CHAPTER ONE

ONTOGENY OF ERYTHROPOIESIS IN THE MAMMALIAN EMBRYO

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Abstract

Red cells are required not only for adult well-being but also for survival and growth of the mammalian embryo beyond early postimplantation stages of development. The embryo’s first “primitive” erythroid cells, derived from a transient wave of committed progenitors, emerge from the yolk sac as immature precursors and differentiate as a semisynchronous cohort in the bloodstream. Surprisingly, this maturational process in the mammalian embryo is characterized by globin gene switching and ultimately by enucleation. The yolk sac also synthesizes a second transient wave of “definitive” erythroid progenitors that enter the bloodstream and seed the liver of the fetus. At the same time, hematopoietic stem cells within the embryo also seed the liver and are the

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presumed source of long-term erythroid potential. Fetal definitive erythroid precursors mature in macrophage islands within the liver, enucleate, and enter the bloodstream as erythrocytes. Toward the end of gestation, definitive erythropoiesis shifts to its final location, the bone marrow. It has recently been recognized that the yolk sac-derived primitive and fetal definitive erythroid lineages, like their adult definitive erythroid counterpart, are each hierarchically associated with the megakaryocyte lineage. Continued comparative studies of primitive and definitive erythropoiesis in mammalian and nonmammalian embryos will lead to an improved understanding of terminal erythroid maturation and globin gene regulation.

1. Introduction

The red cells of mammals are unique in the animal kingdom because they circulate as enucleated cells, while the red cells of fish, amphibians, and birds remain nucleated (Gulliver, 1875). Nearly 100 years ago, it was recognized that two distinct populations of red cells circulate in the bloodstream of early mammalian embryos (Maximow, 1909). The first population consisted of extremely large, nucleated red cells that originated in the yolk sac (Fig. 1.1). These “primitive” red cells were subsequently superseded by a second “definitive” population of smaller, enucleated red cells

Figure 1.1 Visual comparison of primitive and definitive erythroid cells in the mouse. Circulating blood cells were isolated both from E9.5 embryos and from adult mice, mixed together, cytospun, and stained with Wright-Giemsa. The nucleated primitive erythroid cells are at the basophilic stage of maturation and markedly larger than the definitive erythrocytes.
that continue to circulate during fetal and postnatal life. Because primitive red cells are nucleated and confined to the embryo, they were thought to share many characteristics with the nucleated red cells of nonmammalian vertebrates (Tavassoli, 1991). However, recent findings have challenged this long-held paradigm and have demonstrated that primitive erythroblasts fully mature, like their definitive counterparts, into enucleated erythrocytes. Furthermore, studies in the murine embryo suggest that definitive erythroblasts are derived from more than one source during embryogenesis. This chapter will discuss the current understanding of the ontogeny of the primitive and definitive erythroid lineages in the mammalian embryo.

Embryonic erythropoiesis is best understood in the context of adult erythropoiesis. In the adult human, maintenance of a steady-state normal red cell mass requires the synthesis of two million erythrocytes every second. This red cell production is sustained by the continued generation from hematopoietic stem cells (HSC) of committed progenitors that are assayed by their ability to form colonies in semisolid media supplemented with cytokines. Clonal colonies containing both erythroid cells and platelet-forming megakaryocytes support the concept that the erythroid and megakaryocyte lineages share a common bipotential progenitor (Debili et al., 1996; McCleod et al., 1976; Suda et al., 1983). Such bipotential progenitors have been prospectively isolated from the bone marrow by flow cytometry (Akashi et al., 2000). Downstream of these bipotential progenitors lay unipotential erythroid and megakaryocyte progenitors. The most immature erythroid-restricted progenitor is the burst-forming unit erythroid (BFU-E) that generates the more mature colony-forming unit erythroid (CFU-E) (Heath et al., 1976; Stephenson et al., 1971). These progenitors, in turn, give rise to nucleated erythroblasts that undergo a limited number of cell divisions as they decrease their cell size, accumulate hemoglobin, and undergo progressive nuclear condensation. Erythroid precursors mature in association with macrophage cells in “erythroblast islands” which serve as a stromal microenvironment within the bone marrow cavity (Bessis et al., 1978). Following enucleation, young erythrocytes remove ribosomes and mitochondria, assume a biconcave shape, and enter the bloodstream where they function to provide oxygen to all tissues of the body. Erythropoiesis in the embryo differs from erythropoiesis in the adult because of two significant dilemmas faced by the embryo. First, functional red cells are required before long-term HSC and their microenvironmental niches are established. Second, the mammalian embryo grows extremely rapidly and embryonic erythropoiesis must generate ever-increasing numbers of red cells to accommodate this growth. In fact, a 70-fold increase in the red cell mass has been estimated to occur in fetal mice between embryonic day 12.5 (E12.5) and E16.5 of gestation (Russell et al., 1968). This contrasts with erythropoiesis in the adult, which is in steady state unless perturbed by some stress such as bleeding or hemolysis.
2. PRIMITIVE ERYTHROPOIESIS

