



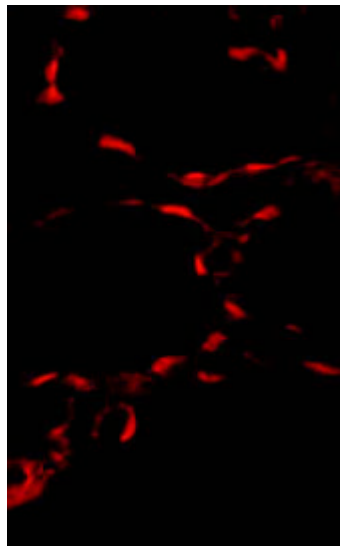
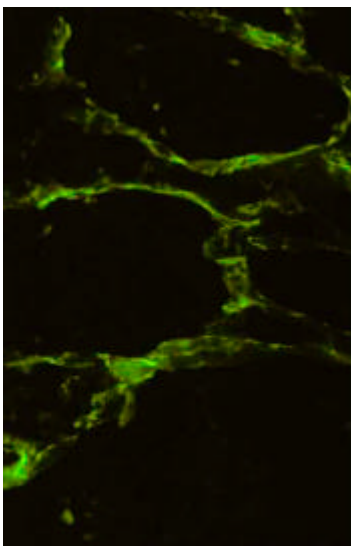
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## **Prox1: A reliable marker for lymphatic endothelial cells**

The ability to discriminate reliably at the histological level between blood and lymphatic capillaries would greatly assist the study of a number of biological and pathological questions and may also be of clinical utility.

During the last years it has been shown by many groups that the homeobox transcription factor Prox1 is a very specific and reliable marker for the lymphatics. Prox1 is the mammalian homolog of the *Drosophila* homeobox gene prospero and is involved in the formation of the lymphatic system during development. It is found in the nucleus. It can be used to stain lymphatic endothelial cells (LEC) of many different species. In opposite to other markers for LECs the protein is also expressed in adult tissues. Recently it has been shown that Prox1 is a constitutive marker of lymphangioma endothelial cells and can be used to distinguish from blood vascular endothelial cells, which are negative for Prox1 but positive for CD34. In LECs, Prox1 is coexpressed with CD31 and VEGFR-3 but not with PAL-E and generally not with CD34.

The rabbit polyclonal antibody is protein-A purified and can be used for several staining techniques. For more information see: **Datasheet**



Double staining of of a section from a human lymphangioma of the skin: CD31 (green) and Prox1 (red). The nuclei of the LECs are stained in red.

Courtesy by Jörg Wilting,  
Göttingen

## **Literature to Prox1**

Cornea 2003 Apr;22(3):273-81

### **Corneal lymphangiogenesis: evidence, mechanisms, and implications for corneal transplant immunology.**

Cursiefen C, Chen L, Dana MR, Streilein JW.

**PURPOSE**The normal cornea is devoid of blood and lymphatic vessels but can become vascularized secondary to a variety of corneal diseases and surgical manipulations. Whereas corneal (hem)angiogenesis, i.e., the outgrowth of new blood vessels from preexisting limbal vessels, is obvious both clinically and histologically, proof of associated corneal lymphangiogenesis has long been hampered by invisibility and lack of specific markers. This has changed with the recent discovery of the lymphatic endothelial markers vascular endothelial growth factor receptor 3, LYVE-1 (a lymphatic endothelium-specific hyaluronan receptor), **Prox 1**, and Podoplanin.**METHODS**We herein summarize the current evidence for lymphangiogenesis in the cornea and describe its molecular markers and mediators. Furthermore, the pathophysiologic implications of corneal lymphangiogenesis for corneal transplant immunology are discussed.**RESULTS**Whereas corneal angiogenesis in vascularized high-risk beds provides a route of entry for immune effector cells to the graft, lymphangiogenesis enables the exit of antigen-presenting cells and antigenic material from the graft to regional lymph nodes, thus inducing alloimmunization and subsequent graft rejection.**CONCLUSIONS**Antilymphangiogenic strategies may improve transplant survival both in the high- and low-risk setting of corneal transplantation.

Microsc Res Tech 2003 Feb 1;60(2):171-80

### **Lymphangiogenesis in tumors: What do we know?**

Reis-Filho JS, Schmitt FC.

Lymphangiogenesis, the growth of new lymphatic vessels, has long been regarded as a putative efficient pathway to neoplastic metastization. However, until recently consistent data regarding reliable lymphatic endothelial cell markers were lacking. Moreover, the presence of new formed lymphatic vessels was considered a largely disputable concept. Now, this scenario has changed significantly, owing to consistent reports describing novel lymphatic endothelial cell (LEC) markers, the demonstration of new formed lymphatic vessels within the bulk of the tumor in animal models and human neoplasms, and the characterization of the VEGF-C/VEGFR-3 pathway. We herein review the major breakthroughs in the field of lymphangiogenesis, with special emphasis on novel and reliable LEC markers, such as **prox-1**, LYVE-1, and podoplanin, as well as on the pathological assessment of lymphangiogenesis as a putative prognostic factor for human neoplasms.

