Integration site specificity

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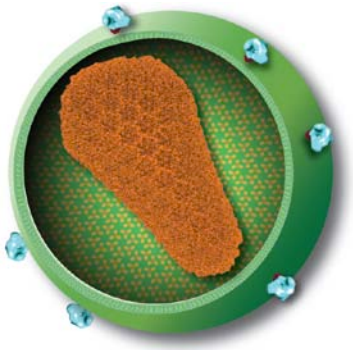
Approaches to controlling integration targeting

- Choice of retroviral or transposon vector
- Modify integrase or transposase (e. g. add DNA binding domains)
- Modify host cell tethering factors
- Site specific DNA cleavage, recombine in new sequences (e. g. zinc finger nucleases with AAV vectors)
- Allow random integration, then fish out cells with integration site in “safe harbor”

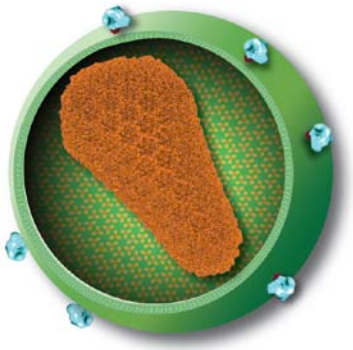
Lentiviral vectors for gene therapy

- No insertional mutagenesis in HIV-positive individuals
- Can infect non-dividing cells
- Other advantages

