Alternative Mechanisms of Retroviral Mutagenesis

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Recombinant DNA Advisory Committee December 9, 2010

Historical: Retrovirus-induced Leukemias

Retrovirus
IP

Newborn

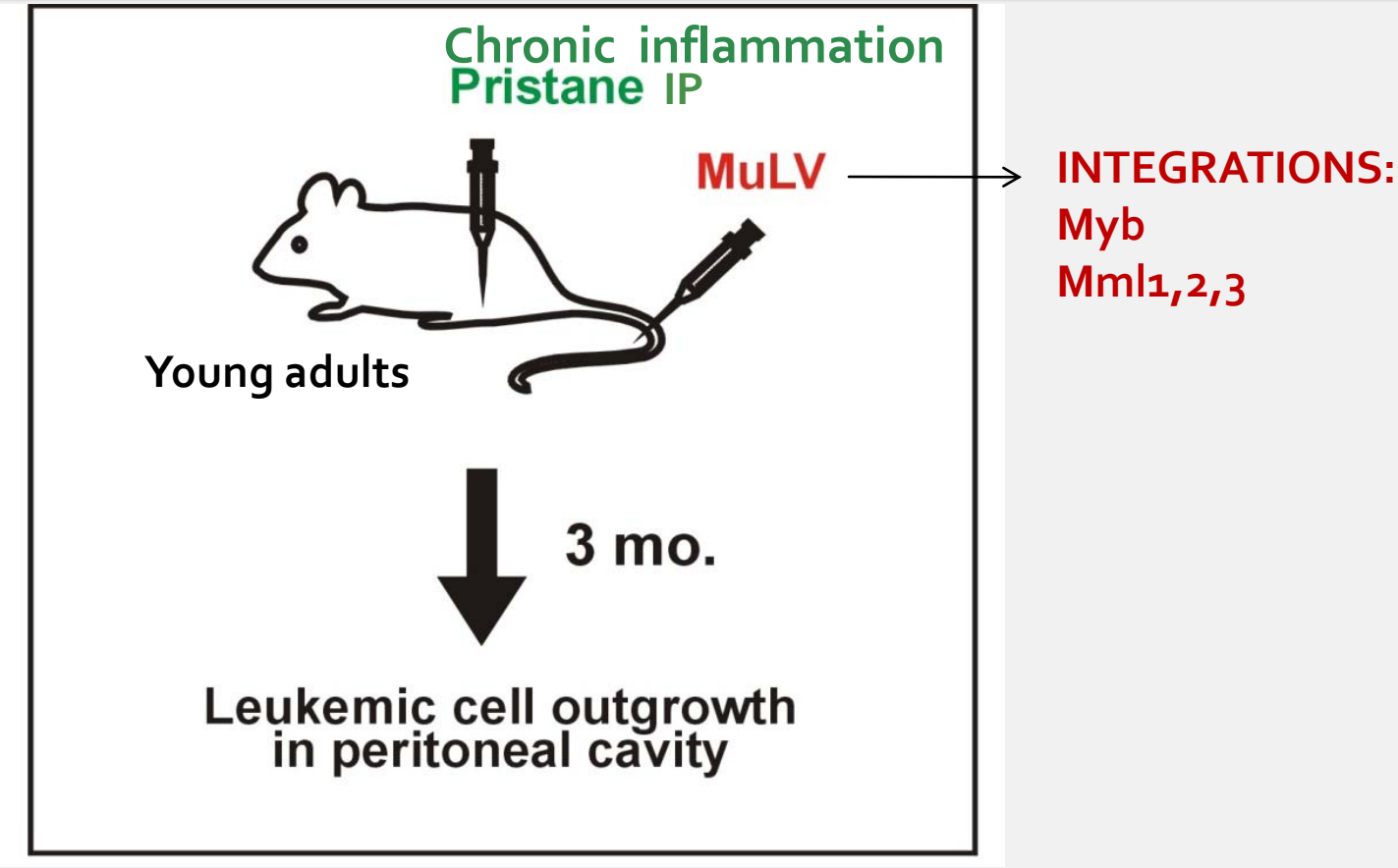
3-12 mo

Leukemia

Genetic mechanisms for leukemia development involve collaborations between

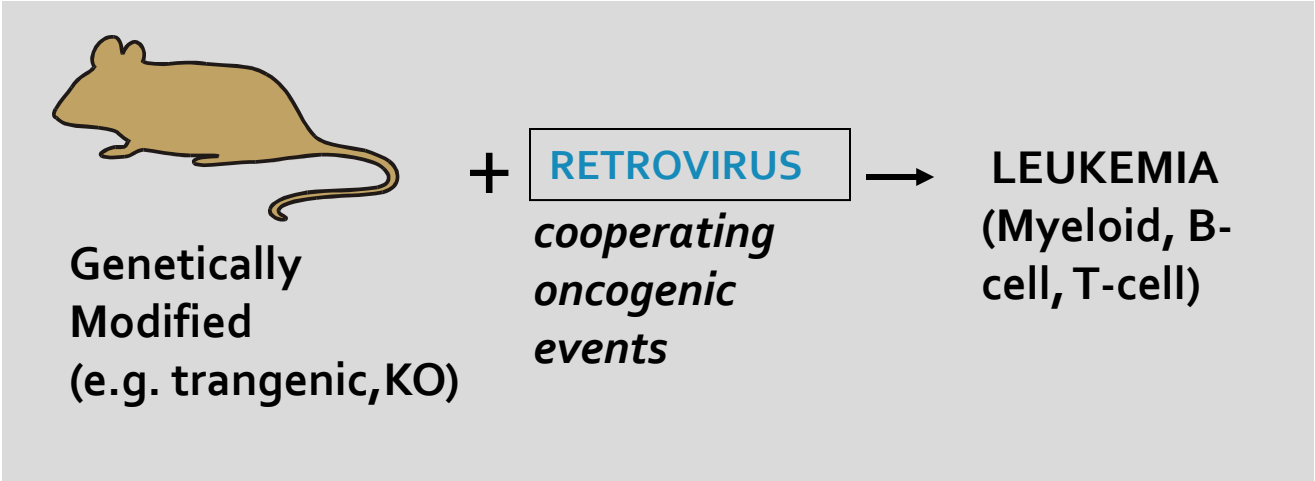
1. Multiple virus integration events
2. Non-viral related mutations

