Current Biology, Vol. 12, 492–497, March 19, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00694-2

Endothelial Signaling in Kidney Morphogenesis: A Role for Hemodynamic Forces

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Summary

The local presence of endothelial cells seems necessary for proper embryonic development of several organs. However, the signals involved are unknown. The glomerulus is generated by the coalescence of podocytes around an ingrowing capillary and is the site of blood ultrafiltration. In the absence of vessels, glomerular assembly does not occur. We describe mutations in the zebrafish that prevent glomerulogenesis. All mutants display cardiac dysfunction. Pharmacological interference with cardiac output and focal laser occlusion of the vessel similarly prevent glomerular formation. The unifying feature of all these perturbations is absence of blood flow. We find that expression of *matrix metalloproteinase-2* (*MMP-2*), known in other systems to be regulated in a stretch-responsive manner, is in renal endothelial cells and is regulated by flow, suggesting that an MMP-2-sensitive event may be downstream of the flow-related signal. In support of this, blockade of MMP-2 activity by injection of TIMP-2 does not perturb circulation but does prevent glomerular assembly. Thus, vascular flow is required for glomerular assembly, most probably acting via a stretch-responsive signaling system in the vessel wall.

Results and Discussion

Vessels are needed in all tissues to deliver oxygen. Recent work suggests that they may also act as signaling centers to help to organize particular organs during embryogenesis, such as the pancreas and liver [1, 2]. The zebrafish mutant *cloche* lacks endothelial cells and does not form a normal pronephric glomerulus [3]. The zebrafish embryo is a useful system in which to examine the effects of vessels on development, because they do not depend upon blood cells to deliver oxygen but rather rely upon diffusion from the environment [4–6]. This means that effects of vessel per se can be more readily distinguished from secondary effects of hypoxia.

The glomerulus of the zebrafish is generated by medial migration of intermediate mesoderm (IM) precursor cells expressing the Wilms' tumor suppressor gene (*wt1*) [7]. These cells form bilateral pronephric primordia at 36 hr postfertilization (hpf, Figure 1A) and later give rise to the midline glomerulus (Figure 1B). The glomerular podocytes coalesce around a vascular sprout from the aorta [3, 8]. Ultrafiltration of blood in the glomerulus commences at 40 hr postfertilization [8].

Through an ENU mutagenesis screen, using *wt1* as a marker, we sought mutations that perturb glomerular assembly without affecting acquisition of the *wt1* fate in the IM. We found five mutations in which *wt1* cells do not migrate medially. We noted that they shared the common feature of impaired or absent circulation. One of these mutations mapped genetically to a small interval containing the *titin* gene. This mutation did not complement an allele of a mutation we have identified as causing a cardiac-specific mutation in *titin*, a component of the myofibril of the heart, and not expressed in the kidney [36]. This suggested that the glomerular defect might arise secondary to the cardiac dysfunction.

We therefore examined pronephric morphogenesis in embryos without a circulation due to other mutations, including *island beat* (*isl*), *valentine* (*vtn*), and *silent heart* (*sih*). An example is shown in Figure 1C. *island beat* embryos have a mutation in the pore-forming α1c subunit of the L-type calcium channel, a gene expressed in the nervous system, pancreas, and heart but not in the kidney [9]. Its cardiac defects include a silent ventricle, fibrillating atrium, and absent circulation. In *isl*, *wt1*-expressing cells remain unmerged and ventral to the somites. Similar unfused primordia were observed in *valentine* embryos (Figure 1D), which have a weak heartbeat and lack circulation, and in *silent heart* embryos (Figure 1E), which fail to initiate a heart beat [10, 11].

Podocytes in *isl* appear to differentiate normally, expressing *wt1* and *vascular endothelial growth factor* (*VEGF*) but remain unmerged (Figures 2A and 2B). By electron microscopy, the glomerulus of a 48 hpf wild-type embryo is a spherical mass ventral to the dorsal aorta containing, at its center, interdigitating endothelial cells (Figure 2C). In *isl*, endothelial cells from the dorsal aorta do not invade the clusters but are found instead surrounding the podocytes (Figure 2D). The endothelial cells are normal in appearance and form normal cell junctions (Figure 2E). Podocytes are also wild-type in appearance, manifesting foot processes, even though they remain in bilateral clusters (Figure 2F). Elsewhere, circulatory anatomy appears normal; vasculogenesis and sprouting angiogenesis, for example, the intersomitic vessels, are apparently unaffected at this stage.