2.1. Emergence of blood islands in the yolk sac

Studies in multiple organisms indicate that the initial generation of blood cells in the embryo depends on the formation of mesoderm cells during gastrulation. In the mouse embryo, mesoderm cells begin to traverse the primitive streak and occupy an intermediate position between primitive ectoderm and visceral endoderm germ layers at E6.5–7.0. Cell tracking studies indicate that mesoderm cells migrate through the posterior streak and contribute to the formation of all the extraembryonic structures, including the yolk sac, the chorion, and the amnion (Kinder et al., 1999). The yolk sac is a bilayer structure, composed of mesoderm-derived and visceral endoderm-derived cell layers. Within the mesoderm layer, pools of primitive erythroid cells, the so-called “blood islands,” rapidly emerge between E7.5 and E8.0 in the mouse conceptus (Ferkowicz et al., 2003; Haar and Ackerman, 1971; Silver and Palis, 1997). These blood islands become enveloped by endothelial cells which form the initial vascular plexus of the yolk sac (reviewed by Ferkowicz and Yoder, 2005). The emergence of primitive erythroid cells and endothelial cells at the same time (early gastrulation) and place (yolk sac mesoderm) within the early conceptus has long suggested that these lineages share a common developmental origin.

Pioneering experiments in the chick embryo led to the concept that signals from the visceral endoderm layer of the yolk sac induce the formation of blood and endothelium in adjacent yolk sac mesoderm (Wilt, 1965). Visceral endoderm was also found to be important for blood vessel formation in yolk sac mesoderm in mouse embryos (Palis et al., 1995). Support for the role of visceral endoderm in endothelial cell and blood cell formation comes from GATA-4-null embryoid bodies that lack visceral endoderm and display markedly reduced blood island formation (Bielinska et al., 1996). Multiple signaling cascades have been implicated in the initiation of both blood cell and blood vessel development. Alterations in vascular endothelial growth factor (VEGF) signaling cause defects in numbers and migration of hematopoietic and vascular precursors (Hamada et al., 2000; Shalaby et al., 1997). Furthermore, Indian hedgehog signaling has been found to be an important component of the induction both for blood cells (Belaousoff et al., 1999; Dyer et al., 2001) and for endothelium (Byrd et al., 2002). The bone morphogenetic protein and Wnt signaling cascades may also combine to specify hematopoietic regions in the vertebrate embryo (Marvin et al., 2001; Wang et al., 2007). Finally, observations in frog and chick embryos suggest that spatial restriction of blood islands within the yolk sac occurs through inhibitory fibroblast growth factor signals (Kumano and Smith, 2000; Nakazawa et al., 2006).
2.2. Primitive erythroid cell maturation