Science 2003 Jan 10;299(5604):247-51

### **Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk.**

Abtahian F, Guerriero A, Sebzda E, Lu MM, Zhou R, Mocsai A, Myers EE, Huang B, Jackson DG, Ferrari VA, Tybulewicz V, Lowell CA, Lepore JJ, Koretzky GA, Kahn ML.

Lymphatic vessels develop from specialized endothelial cells in preexisting blood vessels, but the molecular signals that regulate this separation are unknown. Here we identify a failure to separate emerging lymphatic vessels from blood vessels in mice lacking the hematopoietic signaling protein SLP-76 or Syk. Blood-lymphatic connections lead to embryonic hemorrhage and arteriovenous shunting. Expression of *slp-76* could not be detected in endothelial cells, and blood-filled lymphatics also arose in wild-type mice reconstituted with SLP-76-deficient bone marrow. These studies reveal a hematopoietic signalling pathway required for separation of the two major vascular networks in mammals.

EMBO J 2002 Sep 2;21(17):4593-9

**Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor.**

Petrova TV, Makinen T, Makela TP, Saarela J, Virtanen I, Ferrell RE, Finegold DN, Kerjaschki D, Yla-Herttuala S, Alitalo K.

Lymphatic vessels are essential for fluid homeostasis, immune surveillance and fat adsorption, and also serve as a major route for tumor metastasis in many types of cancer. We found that isolated human primary lymphatic and blood vascular endothelial cells (LECs and BECs, respectively) show interesting differences in gene expression relevant for their distinct functions *in vivo*. Although these phenotypes are stable *in vitro* and *in vivo*, overexpression of the homeobox transcription factor **Prox-1** in the BECs was capable of inducing LEC-specific gene transcription in the BECs, and, surprisingly, Prox-1 suppressed the expression of approximately 40% of the BEC-specific genes. Prox-1 did not have global effects on the expression of LEC-specific genes in other cell types, except that it up-regulated cyclin E1 and E2 mRNAs and activated the cyclin e promoter in various cell types. These data suggest that Prox-1 acts as a cell proliferation inducer and a fate determination factor for the LECs. Furthermore, the data provide insights into the phenotypic diversity of endothelial cells and into the possibility of transcriptional reprogramming of differentiated endothelial cells.

FASEB J 2002 Aug;16(10):1271-3

**The transcription factor Prox1 is a marker for lymphatic endothelial cells in normal and diseased human tissues.**

Wilting J, Papoutsi M, Christ B, Nicolaidis KH, von Kaisenberg CS, Borges J, Stark GB, Alitalo K, Tomarev SI, Niemeyer C, Rossler J.

Detection of lymphatic endothelial cells (LECs) has been problematic because of the lack of specific markers. The homeobox transcription factor **Prox1** is expressed in LECs of murine and avian embryos. We have studied expression of Prox1 in human tissues with immunofluorescence. In 19-wk-old human fetuses, Prox1 and vascular endothelial growth factor receptor-3 (VEGFR-3) are coexpressed in LECs of lymphatic trunks and lymphatic capillaries. Prox1 is located in the nucleus, and its expression is mutually exclusive with that of the blood vascular marker PAL-E. Prox1 is a constitutive marker of LECs and is found in tissues of healthy adults and lymphedema patients. Blood vascular endothelial cells (BECs) of hemangiomas express CD31 and CD34, but not Prox1. A subset of these cells is positive for VEGFR-3. Lymphatics in the periphery of hemangiomas express Prox1 and CD31, but not CD34. In lymphangiomas, LECs express Prox1, CD31, and

VEGFR-3, but rarely CD34. In the stroma, spindle-shaped CD34-positive cells are present. We show that Prox1 is a reliable marker for LECs in normal and pathologic human tissues, coexpressed with VEGFR-3 and CD31. VEGFR-3 and CD34 are less reliable markers for LECs and BECs, respectively, because exceptions from their normal expression patterns are found in pathologic tissues.

Semin Cell Dev Biol 2002 Feb;13(1):9-18

### **Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis.**

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Vascular endothelial growth factor receptor-3 (VEGFR-3) mediates lymphatic endothelial cell (LEC) growth, migration, and survival by binding VEGF-C and VEGF-D. Recent studies have revealed new regulators of the lymphatic endothelium, such as the transcription factor Prox1, and the cell surface proteins podoplanin and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). Furthermore, the isolation of LECs now allows detailed molecular studies of the factors regulating the lymphatic vasculature. These studies are aimed at targeting the lymphatic vasculature in the treatment of various diseases, such as tumour metastasis and lymphoedema.

Genes Dev 2002 Apr 1;16(7):773-83

### **The rediscovery of the lymphatic system: old and new insights into the development and biological function of the lymphatic vasculature .**

Oliver G, Detmar M.  
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EMBO J 2002 Apr 2;21(7):1505-13

### **An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype.**

Wigle JT, Harvey N, Detmar M, Lagutina I, Grosveld G, Gunn MD, Jackson DG, Oliver G.

