JCI The Journal of Clinical Investigation

Notch: a mastermind of vascular morphogenesis

Leonard M. Anderson, Gary H. Gibbons

J Clin Invest. 2007;117(2):299-302. https://doi.org/10.1172/JCI31288.

Commentary

The way in which multiple cell types organize themselves into a carefully sculpted, 3D labyrinth of vessels that regulate blood flow throughout the body has been a longstanding mystery. Clinicians familiar with congenital cardiovascular disease recognize how genetic variants and modest perturbations in this complex set of spatiotemporal interactions and stochastic processes can result in life-threatening anomalies. Although the mystery is not yet fully solved, we are poised at an exciting juncture, as insights from murine disease models are converging with advances in human genetics to shed new light on puzzling clinical phenotypes of vascular disease. The study by High et al. in this issue of the *JCI* establishes a model system that mimics clinical features of congenital cardiovascular disease and further defines the role of the Notch signaling pathway in the neural crest as an essential determinant of cardiovascular structure (see the related article beginning on page 353).

an understanding of these relationships would be required before attempting to alter ODC stress responses in MS patients for therapeutic benefit.

Acknowledgments

We would like to acknowledge John H. Russell for his helpful review and discussion of this commentary.

Address correspondence to: Anne H. Cross, Department of Neurology and Neurosurgery, Washington University School of Medicine, 660 S. Euclid Avenue, Box 8111, St. Louis, Missouri 63110, USA. Phone: (314) 362-3293; Fax: (314) 747-1345; E-mail: crossa@neuro.wustl.edu.

1. Giovannoni, G., and Hartung, H.P. 1996. The immunopathogenesis of multiple sclerosis and Guillain-Barre syndrome. *Curr. Opin. Neurol.* **9**:165–177.
2. Lucchinetti, C., et al. 1999. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 113 cases. *Brain.* **122**:2279–2295.
3. Liblau, R.S., Singer, S.M., and McDevitt, H.O. 1995. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today.* **16**:34–38.
4. Zamvil, S.S., and Steinman, L. 1990. The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* **8**:579–621.
5. Soos, J.M., et al. 2002. Cutting edge: oral type I IFN-tau promotes a Th2 bias and enhances suppression of autoimmune encephalomyelitis by oral glatiramer acetate. *J. Immunol.* **169**:2231–2235.
6. Youssef, S., et al. 2002. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature.* **420**:78–84.
7. Panitch, H.S., Hirsch, R.L., Schindler, J., and Johnson, K.P. 1987. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology.* **37**:1097–1102.
8. Furlan, R., et al. 2001. Intrathecal delivery of IFN-gamma protects C57BL/6 mice from chronic-progressive experimental autoimmune encephalomyelitis by increasing apoptosis of central nervous system-infiltrating lymphocytes. *J. Immunol.* **167**:1821–1829.
9. Willenborg, D.O., Fordham, S., Bernard, C.C., Cowden, W.B., and Ramshaw, I.A. 1996. IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol.* **157**:3223–3227.
10. Willenborg, D.O., Fordham, S.A., Staykova, M.A., Ramshaw, I.A., and Cowden, W.B. 1999. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. *J. Immunol.* **163**:5278–5286.
11. Gao, X., et al. 2000. Interferon-gamma protects against cuprizone-induced demyelination. *Mol. Cell. Neurosci.* **16**:338–349.
12. Komiyama, Y., et al. 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J. Immunol.* **177**:566–573.
13. Lin, W., et al. 2007. The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. *J. Clin. Invest.* **117**:448–456. doi:10.1172/JCI29571.
14. Rao, R.V., Ellerby, H.M., and Bredesen, D.E. 2004. Coupling endoplasmic reticulum stress to the cell death program. *Cell Death Differ.* **11**:372–380.
15. Oyadomari, S., Araki, E., and Mori, M. 2002. Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. *Apoptosis.* **7**:335–345.
16. Harding, H.P., et al. 2003. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell.* **11**:619–633.
17. Lu, P.D., et al. 2004. Cytoprotection by pre-emptive conditional phosphorylation of translation initiation factor 2. *EMBO J.* **23**:169–179.
18. Lin, W., et al. 2006. Interferon-gamma inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. *Brain.* **129**:1306–1318.
19. Lin, W., Harding, H.P., Ron, D., and Popko, B. 2005. Endoplasmic reticulum stress modulates the response of myelinating oligodendrocytes to the immune cytokine interferon-gamma. *J. Cell Biol.* **169**:603–612.