Primitive red cells arise in yolk sac blood islands shortly after gastrulation, beginning at E7.5 in the mouse (Haar and Ackerman, 1971; Silver and Palis, 1997). Primitive erythroblasts are produced by unique progenitors, termed primitive erythroid colony-forming cells (EryP-CFC), that generate colonies distinguishable from definitive erythroid colonies by their intermediate maturation time, colony morphology, and the unique pattern of globin gene expression (Palis et al., 1999; Wong et al., 1986; Table 1.1). EryP-CFC are first found in the murine yolk sac at E7.25, soon after the start of gastrulation but before the formation of blood islands. EryP-CFC then rapidly expand in numbers peaking at E8.0, after which they decrease until no longer found by E9.0 (Palis et al., 1999). The transient nature of primitive erythropoiesis is because EryP-CFC emerge in such a narrow temporal wave. The progenies of these progenitors are the exclusive red cells in the embryo until the newly formed fetal liver releases the first definitive red cells into the circulation at E12 (Brotherton et al., 1979; Kingsley et al., 2004; Steiner and Vogel, 1973). Therefore, anemia observed in the fetus before E13 must be due to loss or decreased synthesis of primitive erythroid cells. Disruption of genes necessary for the emergence (SCL, LMO2) or maturation (GATA-1) of the primitive erythroid lineage indicates that primitive erythroblasts are necessary for survival of the embryo beyond E9.5–10.5 (Fujiwara et al., 1996; Shivdasani et al., 1995; Warren et al., 1994). In contrast, mouse embryos specifically lacking definitive erythrocytes, as occurs following the targeted disruption of the c-myb transcription factor, survive until E15.5 (Mucenski et al., 1991). These results indicate that the primitive erythroid lineage provides a sufficient source of red cells to ensure embryonic survival until relatively late stages of development.

Morphologic analysis indicates that primitive erythroblasts mature in a semisynchronous cohort as they circulate in the bloodstream (Fraser et al., 2007; Kingsley et al., 2004; Morioka and Minamikawa-Tachino, 1993). Within 24 h of the appearance of EryP-CFC, primitive proerythroblasts can be observed ensheathed by a primary vascular plexus. These immature primitive erythroblasts begin to circulate at E8.25 coincident with, or soon after, the onset of cardiac contractions (Ji et al., 2003; Lucitti et al., 2007; McGrath et al., 2003). They continue to divide in the bloodstream until E13, as evidenced by the presence of circulating mitotic figures (Bethlenfalvay and Block, 1970), thymidine incorporation (de la Chapelle et al., 1969), and cell cycle studies (Sangiorgi et al., 1990). Primitive erythroblasts accumulate increasing amounts of hemoglobin and become progressively less basophilic (Sasaki and Matsumura, 1986; Steiner and Vogel, 1973). Hemoglobin synthesis continues until replication ceases (Fantoni et al., 1968), and primitive red cells reach their steady-state hemoglobin content of 80–100 pg per cell, approximately six times the amount of hemoglobin found in adult murine
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|                      | EryP-CFC  | CFU-E (2  | CFU-E (2)
| Progenitors<sup>a</sup> (colony | (5 days)   | days)      | days) |
|                      | formation n days) | BFU-E (7–10 days) | BFU-E (7–10 days) |
| Sites of maturation  | Yolk sac, bloodstream | Fetal liver | Bone marrow, spleen |
| Erythroblast islands | No        | Yes        | Yes   |
| Cell size<sup>b</sup> (MCV) | 400 fl  | 150 fl    | 70 fl |
| Hemoglobin (Hb) accumulation<sup>c</sup> | 80–100 pg | 25 pg     | 12 pg |
| β-Globin transcription<sup>d</sup> | βH1, εy, β1, β2 | β1, β2 | β1, β2 |
| α-Globin transcription<sup>d</sup> | ζ, α1, α2 | α1, α2 | α1, α2 |
| Cytokines            | EPO (relative) | EPO (absolute), SCF | EPO (absolute), SCF |
| Transcription factors<sup>e</sup> | SCL, LMO2, GATA-2, GATA-1, EKLF, KLF-2 | SCL, LMO2, GATA-2, GATA-1, EKLF, c-myb, Gfi-1b | GATA-1, EKLF, c-myb |

<sup>a</sup> CFU-E, BFU-E (< Heath et al., 1976; Stephenson et al., 1971), EryP-CFC (Palis et al., 1995).

<sup>b</sup> Kingsley et al. (2004).

<sup>c</sup> Steiner and Vogel (1973).

<sup>d</sup> Trimborn et al. (1999); Kingsley et al. (2004).

<sup>e</sup> GATA-1 (< Fujiwara et al., 1996; Pevny et al., 1991), EKLF (< Nuez et al., 1995; Perkins et al., 1995), KLF2 (Basu et al., 2005), c-myb (< Mucenski, 1991), Gfi-1b (< Saleque et al., 2002), SCL (< Porcher et al., 1996; Robb et al., 1995; Shivdasani et al., 1995), LMO2 (< Warren et al., 1994; Yamada et al., 1998), GATA-2 (< Tsai et al., 1994).
erythrocytes (Table 1.1; Steiner and Vogel, 1973). This correlates with the finding that primitive erythroblasts are approximately six times larger than adult erythrocytes (Fig. 1.1; Kingsley et al., 2004).