If blood flow is directly involved in the triggering of glomerular assembly, its effect should be maximal at the time of medial migration of the *wt1*-expressing cells. The heart beat starts at 24 hpf, and glomerular assembly occurs between 36 and 48 hpf [7, 8]. We therefore used 2,3-butanedione monoxime (BDM), an inhibitor of the myofibrilar ATPases [12], to stop the heart reversibly during this time window and showed that the glomerulus fails to form (Figure 3A). When BDM is withdrawn from the embryo media, glomerular morphogenesis is restored, provided that BDM is removed prior to 55 hr of

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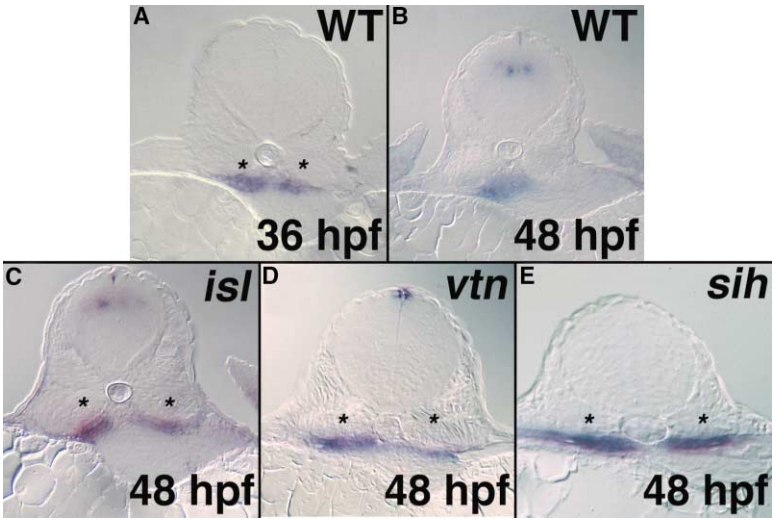

Figure 1. Glomerular Morphogenesis in Mutant Embryos with Cardiovascular Dysfunction Is Disrupted

(A) Cross-section of a 36 hpf embryo. The pronephric primordia, marked by *wt1* expression, have not yet merged at the midline (asterisks). (B) At 48 hpf, the primordia have fused, and *wt1* marks the glomerular podocytes found at the midline. (C) *wt1*-expressing cells form normally but remain bilateral (asterisks) in embryos with no cardiac output due to a mutation in the pore-forming subunit of the L-type calcium channel gene (*isl*). Unfused pronephric primordia are also observed in (D) *valentine* (*vtn*) and (E) *silent heart* (*sih*) mutants, which also perturb cardiovascular function.

development. This recovery indicates that there is no significant cellular damage caused by circulatory failure.

In order to examine whether the apparent flow-related signal derives from the vessel, we focally occluded the aorta, using a laser to generate a clot at the radix (Figure 3B), a region just rostral to the point near which podocytes assemble [13]. In these embryos, a normal heart beat and cranial circulation are retained, but there is no glomerular assembly.

Embryos that lack flow do not appear to be delayed in development. Even as late as 72 hpf, the *wt*1-labeled primordia remain bilateral, and glomerular morphogenesis fails to occur (Figure 4A). We assayed the development of the emerging liver bud, a neighboring structure, as well as the size of the growing pectoral fin buds in both wild-type and *isl* mutants. The forkhead gene *fkd2* labels the emerging liver bud at 48 hpf [14]. Expression of *fkd2* in *isl* mutants indicates that liver bud formation initiates at the proper developmental stage (Figure 4B). The apical ectodermal ridge of the fin buds was assayed by expression of *fgf8*/*acerebellar* [15]. The size of the fin buds in *isl* mutants appear indistinguishable from wild-type siblings (Figure 4B). The size of the brain in *isl* mutants is typically smaller than that of wild-type siblings. This observation has been previously noted for mutants that lack circulation and may reflect a failure of the brain ventricles to inflate [16].

The genetic, pharmacological, and physical obstruction experiments all indicate that blood flow to the aorta is essential for glomerulogenesis. Hemodynamic forces seemed the most likely stimulant, but, alternatively, it could be a circulating factor. To examine this latter possibility, we developed a means to replace all blood components with saline. In these bloodless embryos, cardiac function is remarkably unperturbed. The process of glomerulogenesis proceeds normally (Figure 4C) with formation of a midline glomerulus by 48 hpf. It seems highly unlikely that a circulating constituent would be replenished over this time frame as an explanation, especially since removal of blood does not delay the formation of the glomerulus. Hence, hemodynamic forces per se seem to be the proximate stimulus.

