# Hematopoietic cell development in the zebrafish embryo

Julien Y. Bertrand[a] and David Traver[a,b]

[a]Division of Biological Sciences and [b]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California, USA

Correspondence to Dr David Traver, 9500 Gilman Drive, Natural Sciences Building Room 6107, La Jolla, CA 92093-0380, USA
Tel: +1 858 822 4593; e-mail: dtraver@ucsd.edu

**Current Opinion in Hematology** 2009, 16:243–248

**Purpose of review**

A wealth of new experimental evidence has been published over the past year that has helped refine our models of blood cell development. We will review this information, discuss the current models of hematopoietic ontogeny and provide perspective on current and future research directions, with an emphasis on how studies in the zebrafish are helping us better understand how hematopoietic stem cells are formed in the vertebrate embryo.

**Recent findings**

Several important studies have been published recently addressing the embryonic development of hematopoietic stem cells. These studies have helped clarify several controversial topics in developmental hematopoiesis, including the concepts of the hemangioblast and hemogenic endothelium. In particular, the postulate that hematopoietic stem cells arise through hemogenic endothelial intermediates has been greatly strengthened by a collection of convincing publications reviewed below.

**Summary**

A precise understanding of how hematopoietic stem cells are patterned during development has important implications for both developmental biology and regenerative medicine. Since hematopoietic stem cells are the only hematopoietic cells capable of lifelong, multilineage blood cell production, understanding the stepwise, molecular processes of their instruction from mesoderm is key to replicating these events *in vitro* from pluripotent embryonic stem cells.

**Keywords**

developmental hematopoiesis, hemangioblast, hematopoietic stem cells, hemogenic endothelium, zebrafish

Curr Opin Hematol 16:243–248

1065-6251

## Introduction

Hematopoietic tissues are comprised of a large array of diversely differentiated cell types including lymphocytes, myeloid cells and erythrocytes that possess limited half-lives. Each, therefore, depends upon continuous replenishment over time. In adult mammals, mature blood cells are constantly generated in the bone marrow from rare hematopoietic stem cells (HSCs). HSCs are multipotent at the single-cell level and have the ability to self-renew. Recent evidence suggests that HSCs arise from an initial pool generated during embryogenesis that seed and populate subsequent hematopoietic sites. Lineage marking of cells expressing an estrogen-inducible Cre recombinase (Cre-ERT) under control of the *scl* promoter demonstrated that the percentage of HSCs marked at embryonic day (E) 10.5 was consistent over 5 months, and that cells transplanted from E14.5 fetal liver into conditioned adult recipients showed similar marked HSC frequency [1]. These findings support the notion that de-novo production of HSCs may occur only during a defined window in development, and that subsequent HSC expansion occurs through self-renewing divisions of HSCs derived from this pool. Similar lineage tracing experiments using VE-Cadherin Cre knock-in mice have provided strong support for the concept of hemogenic endothelium as the birthplace of HSCs. Zovein *et al.* [2•] recently reported that a constitutive VE-Cadherin Cre transgene, limited to expression in endothelial cells, resulted in the labeling of approximately half of adult bone marrow cells. Subsequent experiments utilizing an inducible VE-Cadherin Cre-ERT transgene showed that induction at E9.5 led to long-term labeling of all major hematopoietic lineages in adult animals. This long-term labeling correlated with marking of presumptive HSCs along the ventral floor of the dorsal aorta at E10.5. Chen *et al.* [3••] have also recently shown that the transcription factor *runx1*, long known to be necessary for specification of HSCs, is required specifically within VE-Cadherin$^+$ endothelium for HSC induction. Together, these data strongly support the hypothesis that nascent HSCs arise from vascular endothelial cells in the mid-gestation