2.3. Globin gene expression

Hemoglobin molecules contain globin chains derived from both the α- and β-globin gene loci. While definitive erythroid cells in the mouse express α1-, α2-, β1-, and β2-globins, primitive erythroid cells in addition express ζ-, βH1-, and ε-globins (Trimborn et al., 1999). These latter embryonic globin genes are differentially expressed in primitive erythroid cells (Farace et al., 1984; Kingsley et al., 2006; Whitelaw et al., 1990). The initially expressed ζ- and βH1-globin genes are superseded by the α1-, α2-, and ε-globin genes, a process termed “maturational” globin switching since this globin switching occurs as primitive erythroid precursors terminally differentiate (Kingsley et al., 2006). These changes in globin transcript levels are associated with changes in RNA polymerase II density at their promoters. Furthermore, the βH1- and ε-globin genes in primitive erythroid cells reside in a single large hyperacetylated domain, suggesting that the maturational globin switching is regulated by altered transcription factor presence instead of chromatin accessibility as postulated in the adult (Kingsley et al., 2006). In contrast, the regions containing these genes are not associated with histone hyperacetylation (Bulger et al., 2003) and they are not expressed in definitive erythroid cells (Kingsley et al., 2006; Trimborn et al., 1999). Primitive erythroid cells in human embryos also appear to undergo maturational globin switching. Both ζ- to α-globin and ε- to γ-globin gene switches have been described between 5 and 7 weeks gestation (Peschle et al., 1985). Differentiating human embryonic stem cells have recently been used to model embryonic hematopoiesis and their study has led to a renewed interest in globin gene expression and regulation in primitive and fetal definitive erythroid cells (Chang et al., 2006; Olivier et al., 2006; Zambidis et al., 2005). A better understanding of the mechanisms regulating embryonic versus fetal/adult globin gene expression may ultimately lead to novel approaches for the treatment of the thalassemia syndromes and sickle cell anemia. Intriguingly, reactivation of the embryonic ζ-globin gene has been shown to ameliorate an adult mouse model of sickle cell disease (He and Russell, 2004).

2.4. Differences and commonalities between primitive and definitive erythropoiesis

As seen with globins, there are additional gene usage differences identified between primitive and definitive erythropoiesis (Table 1.1). In particular, mice lacking c-myb fail to generate definitive erythrocytes but appear to
have a normal primitive red cell mass (Mucenski et al., 1991). Targeted disruption of the transcriptional repressor Gfi-1b causes a block in the synthesis of definitive erythroid cells, while primitive erythroid cells are present but have a delay in maturation (Saleque et al., 2002). While Runx1 is expressed both by primitive and by definitive erythroid cells, targeted disruption of Runx1 and its partner core binding factor β each leads to defects only in the latter (Okuda et al., 1996; Sasaki et al., 1996; Wang et al., 1996). Targeted disruption of erythroid Kruppel-like factor (EKLF) leads to a complete block in definitive erythroid cell maturation in the fetal liver and fetal death at E15.5 (Nuez et al., 1995; Perkins et al., 1995). However, it has recently been recognized that EKLF regulates many erythroid-specific genes and EKLF-null fetuses display significant abnormalities of primitive erythroblasts (Hodge et al., 2006).

Erythropoiesis in the adult is critically dependent on erythropoietin, a cytokine that promotes late-stage erythroid progenitor and immature precursor survival (reviewed by Koury, 2005). Addition of erythropoietin to yolk sac tissues explanted in vitro leads to an expansion of primitive erythroid cells containing hemoglobin and an increase in embryonic globin transcripts (Kimura et al., 2000; McGann et al., 1997). Furthermore, immature primitive erythroblasts express erythropoietin receptor transcripts (McGann et al., 1997) and protein on their cell surface (Boussios et al., 1989). Exogenous erythropoietin abrogates apoptosis of immature primitive erythroid cells cultured in vitro (Kimura et al., 2000). Targeted disruption of erythropoietin or the erythropoietin receptor in mice leads to a 5- to 20-fold reduction in primitive erythroid cells by E11.5 and fetal demise from severe anemia by E13.5 (Kieran et al., 1996; Lin et al., 1996; Wu et al., 1995). These results, taken together, indicate that erythropoietin signaling is critical for the survival and maturation of primitive erythroid precursors.

