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lyl-1 and *tal-1/scl*, two genes encoding closely related bHLH transcription factors, display highly overlapping expression patterns during cardiovascular and hematopoietic ontogeny

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Abstract

The *TAL-1/SCL* and *LYL-1* genes encode two closely related basic helix–loop–helix transcription factors involved in child T-acute lymphoblastic leukemia through chromosomal rearrangements and transcriptional deregulation. During ontogeny, Tal-1/SCL is required for hematopoietic cell generation, both in the yolk sac, where erythro-myeloid cells are first produced, then in the intra-embryonic compartment, where hematopoietic stem cells independently arise. We describe here the expression pattern of *lyl-1* in mouse embryos from 7 to 14 days post coitus using *in situ* hybridization, as well as β-Galactosidase (β-Gal) expression in *lyl-1-lacZ* knock-in embryos, which express a C-terminally truncated Lyl-1 protein fused to the β-Galactosidase (Lyl-1Δ/β-Gal). In addition, we compare *lyl-1* expression pattern with that of *tal-1/scl*. Similar to Tal-1/SCL, Lyl-1 mRNA expression occurs in the developing cardiovascular and hematopoietic systems. However, contrary to *tal-1/scl*, *lyl-1* is not expressed in the developing nervous system. In *lyl-1-lacZ* knock-in heterozygous and homozygous embryos, β-Gal expression completely correlates with Lyl-1 mRNA expression in the intra-embryonic compartment and is present: (1) in the developing hematopoietic system, precisely where hematopoietic stem cells emerge, and thereafter in the fetal liver; (2) in the developing vascular system; and (3) in the endocardium. In contrast, whereas Lyl-1 mRNA is expressed in yolk sac-derived endothelial and hematopoietic cells, Lyl-1Δ/β-Gal is either absent or poorly expressed in these cell types, thus differing from Tal-1/SCL, which is highly expressed there at both mRNA and protein levels.
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Abbreviations: AGM, aorta–gonads–mesonephros; dpc, days post-coitum; β-Gal, β-galactosidase; bHLH, basic helix–loop–helix; FL, fetal Liver; HIAC, hematopoietic intra-aortic clusters; HSC, hematopoietic stem cells; LTR, long-term reconstitution (activity); P-Sp, para-aortic splanchnopleura; S, pairs of somites; SAP, sub-aortic patches; YS, yolk sac.

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1. Results and discussion

The basic helix–loop–helix (bHLH) transcription factor Lyl-1 was first identified due to its translocation in T-cell leukemia (Mellentin et al., 1989). Lyl-1 bHLH domain is highly homologous to that of Tal-1/SCL, another transcription factor involved in the development of T-cell leukemia (Visvader et al., 1991). *tal-1/scl* is one of the major genes involved in the generation of hematopoietic precursors in the embryo since its deletion leads to a complete absence of hematopoiesis (Robb et al., 1995; Shivdasani et al., 1995; Porcher et al., 1996; Robb et al., 1996). Contrary to *tal-1/scl* which expression pattern has been extensively analyzed (Kallianpur et al., 1994; Elefanty et al., 1998, 1999), *lyl-1* embryonic expression has been so far only described in the vascular system of 9.5 days post-coitus (dpc) mouse embryos (Chapman et al., 2003). In the present study, we thoroughly examined *lyl-1* expression pattern in 7–14 dpc mouse embryos using both *in situ* hybridization, in comparison with *tal-1/scl*, and β-Galactosidase expression in *lyl-1*$^{+/LacZ}$ and *lyl-1*$^{LacZ/LacZ}$ embryos (see below), with particular emphasis given to the development of hematopoiesis.

Hematopoiesis in vertebrate embryos results from the contribution of two distinct, independently generated, precursor pools (for review, see Godin and Cumano, 2002). In the mouse, the first wave of hematopoietic precursor production takes place in the yolk sac (YS)-blood islands from 7.5 dpc (Palis et al., 1999; Bertrand et al., 2005a). YS-derived precursors do not display the multipotentiality and capacity to sustain in the long-term the hematopoiesis of irradiated recipients (LTR activity), which typify hematopoietic stem cells (HSC) (Cumano et al., 1996, 2001). HSC develop from a second generation event, which takes place in the intra-embryonic compartment from 8.5 dpc, in a site named first para-aortic splanchnopleura (P-Sp: 8.5–9 dpc), then AGM from 10 to 11.5 dpc, as the aorta, gonads and mesonephros have developed from the P-Sp (Medvinsky and Dzierzak, 1996; Godin et al., 1999). HSC generation can be ascribed to the aorta and underlying mesenchyme (Godin et al., 1999; de Bruijn et al., 2000; Bertrand et al., 2005b). Cytological analyses of the AGM, performed at the time when HSC production peaks at 10.5–11.5 dpc, uncovered the presence of these cells in two distinct locations (for review, see Godin and Cumano, 2002: (1) within cells clusters located in the ventro-lateral part of the aorta. These hematopoietic intra-aortic clusters (HIAC) have been described in all investigated vertebrate species; (2) within cellular patches localized in the mesenchyme below the aortic clusters, which express, amongst other markers, the AA4.1 antigen and the transcription factors GATA-2 and GATA-3 (Manaia et al., 2000; Bertrand et al., 2005b). These sub-aortic patches (SAP) have, up to now, only be characterized in mouse (Manaia et al., 2000) and human (Marshall et al., 1999) embryos.

