

Medical sciences / Sciences médicales

# Emergence of haematopoietic stem cells during development

Françoise Dieterlen-Lièvre

UPR 2197 CNRS, 33, av. de la Terrasse, 91498 Gif-sur-Yvette cedex, France

Received 20 December 2006; accepted after revision 4 May 2007

Available online 12 June 2007

Presented by Jean-François Bach and Nicole Le Douarin

## Abstract

Self-renewable haematopoietic stem cells (HSCs) become segregated during development into a finite pool, from which they are mobilized upon physiological requirement. A central feature characterizing developmental haematopoiesis is that definitive organs become colonized by HSCs originating from a central source. The emission of HSCs occurs more or less continuously during a protracted period in parallel or successive sites. The most recently discovered of these sites is the placenta. The allantois, which is one of the components of the placenta, probed before it becomes vascularised, turns out to be a location where clonogenic precursors become committed. The placenta is thus a site of intrinsic haematopoiesis. Until this finding, the aorta and periaortic tissues were held to be the sites of definitive HSC commitment. The haematopoietic process in the aorta is prominent, particularly in avian embryos, and displays striking anatomical relationships between endothelial and haematopoietic cells. This made it possible to investigate the cytological and molecular relationship between the two types of cells. Somite exchanges between quail and chicken disclosed two distinct lineages, a dorsal one, purely endothelial, and a ventral one, hemangioblastic. The latter, also termed hemogenic endothelium, builds at first the whole inside lining of the aorta, and is then progressively replaced by cells of somitic origin, beginning with the aortic roof; it emits haematopoietic cells when located in the floor of the aorta and disappears. These events involve a changing molecular pattern, with expressions of transcription factor Runx1 and receptor VEGF-R2 as faithful markers of the lineage switch. Taking advantage of the stereotyped anatomical arrangement at the aortic level, which is favourable to dissect the mechanisms of HSC commitment, the analysis of developmental haematopoiesis should progress still further. **To cite this article:** F. Dieterlen-Lièvre, C. R. Biologies 330 (2007).

© 2007 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

## Résumé

**Émergence des cellules souches hématopoïétiques pendant le développement.** Les organes hématopoïétiques doivent être colonisés au cours du développement par des cellules souches extrinsèques (cellules souches hématopoïétiques = CSH). Ces CSH autorenewables se ségrègent pour constituer une réserve, d'où elles se mobilisent lors des besoins physiologiques. L'émission de CSH se produit plus ou moins continûment pendant une certaine période du développement, dans des sites parallèles ou successifs. Le plus récemment découvert de ces sites est le placenta. L'ébauche de l'allantoïde donne la partie fœtale de l'arbre vasculaire du placenta. Testée avant vascularisation, cette annexe de l'embryon contient des progéniteurs clonogéniques, qui se sont donc formées in situ. On peut en conclure que le placenta est un site de détermination de CSH, rôle jusqu'à présent considéré comme dévolu à l'aorte ou aux tissus péri-aortiques à cette période du développement. Par ailleurs, les relations de développement entre lignages hématopoïétique et endothélial ont été analysées au niveau de l'hématopoïèse aortique dans le modèle aviaire. L'échange de somites entre caille et poulet a révélé l'existence de deux lignages distincts, l'un dorsal et purement endothélial, l'autre ventral,

E-mail address: [francoise.dieterlen@club-internet.fr](mailto:francoise.dieterlen@club-internet.fr).

qui constitue le revêtement primitif de l'aorte, émet des cellules hématopoïétiques, puis disparaît, remplacé par des angioblastes d'origine somitique. Le processus hématopoïétique, qui a lieu dans le plancher de l'aorte, est précédé d'une évolution moléculaire, qui met en jeu notamment le facteur de transcription Runx1 et le récepteur VEGF-R2. **Pour citer cet article : F. Dieterlen-Lièvre, C. R. Biologies 330 (2007).**

