wang et al. (*257*) also identified miR-24 as a repressor of the ALK4 activin type I receptor, which also antagonizes erythroid differentiation when overepxressed. In a study by Kosaka et al. (*258*), miR-210 was found to be upregulated in erythropoietin-stimulated erythroid differentiation. Knocking down miR-210 in these cells led to apoptosis (*258*), thus indicating a regulatory role for miR-210, though it is not yet clear what its mRNA targets are. The significance of miRNA function in leukemia has also been recently highlighted in the case of miR-29b, which was found to induce global hypomethylation and tumor suppressor gene reactivation in acute myeloid leukemia by targeting directly DNA methyltransferases DNT1, DNT3A, and 3B (*259*). Furthermore, in a number

of studies, miR-451 was found to be erythroid restricted and highly upregulated during erythroid maturation (*260–264*), consistent with the recent observation that the miR-451 gene locus is under the transcriptional control of GATA-1 (*176*). Significantly, knocking down miR-451 in MEL cells interfered with hemoglobinization (*262*), whereas miR-451 knockdowns in zebrafish blocked erythroid maturation (*176*). Significantly, a recent study in zebrafish identified the GATA-2 mRNA as a miR-451 target, thus implicating miR-451 in the GATA-2 downregulation that is required for terminal erythroid maturation to proceed (*265*). Furthermore, bioinformatic and microarray expression analyses in mouse predicted several transcriptional regulators as being amongst its targets (*176*). Taken together, these observations demonstrate an important and evolutionarily conserved role for miR-451 in erythroid differentiation.

Regulatory roles for miRNAs in steps that precede commitment to the erythroid lineage have also been described. Lu et al. (*264*) showed that expression of miR-150 plays a pivotal role in the commitment of megakaryocyte-erythrocyte progenitors (MEPs) toward the megakaryocytic lineage. Thus, high miR-150 expression levels are associated with a megakaryocytic fate, whereas low expression levels are associated with an erythroid fate. Significantly, miR-150 appears to exert its effect at the cellular commitment level, tipping the balance toward a megakaryocytic fate by targeting the Myb mRNA for repression (*264*). Myb is a key hematopoietic transcription factor, the functions of which also include promoting commitment of progenitors to the erythroid lineage (*e.g.* ref. *266*). Recently, another study by Zhao et al. (*267*) also reported the repression of Myb mRNA by miR-15a in a manner which is again antagonistic to erythroid commitment and differentiation, because overexpression of miR-15a in $CD34^+$ progenitors led to a decline in Myb expression and a block in erythroid colony formation *in vitro* (*267*). These observations led to a model whereby commitment to the erythroid lineage can be regulated in an inverse manner by expression of miRs -150 and -15a which, in turn, downregulate Myb mRNA expression. It would thus be of great interest to identify the way(s) by which Myb mRNA escapes miRNA suppression in committing progenitors to the erythroid lineage. The fact that Myb binds to and represses the promoter of the miR-15a gene suggests a negative regulatory loop for the fine tuning of Myb expression in hematopoiesis (*267*). Alternatively, or in addition, there may be a hierarchy in the order by which different miRNAs (*e.g.* miR-150 or miR-15a) bind to the 3′-UTR of the Myb mRNA that may influence the fine tuning of Myb's expression levels.

THE DEVELOPMENTAL PROGRAM OF ERYTHROPOIESIS: COMMITMENT AND HEMOGLOBINIZATION

The observation that committed MEL cells undergo irreversible loss of proliferation while they continue to synthesize erythroid markers has prompted several studies aimed to demonstrate whether commitment to erythroid maturation can occur without expression of erythroid markers and vice versa. The use of powerful inhibitors of hemoglobin synthesis indicated that commitment during hematopoietic erythroid cell differentiation occurs separately and independently from hemoglobin biosynthesis (*268*). These findings suggested that the developmental erythroid program of MEL consists of at least two major subprograms. One is responsible for commitment to loss of proliferation upon maturation and the other for biosynthesis of hemoglobin and other hemoproteins (see Fig. 3) (*17, 18, 268*).

Commitment to Maturation and Expression of "Memory"

The analysis of the differentiation program of MEL to terminal maturation has shown that commitment represents a central event in erythropoiesis leading to irreversible growth arrest and discrete patterns of gene expression (*17, 18*). Experimental evidence accumulated thus far by using permanent erythroleukemia cell lines indicates that the products of some oncogenes, including *c-myc, c-myb, c-jun*, and PU.1 transcription factor, are involved in initiating or blocking erythroid differentiation (*269–276*). As a matter of fact, the complex expression pattern of different oncogenes upon induction of MEL cell differentiation (see Fig. 3) with chemical inducers implies their crucial role in the regulatory machinery that triggers cells to erythroid maturation with parallel cessation of proliferation (*18*). To this direction, it has been shown that the observed transcriptional repression of c-myc seen upon over-expression of PU.1 in MEL cells may be mediated through complex formation of PU.1 with histone deacetylases (HDACs) (*277*). Interestingly, a negative cross-talk between *c-myb* and the CREB-binding protein (CBP), that is a coactivator of hematopoietic *Ets* transcription factor Spi-B, has been established giving new insights into hematopoietic cell differentiation program (*278*). Equally important, involvement of *c-myb* in maintaining MEL cells in an immature and proliferating state has been recorded (*276*).

C-Myc is a transcriptional regulator that controls the expression of distinct sets of target genes, thus affecting cellular differentiation and apoptosis in hematopoiesis (*279*). It is not clear, however, whether the functions of *c-myc* in erythropoiesis and apoptosis are mediated by the same or different set of genes. Sensitization of cells to apoptosis induced by *c-myc* is mediated through the release of cytochrome c from mitochondria to cytosol via the involvement of CD95/Fas, p53, and caspases (*280*). This observation is of great interest and in agreement with earlier studies indicating loss of mitochondrial transmembrane potential very early in MEL cells induced to differentiate by chemical inducers (*281, 282*). The working hypothesis that differentiation is accompanied by apoptosis in a small proportion of cells undergoing erythropoiesis (*17*) and a decrease in mitochondrial transmembrane potential independently of the function of Bcl-2 and caspases is further supported by studies in K562 treated with hemin (*283*).

Induction of commitment to MEL cell differentiation has been reported to be associated with prolongation of G_1 phase of

the cell cycle (*284, 285*) and that terminal differentiation is accompanied with arrest of the cell cycle in the G_1/G_0 phase (*17, 18*). Moreover, evidence now exists to indicate that cyclin-dependent kinases (serine/threonine protein kinases) (CDK2, CDK4, and CDK6) play crucial role in cell cycle regulation of eukaryotic cells and that CDKs are involved in the initiation of commitment of MEL cells into terminal maturation (*286–290*). The expression level of CDK2 and CDK4 remained almost constant even at late stages of differentiation, whereas that of CDK6 declined very early before commitment of cells to terminal maturation occurs. These observations are quite interesting in light of evidence that CDK6 plays a key role in the blockade of differentiation (*289*) and that the onset of cell cycle exit in differentiated MEL cells is regulated by CDK2, CDK4, and CDKIs (*286*). Interestingly enough, as it has been recently shown, constitutive expression of ribosomal protein (rp) S5 in MEL cells affected cell cycle arrest at G_1/G_0 and delayed initiation of erythroid differentiation of MEL cells. Such effects were accompanied by changes in the level of both rpS5 protein and *rpS5* mRNA as well as alterations in CDK2, CDK4, and CDK6 (*291*). Deregulation of cancer cell growth with significant G_1 and G_2/M arrest of the cell cycle upon RNAi-mediated gene silencing of *prL13* has been also reported (*292*). Furthermore, alteration in the intracellular level of PU.1 transcription factor via RNAi-mediated silencing has driven MEL cells into erythroid maturation and growth arrest (*293*).

Initiation of commitment is consistent with the ability of inducer-primed MEL cells to "remember" the "original trigger" and enter commitment to erythroid differentiation. In other words, the inducer-treated cells acquire a new property to "remember" the first stimulus and to continue to differentiate after a discontinuous exposure to inducer. This phenomenon so called "memory to the previous exposure" suggested for the first time that the inducing agent used triggers a response before an individual cell commits irreversibly to terminal maturation. The fact that "memory" can last more than one or two cell generations indicates that this specific ability of cells to "remember" the previous exposure to inducer is transmitted into their offspring, and therefore, it is unaffected by cellular replication and DNA synthesis (*294, 295*). Although the molecular basis of "memory" is not known, experiments with metabolic inhibitors indicated that "memory" is erased by treatment with several structurally unrelated agents including inhibitors of new protein and RNA synthesis (*296*), inhibitors of posttranscriptional methylation of RNA (*297*) and even glycocorticoids (*298*) (Fig. 3). Even today, the question whether "memory" is a general response of cells to different growth factors or differentiators and how this occurs remain unanswered. It is known however, that both "memory" and commitment are general properties of differentiated MEL cells, because both are initiated by one inducer and can be terminated by another. "Memory" and commitment have also been observed in RD/TE-671 cells, a human neoplastic cell line of neuroectodermal origin that respond to same inducing agents as MEL cells (*299*).

Number of biochemical events that are needed to contribute for MEL cells to commit is still unknown. The order by which these events occur before commitment it is also unknown. Earlier studies with inhibitors of commitment that act at different levels, for example, modulating Ca^{++} transport, protein synthesis, DNA methylation, and RNA polyadenylation, have made clear that the events which contribute to initiation of commitment it is likely to occur at parallel rather than in sequential fashion, because inhibition of one event does not necessarily abrogate the others as expected if the events had occurred in sequential fashion (*17, 18*).

Regulation of Globin Gene Expression and Production of Hemoglobin

Elucidating how do the α-like globin and the β-like globin genes are normally regulated and contribute via inherited mutations in thalassemias has played a major role in our current understanding of hemoglobin switching during erythropoiesis (see for review refs. *8* and *9*). The local cis-acting sequences controlling globin gene expression (*e.g.* promoters, splicing donor and acceptors, polyA- addition sites) were first identified and characterized throughout the 1980s. In the late 1980s and 1990s, it emerged that both the α- and β-globin gene clusters appeared to be regulated by elements located at a considerable distance (~20–60 kb) from the genes themselves (Fig. 5) (see for review refs. *300* and *301*).

Changes and interactions in α- and β-globin clusters are also subjected to epigenetic regulation, (a process by which phenotype is modified without alterations in genotype), where DNA methylation and histone modifications in chromatin play crucial role in the developmentally-regulated activation and switching of globin gene transcription (reviewed in ref. *302*). Nuclear condensation and irreversible cessation of DNA replication are among the most prominent events occurring in terminally committed and differentiated MEL cells (*303*). Previous studies examining the structure of chromatin by digestion with nucleases have shown that induction of MEL erythroid differentiation correlates with an increase in DNAse I hypersensitivity at sequences located near the α- and β-globin genes (*304, 305*). However, these changes seen in the superfine structure of chromatin have been found not to be directly related with alterations in DNA methylation of these genes at CpG islands. Although the impact of these changes in chromatin structure of the globin genes is still elusive, it is reasonable to postulate that such alterations could reveal cis-acting elements that influence the interactions of cell lineage restricted transcription factors to globin gene promoters (*306–308*). The recent finding that the methyl-CpG binding protein MeCP2, interacts with the histone deacetylase-involved complex, provides evidence on the connection between DNA methylation, chromatin structure remodeling, and gene transcription silencing (*309, 310*). Although the analysis of such epigenetic regulation of globin gene clusters is beyond the scope of this review, it is of importance that differences in the molecular mechanisms of epigenetic regulation of the α- and β-like globin clusters have been recently