Notch: a mastermind of vascular morphogenesis

Leonard M. Anderson and Gary H. Gibbons

Cardiovascular Research Institute, Morehouse School of Medicine, Atlanta, Georgia, USA.

The way in which multiple cell types organize themselves into a carefully sculpted, 3D labyrinth of vessels that regulate blood flow throughout the body has been a longstanding mystery. Clinicians familiar with congenital cardiovascular disease recognize how genetic variants and modest perturbations in this complex set of spatiotemporal interactions and stochastic processes can result in life-threatening anomalies. Although the mystery is not yet fully solved, we are poised at an exciting juncture, as insights from murine disease models are converging with advances in human genetics to shed new light on puzzling clinical phenotypes of vascular disease. The study by High et al. in this issue of the *JCI* establishes a model system that mimics clinical features of congenital cardiovascular disease and further defines the role of the Notch signaling pathway in the neural crest as an essential determinant of cardiovascular structure (see the related article beginning on page 353).

The process of vascular morphogenesis

In the embryo, endothelial precursors initially assemble into a primitive plexus of channels that expand by sprouting and remodeling into a highly organized arborization of vessels that ramify throughout the body. Studies in various knockout mouse models suggest that VEGF, angiopoietins, and PDGF provide local cues that promote vascular morphogenesis and the investment of the endothelial channels with a VSMC layer. The architecture of the vascular tree is further refined to very precise dimensions in accordance with biomechanical parameters of shear and radial stresses (1, 2).

In a classic series of seminal studies involving quail-chick chimeras and tissue ablation experiments, Kirby and colleagues established that the cardiac neural crest plays an essential role in establishing the pattern of the vertebrate vascular system (3). The molecular mechanisms underlying these phenomena are gradually being elucidated (Table 1). The cardiac neural crest (an ectodermal cell population arising from the dorsal neural tube) migrates to populate the aortic arch arteries and cardiac outflow tract. The migrating cardiac neural crest cells contribute to the septation of the truncus arteriosus into a separate pulmonary artery and aorta. Similarly, a subpopulation of these neural crest cells becomes part of the mass of VSMCs that contribute to the formation of the pulmonary trunk, ductus arteriosus, carotid arteries, and proximal subclavian arteries (3–5). The study reported by High et al. in this issue of the *JCI* (6) provides the first demonstration to our knowledge that the Notch transcriptional cascade within the neural crest plays an essential mediator role in VSMC differ-

Nonstandard abbreviations used: HERP, HES-related repressor protein; HES, hairy and enhancer of split; MAML, mastermind-like; Tbx1, T-box 1.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:299–302 (2007). doi:10.1172/JCI31288.

Table 1
Factors implicated in the neural crest contribution to vascular morphogenesis and the process of vascular remodeling during development as well as in adult vascular disorders

	Transcription factors	Autocrine-paracrine factors	Translation to clinical context
Premigratory neural crest	PAX3, TBX1, FOXC2, RAR	WNT, sonic hedgehog, FGF8	Retinoic acid deficiency/excess, DiGeorge syndrome
Postmigration neural crest	Notch pathway, GATA-6, PITX2	Semaphorin 3C, TGF-β /ALK2, PDGF, endothelin	Alagille syndrome
VSMC differentiation/remodeling	MEF2C, myocardin, Kruppel-like factor, serum response factor	TGF-β /ALK2, mechanical stress, angiotensin, fibrillin, elastin	Loeys-Dietz syndrome, CADASIL syndrome, Marfan syndrome, Williams-Beuren syndrome

This list of factors is not exhaustive. ALK2, type I activin–like kinase receptor–2; FOXC2, forkhead box protein C2; MEF2C, myocyte enhancer factor 2C; PAX3, paired box protein 3; RAR, retinoic acid receptor; WNT, wingless-related MMTV integration site protein.

entiation during the formation of the aortic arch and pharyngeal arteries.