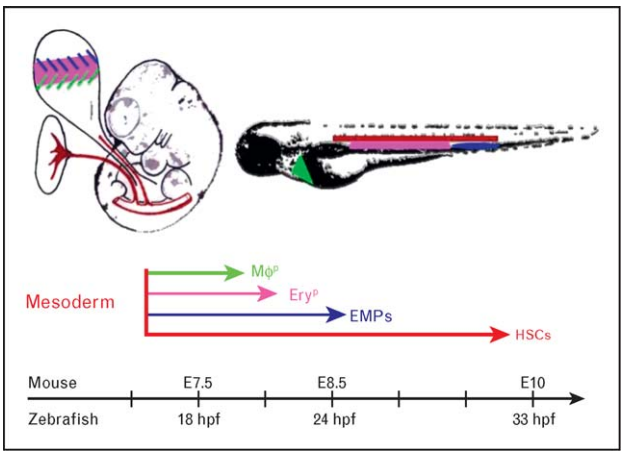
mouse embryo, and that *runx1* is required to confer HSC fate upon a subset of VE-Cadherin$^{+}$ cells.

These data corroborate previous findings in chicken embryos that established the concept of hemogenic endothelium via blood cell production from endothelial cells previously labeled with fluorescently tagged AcLDL molecules [4–7]. Whereas recent results support HSC birth via hemogenic endothelium, the precise locations of HSC emergence remain to be determined. Furthermore, the exact nature of mesodermal commitment to the hematopoietic fates remains imprecisely understood. For example, does hemogenic endothelium arise from an earlier bipotent precursor of blood and vessels (the hemangioblast), or are specific regions of endothelium instructed to adopt hematopoietic fates? Do different mechanisms of lineage commitment exist in different regions of the embryo? This latter possibility seems likely, since many examples of hemangioblastic intermediates have been documented in the literature in the extraembryonic yolk sac. Keller and colleagues have shown clonal differentiation to both the hematopoietic and endothelial lineages from embryonic stem cells [8,9]. More recently, this group has also demonstrated that hemangioblasts can also be purified prospectively from the primitive streak of the early mouse embryo [10]. On the contrary, direct visualization of embryonic stem cell behavior *in vitro* through timelapse imaging has suggested that blood cells emerge from adherent endothelial-like cells [11•]. Similarly, a recent study focused on hematopoietic differentiation from embryonic stem cells suggests that hemogenic endothelium arises directly from hemangioblastic intermediates [12••], which may unify the two concepts in early blood cell development. Many questions thus remain to be answered regarding hematopoietic specification during embryogenesis.

### The zebrafish embryo to study hematopoiesis

Our laboratory and others have been utilizing the unique strengths of zebrafish to address hematopoietic ontogeny in alternate ways. Although a relatively new model system, many studies over the past decade have demonstrated that the key genetic regulators of hematopoiesis have been highly conserved throughout evolution. For example, zebrafish erythroid development relies upon the transcription factor *gata1* [13], myeloid development upon *pu.1* [14], and HSC specification upon *cmyb* and *runx1* [15,16], similar to previous findings in the mouse [17–19]. In addition, the sequential waves of hematopoietic cell development perfectly match the distinct waves of blood cell generation observed in the mammalian embryo. Embryonic hematopoiesis occurs through two major waves as defined by the characteristics of cell types produced. Primitive hematopoiesis generates the first blood cells in development to meet the needs of the rapidly growing embryo. These first hematopoietic waves

**Figure 1 Similarity of hematopoietic development in zebrafish and mouse**




In mouse (upper left panel), primitive hematopoiesis initiates in the yolk sac, producing primitive macrophages (green) and erythroid cells (pink). Later, definitive EMPs emerge in the yolk sac (blue). HSCs are specified in the aorta, gonad, and mesonephros (AGM, red) region. Zebrafish hematopoiesis is similar: primitive macrophages arise from cephalic mesoderm and migrate onto the yolk ball (green). Primitive erythrocytes develop in the intermediate cell mass (ICM; pink). The first definitive progenitors are EMPs (blue), which develop in the PBI. Later, HSCs arise in the AGM region (red). Similar hematopoietic events in mouse and fish are color-matched between upper right and left panels. Timeline comparing developmental events is shown at bottom of figure. E, embryonic day; EMP, erythromyeloid progenitor; hpf, hours post fertilization; HSC, hematopoietic stem cell; PBI, posterior blood island.