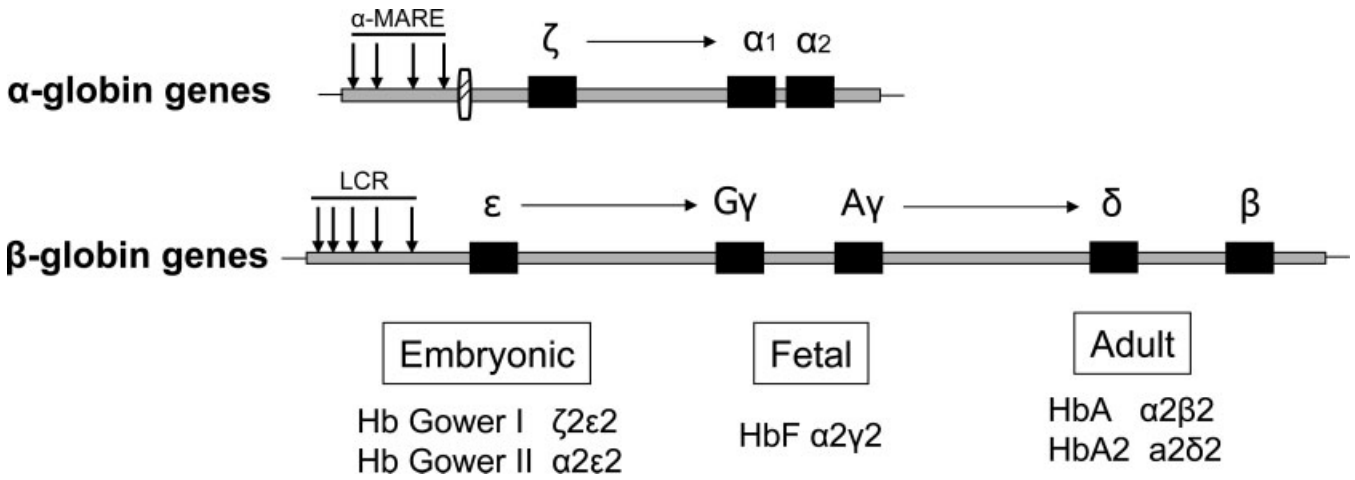


Figure 5. Structure and organization of human α- and β-globin gene family loci. The black boxes depict the corresponding globin genes, whereas the horizontal arrows above represent a switching event leading to expression of the next downstream gene in the locus. The vertical arrows denote the position of upstream DNase I hypersensitive sites identified as the locus control region (LCR) in β-globin and as the α-major regulatory element (MARE) in α-globin gene family. The composition of embryonic, fetal, and adult hemoglobins produced at each developmental stage during erythropoiesis is also indicated.

reported (*311*). This line of investigation implies that the delineation of complexity underlying the molecular mechanisms of epigenetic regulation of globin gene clusters could also contribute to better understanding of erythropoiesis-related transcriptional modulation of gene expression.

Beta-Globin Cluster

Erythroid differentiation is accompanied by a dramatic increase in transcription rates of the β-globin cluster. Regulatory elements of β-like globin genes can be detected as DNase I hypersensitive sites (HSs) in the chromatin of erythroid cells and include cis-elements, (promoters, enhancers, and silencers), which flank the genes and are sufficient and necessary for developmentally-regulated and tissue-specific expression profile of the individual genes (Fig. 5). The globin gene promoters contain, in addition to a TATA box, different recognition sites for erythroid-specific transcription factors like GATA-1, nuclear factor-erythroid 2 (NF-E2), and erythroid Krüppel-like factor (EKLF), which provide part of the tissue-specificity to the promoter (reviewed in ref. *301*). In erythroid progenitor cells, the β-globin genes are expressed at levels that are comparable to those seen for most housekeeping genes. Later, during differentiation, β-globin transcription efficiency is increased up to a 100-fold, reaching expression levels hardly ever observed with other genes. The β-globin genes need the LCR activity to reach these exceptionally high transcription rates (*312*). Recent data support the notion that spatial organization of the β-globin locus dynamically changes in relation to gene expression in a way that it adopts a different position in the nucleus during development and erythroid maturation (*312, 313*). The structural transition occurs during erythroid differentiation and establishes contacts between the LCR and the active genes. This coincides with an increase in β-globin gene expression levels, going from basal levels, which are LCR-independent, to extremely high levels that are LCR-dependent (*314*). It is therefore interesting to further impinge on the functional significance of long-range contacts between regulatory DNA elements and the formation of an active chromatin hub (ACH) spatial structure, in which intervening DNA containing inactive genes would be looped out from this configuration (*301*).

Moreover, enhancers, promoters, and other transcription regulatory DNA elements have in common that they bind often partially overlapping sets of transcription factors that can locally disrupt the nucleosome fiber, rendering these sites hypersensitive to nuclease digestion (*312*). In the case of the β-globin locus, well-known erythroid-specific transcription factors that bind to the β-globin LCR and gene promoters and that are required for or have been implicated in β-globin gene expression are GATA-1 (*153*), EKLF (*210, 315*), and NF-E2 (*316*), as mentioned earlier. In a recent study designed to address the chromatin structure of human β-globin LCR-HSs in erythroid cells expressing embryonic and fetal globin genes and to what extent nucleosomes and activators occupy LCR-HSs together or exclusively, it has been shown that erythroid–specific GATA-1 resided at HS1, HS2 and HS4, while the NF-E2 heterodimer was limited to HS2 (*317*). These data suggested that each HS in the human β-globin LCR has distinct structural features, that may confer different occupation capability upon the binding of erythroid transcription factors. The latter, has been indeed shown to occur by defining also distinct roles of erythroid activators GATA-1 and NF-E2 upon their direct interaction at the LCR HS2 enhancer to activate chromatin structure and transcription of the embryonic ε-globin gene (*318*). In particular, by using a construct model of HS2 linked to a complete ε-globin

gene (HS2/ε-globin locus) in K-562 cells, it has been indicated that recruitment of NF-E2 activator was independent of GATA-1 binding. Furthermore, spatial clustering of transcription regulatory DNA elements results in a high local concentration of binding sites for cognate transcription factors, which consequently accumulate at the site. Efficiency of transcription is proportional to the concentration of transcription factors involved (*319*), and the DNA contacts formed in the context of the β-globin active ACH may therefore be necessary to drive efficient transcription of β-globin genes (*312*). Overall, the clustering of the cis-regulatory elements and active genes of the β-globin locus in an ACH, which is referred to spatial clustering of transcriptional regulatory elements, would result in increased concentration of transcription factors essential for the high transcription rate of the β-globin genes (reviewed in ref. *301*).

Alpha-Globin Cluster

Detailed analysis of the human and mouse α-globin clusters has revealed a set of conserved, erythroid-specific elements that include the promoters of the ζ- and α-globin genes and four MCS (multispecies conserved sequences) elements (MCS-R1 to 4) lying 30–70 kb upstream of the α-globin genes suggesting that these long-range elements may be involved in the regulation of globin gene expression (Fig. 5). In mouse, an additional, nonconserved region associated with an erythroid-specific DHS (DNase I hypersensitive site) (HS-12) has been identified (*320–323*). The chromosome conformation capture (3C) technology (*324*) has been recently applied to delineate the underlying mechanisms by which the long-range upstream elements regulate the recruitment of the preinitiation complex (PIC) to the α- and β-globin promoters. In these experiments, formaldehyde is used to cross-link protein–DNA and protein–protein interactions in intact nuclei. The cross-linked chromatin is then digested by a restriction enzyme followed by ligation. If an interaction between a remote regulatory sequence and a promoter occurs, new, hybrid fragments containing these two elements are generated (at the ligation step), and polymerase chain reaction (PCR) reactions can be used to detect these newly-combined elements. By this approach, it has been shown that the α- and β-globin genes (in human and mouse) interact with all of the upstream MCS elements in late erythroid cells but not in early hematopoietic cells, pluripotent ES cells, or nonerythroid cells, a fact coinciding with the onset of transcription (*301, 323, 325, 326*). These experimental data allowed several models to be proposed on how such large-distance spatial interactions between the upstream sequences and that of promoters and transcription factors may occur in the nucleus of erythroid precursors upon the developmental transcriptional regulation of globin genes (reviewed in refs. *301* and *323*). On the basis of all available data, it has been recently attempted to reconstruct the order of events that occur upon the developmental regulation of α-globin cluster (*323*). As it has been postulated by these authors, the absence of the key hematopoietic transcription factors in pluripotent ES cells causes no association of any MCS elements with DHSs in contrast to what happens in multipotent hematopoietic cells, in which some early stage hematopoietic factors are expressed. In the later case, MCS-R2 is bound by GATA2, NF-E2, and the pentameric SCL complex. This event subsequently leads to GATA2 replacement by GATA1 at MCS-R2 (possibly facilitated by FOG-1), and as the cells become fully committed (pro-erythroblasts) binding is also seen at MCS-R1 and -R4, that represents a status where the α-globin promoters also become activated as judged by the appearance of DHSs. Following this, during terminal differentiation and maturation, the levels of GATA1, NF-E2, and SCL mRNA increase along with their binding to specific motifs and MCS-R3 is bound and activated (*323*)

CONCLUSIONS AND PERSPECTIVES

The discovery of the cellular and molecular events involved in erythropoiesis is essential for understanding the pathogenesis and design therapies of RBC disorders (anemias, thalassemias) and hematological malignancies (leukemias, myelodysplastic syndromes). Experiments carried out with knockout animals (mice, zebrafish, and xenopus), permanent cell lines, and HSCs (embryonic and adult) provided new insights of the homeostatic regulation of erythropoiesis and led to the identification of genes and proteins involved. Among them, genes encoding transcription factors and signaling intermediate effector proteins were found to regulate the self-renewal and differentiation of proerythroid progenitors. Interestingly, committed erythroid progenitors are programmed into terminal erythroid maturation and hemoglobin production by a coordinated network action of transcription factors (GATA-1, FOG-1, TAL-1/SCL/MO2/Ldb1/E2A, EKLF, BCL11A and Gfi-1b), which form active and/or repressive complexes governing gene expression patterns in differentiated erythroid cells. It is still under investigation how these transcriptional complexes are formed, how their function is affected by histone deacetylation and chromatin remodeling, and how do they bind to consensus DNA sequences located within the promoter regions of various genes. Erythroid differentiation was also found to be regulated by miRNAs, a class of small (∼22 nt) RNAs that act posttranscriptionally to selectively regulate gene expression products. The multilevel regulation of erythropoiesis reveals several druggable molecular targets that can be exploited therapeutically for treatment of either anemias, erythroleukemias, and other hematological malignancies. HIF, SCF/c-kit, EpoR, Wnt and Notch-1 signaling, transcription proteins, and chromatin modification can serve as fruitful platforms to develop agents that will induce erythropoiesis or even reverse hematological malignancies by promoting differentiation and/or apoptosis.

REFERENCES

1. Weissman, I. L. (2000) Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **100**, 157–168.
2. Orkin, S. H. and Zon, L. I. (2008) Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* **132**, 631–464.