While definitive erythropoiesis in the fetus liver is completely blocked by the lack of erythropoietin signaling, some primitive erythroid cells continue to mature, suggesting that other cytokine signaling cascades may be differentially active in primitive versus definitive erythropoiesis. The cytokine stem cell factor (SCF), by signaling through the c-kit receptor, potentiates erythropoietin signaling and plays an important role in the proliferation of definitive erythroid precursors (reviewed by Munugalavadla and Kapur, 2005). Mice lacking c-kit signaling die of severe anemia between E14.5 and E16.5. These mice have defects in definitive hematopoiesis; however, the role of c-kit signaling in primitive erythroid cell maturation is unclear (Goldman et al., 2006; Russell et al., 1968). It remains to be determined which other cytokines regulate primitive erythroid cell maturation.

Despite these biological differences in transcriptional regulation and cytokine dependence, it is important to note that primitive and definitive erythropoiesis share many fundamental characteristics of mammalian erythroid
differentiation. Both originate from unipotential progenitors and depend on the action of multiple transcription factors for maturation, including SCL, LMO2, and GATA-1 (Table 1.1 and references therein). The maturation process in both primitive and definitive erythropoiesis is characterized by downregulation of vimentin intermediate filaments (Sangiorgi et al., 1990), the accumulation of hemoglobin at similar rates (Steiner and Vogel, 1973), and the upregulation of bcl-x to prevent apoptosis (Motoyama et al., 1999). Finally, it has recently been shown that primitive erythroblasts in the mouse ultimately enucleate and, like definitive cells, circulate as erythrocytes (Kingsley et al., 2004). Studies using embryonic-specific globin antibodies that distinguish primitive from definitive erythroid cells revealed that primitive erythroid cells enucleate between E12.5 and E17.5. These findings have recently been corroborated in mice with GFP expressed in primitive erythroid cells under control of an embryonic globin promoter (Fraser et al., 2007). It is not known where and by what mechanism late-stage primitive erythroblasts enucleate since they are actively circulating, unlike their adult counterparts that mature and enucleate extravascularly attached to macrophage cells in erythroblast islands of the fetal liver and postnatal bone marrow (reviewed by Chasis, 2006, and by Manwani and Bieker, Chapter 2, in this volume).

3. “Definitive” Erythropoiesis in the Fetus

3.1. Characteristics of definitive erythropoiesis in the fetus

Primitive erythropoiesis fulfills the erythroid functions critical for early postimplantation embryonic survival and growth; however, the fetus requires increasing numbers of red cells throughout gestation. Prior to the formation of the bone marrow cavity, the liver serves as the site of maturation of definitive erythroid cells in the fetus. Soon after the liver begins to form as an organ at E9.5, it is colonized by external hematopoietic elements. Experiments with carefully staged embryos indicate that hematopoietic progenitors enter the liver at 28–30 sp (Houssaint, 1981; Johnson and Moore, 1975). BFU-E and CFU-E are found in the early fetal liver and their numbers expand exponentially for several days and peak at E14.5–15.5 (Kurata et al., 1998; Palis et al., 1999; Rich and Kubanek, 1979). Subsequently, there is gradual transition of hematopoietic activity to the bone marrow cavity and the liver ceases to be a hematopoietic organ in both the mouse and the human soon after birth. While fundamentally similar, there are some differences between fetal progenitors and their adult bone marrow counterparts. CFU-E in the murine fetus are more sensitive to erythropoietin (Rich and Kubanek, 1976). Fetal BFU-E have a greater and more rapid proliferative capacity. Unlike adult marrow-derived BFU-E, fetal
liver-derived BFU-E are capable of proliferating in response to erythropoietin in the absence of added colony-stimulating factors (Emerson et al., 1989; Migliaccio and Migliaccio, 1988; Valtieri et al., 1989).

