What can you do with a luciferase Reporter Assay?

Promoter Dissection Application

Presented Fall 2009

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Have a question?
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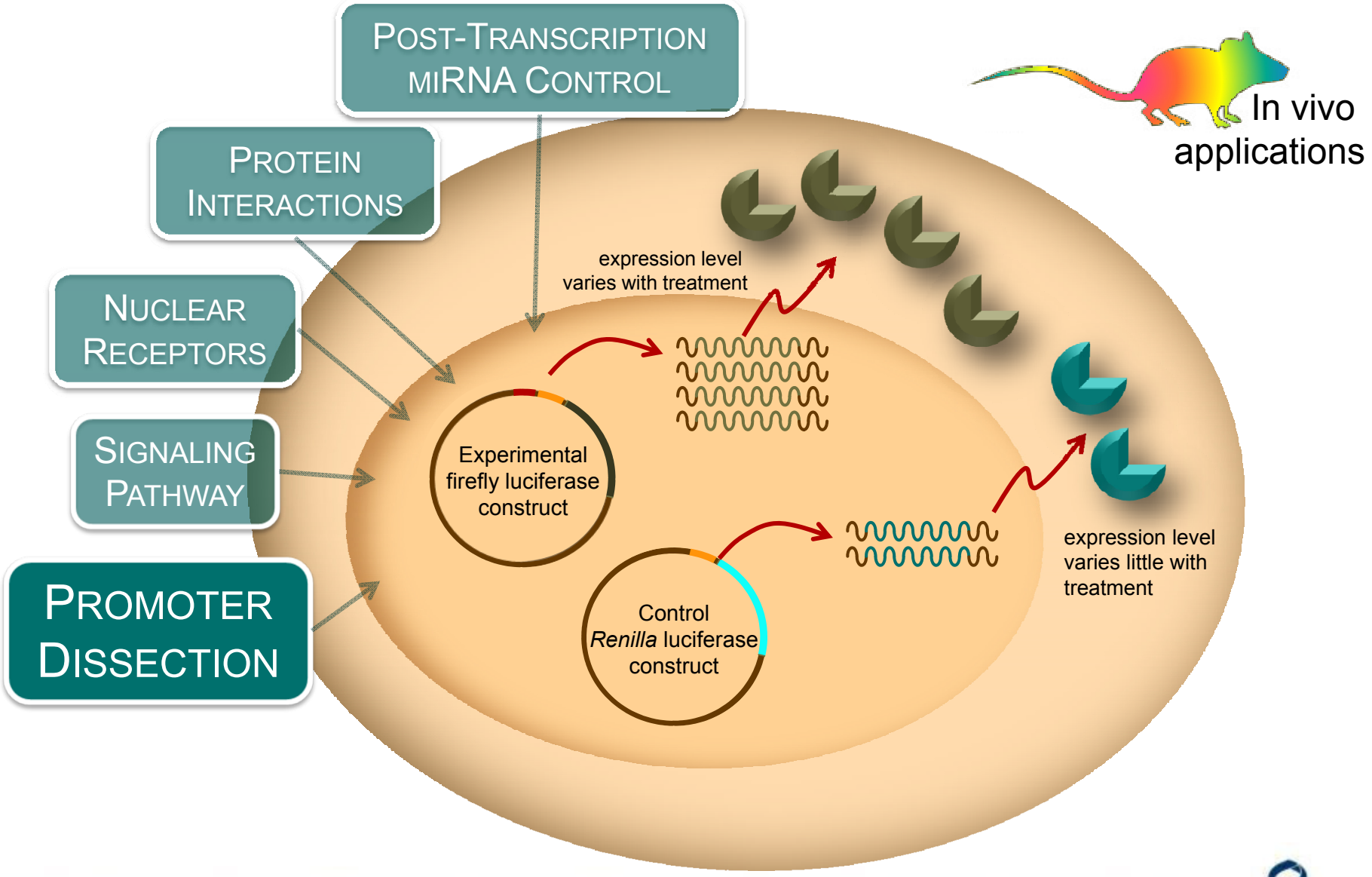
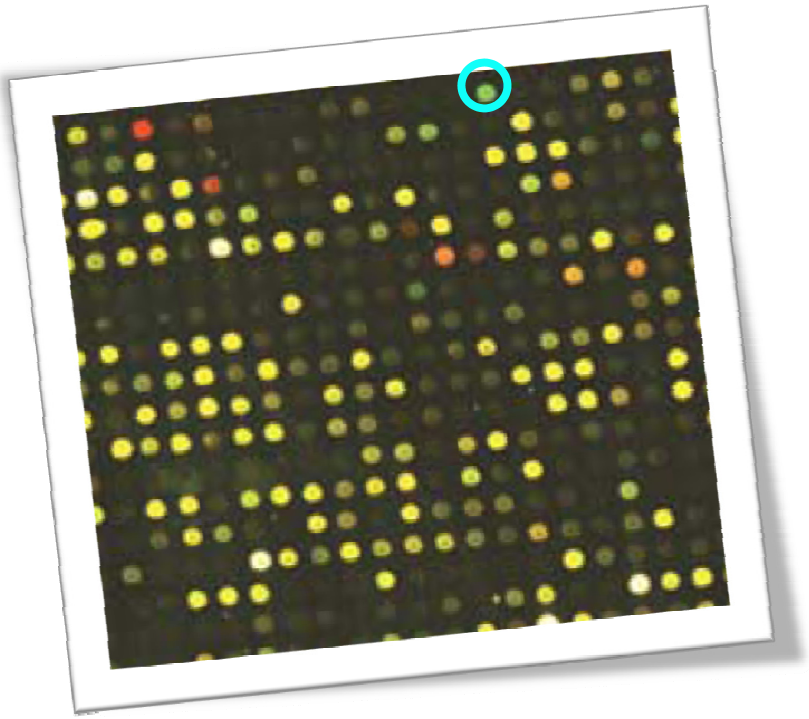
Application Overview