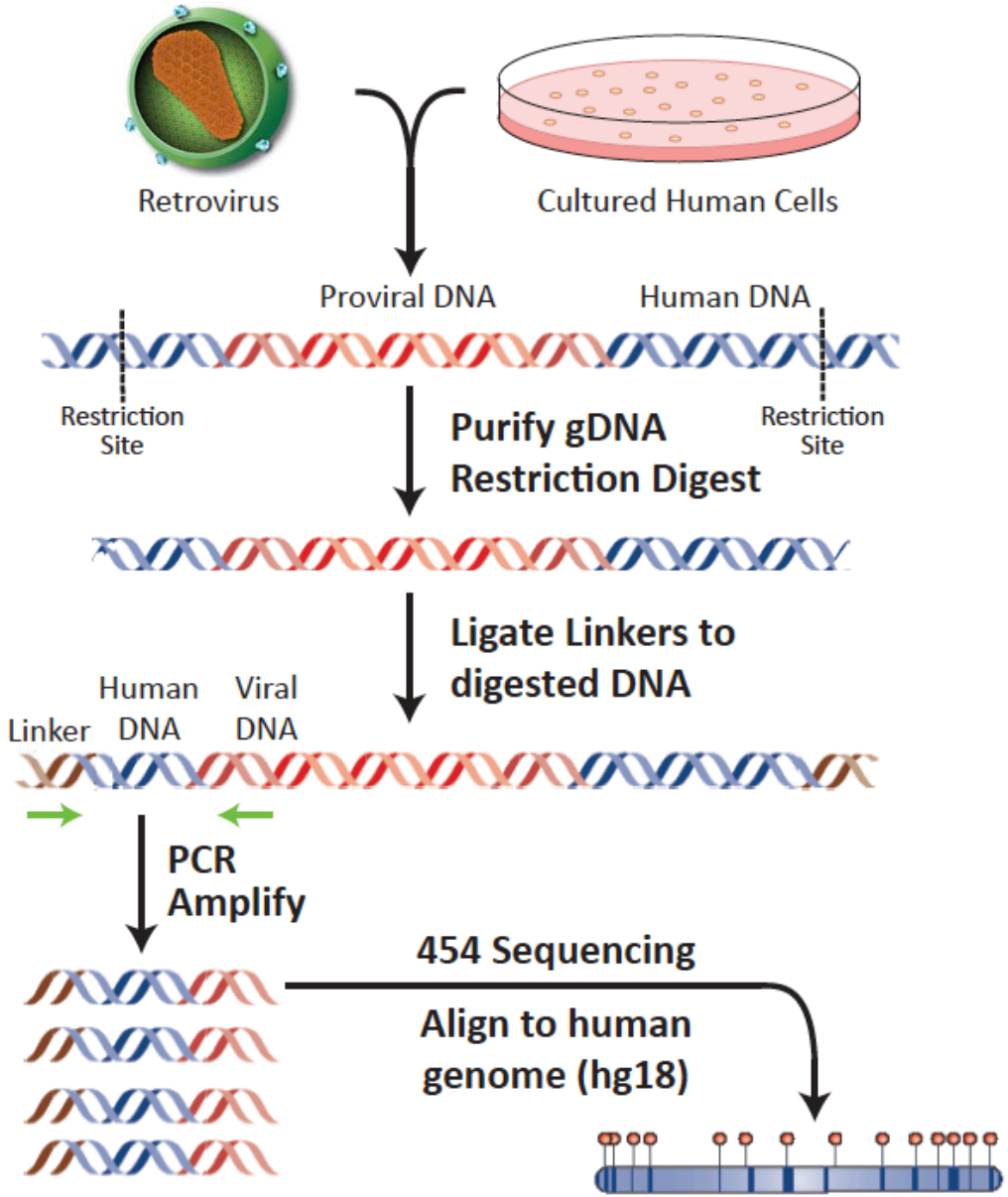


- **HIV integration targeting by LEDGF/p75**

- **Retargeting lentiviral integration with LEDGF/p75 fusions**

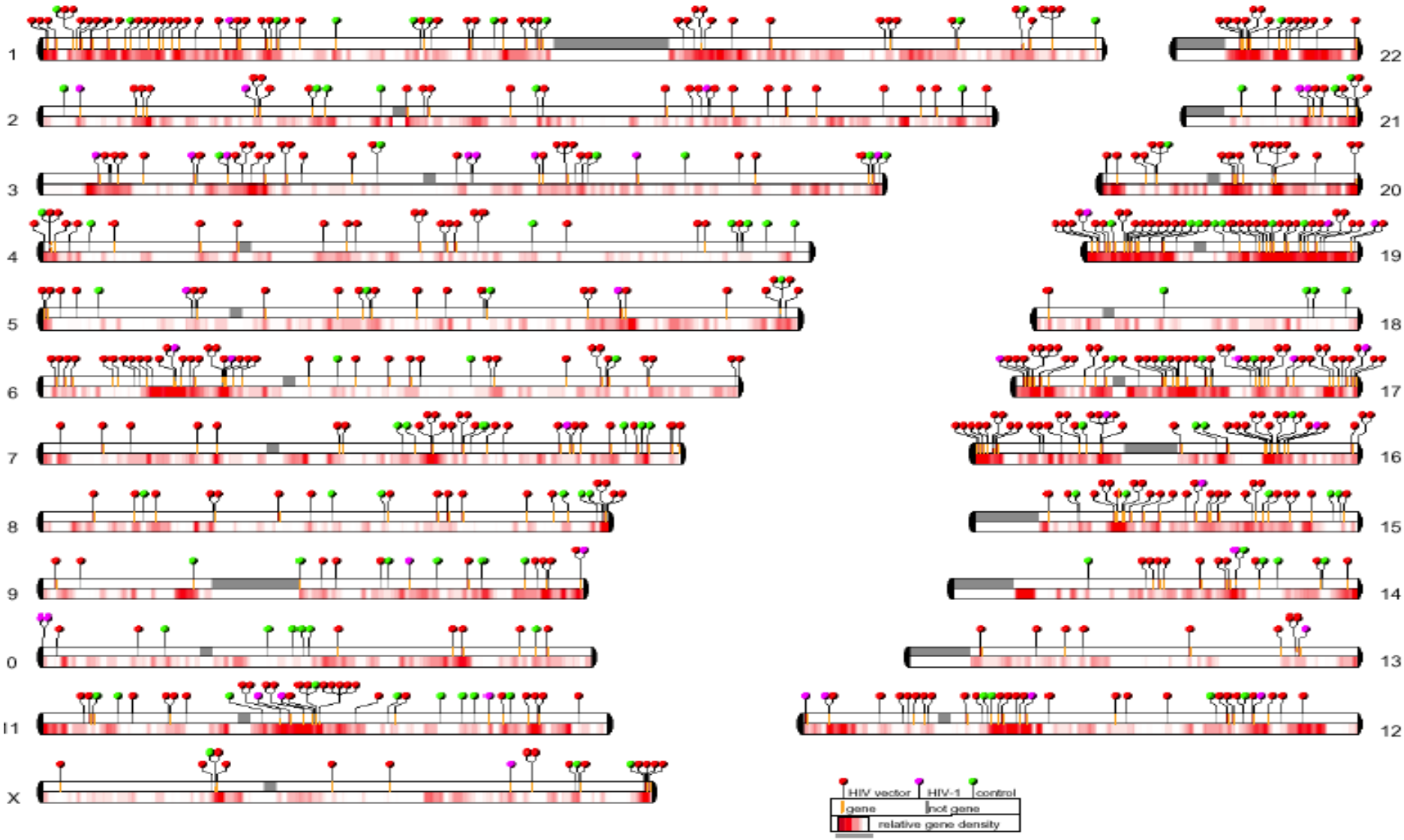


Technology for analyzing integration sites