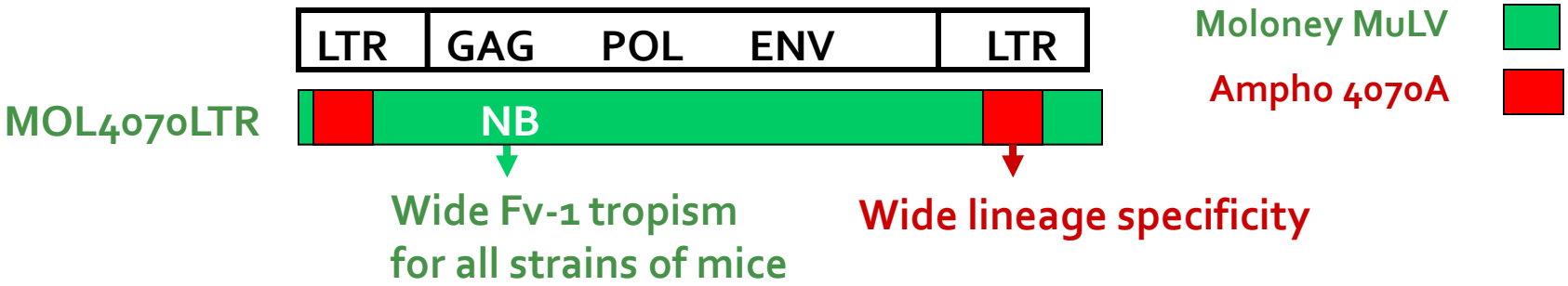
Acute Myeloid Leukemia Model Involving Insertional Mutagenesis and Inflammation



Wolff et al, J Immunol. 141:688,1988 ; Wolff and Nason-Burchenal, Curr. Topics in Immunol. 149:79,1989; Koller et al Virology 224:224, 1996; Haviernik et al. J .Gen .Virol.83:819, 2002

Random Mutagenic Approaches in Mice That are Genetically Engineered To Be Susceptible to Leukemia





Wolff et al. J.Virol. *2003*

Alternative Mechanisms of Mutagenesis

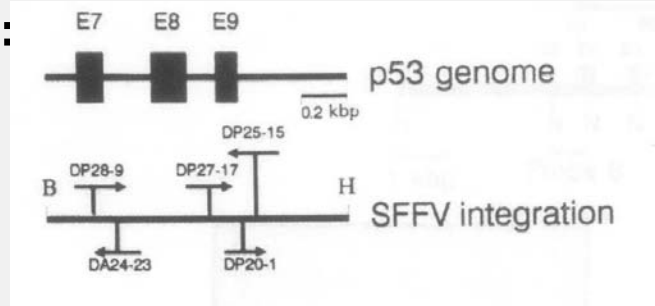
- **Gene inactivation or loss of expression**
  - **Disruption of coding region (p53 and Nf-1)**
  - Alternative splicing
  - Activation of miRNA

- **Gene activation or change in protein function**
  - Truncation of mRNAs or protein
  - Removal of miRNA binding sites
  - Long distance epigenetic influences

Inactivation of Tumor Suppressors-p53 Friend MLV-induced Erythroleukemias

- p53, a gene involved in cell cycle arrest and apoptosis is commonly inactivated by mutation in human cancers

- Retroviral insertions within coding region of p53 in retroviral induced murine erythroleukemias in wt mice resulted in:
  - no RNA and protein expression
  - aberrant p53 mRNA expression



- Proposed mechanisms:
  1) Early termination by polyadenylation signals contained in the virus

  or 2) Formation of fusion transcripts containing viral sequences and p53

  Reduction to homozygosity following virus insertion

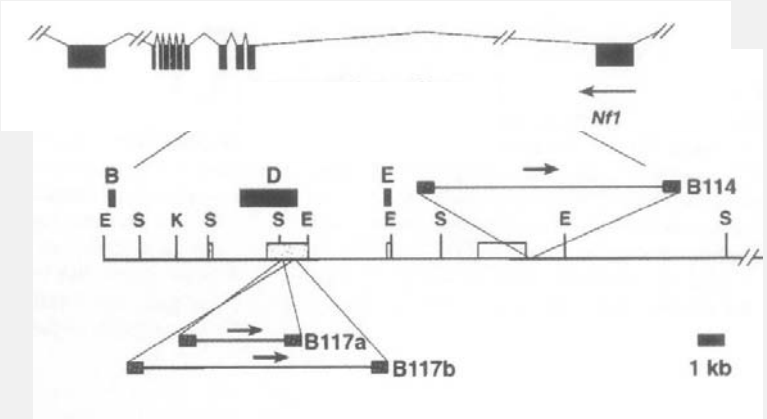
Ben-David et al 1988, 1990

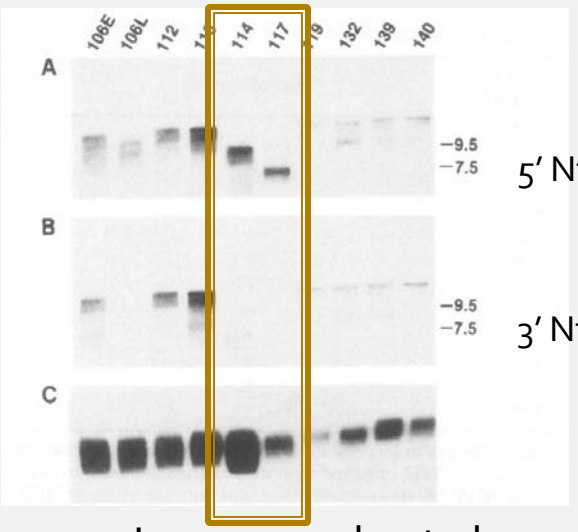
Inactivation of Tumor Suppressors Neurofibromatosis 1 gene (Nf1)

