

RETROVIRUSES AND INSERTIONAL MUTAGENESIS

- 1. Introduction**
- 2. Mechanisms of Gene Activation and Inactivation**
- 3. Consequences of Gene Activation and Inactivation**
- 4. Virus and Host Factors Affecting Insertional Mutagenesis**
- 5. Summary and Conclusions**

RETROVIRUSES AND INSERTIONAL MUTAGENESIS

1. Introduction

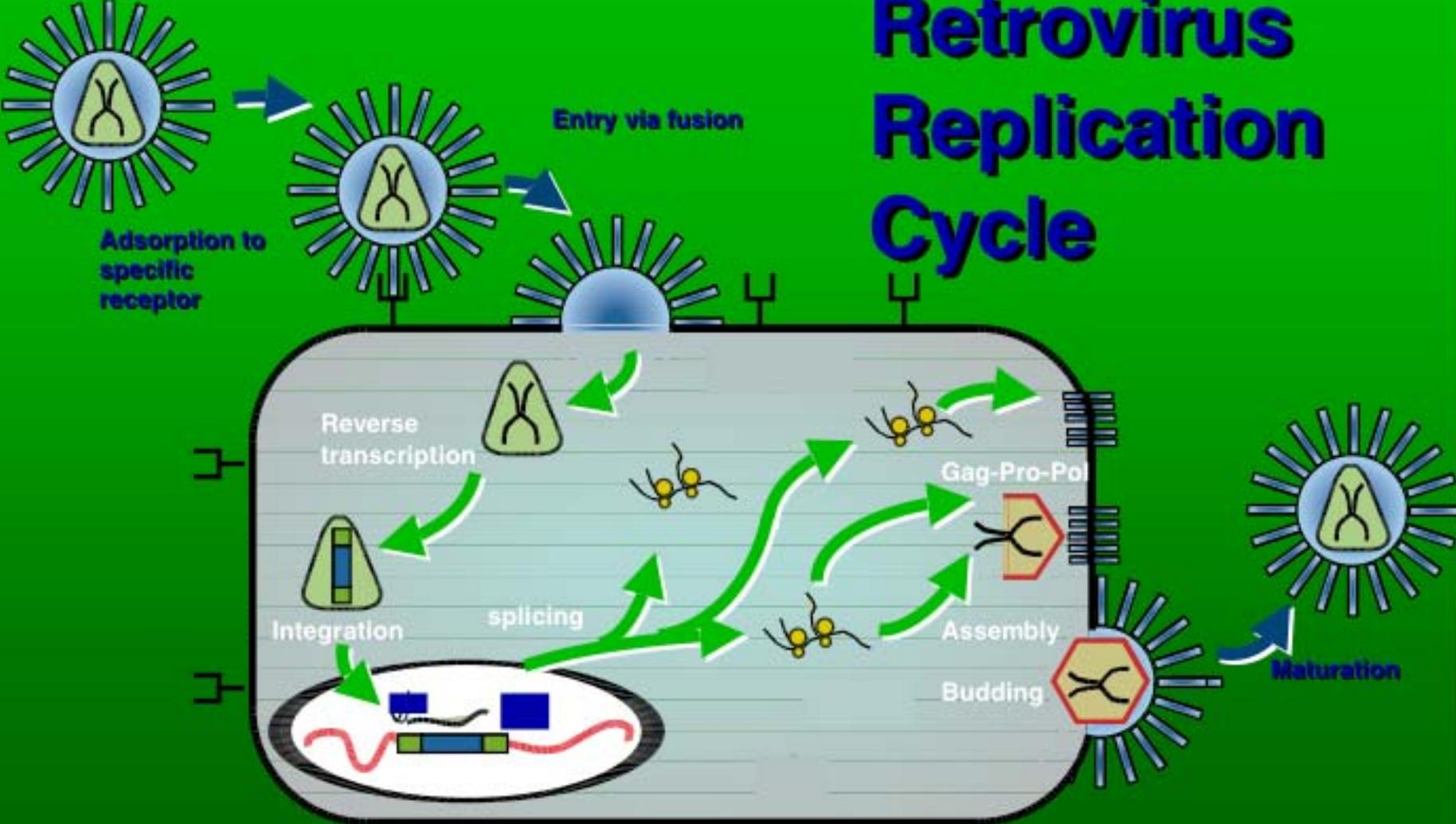
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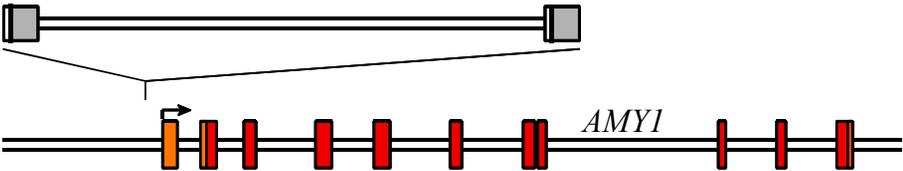
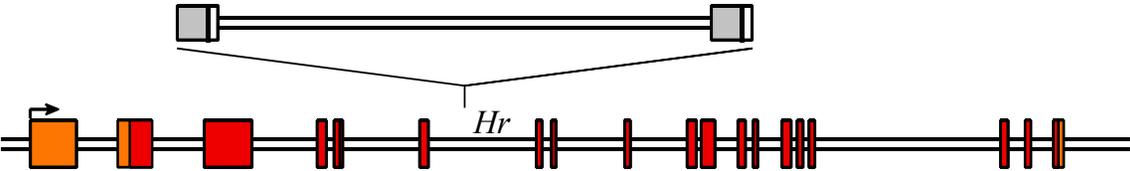
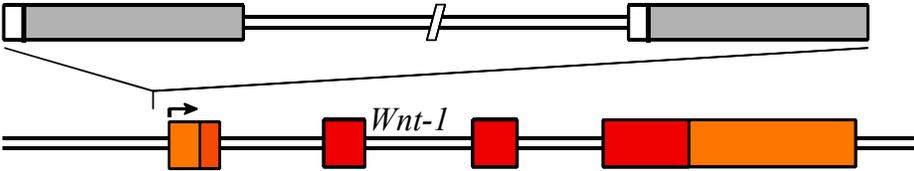
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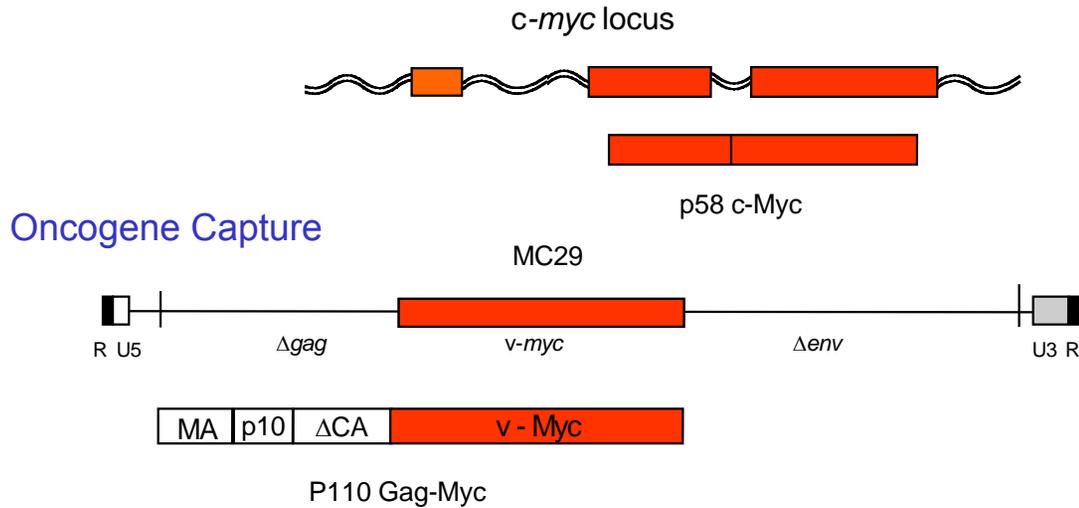
The Retrovirus Replication Cycle