© 2007 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

**Keywords:** Haematopoietic stem cells; Placenta; Allantois; Aorta

**Mots-clés :** Cellules souches hématopoïétiques ; Placenta ; Allantoïde ; Aorte

## 1. Introduction

Self-renewable haematopoietic stem cells (HSCs) make up a finite pool, from which they are mobilized during the whole lifetime upon physiological requirement. This pool is supposed to segregate during development. The circumstances of this segregation, an active field of study for nearly a century, are still fraught with enigmas. Originally, it was thought that this event occurred during a short period of the embryonic life; it was since found that commitment of HSCs is a more durable process that moves from a first site, the yolk sac, to a succession of others during embryonic and early foetal life (Fig. 1). Whether the whole span of sites and the developmental period involved has now been described is, in my mind, an open question. The mechanisms of HSC commitment during development include tissue interactions, intercellular signalling, and activation of transcription factors. Understanding the whole sequence would undoubtedly bring about information on the possibilities of waking this process up in later life, an interesting prospect for cell therapy.

## 2. Sites of HSC emergence

Sites where HSCs expand and differentiate are usually distinct from sites where they become committed. This feature was uncovered through experimental approaches in which organ rudiments were retrieved from an embryo and grafted into another, donor and host differing by a marker to diagnose the origin of cell types in the differentiated organ. This approach was originally devised in a chicken/chicken model and perfected in the quail/chicken model [1]. In the chicken model, the marker relied on the sexual chromosomes in male/female combinations. In the quail/chicken model [2], the marker was provided by the difference in heterochromatin appearance. Thymus, avian bursa of Fabricius, mammalian foetal liver, spleen, bone marrow, were shown to include a stromal compartment derived from the rudiment, and a haematopoietic compartment entirely derived from blood-borne progenitors. The

only organ originally recognized as producing HSCs was the yolk sac, hence the hypothesis attributing to this appendage a unique role in the formation of the haematopoietic system [3].

However, a yolk sac-independent source of HSCs was uncovered by means of chimeric embryos, in which a quail body was engrafted onto a chicken yolk sac [4]. The region of the aorta was then shown, both in the avian and mammalian embryo, to produce HSCs for a short period [5], and was considered until recently as the ultimate site of HSC emergence.

The avian model recently permitted the discovery of a similar role for the allantois. In the avian embryo, the allantoic rudiment is a late-forming bud, emitted by the caudal intestine 24 h after gastrulation has ended. It becomes vascularised by in-situ-committed angioblasts [6] and displays haemoglobinised cell groups that look very much like blood islands. When the still unvascularised quail allantoic rudiment was grafted in a chicken host coelom, the bone marrow of the host became significantly colonized by quail haematopoietic cells (up to 8% of the total haematopoietic population). Furthermore, quail endothelial cells also appeared in the host medullary vascular network [6,7]. In this set of experiments, quail cells were sorted out or stained by means of the hemangioblastic quail-specific antibody QH1 [8].

These data prompted the search for a haematopoietic role of the mammalian placenta, since this appendage forms through the fusion of the allantois with the ectoplacental cone (Fig. 2). In a first approach, in vitro clonogenic progenitors were shown to be abundant in the placenta [9]. They were found consistently from day 10 of gestation, until day 17. Interestingly, in comparison with the foetal liver – a rudiment in which, as seen above, the haematopoietic population is entirely of extrinsic origin –, the quantitative and qualitative composition of the placental haematopoietic population displays specific traits:

- it is first detected 24 h earlier than in the liver;
- the proportion of early versus late progenitors is much higher at all stages than in the liver (Fig. 3).