- NF1 mutations in man result in NF1 syndrome which includes benign subcutaneous neurofibromas and susceptibility to malignancies

- Insertions were found in recombinant inbred BXH-2 mice that have spontaneous retroviral-induced myeloid leukemia due to a lifelong viremia.

- Integrated virus in 1 or 2 alleles giving rise to small transcripts and either no protein or aberrant proteins





5′ Nfi probe

3′ Nfi probe

Largaespada et al.1995, 1996

A large retrovirus mutagenesis screen by Anton Berns' group led to candidate tumor suppressor genes hit by retrovirus integration

- Large-scale mutagenesis in $p19^{ARF}$ and p53-deficient mice

- Postulated: multiple intragenic insertions within both alleles of a gene is more likely for tumor suppressors

- Identified a large number of known or candidate tumor suppressors with more hits per tumor than expected by chance

Uren et al. Cell 133: 727, 2008

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  - Disruption of coding region
  - **Alternative splicing (Ikaros)**
  - Activation of miRNA

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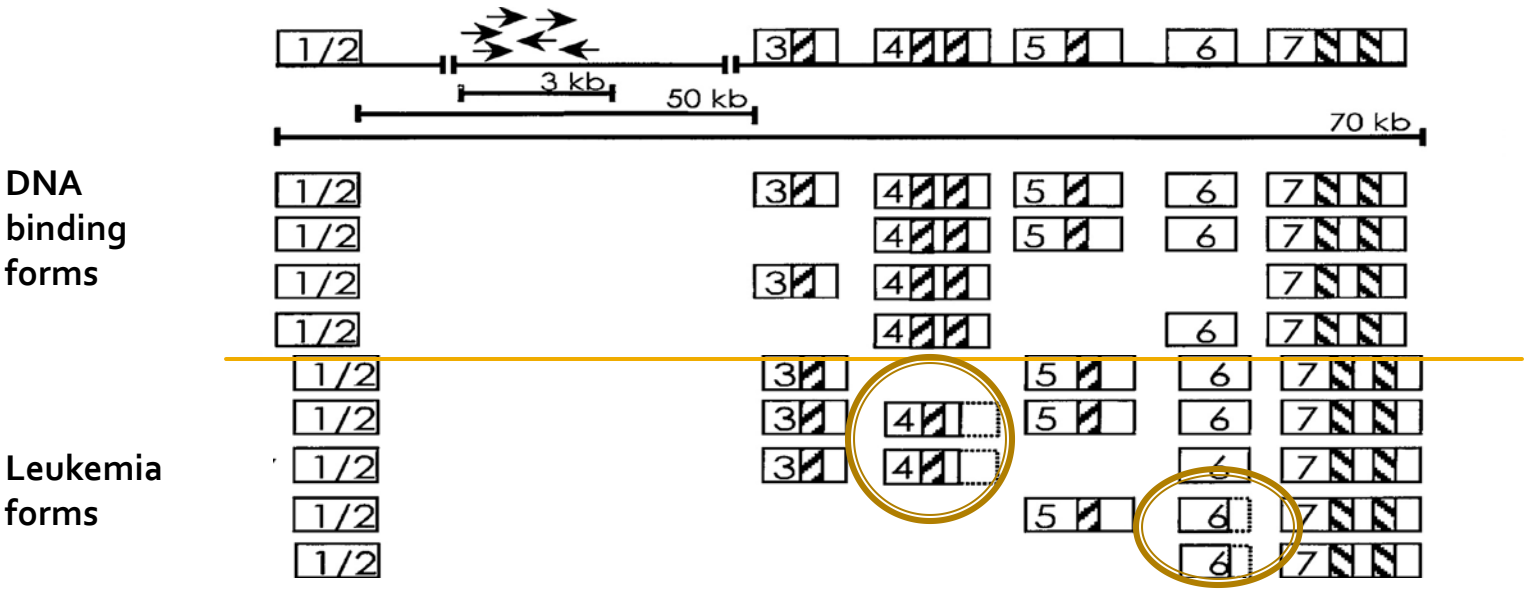
  - Removal of miRNA binding sites
  - Long distance epigenetic influences

Retrovirus-induced Aberrant Isoform Selection

Two groups have found integrations in intron 3 of Ikaros that results in skewed isoform selection:

- MLV integration as a cooperating event in intracellular Notch domain-induced T cell leukmogenesis (Beverly and Capobianco Cancer Cell 3:551, 2003)
- MOL4070LTR mutagenesis in Mx1-Cre, Kras^{G12D}mice (Dail et al. PNAS 107:5106, 2010)