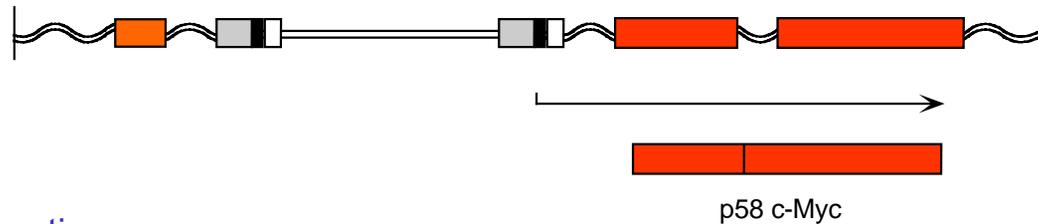
EFFECTS OF PROVIRUS INTEGRATION



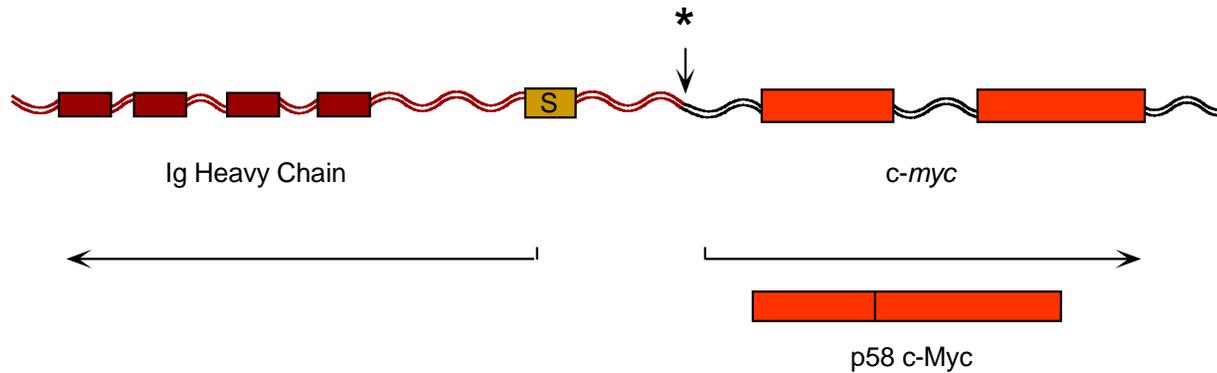
MECHANISMS OF ONCOGENE "ACTIVATION"



Proviral Insertion



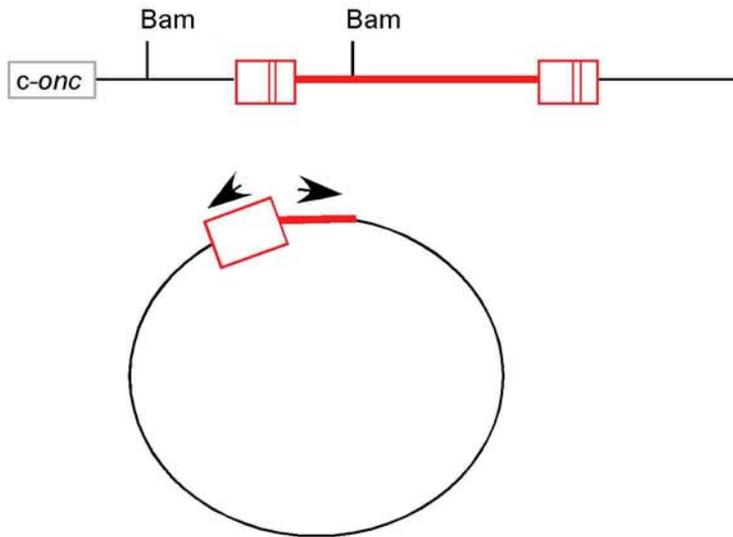
Translocation



INSERTIONAL ACTIVATION OF PROTO-ONCOGENES

- 1. Most common mechanism of activation of proto-oncogenes by retroviruses**
- 2. Almost 100 proto-oncogenes first identified as targets of insertion**
- 3. High-throughput screening suggests many more**
- 4. Genes encoding transcription factors, chromatin remodeling proteins, growth signaling molecules, growth factors and proteins affecting apoptosis are common targets**

COMMON INTERGRATION SITES IN HEMATOPOIETIC TUMORS*



**Clone & Identify Gene(s) Nearby
the CIS (Common Site)**

1. 30 - 60% contain 1 common insertion
2. Many (>100) new sites identified
3. Assuming random distribution, in a set 500 CIS, only 2 clusters of 2 integrations would occur randomly in any ~25 kb region

* Copeland, Jenkins, et al.
* Berns, et al.
* Lenz, et al.