454 Sequencing

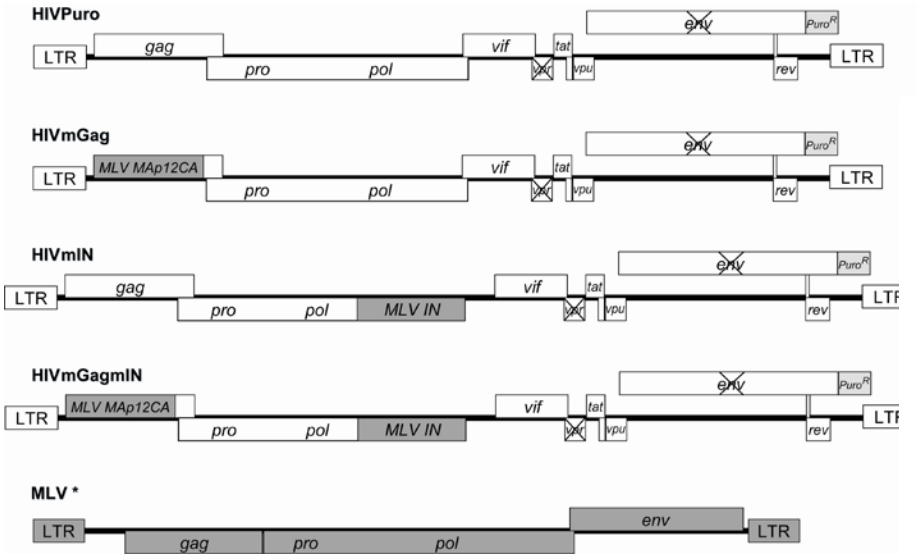
Sites of HIV-1 cDNA Integration in SupT1 Cells



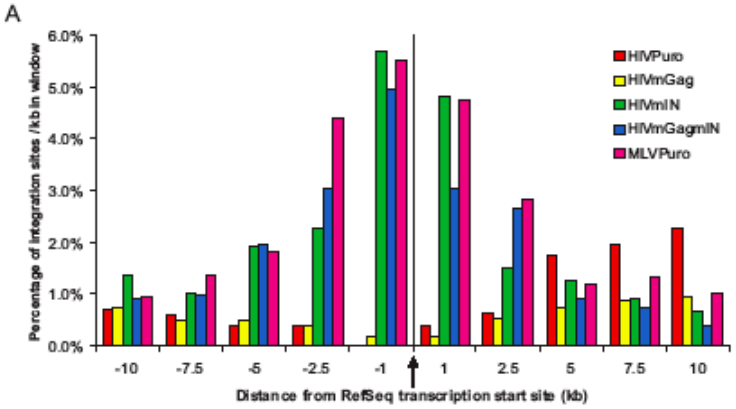
Schroder, Shinn, Chen, Berry, Ecker and Bushman, Cell, 2002

