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.

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An intrinsic host defense against HIV-1 integration?

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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.

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APOBEC3G genes (3, 4), which, based on their potency, specificity, and evident species-specific positive selection as well as the clear existence of viral countermeasures, seem likely to have evolved for the sole purpose of combating viral infection.

In addition to a number of known host-encoded antiretroviral defense mechanisms, an increasing number of genes that can reduce the sensitivity of cultured cells to retrovirus infection in a less obviously directed way have also been identified (5–9). It can be shown that the overexpression of these genes can inhibit susceptibility to retroviruses or that their underexpression can enhance susceptibility to infection. However, whether these genes directly block some critical step in the retrovirus life cycle or affect virus growth indirectly by affecting some aspect of cell physiology is not apparent. Some of these genes play important and obvious roles in the life of cells independent of retroviral infection, and so it is not clear whether their apparent antiretroviral activity is incidental.

A role for $p21^{Waf1/Cip1/Sdi1}$ in inducing HIV-1 resistance

In this issue of the *JCI* (10) and in a previous study (11), Zhang et al. have identified $p21^{Waf1/Cip1/Sdi1}$ (p21) as a gene product that can influence the sensitivity of HSCs to HIV-1 infection. HSCs are one of a few cell types, others being undifferentiated monocytes and unstimulated $CD4^+$ T cells, that resist HIV-1 infection despite the presence of HIV-1 receptors (12–14). In the case of HSCs, CD4 expression appears low, and previous work indicates a major block to HIV-1 infection of HSCs is at virus entry, since infection can be achieved using pseudotyped HIV-1 virions that carry a different viral envelope (14). However, later work also suggests that a second block to HIV-1 replication is imposed by p21 because its depletion could enhance sensitivity to pseudotyped HIV-1 vectors by about 2- to 4-fold (11). The new work in this issue extends this finding and shows that low-level–spreading HIV-1 replication can be obtained in HSCs if these cells are transfected with siRNAs that deplete p21. Additionally, Zhang et al. present a number of experiments designed to illuminate the molecular mechanism underlying the enhancement of HIV-1 replication in HSCs upon p21 depletion (10).

So, is the effect of p21 on HIV-1 infection a direct effect on the incoming virus or a secondary effect of modulating cell physiology? The normal cellular functions of the p21 protein suggest that either could be the case. Notably, p21 is a cyclin-dependent kinase (CDK) inhibitor (15) and particularly targets CDKs that are active in the G1 phase of the cell cycle. In doing so, p21 inhibits cell-cycle progression in some cellular types, including HSCs (16). Although cell-cycle progression is not absolutely required for HIV-1 infection, quiescent cells (in G0 phase) are known to be very poor targets for HIV-1 infection, at least in part because reverse transcription is not completed (17). Additionally, p21 binds to factors involved in DNA repair pathways (18). The requirement for DNA repair activities in HIV-1 infection, particularly at integration, has been debated for some years, and recent findings suggest that some DNA repair activities may actually inhibit HIV-1 infection, perhaps by targeting viral DNA prior to integration into the target cell genome (19). Overall, therefore, there are at least 2 plausible models by which p21 could inhibit HIV-1 infection.

Zhang et al. (10) argue for a direct effect of p21 on incoming HIV-1. Data in the study hint that p21 binds to an incoming subviral complex containing HIV-1 matrix and integrase, known as the HIV-1 preintegration complex (PIC). This is consistent with the notion that the effect could be a direct one on viral components. However, caution is warranted here because this type of experiment is notoriously difficult and artifact prone. Nonetheless, it appears that the effect of p21 depletion is specific to HIV-1 infection, as low-level replication in HSCs of the simian immunodeficiency virus SIVmac251 is unaffected by p21 depletion. The lack of effect on a related virus is more difficult, albeit not impossible, to reconcile with a model in which the effect of p21 depletion on HIV-1 replication has an indirect effect on cell physiology. It would be useful to know whether SIVmac251 PICs associate with p21 and what viral determinants govern the apparently discordant effects of p21 on HIV versus SIVmac251 replication — this would provide strong clues as to an underlying mechanism. If the effects of p21 are direct, it might be possible to show that resistance can be induced in other cellular contexts by ectopic expression of p21. For now, the central role of p21 in cell physiology, and likely a complex cascade of events that accompany its depletion, urge circumspection in formulating models to explain why it appears to inhibit HIV-1 replication. The authors do show that p21-depleted cells do not begin to proliferate until some time after siRNA transfection, but this does not compellingly refute the possibility that p21's effects on HIV-1 infection are indirect because the physiological changes that lead to DNA synthesis and cell division obviously precede the events themselves.