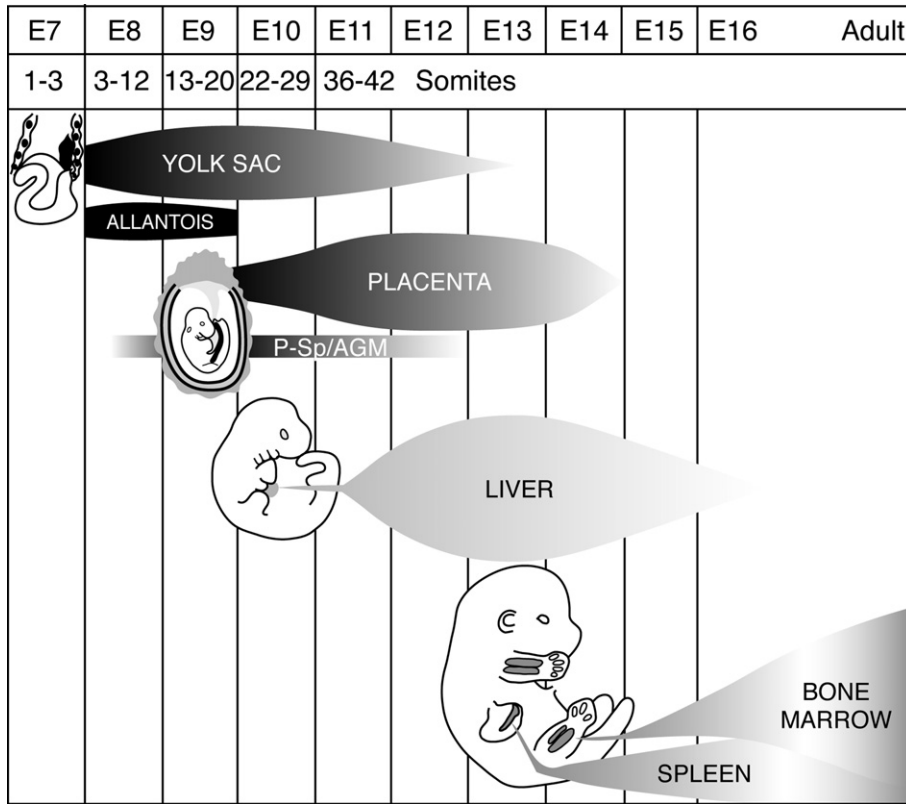


Fig. 1. Synopsis of the development of the haematopoietic system in the mouse embryo and foetus. Extra-embryonic appendages and intra-embryonic organs are involved successively or in parallel in the emission of HSCs (yolk sac, allantois, aortic region – designated as P-SP/AGM for para-aortic splanchnopleura/aorta-gonad-mesonephros –, placenta), and others in the amplification and differentiation of this cell population (liver, spleen, bone marrow). A sequence of sites, exemplified here in the mouse, is the rule in all vertebrates.

