Lyca\textit{t} and \textit{cloche} at the Switch Between Blood Vessel Growth and Differentiation?

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The formation of the cardiovascular system starts in the mouse embryo at approximately embryonic day (E)7.0 to E7.5. The first blood vessels in the extraembryonic membranes, the major intraembryonic vessels, and the heart form by vasculogenesis, the in situ differentiation of mesodermal cells that give rise to “blood-islands.” The latter are composed of hemangioblasts, the common precursors of endothelial and blood cells. Hemangioblasts situated in the lumen of the blood islands will further differentiate into hematocytes, the precursors of all 3 lineages of blood cells. In contrast, hemangioblasts lining the walls of the blood islands will give rise to angioblasts that form endothelial cells.\textsuperscript{1}

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Migrating angioblasts from the proximal lateral mesoderm assemble symmetrically at the lateral sides of the embryo to establish 2 preendo-cardial tubes. They fuse to give rise to the primordial heart.\textsuperscript{2} While vasculogenesis is still proceeding, the uniform blood islands begin to remodel to a network of large and small vessels by the process of angiogenesis, preferentially intussusceptive microvascular growth.\textsuperscript{3,4}

Gene expression and targeting studies have identified vascular endothelial growth factor and its 2 receptors, KDR/\textit{flk-1} and \textit{flt-1}, as critical for the formation and early remodeling of the blood islands. Vascular endothelial growth factor is produced by endodermal and mesodermal cells at the onset of hemangioblast formation, whereas its receptors are expressed in the future endothelial cells lining the blood islands.\textsuperscript{5} \textit{Flk-1} and \textit{flt-1} embryos lack blood islands throughout the embryo and yolk sac.\textsuperscript{6} In \textit{flt-1} embryos, blood islands do not properly remodel but form large blood channels.\textsuperscript{7} Inactivation of a single vascular endothelial growth factor allele caused multiple embryonic malformations including the heart, rudimentary dorsal aortae, and a reduced number of blood cells.\textsuperscript{8,9} All deletions were lethal between days E8.5 and E11 to E12. Angiopoietin (Ang)-1, expressed by mesodermal cells, and its corresponding tie-1 and tie-2 receptors, located on the endothelium, form another important endothelial specific regulatory system that is critical for the mechanisms of intussusceptive microvascular growth.\textsuperscript{10-12}

Recently, the zebrafish mutation \textit{cloche} has been characterized to affect blood vessel and blood cell formation at a very early stage.\textsuperscript{13} In this issue of \textit{Circulation Research}, Xiong et al\textsuperscript{14} report the isolation of lysocardiolipin acyltransferase, \textit{lycat}, from the deletion interval of \textit{cloche}, in the attempt to determine the molecular components of the \textit{cloche} gene in zebrafish. Morpholino-mediated \textit{lycat} knockdown results in a strikingly similar phenotype as compared with the \textit{cloche} mutation. The central embryonic vascular network is established; however, the intersegmental vessel loops are approximately one-third longer and reduced in number with largely increased intervascular spaces as compared with controls. These vessels, as well as the central large vessels, the dorsal aorta, and the axial vein, express \textit{flk-1} and \textit{tie-1} in a mosaic pattern, in the way that some endothelial cells exhibit normal levels, whereas others produce none or very low amounts. The \textit{lycat}-deficient embryos lack cranial blood vessels and possess an extremely thin common endocardial–myocardial layer, comparable to the \textit{cloche} mutation. \textit{Flk-1}, \textit{sc1}, \textit{gata1}, \textit{etsrp}, and \textit{fli1} act in hemangioblasts downstream of \textit{lycat}, as was previously demonstrated for \textit{cloche}. This establishes \textit{lycat} as one of the earliest known regulators of hemangioblasts.\textsuperscript{14}

