properties. Simultaneous isolation of DNA from amoebae and their bacterial endosymbionts was performed using the FastDNA-Kit (BIO 101) according to the protocols recommended by the manufacturer. $ntt2$ and $ntt4$ were amplified with the Extensor hi-fidelity PCR enzyme mix (ABgene) using forward primers introducing a NdeI restriction site instead of the start codon (5′-CAGCATATGTCTCAACAAGAATCAGA GTTTGGT-3′ for $ntt2$, and 5′-CAGGGCCATATGAGTAAAACAAACCAGGTA-3′ for $ntt4$) and reverse primers containing a BamHI restriction site after the stop codon (5′-TTGGGATCCTTAAGATGCAGCTTTTAAGGGATG-3′ for $ntt2$, and 5′-CTAGCAGG ATCCTTATTTTTTTTATAAAAGCGCT-3′ for $ntt4$). The resulting amplification products were digested with BamHI and NdeI, purified and inserted into the expression vector pET16b (Novagen). The newly constructed plasmids (pNTT2, pNTT4) were transformed into and maintained in $E.\ coli$ XL1Blue (Stratagene). Identity of the cloned genes was checked by sequencing.

To analyse transport specificity of NTT2 and NTT4, 100 μl of induced $E.\ coli$ cells harbouring either the plasmid pNTT2 or pNTT4 were added to 100 μl of incubation medium (50 mM P_i buffer medium, pH 7.2) containing the [^{32}P]-labelled nucleotides [α-^{32}P]-ATP, -GTP, -UTP, or [adenylate-^{32}P]-NAD$^+$ (NEN). [α-^{32}P]-labelled ADP was synthesized as described previously[16]. Specific activities of all nucleotides ranged between 80 and 250 μCi mmol^{-1}. Uptake of nucleotides was carried out at 30 °C[14] and terminated after the indicated time periods by transfer of the cells onto a 0.45-μm nitrocellulose filter (Schleicher und Schüll), pre-wetted with incubation medium and set under vacuum. Cells were immediately washed by addition of 2 × 1.5 ml ice-cold incubation medium[16]. The filters were subsequently transferred into 20-ml scintillation vessels, containing 5 ml water, and radioactivity in these samples was quantified in a scintillation counter (Canberra-Packard Tricarb 2500).

Back-exchange studies and thin-layer chromatography

$Escherichia\ coli$ cells synthesizing either NTT2 or NTT4 from UWE25 were incubated for an appropriate time span in phosphate buffer medium containing radioactively labelled ADP or NAD$^+$. Subsequently, cells were collected by centrifugation and re-suspended in ice-cold phosphate buffer medium to remove non-imported radioactivity. After a second centrifugation step cells were re-suspended in phosphate buffer, with or without the indicated counter exchange substrates. For thin-layer chromatography $E.\ coli$ cells were collected by centrifugation and an aliquot of the supernatant was loaded onto a 0.5-mm polyethylene amine-cellulose thin-layer chromatography plate and dried with a fan[16]. Separation of adenine nucleotides and NAD$^+$ was conducted according to a standard protocol[16,27]. RF values of radioactively labelled compounds were determined after radioautography and corresponded to RF values of both unlabelled nucleotides visualized under ultraviolet light and radioactively labelled standards[14].

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Haemangioblast commitment is initiated in the primitive streak of the mouse embryo

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Haematopoietic and vascular cells are thought to arise from a common progenitor called the haemangioblast. Support for this concept has been provided by embryonic stem (ES) cell differentiation studies that identified the blast colony-forming cell (BL-CFC), a progenitor with both haematopoietic and vascular potential[1,2]. Using conditions that support the growth of BL-CFCs, we identify comparable progenitors that can form blast cell colonies (displaying haematopoietic and vascular potential) in gastrulating mouse embryos. Cell mixing and limiting dilution analyses provide evidence that these colonies are clonal, indicating that they develop from a progenitor with haemangioblast potential. Embryo-derived haemangioblasts are first detected at the mid-streak stage of gastrulation and peak in number during the neural plate stage. Analysis of embryos carrying complementary DNA of the green fluorescent protein targeted to the brachyury locus demonstrates that the haemangioblast is a subpopulation of mesoderm that co-expresses brachyury (also known as T) and Flk-1 (also known as Kdr). Detailed mapping studies reveal that haemangioblasts are found at highest frequency in the posterior region of the primitive streak, indicating that initial stages of haematopoietic and vascular commitment occur before blood island development in the yolk sac.