How might physical forces be transduced into molecular signals guiding glomerular morphogenesis? Endothelial genes have been shown to be sensitive to mechanical stretch in their expression levels, and several are in known signaling pathways [17–19]. *smad6* and *smad7*, in the BMP pathway [20], are not detectable by in situ hybridization using expressed sequence tags in the embryonic vessels at these stages. Pharmacological blockade of the nitric oxide and angiotensin converting enzyme signaling pathways do not perturb glomerular assembly (unpublished data).

MMP-2 is expressed in vascular smooth muscle cells and endothelium in a stretch-sensitive fashion [21–23]. MMP-2 acts to degrade collagen type IV and is involved in permitting cell migration through tissues [24]. We find that a zebrafish ortholog of *matrix metalloproteinase-2* (*mmp-2*) is expressed in endothelium of the trunk but not in podocytes or podocyte precursors. Its expression is dramatically reduced in *isl* mutant embryos and in those with cardiac silence induced by BDM (Figures 5A–5C and the Supplementary Material available with this article online). The recovery of *mmp-2* expression after removal of BDM anticipates the restoration of normal glomerular morphogenesis (Figures 5D and 5E).

The activity of MMP-2 can be abrogated by tissue inhibitor of metalloproteinase-2 (TIMP-2), a stochiometric MMP inhibitor with a relatively high affinity for MMP-2 among MMPs [25]. We therefore injected TIMP-2 by micropipette into the circulation of the 36 hpf embryo. Injected embryos maintain their circulation and are motile. Notably, TIMP-2-treated embryos exhibit mild edema, suggesting a defect in osmoregulation. Examination at 48–60 hpf shows that these embryos, despite a normal circulation, cannot form the glomerulus (Figures 5F and 5G). This demonstrates that MMP-2 activity or that of a closely related MMP (such as MMP-9) is essential for assembly of the glomerulus. The sensitivity to TIMP-2 does not require blood. Even in the bloodless embryos, TIMP-2 injection prevents fusion of the kidney primordia ($n = 8$).

This work indicates that local flow to the aortic region adjacent to the nascent glomerulus drives glomerular

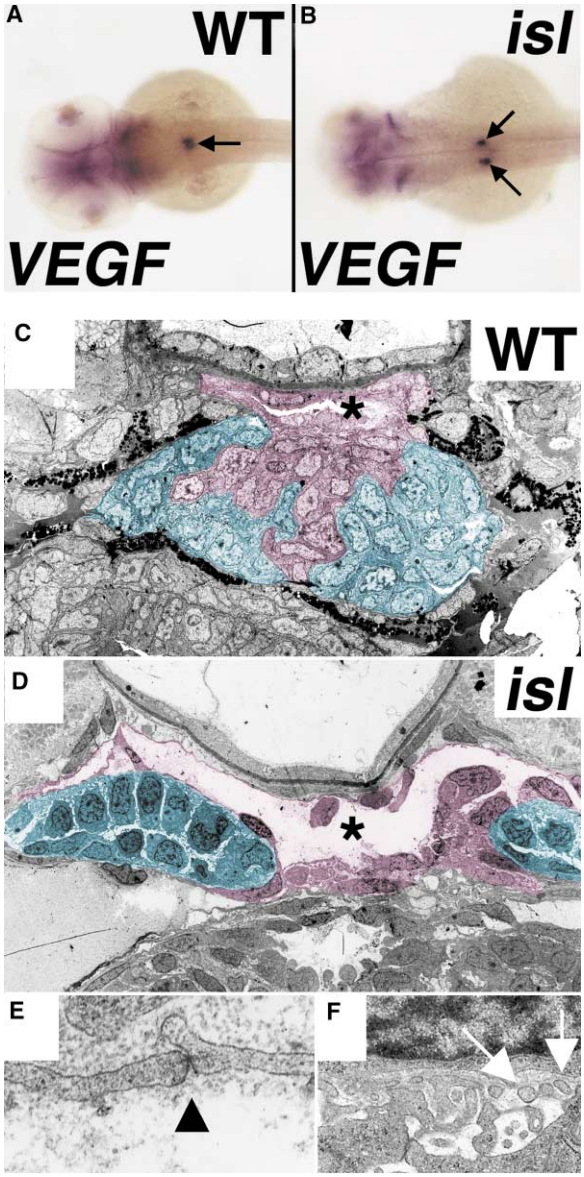

Figure 2. Endothelial Cells and Podocytes Differentiate but Do Not Assemble into a Glomerulus in *isl* Mutant Embryos