Integrase is a viral determinant of integration targeting

HIV/MLV chimeras studied



Chimeras with MLV IN show favored integration near transcription start sites



MK Lewinski, M Yamashita, M Emerman, A Ciuffi, H Marshall,G Crawford, F Collins, P Shinn, J Leipzig, JR Ecker, FD Bushman, PLOS Pathogens, 2006

PSIP1/LEDGF/p75: a host cell factor involved in HIV integration targeting

Transcriptional coactivator

Ge et al. 1988

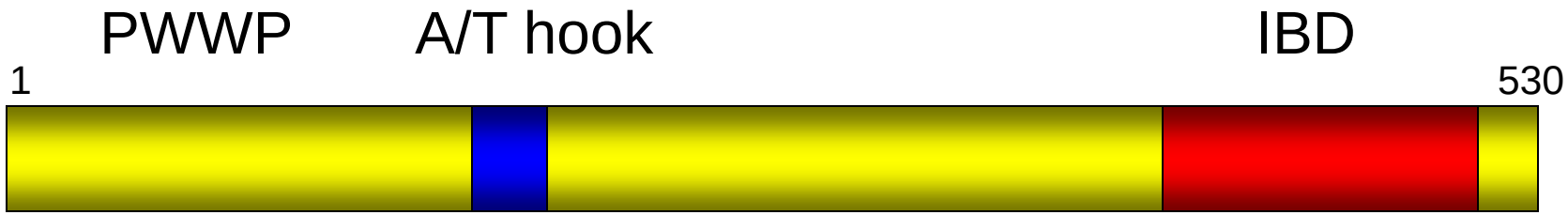
Binds tightly to HIV IN

Cherepanov et al. 2003, Maertens et al. 2003, Llano et al., 2004, Turlure et al., 2004

Promotes integration

Emiliani et al., 2005, *Llano et al., 2006, Shun et al. 2007, Marshall et al., 2007*