are unique in that only unipotent precursors are specified from mesoderm to generate either primitive erythrocytes, which provide oxygen to expanding organs, or macrophages, which remodel developing tissues through removal of apoptotic corpses. Whereas both lineages arise in the extraembryonic yolk sac between E8-9 in mammals, each primitive cell subset arises in different locations in the zebrafish embryo (Fig. 1). Primitive erythrocytes are formed in the intermediate cell mass in the trunk of the embryo [20,21], whereas primitive macrophages derive from cephalic mesoderm [22]. Following the birth of these two embryonic lineages, definitive hematopoiesis arises and is characterized by the generation of multipotent precursors. Similar to primitive hematopoiesis, definitive hematopoiesis also occurs in two independent phases. Erythromyeloid progenitors (EMPs) are formed in the murine yolk sac [23,24] or in the posterior blood island (PBI) of the zebrafish embryo [25] (Fig. 1). EMPs have a restricted differentiation program in that they can only generate erythroid and myeloid cells [23,25–27]. EMPs appear to be a transient population because they cannot self-renew when transplanted [25,28]. Palis and colleagues have speculated that the EMP likely evolved to provide innate immune protection via the production of myelomonocytic cells and hemostatic protection via the production of

platelets [29] before HSCs are first produced. These are excellent points, since production of these mature, effector cells from the first HSCs does not occur for another several days in either mouse or zebrafish development. In mammalian and avian embryos, HSCs are generated *de novo* in the aorta-gonads-mesonephros (AGM) region [30,31], specifically arising in the ventral floor of the aorta [32]. It has been argued that the AGM is the exclusive site of HSC production in the embryo, but recent findings have suggested that HSC production is likely more widespread throughout embryonic and extraembryonic tissues (see Fig. 1). Yoder and colleagues [33,34] have demonstrated that HSC activity can be found in the yolk sac slightly before or concurrent with the start of circulation. Similarly, the placenta has recently been identified to harbor a large number of HSCs, and the finding that placental HSCs are present in embryos lacking a heartbeat, and therefore circulation, strongly suggests that these cells arise *de novo* [35,36]. In the zebrafish, which lacks a yolk sac and placenta, the first HSCs are detectable along the ventral wall of the aorta [15,37•,38,39]. Whereas the presence of HSCs in this region had been long suspected based upon the expression of *c-myb* and *runx1* in cells along the ventral aorta, functional support for this postulate was lacking until recently.

## Tracing hematopoietic progenitor clones

The zebrafish embryo remains translucent for several days of development and, with the creation of fluorescent transgenic lines, presents an optimal system to image the behavior of hematopoietic precursors in their natural environments. A variety of transgenic animals expressing fluorescent proteins under control of hematopoietic promoters now exist (Table 1), many of which are useful to visualize hematopoietic stem and progenitor cells in the developing embryo. Whole embryo imaging can prove powerful to follow the overall behavior of a population of cells, but tracking a single cell or clone of cells can be challenging. Another challenge is that there are very few genes that are expressed only within a particular cell type; this is especially problematic in HSCs in which no single marker can be used to isolate or follow HSCs specifically. To circumvent these issues, we and others have employed laser activation of caged fluorochromes in single or defined numbers of hematopoietic precursor cells to follow their migration and proliferation over time.

**Table 1 Fluorescent transgenic lines available for the study of zebrafish hematopoiesis**

| Transgenic line | Blood cell type | Reference |
|---|---|---|
| gata1:gfp, gata1:DsRed | Erythrocytes | [40,41] |
| mpx:gfp | Neutrophils, monocytes | [42] |
| lysC:gfp, lysC:DsRed | Neutrophils, macrophages | [43] |
| cd45:DsRed | Myeloid cells | [37•] |
| rag2:gfp | Lymphoid precursors | [44] |
| lck:gfp | T cells | [45] |
| cd41:gfp | Thrombocytes, EMPs, HSCs | [46] |
| scl:gfp | HSCs | [47] |
| c-myb:gfp | HSCs | [48] |
| runx1-P2:gfp | HSCs | [49••] |
| runx1-P1:gfp | EMPs | [49••] |
| lmo2:gfp x gata1:DsRed | EMPs | [25] |
| flk1:gfp, flk1:rfp | Vasculature, prehematopoietic mesoderm | [50] |
| fli1:gfp, fli1:DsRed | Vasculature, prehematopoietic mesoderm | [51] |
| lmo2:gfp, lmo2:DsRed | Vasculature, prehematopoietic mesoderm | [52] |