To determine whether haematopoietic and vascular lineages in the early mouse embryo develop from a haemangioblast, cells from pools of late streak to neural plate stage concepti (presumptive yolk sac and embryo proper, embryonic day (E) 7.0–7.5) were plated under conditions similar to those that support the development of embryoid body-derived BL-CFCs. Colonies with the morphology of the BL-CFC-derived blast colonies developed within 3–4 days of culture[1] (Fig. 1a, b). Molecular analysis of these embryo-derived colonies indicated that they expressed genes associated with haematopoietic and vascular development including *Flk-1* (refs 3–5), *Scl*[6,7] (also known as *Tal1*), VE-cadherin[8] (*Cdh5*), *Gata1* (refs 7, 9), *βH1 globin* (*Hbb-bh1*) and *β-major globin* (*Hbb-b1*), with patterns similar to those found in embryoid body-derived blast colonies[2,10] (Fig. 1c). The lack of expression of the mesodermal gene *brachyury*[11]
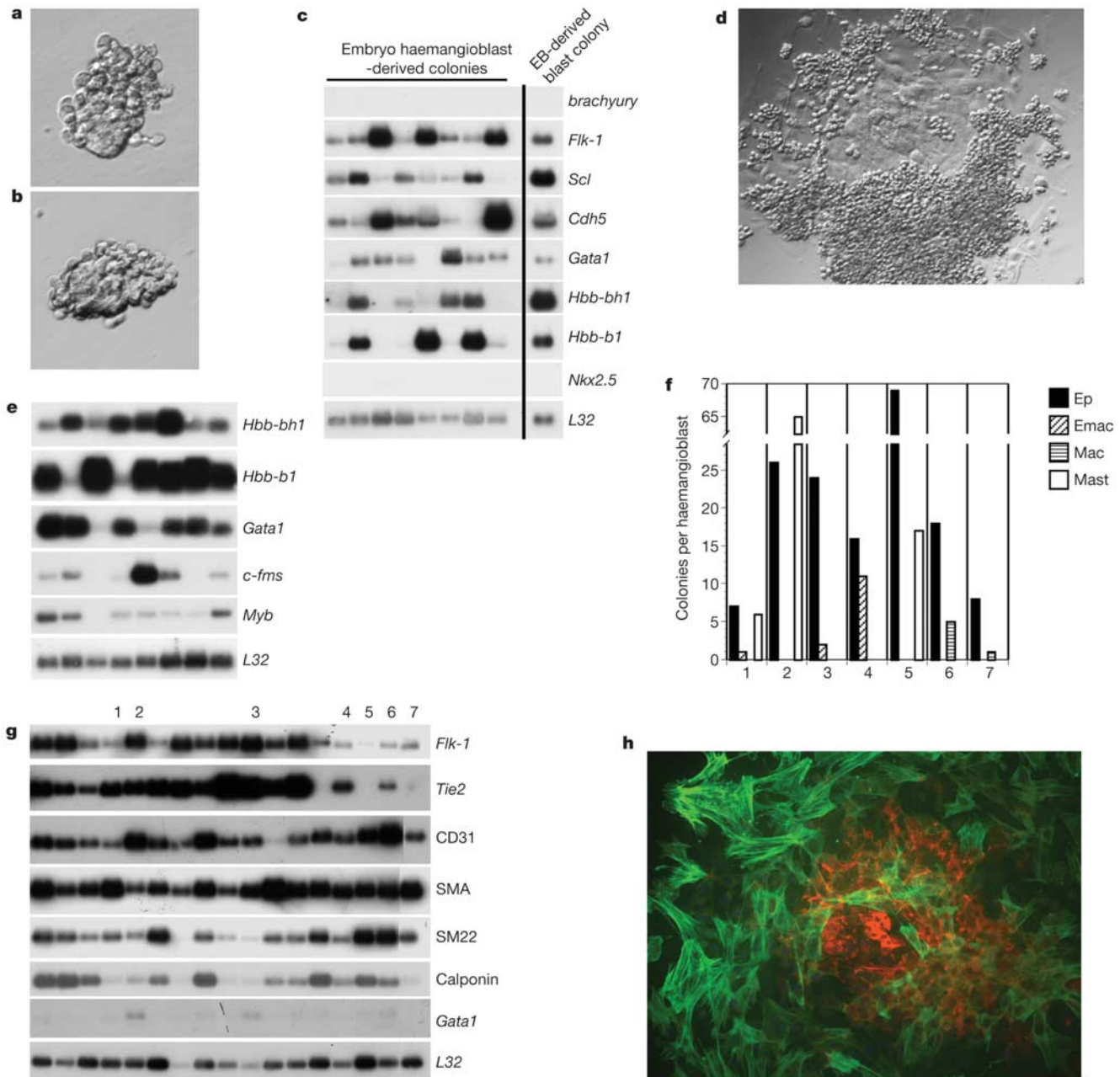
Figure 1 Characteristics and cell lineage potentials of the mouse haemangioblast. **a**, A haemangioblast-derived colony from an E7.5 embryo. **b**, A blast colony from an E3.25 embryoid body. **c**, Gene expression analysis of eight haemangioblast-derived colonies contrasted with that of a representative blast colony derived from an embryoid body (EB). L32 encodes a component of the 60S ribosomal protein, and was used as a control for the amount of cDNA loaded in each lane. **d**, Adherent and non-adherent cells resulting from the expansion of a haemangioblast-derived colony in liquid medium. Original magnification × 200. **e**, Expression analysis of non-adherent cells from eight expanded haemangioblast-derived colonies. **f**, The haematopoietic progenitor potential of the non-adherent cells from seven expanded haemangioblast-derived colonies. Ep, primitive erythroid; mac, macrophage; emac, bipotential erythroid macrophage; mast, mast cell. **g**, Gene expression analysis of adherent cells from expanded haemangioblast-derived colonies. The labelled lanes represent adherent cells obtained from the same colonies as the non-adherent cells used for the haematopoietic progenitor analysis shown in **f**. SMA, smooth muscle actin. **h**, The adherent cells from a haemangioblast-derived colony stained for the expression of smooth muscle actin (green, vascular smooth muscle) and CD31 (red, endothelial cells). Original magnification × 200.