The process of angiogenesis has been well documented, but little is known about the biology of lymphatic endothelial cells and the molecular mechanisms controlling lymphangiogenesis. The homeobox gene **Prox1** is expressed in a subpopulation of endothelial cells that, after budding from veins, gives rise to the mammalian lymphatic system. In Prox1(-)/(-) embryos, this budding becomes arrested at around embryonic day (E)11.5, resulting in embryos without lymphatic vasculature. Unlike the endothelial cells that bud off in E11.5 wild-type embryos, those of Prox1-null embryos did not co-express any lymphatic markers such as VEGFR-3, LYVE-1 or SLC. Instead, the mutant cells appeared to have a blood vascular phenotype, as determined by their expression of laminin and CD34. These results suggest that Prox1 activity is required for both maintenance of the budding of the venous endothelial cells and differentiation toward the lymphatic phenotype. On the basis of our findings, we propose that a blood vascular phenotype is the default fate of budding embryonic venous endothelial cells; upon expression of Prox1, these budding cells adopt a lymphatic vasculature phenotype.

**Prox1 is differentially localized during lens development.**

Duncan MK, Cui W, Oh DJ, Tomarev SI.  
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Prox1, the vertebrate cognate of *Drosophila* Prospero, is a homeodomain protein essential for the development of the lens, liver and lymphatic system. While it is well established that the subcellular distribution of Prospero changes during development, this had not been demonstrated for Prox1. Here, high-resolution confocal microscopy demonstrated that **Prox1** protein is predominately cytoplasmic in the lens placode as well as the lens epithelium and germinative zone throughout development. However during fiber cell differentiation, Prox1 protein redistributes to cell nuclei. Finally, as lens fiber cells condense their chromatin in response to lens denucleation, Prox1 remains in the nucleus but does not appear to interact with DNA. Thus, it appears that the function of Prox1, like that of its *Drosophila* cognate Prospero, is at least partially controlled by changes in its subcellular distribution during development.

**Prox1 is a marker of ectodermal placodes, endodermal compartments, lymphatic endothelium and lymphangioblasts.**

Rodriguez-Niedenfuhr M, Papoutsi M, Christ B, Nicolaidis KH, von Kaisenberg CS, Tomarev SI, Wilting J.

The lymphatic endothelium has mostly been thought to be derived by sprouting from specialized veins. Recently it has been shown that mice deficient for the homeobox transcription factor Prox1 are practically devoid of lymphatics. We have studied the expression of **Prox1** mRNA and protein in chick embryos and human fetuses. In the chick, Prox1 is expressed in specific compartments of all germ layers. In the ectoderm, it is found in the neural tube, trigeminal, spinal and sympathetic ganglia and the retina, and also in placodal structures such as the lens, olfactory, otic, facial, glossopharyngeal and vagal placodes, and the apical ectodermal ridge. In the endoderm, Prox1 is a marker of hepatocytes, bile duct and pancreatic epithelium. In the mesoderm, weak expression is observed in cardiomyocytes, and strong expression in lymphatic endothelium. Identical expression domains are found in 19-week-old human fetuses. In day 6.5 chick embryos, there are several sites of contact of lymphatics with the jugular vein, which has a mixed endothelium of Prox1-positive and -negative cells. The only non-lymphatic endothelial cells expressing Prox1 are found on the concave side of the cardiac valves. To further analyse development of lymphatics, we studied early chick embryos and observed scattered Prox1-positive cells in the dermatome, giving rise to Prox1-positive lymphatic networks during subsequent development. Furthermore, the anlagen of the posterior lymph sacs and the paired thoracic duct can already be observed in day-4 chick embryos. Our studies show that lymphatics develop much earlier than previously described, and they mostly do not seem to be derived by sprouting from veins. In contrast, lymphangioblasts are present in the deep and superficial compartments of the early mesoderm, independently giving rise to the deep and superficial lymphatics.

**LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis.**

Mouta Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, Jain RK.

Lymphatic vessel endothelial hyaluronan receptor (LYVE)-1 is thought to be restricted to lymph vessels and has been used as such to show that tumor lymphangiogenesis occurs on overexpression of lymphangiogenic factors in mouse tumor models. However, these studies have not yet been corroborated in human tumors. Here we show, first, that LYVE-1 is not exclusive to the lymph vessels. Indeed, LYVE-1 is also present in normal hepatic blood sinusoidal endothelial cells in mice and humans. Surprisingly, LYVE-1 is absent from the angiogenic blood vessels of human liver tumors and only weakly present in the microcirculation of regenerative hepatic nodules in cirrhosis, though both vessels are largely derived from the liver sinusoids. Second, we propose a novel approach to identify lymphatics in human and murine liver. By combining LYVE-1 and **Prox 1** (a transcription factor) immunohistochemistry, we demonstrate that lymphatics are abundant in cirrhosis. In contrast, in human hepatocellular carcinoma and liver metastases, they are restricted to the tumor margin and surrounding liver. The absence of intratumor lymphatics in hepatocellular carcinomas and liver metastases may impair molecular and cellular transport in these tumors. Finally, the presence of LYVE-1 in liver sinusoidal endothelia suggests that LYVE-1 has functions beyond the lymph vascular system.

**Endogenous origin of the lymphatics in the avian chorioallantoic membrane.**

Papoutsi M, Tomarev SI, Eichmann A, Prols F, Christ B, Wilting J.