List of relevant transgenic animals currently available in the laboratory, indicating the promoter driver, the reporter fluorophore and the cell population identified.

### Tracking clones by photoactivation of caged fluorophores

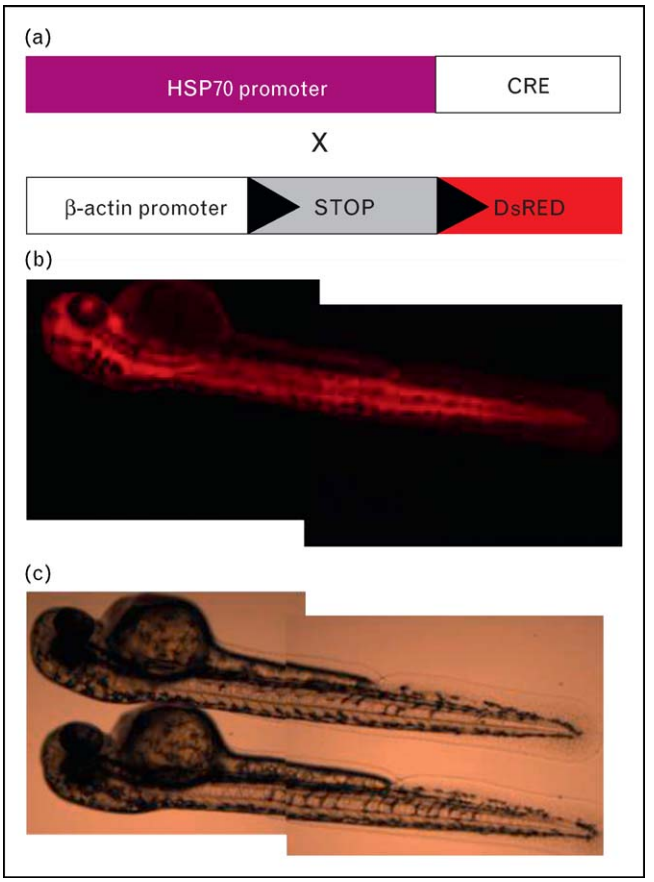
Photoactivation requires the use of caged fluorescent compounds and a microscope-based laser emitting an output frequency sufficient to break the covalent bonds formed by the caging reagent. This technique also requires that every cell contain a photoactivable dye, such as caged rhodamine or caged fluorescein, whose fluorescence is inactivated by the caging process. In order to guarantee uniform distribution of the caged dye in all the cells of the embryo, zygotes are injected. A laser tuned to 365 nm is used to uncage either caged Q-rhodamine, resulting in red fluorescence emission, or caged fluorescein, resulting in green fluorescence emission. In the translucent zebrafish embryo, cells can be targeted by their anatomic location, by expression of a fluorescent transgene, or both. Vogeli *et al.* [53] have recently utilized this technique, based on embryonic staging and anatomy, to demonstrate hemangioblastic activity of single cells targeted in gastrula stage embryos through retrospective analyses. In older embryos at 21 hours post fertilization (hpf), Jin *et al.* [38] uncaged fluorescein in cells marked by a Fli-1:eGFP transgene along the ventral wall of the aorta. Five days later, cells coexpressing uncaged fluorescein and *rag-2* were observed in the thymus [38]. Interestingly, fli-1 is normally considered a vascular-specific marker, suggesting that the targeted cells may represent hemogenic endothelium. We recently utilized this technique to follow the behavior of cells expressing a CD41:eGFP transgene in the zebrafish embryo at later stages. CD41 is a target of the *scl* transcription factor [54], and is perhaps the first marker of mesodermal commitment to the definitive hematopoietic fates [54–56]. In zebrafish embryos at 40 hpf, CD41:eGFP$^{+}$ cells are present in the posterior blood island, and within the ventral wall of the aorta. By uncaging rhodamine in aortic CD41:eGFP$^{+}$ cells, we demonstrated that the progeny of targeted cells robustly colonized the thymus to generate *rag-2*$^{+}$ thymocytes, a trait that only HSCs should possess in the early embryo. By contrast, targeting of CD41:eGFP+ cells in the PBI never resulted in thymic progeny [25]. Transplantation,