shows that they had progressed beyond the mesoderm stage of development, and the finding that they did not express the cardiac-specific gene *Nkx2.5* (ref. 12) indicates that they were not undergoing cardiac development. Cardiac mesoderm derived from ES cells will form colonies that express *Nkx2.5* in the blast cell colony conditions, demonstrating that these conditions are not inhibitory to the development of this lineage (S. Kattman and G.K., unpublished observations).

When plated in liquid culture on a thin layer of matrigel in the presence of haematopoietic and vascular cytokines, individual embryo-derived colonies generated both adherent and non-adherent cells (Fig. 1d). The non-adherent population expressed the haematopoietic markers *βH1* and *β-major globin*, as well as *Gata1*, *c-fms* (also known as *Csf1r*) and *Myb* (Fig. 1e), indicative of the presence of primitive erythroid, macrophage and immature haematopoietic cells[13]. When cultured in methylcellulose, the non-adherent populations of single expanded colonies generated both primitive erythroid and definitive haematopoietic (macrophage and mast) colonies, confirming the potential predicted by the molecular analysis (Fig. 1f). From a total of 118 replated colonies in eight separate experiments, 73 generated haematopoietic progenitors with the following potentials: 37% restricted to primitive erythroid potential, 47% with primitive erythroid and definitive potential and 16% with only definitive potential. These findings indicate that there is heterogeneity in the haematopoietic potential of these colonies.

The adherent cells from the expanded colonies expressed genes representing endothelial and vascular smooth muscle development (Fig. 1g), including *Flk-1*, *Tie2* and CD31 (endothelial; *Pecam1*), and smooth muscle actin, SM22 (*Tagln*) and calponin (vascular smooth muscle)[14,15]. Endothelial and vascular smooth muscle cell lineages are known to develop from the blast colony[1,16]. Immunostaining of the adherent population generated from single colonies demonstrated the presence of a cluster of CD31+ endothelial cells, surrounded by an outgrowth of larger vascular smooth muscle cells expressing smooth muscle actin (Fig. 1h). Contracting cells indicative of cardiac development have not been observed in the expanded cultures, consistent with the above expression analysis indicating that this other mesoderm derivative does not develop from the blast colonies. The conditions used for expansion will support contracting cells from embryoid body-derived cardiac mesoderm (data not shown), demonstrating that the conditions are not inhibitory to this population. Taken together, these findings demonstrate that the embryo-derived colonies display primitive and definitive haematopoietic, endothelial and vascular smooth muscle potential and in this regard are similar, if not identical, to the BL-CFC colonies derived from embryoid bodies[1,16].

As an initial assessment of the clonality of the embryo-derived colonies, cells from embryos carrying either a neomycin or hygromycin transgene were mixed and plated under conditions appropriate for haemangioblast development. The non-adherent (haematopoietic) and adherent (vascular) populations of 38 haemangioblast-derived colonies expanded in liquid culture were harvested for genomic DNA and analysed for the neomycin and hygromycin genes by polymerase chain reaction (PCR, Fig. 2a). Twenty colonies gave non-adherent and adherent cell populations that carried the neomycin transgene only, whereas 17 colonies gave cell populations that carried the hygromycin transgene only. In one colony, neomycin and hygromycin DNA was found in both adherent and non-adherent populations. The presence of only one transgene in adherent and non-adherent cell populations from most of the embryo haemangioblast-derived colonies strongly suggests that they originate from a single cell. In addition, these findings argue against the occurrence of cell fusion in our cultures as this would have resulted in a higher frequency of haematopoietic and vascular cells containing both neomycin and hygromycin genes. Given the haematopoietic and vascular potential of these colonies,

the cell that gives rise to these colonies can be considered the haemangioblast.