(A) In wild-type embryos, mature podocytes at the midline express *VEGF* at the 48 hpf stage [3]. (B) In *isl* embryos, expression of *VEGF* is normal, indicating that differentiation is not affected; however, the podocytes are found in a lateral position. Electron microscopy of (C) wild-type and (D) *isl* mutant embryos. In order to clearly demarcate zones of endothelial cells and glomerular podocytes, the glomerular basement membrane was traced, and the image was digitally painted to indicate endothelial cells in pink and podocytes in blue. (C) Endothelial cells from the dorsal aorta normally sprout from its ventral surface and invade the kidney at the midline, ventral to the notochord. The lumen of the aorta is marked by an asterisk. (D) In *isl* mutants, the podocyte clusters remain in their primitive lateral positions, although they appear well differentiated, with endothelial cells surrounding but not invading the clusters. (E) High-magnification view of *isl* endothelial cells forming cell junctions (arrowhead) that appear as in wild-type [8]. (F) Foot processes (marked by arrows) of the glomerular podocytes are also normal in *isl* embryos [8].

assembly. The absence of flow does not appear to interfere with normal cellular differentiation. Both endothelial and podocytic cell fates are achieved. Rather, it is the medial migration and midline coalescence of these cells that is affected. The dependence upon flow might contribute to the absence of vascularization noted previously in cultured mouse metanephric kidney rudiments when explanted [26].

Our data strongly implicate MMP-2 as the transducing signal between mechanical force of the circulation and glomerular morphogenesis. This is compatible with the known sensitivity of *MMP-2* expression to stretch [21–23]. The proteolytic activity of MMP-2 upon collagen could serve to remodel components of the glomerular basement membrane that separates the podocytes from the capillary endothelium and thereby would facilitate medial migration and vessel invasion [27]. Metanephric glomerular formation is also disrupted in the absence of the laminin α 5 chain, another component of the basement membrane [28]. Alternatively, MMP-2 may act to release stores of inactive signals in the extracellular matrix. For example, a related gelatinase, MMP-9, can release bioactive VEGF from a storage pool in pancreatic tumors [29].

Why might endothelial cell signaling during organogenesis be dependent upon intravascular flow or pressure? In the case of the kidney of fresh water fish, it may be critical to coordinate glomerulogenesis with blood flow. It would be detrimental to develop the fenestrated endothelium of glomerular vessels, designed to enhance fluid loss from the vessel, without having the glomerulus assembled to receive the ultrafiltrate.

Clearly, vascular stretch plays critical roles in adult cardiovascular pathology, with increased pressure exacerbating atherosclerosis and cardiac hypertrophy [30–32]. To our knowledge, this report is the first evidence that circulatory force per se drives organogenesis in the embryo.

Experimental Procedures

Zebrafish Lines

Wild-type zebrafish were maintained and raised as described [33]. Dechorionated embryos were kept in egg water and staged according to somite number or hours postfertilization [34]. The wild-type lines were of the TL or Tübingen backgrounds obtained from the laboratory of Dr. Christianne Nusslein-Volhard in Tübingen, Germany. Mutant lines for *island beat*^{m458}, *silent heart*^{tc300b}, and *valentine*^{m201} have been described elsewhere [9–11].

2,3-Butanedione Monoxime Treatment

Embryos were placed in egg water [33] containing 40 mM 2,3-butanedione monoxime (BDM) for 2–3 min until the heart stopped beating. The embryos were then transferred to egg water containing 20 mM BDM for the desired length of time. Control embryos received the 40 mM pulsed dose only.

Laser Ablation and Diaminofluorene Staining

Tissue ablations were carried out as described in Serluca and Fishman (2001). We focused upon the left and right dorsal aortae, immediately anterior to the radix. Ablation of vascular tissue and circulating blood cells creates a clot which prevents trunk circulation. The embryos were monitored every 2 hr to make sure circulation was not reestablished. Blood cells were visualized by staining with diaminofluorene (DAF). Embryos were fixed in 4% paraformaldehyde overnight at 4°C, rinsed three times with PBS, and incubated with 0.01% DAF, 200 mM Tris (pH 7.4), and 0.05% Tween-20 for 60 min