Role of the Notch pathway in vasculogenesis and VSMC differentiation

The 4 mammalian Notch receptors (Notch 1–4) and 5 ligands (Jagged1 and -2; Delta-like1, -3, and -4) all contain transmembrane domains such that ligand-receptor signaling occurs between adjacent cells (Figure 1). The ligand-receptor binding triggers a γ-secretase–dependent cleavage that releases the intracellular domain of Notch to the nucleus and facilitates an association with the transcription factor CBF-1 (also known as RBP-Jκ or CSL). The subsequent recruitment of the coactivator, mastermind-like (MAML) protein, promotes transcriptional activation of downstream effectors such as hairy and enhancer of split (HES) and the HES-related repressor protein (HERP) family of transcription factors (7). Given that MAML is a common signaling node employed by all Notch receptors, High and colleagues' use of the dominant-negative MAML construct (6) is an effective strategy for inducing broad-based inhibition of the Notch transcriptional cascade.

In murine models, selective knockout of the ligands Jagged1 and Delta-like4 or the receptor Notch1 results in striking abnormalities in vascular development (8). Similarly, the combined deletion of the downstream Notch effectors Herp1 and Herp2 leads to similar vascular malformations. Thus, there is compelling evidence that the Notch signaling pathway plays a key mediator role in vascular morphogenesis (4, 7). However, these previous studies failed to isolate and clarify the specific role of Notch signaling in VSMC differentiation. By focusing on neural crest–derived VSMCs, the study by High and colleagues is among the first that addresses the direct role of Notch in VSMC differentiation in vivo (6).

Molecular pathways in vascular development: implications for genomic medicine

As progress is made in the dissection of the gene regulatory networks that govern vascular morphogenesis, it is important to translate these insights into an understanding of genetic factors that increase susceptibility to clinical phenotypes of vascular malformation. DiGeorge syndrome is a clinically heterogeneous disease caused by a multigene deletion on chromosome 22q11 that is manifested by cardiovascular anomalies reminiscent of neural crest ablation such as tetralogy of Fallot and interrupted aortic arch. A systematic series of complementation experiments in mice have characterized a T-box transcription factor, T-box 1 (Tbx1), as a major mediator in the neural crest during cardiovascular development (9). Indeed, patients that lack the chromosome 22q11 deletion, yet have mutations in the *TBX1* gene, retain features of DiGeorge syndrome (10, 11). Thus, an important paradigm of this unfolding mystery is that highly penetrant clinical phenotypes appear to emerge in the context of mutations of factors (e.g., TBX1) that play a central role in the vascular development gene regulatory network.

Alagille syndrome is a heritable multisystem disease that is clinically characterized by bile duct insufficiency and cardiovascular manifestations that are reminiscent of other neural crest deficiency phenotypes (e.g., pulmonary stenosis, tetralogy of Fallot, and coarctation of the aorta). In accordance with the emerging paradigm, the vast majority of patients with Alagille syndrome have mutations in the Notch ligand Jagged1 (7, 11). Moreover, a recent report suggests that the small percentage of Alagille patients that are negative for Jagged1 mutations have significant mutations in the gene coding for Notch2 (12). Thus, the animal model of neural crest–selective Notch inactivation demonstrated by High et al. (6) appears to translate to the clinical context with high fidelity.

Developmental gene regulatory networks: reactivation in adult vascular disease

Although the study by High et al. (6) focuses on the role of the Notch pathway in cardiovascular development, the implications of this model extend beyond the realm of congenital heart disease to the context of vascular disease in adulthood. Unlike skeletal and cardiac muscle cells, VSMCs are not terminally differentiated and exhibit substantial plasticity in phenotypic modulation within adult vessels. An emerging pathobiological paradigm suggests that the vascular development gene regulatory network is often reactivated in the context of vascular remodeling and repair in adult vascular disease (2).

Studies in our laboratories and by others have demonstrated that elements of the Notch transcriptional cascade are activated in the context of vascular lesion formation and that the Notch pathway is coupled to the regulation of growth, apoptosis, migration, and differentiation of adult VSMCs (2, 13–15). The essential mediator role of the Notch pathway in vascular disease is evidenced by the finding that Herp2-knockout mice exhibit attenuated neointima lesion formation in response to vascular injury (15).