Morphologic examination of the fetal liver in the mouse reveals the presence of immature erythroid precursors at E11.5–12.5 (Marks and Rifkind, 1972). As development proceeds, these precursors associate with macrophage cells to form erythroblast islands similar to those in the bone marrow (Sasaki and Sonoda, 2000). Furthermore, PDGFR-alpha-expressing stromal cells appear to play a role in the fetal liver but not in the embryonic yolk sac microenvironment (Li et al., 2006). There is a gradual transition to more mature erythroid precursor populations as development proceeds (Marks and Rifkind, 1972). Enucleated definitive red cells begin to emerge from the liver at E12 of mouse gestation (Kingsley et al., 2004; Rifkind et al., 1969). Over the next several days, the number of definitive erythroid cells expands exponentially in the circulation concomitant with the continued rapid growth of the fetus (Kingsley et al., 2004; Russell et al., 1968). Fetal erythrocytes in the mouse are approximately twice as large and contain twice the hemoglobin compared with their adult counterparts (Kingsley et al., 2004; Steiner and Vogel, 1973). In the human, fetal erythrocytes can also be distinguished from adult erythrocytes by the accumulation of fetal hemoglobin (HbF, α2γ2) rather than adult hemoglobin (HbA, α2β2). A “switch” from fetal to adult hemoglobin synthesis begins at 32 weeks gestation and is completed after birth. Unlike primates, rodent red cells do not synthesize a distinct fetal form of hemoglobin (Fantoni et al., 1967; Wong et al., 1983). Even though the mouse does not have a unique fetal globin, human fetal globin genes are accurately expressed when introduced into the mouse fetus (Enver et al., 1990; Stamatoyannopoulos, 2005). These results suggest that a conserved transcriptional difference exists during fetal erythropoiesis that regulates other fetal-specific characteristics and has been co-opted by primates to specify globin expression.

3.2. Fetal erythropoiesis and “stress” erythropoiesis

The mechanisms responsible for these differences observed in fetal and adult erythropoiesis remain unclear. Possibilities include hematopoietic cell intrinsic differences or microenvironmental differences between the fetal liver and the postnatal marrow (Muench and Namikawa, 2001). Critical environmental differences may be the relative hypoxia of the fetus, coupled with the need to increase red cell mass due to the expanding blood volume from growth. These factors may create signals and responses similar to those found in the adult where acute hypoxia elicits a “stress” response characterized by the rapid synthesis of large erythrocytes expressing increased amounts of fetal hemoglobin (Alter, 1979). The link between stress and fetal erythropoiesis is further supported by the phenotype of Stat5-null and

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flexed tail mice that each have normal steady-state adult erythropoiesis but display a transient fetal anemia and a blunted response as adults to acute erythroid stress induced by acute anemia (Lenox et al., 2005; Socolovsky et al., 1999). Therefore, adult stress erythropoiesis in adults may represent a reactivation of a fetal erythroid program that is distinct from adult steady-state erythropoiesis and is first used to rapidly expand the number of definitive erythrocytes during embryogenesis. Analysis of the flexed tail mutant implicates BMP4 signaling in stress erythropoiesis (Lenox et al., 2005). It is not known if BMP4 signaling also plays a role in fetal erythropoiesis.

4. Developmental Origins of Erythropoiesis

4.1. Hemangioblast

The concept that the hematopoietic and vascular lineages emerge from common “hemangioblast” precursors has existed for over 100 years and is based, in part, on the close spatial and temporal emergence of primitive erythroid and endothelial cells in the yolk sac. These lineages also share the expression of many genes, including transcription factors and cell surface proteins (reviewed by Park et al., 2005). Recent evidence suggests that hematopoietic potential arises from mesoderm cells expressing many markers associated with endothelium (Ema et al., 2006). A unique blast colony-forming cell (blast-CFC) containing both hematopoietic and endothelial cell potential has been identified both in cultured embryonic stem cells and in mouse embryos (Choi et al., 1998; Huber et al., 2004). Consistent with this unique potential, blast-CFC express Flk-1 and are regulated by several transcription factors, including endoglin and GATA-2, expressed by hematopoietic and endothelial lineages (Perlingeiro, 2007; Lugus et al., 2007).