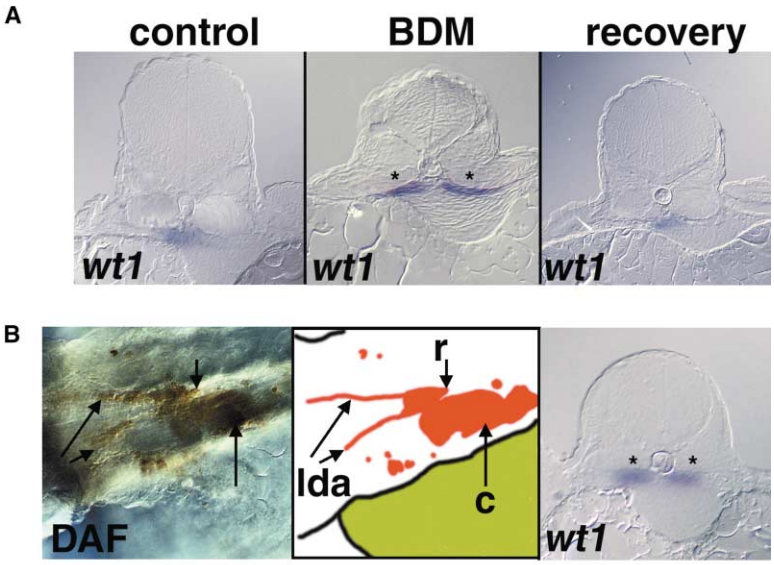

Figure 3. Pharmacological and Surgical Obstruction of Vascular Flow in the Aorta Phenocopies the Kidney Phenotype of Cardiovascular Mutants

(A) 2,3 butanedione monoxime (BDM) was used to reversibly stop the heart between the 36 hpf and 48 hpf stages. Embryos were immersed in 40 mM BDM for 2–3 min to stop the heart at 36 hpf and then maintained in a 20 mM BDM solution in embryo media (E3) until 48 hpf. Control embryos, receiving only the 40 mM pulse, form a normal midline glomerulus (left panel). In embryos kept in BDM between the 36 hpf and 48 hpf stages, the pronephric primordia remain in their lateral positions (middle panel, asterisks, $n > 100$). Recovery following BDM withdrawal 12 hr later (right panel, $n = 46$).

(B) Physical obstruction of the aorta using a laser. The embryo, shown in a dorsolateral view, is stained with diaminofluorene (brown, left panel) to visualize hemoglobin and shown diagrammatically in the center panel (red, blood; green, yolk). Blood can be seen in the left and right lateral dorsal aorta (lda) and in a large clot (c) adjacent to the radix (r). No circulating blood cells are observed in the midline dorsal aorta which lies caudal to the radix. Formation of a midline glomerulus is also blocked by this aortic obstruction (right panel, $n = 17$).

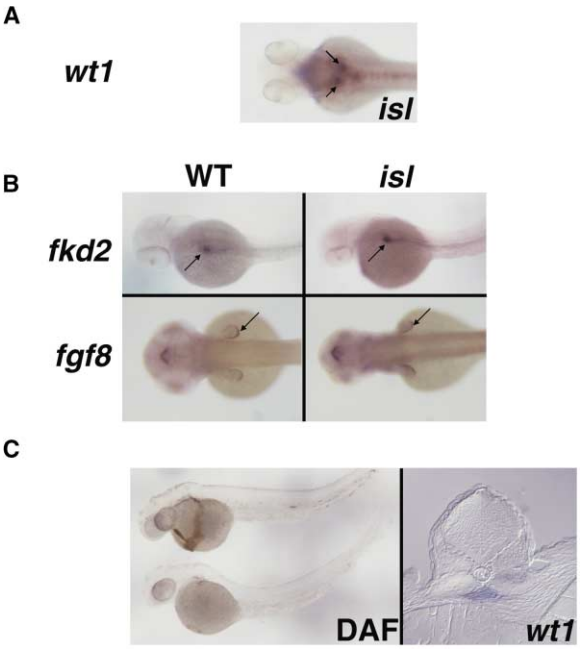

Figure 4. Failure of Glomerular Morphogenesis Is Not Due to Developmental Delay or a Circulating Blood Component

(A) Kidney primordia (visualized by *wt1* staining) in *island beat* remain in their lateral positions at 72 hpf.

(B) *island beat* and wild-type sibling embryos stained with the *fkd2* probe marking the emerging liver bud at 48 hpf and with *fgf8* marking the apical ectodermal ridge, also at 48 hpf. The size of the growing pectoral fin buds appear normal in *isl*.

(C) Exsanguination of embryos at 36 hpf does not affect glomerular formation. The left panel shows a wild-type control (top) and surgically exsanguinated embryo (bottom) stained with DAF as a blood marker. The left panel is a *wt1*-stained exsanguinated embryo. A normal midline glomerulus can form in the absence of blood constituents ($n = 15$).

at room temperature and protected from light. Hydrogen peroxide was added to a concentration of 0.3% to start the reaction. When the desired staining intensity was achieved, the embryos were rinsed in PBS and kept in 1% paraformaldehyde.