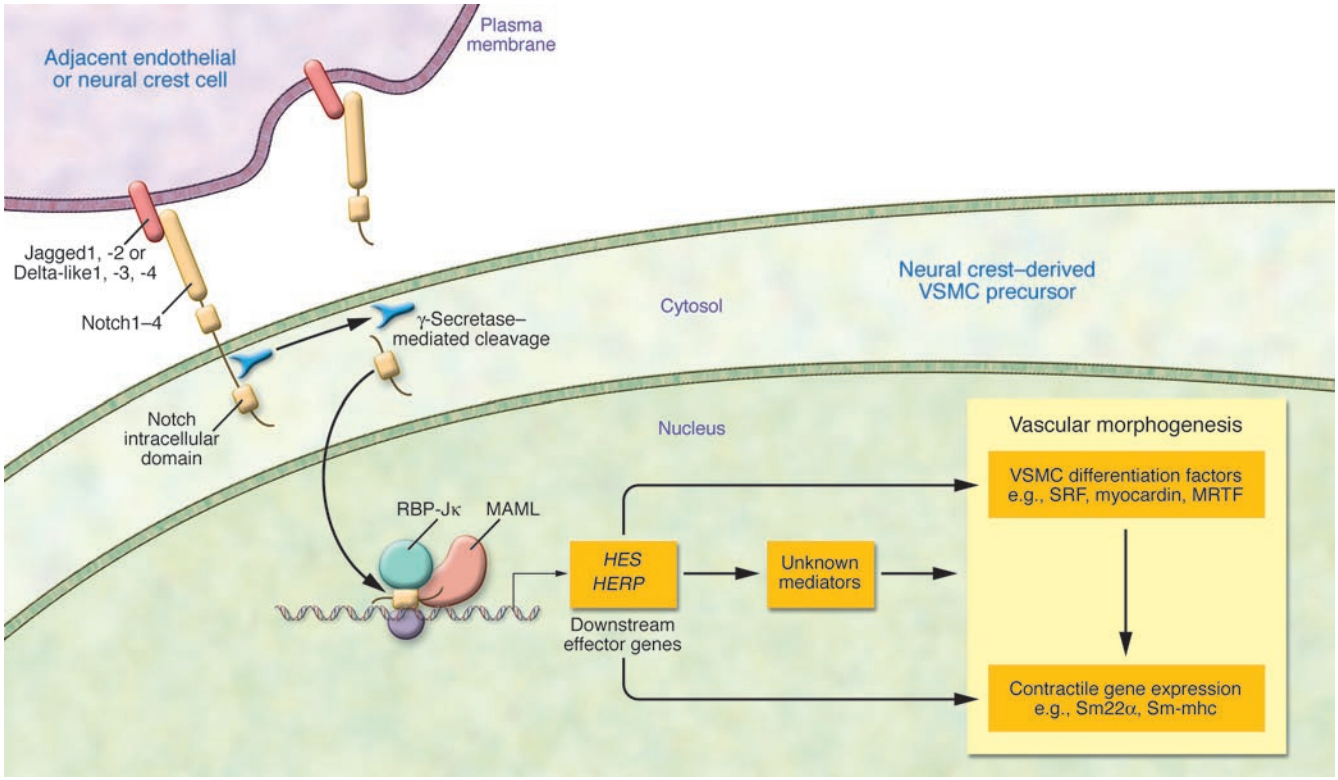

Figure 1
Schematic representation of Notch signaling in neural crest cells that differentiate into VSMCs within the aortic arch during embryonic development. Notch receptors are transmembrane proteins that can transduce cell-cell interactions into cell fate determinations. Upon the binding of Notch to a ligand such as Jagged or Delta, the Notch carboxyterminal fragment is cleaved between Gly1743 and Val1744 by a γ-secretase. The cleaved intracellular domain translocates to the nucleus to form a heterocomplex with the transcription factor RBP-Jκ and coactivators such as MAML, resulting in transactivation of target effector genes (e.g., *HES* and *HERP*). The study by High et al. (6) in this issue of the *JCI* supports the model illustrated in this schema in which cell contact may occur between adjacent endothelial or neural crest cells to initiate VSMC lineage commitment through Notch-induced lateral specification. Downstream activation of target genes results in SMC lineage commitment by: (a) activation of known "master regulators" of VSMC differentiation (e.g., myocardin, myocardin-related transcription factors [MRTFs], or serum response factor [SRF]); (b) direct activation of contractile protein expression (e.g., *smooth muscle myosin heavy chain* [*Sm-mhc*]); or (c) activation of unknown effectors that can transactivate expression of genes in scenarios a and b (19, 20). *Sm22α*, *smooth muscle protein 22–α*.