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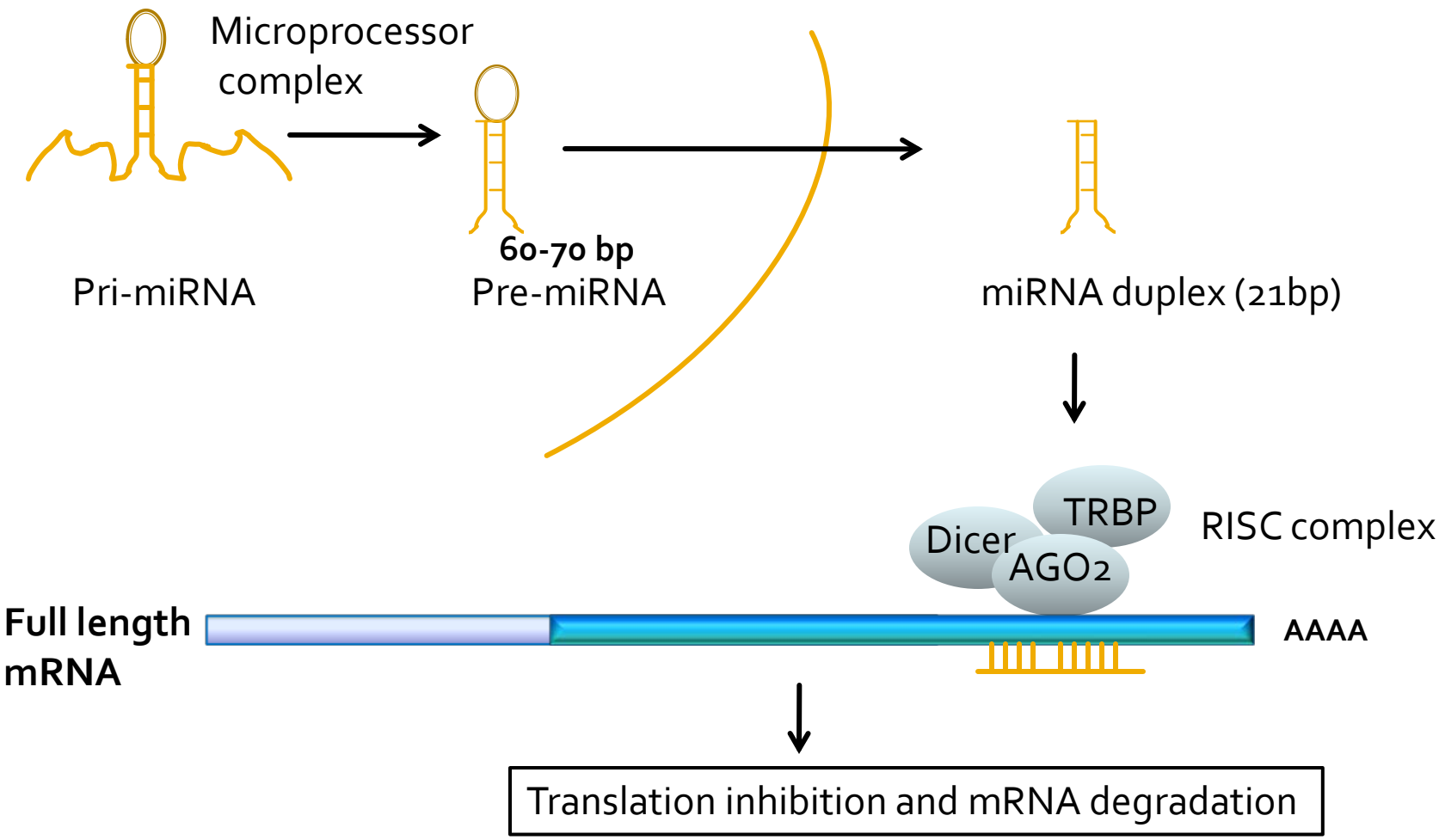
miRNAs

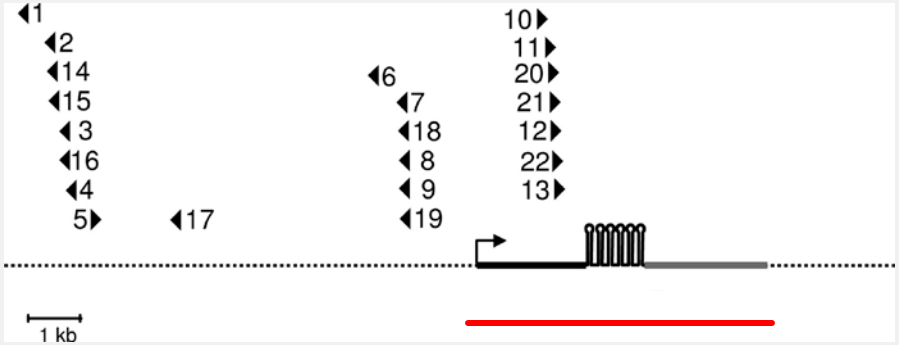

Microprocessor complex
Pri-miRNA
60-70 bp
Pre-miRNA
miRNA duplex (21bp)
TRBP
Dicer
AGO2
RISC complex
Full length mRNA
AAAA
Translation inhibition and mRNA degradation

