Notch input to blood stem cell programming during Xenopus ontogeny
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Multipotent haematopoietic stem cells (HSCs) originate in the dorsal aorta (DA) during vertebrate embryogenesis and after migrating to a permanent niche in the bone marrow or equivalent, give rise to a continuous supply of mature blood cells of all lineages throughout adult life. Previous cell tracing experiments have shown that the cells of the DA and the HSCs, migrate here from an early collection of haemangioblasts (bi-potential precursors of blood and endothelial cells) which reside in the dorsolateral plate (DLP) mesoderm. Development of HSCs is tightly regulated by a number of key signalling pathways in both the DLP and once the cells reach the midline. In particular, notch signalling is considered an important factor in HSC development and we know from studies in mice and zebrafish that notch is required for both arterial and HSC development. Here, we have used the relatively slow development and the spatial separation of definitive haematopoiesis from primitive haematopoiesis in *Xenopus laevis* embryos to reveal the first defect of reduced notch signalling in the haematopoietic system. Morpholo- lino knock-down of notch1 or the notch target genes esr1 and hesr1, or the application of a potent γ-secretase inhibitor, DAPM, to *Xenopus* embryos leads to loss of HSC marker expression. Accompanying the loss of HSC expression we see maintenance of expression of specific endothelial genes, including the VEGF receptor, flk1. We show that notch1 is specifically required for the development of HSCs without affecting arterial or haemangioblast programming and thereby uncouple the notch contributions to arterial and HSC development. Here, for the first time, we also show that esr1 and hesr1 are required for the HSC programme.

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els1, an evolutionarily conserved and functionally uncharacterized gene, is required for zebrafish embryonic haematopoiesis
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Embryonic haematopoiesis is an excellent model for understanding the complex decisions made by multi-potent progenitor cells toward precise and specific lineage differentiation. In zebrafish, Tbx16, a T-box transcription factor, is essential for appropriate embryonic erythropoiesis, as its loss-of-function mutant known as spadetail (spt) has virtually no embryonic erythrocytes. However, what genes lie downstream of Tbx16 and mediate its requirement for erythropoiesis is unknown. Here we report one such potential candidate els1, which is conserved in all vertebrates we investigated, but none of whose homologs have been experimentally examined for their function. els1 is expressed specifically in the intermediate mesoderm (IM), a transient embryonic tissue that gives rise to future pronephros, vasculature and blood, including embryonic erythrocytes; the expression peaks during early somitogenesis. This suggests that els1 is expressed in the right place and time for a role in erythropoiesis. This IM expression is virtually absent in a spt mutant, suggesting that els1 functions downstream of tbx16. Importantly, loss of els1 function (by way of morpholino injection) leads to a dramatic reduction of expression of the erythroid progenitor marker gene gata1, suggesting for the first time that els1 is required for appropriate embryonic erythropoiesis. The Els1 protein domain organization raises the possibility that Els1 may be a novel signaling molecule. In the near future, we propose to perform a structure–function study of the Els1 protein in vivo to shed light on the mechanism of its requirement for embryonic haematopoiesis.

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Effect of wnt signaling on the formation of embryonic blood cells in zebrafish
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We have previously shown that the formation of embryonic red blood cells (RBCs) in the zebrafish embryo is dependent upon interactions between embryonic trunk intermediate mesoderm (from which RBCs are derived) and trunk paraxial mesoderm. The spadetail mutant has been shown to lack trunk paraxial mesoderm and RBCs, even though the intermediate mesoderm in spadetail mutants appears to be otherwise normal. A micro-array analysis between WT and spadetail mutants has identified wnt family members as candidate molecules used to signal between the paraxial mesoderm and the RBC producing intermediate mesoderm. The role of wnt family members in blood development has only recently been recognized and we report here upon the expression and inhibition of wnt family members within the paraxial mesoderm that play a required role during embryonic blood formation in the intermediate mesoderm. We also show that wnt signalling plays a dual role in development of erythroid and myeloid cells and this signalling is required during blastula and early gastrula stages with regard to blood development.

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The Aplnr GPCR signals independently of Gα/o proteins and cell-non-autonomously in the development of myocardial progenitor cells
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Myocardial progenitor development involves the migration of cells to the anterior lateral plate mesoderm (ALPM) where they are exposed to the necessary signals for differentiation. Whether the arrival of cells to this location is sufficient, or whether earlier signaling events are required, for progenitor development is poorly understood. Here we demonstrate that in the absence of Aplnr signaling, cells fail to migrate to the heart-forming region of the ALPM. Our work uncovers a previously uncharacterized cell-non-autonomous function for Aplnr signaling in cardiac development.