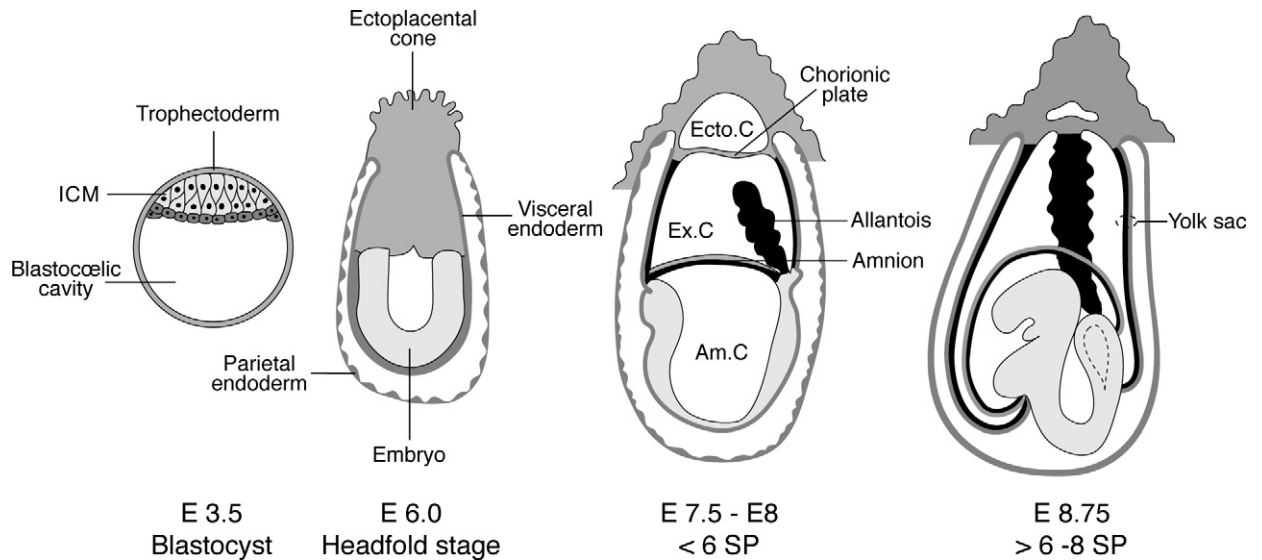


Fig. 2. Development of the mouse embryo. The placenta forms through the fusion of the allantois to the chorionic plate occurring between the stages of six and eight pairs of somites, and becomes vascularised at the same time. Am.C: amniotic cavity. Ecto.C: ectoplacental cavity. Ex.C: exocoelom. ICM: inner cell mass. SP: somite pairs.

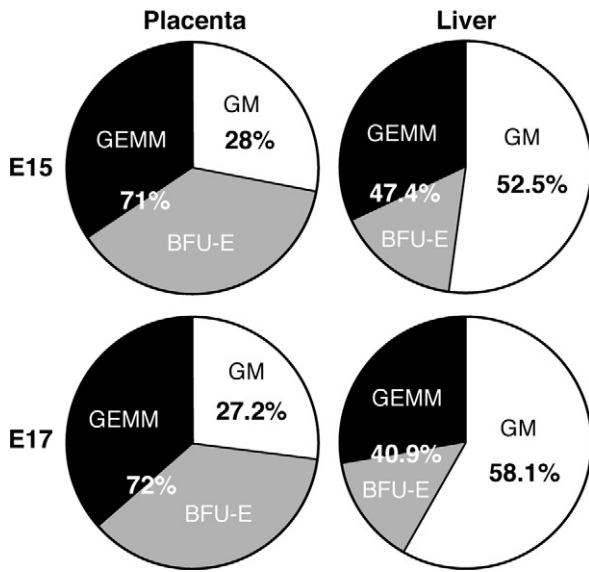


Fig. 3. The placenta is rich in early clonogenic progenitors (GEMM: granulocyte/erythrocyte/macrophage/monocyte colonies; BFU-E: burst forming unit/erythroid) compared to the liver, where committed GM (granulocyte/macrophage) colonies are more abundant. E15, 17: days of gestation.

These data point to an important role of the placenta in the development of the haematopoietic system. However, in order to appreciate its true role, two crucial facts had to be ascertained: (i) whether bona fide HSCs are present (namely Long-Term Repopulating HSCs, which display self-renewal potential for at least 6 months); (ii) whether these cells emerge in situ. LTR HSCs, detected by injection into irradiated adults, were found in the placenta from day 10.5–11 to day 14 [10], i.e., for a shorter period than clonogenic progenitors; like the latter, LTR HSCs were found earlier in the placenta than in the liver. The belated detection of LTR HSCs, by reference to clonogenic progenitors, is explained by the larger number of cells required for in vivo reconstitution, compared to in vitro seeding. The more abrupt disappearance has probably two causes: the progenitors emigrate, and if commitment ceases, the ones still present evolve to more mature states, that is, still capable of yielding colonies in vitro, but no more endowed with LTR potential.