To determine when haemangioblasts are generated during early embryogenesis, a kinetic analysis was performed on embryos ranging from the early streak to the head fold stage of development (E6.75 to E8.0.) (Fig. 2b). No haemangioblast-derived colonies were detected in the early streak stage embryos. Approximately 28% of the mid-streak embryos contain haemangioblasts, whereas 59% of those at the late streak/early neural plate stage and 88% of the embryos at neural plate stage were found to contain these progenitors. Most of the embryos at the late streak/early neural plate and neural plate stages were found to have between one and five haemangioblasts per embryo, but some had more than ten (the maximum number found was 33). Haemangioblast activity seems to decline after the neural plate stage as only 47% of those at the
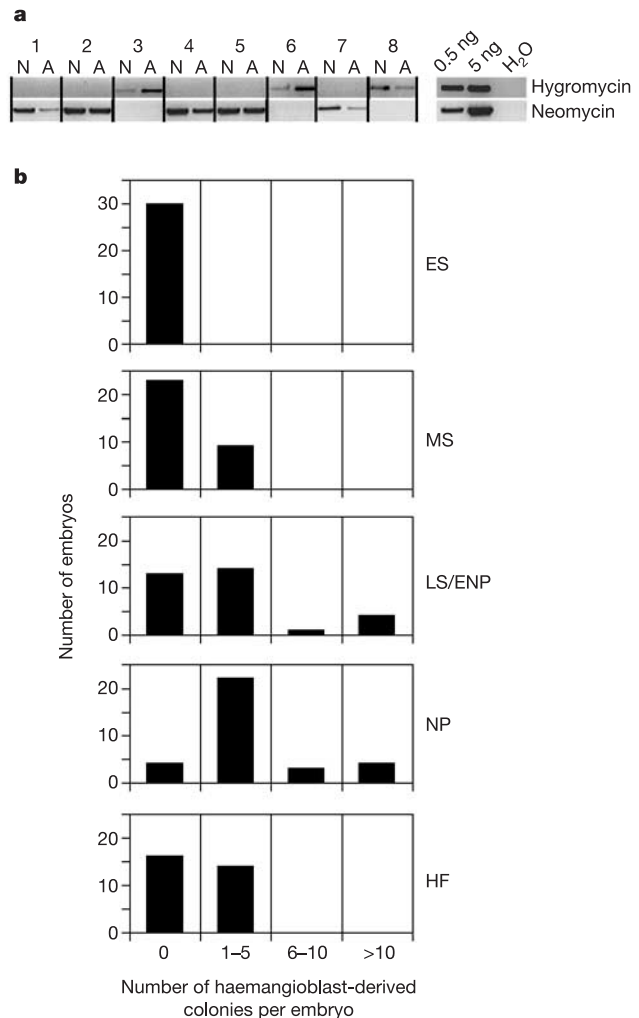
Figure 2 Clonality of haemangioblast-derived colonies and kinetics of haemangioblast development. **a**, PCR analysis of the non-adherent (N) or adherent (A) outgrowths from eight haemangioblast-derived colonies generated from a mixture of E7.5 embryos carrying either the neomycin or hygromycin gene. The controls represent 0.5 ng and 5 ng of genomic cDNA from embryonic stem cells containing either the neomycin or hygromycin gene. **b**, The haemangioblast potential of 30–33 embryos analysed at the indicated stages: ES, early streak (30 embryos); MS, mid-streak (32 embryos); LS/ENP, late streak/early neural plate (32 embryos); NP, neural plate (33 embryos); HF, head fold (30 embryos).

head fold stage contain these progenitors. These findings indicate that haemangioblast development within the mouse embryo is a dynamic process that is initiated at the mid-streak stage, peaks at the late streak/early neural plate and neural plate stages, and sharply declines at the head fold stage. This pattern defines the haemangioblast stage, which represents a narrow developmental window spanning approximately 12–18 h of mouse gestation.