The role of the Notch pathway in adult vascular disease is also indicated by the observation that mutations in the gene coding for Notch3 results in CADASIL syndrome, an autosomal-dominant arteriopathy characterized by increased susceptibility to stroke and dementia in middle-age adults (16). Moreover, recent animal studies suggest that the Notch signaling cascade is activated during ischemic stroke, such that the inhibition of Notch signaling attenuates brain injury after stroke (17). It is exciting to consider the potential clinical utility of selective inhibition of the Notch pathway to ameliorate the course of adult vascular disease.

The vascular development gene regulatory network: future directions

The study by High et al. (6) provides compelling evidence that neural crest–directed blockade of Notch signaling with a dominant-negative *MAML* gene construct inhibits VSMC differentiation and results in aortic patterning defects reminiscent of clinical phenotypes. However, a limitation of this approach is the potential for forced expression of dominant-negative constructs to yield spurious results due to "off-target" effects that may extend beyond the Notch pathway. In fact, recent reports indicate that MAML can also act as a coactivator of myocyte enhancer factor 2C (MEF2C) (18), a well-established mediator of cardiovascular morphogenesis. It remains an open question which additional pathways are engaged in parallel to Notch and what downstream effectors mediate the direct coupling of Notch signaling to VSMC differentiation programs. In addition, the current model fails to reconcile the conflicting data derived from in vitro models that suggest that Notch signaling may inhibit myocardin-induced VSMC differentiation (19, 20). Unfortunately, a common limitation of these in vitro studies is the failure to capture the subtle physiologic interplay among epigenetics, coactivator/corepressor complexes that exist in vivo, and the combinatorial effect of several downstream Notch effectors working in concert to orchestrate the VSMC differentiation program. It is becoming clear that the capacity of Notch to elicit lineage specification in a wide spectrum of developmental processes reflects the exquisite sensitivity of the pathway to the local milieu and the combinatorial interplay with other elements of the gene regulatory network peculiar to a given cellular context. The in vivo model used by High and colleagues captures the complex nuances of these gene regulatory circuits and provides a way forward toward the identification of

novel downstream mediators of the Notch pathway in VSMC differentiation.

It is anticipated that the growing application of genomic approaches to define signature patterns in gene expression profiles during lineage commitment will lead to the discovery of new members of the vascular development gene regulatory network, advancing our understanding of human disease (21). This convergence of genomic strategies is exemplified by the recent discovery that mutations in the TGF-β signaling pathway (a key mediator in the vascular development gene circuitry) result in a newly defined form of aortic disease (Loeys-Dietz syndrome) (22), and may foster a novel therapeutic strategy for adult vascular disease (23). Likewise, the growing integration of systems biology approaches (21) into the analysis of cardiovascular development holds promise for unlocking the remaining mysteries of the complex gene regulation circuitry governing vascular morphogenesis.

Address correspondence to: Gary H. Gibbons, 720 Westview Dr., Morehouse School of Medicine Cardiovascular Research Institute, Atlanta, Georgia 30310, USA. Phone: (404) 752-1545; Fax: (404) 752-1042; E-mail: ggibbons@msm.edu.