Effects of p21 on HIV-1 integration

These caveats aside, the most striking and provocative finding in the current study (10), and one that suggests a specific effect on the incoming HIV-1 PIC, is the rather dramatic difference in the fate of nascent viral DNA that was observed in HSCs upon p21 depletion. In fact, the effects of p21 depletion on the fate of HIV-1 DNA appear much greater than the effects of p21 depletion on infection (as measured by the expression of a reporter gene embedded in the HIV genome). Specifically, control-infected HSCs appear to accumulate circular forms of HIV-1 DNA while infection of p21-depleted cells results in far more integrated proviruses and very low–level circle formation. Excessive circle formation is a hallmark of failed integration; pharmacological or mutational inhibition of HIV-1 integrase catalytic activity induces precisely this phenotype. Reasonably, the authors speculate that DNA repair systems that interact with p21 (18) might be responsible for this phenomenon. The fate of HIV-1 DNA following its entry into the nucleus is a step in the viral life cycle that is increasingly recognized to be influenced by host factors; for example, LEDGF, a chromatin-associated host protein that directly binds HIV-1 integrase, appears to be rather important for integration (20). DNA repair pathways are also increasingly recognized as inhibiting retroviral infection (19); thus, there are a number of potential mechanisms by which p21 depletion could promote integration at the expense of circle formation.

Conclusions

Based on the current findings (10), it would seem premature to dub p21 a bona fide restriction factor. Nonetheless, it does join the growing list of gene products that can influence cellular sensitivity to HIV-1 infection, and its effects on nascent HIV-1 DNA are unique and interesting. Further work will be required to determine precisely how p21's effects on HIV-1 DNA are mediated and how p21's effects relate to the function

of other host proteins that determine the fate of HIV-1 DNA once it has entered the target cell nucleus.

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Transcriptional regulation of epithelial-mesenchymal transition

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It has become increasingly obvious that the notion of a terminally differentiated cell is likely a simplified concept. Epithelial-mesenchymal transition (EMT), during which epithelial cells assume a mesenchymal phenotype, is a key event occurring during normal development and pathological processes. Multiple extracellular stimuli and transcriptional regulators can trigger EMT, but how such distinct signaling pathways orchestrate the complex cellular events that facilitate EMT is not well understood. In this issue of the *JCI*, Venkov et al. report on their examination of fibroblasts resulting from EMT and describe a novel protein-DNA complex that is essential for transcription of *fibroblast-specific protein 1* (*FSP1*) and sufficient to induce early EMT events (see the related article beginning on page 482). Collectively, their results suggest that this complex is an important regulator of the EMT transcriptome.

During development and adult organ pathogenesis, cells are in a constant state of phenotypic transition. In pathological settings, differentiated adult cells from the kidney, lung, liver, or heart can undergo drastic phenotypic transitions. Such acts are likely undertaken to avoid cell death in a hostile environment. But if an insult, such as organ fibrosis, persists, then such transitions likely become semipermanent.

During epithelial-mesenchymal transition (EMT), epithelial cells gradually lose their epithelial signatures while acquiring the characteristics of mesenchymal cells. EMT is regarded as a critical regulator of metazoan embryogenesis and physiological processes such as wound healing. EMT also contributes significantly in pathologies such as tissue fibrosis and cancer metastasis. Hallmarks of EMT include: (a) the downregulation of cell adhesion molecules such as E-cadherin; (b) the increased expression of MMPs to assist in the degradation of the basement membrane; (c) the activation of the Rac/Rho/Cdc42 family small GTPase to bring about cytoskeleton rearrangement; and (d) the nuclear translocation of several transcription factors including β-catenin and the T cell factor/lymphocyte enhancer factor 1 (TCF/LEF1) complex, Snail1, Snail2, and Twist (1, 2). The adoption of a fibroblast-like transcription profile is crucial for the survival of the cells undergoing EMT. Several key transcription factors have been described (1); however, it is now clear that more such transcriptional regulators are required to govern the complex EMT transcriptome.

Nonstandard abbreviations used: CBF-A, CArG box–binding factor–A; EMT, epithelial-mesenchymal transition; FSP1, fibroblast-specific protein 1; FTS-1, fibroblast transcription site–1; KAP-1, KRAB-associated protein 1.

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