Recent studies using an ES cell line with green fluorescence protein (GFP) cDNA targeted to the *brachyury* locus demonstrated that the ES-derived BL-CFC expresses *Flk-1* and *brachyury*, and thus represents a subset of mesoderm undergoing commitment to the haemangioblast lineages[17]. To determine whether the embryo-derived haemangioblast represents a similar stage of development, embryos from transgenic mice generated using the GFP–Bry ES cells were used. GFP–Bry$^{+/-}$ embryos (E7.5) express GFP in the primitive streak where *brachyury* is normally expressed[18] (Fig. 3a). Flow cytometric analysis of pooled embryos (E7.5) from matings of GFP–Bry$^{+/-}$ males and wild-type females revealed the presence of two GFP-expressing populations: GFP$^+$Flk-1$^+$ and GFP$^+$Flk-1$^-$, comparable to the populations present in embryoid bodies that have been differentiated for 3.5 days[17] (Fig. 3b). It is worth noting that the GFP-positive cells represent only 50% of the cells that express *brachyury*, as theoretically only half of the embryos are transgenic. Molecular analysis of the GFP-expressing populations showed that the GFP$^+$Flk-1$^+$ cells expressed *Flk-1*, *brachyury* and *Scl*. The GFP$^+$Flk-1$^-$ cells did not express appreciable levels of *Flk-1* or *Scl*, but did express *brachyury* at levels higher than in the GFP$^+$Flk-1$^+$ fraction (Fig. 3c). Haemangioblasts were found at a higher frequency in the GFP$^+$Flk-1$^+$ population compared with the GFP$^+$Flk-1$^-$ population (Fig. 3d). By using only GFP–Bry$^{+/-}$ embryos, we found that as in the embryoid body studies, the GFP$^-$Flk-1$^-$ population did not give rise to haemangioblast-derived colonies (data not shown). When the relative size of the two GFP$^+$ populations is taken into consideration, the GFP$^+$Flk-1$^+$ and GFP$^+$Flk-1$^-$ fractions contain 75% and 25% of the total number of haemangioblasts assayed, respectively (Fig. 3e). These findings demonstrate that most embryo haemangioblasts co-express Flk-1 and brachyury, similar to the embryoid body-derived BL-CFCs. These findings indicate that these progenitors probably represent a subset of mesoderm committed to the haematopoietic and vascular lineages. The activity observed in the GFP$^+$Flk-1$^-$ fraction may represent progenitors that are already committed to the haemangioblast programme but do not yet express Flk-1 protein on the cell surface. Maturation of these progenitors within the methylcellulose cultures to the stage at which they express cell surface Flk-1 would enable them to respond to vascular endothelial growth factor (VEGF) and generate a typical haemangioblast-derived colony. The co-expression of *Flk-1* and *brachyury* can be considered to be the signature of the haemangioblast, because the expression patterns of these genes in embryoid bodies segregate rapidly after this developmental stage (M. Kennedy and G.K., unpublished observations).

The presence of haemangioblasts in the Flk-1- and GFP-expressing fractions enables us to isolate sufficient numbers of cells (free of non-mesodermal cells; that is, the GFP$^-$Flk-1$^-$ fraction) for dose-response experiments that can further validate the clonal nature of the colonies. For this analysis, the total Flk-1$^+$ and GFP$^+$ cell populations (Flk-1$^+$GFP$^-$, Flk-1$^+$GFP$^+$ and Flk-1$^-$GFP$^+$) from 59 to 69 embryos were isolated by sorting and cultured at different concentrations. As indicated in Fig. 3f, the number of colonies was directly proportional to the number of cells plated when compared on a logarithmic scale. The relationship was linear with a slope of 1, indicating that a single cell accounts for the growth of an individual colony.