1. Ferguson, J.E., Kelley, R.W., and Patterson, C. 2005. Mechanisms of endothelial differentiation in embryonic vasculogenesis. *Arterioscler. Thromb. Vasc. Biol.* **25**:2246–2254.
2. Gibbons, G.H., and Dzau, V.J. 1994. The emerging concept of vascular remodeling. *New Engl. J. Med.* **330**:1431–1438.
3. Creazzo, T.L., Godt, R.E., Leatherbury, L., Conway, S.J., and Kirby, M.L. 1998. Role of cardiac neural crest in cardiovascular development. *Annu. Rev. Physiol.* **60**:267–286.
4. Hirschi, K.K., and Majesky, J.W. 2004. Smooth muscle stem cells. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **276**:22–33.
5. Stoller, J.Z., and Epstein, J.A. 2005. Cardiac neural crest. *Semin. Cell Dev. Biol.* **16**:704–715.
6. High, F.A., et al. 2007. An essential role for Notch in neural crest during cardiovascular development and smooth muscle differentiation. *J. Clin. Invest.* **117**:353–363. doi:10.1172/JCI30070.
7. Alva, J.A., and Iruela-Arispe, M.L. 2004. Notch signaling in vascular morphogenesis. *Curr. Opin. Hematol.* **11**:278–283.
8. Xue, Y., et al. 1999. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.* **8**:723–730.
9. Zhang, Z., Tuong, H., and Baldini, A. 2006. Mesodermal expression of Tbx1 is necessary and sufficient for pharyngeal arch and cardiac outflow tract development. *Development.* **133**:3587–3595.
10. Baldini, A. 2005. Dissecting contiguous gene defects: TBX1. *Curr. Opin. Genet. Dev.* **15**:279–284.
11. Epstein, J.A., and Parmacek, M.S. 2005. Recent advances in cardiac development with therapeutic implications for adult cardiovascular disease. *Circulation.* **112**:592–597.
12. McDaniell, R., et al. 2006. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the Notch signaling pathway. *Am. J. Hum. Genet.* **79**:169–173.
13. Campos, A.H., Wang, W., Pollman, M.J., and Gibbons, G.H. 2002. Determinants of Notch-3 receptor expression and signaling in vascular smooth muscle cells: implications in cell-cycle regulation. *Circ. Res.* **91**:999–1006.
14. Morrow, D., et al. 2005. Cyclic strain inhibits Notch receptor signaling in vascular smooth muscle cells in vitro. *Circ. Res.* **96**:567–575.
15. Sakata, Y., et al. 2004. Transcription factor CHF1/Hey2 regulates neointimal formation in vivo and vascular smooth muscle proliferation and migration in vitro. *Arterioscler. Thromb. Vasc. Biol.* **24**:2069–2074.
16. Domenga, V., et al. 2004. Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. *Genes Dev.* **18**:2730–2735.
17. Arumugam, T.V., et al. 2006. Gamma secretase–mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. *Nat. Med.* **12**:621–623.
18. Shen, H., et al. 2006. The Notch coactivator, MAML1, functions as a novel coactivator for MEF2C-mediated transcription and is required for normal myogenesis. *Genes Dev.* **20**:675–688.
19. Doi, H., et al. 2006. Jagged1-selective Notch signaling induces smooth muscle differentiation via a RBP-J–dependent pathway. *J. Biol. Chem.* **281**:28555–28564.
20. Proweller, A., Pear, W.S., and Parmacek, M.S. 2005. Notch signaling represses myocardin-induced smooth muscle cell differentiation. *J. Biol. Chem.* **280**:8994–9004.
21. Olson, E.N. 2006. Gene regulatory networks in the evolution and development of the heart. *Science.* **313**:1922–1927.
22. Loeys, B.L., et al. 2006. Aneurysm syndromes caused by mutations in the TGF-β receptor. *New Engl. J. Med.* **355**:788–798.
23. Habashi, J.P., et al. 2006. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science.* **312**:117–121.

An intrinsic host defense against HIV-1 integration?

Paul D. Bieniasz

Aaron Diamond AIDS Research Center and Laboratory of Retrovirology, The Rockefeller University, New York, New York, USA.

HSCs are one of only a few cell types that resist HIV-1 infection despite the presence of HIV-1 receptors. An increasing number of genes have been identified that can reduce the sensitivity of cultured cells to retrovirus infection, and in this issue of the *JCI*, Zhang et al. identify p21$^{Waf1/Cip1/Sdi1}$ (p21) as a gene product that can influence the sensitivity of HSCs to HIV-1 infection (see the related article beginning on page 473). Strikingly, p21 appears to alter the fate of nuclear HIV-1 DNA, promoting the formation of circular viral DNA forms rather than functional proviruses.

For many years, the ability of a particular retrovirus to colonize a given target cell type or species was thought to be governed solely by its ability to exploit required cellular cofactors provided to it by a candidate target cell. HIV-1, for example, can only infect cells that express CD4 and a chemokine receptor because those molecules are required to mediate the fusion of virion and target cell membranes. Similarly, HIV cannot replicate in rodent fibroblasts even when they are engineered to express HIV-1 receptors because of an incompatibility between the viral and host factors required for efficient gene expression. These host cell–specific blocks have proved extremely useful in enabling researchers to infer and subsequently discover and validate the existence of host cell factors that are required for HIV-1 replication.

However, what was not appreciated until quite recently is that evolution has equipped cells with a variety of genes whose major and perhaps only role is to prevent retrovirus replication (1, 2). The products of these inhibitory genes, termed *restriction factors*, are nearly as important as required cofactors in determining the cellular host range of HIV-1 and other retroviruses. The best known and characterized of the restriction factors are encoded by the *TRIM5* and

Nonstandard abbreviations used: p21, p21$^{Waf1/Cip1/Sdi1}$; PIC, HIV-1 preintegration complex.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:302–304 (2007). doi:10.1172/JCI31290.