LEDGF/p75 protein

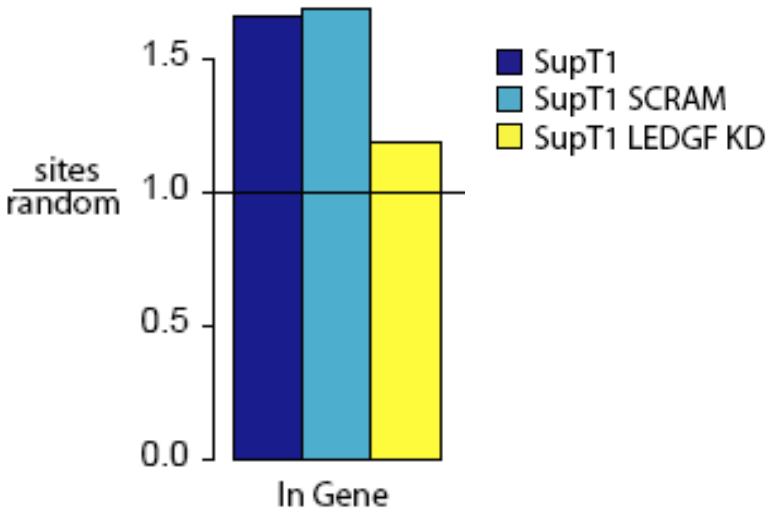


Data from labs of Poeschla, Engelman, Debyser, Cherepanov, Benharous, Bushman…

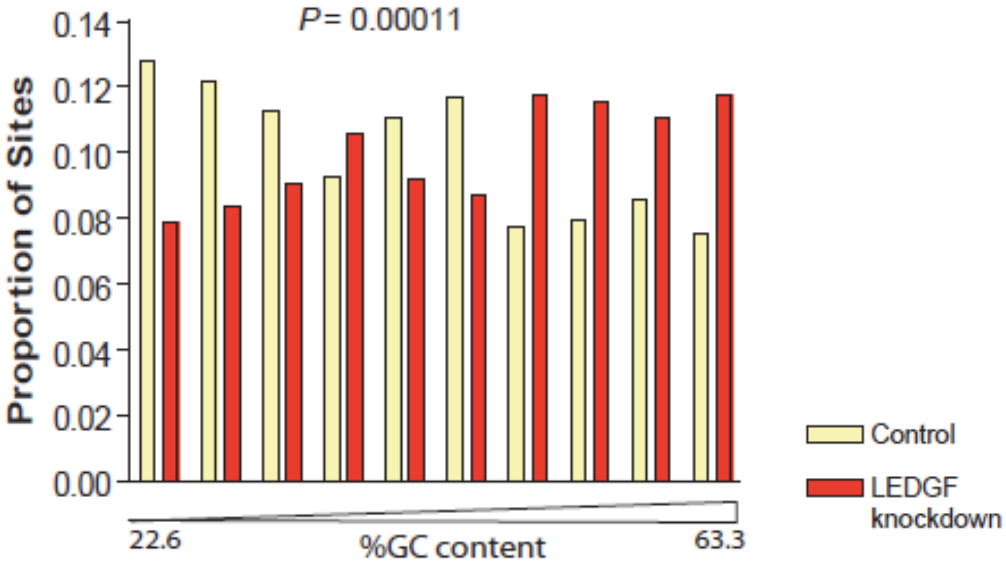
Depletion of PSIP1/LEDGF/p75 Redirects Integration

Data from SupT1 knockdown (human), MEF gene trap (murine)
Studied HIV and equine infectious anemia virus (another lentivirus)

Integration in transcription units

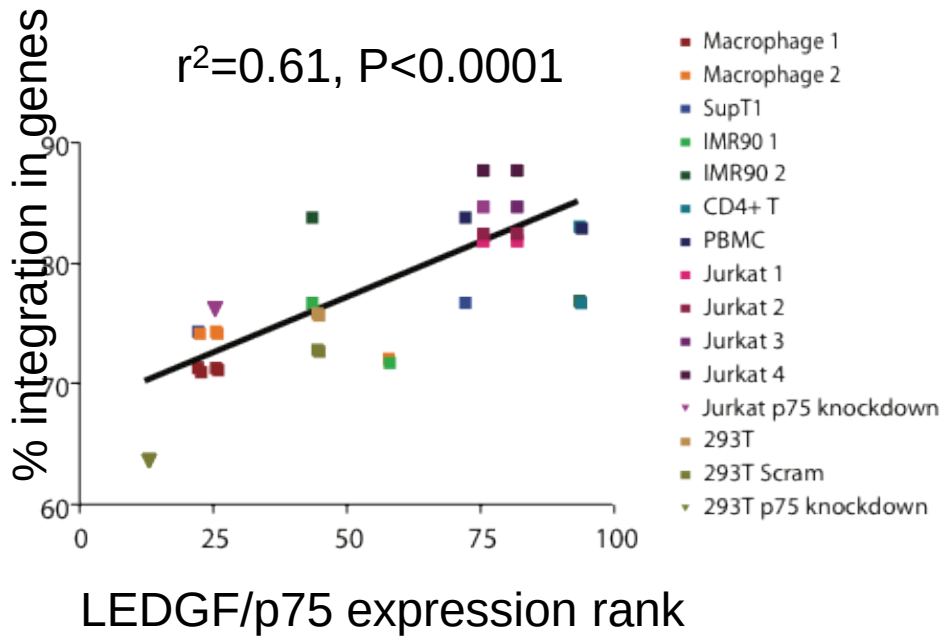


Integration in GC-rich regions



Ciuffi, M. Llano, E. Poeschla, H. Marshall, C. Hoffmann, P. Shinn, S. Hannenhalli, J. Ecker, F. Bushman. Nature Medicine, 2005.
Shun, Cherepanov, Engelman et al. Genes and Dev. 2007
Marshall, Ronen, Bickmore, Poeschla, Bushman et al., PLoS One, 2007