3. Palis, J. (2008) Ontogeny of erythropoiesis. *Curr. Opin. Hematol.* **15**, 155–161.
4. Adams, G. B. and Scadden, D. T. (2006) The hematopoietic stem cell in its place. *Nat. Immunol.* **7**, 333–337.
5. Metcalf, D. (2008) Hematopoietic cytokines. *Blood* **111**, 485–491.
6. Koury, M. J., Sawyer, S. T., and Brandt, S. J. (2002) New insights into erythropoiesis. *Curr. Opin. Hematol.* **9**, 93–100.
7. Ingley, E., Tilbrook, P. A., and Klinken, S. P. (2004) New insights into the regulation of erythroid cells. *IUBMB Life* **56**, 177–184.
8. Stamatoyannopoulos, G. (2005) Control of globin gene expression during development and erythroid differentiation. *Exp. Hematol.* **33**, 259–271.
9. Bank, A. (2006) Regulation of human fetal hemoglobin: new players, new complexities. *Blood* **107**, 435–443.
10. McGrath, K. E., Kingsley, P. D., Koniski, A. D., Porter, R. L., Bushnell, T. P., and Palis, J. (2008) Enucleation of primitive erythroid cells generates a transient population of "pyrenocytes" in the mammalian fetus. *Blood* **111**, 2409–2417.
11. Dzierzak, E. and Speck, N. A. (2008) Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nat. Immunol.* **9**, 129–136.
12. Bessis, M. (1958) L'ilot eryhtroblastique: Unite functionelle de la moelle osseuse. [Erythroblastic island, functional unity of bone marrow]. *Rev. Hematol.* **13**, 8–11.
13. Allen, T. D. and Dexter, T. M. (1982) Ultrastructural aspects of erythropoietic differentiation in long-term bone marrow culture. *Differentiation* **21**, 86–94.
14. Chasis, J. A. and Mohandas, N. (2008) Erythroblastic islands: niches for erythropoiesis. *Blood* **112**, 470–478.
15. Eshghi, S., Vogelezang, M. G., Hynes, R. O., Griffith, L. G., and Lodish, H. F. (2007) Alpha4beta1 integrin and erythropoietin mediate temporally distinct steps in erythropoiesis: integrins in red cell development. *J. Cell Biol.* **177**, 871–880. [Erratum in: *J. Cell Biol.* (2008) **181**, 395].
16. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971) Hemoglobin synthesis in murine virus-induced leukemic cells in vitro: stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc. Natl. Acad. Sci. USA* **68**, 378–382.
17. Marks, P. A. and Rifkind, R. A. (1978) Erythroleukemic differentiation. *Annu. Rev. Biochem.* **47**, 419–448.
18. Tsiftsoglou, A. S., Pappas, I. S., and Vizirianakis, I. S. (2003) Mechanisms involved in the induced differentiation of leukemia cells. *Pharmacol. Ther.* **100**, 257–290.
19. Tsiftsoglou, A. S., Barrnett, R. J., and Sartorelli, A. C. (1979) Enucleation of differentiated murine erythroleukemia cells in culture. *Proc. Natl. Acad. Sci. USA* **76**, 6381–6385.
20. Tsiftsoglou, A. S., Tsamadou, A. I., and Papadopoulou, L. C. (2006) Heme as key regulator of major mammalian cellular functions: molecular, cellular, and pharmacological aspects. *Pharmacol. Ther.* **111**, 327–345.
21. Weiss, M. J., Yu, C., and Orkin, S. H. (1997) Erythroid-cell-specific properties of transcription factor GATA-1 revealed by phenotypic rescue of a gene targeted cell line. *Mol. Cell. Biol.* **17**, 1642–1651.
22. Gregory, T., Yu, C., Ma, A., Orkin, S. H., Blobel, G. A., and Weiss, M. J. (1999) GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression. *Blood* **94**, 87–96.
23. Kapur, R. and Zhang, L. (2001) A novel mechanism of cooperation between c-Kit and erythropoietin receptor: stem cell factor induces the expression of Stat5 and erythropoietin receptor, resulting in efficient proliferation and survival by erythropoietin. *J. Biol. Chem.* **276**, 1099–1106.
24. Rylski, M., Welch, J. J., Chen, Y. Y., Letting, D. L., Diehl, J. A., Chodosh, L. A., Blobel, G. A., and Weiss, M. J. (2003) GATA-1-mediated proliferation arrest during erythroid maturation. *Mol. Cell. Biol.* **23**, 5031–5042.
25. Manugalavadla, V. and Kapur, R. (2005) Role of c-Kit and erythropoietin receptor in erythropoiesis. *Crit. Rev. Oncol. Hematol.* **54**, 63–75.
26. Welch, J. J., Watts, J. A., Vakoc, C. R., Yao, Y., Wang, H., Hardison, R. C., Blobel, G. A., Chodosh, L. A., and Weiss, M. J. (2004) Global regulation of erythroid gene expression by transcription factor GATA-1. *Blood* **104**, 3136–3147.
27. Olsen, A. L., Stachura, D. L., and Weiss, M. J. (2006) Designer blood: creating hematopoietic lineages from embryonic stem cells. *Blood* **107**, 1265–1275.
28. Wiles, M. V. and Keller, G. (1991) Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development* **111**, 259–267.
29. Keller, G., Kennedy, M., Papayannopoulou, T., and Wiles, M. V. (1993) Hematopoietic commitment during embryonic stem cell differentiation in culture. *Mol. Cell. Biol.* **13**, 473–486.
30. Carotta, S., Pilat, S., Mairhofer, A., Schmidt, U., Dolznig, H., Steinlein, P., and Beug, H. (2004) Directed differentiation and mass cultivation of pure erythroid progenitors from mouse embryonic stem cells. *Blood* **104**, 1873–1880.
31. Nakano, T. (1996) In vitro development of hematopoietic system from mouse embryonic stem cells: a new approach for embryonic hematopoiesis. *Int. J. Hematol.* **65**, 1–8.
32. Kitajima, K., Tanaka, M., Zheng, J., Sakai-Ogawa, E., and Nakano, T. (2003) In vitro differentiation of mouse embryonic stem cells to hematopoietic cells on an OP9 stromal cell monolayer. *Methods. Enzymol.* **365**, 72–83.
33. Weiss, M. J., Keller, G., and Orkin, S. H. (1994) Novel insights into erythroid development revealed through in vitro differentiation of GATA-1 embryonic stem cells. *Genes Dev.* **8**, 1184–1197.
34. Kennedy, M., Firpo, M., Choi, K., Wall, C., Robertson, S., Kabrun, N., and Keller, G. (1997) A common precursor for primitive erythropoiesis and definitive haematopoiesis. *Nature* **386**, 488–493.
35. Choi, K., Kennedy, M., Kazarov, A., Papadimitriou, J. C., and Keller, G. (1998) A common precursor for hematopoietic and endothelial cells. *Development* **125**, 725–732.
36. Thompson, M. A., Ransom, D. G., Pratt, S. J., MacLennan, H., Kieran, M. W., Detrich, H. W. III., Vail, B., Huber, T. L., Paw, B., Brownlie, A. J., Oates, A. C., Fritz, A., Gates, M. A., Amores, A., Bahary, N., Talbot, W. S., Her, H., Beier, D. R., Postlethwait, J. H., and Zon, L. I. (1998) The cloche and spadetail genes differentially affect hematopoiesis and vasculogenesis. *Dev. Biol.* **197**, 248–269.
37. Yu, J. and Thomson, J. A. (2008) Pluripotent stem cell lines. *Genes Dev.* **22**, 1987–1997.
38. Wang, L., Menendez, P., Cerdan, C., and Bhatia, M. (2005) Hematopoietic development from human embryonic stem cell lines. *Exp. Hematol.* **33**, 987–996.
39. Chang, K. H., Nelson, A. M., Cao, H., Wang, L., Nakamoto, B., Ware, C. B., and Papayannopoulou, T. (2006) Definitive-like erythroid cells derived from human embryonic stem cells coexpress high levels of embryonic and fetal globins with little or no adult globin. *Blood* **108**, 1515–1523.
40. Ma, F., Ebihara, Y., Umeda, K., Sakai, H., Hanada, S., Zhang, H., Zaike, Y., Tsuchida, E., Nakahata, T., Nakauchi, H., and Tsuji, K. (2008) Generation of functional erythrocytes from human embryonic stem cell-derived definitive hematopoiesis. *Proc. Natl. Acad. Sci. USA* **105**, 13087–13092.
41. Chang, K. H., Nelson, A. M., Fields, P. A., Hesson, J. L., Ulyanova, T., Cao, H., Nakamoto, B., Ware, C. B., and Papayannopoulou, T. (2008) Diverse hematopoietic potentials of five human embryonic stem cell lines. *Exp. Cell Res.* **314**, 2930–2940.
42. Zambidis, E. T., Peault, B., Park, T. S., Bunz, F., and Civin, C. I. (2005) Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and defini-

tive stages resembling human yolk sac development. *Blood* **106**, 860–870.
43. Olivier, E. N., Qiu, C., Velho, M., Hirsch, R. E., and Bouhassira, E. E. (2006) Large-scale production of embryonic red blood cells from human embryonic stem cells. *Exp. Hematol.* **34**, 1635–1642.
44. Lu, J., Guo, S., Ebert, B. L., Zhang, H., Peng, X., Bosco, J., Pretz, J., Schlanger, R., Wang, J. Y., Mak, R. H., Dombkowski, D. M., Preffer, F. I., Scadden, D. T., and Golub, T. R. (2008) MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. *Dev. Cell.* **14**, 843–853.
45. Baek, E. J., Kim, H. S., Kim, S., Jin, H., Choi, T. Y., and Kim, H. O. (2008) In vitro clinical-grade generation of red blood cells from human umbilical cord blood $CD34^+$ cells. *Transfusion* **48**, 2235–2245.
46. Baron, M. H. and Fraser, S. T. (2005) The specification of early hematopoiesis in the mammal. *Curr. Opin. Hematol.* **12**, 217–221.
47. McGrath, K. and Palis, J. (2008) Ontogeny of erythropoiesis in the mammalian embryo. *Curr. Top. Dev. Biol.* **82**, 1–22.
48. Fraser, S. T., Isern, J., and Baron, M. H. (2007) Maturation and enucleation of primitive erythroblasts during mouse embryogenesis is accompanied by changes in cell-surface antigen expression. *Blood* **109**, 343–352.
49. Kingsley, P. D., Malik, J., Emerson, R. L., Bushnell, T. P., McGrath, K. E., Bloedorn, L. A., Bulger, M., and Palis, J. (2006) "Maturational" globin switching in primary primitive erythroid cells. *Blood* **107**, 1665–1672.
50. Kingsley, P. D., Malik, J., Fantauzzo, K. A., and Palis, J. (2004) Yolk sac-derived primitive erythroblasts enucleate during mammalian embryogenesis. *Blood* **104**, 19–25.
51. Whitelaw, E., Tsai, S. F., Hogben, P., and Orkin, S. H. (1990) Regulated expression of globin chains and the erythroid transcription factor GATA-1 during erythropoiesis in the developing mouse. *Mol. Cell. Biol.* **10**, 6596–6606.
52. Lux, C. T., Yoshimoto, M., McGrath, K., Conway, S. J., Palis, J., and Yoder, M. C. (2008) All primitive and definitive hematopoietic progenitor cells emerging before E10 in the mouse embryo are products of the yolk sac. *Blood* **111**, 3435–3438.
53. Isern, J., Fraser, S. T., He, Z., and Baron, M. H. (2008) The fetal liver is a niche for maturation of primitive erythroid cells. *Proc. Natl. Acad. Sci. USA* **105**, 6662–6667.
54. Carradice, D. and Lieschke, G. J. (2008) Zebrafish in hematology: sushi or science? *Blood* **111**, 3331–3342.
55. Burns, C. E., Traver, D., Mayhall, E., Shepard, J. L., and Zon, L. I. (2005) Hematopoietic stem cell fate is established by the Notch-Runx pathway. *Genes Dev.* **19**, 2331–2342.
56. Gering, M. and Patient, R. (2005) Hedgehog signaling is required for adult blood stem cell formation in zebrafish embryos. *Dev. Cell.* **8**, 389–400.
57. Stainier, D. Y., Weinstein, B. M., Detrich, H. W., Zon, L. I. III., Fishman, M. C. (1995) Cloche, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. *Development* **121**, 3141–3150.
58. Huber, T. L. and Zon, L. I. (1998) Transcriptional regulation of blood formation during Xenopus development. *Semin. Immunol.* **10**, 103–109.
59. Walmsley, M., Ciau-Uitz, A., and Patient, R. (2005) Tracking and programming early hematopoietic cells in Xenopus embryos. *Methods Mol. Med.* **105**, 123–136.
60. Zon, L. I. (1995) Developmental biology of hematopoiesis. *Blood* **86**, 2876–2891.
61. Sadlon, T. J., Lewis, I. D., and D'Andrea, R. J. (2004) BMP4: its role in development of the hematopoietic system and potential as a hematopoietic growth factor. *Stem Cells* **22**, 457–474.
62. Dieterlen-Lievre, F. (1975) On the origin of haemopoietic stem cells in the avian embryo: an experimental approach. *J. Embryol. Exp. Morphol.* **33**, 607–619.
63. Caprioli, A., Jaffredo, T., Gautier, R., Dubourg, C., and Dieterlen-Lievre, F. (1998) Blood-borne seeding by hematopoietic and endothelial precursors from the allantois. *Proc. Natl. Acad. Sci. USA* **95**, 1641–1646.
64. Schroeder, C., Gibson, L., Nordstrom, C., and Beug, H. (1993) The estrogen receptor cooperates with the TGF alpha receptor (c-erbB) in regulation of chicken erythroid progenitor self-renewal. *EMBO J.* **12**, 951–960.
65. Steinlein, P., Wessely, O., Meyer, S., Deiner, E. M., Hayman, M. J., and Beug, H. (1995) Primary, self-renewing erythroid progenitors develop through activation of both tyrosine kinase and steroid hormone receptors. *Curr. Biol.* **5**, 191–204.
66. Wessely, O., Deiner, E. M., Beug, H., and von Lindern, M. (1997) The glucocorticoid receptor is a key regulator of the decision between self-renewal and differentiation in erythroid progenitors. *EMBO J.* **16**, 267–280.
67. Wessely, O., Bauer, A., Quang, C. T., Deiner, E. M., von Lindern, M., Mellitzer, G., Steinlein, P., Ghysdael, J., and Beug, H. (1999) A novel way to induce erythroid progenitor self renewal: cooperation of c-Kit with the erythropoietin receptor. *Biol. Chem.* **380**, 187–202.
68. Hayman, M. J., Meyer, S., Martin, F., Steinlein, P., and Beug, H. (1993) Self-renewal and differentiation of normal avian erythroid progenitor cells: regulatory roles of the TGF alpha/c-ErbB and SCF/c-kit receptors. *Cell* **74**, 157–169.
69. Beug, H., Bauer, A., Dolznig, H., von Lindern, M., Lobmayer, L., Mellitzer, G., Steinlein, P., Wessely, O., and Mullner, E. (1996) Avian erythropoiesis and erythroleukemia: towards understanding the role of the biomolecules involved. *Biochim. Biophys. Acta* **1288**, M35–M47.
70. McNagny, K. M. and Graf, T. (1996) Acute avian leukemia viruses as tools to study hematopoietic cell differentiation. *Curr. Top. Microbiol. Immunol.* **212**, 143–162.
71. Nielsen, J. S., Doyonnas, R., and McNagny, K. M. (2002) Avian models to study the transcriptional control of hematopoietic lineage commitment and to identify lineage-specific genes. *Cells Tissues Organs* **171**, 44–63.
72. Kim, S. I. and Bresnick, E. H. (2007) Transcriptional control of erythropoiesis: emerging mechanisms and principles. *Oncogene* **26**, 6777–6794.
73. Smith, T. G., Robbins, P. A., and Ratcliffe, P. J. (2008) The human side of hypoxia-inducible factor. *Br. J. Hematol.* **141**, 325–334.
74. Jelkmann, M. (2004) Molecular biology of erythropoietin. *Intern. Med.* **43**, 649–659.
75. Rogers, H. M., Yu, X., Wen, J., Smith, R., Fibach, E., and Noguchi, C. T. (2008) Hypoxia alters progression of the erythroid program. *Exp. Hematol.* **36**, 17–27.
76. Freeburg, P. B. and Abrahamson, D. R. (2003) Hypoxia-inducible factors and kidney vascular development. *J. Am. Soc. Nephrol.* **14**, 2723–2730.
77. Semenza, G. L., Nejfelt, M. K., Chi, S. M., and Antonarakis, S. E. (1991) Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc. Natl. Acad. Sci. USA* **88**, 5680–5684.
78. Semenza, G. and Wang, G. (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol. Cell. Biol.* **12**, 5447–5454.
79. Wang, G. L. and Semenza, G. L. (1995) Purification and characterization of HIF-1. *J. Biol. Chem.* **270**, 1230–1237.
80. Hoffman, E. C., Reyes, H., Chuff, E. J., Sander, F., and Conley, L. H. (1991) Cloning of a factor required for activation of the Ah (dioxin) receptor. *Science* **252**, 954–958.
81. Mylonis, I., Chachami, G., Paraskeva, E., and Simos, G. (2008) Atypical CRM1-dependent nuclear export signal mediates regulation of hypoxia-inducible factor-1alpha by MAPK. *J. Biol. Chem.* **283**, 27620–27627.