The second aspect concerns the origin of the HSCs or progenitors that are present in the placenta: do they become committed in situ or do they colonize the placenta? In order to determine this point, experiments must be carried out at very early stages, prior to vascularisation, which occurs in the allantois between the stages of six and eight pairs of somites, prior to the formation of the placenta. In view of the minute number of

Table 1

Comparison of LTR HSC numbers between placenta (PI) and aortic region (AGM). At E10.5, the caudal half of the embryo, which contains the AGM, was used. The figures are the numbers of 'long-term reconstituting units per embryo equivalent' = RU. ee (number of reconstituted receivers/total number of receivers/transplanted dose; the number of cells obtained at these stages from the pool of embryos used for reconstitution is too small to be counted). The number of RU. ee is similar in the two sites at E10.5 and E11.5. It is multiplied by 20 in the placenta at E12.5, while it remains small in the AGM. RU. ee disappear from the AGM at E13.5 and from the placenta at E15.5. The liver contains RU. ee only from E11.5 onwards, the delay corresponding to the requirement for colonization. During the following days, the overall haematopoietic population in the liver amplifies considerably, so that RU. ee become twice as numerous as in the placenta. However, as seen in Fig. 3, they are significantly outgrown by the progenitor population

	P1	Caudal half or AGM
E10.5	0.103	0.107
E11	0.48	0.31
E11.5	2.5	2.0
E12.5	50	3.3

cells that can be obtained from these early rudiments, the clonogenic methodology was again applied [11]. As recorded above, progenitors were first detected in the placenta at the stage of 20 pairs of somites. It was thus unlikely to find them in the allantois prior to the 6-somite stage. In order to allow the cell interactions required for HSC commitment, allantoic buds retrieved between one and six pairs of somites were pre-cultured in toto prior to dissociation. In these conditions, colonies developed from these early rudiments, while the possibility of colonization was excluded. Two studies carried out in parallel, with protocols differing only in slight detail, arrived at the same conclusion [11,12].

Thus, it can be concluded that the placenta is a site where HSCs become committed. Indeed, it is likely that this organ plays a major role in the development of the haematopoietic system, probably more important than the aortic region. The period of placental activity lasts 4 days, versus 36 h in the aortic region; furthermore, the number of progenitors or HSCs found is much larger in the placenta (Table 1).

### 3. Intra-aortic haematopoiesis

In the case of the yolk sac, tissue dissociation and re-association experiments have shown that, to carry out haematopoietic activity, mesoderm must have a contact with endoderm [13–15]. In the aorta, which is the next site engaged in haematopoiesis, this activity displays a stereotyped aspect in all vertebrates studied: clusters of haematopoietic cells are appended to the aortic ven-

tral endothelium. This unique anatomical arrangement offers an opportunity to study the origin of the cells involved and the relationship between blood cells and endothelial cells, a question that divides the community of blood and vessel specialists. Efficient experimental approaches could be carried out *in vivo* in the avian embryo [16–18]. Since a corpus of data had established the existence of somitic progenitors of endothelial cells, quail somites were grafted orthotopically in place of chicken somites on day 2 (E2) of development, when the aorta is represented by two distinct rudiments. As the two rudiments fuse, cells derived from the grafted-quail somitic material are seen to integrate into the roof of the aorta. From there on, these quail cells trickle down along the sides of the aorta, finally replacing the original host endothelial cells, which disappear altogether. The ventral haematopoietic cell clusters are all chicken ones in these chimeras, *i.e.*, host in origin; as they pursue their evolution, either by entering the aortic blood flow or by migrating into the mesentery, they disappear from the ventral endothelium, and are replaced by quail cells. In other words, cells derived from splanchnopleural mesoderm compose the early aortic endothelium and they are progressively replaced by somite-derived angioblasts, when they switch their fate to haematopoiesis.