Given that the haemangioblast represents a subpopulation of mesoderm, we wanted to define its location in the gastrulating
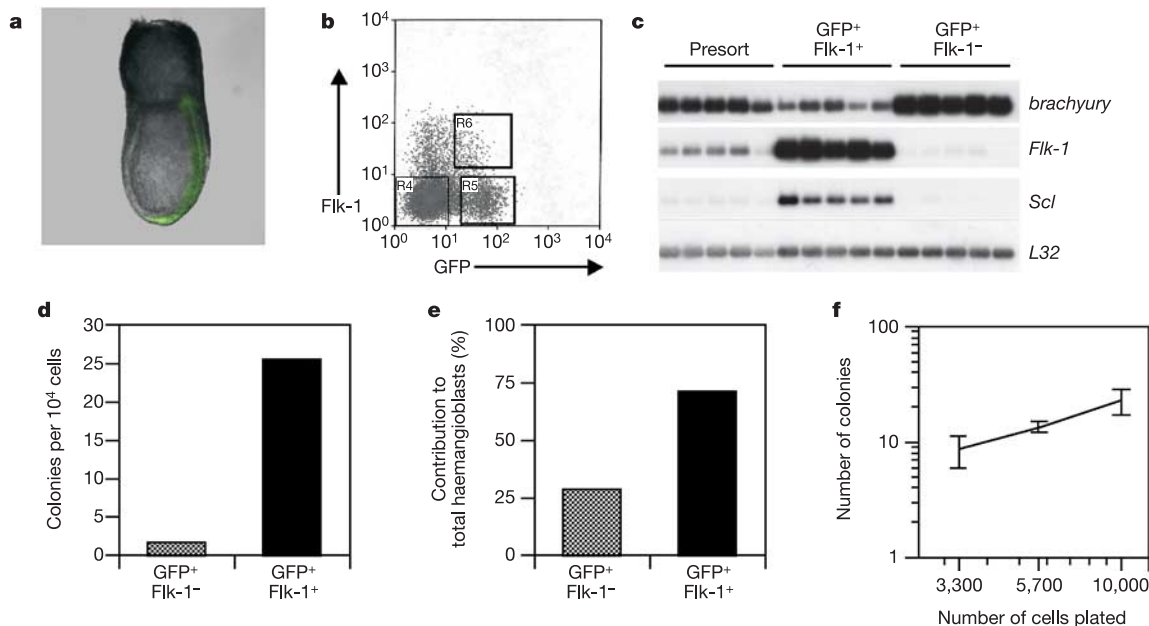
Figure 3 Isolation and characterization of GFP- and Flk-1-expressing cell populations from E7.5 GFP–Bry$^{+/-}$ embryos. **a**, GFP expression in the primitive streak of an GFP–Bry$^{+/-}$ embryo at the neural plate stage. **b**, Flow cytometric analysis of pooled embryos from matings of GFP–Bry$^{+/-}$ males with wild-type females. The gates used to isolate the GFP$^+$Flk-1$^-$ and GFP$^+$Flk-1$^+$ populations are indicated. **c**, Expression analysis of the GFP$^+$Flk-1$^-$ and GFP$^+$Flk-1$^+$ sorted populations. Each lane represents 1,000 cells of the indicated populations. **d**, Frequency of haemangioblasts in the GFP$^+$Flk-1$^-$ and GFP$^+$Flk-1$^+$ populations. **e**, Percentage contribution of the GFP$^+$Flk-1$^-$ and GFP$^+$Flk-1$^+$ populations to the haemangioblast potential of the total GFP population. **f**, Linear relationship of sorted Flk-1$^+$ and/or GFP$^+$ cells (includes GFP$^-$Flk-1$^+$, GFP$^+$Flk-1$^+$ and GFP$^+$Flk-1$^-$ cells) from E7.5 embryos to number of haemangioblast-derived colonies. The experiment was performed three times. Results are presented as means ± s.e.m.

embryo. GFP–Bry$^{+/-}$ embryos (E7.5) were dissected into fragments containing the yolk sac, posterior primitive streak, distal primitive streak, anterior region and lateral domains (Fig. 4a). The haemangioblast content of each portion of each embryo was determined. Most of the haemangioblasts (66%) were found in the posterior primitive streak, with little contribution from the distal primitive streak, the anterior region or the lateral domains. The yolk sac did contain some haemangioblasts, possibly reflecting progenitors that had migrated from the streak region and were undergoing differentiation. Gene expression analysis demonstrated the presence of *Flk-1* in the posterior primitive streak but not the distal primitive streak, consistent with the observed segregation of haemangioblast activity between these regions (Fig. 4b). The higher levels of the anterior marker cerberus-like (*Cer1*, refs 19, 20) in the distal compared to the proximal primitive streak and the presence of the posterior marker *Mesp1* (ref. 21) in the proximal but not in the distal primitive streak confirm the accuracy of the dissections. As expected, expression of *brachyury* was found in the primitive streak and yolk sac, indicating good separation of these regions from the lateral and anterior regions. *Gata1* expression is only found in the yolk sac, consistent with the initiation of hematopoiesis in this region.

Contribution to haemangioblast potential (%)