LEDGF/p75 expression and integration frequency in transcription units



- Different cell types show reproducible differences in the frequency of HIV integration in transcription units
- These differences correlate with the relative expression level of LEDGF/p75

The more LEDGF/p75, the greater fraction of integration events in transcription units

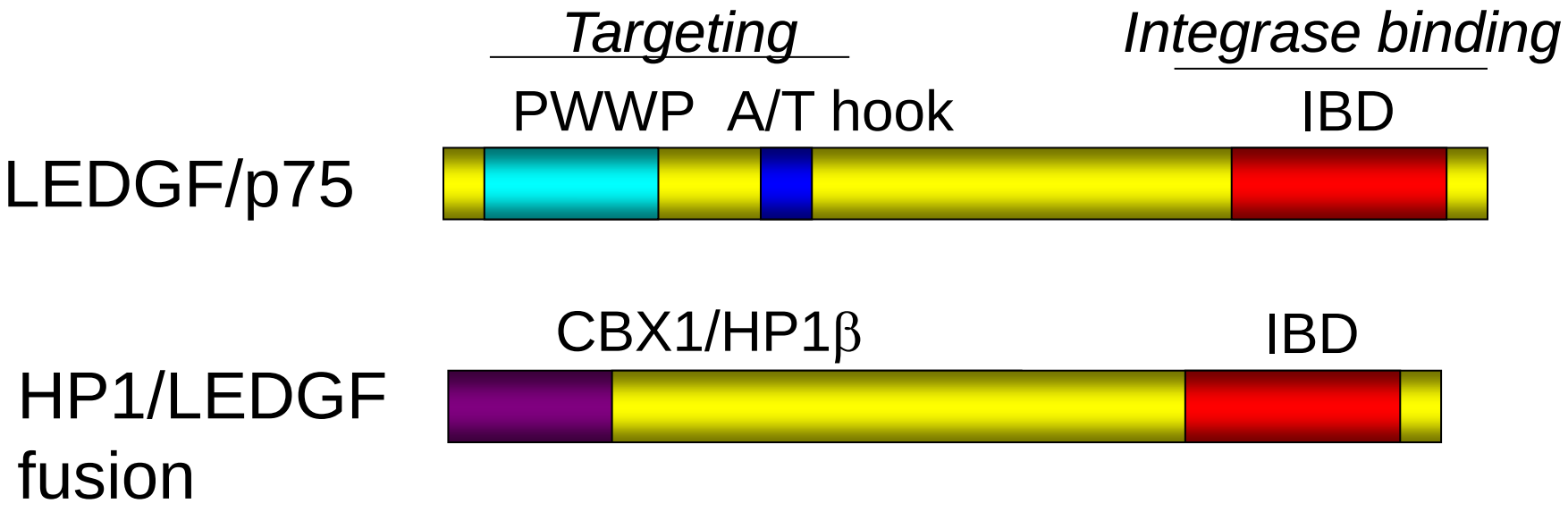
Retargeting lentiviral integration away from genes

Rik Gijsbers, Keshet Ronen, Sofie Vets, Nirav Malani, Jan De Rijck, Melissa McNeely, Frederic D. Bushman, and Zeger Debyser