These experiments demonstrate that the blood/vascular system derives from two distinct mesodermal lineages, a ventral one, which is hemangioblastic (endowed with blood and endothelial potentials), and a dorsal one, which is purely angioblastic. The dorsal lineage is also responsible for the vascularisation of the body wall, while the viscera are vascularised by the ventral mesoderm. In the transplantation experiments, the somitic-derived endothelial cells prove incapable of entering the viscera. The haematopoietic process occurring at the expense of the primary endothelial cells is detected at a very early stage by the activation of haematopoietic specific transcription factors such as *Runx1* and *c-myb* and the inactivation of the hemangioblastic–angioblastic specific receptor VEGFR2 [19].

It has been possible to manipulate these distinct dorsal and ventral potentials by treating the respective mesodermal layers, either by tissue contact with tissues, or by growth factors. Ventral mesoderm associated with ectoderm for 12 h prior to grafting or cultured on a semi-solid medium containing EGF or TGF $\alpha$  loses the potential to invade visceral mesoderm as well as to integrate into the floor of the aorta and undergo haematopoiesis. Inversely, dorsal mesoderm associated with endoderm or treated with VEGF or TGF $\beta$  acquires this potential [16]. The signals at work in normal development still

have to be worked out. In particular, it will be interesting to know how long the endodermal signal is necessary. Paradoxically, the mammalian allantois, which forms at a very early stage as gastrulation proceeds, is a purely mesodermal rudiment. In contrast, the avian allantoic bud is emitted by the caudal intestine and has the same germ layer makeup, *i.e.*, mesoderm lined with endoderm. It is likely that a short contact with nascent endoderm occurs as gastrulation is ongoing.

#### 4. Conclusions

The present review emphasises recent findings obtained from both avian and mammalian models. In the continuity of previous investigations, several further aspects of the early development of the haematopoietic and endothelial systems were unravelled in the avian embryo by means of *in vivo* experiments and were then extended to the mammalian embryo. Classical and novel features are summarized below:

- extrinsic HSCs colonize haematopoietic organ rudiments;
- contact with endoderm allows mesoderm to become haematopoietic; this requirement, known in the case of the yolk sac, has been extended to lateral plate mesoderm, by the identification of a dorsal angioblastic lineage and a ventral hemangioblastic lineage, the first deriving from somites and the second from lateral plate splanchnopleural mesoderm;
- HSCs and angioblasts are clearly linked in development, since a flip flop between angioblasts and HSCs can be rather easily brought about by several growth factors. The precise signalling network responsible *in vivo* is not worked out; it should be pointed in this regard that the production of haematopoietic cells by the floor of the aorta depends on precise cell peregrinations: cells from the ventral lateral mesoderm migrate centripetally to form the two aortic rudiment endothelia; as the two rudiments fuse, these cells are progressively driven ventrally by the insertion of somite-derived angioblasts; they enter the haematopoietic pathway exclusively when located in the floor of the aorta. Whether this precise pattern is chronological or due to the availability of local signals in the floor is an interesting question for future analysis;
- evidence of circulating angioblasts in the avian embryo, yielded by allantoic grafts, converges with the experimental data in the adult mouse, and points to the capacity of progenitors with hemangioblastic potential to migrate in the blood flow. However, an

approach involving cross circulation between early quail and chicken embryos indicates that circulating angioblasts are rare and thus not very likely to play a significant role during normal development [20].