82. Percy, M. L. (2007) Genetically heterogeneous origins of idiopathic erythrocytosis. *Hematology* **12**, 131–139.
83. Ang, S., Chen, H., Hirota, K., Gordeuk, V., Jelinek, J., Guan, Y., Liu, E., Sergueeva, A., Miasnikova, G., Mole, D., Maxwell, P., Stockton, D., Semenza, G., and Prchal, J. (2002) Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat. Genet.* **32**, 614–621.
84. Percy, M. J., Furlow, P. W., Beer, P. A., Lappin, T. R., McMullin, M. F., and Lee, F. S. (2007) A novel erythrocytosis-associated PHD2 mutation suggests the location of a HIF binding groove. *Blood* **110**, 2193–2196.
85. Scortegagna, M., Ding, K., Zhang, Q., Oktay, Y., Bennett, M. J., Bennett, M., Shelton, J. M., Richardson, J. A., Moe, O., and Garcia, J. A. (2005) HIF-2α regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood* **105**, 3133–3140.
86. Rankin, E. B., Biju, M. P., Liu, Q., Unger, T. L., Rha, J., Johnson, R. S., Simon, M. C., Keith, B., and Haase, V. H. (2007) Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. *J. Clin. Invest.* **117**, 1068–1077.
87. Percy, M. J., Furlow, P. W., Lucas, G. S., Li, X., Lappin, T. R., McMullin, M. F., and Lee, F. S. (2008) A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. *N. Engl. J. Med.* **358**, 162–168.
88. Yamashita, T., Ohneda, O., Sakiyama, A., Iwata, F., Ohneda, K., and Fujii-Kuriyama, Y. (2008) The microenvironment for erythropoiesis is regulated by HIF-2α through VCAM-1 in endothelial cells. *Blood* **112**, 1482–1492.
89. Fandrey, J. (2008) Erythropoiesis-once more HIF. *Blood* **112**, 931–932.
90. Fruehauf, J. P. and Meyskens, F. L. Jr. (2007) Reactive oxygen species: a breath of life or death? *Clin. Cancer Res.* **13**, 789–794.
91. Hattangadi, S. M. and Lodish, H. F. (2007) Regulation of erythrocyte lifespan: do reactive oxygen species set the clock? *J. Clin. Invest.* **117**, 2075–2077.
92. Marinkovic, D., Zhang, X., Yalcin, S., Luciano, J. P., Brugnara, C., Huber, T., and Ghaffari, S. (2007) Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J. Clin. Invest.* **117**, 2133–2144.
93. Bakker, W. J., Blázquez-Domingo, M., Kolbus, A., Besooyen, J., Steinlein, P., Beug, H., Coffer, P. J., Löwenberg, B., von Lindern, M., and van Dijk, T. B. (2004) FoxO3a regulates erythroid differentiation and induces BTG1, an activator of protein arginine methyl transferase 1. *J. Cell Biol.* **164**, 175–184.
94. Bakker, W. J., van Dijk, T. B., Parren-van Amelsvoort, M., Kolbus, A., Yamamoto, K., Steinlein, P., Verhaak, R. G., Mak, T. W., Beug, H., Löwenberg, B., and von Lindern, M. (2007) Differential regulation of Foxo3a target genes in erythropoiesis. *Mol. Cell. Biol.* **27**, 3839–3854.
95. Nemeth, E. (2008) Iron regulation and erythropoiesis. *Curr. Opin. Hematol.* **15**, 169–175.
96. Andrews, N. C. (2008) Forging a field: the golden age of iron biology. *Blood* **112**, 219–230.
97. De Domenico, I., Ward, D. M., and Kaplan, J. (2007) Hepcidin regulation: ironing out the details. *J. Clin. Invest.* **117**, 1755–1758.
98. Nemeth, M. J. and Bodine, D. M. (2007) Regulation of hematopoiesis and the hematopoietic stem cell niche by Wnt signaling pathways. *Cell Res.* **17**, 746–758.
99. Wrighting, D. M. and Andrews, N. C. (2006) Interleukin-6 induces hepcidin expression through STAT3. *Blood* **108**, 3204–3209.
100. Babitt, J. L., Huang, F. W., Wrighting, D. M., Xia, Y., Sidis, Y., Samad, T. A., Campagna, J. A., Chung, R. T., Schneyer, A. L., Woolf, C. J., Andrews, N. C., and Lin, H. Y. (2006) Bone morphogenetic protein signalling by hemojuvelin regulates hepcidin expression. *Nat. Genet.* **38**, 531–539.
101. Babitt, J. L., Huang, F. W., Xia, Y., Sidis, Y., Andrews, N. C., and Lin, H. Y. (2007) Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. *J. Clin. Invest.* **117**, 1933–1939.
102. Lin, L., Valore, E. V., Nemeth, E., Goodnough, J. B., Gabayan, V., and Ganz, T. (2007) Iron transferrin regulates hepcidin synthesis in primary hepatocyte culture through hemojuvelin and BMP2/4. *Blood* **110**, 2182–2189.
103. Peyssonnaux, C., Zinkernagel, A. S., Schuepbach, R. A., Rankin, E., Vaulont, S., Haase, V. H., Nizet, V., and Johnson, R. S. (2007) Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J. Clin. Invest.* **117**, 1926–1932.
104. Muckenthaler, M. U., Galy, B., and Hentze, M. W. (2008) Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. *Annu. Rev. Nutr.* **28**, 197–213.
105. Hentze, M. W. and Kühn L. C. (1996) Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proc. Natl. Acad. Sci. USA* **93**, 8175–8182.
106. Wang, J., Chen, G., Muckenthaler, M., Galy, B., Hentze, M. W., and Pantopoulos, K. (2004) Iron-mediated degradation of IRP2, an unexpected pathway involving a 2-oxoglutarate-dependent oxygenase activity. *Mol. Cell. Biol.* **24**, 954–965.
107. Wang, J. and Pantopoulos, K. (2005) The pathway for IRP2 degradation involving 2-oxoglutarate-dependent oxygenase(s) does not require the E3 ubiquitin ligase activity of pVHL. *Biochem. Biophys. Acta* **1743**, 79–85.
108. Sanchez, M., Galy, B., Muckenthaler, M. U., and Hentze, M. W. (2007) Iron-regulatory proteins limit hypoxia-inducible factor-2a expression in iron deficiency. *Nat. Struct. Mol. Biol.* **14**, 420–426.
109. Kerenyi, M. A., Grebien, F., Gehart, H., Schifrer, M., Artaker, M., Kovacic, B., Beug, H., Moriggl, R., and Mullner, E. W. (2008) Stat 5 regulates cellular iron uptake of erythroid cells via IRP-2 and TfR-1. *Blood* **112**, 3878–3888.
110. Grebien, F., Kerenyi, M. A., Kovacic, B., Kolbe, T., Becker, V., Dolznig, H., Pfeffer, K., Klingmüller, U., Müller, M., Beug, H., Müllner, E. W., and Moriggl, R. (2008) Stat5 activation enables erythropoiesis in the absence of EpoR and Jak2. *Blood* **111**, 4511–4522.
111. Dos Santos, C. O., Dore, L. C., Valentine, E., Shelat, S. G., Hardison, R. C., Ghosh, M., Wang, W., Eisenstein, R. S., Costa, F. F., and Weiss, M. J. (2008) An iron responsive element-like stem-loop regulates α-hemoglobin-stabilizing protein mRNA. *J. Biol. Chem.* **283**, 26956–26964.
112. Elorza, A. A., Hyde, B. B., Mikkola, H. K., Collins, S., and Shirihai, O. S. (2008) UCP2 modulates cell proliferation through the MAPK/ERK pathway during erythropoiesis and has no effect on heme biosynthesis. *J. Biol. Chem.* **283**, 30461–30470.
113. Blank, U., Karlsson, G., and Karlsson, S. (2008) Signaling pathways governing stem-cell fate. *Blood* **111**, 492–503.
114. Campbell, C., Risueno, R. M., Salati, S., Guezguez, B., and Bhatia, M. (2008) Signal control of hematopoietic stem cell fate: Wnt, Notch, and Hedgehog as the usual suspects. *Curr. Opin. Hematol.* **15**, 319–325.
115. Zhang, C. C. and Lodish, H. F. (2008) Cytokines regulating hematopoietic stem cell function. *Curr. Opin. Hematol.* **15**, 307–311.
116. Edling, C. E. and Hallberg, B. (2007) c-Kit: a hematopoietic cell essential receptor tyrosine kinase. *Int. J. Biochem. Cell Biol.* **39**, 1995–1998.
117. Kent, D., Copley, M., Benz, C., Dykstra, B., Bowie, M., and Eaves, C. (2008) Regulation of hematopoietic stem cells by the steel factor/KIT signaling pathway. *Clin. Cancer Res.* **14**, 1926–1930.
118. Scholl, C., Gilliland, D. G., and Fröhling, S. (2008) Deregulation of signaling pathways in acute myeloid leukemia. *Semin. Oncol.* **35**, 336–345.
119. Roskoski, R. Jr. (2005) Signaling by Kit protein-tyrosine kinase--the stem cell factor receptor. *Biochem. Biophys. Res. Commun.* **337**, 1–13.
120. Sattler, M. and Salgia, R. (2004) Targeting c-Kit mutations: basic science to novel therapies. *Leuk. Res.* **28**, S11–S20.