Why is my gene responding to a treatment?

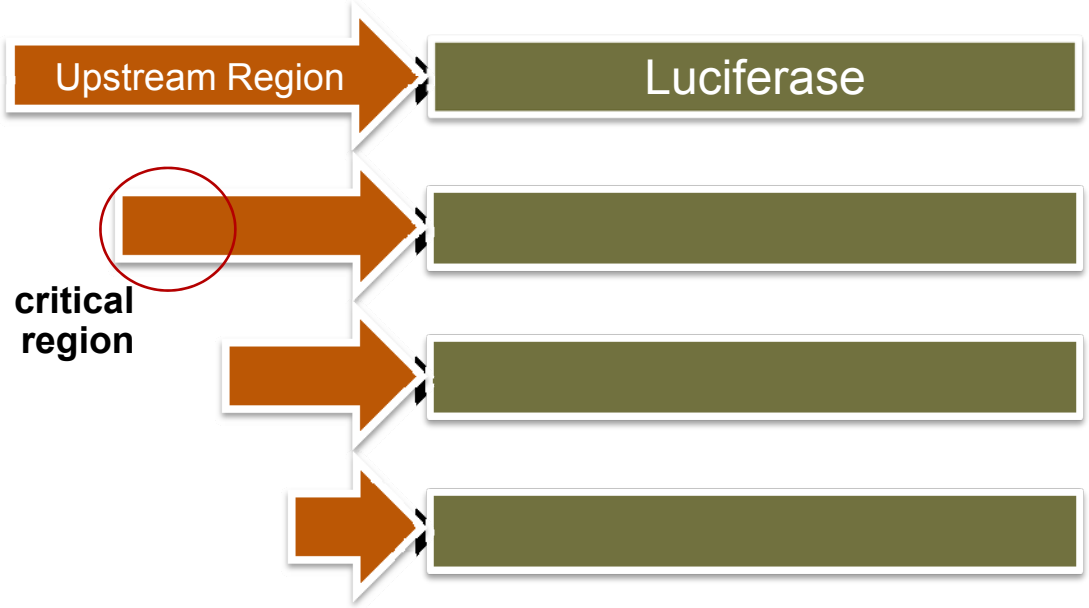


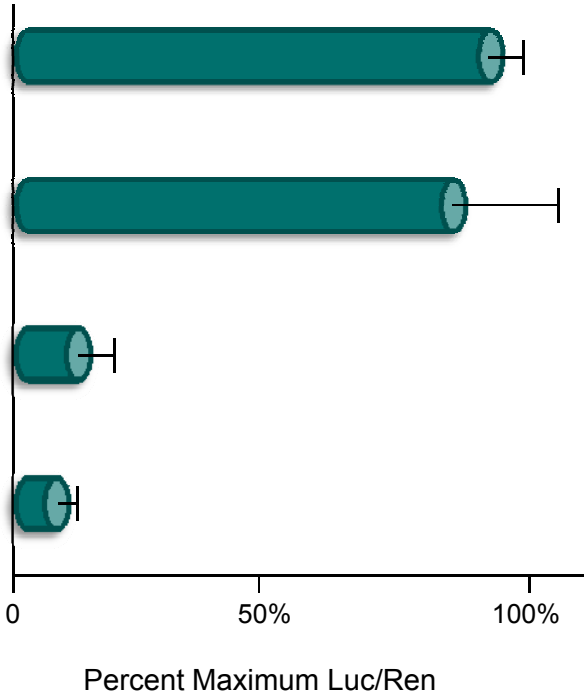
Bioinformatic analysis of promoter region shows many potential transcription factor binding sites that could be mediating the response.