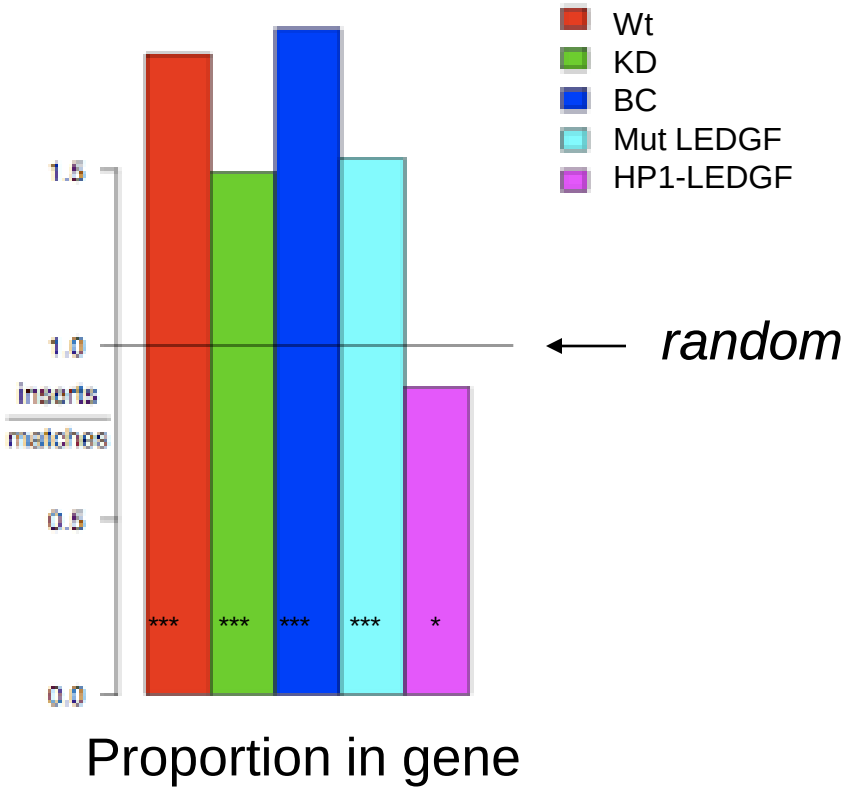
See also papers from Daniel, Engelman, Hughes and colleagues

Retargeting lentiviral integration using fusions of HP1β(CBX) to LEDGF/p75

- HP1 enriched in heterochromatic regions particularly around centromeres
- Binds to H3K9me2&3