YS	Ant	L	DPS	PPS
8%	3%	14%	9%	66%
(8)	(3)	(15)	(10)	(70)
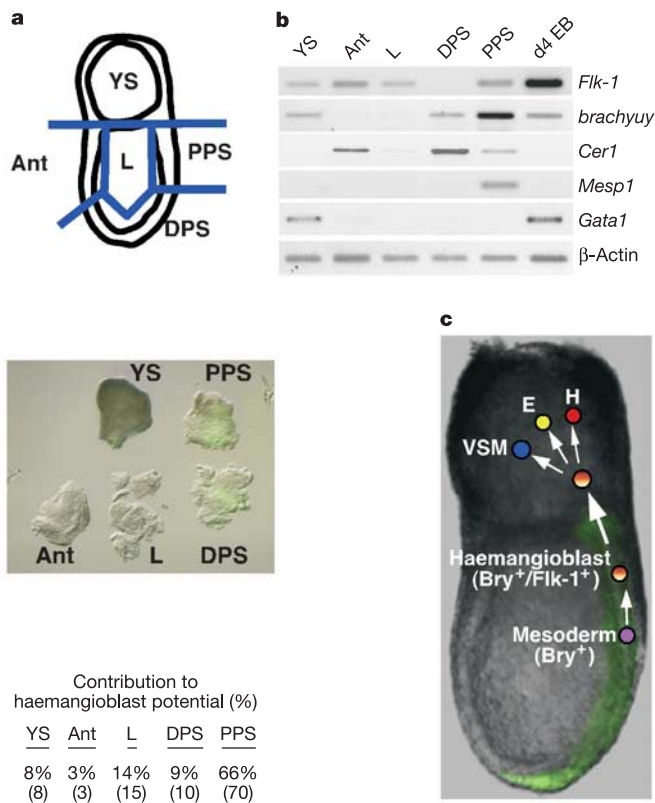
Figure 4 Localization of the haemangioblast in the embryo. **a**, GFP–Bry$^{+/-}$ embryos at E7.5 were dissected into the yolk sac (YS), anterior (Ant), lateral (L), posterior primitive streak (PPS) and distal primitive streak (DPS) and cultured in haemangioblast conditions. The numbers of haemangioblast-derived colonies from fragments (shown in brackets) obtained from 23 embryos were summed (106 haemangioblasts total) and the percentage contribution each fragment made to the total haemangioblast potential of these embryos is indicated. **b**, Expression analysis on the dissected fragments. **c**, A model of haemangioblast development in the mouse embryo. The haemangioblast is a brachyury$^+$ and Flk-1$^+$ cell that arises in the primitive streak and migrates onto the yolk sac where it differentiates into haematopoietic (H), endothelial (E) and vascular smooth muscle (VSM) progenitor cells.

The localization of the haemangioblast to the posterior region of the primitive streak supports a model in which the earliest stages of haematopoietic and vascular commitment are initiated at this site, before migration of cells to the region of the presumptive yolk sac (Fig. 4c). The fact that relatively few progenitors are detected in the developing yolk sac suggests that the haemangioblasts rapidly differentiate into restricted haematopoietic and vascular progenitors as they egress from the streak. This model of haemangioblast development is consistent with previous findings indicating that one of the earliest deficiencies in *Flk-1* null embryos is the inability of primitive streak progenitors to migrate to the yolk sac[22]. These progenitors may be the equivalent of the haemangioblast identified in our study. The embryo haemangioblasts represent a transient progenitor population that is generated between the mid-streak and head fold stages of development. The observed heterogeneity in haemangioblast numbers between embryos probably reflects the dynamic nature of their development and indicates that these progenitors undergo rapid differentiation after their induction.

Lineage tracing studies have so far failed to identify a cell with haematopoietic and vascular potential in the early embryo[23]. However, given the fact that the frequency of the haemangioblast is low (approximately 1 in 400 GFP$^+$Flk-1$^+$ cells, Fig. 3d), this progenitor could have been easily missed. Most evidence now suggests that haematopoiesis is initiated independently in the yolk sac and in the para-aortic splanchnopleura during early embryonic development and that it is different at each site[24,25]. The yolk sac gives rise to the primitive erythroid lineage and a restricted subset of definitive haematopoietic progenitors but does not generate lymphoid progenitors and haematopoietic stem cells (HSCs) capable of long-term repopulation in recipient adult animals[25,26]. The para-aortic splanchnopleura region supports the development of multilineage hematopoiesis, including lymphoid progenitors and HSCs, but does not give rise to primitive erythrocytes. The potential of the haemangioblast described here is consistent with the interpretation that it is the progenitor of yolk sac hematopoiesis. The fact that the highest number of haemangioblasts detected in a single embryo was 33 suggests that this number may be sufficient to establish this haematopoietic programme. As the para-aortic splanchnopleura represents a site of haematopoietic development in close proximity to blood vessels (dorsal aorta), it should also contain haemangioblasts. These haemangioblasts would represent the progenitors of the HSCs. Future studies using the GFP–Bry$^{+/-}$ embryos should be able to elucidate whether haemangioblasts co-expressing *Flk-1* and *brachyury* also exist in the para-aortic splanchnopleura. Access to site-specific haemangioblasts would provide an opportunity to study how different haematopoietic programmes arise during development. □

Methods

Dissection and culture of mouse embryos

Haemangioblast and haematopoietic progenitor assays

The haemangioblast assay used was a modification of the blast assay developed in the