Electron Microscopy

Homozygous *isl* embryos and wild-type siblings were fixed overnight at 4°C in 1.5% glutaraldehyde, 1% paraformaldehyde, 3% sucrose in 70 mM sodium phosphate buffer (pH 7.2). The tissue was washed several time in 0.1 M cacodylate buffer (pH 7.4), postfixed for 1 hr (4°C) in 1% OsO_4, 40 mM $K_4Fe(CN)_6$, and rinsed twice in cacodylate buffer. The embryos were embedded and sectioned as previously described [3].

Exsanguination

Electrolytically sharpened tungsten needles were used to make an incision in the ventral portion of a 36 hpf embryo, posterior to the cloaca. Cardiac function is unperturbed and forces all blood into the buffered solution [1× Danieau's: 58 mM NaCl, 0.7 mM KCl, 0.6 mM $Ca(NO_3)_2$, 0.4 mM $MgSO_4$, 5 mM HEPES (pH 7.6)]. Embryos were allowed to heal for 45–60 min and then injected by microcatheter [35] with Hank's modified saline [33]. The embryos were allowed to develop until 48 hpf and assayed for *wt1* expression.

Cloning of *matrix metalloproteinase-2* and Injections

An expressed sequence tag (EST) coding for a zebrafish gene homologous to human *MMP-2* was used to design a primer (TCTGTCCCCATGAGCTCCTGTTCACA) to amplify the 3′ portion of the gene using a 3′ Rapid Amplification of cDNA Ends (RACE) kit (Clontech) from 2 day embryonic mRNA. The DNA fragment was cloned into pCRII (Invitrogen). The cDNA used in this study encodes the C-terminal half of the zebrafish *mmp-2* gene. *Spe I* was used to linearize the template and T7 RNA polymerase to transcribe the antisense riboprobe. Purified TIMP-2 was purchased from Calbiochem and used as a 7 μg/ml solution. Injection of inhibitors was carried out using a previously described microangiography method [35].

Supplementary Material

Supplementary Material including the predicted amino acid alignment of the zebrafish MMP-2 clone used in this study, with gelatinase (MMP-2 and MMP-9) homologs from fish and mammalian species, can be found online at http://images.cellpress.com/supmat/supmatin.htm.

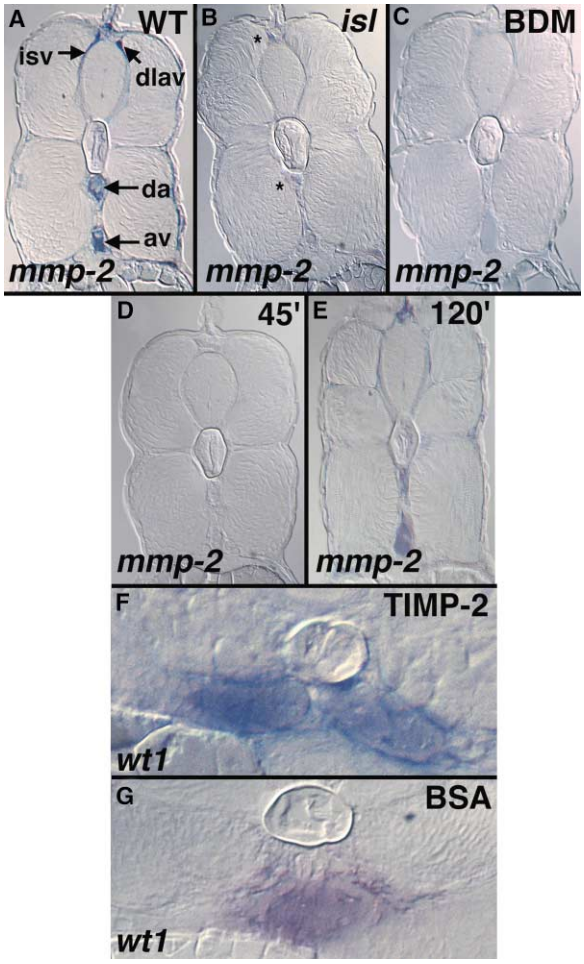

Figure 5. Transduction of the Biomechanical Signal from the Vasculature Requires *matrix metalloproteinase-2*

(A) Cross-section of a wild-type embryo expressing *MMP-2* RNA in the trunk vasculature including the dorsal aorta (da), axial vein (av), intersomitic vessels (isv), and dorsal longitudinal anastomotic vessel (dlav). *mmp-2* expression is absent in the vasculature of (B) *isl* mutants and (C) BDM-treated embryos. (D) and (E) expression of *mmp-2* assayed 45 min and 2 hr following the removal the BDM block at 48 hpf. Normal expression is observed after 2 hr of restored circulation. (F) TIMP-2 injected into the circulation of a 36 hpf embryo by microcatheterization blocks glomerular assembly. (G) Embryos injected identically with a 1% BSA dose form a normal glomerulus.