Biologically Relevant miRNAs in Hematopoiesis that Negatively Regulate Genes

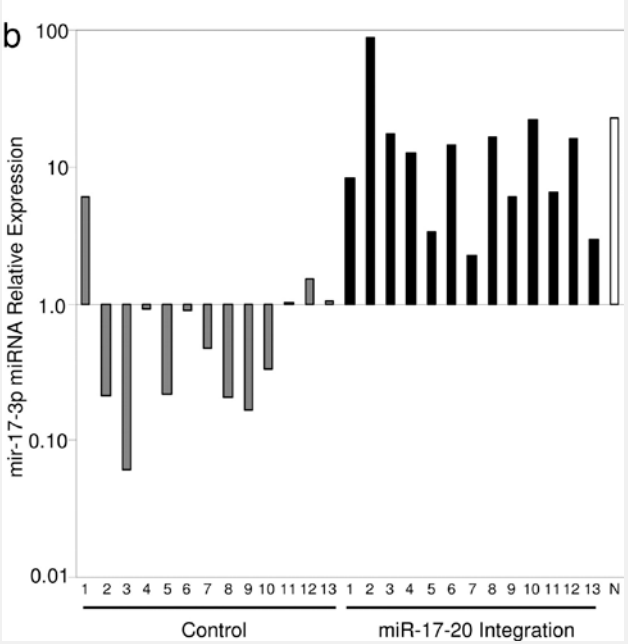
miRNA	Hematopoietic Lineage	Hematopoietic Malignancy	Relevant Targets
miR-223	Myelocytes	ALL, CLL	*NFI-A, E2F1, MEF2c*
miR-181	Lymphocytes	B-CLL	*Bcl2, CD69, TCR AID, SHP-2, PTPN22, Tcl1, PLAG1*
miR-150	Lymphocytes, MKs	CLL	*MYB*
miR-155	Lymphocytes, Myelocytes	CLL, AML, Lymphoma	*SHIP1, C/EBPβ, AID, SOCS1, PU.1, Cepbb, Csfr1, HIF , TAB2*
miR-17 92	Lymphocytes	CML, Lymphoma	*Bim, E2F1, PTEN*

Navarro and Leiberman, 2010

Activation of mir-17-92 Cistron in Mouse T Lymphomas Induced Retrovirus SL3-3.



mir-17-92 cistron



Matthias Wabl lab
Wang C L et al. PNAS 2006;103:18680

Mir-17-92 was shown to accelerate tumors in a c-myc-induced B-cell lymphoma model

He et al Nature 2005 435:8282

Retrovirus Insertions in the miR-106a Cistron and miR29/miR 29b1 Locus

- Eric Rassart's and Matthias Wabl's groups found in RadLV and Sl3-3 MLV-induced lymphomas integrations 5' to the mir106a -363 cluster with overexpression of miRNAs

Lum et al., Retrovirology, 4:5, 2007
Landais et al.,Cancer Res.67:5699, 2007

- Peter Aplan's group using MOL4070LTR in NUP98-HOXD13 transgenic mice found integrations near the miR29/miR29b1 locus (chr 6qA3.3) and demonstrated overexpression.