The lymphatics of the intestinal organs have important functions in transporting chyle toward the jugulosubclavian junction, but the lymphangiogenic potential of the splanchnic mesoderm has not yet been tested. Therefore, we studied the allantoic bud of chick and quail embryos. It is made up of endoderm and splanchnic mesoderm and fuses with the chorion to form the chorioallantoic membrane (CAM) containing both blood vessels and lymphatics. In day 3 embryos (stage 18 of Hamburger and Hamilton [HH]), the allantoic mesoderm consists of mesenchymal cells that form blood islands during stage 19 (HH). The endothelial network of the allantoic bud, some intraluminal and some mesenchymal cells express the hemangiopoietic marker QH1. The QH1-positive endothelial cells also express the vascular endothelial growth factor receptor-3 (VEGFR-3), whereas the integrating angioblasts and the round hematopoietic cells are QH1-positive/VEGFR-3-negative. The ligand, VEGF-C, is expressed ubiquitously in the allantoic bud, and later predominantly in the allantoic epithelium and the wall of larger blood vessels. Allantoic buds of stage 17-18 (HH) quail embryos were grafted homotopically into chick embryos and reincubated until day 13. In the chimeric CAMs, quail endothelial cells are present in blood vessels and lymphatics, the latter being QH1 and VEGFR-3 double-positive. QH1-positive hematopoietic cells are found at many extra- and intraembryonic sites, whereas endothelial cells are confined to the grafting site. Our results show that the early allantoic bud contains hemangioblasts and lymphangioblasts. The latter can be identified with **Prox1** antibodies and mRNA probes in the allantoic mesoderm of day 4 embryos (stage 21 HH). Prox1 is a specific marker of the lymphatic endothelium throughout CAM development.

## **Lymphatic versus blood vascular endothelial growth factors and receptors in humans.**

Partanen TA, Paavonen K.

Three different growth factor systems have been described acting via endothelial cell-specific receptor tyrosine kinases (RTKs). These are vascular endothelial growth factors (VEGFs), angiopoietins, and ephrins. Recent studies on gene targeting suggest that they play critical roles in embryonic development and contribute to the integrity and responses to environmental factors in the adult vasculature. Coagulation, inflammation, immune response regulation, vascular tone, stromal component synthesis, and angiogenesis are all dependent on the physiological and pathological events that affect endothelial cells in the heart, arteries, veins, and lymphatic vessels. Angiogenesis, the formation of new blood vessels from preexisting ones, takes place in adults only during hormonal control of female reproduction. All other activation of angiogenesis in adulthood occurs in response to injury or pathological processes such as tumorigenesis, diabetes, or inflammatory conditions. Insufficient growth of collateral vessels is a major problem in atherosclerotic cardiovascular disease. Controlled stimulation of angiogenesis would be of therapeutic value. Lymphangiogenesis, the mechanisms involved in the development of lymphatic vessels, was studied intensively nearly a century ago, although since then it has been neglected, perhaps because, unlike the disorders of blood vessels, those of the lymphatic vessels are seldom life-threatening. Interrupting this one-way system can cause severe disorders, including liver dysfunction, genetic disease (e.g., Milroy's disease), and degenerative disease (e.g., primary lymphangiosclerosis). Recently, novel growth factors, receptors, cell surface proteins, and transcription factors have been found which play a role in the lymphatic endothelium. These are VEGF-C, VEGF-D, VEGFR-3, LYVE-1, podoplanin, and **Prox-1**. Until recently lymphatic vessels have been difficult to study due to a lack of appropriate tools. Monoclonal antibodies raised against VEGFR-3 and against its ligands, VEGF-C and VEGF-D, have offered an insight into expression studies in tissues. In this review, we summarize the recent data on VEGFs in the human vasculature.

## Development of the avian lymphatic system.

Wilting J, Papoutsi M, Othman-Hassan K, Rodriguez-Niedenfuhr M, Prols F, Tomarev SI, Eichmann A.  
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Recently, highly specific markers of the lymphatic endothelium have been found enabling us to reinvestigate the embryonic origin of the lymphatics. Here we present a review of our studies on the development of the lymphatic system in chick and quail embryos. We show that the lymphatic endothelium is derived from two sources: the embryonic lymph sacs and mesenchymal lymphangioblasts. Proliferation studies reveal a BrdU-labeling index of 11.5% of lymph sac endothelial cells by day 6.25, which drops to 3.5% by day 7. Lymphangioblasts are able to integrate into the lining of lymph sacs. Lymphatic endothelial cells express the vascular endothelial growth factor (VEGF) receptors-2 and -3. Their ligand, VEGF-C, is expressed almost ubiquitously in embryonic and fetal tissues. Elevated expression levels are found in the tunica media of large blood vessels, which usually serve as major routes for growing lymphatics. The homeobox gene, Prox1, is

expressed in lymphatic but not in blood vascular endothelial cells throughout all stages examined, namely, in developing lymph sacs of day 6 embryos and in lymphatics at day 16. Experimental studies show the existence of lymphangioblasts in the mesoderm, a considerable time before the development of the lymph sacs. Lymphangioblasts migrate from the somites into the somatopleure and contribute to the lymphatics of the limbs. Our studies indicate that these lymphangioblasts already express **Prox1**.