Promoter dissection can help identify promoter elements involved in the response

Traditional Promoter Dissection

Use of deletion mutagenesis to find functional elements responsible for regulation of gene transcription





Commonly called “promoter bashing”

Case Study: ΔVII-Ets-1 activates HIV-1 provirus

Isolation of a cellular factor that can reactivate latent HIV-1 without T cell activation

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HIV-1 latency in resting CD4+ T cells represents a major barrier to virus eradication in patients on highly active antiretroviral therapy (HAART). Eliminating the latent HIV-1 reservoir may require the reactivation of viral gene expression in latently infected cells. Most approaches for reactivating latent HIV-1 require nonspecific T cell activation, which has potential toxicity. To identify factors for reactivating latent HIV-1 without inducing global T cell activation, we performed a previously undescribed unbiased screen for genes that could activate transcription from the HIV-1 LTR in an NF-κB-independent manner, and isolated an alternatively spliced form of the transcription factor Ets-1, ΔVII-Ets-1. ΔVII-Ets-1 activated HIV-1 transcription through 2 conserved regions in the LTR, and reactivated latent HIV-1 in cells from patients on HAART without causing significant T cell activation. Our results highlight the therapeutic potential of cellular factors for the reactivation of latent HIV-1 and provide an efficient approach for their identification.

antiretroviral therapy | ΔVII-Ets-1 | expression cloning | long terminal repeat | viral reservoir

Advances in antiretroviral therapy have dramatically reduced mortality among patients with HIV-1 infection (1). However, there is still no therapeutic regimen to cure chronic HIV-1 infection. Although highly active antiretroviral therapy (HAART) can suppress plasma viral load to undetectable levels, viremia rebounds within weeks after discontinuation of HAART. The major barrier to eradication of HIV-1 infection is the existence of viral reservoirs. Among them, the best characterized is a small pool of latently-infected resting memory CD4+ T cells harboring an integrated provirus (2–4). Previous studies have demonstrated the stability of this latent reservoir in patients on HAART (5). The half-life of this reservoir was estimated to be >44 months. At this rate of decay, it is expected to take >60 years to purge HIV-1 from infected patients on HAART. Thus, this reservoir necessitates the lifetime use of HAART, and strategies are needed for eradication of latently infected cells (6, 7).

Recently, reactivation of latent virus has gained wide interest as a potential strategy to eradicate the viral reservoirs (8–11). It is assumed that latently infected cells can be killed either by immune attack or direct viral cytopathic effects after reactivation of latent HIV-1. A reactivation strategy, along with simultaneous efficient suppression of viral spread by HAART, might reduce and ultimately eliminate the latent reservoirs (6, 7). Although logical, this approach has practical limitations. Because signals that cause T cell activation also activate HIV-1 replication, some studies have focused on strategies to induce some level of T cell activation as a means of reactivating latent HIV-1 (10, 11). Unfortunately, the potential toxicity of such nonspecific T cell activation has severely complicated this approach (10, 11). For example, patients treated with agonistic anti-CD3 monoclonal antibody and IL-2 suffered from severe side effects, transient renal failure, and seizure. An ideal reactivation strategy for virus eradication might allow activation of HIV-1 without inducing global T cell activation.

The HIV-1 provirus responds to various extracellular stimuli, including T cell activation signals and some proinflammatory cytokines (12–14). The HIV-1 promoter, located within the U3 region of the LTR, contains an array of *cis*-acting transcription factor binding sites (15). The interaction between these diverse signals and the various binding sites in the LTR forms a complex regulatory network. In particular, the host transcription factor NF-κB is important for activating HIV-1 gene expression through 2 conserved κB sites in the core enhancer region of the HIV-1 LTR (12, 13). However, HIV-1 can replicate in the absence of κB sites in the LTR (16), consistent with the existence of NF-κB-independent pathways in the activation of HIV-1 (17, 18). NF-κB also has a critical role in innate and adaptive immune responses, and regulates genes that have important roles during T cell activation (19). Because of the central role of NF-κB in T cell activation, we reasoned that to find genes that could uncouple the activation of latent HIV-1 from T cell activation it would be desirable to identify factors that could activate the HIV-1 LTR in an NF-κB-independent manner.