Slape et al, Cancer Res 67:5148, 2007

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  - Removal of miRNA binding sites
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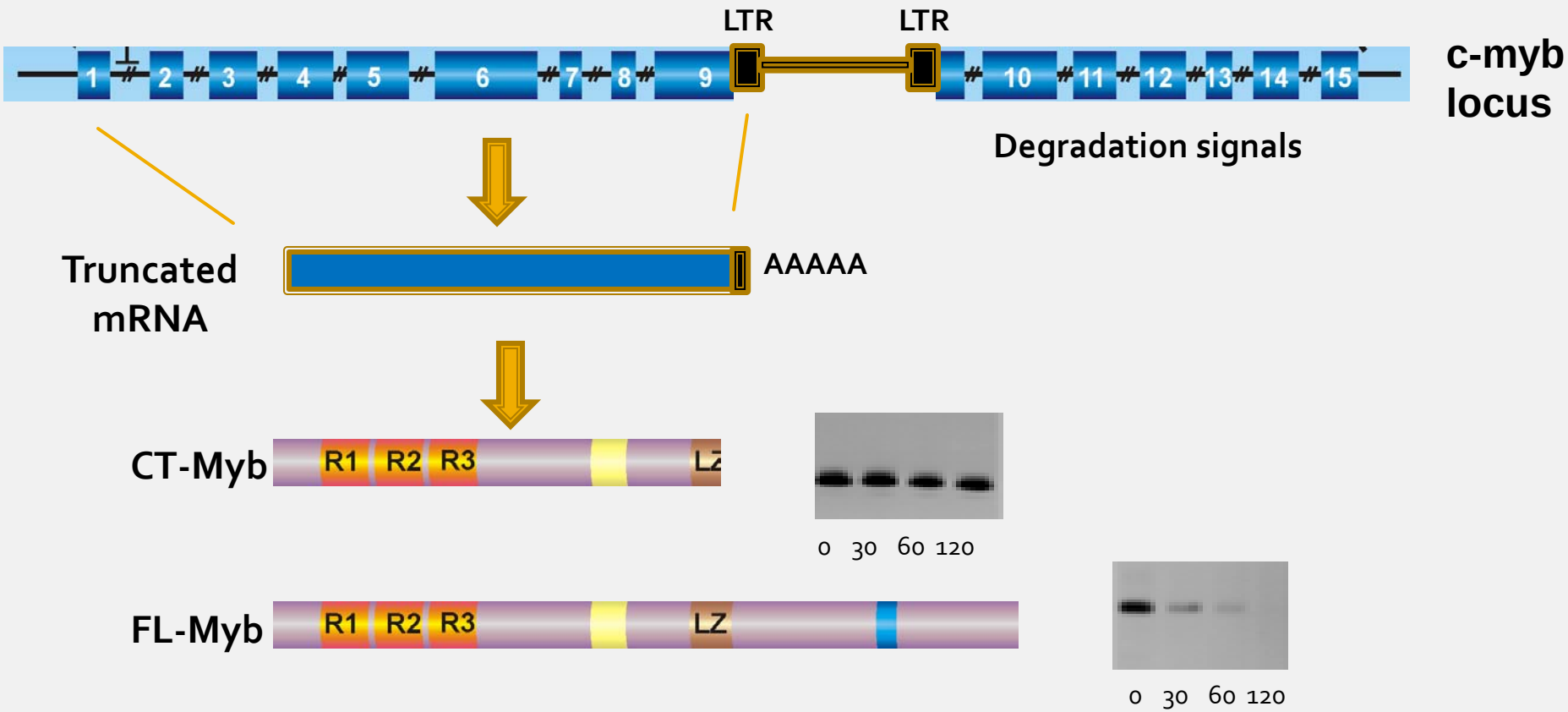
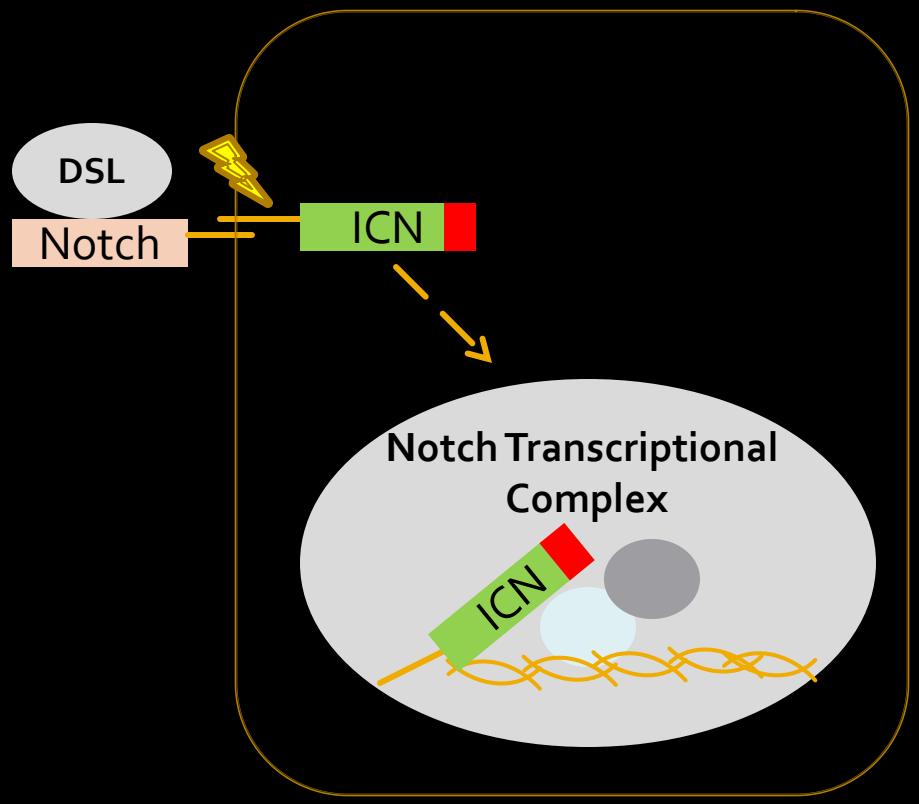
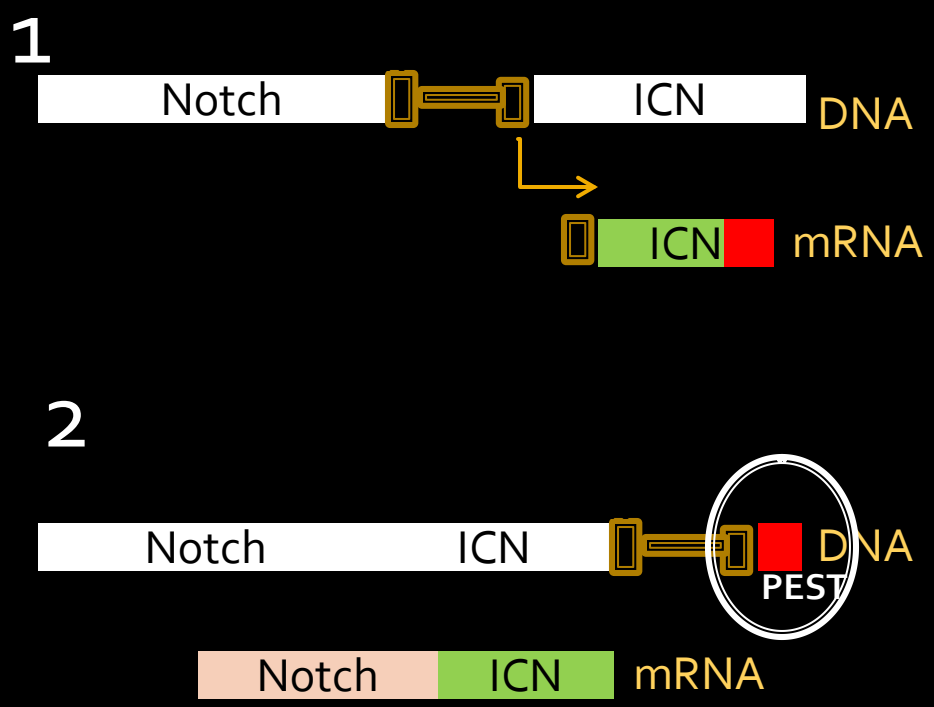
Activation of Murine c-Myb by Integration Within the Coding Region-Induces Stability



Bies et al. Oncogene 14:203, 1997; Bies et al. Oncogene 19:2846, 2000

Two Types of Mutations of the Notch1 gene Lymphomas Induced by Mo-MuLV Proviral Integrations in *c-myc* Transgenic Mice