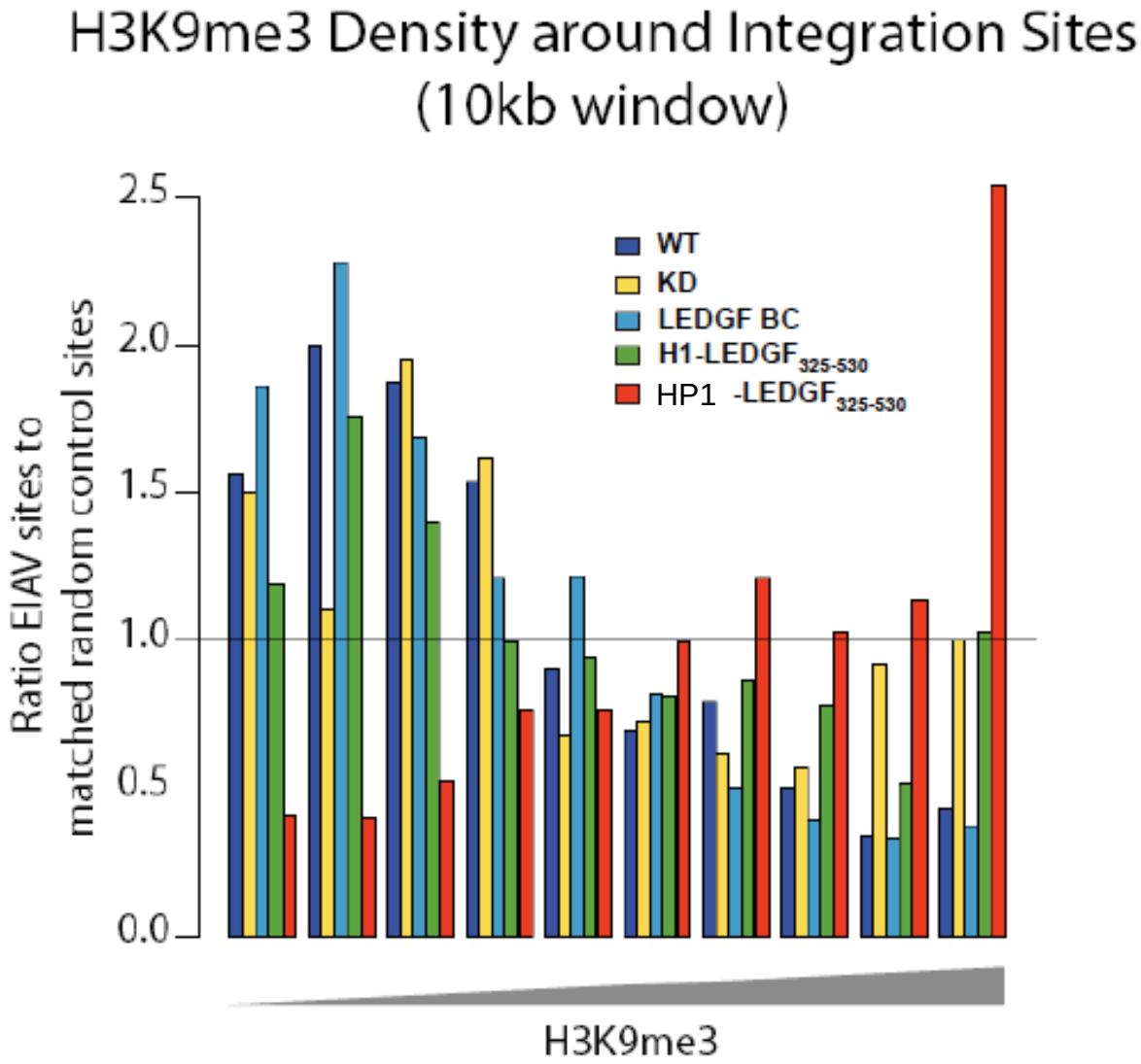


Integration in Transcription Units



Expression of HP1-LEDGF fusion directs integration away from genes

Integration in presence of HP1-LEDGF fusion favored near sites of H3K9me3



Summary

- HIV favors integration in active transcription units
- Other retroviruses have different favored targets
- PSIP1/LEDGF/p75 tethers HIV integration complexes to DNA
- Method for controlling integration inside cells?

Credits

Bushman laboratory	Former Bushman Lab	Collaborators
Troy Brady	Rick Mitchell	Charles Berry
Kyle Bittinger	Brett Beitzel	Sumit Chanda
Rohini Sinha	Mary Lewinski	John Young
Keshet Ronen	Astrid Schroder	Renate Koenig
Karen Ocwieja	Angela Ciuffi	Joe Ecker
Christian Hoffmann	Heather Marshall Rose	Craig Hyde
Nirav Malani	Jeremy Leipzig	Mark Yeager
Rithun Mukherjee	Matt Culyba	Kushol Gupta
Young Hwang	Gary Wang	Greg Van Duyne
Rebecca Custers-Allen	Tracy Diamond	Masahiro Yamashita
Serena Dollive	Scott Sherrill-Mix	Mike Emerman
Jennifer Hwang	Nana Minkah	Francis Collins
Jimmy Hu		Nam Lee
Sam Minot		Paul Bieniasz
Greg Peterfreund		Philippe Leboulch
Shannah Roth		Alain Fischer
Frances Male		Marina Cavazzana-Calvo
Emily Charlson		Zeger Debyser
		Rik Gijsbers