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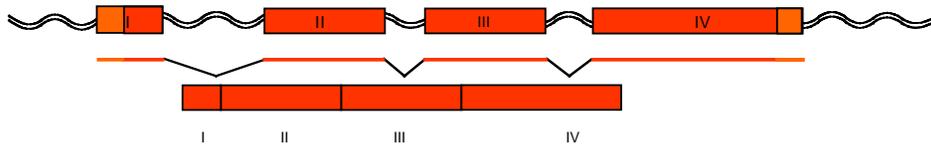
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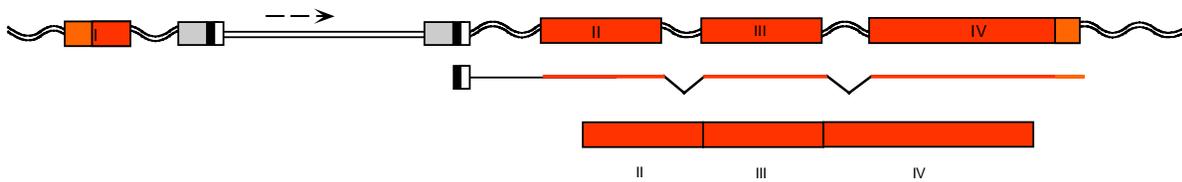
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MECHANISMS OF INSERTIONAL ACTIVATION