As stated before, *tal-1/scl* invalidation disrupts the generation of both extra- and intra-embryonic hematopoietic precursors: erythro-myeloid precursors in the early YS are not produced (Robb et al., 1995; Shivdasani et al., 1995) and HSC generation is also impaired, since *tal-1/scl*$^{-/-}$ ES cells do not contribute to definitive hematopoiesis of chimaeric embryos (Porcher et al., 1996; Robb et al., 1996). Tal-1/SCL is expressed by embryonic hematopoietic precursors in the yolk sac (Kallianpur et al., 1994; Elefanty et al., 1998) and also in the aortic region where HSC are generated, namely in the HIAC (Elefanty et al., 1999). In addition to the hematopoietic system, embryonic expression of Tal-1/SCL includes the endothelial (Kallianpur et al., 1994) and central nervous system (Elefanty et al., 1999; van Eekelen et al., 2003).

We used mice in which the *lyl-1* gene has been modified through homologous recombination (Capron et al., 2006). In these mice, part of *lyl-1* coding region was replaced by the *lacZ* reporter gene. The *lacZ* insertion occurs within the sequence encoding the first helix of Lyl-1 HLH domain, in exon four. Consequently, this *lyl-1*^{lacZ} allele encodes a C-terminally truncated Lyl-1 protein fused to β-Gal (Capron et al., 2006), thereafter referred to as Lyl-1Δ/β-Gal. As *lyl-1*^{lacZ} keeps all the 5′ cis-regulatory region of *lyl-1*, the detection of β-Gal expression and/or activity in any cells is likely to reveal the transcriptional activation of the *lyl-1* locus in wild-type mice. Unfortunately, the *bona fide* Lyl-1 protein expression pattern could not be assessed due to the absence of an anti-Lyl-1 antibody suitable for immunolabeling of embryo sections.

Null homozygous *lyl-1*$^{LacZ/LacZ}$ mice are viable and display a reduced number of B cells resulting from a partial block after the pro-B stage. Moreover, both *lyl-1*$^{LacZ/LacZ}$ fetal liver and bone marrow HSC, which exhibit high β-Gal activity levels, display a severely impaired long-term reconstitution capacity (Capron et al., 2006). No morphological alteration of development was observed in *lyl-1*$^{+/LacZ}$ and *lyl-1*$^{LacZ/LacZ}$ embryos, compared to wild type.

Fig. 1. *lyl-1* and *tal-1/scl* expression in the extra-embryonic compartment. *Abbreviations:* Al, allantois; Am, amnios; H, heart; NF, neural folds; YS, yolk sac; (A–C) 7–7.5 dpc: Tal-1/SCL (A) and Lyl-1 (B) mRNA are present in YS mesoderm (arrowheads). (C) No β-Gal activity is detected in the mesoderm (arrowheads) of *lyl-1*$^{LacZ/LacZ}$embryos. (D,E) 7.5 dpc: Tal-1/SCL (D) and Lyl-1 (E) mRNA are detected in YS-blood islands (arrowheads). (F–H) 8 dpc: YS-blood islands (arrowheads) express both Tal-1/SCL (F) and Lyl-1 (G) mRNA. In *lyl-1*$^{LacZ/LacZ}$ embryos (H), β-Gal activity is absent in YS-blood islands (arrowheads), but can be detected in nascent vessels in the cephalic region. Tal-1/SCL (F) and Lyl-1 (G) mRNA and β-Gal activity (H) are detected in the allantois. (I–M) Sections of wholemount 8 dpc embryos. Tal-1/SCL (I) and Lyl-1 (J) mRNA is expressed in YS-blood islands (arrowheads). In *lyl-1*$^{+/LacZ}$ embryos (K), endodermal cells display a non specific β-Gal activity (arrows), whereas a specific staining is present in endothelial cells from the neural folds and allantois. Neither peripheral endothelial cells nor inner hematopoietic cells of the YS-blood islands display X-Gal staining (L) whereas Lyl-1 mRNA is detected (M). (N,O) 8.5 dpc (10S): Lyl-1 mRNA (N) is expressed by intra-embryonic endothelial cells (arrows). In circulating cells, the expression is similar to that of βH1 globin mRNA (O), expressed in YS-derived erythrocytes (open arrows). Scale bars: (A–L) 100 μm; (M) 20 μm; and (N,O) 200 μm.

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