121. Lennartsson, J. and Rönnstrand, L. (2006) The stem cell factor receptor/c-Kit as a drug target in cancer. *Curr. Cancer Drug Targets* **6**, 65–75.
122. Staal, F. J. and Clevers, H. C. (2005) Wnt signaling and haematopoiesis: a Wnt-Wnt situation. *Nat. Rev. Immunol.* **5**, 21–30.
123. Austin, T. W., Solar, G. P., Ziegler, F. C., Liem, L., and Matthews, W. (1997) A role for the Wnt gene family in hematopoiesis: expansion of multi-lineage progenitor cells. *Blood* **89**, 3624–3635.
124. Van Den Berg, D. J., Sharma, A. K., Bruno, E., and HoVman, R. (1998) Role of members of the Wnt gene family in human hematopoiesis. *Blood* **92**, 3189–3202.
125. Reya, T., Duncan, A. W., Ailles, L., Domen, J., Scherer, D. C., Willert, K., Hintz, L., Nusse, R., and Weissman, I. L. (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409–414.
126. Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R., and Nusse, R. (2003) Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448–452.
127. Ohishi, K., Varnum-Finney, B., and Bernstein, I. D. (2002) The Notch pathway: modulation of cell fate decisions in hematopoiesis. *Int. J. Hematol.* **75**, 449–459.
128. Cheng, X., Huber, T. L., Chen, V. C., Gadue, P., and Keller, G. M. (2008) Numb mediates the interaction between Wnt and Notch to modulate primitive erythropoietic specification from the hemangioblast. *Development* **135**, 3447–3458.
129. Shelly, S. L., Fuchs, C., and Miele, L. (1999) Notch-1 inhibits apoptosis in murine erythroleukemia cells and is necessary for differentiation induced by hybrid polar compounds. *J. Cell Biochem.* **73**, 164–175.
130. Jang, M. S., Miao, H., Carlesso, N., Shelly, L., Zlobin, A., Darack, N., Qin, J. Z., Nickoloff, B. J., and Miele, L. (2004) Notch-1 regulates cell death independently of differentiation in murine erythroleukemia cells through multiple apoptosis and cell cycle pathways. *J. Cell Physiol.* **199**, 418–433.
131. Duncan, A. W., Rattis, F. M., DiMascio, L. N., Congdon, K. L., Pazianos, G., Zhao, C., Yoon, K., Cook, J. M., Willert, K., Gaiano, N., and Reya, T. (2005) Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. *Nat. Immunol.* **6**, 314–322.
132. Ganapati, U., Tan, H. T., Lynch, M., Dolezal, M., de Vos, S., and Gasson, J. C. (2007) Modelling Notch signaling in normal and neoplastic hematopoiesis: global gene expression profiling in response to activated notch expression. *Stem Cells* **25**, 1872–1880.
133. Hayward, P., Brennan, K., Sanders, P., Balayo, T., DasGupta, R., Perrimon, N., and Martinez Arias, A. (2005) Notch modulates Wnt signalling by associating with Armadillo/beta-catenin and regulating its transcriptional activity. *Development* **132**, 1819–1830.
134. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ihle, J. N. (1993) JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. *Cell* **74**, 227–236.
135. Miura, O., Nakamura, N., Quelle, F. W., Witthuhn, B. A., Ihle, J. N., and Aoki, N. (1994) Erythropoietin induces association of the JAK2 protein tyrosine kinase with the erythropoietin receptor in vivo. *Blood* **84**, 1501–1507.
136. Elliott, S., Pham, E., and Macdougall, I. C. (2008) Erythropoietins: a common mechanism of action. *Exp. Hematol.* **36**, 1573–1584.
137. Richmond, T. D., Chohan, M., and Barber, D. L. (2005) Turning cells red: signal transduction mediated by erythropoietin. *Trends Cell Biol.* **15**, 146–155.
138. Tong, W., Zhang, J., and Lodish, H. F. (2005) Lnk inhibits erythropoiesis and Epo-dependent JAK2 activation and downstream signaling pathways. *Blood* **105**, 4604–4612.
139. Cantor, A. B. and Orkin, S. H. (2002) Transcriptional regulation of erythropoiesis: an affair involving multiple partners. *Oncogene* **21**, 3368–3376.
140. Perry, C. and Soreq, H. (2002) Transcriptional regulation of erythropoiesis. Fine tuning of combinatorial multi-domain elements. *Eur. J. Biochem.* **269**, 3607–3618.
141. Evans, T. and Felsenfeld, G. (1989) The erythroid-specific transcription factor Eryf1: a new finger protein. *Cell* **58**, 877–885.
142. Tsai, S. F., Martin, D. I., Zon, L. I., D'Andrea, A. D., Wong, G. G., and Orkin, S. H. (1989) Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature* **339**, 446–451.
143. Akashi, K., He, X., Chen, J., Iwasaki, H., Niu, C., Steenhard, B., Zhang, J., Haug, J., and Li, L. (2003) Transcriptional accessibility for genes of multiple tissues and hematopoietic lineages is hierarchically controlled during early hematopoiesis. *Blood* **101**, 383–389.
144. Cross, M. A. and Enver, T. (1997) The lineage commitment of haemopoietic progenitor cells. *Curr. Opin. Genet. Dev.* **7**, 609–613.
145. Molkentin, J. D. (2000) The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. *J. Biol. Chem.* **275**, 38949–38952.
146. Patient, R. K. and McGhee, J. D. (2002) The GATA family (vertebrates and invertebrates). *Curr. Opin. Genet. Dev.* **12**, 416–422.
147. Weiss, M. J. and Orkin, S. H. (1995) GATA transcription factors: key regulators of hematopoiesis. *Exp. Hematol.* **23**, 99–107.
148. Ferreira, R., Ohneda, K., Yamamoto, M., and Philipsen, S. (2005) GATA1 function, a paradigm for transcription factors in hematopoiesis. *Mol. Cell. Biol.* **25**, 1215–1227.
149. Gutierrez, L., Nikolic, T., van Dijk, T. B., Hammad, H., Vos, N., Willart, M., Grosveld, F., Philipsen, S., and Lambrecht, B. N. (2007) Gata1 regulates dendritic-cell development and survival. *Blood* **110**, 1933–1941.
150. Orkin, S. H. (1992) GATA-binding transcription factors in hematopoietic cells. *Blood* **80**, 575–581.
151. Trainor, C. D., Omichinski, J. G., Vandergon, T. L., Gronenborn, A. M., Clore, G. M., and Felsenfeld, G. (1996) A palindromic regulatory site within vertebrate GATA-1 promoters requires both zinc fingers of the GATA-1 DNA-binding domain for high-affinity interaction. *Mol. Cell. Biol.* **16**, 2238–2247.
152. Fujiwara, Y., Browne, C. P., Cunniff, K., Goff, S. C., and Orkin, S. H. (1996) Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. *Proc. Natl. Acad. Sci. USA* **93**, 12355–12358.
153. Pevny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S. F., D'Agati, V., Orkin, S. H., and Costantini, F. (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. *Nature* **349**, 257–260.
154. Pevny, L., Lin, C. S., D'Agati, V., Simon, M. C., Orkin, S. H., and Costantini, F. (1995) Development of hematopoietic cells lacking transcription factor GATA-1. *Development* **121**, 163–172.
155. Pan, X., Ohneda, O., Ohneda, K., Lindeboom, F., Iwata, F., Shimizu, R., Nagano, M., Suwabe, N., Philipsen, S., Lim, K. C., Engel, J. D., and Yamamoto, M. (2005) Graded levels of GATA-1 expression modulate survival, proliferation, and differentiation of erythroid progenitors. *J. Biol. Chem.* **280**, 22385–22394.
156. Heyworth, C., Pearson, S., May, G., and Enver, T. (2002) Transcription factor-mediated lineage switching reveals plasticity in primary committed progenitor cells. *EMBO J.* **21**, 3770–3781.
157. Iwasaki, H., Mizuno, S., Wells, R. A., Cantor, A. B., Watanabe, S., and Akashi, K. (2003) GATA-1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte lineages. *Immunity* **19**, 451–462.
158. Graf, T. (2002) Differentiation plasticity of hematopoietic cells. *Blood* **99**, 3089–3101.
159. Ney, P. A. (2006) Gene expression during terminal erythroid differentiation. *Curr. Opin. Hematol.* **13**, 203–208.
160. Bresnick, E. H., Martowicz, M. L., Pal, S., and Johnson, K. D. (2005) Developmental control via GATA factor interplay at chromatin domains. *J. Cell Physiol.* **205**, 1–9.

161. Rodriguez, P., Bonte, E., Krijgsveld, J., Kolodziej, K. E., Guyot, B., Heck, A. J., Vyas, P., de Boer, E., Grosveld, F., and Strouboulis, J. (2005) GATA-1 forms distinct activating and repressive complexes in erythroid cells. *EMBO J.* **24**, 2354–2366.
162. Lowry, J. A. and Mackay, J. P. (2006) GATA-1: one protein, many partners. *Int. J. Biochem. Cell Biol.* **38**, 6–11.
163. Crossley, M. and Orkin, S. H. (1994) Phosphorylation of the erythroid transcription factor GATA-1. *J. Biol. Chem.* **269**, 16589–16596.
164. Rooke, H. M. and Orkin, S. H. (2006) Phosphorylation of Gata1 at serine residues 72, 142, and 310 is not essential for hematopoiesis in vivo. *Blood* **107**, 3527–3530.
165. Boyes, J., Byfield, P., Nakatani, Y., and Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* **396**, 594–598.
166. Hung, H. L., Lau, J., Kim, A. Y., Weiss, M. J., and Blobel, G. A. (1999) CREB-Binding protein acetylates hematopoietic transcription factor GATA-1 at functionally important sites. *Mol. Cell. Biol.* **19**, 3496–3505.
167. Lamonica, J. M., Vakoc, C. R., and Blobel, G. A. (2006) Acetylation of GATA-1 is required for chromatin occupancy. *Blood* **108**, 3736–3738.
168. Hernandez-Hernandez, A., Ray, P., Litos, G., Ciro, M., Ottolenghi, S., Beug, H., and Boyes, J. (2006) Acetylation and MAPK phosphorylation cooperate to regulate the degradation of active GATA-1. *EMBO J.* **25**, 3264–3274.
169. Collavin, L., Gostissa, M., Avolio, F., Secco, P., Ronchi, A., Santoro, C., Del Sal, G. (2004) Modification of the erythroid transcription factor GATA-1 by SUMO-1. *Proc. Natl. Acad. Sci. USA* **101**, 8870–8875.
170. De Maria, R., Zeuner, A., Eramo, A., Domenichelli, C., Bonci, D., Grignani, F., Srinivasula, S. M., Alnemri, E. S., Testa, U., and Peschle, C. (1999) Negative regulation of erythropoiesis by caspase-mediated cleavage of GATA-1. *Nature* **401**, 489–493.
171. Whyatt, D., Lindeboom, F., Karis, A., Ferreira, R., Milot, E., Hendriks, R., de Bruijn, M., Langeveld, A., Gribnau, J., Grosveld, F., and Philipsen, S. (2000) An intrinsic but cell-nonautonomous defect in GATA-1-overexpressing mouse erythroid cells. *Nature* **406**, 519–524.
172. Gutierrez, L., Lindeboom, F., Langeveld, A., Grosveld, F., Philipsen, S., and Whyatt, D. (2004) Homotypic signalling regulates Gata1 activity in the erythroblastic island. *Development* **131**, 3183–3193.
173. Munugalavadla, V., Dore, L. C., Tan, B. L., Hong, L., Vishnu, M., Weiss, M. J., and Kapur, R. (2005) Repression of c-kit and its downstream substrates by GATA-1 inhibits cell proliferation during erythroid maturation. *Mol. Cell. Biol.* **25**, 6747–6759.
174. Choe, K. S., Radparvar, F., Matushansky, I., Rekhtman, N., Han, X., and Skoultchi, A. I. (2003) Reversal of tumorigenicity and the block to differentiation in erythroleukemia cells by GATA-1. *Cancer Res.* **63**, 6363–6369.
175. Hino, M., Nishizawa, Y., Tatsumi, N., Tojo, A., and Morii, H. (1995) Down-modulation of c-kit mRNA and protein expression by erythroid differentiation factor/activin A. *FEBS Lett.* **374**, 69–71.
176. Dore, L. C., Amigo, J. D., Dos Santos, C. O., Zhang, Z., Gai, X., Tobias, J. W., Yu, D., Klein, A. M., Dorman, C., Wu, W., Hardison, R. C., Paw, B. H., and Weiss, M. J. (2008) A GATA-1-regulated microRNA locus essential for erythropoiesis. *Proc. Natl. Acad. Sci. USA* **105**, 3333–3338.
177. Tsang, A. P., Visvader, J. E., Turner, C. A., Fujiwara, Y., Yu, C., Weiss, M. J., Crossley, M., and Orkin, S. H. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell* **90**, 109–119.
178. Fox, A. H., Liew, C., Holmes, M., Kowalski, K., Mackay, J., and Crossley, M. (1999) Transcriptional cofactors of the FOG family interact with GATA proteins by means of multiple zinc fingers. *EMBO J.* **18**, 2812–2822.
179. Tsang, A. P., Fujiwara, Y., Hom, D. B., and Orkin, S. H. (1998) Failure of megakaryopoiesis and arrested erythropoiesis in mice lacking the GATA-1 transcriptional cofactor FOG. *Genes Dev.* **12**, 1176–1188.
180. Crispino, J. D., Lodish, M. B., MacKay, J. P., and Orkin, S. H. (1999) Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG complex. *Mol. Cell* **3**, 219–228.
181. Nichols, K. E., Crispino, J. D., Poncz, M., White, J. G., Orkin, S. H., Maris, J. M., and Weiss, M. J. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. *Nat. Genet.* **24**, 266–270.
182. Hong, W., Nakazawa, M., Chen, Y. Y., Kori, R., Vakoc, C. R., Rakowski, C., and Blobel, G. A. (2005) FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by GATA-1. *EMBO J.* **24**, 2367–2378.
183. Vakoc, C. R., Letting, D. L., Gheldof, N., Sawado, T., Bender, M. A., Groudine, M., Weiss, M. J., Dekker, J., and Blobel, G. A. (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol. Cell* **17**, 453–462.
184. Cantor, A. B., Iwasaki, H., Arinobu, Y., Moran, T. B., Shigematsu, H., Sullivan, M. R., Akashi, K., and Orkin, S. H. (2008) Antagonism of FOG-1 and GATA factors in fate choice for the mast cell lineage. *J. Exp. Med.* **205**, 611–624.
185. Querfurth, E., Schuster, M., Kulessa, H., Crispino, J. D., Doderlein, G., Orkin, S. H., Graf, T., and Nerlov, C. (2000) Antagonism between C/EBPbeta and FOG in eosinophil lineage commitment of multipotent hematopoietic progenitors. *Genes Dev.* **14**, 2515–2525.
186. Buck, I., Morceau, F., Cristofanon, S., Heintz, C., Chateauvieux, S., Reuter, S., Dicato, M., and Diederich, M. (2008) Tumor necrosis factor alpha inhibits erythroid differentiation in human erythropoietin-dependent cells involving p38 MAPK pathway, GATA-1 and FOG-1 downregulation and GATA-2 upregulation. *Biochem. Pharmacol.* **76**, 1229–1239.
187. Jing, H., Vakoc, C. R., Ying, L., Mandat, S., Wang, H., Zheng, X., and Blobel, G. A. (2008) Exchange of GATA factors mediates transitions in looped chromatin organization at a developmentally regulated gene locus. *Mol. Cell* **29**, 232–242.
188. Porcher, C., Swat, W., Rockwell, K., Fujiwara, Y., Alt, F. W., and Orkin, S. H. (1996) The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell* **86**, 47–57.
189. Robb, L., Lyons, I., Li, R., Hartley, L., Kontgen, F., Harvey, R. P., Metcalf, D., and Begley, C. G. (1995) Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc. Natl. Acad. Sci. USA* **92**, 7075–7079.
190. Shivdasani, R. A., Mayer, E. L., and Orkin, S. H. (1995) Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature* **373**, 432–434.
191. Mikkola, H. K., Klintman, J., Yang, H., Hock, H., Schlaeger, T. M., Fujiwara, Y., and Orkin, S. H. (2003) Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. *Nature* **421**, 547–551.
192. Goardon, N., Lambert, J. A., Rodriguez, P., Nissaire, P., Herblot, S., Thibault, P., Dumenil, D., Strouboulis, J., Romeo, P. H., and Hoang, T. (2006) ETO2 coordinates cellular proliferation and differentiation during erythropoiesis. *EMBO J.* **25**, 357–366.
193. Schuh, A. H., Tipping, A. J., Clark, A. J., Hamlett, I., Guyot, B., Iborra, F. J., Rodriguez, P., Strouboulis, J., Enver, T., Vyas, P., and Porcher, C. (2005) ETO-2 associates with SCL in erythroid cells and megakaryocytes and provides repressor functions in erythropoiesis. *Mol. Cell. Biol.* **25**, 10235–10250.
194. Wadman, I., Li, J., Bash, R. O., Forster, A., Osada, H., Rabbitts, T. H., and Baer, R. (1994) Specific in vivo association between the bHLH and LIM proteins implicated in human T cell leukemia. *EMBO J.* **13**, 4831–4839.