What does the \textit{lycat} phenotype suggest concerning its function in endothelial cells, as well as in blood vessel and heart morphogenesis? For the formation of the heart, it is essential that the endocardial tube and the surrounding mesoderm that differentiates to myocardium interact. This communication is likely disturbed in the \textit{lycat} mutants, and a normal heart therefore cannot form. Mice deficient of Ang-1, or its tie-2 receptor, were unable to recruit mesodermal cells to the endocardium resulting in a pathological heart morphology with a thinned myocardium that remained distant to the endocardium.\textsuperscript{10,11} It is not known presently whether Ang-1/tie-2 signaling is affected by the \textit{lycat} knockout. In their video file (available in the online data supplement to the article at http://circres.ahajournals.org), Xiong et al show impressively “endocardial” contractions that are, however, less effective, because the myocardium forms no separate thick layer.\textsuperscript{14} This suggests, that mesodermal cells are recruited and likely incorporated into the endocardium to give rise to a common 1-layered, enlarged endocardial–myocardial structure instead of forming the adjacent myocardium. Low expression levels of tie-1 could support this inclusion of mesodermal cells into the endocardium. Tie-1 was identified as a counter player of tie-2, and low tie-1 expression would thus support tie-2–mediated periendothelial recruitment. (Figure A).\textsuperscript{10,11} Alternatively, it could be argued that the endocardium never forms. However, the supplemental video in the article by Xiong et al shows that the circulation is intact.\textsuperscript{14} This means that the “myocardium” is properly connected to the large vessels, indicating that the sinus venosus and...
The integration of periendothelial cells into the endothelial lining, could cause the formation of elongated intersegmental vessels in the central region of the embryo. Periendothelial cells are critical for vessel lumen division to multiply vascular segments by the insertion of transluminal tissue folds that form pillars, spanning between opposite vessel walls. The analysis of Ang-1– and tie-2–deficient embryos has proven the pivotal role of periendothelial cells for the stabilization of folds and pillars and subsequently successful vessel division resulting in normal vessel growth and remodeling via intussusceptive microvascular growth. Vascular loops can form “in situ” in the wall of larger vessels in a similar way (Figure, B). The integration of periendothelial cells into the endothelial lining in the cloche knockdown will reduce the formation of transluminal tissue folds necessary for lumen division or loop systems, which explains the coarse vascular network composed of large vessel loops and big spaces between them. Some authors suggest the intersegmental vessels form by endothelial sprouting; however, a careful analysis of thin serial sections that would confirm the existence of blind ending segments was not performed. Indeed, such an analysis demonstrated that the perineural (intersomitic) loop plexus of mouse embryos that corresponds to the intersegmental loop system in the zebrafish is formed by in situ loop formation.

The detailed analysis of the vascular plexus in the chicken chorioallantoic membrane, and in mouse embryos, has demonstrated the constant existence of a periendothelial cell layer around all growing vessels. The periendothelial cells differentiate later to form smooth muscle cells and pericytes. As long as the network is expanding, periendothelial cells exhibit a striking similarity to endothelial cells, with which they form common intercellular junctions. Tracing periendothelial cells by analysis of serial sections has shown, at the ultrastructural level, that their extensions are frequently incorporated into the endothelial layer. These cells migrate from the surrounding mesenchyme toward vessel walls under the regulation of the Ang-1/tie-2 system and can thus readily contribute to expand the endothelial lining. Endothelial cell mitosis has only rarely been observed. Therefore, incorporation of periendothelial cells into the endothelial layer appears to be the major mechanism for the growth of vessel segments. The expression of differing amounts of cloche could regulate vessel wall expansion by periendothelial incorporation versus lumen division by periendothelial stabilization of transluminal tissue folds and pillars. Further investigation will deepen our insight into this possible switch that may determine cardiovascular development.

**Acknowledgments**

The author thanks Max Patan for assistance with the graphical art work.
Sources of Funding
The work of the author cited in this editorial was funded by the Swiss National Science Foundation, the German Research Foundation, and the American Heart Association.

Disclosures
None.

References

KEY WORDS: *cloche* ■ *lycat* ■ angiogenesis ■ vasculogenesis ■ intussusceptive microvascular growth
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Circ Res. 2008;102:1005-1007
doi: 10.1161/CIRCRESAHA.108.176446

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