gene expression analyses, and in-vitro differentiation of CD41$^+$, LMO2$^+$, GATA-1$^+$ cells isolated from the PBI showed them to be committed EMPs. Additional fate mapping of earlier LMO2:eGFP$^+$ cells in the converging stripes of posterior mesoderm demonstrated that uncaged cells gave rise to small, round cells that localized to perivascular regions within the labyrinth of vessels in the PBI. This is precisely the location in which the first CD41:eGFP$^+$ or LMO2:eGFP$^+$, GATA-1:DsRed$^+$ EMPs are observed, suggesting that this population arises *de novo* within the PBI. Herbomel and colleagues have also used photoactivation to trace the migration of HSCs within the ventral wall of the aorta to the ventral region of the tail, as the PBI is later remodeled into caudal hematopoietic tissue (CHT). Interestingly, these experiments have suggested that this homing to the CHT is a prerequisite to the colonization of the thymus or pronephros by HSCs [39,57•].

### Towards the permanent labeling of hematopoietic stem cell clones

Labeling by photoactivation of caged fluorescent reporters is a powerful technique, but is limited to the first several days of development since the dye is diluted by cell division. In order to circumvent this issue, we have generated a transgenic animal that can be switched from a silenced state to a fluorescent reporter following Cre-mediated recombination (Fig. 2). In this line, a 12 kb β-actin promoter controls the expression of the DsRed fluorescent reporter, which can only be transcribed following excision of a floxed repressor sequence by the Cre recombinase. When combined with a laser-inducible Cre transgene, this system should allow the clonal tracking of targeted cells over their lifetime. Towards this goal, we have been utilizing transgenic animals carrying the CRE recombinase under transcriptional control of the inducible *hsp70* promoter. When hsp70:CRE and β-actin:Switch adults are mated, they generate embryos carrying both transgenes. Ubiquitous expression of the DsRed reporter gene is observed in double transgenic embryos following heat shock at 39°C for 30 min (Fig. 2, Santoso and Traver, unpublished results). We have also observed, however, low level transgene expression in double transgenic animals in the absence of heat shock, demonstrating that the *hsp70* promoter is leaky. We are currently working to overcome this issue by using new transgenic lines with inducible Cre protein fused to the estrogen receptor. This should provide improved reliability by allowing a second level of control via restricted nuclear access of Cre only following tamoxifen administration [58]. Thus, in the very near future, we should have the means to target any cell of interest, using the same combination of GFP expression and anatomic location described above to assess the fate potential of any cell in the embryo. This system will allow the determination of lifespan, proliferative potential and migratory routes of nascent HSCs, or any other cell type of interest, in the developing embryo. Furthermore, since each clone will express a fluorescent tag, it will be possible to determine the lineages present following purification by flow cytometry. Using this system, we hope to determine precisely in which location mesoderm first commits to the HSC fate. These methods will also allow the dissection of how mesodermal commitment occurs, providing another model to test whether HSCs are necessarily born from hemangioblastic intermediates, hemogenic endothelium, or whether the definitive hematopoietic program is distinct from vascular patterning.

**Figure 2 Strategy to permanently label hematopoietic clones**




(a) We generated two transgenic lines in which the CRE recombinase is placed under the control of the inducible HSP70 promoter, and a switchable construct is placed under the control of the 12 kb β-actin promoter. (b, c) Fluorescent (b) and DIC (c) images of the progeny of these lines. After heat shock at 38°C, double transgenic animals (upper panel in b and c) turn on DsRed expression ubiquitously, whereas single transgenic embryos (lower panel in b and c) for the switch construct only do not turn red (photographs courtesy of Dr Buyung Santoso).