Hoemann et al. Mol. Cell. Biol. 20:3831, 2000

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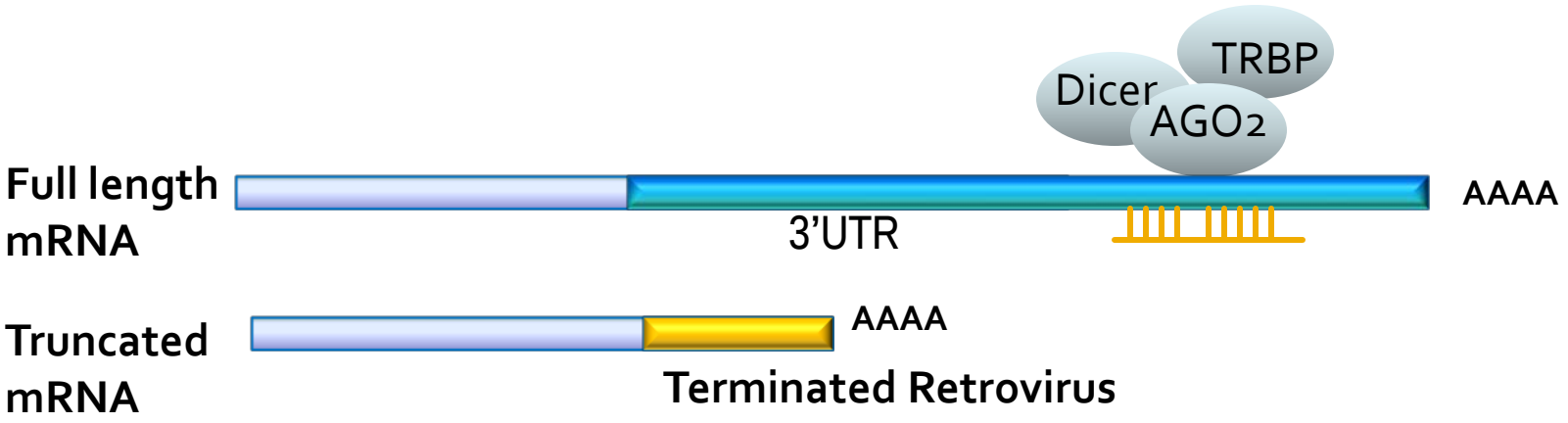
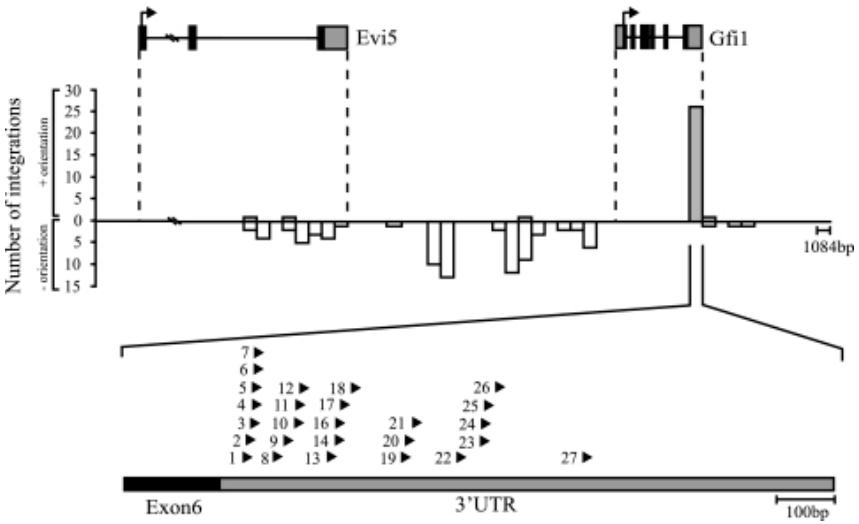
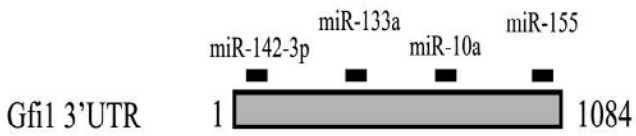
Activation of Murine Gfi-1 by Removal of Micro RNA Target Sites in SL3-3 Lymphomas







Dabrowska, et al J Virol 83:8051, 2009

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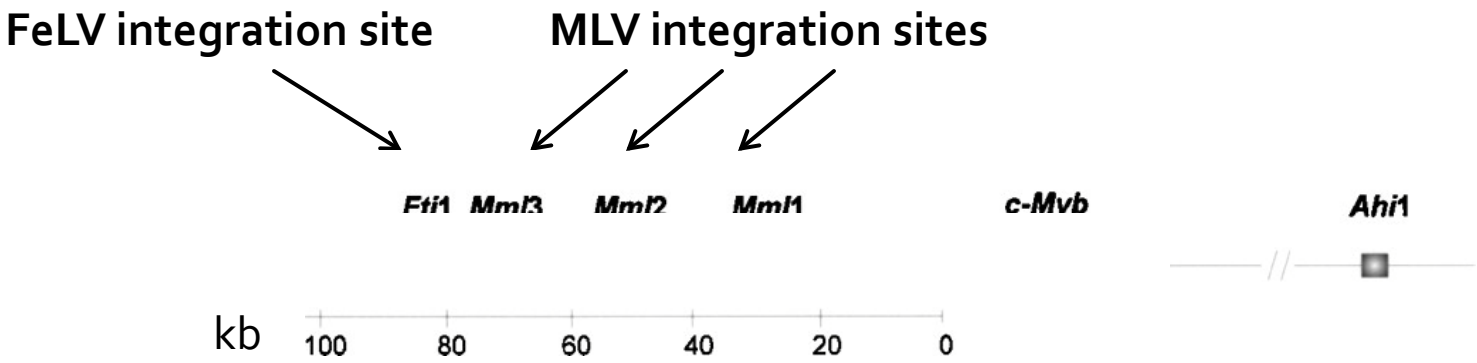
Long Distance Epigenetic Influences of Common Integration Sites Upstream of c-Myb



- Increases in histone methylation marks normally associated with transcription activation (H3K4me1, H3K4me3)
- Mml1, 2 ,3 are found near potential CTCF binding sites
- Alterations in DNA looping between upstream sites and c-myb promoter

Barr et al. Mamm. Genome 10:556-559, 1999
Haviernik et al. J. Gen. Virol. 83:819, 2002
Recent unpublished data from the Wolff lab