## References

- [1] N.M. Le Douarin, F. Dieterlen-Lièvre, P.D. Oliver, Ontogeny of primary lymphoid organs and lymphoid stem cells, *Am. J. Anat.* 170 (3) (1984) 261–299.
- [2] N.M. Le Douarin, A Feulgen-positive nucleolus, *Exp. Cell Res.* 77 (1) (1973) 459–468.
- [3] M.A.S. Moore, J.J.T. Owen, Stem cell migration in developing myeloid and lymphoid systems, *Lancet* 2 (1967) 658–659.
- [4] F. Dieterlen-Lièvre, On the origin of haemopoietic stem cells in the avian embryo: an experimental approach, *J. Embryol. Exp. Morphol.* 33 (3) (1975) 607–619.
- [5] F. Dieterlen-Lièvre, Commitment of hematopoietic stem cells in avian and mammalian embryos: an ongoing story, *Int. J. Dev. Biol.* 49 (2005) 125–130.
- [6] A. Caprioli, T. Jaffredo, R. Gautier, C. Dubourg, F. Dieterlen-Lièvre, Blood-borne seeding by hematopoietic and endothelial precursors from the allantois, *Proc. Natl Acad. Sci. USA* 95 (1998) 1641–1646.
- [7] A. Caprioli, K. Minko, C. Drevon, A. Eichmann, F. Dieterlen-Lièvre, T. Jaffredo, Hemangioblast commitment in the avian allantois: cellular and molecular aspects, *Dev. Biol.* 238 (1) (2001) 64–78.
- [8] L. Pardanaud, C. Altmann, P. Kitos, F. Dieterlen-Lièvre, C.A. Buck, Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells, *Development* 100 (2) (1987) 339–349.
- [9] M. Alvarez-Silva, P. Belo-Diabangouaya, J. Salaün, F. Dieterlen-Lièvre, Mouse placenta is a major hematopoietic organ, *Development* 130 (22) (2003) 5437–5444.
- [10] C. Gekas, F. Dieterlen-Lièvre, S.H. Orkin, H.K.A. Mikkola, The placenta is a niche for hematopoietic stem cells, *Dev. Cell* 8 (3) (2005) 365–375.
- [11] C. Corbel, J. Salaün, P. Belo-Diabangouaya, F. Dieterlen-Lièvre, Hematopoietic potential of the pre-fusion allantois, *Dev. Biol.* 301 (2) (2007) 478–488.
- [12] B.M. Zeigler, D. Sugiyama, M. Chen, Y. Guo, K.M. Downs, N.A. Speck, The allantois and chorion, when isolated before circulation or chorio-allantoic fusion, have hematopoietic potential, *Development* 133 (21) (2006) 4183–4192.
- [13] Y. Miura, F.H. Wilt, Tissue interaction and the formation of the first erythroblasts of the chick embryo, *Dev. Biol.* 19 (2) (1969) 201–211.
- [14] J. Kessel, B. Fabian, Inhibitory and stimulatory influences on mesodermal erythropoiesis in the early chick blastoderm, *Development* 101 (1) (1987) 45–49.
- [15] I. Flamme, Is extraembryonic angiogenesis in the chick embryo controlled by the endoderm? A morphology study, *Anat. Embryol. (Berl.)* 180 (3) (1989) 259–272.
- [16] L. Pardanaud, F. Dieterlen-Lièvre, Manipulation of the angiopoietic/hemangiopoietic commitment in the avian embryo, *Development* 126 (4) (1999) 617–627.
- [17] L. Pardanaud, D. Luton, M. Prigent, L.M. Bourcheix, L.M. Catala, F. Dieterlen-Lièvre, Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis, *Development* 122 (1996) 1363–1371.
- [18] C. Pouget, R. Gautier, M.-A. Teillet, T. Jaffredo, Somite-derived cells replace ventral aortic hemangioblasts and provide aortic smooth muscle cells of the trunk, *Development* 133 (6) (2006) 1013–1022.
- [19] F. Dieterlen-Lièvre, C. Pouget, K. Bollérot, T. Jaffredo, Are intra-aortic hemopoietic cells derived from endothelial cells during ontogeny?, *Trends Cardiovasc. Med.* 16 (4) (2006) 128–139.
- [20] L. Pardanaud, A. Eichmann, Identification, emergence and mobilization of circulating endothelial cells or progenitors in the embryo, *Development* 133 (13) (2006) 2527–2537.