Dev Genes Evol 2000 May;210(5):223-30

### **Prox 1 in eye degeneration and sensory organ compensation during development and evolution of the cavefish *Astyanax*.**

Jeffery W, Strickler A, Guiney S, Heyser D, Tomarev S.  
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We have investigated expression of the homeobox gene Prox 1 during eye degeneration and sensory organ compensation in cavefish embryos. The teleost *Astyanax mexicanus* consists of sighted surface-dwelling forms (surface fish) and several populations of blind cave-dwelling forms (cavefish), which have evolved independently. Eye formation is initiated during cavefish development, but the lens vesicle undergoes apoptosis, and the eye subsequently arrests and degenerates. The requirement of **Prox 1** for lens fiber differentiation and gamma-crystallin expression in the mouse suggests that changes in the expression of this gene could be involved in cavefish eye degeneration. Surface fish and cavefish embryos stained with a Prox 1 antibody showed Prox 1 expression in the lens, neuroretina, myotomes, heart, hindbrain, and gut, as reported in other vertebrates. We found that Prox 1 expression is not altered during cavefish lens development. Prox 1 protein was detected in the lens vesicle as soon as it formed and persisted until the time of lens degeneration in each cavefish population. The cavefish lens vesicle was also shown to express a gamma-crystallin gene, suggesting that Prox 1 is functional in cavefish lens development. In addition to the tissues described above, Prox 1 is expressed in developing taste buds and neuromasts in cavefish, which are enhanced to compensate for blindness. It is concluded that the Prox 1 gene is not involved in lens degeneration, but that expansion of the Prox 1 expression domain occurs during taste bud and neuromast development in cavefish.

Nucleic Acids Res 2001 Jan 15;29(2):515-26

### **Antagonistic action of Six3 and Prox1 at the gamma-crystallin promoter.**

Lengler J, Krausz E, Tomarev S, Prescott A, Quinlan RA, Graw J.

Gamma-crystallin genes are specifically expressed in the eye lens. Their promoters constitute excellent models to analyse tissue-specific gene expression. We investigated murine CRYGE/f promoters of different length in lens epithelial cell lines. The most active fragment extends from position -219 to +37. Computer analysis predicts homeodomain and paired-domain binding sites for all rodent CRYG /e/f core promoters. As examples, we analysed the effects of Prox1 and Six3, which are considered important transcription factors involved in lens development. Because of endogenous Prox1 expression in N/N1003A cells, a weak stimulation of CRYGE/f promoter activity was found for **PROX1**. In contrast, PROX1 stimulated the CRYGF promoter 10-fold in CD5A cells without endogenous PROX1. In both cell lines Six3 repressed the CRYGF promoter to 10% of its basal activity. Our cell transfection experiments indicated that CRYG expression

increases as Six3 expression decreases. Prox1 and Six3 act antagonistically on regulation of the CRYGD/e/f promoters. Functional assays using randomly mutated gammaF-crystallin promoter fragments define a Six3-responsive element between -101 and -123 and a Prox1-responsive element between -151 and -174. Since Prox1 and Six3 are present at the beginning of lens development, expression of CRYGD/e/f is predicted to remain low at this time. It increases as Six3 expression decreases during ongoing lens development.

Nat Genet 2000 Jul;25(3):254-5

### **Hepatocyte migration during liver development requires Prox1.**

Sosa-Pineda B, Wigle JT, Oliver G.

Several genes are required during the early phases of liver specification, proliferation and differentiation. Here we report that **Prox1** is required for hepatocyte migration. Loss of Prox1 leads to formation of a smaller liver with a reduced population of clustered hepatocytes surrounded by a laminin-rich basal membrane.

Differentiation 1999 Nov;65(3):141-9

### **Pax-6 and Prox 1 expression during lens regeneration from Cynops iris and Xenopus cornea: evidence for a genetic program common to embryonic lens development.**

Mizuno N, Mochii M, Yamamoto TS, Takahashi TC, Eguchi G, Okada TS.  
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Lens regeneration from non-lens ocular tissues has been well documented in amphibians, from the dorsal iris in the newt and from the outer cornea in Xenopus. To understand the early molecular events which govern lens regeneration, we examined the expression of two early marker genes of normal lens development, Pax-6 and **Prox 1**. In both Cynops (newt) iris and Xenopus cornea, Pax-6 is expressed soon after lentectomy in a region broader than that giving rise to the regenerating lens, indicative of an important role for Pax-6 in determination of the regeneration potential. Then Prox 1 expression begins within the Pax-6-expressing tissue, and these Prox 1-expressing cells give rise to the regenerating lens. This sequence of events also takes place in the lens placode of the embryo, indicating that the presence of the same genetic program operates in both embryonic lens development and lens regeneration, at least partly. In the Cynops iris, Pax-6 expression occurs initially in the entire marginal region of the iris after lentectomy but then becomes restricted to the dorsal region. Further studies are expected to elucidate the mechanism of this long-standing problem of the dorsal-restriction of lens regeneration from the newt iris.