195. Wadman, I. A., Osada, H., Grutz, G. G., Agulnick, A. D., Westphal, H., Forster, A., and Rabbitts, T. H. (1997) The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. *EMBO J.* **16**, 3145–3157.
196. Cohen-Kaminsky, S., Maouche-Chretien, L., Vitelli, L., Vinit, M. A., Blanchard, I., Yamamoto, M., Peschle, C., and Romeo, P. H. (1998) Chromatin immunoselection defines a TAL-1 target gene. *EMBO J.* **17**, 5151–5160.
197. Anderson, K. P., Crable, S. C., and Lingrel, J. B. (1998) Multiple proteins binding to a GATA-E box-GATA motif regulate the erythroid Krüppel-like factor (EKLF) gene. *J. Biol. Chem.* **273**, 14347–14354.
198. Valverde-Garduno, V., Guyot, B., Anguita, E., Hamlett, I., Porcher, C., and Vyas, P. (2004) Differences in the chromatin structure and cis-element organization of the human and mouse GATA1 loci: implications for cis-element identification. *Blood* **104**, 3106–3116.
199. Warren, A. J., Colledge, W. H., Carlton, M. B., Evans, M. J., Smith, A. J., and Rabbitts, T. H. (1994) The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. *Cell* **78**, 45–57.
200. Mead, P. E., Kelley, C. M., Hahn, P. S., Piedad, O., and Zon, L. I. (1998) SCL specifies hematopoietic mesoderm in Xenopus embryos. *Development* **125**, 2611–2620.
201. Mead, P. E., Deconinck, A. E., Huber, T. L., Orkin, S. H., and Zon, L. I. (2001) Primitive erythropoiesis in the Xenopus embryo: the synergistic role of LMO-2, SCL and GATA-binding proteins. *Development* **128**, 2301–2308.
202. Meier, N., Krpic, S., Rodriguez, P., Strouboulis, J., Monti, M., Krijgsveld, J., Gering, M., Patient, R., Hostert, A., and Grosveld, F. (2006) Novel binding partners of Ldb1 are required for haematopoietic development. *Development* **133**, 4913–4923.
203. Perkins, A. (1999) Erythroid Kruppel like factor: from fishing expedition to gourmet meal. *Int. J. Biochem. Cell Biol.* **31**, 1175–1192.
204. Bieker, J. J. (2005) Probing the onset and regulation of erythroid cell-specific gene expression. *Mt Sinai J. Med.* **72**, 333–338.
205. Drissen, R., von Lindern, M., Kolbus, A., Driegen, S., Steinlein, P., Beug, H., Grosveld, F., and Philipsen, S. (2005) The erythroid phenotype of EKLF-null mice: defects in hemoglobin metabolism and membrane stability. *Mol. Cell. Biol.* **25**, 5205–5214.
206. Gregory, R. C., Taxman, D. J., Seshasayee, D., Kensinger, M. H., Bieker, J. J., and Wojchowski, D. M. (1996) Functional interaction of GATA1 with erythroid Krüppel-like factor and Sp1 at defined erythroid promoters. *Blood* **87**, 1793–1801.
207. Merika, M. and Orkin, S. H. (1995) Functional synergy and physical interactions of the erythroid transcription factor GATA-1 with the Krüppel family proteins Sp1 and EKLF. *Mol. Cell. Biol.* **15**, 2437–2447.
208. Feng, W. C., Southwood, C. M., and Bieker, J. J. (1994) Analyses of beta-thalassemia mutant DNA interactions with erythroid Krüppel-like factor (EKLF), an erythroid cell-specific transcription factor. *J. Biol. Chem.* **269**, 1493–1500.
209. Orkin, S. H., Kazazian, H. H. Jr., Antonarakis, S. E., Goff, S. C., Boehm, C. D., Sexton, J. P., Waber, P. G., and Giardina, P. J. (1982) Linkage of beta-thalassaemia mutations and beta-globin gene polymorphisms with DNA polymorphisms in human beta-globin gene cluster. *Nature* **296**, 627–631.
210. Nuez, B., Michalovich, D., Bygrave, A., Ploemacher, R., and Grosveld, F. (1995) Defective haematopoiesis in fetal liver resulting from inactivation of the EKLF gene. *Nature* **375**, 316–318.
211. Perkins, A. C., Sharpe, A. H., and Orkin, S. H. (1995) Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. *Nature* **375**, 318–322.
212. Wijgerde, M., Gribnau, J., Trimborn, T., Nuez, B., Philipsen, S., Grosveld, F., and Fraser, P. (1996) The role of EKLF in human beta-globin gene competition. *Genes Dev.* **10**, 2894–2902.
213. Drissen, R., Palstra, R. J., Gillemans, N., Splinter, E., Grosveld, F., Philipsen, S., de Laat, W. (2004) The active spatial organization of the beta-globin locus requires the transcription factor EKLF. *Genes Dev.* **18**, 2485–2490.
214. Hodge, D., Coghill, E., Keys, J., Maguire, T., Hartmann, B., McDowall, A., Weiss, M., Grimmond, S., and Perkins, A. (2006) A global role for EKLF in definitive and primitive erythropoiesis. *Blood* **107**, 3359–3370.
215. Pilon, A. M., Arcasoy, M. O., Dressman, H. K., Vayda, S. E., Maksimova, Y. D., Sangerman, J. I., Gallagher, P. G., and Bodine, D. M. (2008) Failure of terminal erythroid differentiation in EKLF-deficient mice is associated with cell cycle perturbation and reduced expression of E2F2. *Mol. Cell. Biol.* **28**, 7394–7401.
216. Bouilloux, F., Juban, G., Cohet, N., Buet, D., Guyot, B., Vainchenker, W., Louache, F., and Morle, F. (2008) EKLF restricts megakaryocytic differentiation at the benefit of erythrocytic differentiation. *Blood* **112**, 576–584.
217. Frontelo, P., Manwani, D., Galdass, M., Karsunky, H., Lohmann, F., Gallagher, P. G., and Bieker, J. J. (2007) Novel role for EKLF in megakaryocyte lineage commitment. *Blood* **110**, 3871–3880.
218. Siatecka, M., Xue, L., and Bieker, J. J. (2007) Sumoylation of EKLF promotes transcriptional repression and is involved in inhibition of megakaryopoiesis. *Mol. Cell. Biol.* **27**, 8547–8560.
219. Gilks, C. B., Bear, S. E., Grimes, H. L., and Tsichlis, P. N. (1993) Progression of interleukin-2 (IL-2)-dependent rat T cell lymphoma lines to IL-2-independent growth following activation of a gene (Gfi-1) encoding a novel zinc finger protein. *Mol. Cell. Biol.* **13**, 1759–1768.
220. Duan, Z. and Horwitz, M. (2003) Gfi-1 oncoproteins in hematopoiesis. *Hematology* **8**, 339–344.
221. Osawa, M., Yamaguchi, T., Nakamura, Y., Kaneko, S., Onodera, M., Sawada, K., Jegalian, A., Wu, H., Nakauchi, H., and Iwama, A. (2002) Erythroid expansion mediated by the Gfi-1B zinc finger protein: role in normal hematopoiesis. *Blood* **100**, 2769–2777.
222. Saleque, S., Cameron, S., and Orkin, S. H. (2002) The zinc-finger proto-oncogene Gfi-1b is essential for development of the erythroid and megakaryocytic lineages. *Genes Dev.* **16**, 301–306.
223. Huang, D. Y., Kuo, Y. Y., and Chang, Z. F. (2005) GATA-1 mediates auto-regulation of Gfi-1B transcription in K562 cells. *Nucleic Acids Res.* **33**, 5331–5342.
224. Jegalian, A. G. and Wu, H. (2002) Regulation of Socs gene expression by the proto-oncoprotein GFI-1B: two routes for STAT5 target gene induction by erythropoietin. *J. Biol. Chem.* **277**, 2345–2352.
225. Tong, B., Grimes, H. L., Yang, T. Y., Bear, S. E., Qin, Z., Du, K., El-Deiry, W. S., and Tsichlis, P. N. (1998) The Gfi-1B proto-oncoprotein represses p21WAF1 and inhibits myeloid cell differentiation. *Mol. Cell. Biol.* **18**, 2462–2473.
226. Liu, P., Keller, J. R., Ortiz, M., Tessarollo, L., Rachel, R. A., Nakamura, T., Jenkins, N. A., and Copeland, N. G. (2003) Bcl11a is essential for normal lymphoid development. *Nat. Immunol.* **4**, 525–532.
227. Menzel, S., Garner, C., Gut, I., Matsuda, F., Yamaguchi, M., Heath, S., Foglio, M., Zelenika, D., Boland, A., Rooks, H., Best, S., Spector, T. D., Farrall, M., Lathrop, M., and Thein, S. L. (2007) A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. *Nat. Genet.* **39**, 1197–1199.
228. Lettre, G., Sankaran, V. G., Bezerra, M. A., Araújo, A. S., Uda, M., Sanna, S., Cao, A., Schlessinger, D., Costa, F. F., Hirschhorn, J. N., and Orkin, S. H. (2008) DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc. Natl. Acad. Sci. USA* **105**, 11869–11874.
229. Uda, M., Galanello, R., Sanna, S., Lettre, G., Sankaran, V. G., Chen, W., Usala, G., Busonero, F., Maschio, A., Albai, G., Piras, M. G., Sestu, N., Lai, S., Dei, M., Mulas, A., Crisponi, L., Naitza, S., Asunis,

I., Deiana, M., Nagaraja, R., Perseu, L., Satta, S., Cipollina, M. D., Sollaino, C., Moi, P., Hirschhorn, J. N., Orkin, S. H., Abecasis, G. R., Schlessinger, D., and Cao, A. (2008) Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. *Proc. Natl. Acad. Sci. USA* **105**, 1620–1625.