Acknowledgments

We thank John Mably, Jordan Shin, and Mykol Larvie for helpful criticisms on the manuscript. *island beat* mutant embryos were kindly provided by Wolfgang Rottbauer. This work was supported in part by National Institutes of Health grants R01DK55383, R01HL63206, and R01HL49579 to M.C.F. and by NIH grants R01DK53093 and P01DK54711 to I.A.D.

Received: October 29, 2001
Revised: December 24, 2001
Accepted: January 16, 2002
Published: March 19, 2002

References

1. Matsumoto, K., Yoshitomi, H., Rossant, J., and Zaret, K.S. (2001). Liver organogenesis promoted by endothelial cells prior to vascular function. Science *294*, 559–563.
2. Lammert, E., Cleaver, O., and Melton, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. Science *294*, 564–567.
3. Majumdar, A., and Drummond, I.A. (1999). Podocyte differentiation in the absence of endothelial cells as revealed in the zebrafish avascular mutant, cloche. Dev. Genet. *24*, 220–229.
4. Weinstein, B.M., Schier, A.F., Abdelilah, S., Malicki, J., Solnica-Krezel, L., Stemple, D.L., Stainier, D.Y., Zwartkruis, F., Driever, W., and Fishman, M.C. (1996). Hematopoietic mutations in the zebrafish. Development *123*, 303–309.
5. Ransom, D.G., Haffter, P., Odenthal, J., Brownlie, A., Vogelsang, E., Kelsh, R.N., Brand, M., van Eeden, F.J., Furutani-Seiki, M., Granato, M., et al. (1996). Characterization of zebrafish mutants with defects in embryonic hematopoiesis. Development *123*, 311–319.
6. Pelster, B., and Burggren, W.W. (1996). Disruption of hemoglobin oxygen transport does not impact oxygen-dependent physiological processes in developing embryos of zebra fish (Danio rerio). Circ. Res. *79*, 358–362.
7. Serluca, F.C., and Fishman, M.C. (2001). Pre-pattern in the pronephric kidney field of zebrafish. Development *128*, 2233–2241.
8. Drummond, I.A., Majumdar, A., Hentschel, H., Elger, M., Solnica-Krezel, L., Schier, A.F., Neuhauss, S.C., Stemple, D.L., Zwartkruis, F., Rangini, Z., et al. (1998). Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. Development *125*, 4655–4667.
9. Rottbauer, W., Baker, K., Wo, Z.G., Mohideen, M.A., Cantiello, H.F. and Fishman, M.C. (2001). Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel a1 subunit. Dev. Cell *1*, 265–275.
10. Chen, J.N., Haffter, P., Odenthal, J., Vogelsang, E., Brand, M., van Eeden, F.J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.P., et al. (1996). Mutations affecting the cardiovascular system and other internal organs in zebrafish. Development *123*, 293–302.
11. Stainier, D.Y., Fouquet, B., Chen, J.N., Warren, K.S., Weinstein, B.M., Meiler, S.E., Mohideen, M.A., Neuhauss, S.C., Solnica-Krezel, L., Schier, A.F., et al. (1996). Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. Development *123*, 285–292.
12. Herrmann, C., Wray, J., Travers, F., and Barman, T. (1992). Effect of 2,3-butanedione monoxime on myosin and myofibrillar ATPases. An example of an uncompetitive inhibitor. Biochemistry *31*, 12227–12232.
13. Isogai, S., Horiguchi, M., and Weinstein, B.M. (2001). The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. Dev. Biol. *230*, 278–301.
14. Chen, J.N., van Bebber, F., Goldstein, A.M., Serluca, F.C., Jackson, D., Childs, S., Serbedzija, G., Warren, K.S., Mably, J.D., Lindahl, P., et al. (2001). Genetic steps to organ laterality in zebrafish. Comp. Funct. Genom. *2*, 60–68.
15. Reifers, F., Bohli, H., Walsh, E.C., Crossley, P.H., Stainier, D.Y., and Brand, M. (1998). Fgf8 is mutated in zebrafish acerebellar (ace) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. Development *125*, 2381–2395.
16. Schier, A.F., Neuhauss, S.C., Harvey, M., Malicki, J., Solnica-Krezel, L., Stainier, D.Y., Zwartkruis, F., Abdelilah, S., Stemple, D.L., Rangini, Z., et al. (1996). Mutations affecting the development of the embryonic zebrafish brain. Development *123*, 165–178.
17. Garcia-Cardena, G., Comander, J., Anderson, K.R., Blackman, B.R., and Gimbrone, M.A., Jr. (2001). Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. Proc. Natl. Acad. Sci. USA *98*, 4478–4485.
18. Topper, J.N., and Gimbrone, M.A., Jr. (1999). Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. Mol. Med. Today *5*, 40–46.
19. Topper, J.N., Cai, J., Falb, D., and Gimbrone, M.A., Jr. (1996). Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. Proc. Natl. Acad. Sci. USA *93*, 10417–10422.