## Conclusion

Many recent publications have demonstrated that mesodermal commitment to the hematopoietic fates is

surprisingly complex, with at least four distinct waves of precursor production during development. A challenge is thus to better understand how HSCs are specifically patterned, since this cellular subset is the only one of the four to remain throughout adulthood. This is an interesting problem from a developmental biology perspective, and an important problem for eventual clinical applications using HSCs derived from embryonic stem cells. With the incredible advances in cellular reprogramming by the induced pluripotency factors, the major challenge for regenerative medicine now lies in the ability to replicate development in the culture dish. We hope to learn the stepwise process through which the embryo instructs mesoderm to adopt the HSC fate through genetic and imaging approaches in the zebrafish, and ultimately translate this knowledge to instruct embryonic stem cell differentiation to HSCs via provision of the same molecular cues.

## Acknowledgements

The work was funded by the California Institute for Regenerative Medicine (J.Y.B. and D.T.), the American Society of Hematology (D.T.), and the National Institutes of Health (D.T.).

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 316).


**1** Gothert JR, Gustin SE, Hall MA, *et al.* In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis. Blood 2005; 105:2724–2732.

**2** • Zovein AC, Hofmann JJ, Lynch M, *et al.* Fate tracing reveals the endothelial origin of hematopoietic stem cells. Cell Stem Cell 2008; 3:625–636.
This work shows that the adult hematopoietic system derives from VE-Cadherin+ endothelial cells in the mid-gestation embryo.

**3** •• Chen MJ, Yokomizo T, Zeigler BM, *et al.* Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. Nature 2009; 457:887–891.
This study also shows that VE-Cadherin+ endothelial cells are the source of adult hematopoiesis. This study also clearly shows the importance of Runx1 for the transition between endothelial and hematopoietic cells, as selective deletion of Runx1 in endothelial cells prevents the development of hematopoietic stem cells.

**4** de Bruijn MF, Ma X, Robin C, *et al.* Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. Immunity 2002; 16:673–683.

**5** Jaffredo T, Gautier R, Eichmann A, Dieterlen-Lievre F. Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny. Development 1998; 125:4575–4583.

**6** Nishikawa SI, Nishikawa S, Kawamoto H, *et al.* In vitro generation of lymphohematopoietic cells from endothelial cells purified from murine embryos. Immunity 1998; 8:761–769.

**7** Oberlin E, Tavian M, Blazsek I, Peault B. Blood-forming potential of vascular endothelium in the human embryo. Development 2002; 129:4147–4157.

**8** Choi K, Kennedy M, Kazarov A, *et al.* A common precursor for hematopoietic and endothelial cells. Development 1998; 125:725–732.

**9** Fehling HJ, Lacaud G, Kubo A, *et al.* Tracking mesoderm induction and its specification to the hemangioblast during embryonic stem cell differentiation. Development 2003; 130:4217–4227.

**10** Huber TL, Kouskoff V, Fehling HJ, *et al.* Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 2004; 432:625–630.

**11** • Eilken HM, Nishikawa S, Schroeder T. Continuous single-cell imaging of blood generation from haemogenic endothelium. Nature 2009; 457:896–900.
The authors observed the development of single cells *in vitro* starting from embryonic stem cells. They showed that development of blood proceeds through an endothelial stage.

**12** •• Lancrin C, Sroczynska P, Stephenson C, *et al.* The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. Nature 2009; 457:892–895.
This study elucidates the different intermediates through which embryonic stem cells proceed, in order to differentiate into blood. This model only applies to yolk sac hematopoiesis.

**13** Lyons SE, Lawson ND, Lei L, *et al.* A nonsense mutation in zebrafish gata1 causes the bloodless phenotype in vlad tepes. Proc Natl Acad Sci U S A 2002; 99:5454–5459.