Cell 1999 Sep 17;98(6):769-78

### **Prox1 function is required for the development of the murine lymphatic system.**

Wigle JT, Oliver G.

The lack of specific markers has raised problems in documenting the precise manner by which the lymphatic system develops. Here we report that the homeobox gene **Prox1** is expressed in a

subpopulation of endothelial cells that by budding and sprouting give rise to the lymphatic system. The initial localization of these cells in the veins and their subsequent budding are both polarized, suggesting that unidentified guidance signals regulate this process. In Prox1 null mice, budding and sprouting is arrested, although vasculogenesis and angiogenesis of the vascular system is unaffected. These findings suggest that Prox1 is a specific and required regulator of the development of the lymphatic system and that the vascular and lymphatic systems develop independently.

Invest Ophthalmol Vis Sci 1999 Aug;40(9):2039-45

### **Regulation of Prox 1 during lens regeneration.**

Del Rio-Tsonis K, Tomarev SI, Tsonis PA.

**PURPOSE:** To determine the expression pattern of **Prox 1** during the process of lens regeneration in the urodele *Notophthalmus viridescens*. **METHODS:** Polymerase chain reaction was performed to amplify a partial newt Prox 1 sequence. In situ hybridization and immunodetection methods were used to detect the Prox 1 mRNA and the Prox 1 protein, respectively. **RESULTS:** Prox 1 mRNA was present in the retina and in the lens (in the epithelium and bow region) of the intact eye. Prox 1 protein was found to be predominantly present in the lens and dorsal iris of the intact eye, although some trace levels of Prox 1 protein were detected in the ventral iris as well. After lensectomy, expression of the mRNA was also pronounced in the dorsal dedifferentiating iris and the regenerating lens. The ventral iris also expressed Prox 1 but seemingly at lower levels. Although Prox 1 protein showed upregulation in the dorsal iris during the process of lens regeneration, trace levels were also detected in the ventral iris. In the retina, Prox 1 protein was distributed in horizontal cells of the inner nuclear layer, whereas the mRNA was expressed in all layers of the retina. **CONCLUSIONS:** Prox 1 was unevenly distributed in the intact cells of the newt iris, with significantly higher levels of Prox 1 protein present in the dorsal versus the ventral margin. This protein was differentially regulated during the process of lens regeneration, with obvious upregulation in the dorsal iris. Prox 1 is the first transcriptional factor to be shown to be regulated in the dorsal versus ventral iris during the process of lens regeneration.

Nat Genet 1999 Mar;21(3):318-22

### **Prox1 function is crucial for mouse lens-fibre elongation.**

Wigle JT, Chowdhury K, Gruss P, Oliver G.

Although insights have emerged regarding genes controlling the early stages of eye formation, little is known about lens-fibre differentiation and elongation. The expression pattern of the Prox1 homeobox gene suggests it has a role in a variety of embryonic tissues, including lens. To analyse the requirement for **Prox1** during mammalian development, we inactivated the locus in mice. Homozygous Prox1-null mice die at mid-gestation from multiple developmental defects; here we describe the specific effect on lens development. Prox1 inactivation causes abnormal cellular proliferation, downregulated expression of the cell-cycle inhibitors Cdkn1b (also known as p27KIP1) and Cdkn1c (also known as p57KIP2), misexpression of E-cadherin and inappropriate apoptosis. Consequently, mutant lens cells fail to polarize and elongate properly, resulting in a hollow lens. Our data provide evidence that the progression of terminal fibre differentiation and elongation is dependent on Prox1 activity during lens development.

Development 1999 Feb;126(3):443-56

**Transcription factors Mash-1 and Prox-1 delineate early steps in differentiation of neural stem cells in the developing central nervous system.**

Torii M, Matsuzaki F, Osumi N, Kaibuchi K, Nakamura S, Casarosa S, Guillemot F, Nakafuku M.

Like other tissues and organs in vertebrates, multipotential stem cells serve as the origin of diverse cell types during genesis of the mammalian central nervous system (CNS). During early development, stem cells self-renew and increase their total cell numbers without overt differentiation. At later stages, the cells withdraw from this self-renewal mode, and are fated to differentiate into neurons and glia in a spatially and temporally regulated manner. However, the molecular mechanisms underlying this important step in cell differentiation remain poorly understood. In this study, we present evidence that the expression and function of the neural-specific transcription factors Mash-1 and Prox-1 are involved in this process. In vivo, Mash-1- and **Prox-1**-expressing cells were defined as a transient proliferating population that was molecularly distinct from self-renewing stem cells. By taking advantage of in vitro culture systems, we showed that induction of Mash-1 and Prox-1 coincided with an initial step of differentiation of stem cells. Furthermore, forced expression of Mash-1 led to the down-regulation of nestin, a marker for undifferentiated neuroepithelial cells, and up-regulation of Prox-1, suggesting that Mash-1 positively regulates cell differentiation. In support of these observations in vitro, we found specific defects in cellular differentiation and loss of expression of Prox-1 in the developing brain of Mash-1 mutant mice in vivo. Thus, we propose that induction of Mash-1 and Prox-1 is one of the critical molecular events that control early development of the CNS.