230. Sedgewick, A. E., Timofeev, N., Sebastiani, P., So, J. C., Ma, E. S., Chan, L. C., Fucharoen, G., Fucharoen, S., Barbosa, C. G., Vardarajan, B. N., Farrer, L. A., Baldwin, C. T., Steinberg, M. H., and Chui, D. H. (2008) BCL11A is a major HbF quantitative trait locus in three different populations with beta-hemoglobinopathies. *Blood Cells Mol. Dis.* **41**, 255–258.
231. Sankaran, V. G., Menne, T. F., Xu, J., Akie, T. E., Lettre, G., Van Handel, B., Mikkola, H. K., Hirschhorn, J. N., Cantor, A. B., and Orkin, S. H. (2008) Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science* **322**, 1839–1842.
232. Chen, Z., Luo, H. Y., Steinberg, M. H., and Chui, D. H. (2009) BCL11A represses HBG transcription in K562 cells. *Blood Cells Mol. Dis.* **42**, 144–149.
233. Bank, A. (2006) Regulation of human fetal hemoglobin: new players, new complexities. *Blood* **107**, 435–443.
234. Yin, B., Delwel, R., Valk, P. J., Wallace, M. R., Loh, M. L., Shannon, K. M., and Largaespada, D. A. (2009) A retroviral mutagenesis screen reveals strong cooperation between Bcl11a overexpression and loss of the Nf1 tumor suppressor gene. *Blood* **113**, 1075–1085.
235. Huang, S., Qiu, Y., Stein, R. W., and Brandt, S. J. (1999) p300 functions as a transcriptional coactivator for the TAL1/SCL oncoprotein. *Oncogene* **18**, 4958–4967.
236. Zhang, W., Kadam, S., Emerson, B. M., and Bieker, J. J. (2001)REFATL>Site-specific acetylation by p300 or CREB binding protein regulates erythroid Krüppel-like factor transcriptional activity via its interaction with the SWI-SNF complex. *Mol. Cell. Biol.* **21**, 2413–2422.
237. Armstrong, J. A., Bieker, J. J., and Emerson, B. M. (1998) A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. *Cell* **95**, 93–104.
238. Kadam, S., McAlpine, G. S., Phelan, M. L., Kingston, R. E., Jones, K. A., and Emerson, B. M. (2000) Functional selectivity of recombinant mammalian SWI/SNF subunits. *Genes Dev.* **14**, 2441–2451.
239. Xu, Z., Meng, X., Cai, Y., Koury, M. J., and Brandt, S. J. (2006) Recruitment of the SWI/SNF protein Brg1 by a multiprotein complex effects transcriptional repression in murine erythroid progenitors. *Biochem J.* **399**, 297–304.
240. Bultman, S. J., Gebuhr, T. C., and Magnuson, T. (2005) A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in beta-globin expression and erythroid development. *Genes Dev.* **19**, 2849–2861.
241. Stumpf, M., Waskow, C., Krotschel, M., van Essen, D., Rodriguez, P., Zhang, X., Guyot, B., Roeder, R. G., and Borggrefe, T. (2006) The mediator complex functions as a coactivator for GATA-1 in erythropoiesis via subunit Med1/TRAP220. *Proc. Natl. Acad. Sci. USA* **103**, 18504–18509.
242. Deconinck, A. E., Mead, P. E., Tevosian, S. G., Crispino, J. D., Katz, S. G., Zon, L. I., and Orkin, S. H. (2000) FOG acts as a repressor of red blood cell development in Xenopus. *Development* **127**, 2031–2040.
243. Katz, S. G., Cantor, A. B., and Orkin, S. H. (2002) Interaction between FOG-1 and the corepressor C-terminal binding protein is dispensable for normal erythropoiesis in vivo. *Mol. Cell. Biol.* **22**, 3121–3128.
244. Chen, X. and Bieker, J. J. (2001) Unanticipated repression function linked to erythroid Krüppel-like factor. *Mol. Cell. Biol.* **21**, 3118–3125.
245. Huang, S. and Brandt, S. J. (2000) mSin3A regulates murine erythroleukemia cell differentiation through association with the TAL1 (or SCL) transcription factor. *Mol. Cell. Biol.* **20**, 2248–2259.
246. Chen, X. and Bieker, J. J. (2004) Stage-specific repression by the EKLF transcriptional activator. *Mol. Cell. Biol.* **24**, 10416–10424.
247. Saleque, S., Kim, J., Rooke, H. M., and Orkin, S. H. (2007) Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. *Mol. Cell* **27**, 562–572.
248. Chang, T. C. and Mendell, J. T. (2007) MicroRNAs in vertebrate physiology and human disease. *Annu. Rev. Genom. Hum. Genet.* **8**, 215–239.
249. O'Carroll, D., Mecklenbrauker, I., Das, P. P., Santana, A., Koenig, U., Enright, A. J., Miska, E. A., and Tarakhovsky, A. (2007) A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. *Genes Dev.* **21**, 1999–2004.
250. Martinez, J. and Busslinger, M. (2007) Life beyond cleavage: the case of Ago2 and hematopoiesis. *Genes Dev.* **21**, 1983–1988.
251. Garzon, R. and Croce, C. M. (2008) MicroRNAs in normal and malignant hematopoiesis. *Curr. Opin. Hematol.* **15**, 352–358.
252. Lawrie, C. H. (2007) MicroRNAs and haematology: small molecules, big function. *Br. J. Haematol.* **137**, 503–512.
253. Kluiver, J., Kroesen, B. J., Poppema, S., and van den Berg, A. (2006) The role of microRNAs in normal hematopoiesis and hematopoietic malignancies. *Leukemia* **20**, 1931–1936.
254. Chen, C. Z. and Lodish, H. F. (2005) MicroRNAs as regulators of mammalian hematopoiesis. *Semin. Immunol.* **17**, 155–165.
255. Chen, C. Z., Li, L., Lodish, H. F., and Bartel, D. P. (2004) MicroRNAs modulate hematopoietic lineage differentiation. *Science* **303**, 83–86.
256. Felli, N., Fontana, L., Pelosi, E., Botta, R., Bonci, D., Facchiano, F., Liuzzi, F., Lulli, V., Morsilli, O., Santoro, S., Valtieri, M., Calin, G. A., Liu, C. G., Sorrentino, A., Croce, C. M., and Peschle, C. (2005) MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. *Proc. Natl. Acad. Sci. USA* **102**, 18081–18086.
257. Wang, Q., Huang, Z., Xue, H., Jin, C., Ju, X. L., Han, J. D., and Chen, Y. G. (2008) MicroRNA miR-24 inhibits erythropoiesis by targeting activin type I receptor ALK4. *Blood* **111**, 588–595.
258. Kosaka, N., Sugiura, K., Yamamoto, Y., Yoshioka, Y., Miyazaki, H., Komatsu, N., Ochiya, T., and Kato, T. (2008) Identification of erythropoietin-induced microRNAs in haematopoietic cells during erythroid differentiation. *Br. J. Haematol.* **142**, 293–300.
259. Garzon, R., Liu, S., Fabbri, M., Liu, Z., Heaphy, C. E., Callegari, E., Schwind, S., Pang, J., Yu, J., Muthusamy, N., Havelange, V., Volinia, S., Blum, W., Rush, L. J., Perrotti, D., Andreeff, M., Bloomfield, C. D., Byrd, J. C., Chan, K., Wu, L. C., Croce, C. M., and Marcucci, G. (2009) MicroRNA -29b induces global DNA hypomethylation and tumor suppressor gene re-expression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* **113**, 6269–6270.
260. Rathjen, T., Nicol, C., McConkey, G., and Dalmay, T. (2006) Analysis of short RNAs in the malaria parasite and its red blood cell host. *FEBS Lett.* **580**, 5185–5188.
261. Bruchova, H., Yoon, D., Agarwal, A. M., Mendell, J., and Prchal, J. T. (2007) Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. *Exp. Hematol.* **35**, 1657–1667.
262. Zhan, M., Miller, C. P., Papayannopoulou, T., Stamatoyannopoulos, G., and Song, C. Z. (2007) MicroRNA expression dynamics during murine and human erythroid differentiation. *Exp. Hematol.* **35**, 1015–1025.
263. Masaki, S., Ohtsuka, R., Abe, Y., Muta, K., and Umemura, T. (2007) Expression patterns of microRNAs 155 and 451 during normal human erythropoiesis. *Biochem. Biophys. Res. Commun.* **364**, 509–514.
264. Lu, S. J., Feng, Q., Park, J. S., Vida, L., Lee, B. S., Strausbauch, M., Wettstein, P. J., Hong, G. R., and Lanza, R. (2008) Biological proper-