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*Peter Haviernik

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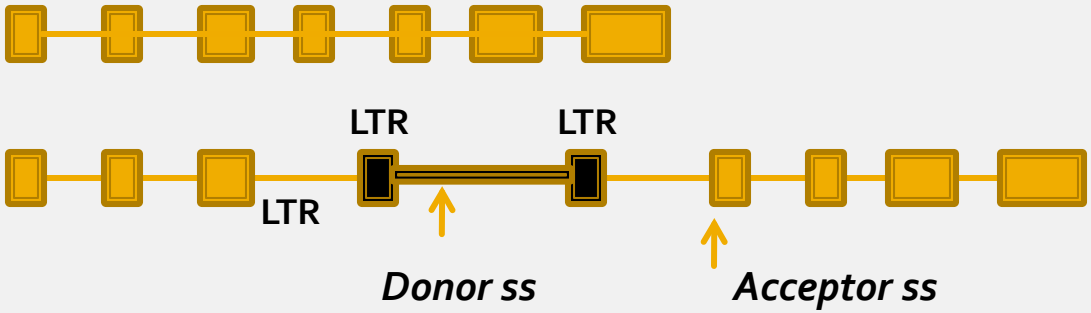
- NHGRI
  Paul Liu
- University of Glasgow
  James Neil
- UCSF
  Kevin Shannon
- Hiroshima University
  Hiroaki Honda

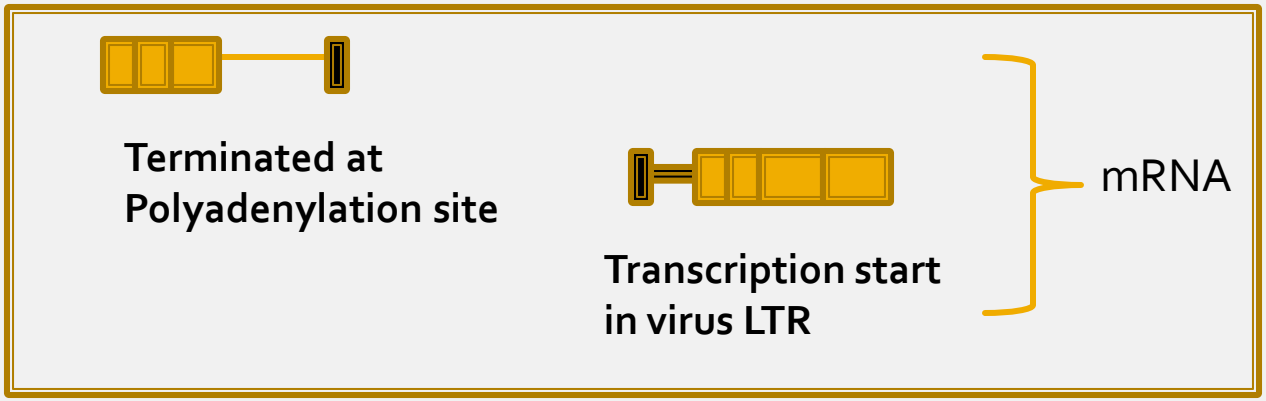
Explanations for truncated mRNAs

Long Terminal Repeat (LTR)

Transcription initiation

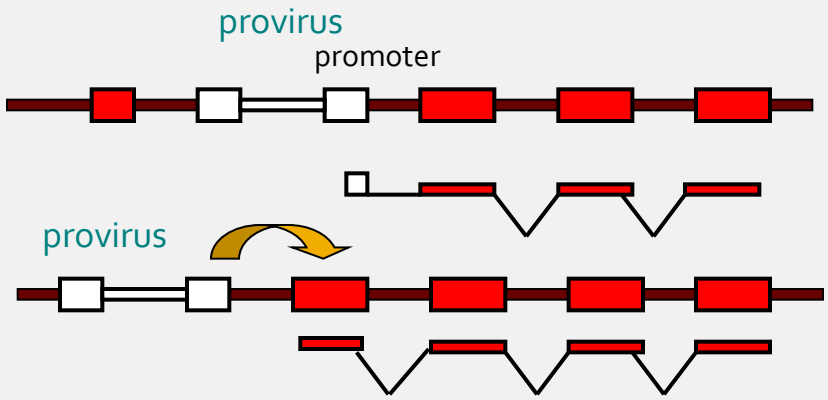
Polyadenylation site



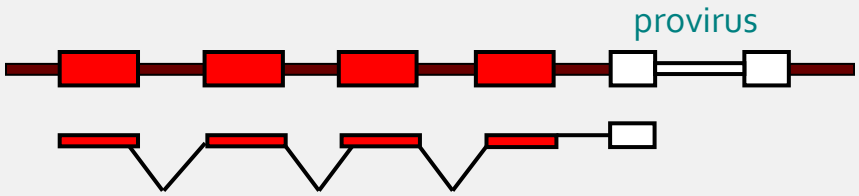


Most Commonly Known Mechanisms of Gene Activation by Retrovirus Insertion

Integrated at the 5' end of gene---**promoter and or enhancer activation**



Integrated at the 3' end of gene---**enhancer activation**



Insertional Mutagenesis in Genetically Engineered Mice Using MOL4070LTR Virus

Knockout	
Mx-Cre, $Nf1^{fl/fl}$ (MPD, AML)	Nature 61:411, 2009
p15Ink4b (AML)	Blood 116:979, 2010
Activation of a mutant allele	
Mx-Cre, $Kras^{LSL\text{-}G12D}$ (T-ALL)	PNAS 107:5106, 2010
Transgenic	
NUP98-HOX13 (MDS,AML)	Cancer Res. 67:5148, 2007
p210BCR/ABL (ALL)	Oncogene 27:3465-, 2008
CALM-AF10 (B-Cell)	Blood 115:1194, 2010
E2A-HLF (B-Cell)	Oncogene 29, 1963, 2010