**14** Rhodes J, Hagen A, Hsu K, *et al.* Interplay of pu.1 and gata1 determines myelo-erythroid progenitor cell fate in zebrafish. Dev Cell 2005; 8:97–108.

**15** Burns CE, Traver D, Mayhall E, *et al.* Hematopoietic stem cell fate is established by the Notch-Runx pathway. Genes Dev 2005; 19:2331–2342.

**16** Kalev-Zylinska ML, Horsfield JA, Flores MV, *et al.* Runx1 is required for zebrafish blood and vessel development and expression of a human RUNX1-CBF2T1 transgene advances a model for studies of leukemogenesis. Development 2002; 129:2015–2030.

**17** Mucenski ML, McLain K, Kier AB, *et al.* A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. Cell 1991; 65:677–689.

**18** North T, Gu TL, Stacy T, *et al.* Cbfa2 is required for the formation of intra-aortic hematopoietic clusters. Development 1999; 126:2563–2575.

**19** Wang Q, Stacy T, Binder M, *et al.* Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc Natl Acad Sci U S A 1996; 93:3444–3449.

**20** Detrich HW 3rd, Kieran MW, Chan FY, *et al.* Intraembryonic hematopoietic cell migration during vertebrate development. Proc Natl Acad Sci U S A 1995; 92:10713–10717.

**21** Thompson MA, Ransom DG, Pratt SJ, *et al.* The cloche and spadetail genes differentially affect hematopoiesis and vasculogenesis. Dev Biol 1998; 197:248–269.

**22** Herbomel P, Thisse B, Thisse C. Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development 1999; 126:3735–3745.

**23** Bertrand JY, Giroux S, Golub R, *et al.* Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proc Natl Acad Sci U S A 2005; 102:134–139.

**24** Bertrand JY, Jalil A, Klaine M, *et al.* Three pathways to mature macrophages in the early mouse yolk sac. Blood 2005; 106:3004–3011.

**25** Bertrand JY, Kim AD, Violette EP, *et al.* Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. Development 2007; 134:4147–4156.

**26** Palis J, Chan RJ, Koniski A, *et al.* Spatial and temporal emergence of high proliferative potential hematopoietic precursors during murine embryogenesis. Proc Natl Acad Sci U S A 2001; 98:4528–4533.

**27** Palis J, Robertson S, Kennedy M, *et al.* Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 1999; 126:5073–5084.

**28** Cumano A, Ferraz JC, Klaine M, *et al.* Intraembryonic, but not yolk sac hematopoietic precursors, isolated before circulation, provide long-term multilineage reconstitution. Immunity 2001; 15:477–485.

**29** Tober J, Koniski A, McGrath KE, *et al.* The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. Blood 2007; 109:1433–1441.

**30** Cumano A, Dieterlen-Lievre F, Godin I. Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. Cell 1996; 86:907–916.

**31** Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 1996; 86:897–906.

**32** Pardanaud L, Luton D, Prigent M, *et al.* Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. Development 1996; 122:1363–1371.

**33** Yoder MC, Hiatt K, Dutt P, *et al.* Characterization of definitive lymphohematopoietic stem cells in the day 9 murine yolk sac. Immunity 1997; 7:335–344.

**34** Yoder MC, Hiatt K, Mukherjee P. In vivo repopulating hematopoietic stem cells are present in the murine yolk sac at day 9.0 postcoitus. Proc Natl Acad Sci U S A 1997; 94:6776–6780.

**35** Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. Dev Cell 2005; 8:365–375.

**36** Rhodes KE, Gekas C, Wang Y, *et al.* The emergence of hematopoietic stem cells is initiated in the placental vasculature in the absence of circulation. Cell Stem Cell 2008; 2:252–263.

**37** • Bertrand JY, Kim AD, Teng S, Traver D. CD41+ cmyb+ precursors colonize the zebrafish pronephros by a novel migration route to initiate adult hematopoiesis. Development 2008; 135:1853–1862.
In this study, we characterized the first HSCs in the zebrafish embryo, and one possible way for them to reach their site of residence in the kidney marrow.