Proto-oncogene

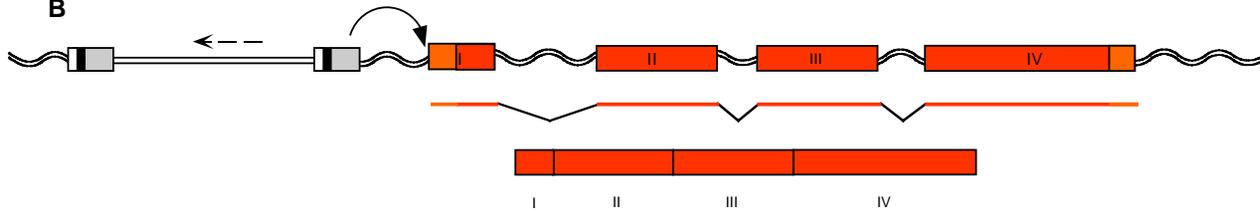


A



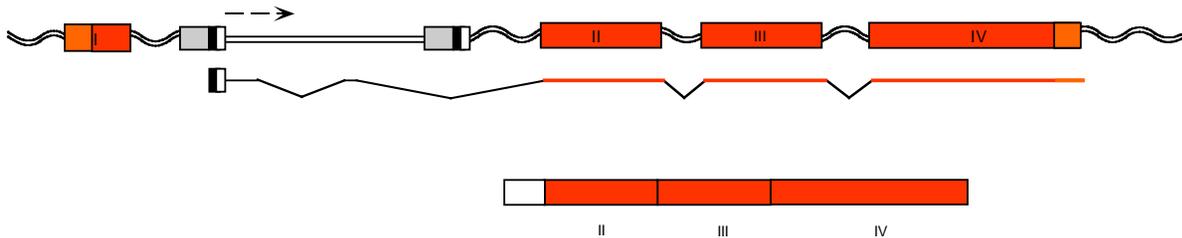
Promoter Insertion

B



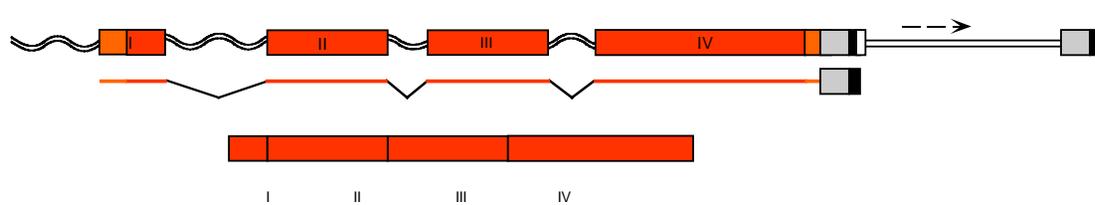
Enhancer Insertion

C



Leader Insertion

D



Terminator Insertion

ENHANCER INSERTION IS THE MOST COMMON MECHANISM

1. Flexibility of site of integration

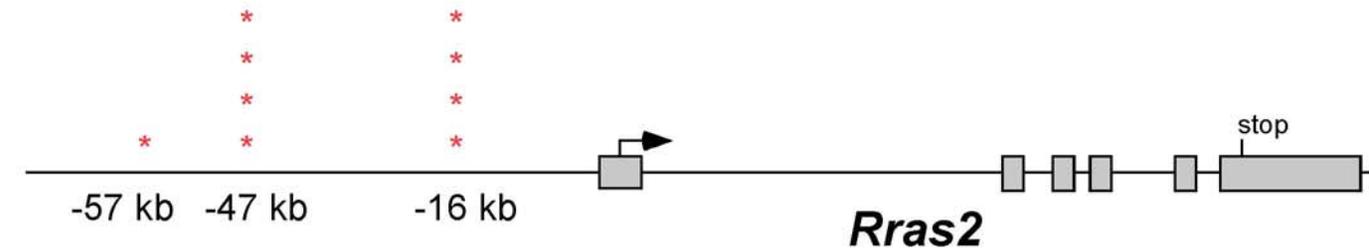
Orientation of provirus does not matter

Distance from gene can be as much as 100 kb

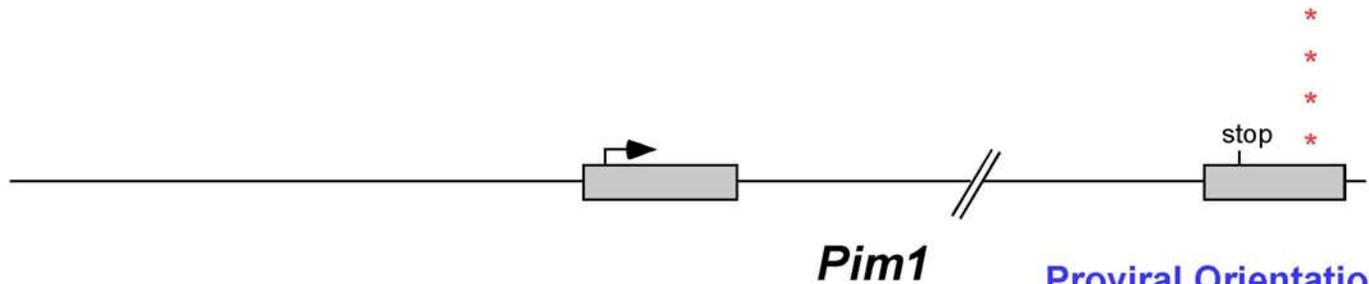
2. Single insertion can activate an oncogene

3. Changes caused by insertion can co-operate with other changes to lead to tumor formation

SITE CLUSTERING AND ORIENTATION BIAS CAN EXIST *



Proviral Orientation - Inverse



Proviral Orientation - Same

* Kim, et al. J. Virol. 77, 2003

HOW RANDOM IS INTEGRATION?

Does Transcription Influence Site Choice?

Schroeder, et al. Cell 110, 2002

- 69% of HIV integrations fell in transcription units
- Introns were favored over exons
- Orientation of provirus and gene did not correlate
- Regional hot spots found
- Integrations tended to fall in transcribed genes

Weidhass, et al. J. Virol. 74, 2000

- Assessed integration into a specific gene with different levels of expression
- Nontranscribed regions preferred

A final answer requires more experimentation

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CONSEQUENCES OF INSERTIONAL ACTIVATION

- 1. Upregulation or dysregulation of expression - too much of the product in the wrong cell type**
- 2. Altered (truncated) gene products missing regulatory sequences**
- 3. Loss of expression - Rare**

WHAT DOES THIS ALL MEAN?

1. Changes in gene expression may be quite common - $\gg 1\%$
2. Most are of no consequence to the cell
3. More rarely the cell acquires a selective growth or survival advantage
4. For proto-oncogenes, the effect is dominant
5. Assuming 200 proto-oncogenes and a target size of 1 kb, ~ 1 in 15,000 integrations may predispose to tumor development

LESSONS (CONTINUED)

6. From model systems, we know that a single integration is usually complemented by other changes
7. But the integration event is the key rate limiting step
8. Because inactivation of tumor suppressors usually requires two events, such changes occur more rarely, but have been observed

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VIRAL FACTORS

1. Virus replication increases chances of critical integration, but is not required

2. LTR sequences can influence tumor type

Friend MLV - erythroleukemia

Moloney MLV - thymic lymphoma

3. LTR sequences do not affect integration sites

4. LTR sequences affect expression in the target tissue

5. Env gene products can directly stimulate cell growth

HOST FACTORS

- 1. Same virus can activate different genes depending on the genetic background of the host**