20. Topper, J.N., Cai, J., Qiu, Y., Anderson, K.R., Xu, Y.Y., Deeds, J.D., Feeley, R., Gimeno, C.J., Woolf, E.A., Tayber, O., et al. (1997). Vascular MADs: two novel MAD-related genes selectively inducible by flow in human vascular endothelium. Proc. Natl. Acad. Sci. USA *94*, 9314–9319.
21. Singhal, P.C., Sagar, S., and Garg, P. (1996). Simulated glomerular pressure modulates mesangial cell 72 kDa metalloproteinase activity. Connect. Tissue Res. *33*, 257–263.
22. Yasuda, T., Kondo, S., Homma, T., and Harris, R.C. (1996). Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. J. Clin. Invest. *98*, 1991–2000.
23. Bassiouny, H.S., Song, R.H., Hong, X.F., Singh, A., Kocharyan, H., and Glagov, S. (1998). Flow regulation of 72-kD collagenase IV (MMP-2) after experimental arterial injury. Circulation *98*, 157–163.
24. Hiraoka, N., Allen, E., Apel, I.J., Gyetko, M.R., and Weiss, S.J. (1998). Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell *95*, 365–377.
25. Olson, M.W., Gervasi, D.C., Mobashery, S., and Fridman, R. (1997). Kinetic analysis of the binding of human matrix metalloproteinase-2 and -9 to tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2. J. Biol. Chem. *272*, 29975–29983.
26. Robert, B., St John, P.L., and Abrahamson, D.R. (1998). Direct visualization of renal vascular morphogenesis in Flk1 heterozygous mutant mice. Am. J. Physiol. *275*, F164–F172.
27. Vu, T.H., and Werb, Z. (2000). Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev. *14*, 2123–2133.
28. Miner, J.H., and Li, C. (2000). Defective glomerulogenesis in the absence of laminin alpha5 demonstrates a developmental role for the kidney glomerular basement membrane. Dev. Biol. *217*, 278–289.
29. Bergers, G., Brekken, R., McMahon, G., Vu, T.H., Itoh, T., Tamaki, K., Tanzawa, K., Thorpe, P., Itohara, S., Werb, Z., et al. (2000). Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat. Cell Biol. *2*, 737–744.
30. Gimbrone, M.A., Jr., Nagel, T., and Topper, J.N. (1997). Biomechanical activation: an emerging paradigm in endothelial adhesion biology. J. Clin. Invest. *100*, S61–S65.
31. Gimbrone, M.A., Jr., Topper, J.N., Nagel, T., Anderson, K.R., and Garcia-Cardena, G. (2000). Endothelial dysfunction, hemodynamic forces, and atherogenesis. Ann. N Y Acad. Sci. *902*, 230–239.
32. Braunwald, E., Zipes, D.P., and Libby, P. (2001). Heart Disease: A Textbook of Cardiovascular Medicine, 6th Edition (Philadelphia: Saunders).
33. Westerfield, M. (1995). The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Brachydanio rerio), 3rd Edition (Eugene, OR: University of Oregon Press).
34. Kimmel, C., Ballard, W., Kimmel, S., Ullmann, B., and Schilling, T. (1995). Stages of embryonic development of the zebrafish. Dev. Dyn. *203*, 253–310.
35. Weinstein, B.M., Stemple, D.L., Driever, W., and Fishman, M.C. (1995). Gridlock, a localized heritable vascular patterning defect in the zebrafish. Nat. Med. *1*, 1143–1147.
36. Xu, X., Meiler, S.E., Zhong, T.P., Mohideen, M., Crossley, D.A., Burggren, W.W., and Fishman, M.C. (2002). Cardiomyopathy in zebrafish due to an alternatively spliced exon of titin. Nat. Genet. *30*, 205–209.