Mech Dev 1998 Aug;76(1-2):175-8

**Restricted expression of the homeobox gene prox 1 in developing zebrafish.**

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**Prox 1** is a vertebrate homeobox gene which is homologous to the Drosophila transcription factor, prospero. We have isolated a prox 1 cDNA from zebrafish, which encodes a protein that has 82%, 84% and 83% amino acid identity with chicken, mouse and human Prox 1, respectively. Antibodies raised against human Prox 1 cross-react with zebrafish Prox 1 and are used here to determine the expression patterns of Prox 1 during zebrafish embryogenesis by whole-mount immunohistochemistry. In the 10-somite embryo, Prox 1 is expressed over the prospective lens placode and over a broad region of epithelium extending from the eye to the otic vesicle. As embryogenesis proceeds, Prox 1 expression in the eye lens becomes intense, and is detected in maturing muscle pioneer cells and superficial muscle cells. In the CNS, Prox 1 is expressed in a stripe along the forebrain-midbrain boundary, in a segmented pattern in the ventral hindbrain, and in selected cells of the ventral spinal cord. Additional sites of Prox 1 expression include the lateral line primordium, the trigeminal ganglia, the otic vesicle and occasional endodermal cells.

Biochem Biophys Res Commun 1998 Jul 30;248(3):684-9

## **Characterization of the mouse Prox1 gene.**

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Prox1, a vertebrate homologue of *Drosophila prospero*, encodes a divergent homeodomain protein. We have isolated and characterized full length mouse **Prox1** cDNA and genomic clones. Mouse Prox1 gene mapped to position 106.3 cM from the centromere of Chromosome 1, which is very close to the retinal degeneration mutation, rd3. Although the coding sequence and exon-intron junctions of the Prox1 genes of wild type and rd3 mutant mice are identical, Northern blot analysis indicated that the ratio of the short (2.3 kb) and long (8 kb) forms of Prox1 mRNA is different in RNA isolated from wild type and rd3 retinas. Immunostaining of the eyes from wild type and rd3 animals also revealed differences in the distribution of Prox1 protein in the retina and lens. These data suggest that the rd3 mutation affects expression of the mouse Prox1 gene.

Int J Dev Biol 1997 Dec;41(6):835-42

## **Pax-6, eyes absent, and Prox 1 in eye development.**

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Eyes in different systematic groups including arthropods, molluscs and vertebrates probably have a common evolutionary origin. As a consequence of this, related genes are used for regulation of the early steps of eye development in different organisms. In this review, I briefly summarize data on three gene families which might be essential for eye development across species: Pax-6/eyeless, Eya/eyes absent and **Prox**/prospero with emphasis on our contribution here. Mechanisms of eye formation and the generation of different types of eyes in the course of evolution are discussed.

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## **Pax-6, Prox 1, and Chx10 homeobox gene expression correlates with phenotypic fate of retinal precursor cells.**

Belecky-Adams T, Tomarev S, Li HS, Ploder L, McInnes RR, Sundin O, Adler R.

**PURPOSE:** To study the expression patterns of the homeobox genes Pax-6, **Prox 1**, and Chx10 during chick retinal development in vivo and in vitro. **METHODS:** Sections of paraformaldehyde-fixed, paraffin-embedded eyes were obtained at a range of developmental stages. In situ hybridization was carried out on tissue sections using digoxigenin-labeled sense and antisense RNA probes that recognize chicken Pax-6 and Prox 1 (whose sequences were already available), and chicken Chx10 (which was cloned and sequenced as part of this study). Selected developmental stages were also studied by immunocytochemistry with antibodies against Pax-6 and Prox 1, and by Northern blot analysis using <sup>32</sup>P-labeled probes. **RESULTS:** Until embryonic day (ED) 5, in situ hybridization shows widespread, diffuse distribution of all three genes. Between ED 6 and ED 8, however, they acquire distinct, topographically specific patterns of expression. The Prox 1 signal is predominantly expressed in the prospective horizontal cell layer of the neuroepithelium, decreases vitreally, and is absent from ganglion cells and the prospective photoreceptor layer. Pax-6 is strongly expressed only in the prospective ganglion-cell and amacrine-cell regions at the same stages, and is not detected in prospective photoreceptors. Chx10 expression becomes concentrated

in the future bipolar-cell region of the inner nuclear layer. Similar patterns are maintained by ED 15 through ED 18, after cell differentiation has taken place. Pax-6 and Prox 1 immunoreactive materials showed nuclear localization and a pattern of laminar distribution equivalent to that seen by in situ hybridization. CONCLUSIONS: These results suggest that the differentiated fate of retinal precursor cells may be influenced by Pax-6, Prox 1, or Chx10, this hypothesis is now being tested using dissociated chick embryo retinal cell cultures.

Mamm Genome 1996 Dec;7(12):877-80

**Characterization and localization of the mProx1 gene directly upstream of the mouse alpha-globin gene cluster: identification of a polymorphic direct repeat in the 5'UTR.**

Kielman MF, Barradeau S, Smits R, Harteveld CL, Bernini LF.