ES/embryoid body system. Embryo cell suspensions were plated in 1% methylcellulose containing 10% FCS (Summit), VEGF (5 ng ml^{-1}), insulin-like growth factor-1 (IGF-1; 50 ng ml^{-1}), leukaemia inhibitory factor (LIF; 1 ng ml^{-1}), interleukin-6 (IL-6; 5 ng ml^{-1}) and 25% D4T endothelial cell conditioned medium[2]. Colonies were grown in either 10 mm dishes or 24-well or 96-well plates in low oxygen incubators (5% oxygen). Haemangioblast colonies were further expanded using previously published expansion conditions[1]. Haematopoietic progenitors were assayed in 1% methylcellulose containing 10% protein-free hybridoma medium (Gibco/BRL), 15% plasma-derived serum (Antech), c-kit ligand (KL; 1% conditioned medium), IL-3 (1% conditioned medium), granulocyte–macrophage colony-stimulating factor (GM-CSF; 3 ng ml^{-1}), IL-11 (5 ng ml^{-1}), erythropoietin (2 U ml^{-1}), IL-6 (5 ng ml^{-1}) and thrombopoietin (1% conditioned medium). Primitive erythroid colonies were counted from day 4–5 whereas definitive macrophage, erythroid–macrophage and mast colonies were counted after 7–10 days of culture.

Gene expression analysis

Expression analyses of haemangioblast/blast colonies, the non-adherent and adherent cell populations from expanded haemangioblast colonies, and sorted cell populations were performed using a modified global cDNA amplification protocol[2,28].

Total RNA from pooled fragments of dissected neural plates stage embryos was harvested using the Absolutely RNA Microprep kit (Stratagene) and reverse transcribed with Omniscript RT (Qiagen) to generate cDNA. Primers used for PCR amplification of *brachyury*, *Flk-1* and β-actin were as previously reported[17]. The following additional primers were also used: *Gata1* (forward) 5′-CATTGGCCCCTTGTGAGGCCAGAGA-3′, *Gata1* (reverse) 5′-ACCTGATGGAGCTTGAAATAGAGGC-3′; *Cer1* (forward) 5′-CCAG GCTTGGAAGATTCTGGAAGA-3′, *Cer1* (reverse) 5′-GTCTTCACCATGCACTGACA CTCT-3′; *Mesp1* (forward) 5′-GTTCCTGTACGCAGAAACAGCATC-3′, *Mesp1* (reverse) 5′-CAAGGAGGGTTGGAATGGTACAGT-3′.

Neomycin and hygromycin gene detection

PCR reactions for the neomycin and hygromycin genes were performed using the Advantage 2 PCR system (BD Biosciences Clontech) on genomic DNA isolated from the non-adherent and adherent cell populations of the liquid-expanded haemangioblast colonies. The primers used were as previously published[2].

Fluorescence-activated cell sorting

Embryo cells at a concentration of 1×10^6 ml^{-1} were incubated with a biotinylated anti-Flk-1 antibody followed by incubation with streptavidin-PE-Cy5 (BD Pharmingen) and sorted on a MoFlo high-speed cell sorter (Cytomation). For the limiting dilution analysis (Fig. 3f), GFP$^-$Flk-1$^+$, GFP$^+$Flk-1$^+$ and GFP$^+$Flk-1$^-$ cells were sorted directly into haemangioblast conditions in 96-well plates. Out of three experiments, one was performed using duplicate wells containing 3,300, 5,700 or 10,000 cells each, and two experiments were performed using only one well for each condition.

Immunofluorescence for CD31 and SMA expression

Haemangioblast colonies were cultured on fibronectin-coated glass coverslips in IMDM medium containing VEGF (5 ng ml^{-1}) and basic fibroblast growth factor (bFGF; 10 ng ml^{-1}) for 5–8 days. The coverslips were fixed with 4% paraformaldehyde for 15 min at room temperature, incubated with biotinylated anti-mouse CD31 (BD Pharmingen) and anti-mouse SMA (NeoMarkers) in 1 × PBS buffer for 1 h, washed five times (10 min washes) and incubated with streptavidin-Cy3 (Sigma) and anti-mouse-FITC (Biosource International) for 1 h. After five washes the coverslips were inverted onto a drop of 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Inc.) on slides and viewed under an inverted fluorescence microscope.

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The role of *barren stalk1* in the architecture of maize

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The architecture of higher plants is established through the activity of lateral meristems—small groups of stem cells formed during vegetative and reproductive development. Lateral meristems generate branches and inflorescence structures, which define the overall form of a plant[1–3], and are largely responsible for the evolution of different plant architectures[3]. Here, we report the isolation of the *barren stalk1* gene, which encodes a non-canonical basic helix–loop–helix protein required for the