**38** Jin H, Xu J, Wen Z. Migratory path of definitive hematopoietic stem/progenitor cells during zebrafish development. Blood 2007; 109:5208–5214.

**39** Murayama E, Kissa K, Zapata A, *et al.* Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. Immunity 2006; 25:963–975.

**40** Long Q, Meng A, Wang H, *et al.* GATA-1 expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. Development 1997; 124:4105–4111.

**41** Traver D, Paw BH, Poss KD, *et al.* Transplantation and in vivo imaging of multilineage engraftment in zebrafish bloodless mutants. Nat Immunol 2003; 4:1238–1246.

**42** Renshaw SA, Loynes CA, Trushell DM, *et al.* A transgenic zebrafish model of neutrophilic inflammation. Blood 2006; 108:3976–3978.

**43** Hall C, Flores MV, Storm T, *et al.* The zebrafish lysozyme C promoter drives myeloid-specific expression in transgenic fish. BMC Dev Biol 2007; 7:42.

**44** Jessen JR, Jessen TN, Vogel SS, Lin S. Concurrent expression of recombination activating genes 1 and 2 in zebrafish olfactory sensory neurons. Genesis 2001; 29:156–162.

**45** Langenau DM, Ferrando AA, Traver D, *et al.* In vivo tracking of T cell development, ablation, and engraftment in transgenic zebrafish. Proc Natl Acad Sci U S A 2004; 101:7369–7374.

**46** Lin HF, Traver D, Zhu H, *et al.* Analysis of thrombocyte development in CD41-GFP transgenic zebrafish. Blood 2005; 106:3803–3810.

**47** Zhang XY, Rodaway AR. SCL-GFP transgenic zebrafish: in vivo imaging of blood and endothelial development and identification of the initial site of definitive hematopoiesis. Dev Biol 2007; 307:179–194.

**48** North TE, Goessling W, Walkley CR, *et al.* Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 2007; 447:1007–1011.

**49** •• Lam EY, Chau JY, Kalev-Zylinska ML, *et al.* Zebrafish runx1 promoter-EGFP transgenics mark discrete sites of definitive blood progenitors. Blood 2009; 113:1241–1249.
This study shows that the two isoforms of Runx1 are differentially expressed in EMPs and HSCs in the early zebrafish embryo. Moreover the authors established two different transgenic lines that reproduce these two patterns of expression, which will be of great interest to study the different signals involved in EMPs versus HSCs specification.

**50** Jin SW, Beis D, Mitchell T, *et al.* Cellular and molecular analyses of vascular tube and lumen formation in zebrafish. Development 2005; 132:5199–5209.

**51** Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. Dev Biol 2002; 248:307–318.

**52** Zhu H, Traver D, Davidson AJ, *et al.* Regulation of the lmo2 promoter during hematopoietic and vascular development in zebrafish. Dev Biol 2005; 281:256–269.

**53** Vogeli KM, Jin SW, Martin GR, Stainier DY. A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. Nature 2006; 443:337–339.

**54** Mikkola HK, Fujiwara Y, Schlaeger TM, *et al.* Expression of CD41 marks the initiation of definitive hematopoiesis in the mouse embryo. Blood 2003; 101:508–516.

**55** Ferkowicz MJ, Starr M, Xie X, *et al.* CD41 expression defines the onset of primitive and definitive hematopoiesis in the murine embryo. Development 2003; 130:4393–4403.

**56** Mitjavila-Garcia MT, Cailleret M, Godin I, *et al.* Expression of CD41 on hematopoietic progenitors derived from embryonic hematopoietic cells. Development 2002; 129:2003–2013.

**57** • Kissa K, Murayama E, Zapata A, *et al.* Live imaging of emerging hematopoietic stem cells and early thymus colonization. Blood 2008; 111:1147–1156.
This study describes beautiful imaging of the emergence of HSCs in th zebrafish AGM and their migration to the thymic anlage.

**58** Feil R, Brocard J, Mascrez B, *et al.* Ligand-activated site-specific recombination in mice. Proc Natl Acad Sci U S A 1996; 93:10887–10890.