These hemangioblast precursors are primarily confined to the region of the primitive streak in gastrulating mouse embryos. Blood islands, composed of primitive erythroid precursors, arise in a ring along the mesometrial edge of the mouse conceptus (Drake et al., 2000; Ferkowicz et al., 2003; McGrath et al., 2003). Since hemangioblast precursors are found primarily in the primitive streak and not in the yolk sac, it is thought that they rapidly commit to hematopoietic and vascular fates soon after their emergence during early gastrulation. There is increasing evidence to suggest that many, if not most, yolk sac vascular cells arise from unilineage angioblast precursors and not from hemangioblasts (Furuta et al., 2006; Ueno and Weissman, 2006). In contrast, hemangioblast precursors contain primitive erythroid, definitive erythroid, and multilineage myeloid potential (Choi et al., 1998; Huber et al., 2004). These findings support the concept that all
primitive erythroid and the first definitive erythroid cells in the embryo are ultimately derived from hemangioblast precursors.

4.2. Hematopoietic stem cell

A hallmark of adult hematopoiesis is the continuous generation of mature blood cells from HSC. The developmental origin of long-term HSC during murine embryogenesis capable of engrafting adult recipients is associated with the appearance of cell clusters arising from the dorsal aorta in the aorta-gonad-mesonephros (AGM) region at E10.5 (de Bruijn et al., 2000; Muller et al., 1994). The placenta serves as a site of HSC expansion (reviewed by Mikkola et al., 2005) and may also be a site of HSC origin given that the allantois and chorion contain hematopoietic potential when cultured in vitro (Zeigler et al., 2006). HSC are first found within the fetal liver at E11, consistent with their migration from these vascular sites of “hemogenic” endothelium (Ema and Nakauchi, 2000; Kumaravelu et al., 2002; Muller et al., 1994). However, definitive erythroid potential is found in the conceptus before long-term HSC formation. Specifically, BFU-E emerge in the yolk sac at E8.25 before the onset of circulation (Palis et al., 1999; Wong et al., 1986). Once circulation begins, BFU-E are found in increasing numbers in the bloodstream and then concentrated in the fetal liver by E10 (Palis et al., 1999). These spatiotemporal kinetics suggest that yolk sac-derived BFU-E colonize the fetal liver. Interestingly, similar kinetics have been described for BFU-E in human embryos that emerge from the yolk sac at 4.5 weeks gestation, enter the bloodstream, and are found in increasing numbers in the liver by 6 weeks gestation (Migliaccio et al., 1986). It is hypothesized that once these reach the liver’s hematopoietic environment, these yolk sac-derived BFU-E complete their maturation to produce the first definitive red cells of the embryo. Co-organ culture of yolk sac and fetal liver primordial taken from <28 sp mouse embryos indicates that the liver contains soluble factors that promote the differentiation of definitive erythroid potential present in the yolk sac (Cudennec et al., 1981). As there is no current method to distinguish the progeny of yolk sac definitive progenitors from those arising from later HSC sources, this hypothesis is not yet proven. However, Ncx1–null mouse embryos, lacking a heartbeat and systemic circulation, synthesize normal numbers of primitive and definitive erythroid progenitors in the yolk sac but fail to redistribute primitive erythroblasts and definitive erythroid progenitors to the embryo proper (Lux et al., 2007). These recent results support the notion that the definitive erythroid progenitors that initially seed the fetal liver are entirely derived from the yolk sac. Furthermore, HSC do not mature at their site of synthesis (Godin et al., 1999) and do not colonize the liver until E11. Thus, they have insufficient time to generate the mature erythrocytes that emerge from the liver beginning at E12.
Taken together, these data support a model of erythroid ontogeny in the embryo whereby three distinct waves of erythroid progenitors generate maturing precursors in three different microenvironments (Fig. 1.2). The first wave consists of EryP-CFC that generate primitive erythroid cells that mature in the bloodstream. The second wave consists of BFU-E that emerge from the yolk sac, colonize the fetal liver, and generate the first fetal definitive erythrocytes that enter the circulation. The third wave consists of long-term HSC-derived BFU-E that are responsible for continued synthesis of fetal erythrocytes within the liver, and ultimately adult erythrocytes within the bone marrow (Fig. 1.2). These three waves of erythropoiesis also are associated with distinct hematopoietic potentials. While the yolk sac-derived primitive erythroid wave was initially thought to be only erythroid, its onset is coincident with that of the megakaryocyte lineage (Palis et al., 1999; Tober et al., 2007; Xu et al., 2001). A hierarchical association of these lineages is supported by the recent discovery of a unique bipotential primitive erythroid/megakaryocyte progenitor (Tober et al., 2007). Like primitive erythroid progenitors, these bipotential primitive erythroid/megakaryocyte progenitors originate from hemangioblast precursors and expand transiently only within the yolk sac (Tober et al., 2007).