The alpha-globin major regulatory element (alpha MRE) positioned far upstream of the gene cluster is essential for the proper expression of the alpha-globin genes. Analysis of the human and mouse alpha-globin Upstream Flanking Regions (alpha UFR) has identified three nonglobin genes in the order Dist1-MPG-**Prox1**-alpha-globin. Further characterization of the whole region indicates that the alpha MRE and several other erythroid DNase HSSs are associated with the transcription unit of the Prox1 gene. In this paper we describe the characterization and localization of the mouse Prox1 cDNA and compare it with its human homolog, the -14 gene, and another human cDNA sequence named hProx1. Our results show a strong conservation between the -14 gene and the mouse Prox1 gene with the exception of the first exon of the mProx1 gene. This exon is absent in the -14 cDNA but is present and conserved in the human Prox1 cDNA, indicating that the human -14/hProx1 gene is alternatively spliced or transcribed. The mProx1 gene encodes a predicted protein of 491 amino acids (aa) whose function is not known. In the 5'UTR of this gene, a 35-bp repeat (VNTR) is positioned, which is highly polymorphic among laboratory inbred mice (*Mus domesticus*). Our results strongly suggest that the mProx1 VNTR arose during the divergence of *M. spretus* and *M. domesticus*. Besides its use in evolutionary studies and positional cloning, the mProx1 VNTR might be invaluable for monitoring the expression of a transgenic mProx1 gene. The cloning of the mProx1 gene will be helpful to analyze its possible role on alpha-globin as well on MPG expression in the mouse.

Dev Dyn 1996 Aug;206(4):354-67

**Chicken homeobox gene Prox 1 related to *Drosophila prospero* is expressed in the developing lens and retina.**

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**Prox 1** is the vertebrate homolog of *Drosophila prospero*, a gene known to be expressed in the lens-secreting cone cells of fly ommatidia. Chicken Prox 1 cDNAs were isolated from 14 day embryonic chicken lenses, and a complete open reading frame encoding an 83 kDa protein was elucidated. The homeodomains of chicken and mouse Prox 1 are identical at the amino acid level and are 65-67% similar to the homeodomains of *Drosophila* and *C. elegans prospero*. The homology between these proteins extends beyond the homeodomain. There is 56% identity between chicken Prox 1 and *Drosophila prospero* in the C-terminal region downstream of the homeodomain, whereas there is little similarity upstream of the homeodomain. Prox 1 is expressed most actively in the developing

lens and midgut and at lower levels in the developing brain, heart, muscle, and retina. cDNA sequencing has established that there are alternatively spliced forms of the single Prox 1 gene, which probably account for the two abundant RNAs of about 2 and 8 kb and two less abundant RNAs close to 3.5 kb in length in the lens. In the lens fibers, only the shortest mRNA was present, whereas, in the epithelial cells, both short and long mRNAs were detected. By using in situ hybridization, expression of the Prox 1 gene was first detected at stage 14 in the early lens placode and slightly preceded the expression of delta 1-crystallin, the first crystallin gene expressed in the developing chicken lens. At later stages of development, Prox 1 mRNA was observed throughout the lens, but it appeared more abundant around the bow region of the equator than in the anterior epithelium or the fibers. In the retina, expression of the Prox 1 gene was detected mainly in the inner nuclear layer during later stages of histogenesis. The conserved pattern of Prox 1/prospero gene expression in vertebrates and *Drosophila* suggests that Prox 1, like Pax-6, may be essential for eye development in different systematic groups.

Genomics 1996 Aug 1;35(3):517-22

### **Structure and chromosomal localization of the human homeobox gene Prox 1.**

Zinovieva RD, Duncan MK, Johnson TR, Torres R, Polymeropoulos MH, Tomarev SI.

The genomic organization and nucleotide sequence of the human homeobox gene **Prox 1** as well as its chromosomal localization have been determined. This gene spans more than 40 kb, consists of at least 5 exons, and encodes an 83-kDa protein. It shows 89% identity with the chicken sequence at the nucleotide level in the coding region, while the human and chicken proteins are 94% identical. Among the embryonic tissues analyzed (lens, brain, lung, liver, and kidney), the human Prox 1 gene is most actively expressed in the developing lens, similar to the expression pattern of the chicken Prox 1 gene. The Prox 1 gene was mapped to human chromosome 1q32.2-q32.3.

Mech Dev 1993 Nov;44(1):3-16

### **Prox 1, a prospero-related homeobox gene expressed during mouse development.**

Oliver G, Sosa-Pineda B, Geisendorf S, Spana EP, Doe CQ, Gruss P.

**Prox 1**, a likely mouse homologue of the *Drosophila* homeobox gene prospero has been cloned and its expression pattern analyzed during development. In *Drosophila*, prospero is expressed in the developing CNS, lens-secreting cone cells of the eye, and midgut. In the mouse, Prox 1 is expressed in many of the same tissues: young neurons of the subventricular region of the CNS, developing eye lens and pancreas. Expression is also detected in the developing liver and heart, as well as transiently in the skeletal muscles. The similarities in protein sequence and expression patterns between the mouse and fly cognate genes suggest that Prox 1 may play, among others, a fundamental role in early development of the murine CNS.