To systematically search for NF-κB-independent pathways for the activation of HIV-1, we performed an expression cloning screen using a reporter containing mutated NF-κB sites in the enhancer region of the HIV-1 LTR. By screening a human splenocyte cDNA expression library, we isolated an alternatively spliced form of the Ets-1 transcription factor, ΔVII-Ets-1. ΔVII-Ets-1 was able to activate the NF-κB site-mutated HIV-1 LTR without stimulating T cell activation and could activate latent HIV-1 from resting CD4+ T cells isolated from patients on HAART. Our results identify a cellular factor that can reactivate latent HIV-1 without inducing T cell activation, and illustrate the potential of this expression cloning strategy to yield novel approaches for eradicating latent reservoirs of HIV-1.

Results

Expression Cloning Screen to Identify NF-κB-Independent Pathways for the Reactivation of Latent HIV-1. To facilitate the identification of NF-κB-independent pathways that could activate the HIV-1 LTR, we generated a luciferase reporter, mκB-LTR-Luc, which contains the HIV-1 LTR from reference strain NL4-3 with mutated κB sites within the core enhancer region (−106 to −83) (Fig. 1*A*) that have been shown to abolish the activity of NF-κB on the HIV-1 LTR (13). We then screened a human splenocyte cDNA expression library for the ability to stimulate the mκB-LTR-Luc reporter. To maximize the number of the cDNAs that could be assayed, we generated cDNA pools with ≈100 cDNAs

Author contributions: H.-C.Y., R.F.S., and J.L.P. designed research; H.-C.Y. and L.S. performed research; H.-C.Y., R.F.S., and J.L.P. analyzed data; and H.-C.Y., R.F.S., and J.L.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0809536106/DCSupplemental.

MICROBIOLOGY

- Latent HIV-1 provirus integrated into T-cells is a real problem.
- Need a way to activate the provirus without activating the T cell (i.e., avoid NF-κB pathways) so cells can be eliminated.
- Indentified **ΔVII-Ets-1** a splice variant of transcription factor Ets-1
- What elements are required for ΔVII-Ets-1 in the HIV long terminal repeat?

Yang, H-.C. *et al.* (2009) *PNAS* **106**,6321-26.

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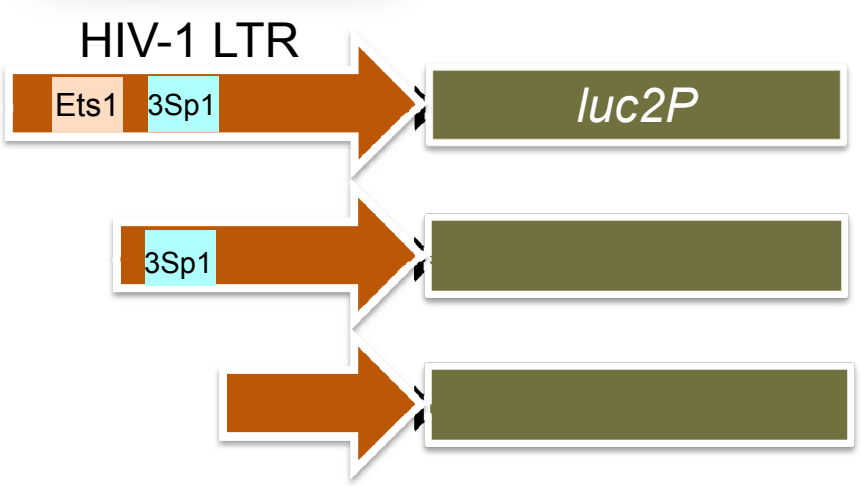
Case Study:
Yang, H-.C., et al.

Dissection identifies ΔVII-Ets-1 sites



% Activity

100%

~50%

~0%

- The ΔVII-Ets-1 requires the Ets-1 Binding Sites and the 3 Sp1 sites to exert full activity
- Activity through both sites requires an intact DNA binding domain within ΔVII-Ets-1

- Studies showed that ΔVII-Ets-1 activates the promoter more effectively than full-length Ets-1
- Exon 7 must be the site of Ets-1 control

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More Information…

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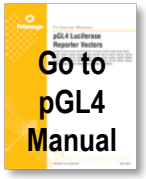

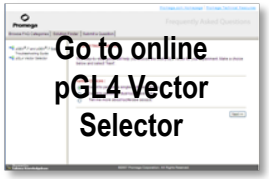

Dual-Luciferase® System citations on HighWire Press®

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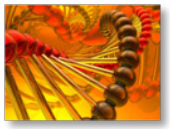

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