ties and enucleation of red blood cells from human embryonic stem cells. *Blood* **112**, 4475–4484.
265. Pase, L., Layton, J. E., Kloosterman, W. P., Carradice, D., Waterhouse, P. M., and Lieschke, G. J. (2009) miR-451 regulates zebrafish erythroid maturation in vivo via its target gata2. *Blood* **113**, 1794–1804.
266. Vegiopoulos, A., Garcia, P., Emambokus, N., and Frampton, J. (2006) Coordination of erythropoiesis by the transcription factor c-Myb. *Blood* **107**, 4703–4710.
267. Zhao, H., Kalota, A., Jin, S., and Gewirtz, A. M. (2008) The c-myb Protooncogene and microRNA (miR)-15a comprise an active autoregulatory feedback loop in human hematopoietic cells. *Blood* **113**, 505–516.
268. Tsiftsoglou, A. S., Nunez, M. T., Wong, W., and Robinson, S. H. (1983) Dissociation of iron transcript and heme biosynthesis form commitment to terminal maturation of murine erythroleukemia cells. *Proc. Natl. Acad. Sci. USA* **80**, 7528–7582.
269. Dmitrovsky, E., Kuehl, W. M., Hollis, G. F., Kirsch, I. R., Bender, T. P., and Segal, S. (1986) Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukaemia cell line. *Nature* **322**, 748–750.
270. Clarke, M. F., Kukowska-Latallo, J. F., Westin, E., Smith, M., and Prochownik, E. V. (1988) Constitutive expression of a c-myb cDNA blocks Friend murine erythroleukemia cell differentiation. *Mol. Cell. Biol.* **8**, 884–892.
271. Smith, M. J., and Prochownik, E. V. (1992) Inhibition of c-jun causes reversible proliferative arrest and withdrawal from the cell cycle. *Blood* **79**, 2107–2115.
272. Francastel, C., Mazouzi, Z., and Robert-Lezenes, J. (1992) Co-induction of c-fos and junB during the latent period preceding commitment of Friend erythroleukemia cells to differentiation. *Leukemia* **6**, 935–939.
273. Rao, G., Rekhtman, N., Cheng, G., Krasikov, T., and Skoultchi, A. I. (1997) Deregulated expression of the PU.1 transcription factor blocks murine erythroleukemia cell terminal differentiation. *Oncogene* **14**, 123–131.
274. Yamada, T., Kondoh, N., Matsumoto, M., Yoshida, M., Maekawa, A., and Oikawa, T. (1997) Overexpression of PU.1 induces growth and differentiation inhibition and apoptotic cell death in murine erythroleukemia cells. *Blood* **89**, 1383–1393.
275. Oikawa, T., Yamada, T., Kihara-Negishi, F., Yamamoto, H., Kondoh, N., Hitomi, Y., and Hashimoto, Y. (1999) The role of Ets family transcription factor PU.1 in hematopoietic cell differentiation, proliferation and apoptosis. *Cell Death Differ*. **6**, 99–608.
276. Chen, J., Kremer, C. S., and Bender, T. P. (2002) A Myb dependent pathway maintains Friend murine erythroleukemia cells in an immature and proliferating state(2002) *Oncogene* **21**, 1859–1869.
277. Kihara-Negishi, F., Yamamoto, H., Suzuki, M., Yamada, T., Sakurai, T., Tamura, T., and Oikawa, T. (2001) *In vivo* complex formation of PU.1 with HDAC1 associated with PU.1-mediated transcriptional repression. *Oncogene* **20**, 6039–6047.
278. Yamamoto, H., Kihara-Negishi, F., Yamada, T., Suzuki, M., Nakano, T., and Oikawa, T. (2002) Interaction between the hematopoietic Ets transcription factor Spi-B and the coactivator CREB-binding protein associated with negative cross-talk with c-myb. *Cell Growth Differ*. **13**, 69–75.
279. Hoffman, B., Amanullah, A., Shafarenko, M., and Liebermann, D. A. (2002) The proto-oncogene *c-myc* in hematopoietic development and leukemogenesis. *Oncogene* **21**, 3414–3421.
280. Juin, P., Hueber, A. O., Littlewood, T., and Evan, G. (1999) c-Myc-induced sensitization to apoptosis is mediated through cytochrome c release. *Genes Dev*. **13**, 1367–1381.
281. Wong, W., Robinson, S. H., and Tsiftsoglou, A. S. (1985) Relationship of mitochondrial membrane potential to hemoglobin synthesis during Friend cell maturation. *Blood* **66**, 999–1001.
282. Hotti, A., Järvinen, K., Siivola, P., and Hölttä, E. (2000) Caspases and mitochondria in c-Myc-induced apoptosis: identification of ATM as a new target of caspases. *Oncogene* **19**, 2354–2362.
283. Diaz, C., Lee, A. T., McConkey, D. J., and Schroit, A. J. (1999) Phosphatidylserine externalization during differentiation-triggered apoptosis of erythroleukemic cells. *Cell Death Differ*. **6**, 218–226.
284. Terada, M., Fried, J., Nudel, U., Rifkind, R. A., and Marks, P. A. (1977) Transient inhibition of initiation of S-phase associated with dimethyl sulfoxide induction of murine erythroleukemia cells to erythroid differentiation. *Proc. Natl. Acad. Sci. USA* **74**, 248–252.
285. Geller, R., Levenson, R., and Housman, D. (1978) Significance of the cell cycle in commitment of murine erythroleukemia cells to erythroid differentiation. *J. Cell. Physiol.* **95**, 213–222.
286. Hsieh, F. F., Barnett, L. A., Green, W. F., Freedman, K., Matushansky, I., Skoultchi, A. I., and Kelley, L. L. (2000) Cell cycle exit during terminal erythroid differentiation is associated with accumulation of $p27^{kip1}$ and inactivation of cdk2 kinase. *Blood* **96**, 2746–2754.
287. Matushansky, I., Radparvar, F., and Skoultchi, A. I. (2000) Manipulating the onset of cell cycle withdrawal in differentiated erythroid cells with cyclin-dependent kinases and inhibitors. *Blood* **96**, 2755–2764.
288. Matushansky, I., Radparvar, F., and Skoultchi, A. I. (2000) Reprogramming leukemic cells to terminal differentiation by inhibiting specific cyclin-dependent kinases in G_1. *Proc. Natl. Acad. Sci. USA* **97**, 14317–14322.
289. Matushansky, I., Radparvar, F., and Skoultchi, A. I. (2003) CDK6 blocks differentiation: coupling cell proliferation to the block to differentiation in leukemic cells. *Oncogene* **22**, 4143–4149.
290. Zhu, L. and Skoultchi, A. I. (2001) Coordinating cell proliferation and differentiation. *Curr. Opin. Genet. Dev.* **11**, 91–97.
291. Matragkou, C. N., Papachristou, E. T., Tezias, S. S., Tsiftsoglou, A. S., Choli-Papadopoulou, T., and Vizirianakis, I. S. (2008) The potential role of ribosomal protein S5 on cell cycle arrest and initiation of murine erythroleukemia cell differentiation. *J. Cell. Biochem.* **104**, 1477–1490.
292. Kobayashi, T., Sasaki, Y., Oshima, Y., Yamamoto, H., Mita, H., Suzuki, H., Toyota, M., Tokino, T., Itoh, F., Imai, K., and Shinomura, Y. (2006) Activation of the ribosomal protein L13 gene in human gastrointestinal cancer. *Int. J. Mol. Med.* **18**, 161–170.
293. Papetti, M. and Skoultch, A. I. (2007) Reprogramming leukemia cells to terminal differentiation and growth arrest by RNA interference of PU.1. *Mol. Cancer Res.* **5**, 1053–1062.
294. Levenson, R. and Housman, D. (1979) Memory of MEL cells to a previous exposure to inducer. *Cell* **17**, 485–490.
295. Tsiftsoglou, A. S. and Wong, W. (1985) Molecular and cellular Mechanisms of Leukemic hemopoietic cell differentiation: an analysis of the fiend system. *Anticancer Res.* **5**, 81–99.
296. Housman, D., Levenson, R., Volloch, V., Tsiftsoglou, A., Gusella, J. F., Parker, D., Kernen, J., Mitrani, A., Weeks, V., Witte, O., and Besmer P. (1980) Control of proliferation and differentiation in cells transformed by Friend virus. *Cold Spring Harbor Symp. Quant. Biol.* **44**, 1177–1185.
297. Vizirianakis, I. S. and Tsiftsoglou, A. S. (1995) N^6-methyladenosine inhibits murine erythroleukemia cell maturation by blocking methylation of RNA and memory via conversion to S-(N^6-methyl)-adenosylhomocysteine. *Biochem. Pharmacol.* **50**, 1807–1814.
298. Tsiftsoglou, A. S., Wong, W., and Housman, D. (1983) Dexamethasone-sensitive and insensitive responses during in vitro differentiation of Friend erythroleukemia cells. *Biochim. Biophys. Acta* **759**, 160–169.
299. Pappas, I. S., Sophianos, D., Tzartos, S., and Tsiftsoglou, A. S. (1996) Expression of memory, differentiation, and repression of c-myc and p53 genes in human RD/TE-671 cells induced by a ureido-derivative of pyridine (UDP-4). *Cell Growth Differ.* **7**, 797–809.

300. Higgs, D. R. and William, G. W. (2008) Long-range regulation of a globin gene expression during erythropoiesis. *Curr. Opin. Hematol.* **15**, 176–183.
301. Palstra, R. J., de Laat, W., and Grosveld, F. (2008) Beta-globin regulation and long-range interactions. *Adv. Genet.* **61**, 107–142.
302. Wozniak, R. J. and Bresnick, E. H. (2008) Epigenetic control of complex loci during erythropoiesis. *Curr. Top. Devel. Biol.* **82**, 55–83.
303. Scher, W., Scher, B. M., and Waxman, S. (1983) In: *Current Concepts of Erythropoiesis*(C. Dunn, D. R., ed.). pp. 301–388, Wiley, New York.
304. Sheffery, M., Rifkind, R. A., and Marks, P. A. (1982) Murine erythroleukemia cell differentiation: DNAse I hypersensitivity and DNA methylation near the globin genes *Proc. Natl. Acad. Sci. USA* **79**, 1180–1184.
305. Yu, J. and Smith, R. D. (1985) Sequential alterations in globin gene chromatin structure during erythroleukemia cell differentiation. *Proc. Natl. Acad. Sci. USA* **260**, 3035–3040.
306. Robertson, K. D. and Wolffe, A. P. (2000) DNA methylation in health and disease. *Nature Rev. Genet.* **1**, 11–19.
307. Jones, P.A. and Baylin, S. B. (2002) The fundamental role of epigenetic events in cancer. *Nature Rev. Genet.* **3**, 415–428.
308. Laird, P. W. (2003) The power and the promise of DNA methylation markers. *Nature Rev. Cancer* **3**, 253–266.
309. Ng, H. H. and Bird, A. (1999) DNA methylation and chromatin modification. *Curr. Opin. Genet. Dev.* **9**, 158–163.
310. Robertson, K. D. (2002) DNA methylation and chromatin—unraveling the target web. *Oncogene* **21**, 5361–5379.
311. Fathallah, H., Portnoy, G., and Atweh, G. F. (2008) Epigenetic analysis of the human α- and β-globin gene clusters. *Blood Cell Mol. Dis.* **40**, 166–173.
312. De Laat, W., Klous, P., Kooren, J., Noordermeer, D., Palstra, R. J., Simonis, M., Splinter, E., and Grosveld, F. (2008) Three-dimensional organization of gene expression in erythroid cells. *Curr. Top. Dev. Biol.* **82**, 117–139.
313. Kosak, S. T., Scalzo, D., Alworth, S. V., Li, F., Palmer, S., Enver, T., Lee, J. S., and Groudine, M. (2007) Coordinate gene regulation during hematopoiesis is related to genomic organization. *PLoS Biol.* **5**, e309.
314. Palstra, R. J., Tolhuis, B., Splinter, E., Nijmeijer, R., Grosveld, F., and de Laat, W. (2003) The beta-globin nuclear compartment in development and erythroid differentiation. *Nat. Genet.* **35**, 190–194.
315. Perkins, A. C., Sharpe, A. H., and Orkin, S. H. (1995) Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. *Nature* **375**, 318–322.
316. Andrews, N. C., Erdjument-Bromage, H., Davidson, M. B., Tempst, P., and Orkin, S. H. (1993) Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. *Nature* **362**, 722–728.
317. Kim, A., Song, S. H., Brand, M., and Dean, A. (2007) Nucleosome and transcription activator antagonism at human β-globin locus control region DNase I hypersensitive sites. *Nucleic Acids Res.* **35**, 5831–5838.
318. Cho, Y., Song, S. H., Lee, J. J., Choi, N., Kim, C. G., Dean, A., and Song, S. H. (2008) The role of transcriptional activator GATA-1 at human β-globin HS2. *Nucleic Acids Res.* **36**, 4521–4528.
319. Droge, P. and Muller-Hill, B. (2001) High local protein concentrations at promoters: strategies in prokaryotic and eukaryotic cells. *Bioessays* **23**, 179–183.
320. Anguita, E., Hughes, J., Heyworth, C., Blobel, G. A., Wood, W. G., and Higgs, D. R. (2004) Globin gene activation during haemopoiesis is driven by protein complexes nucleated by GATA-1 and GATA-2. *EMBO J.* **23**, 2841–2852.
321. Hughes, J. R., Cheng, J. F., Ventress, N., Prabhakar, S., Clark, K., Anguita, E., de Gobbi, M., de Jong, P., Rubin, E., and Higgs, D. R. (2005) Annotation of cis-regulatory elements by identification, subclassification, and functional assessment of multispecies conserved sequences. *Proc. Natl. Acad. Sci. USA* **102**, 9830–9835.
322. Kielman, M. F., Smits, R., Hof, I., and Bernini, L. F. (1996) Characterization and comparison of the human and mouse Dist1/alpha-globin complex reveals a tightly packed multiple gene cluster containing differentially expressed transcription units. *Genomics* **32**, 341–351.
323. Higgs, D. R., Vernimmen, D., and Wood, B. (2008) Long-range regulation of α-globin gene expression. *Adv. Genet.* **61**, 143–173.
324. Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002) Capturing chromosome conformation. *Science* **295**, 1306–1311.
325. Splinter, E., Heath, H., Kooren, J., Palstra, R. J., Klous, P., Grosveld, F., Galjart, N., and de Laat, W. (2006) CTCF mediates long-range chromatin looping and local histone modification in the β-globin locus. *Genes Dev.* **20**, 2349–2354.
326. Vernimmen, D., de Gobbi, M., Sloane-Stanley, J. A., Wood, W. G., and Higgs, D. R. (2007) Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J.* **26**, 2041–2051.