![Figure 1.2](image)

**Figure 1.2** Simplified model of erythroid ontogeny in the mammalian embryo. Current data support a model whereby three waves of erythroid progenitors emerge in the mammalian embryo. The first wave consists of primitive erythroid progenitors (EryP-CFC) that originate in the yolk sac during early gastrulation and generate primitive erythroid precursors that mature to become enucleated erythrocytes in the bloodstream. The second wave consists of definitive erythroid progenitors (BFU-E) that emerge from the yolk sac and seed the emerging fetal liver. There they generate maturing definitive erythroid precursors that enucleate to become the first circulating definitive erythrocytes of the fetus. The third wave consists of definitive erythroid progenitors that originate from long-term hematopoietic stem cells (HSC) and mature initially in the fetal liver and subsequently in the postnatal bone marrow to later generate fetal and adult red blood cells (RBC). AGM, aorta-gonad-mesonephros region.
Macrophage progenitors first emerge within the yolk sac at the same developmental time as the primitive erythroid and megakaryocyte lineages (Palis et al., 1999), suggesting that “primitive” hematopoiesis in mammals is in fact trilineage in nature. The second wave of yolk sac-derived erythropoiesis consists of the definitive erythroid lineage, which arises temporally and spatially in conjunction with the macrophage, mast cell, granulocyte and megakaryocyte lineages, as well as multipotential high-proliferative potential colony-forming cells (HPP-CFC; Palis et al., 1999, 2001; Xie et al., 2003). Furthermore, recent evidence indicates that the definitive erythroid lineage emerging from the yolk sac shares a common bipotential progenitor with the megakaryocyte lineage (Tober et al., 2007). Thus, both primitive and definitive erythropoiesis arising in the early mammalian embryo during gastrulation, like later definitive erythropoiesis in the marrow, are each hierarchically associated with the megakaryocyte lineage. It remains controversial whether this second wave of erythropoiesis is associated with B lymphoid potential (Cumano et al., 1993, 1996; Sugiyama et al., 2007; Yokota et al., 2006) or HSC capable of engrafting newborn but not adult mice (Yoder et al., 1997). Interestingly, the contribution of yolk sac-derived hematopoietic potential to adult hematopoiesis has recently received some experimental support (Samokhvalov et al., 2007). Finally, the third wave of perinatal and postnatal erythropoiesis is associated with the complete myeloid and lymphoid potential of the HSC.

5. Conclusions

The paradigm of embryonic erythropoiesis has been extensively modified from the simple two-tiered system of an evolutionarily primitive erythroid cell replaced by the adult type of definitive erythroid cell. First, there is a complexity of the definitive forms between the fetal and the adult states that may reflect the erythropoietic stress of the fetus. Second, the yolk sac-derived primitive erythroid lineage has all the hallmarks of mammalian erythropoiesis, including enucleation. However, primitive erythroid precursors begin to circulate and function as immature forms and mature in the bloodstream. Third, the yolk sac also provides a wave of definitive erythroid progenitors that are proposed to colonize the fetal liver and mature there. Thus, the yolk sac appears to provide needed erythropoietic functions to the embryo before HSC-derived hematopoiesis is fully functional. Fourth, the primitive and the definitive erythroid waves that emerge in the yolk sac are each hierarchically associated with megakaryocyte potential. It is not known if embryonic erythropoiesis in nonmammalian organisms is closely associated with thrombopoiesis. It is also not known whether there are two distinct waves of definitive erythropoiesis in nonmammalian organisms,
such as the much-studied zebrafish, frog, and chick embryos, because of the lack of readily available CFC assays in these systems (Samarut et al., 1979). Caution must be exercised when interpreting definitive erythroid potential as inherently downstream of an HSC in these organisms. Similarly, definitive erythropoiesis observed in murine and human embryonic stem cell maturation systems likely reflects the second wave of yolk sac-derived definitive erythropoiesis. Ultimately, a better understanding of the ontogeny of erythropoiesis in mammalian and nonmammalian species will continue to lead to novel insights regarding globin regulation and erythroid maturation.

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**REFERENCES**


Ontogeny of Erythropoiesis in the Mammalian Embryo