Different *wnt* genes and MMTV in mammary tumors
myc vs *erbB* and ALV in chicken hematopoietic tumors

- 2. Age of host at the time of infection can change the integration pattern and disease**

Embryos vs newborns

- 3. Some oncogenes stimulate growth in a restricted set of cell types**

GENOTYPE CAN AFFECT COMMON INTEGRATION SITES

1. Different strains have partially distinct common integration sites
2. Use of transgenics already expressing one oncogene has revealed co-operating genes
3. Use of knock-out mice reveals changes in targets of insertion

Cdkn2a null mice - *Myc* the same, *Tpl2* not targeted, new CIS

p27Kip null mice - *Myc* overrepresented, new CIS

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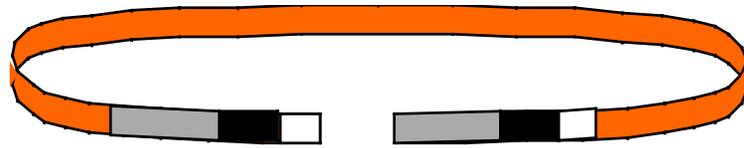
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CONCLUSIONS

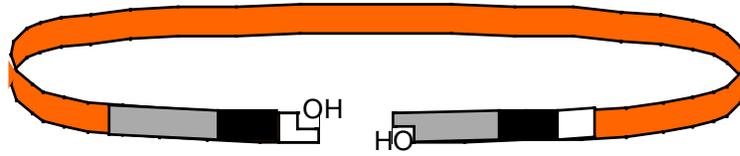
- 1. Integration is an inevitable consequence of retrovirus-based gene therapy**
- 2. We have yet to learn how to target integration**
- 3. Insertional mutagenesis and proto-oncogene activation is almost bound to occur**

WHAT CAN BE DONE?

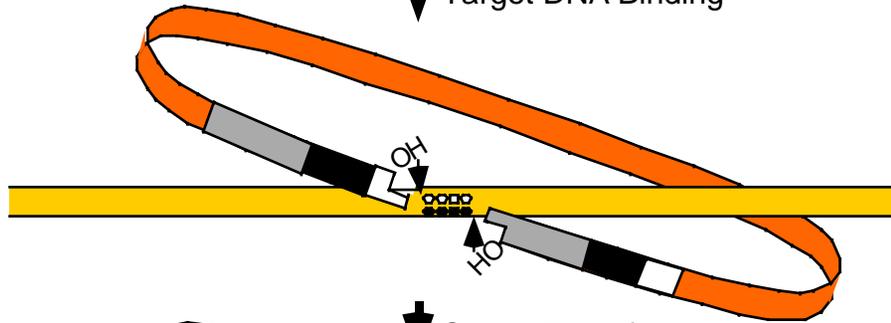
1. **Keep the number of infected cells to the absolute minimum needed**
2. **Avoid LTRs selected for high leukemogenicity**
3. **Use a highly cell-type specific promoter, not an LTR**
4. **Develop better mouse models to test viral parameters**
5. **Consider alternative vector designs**
 - Lentivirus-based
 - SIN and insulators



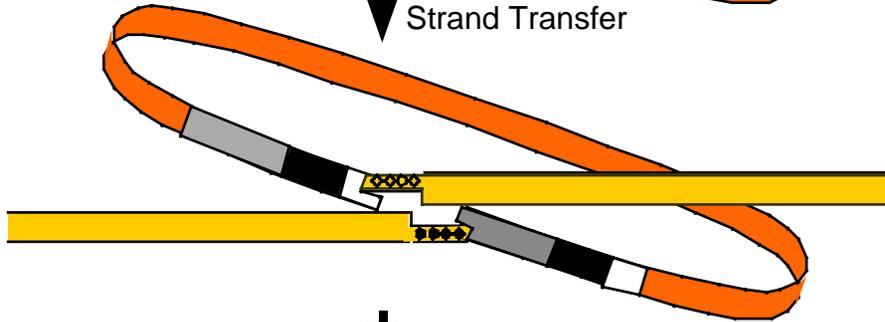
3' End Cleavage



Target DNA Binding



Strand Transfer



Gap Repair

