LMO1/2

- Initially identified at the site of 11p15 (LMO1) or 11p13 (LMO2) chromosomal translocation in T-ALL patients
- Chromosomal translocations juxtapose TCRA/D to LMO1/2
- LMO3 (12p12), LMO4 (1p22) genes not known to be involved in T-ALL.
- Initially named rhombotin (RBTN) 1 and 2, or TTG1 and 2.
- Encode small 158 aa proteins that are 58 % identical
- Mouse and human LMO2 are 99% identical
- LMO = LIM only; contain two zinc-binding LIM domains
  - LIM =cysteine-rich domain originally identified in lin11, ISL3, mec-3
  - LIM + homeodomain (LIM-HD), LIM + protein kinase (LIM-PK), LIM domain only (LMO)

LMO1/2—normal function

- LMO2 – ubiquitous, low level expression
- LMO1 – expressed in rhombomere, pancreatic islet cells, testis
- LMO2 -/- mice embryonic lethal due to lack of yolk sac erythropoiesis
- LMO2 -/- ES cells do not contribute to adult hematopoiesis; suggests LMO2 required for adult hematopoiesis
- An oligomeric complex containing LMO2, LDB1, SCL, E2A, and GATA1 can be detected in erythroid cells.
- LMO2 is thought to serve as a “bridge” in this complex; E2A/SCL and GATA1 directly contact DNA.
- LMO1 also forms a complex with E2A and SCL

E2A

- Immunoglobulin enhancer binding protein.
- "Founding member" of the basic domain, helix-loop-helix (bHLH) family of transcription factors.
- Two isoforms (E12, E47), two related genes (HEB, ITF2), together form a class of "E-proteins".
- Binds specific DNA recognition sequence, CANNTG, as a heterodimer with other bHLH proteins or as a homodimer.
- E2A (both isoforms) and HEB expressed in thymus and critical for normal lymphocyte development.
- Relevant target genes: IgH, TCRB, CD4, and p21.

SCL (TCL5, TAL1)

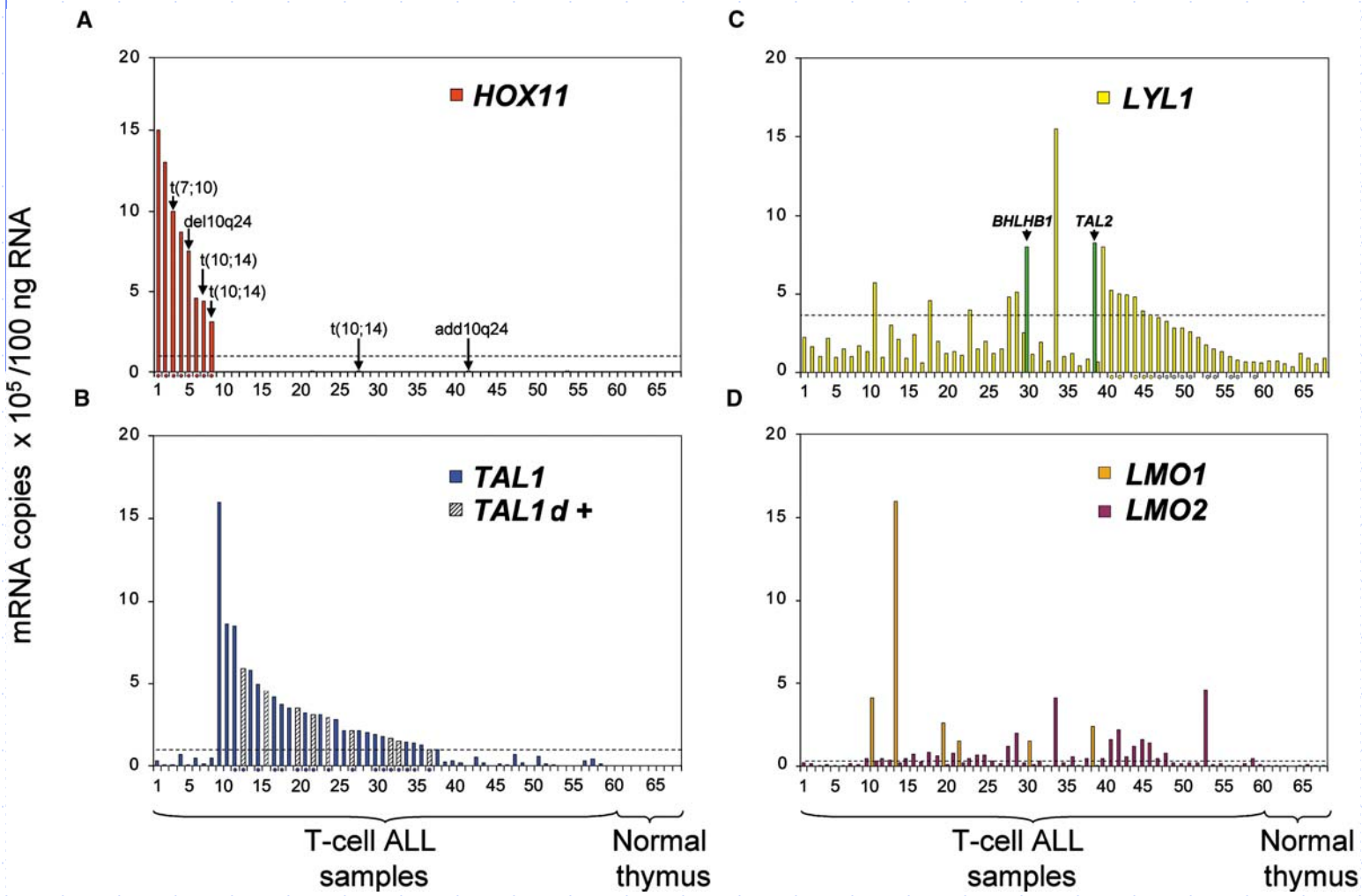
- Initially cloned at the site of a t(1;14) translocation breakpoint present in a stem cell leukemia.
- Encodes a bHLH protein that binds E2A and LMO proteins, and is expressed primarily in bone marrow and endothelial cells.
- Not normally expressed in thymocytes, but over 60% of T-ALL express *SCL* mRNA.
- Activated by either chromosomal translocation or site-specific interstitial deletion in 30% of T-ALL
- Site-specific interstitial deletion places *SCL* coding sequences under control of *SIL* regulatory elements.

LMO1/2 and T-cell leukemia

- Ectopic expression of LMO1/2 in thymocytes caused by chromosomal translocation (LMO1/2 expression low or absent in normal thymocytes). Ectopic expression caused by TCR regulatory elements or loss of LMO P1?
- Additional mechanisms for LMO1/2 activation likely; many T-ALL patients lacking translocations involving LMO1/2 show high levels of LMO1 or LMO2 expression.
- MSH2 deficient mice develop T-cell leukemia; the leukemic cells have activated both LMO2 and SCL.
- Gene expression profiling separates T-ALL into 3 groups (HOX11 activation, SCL activation, and LYL1 activation; many of the leukemias in the SCL and LYL1 groups have activated LMO2.

Co-expression of SCL and LMO proteins in T-ALL samples

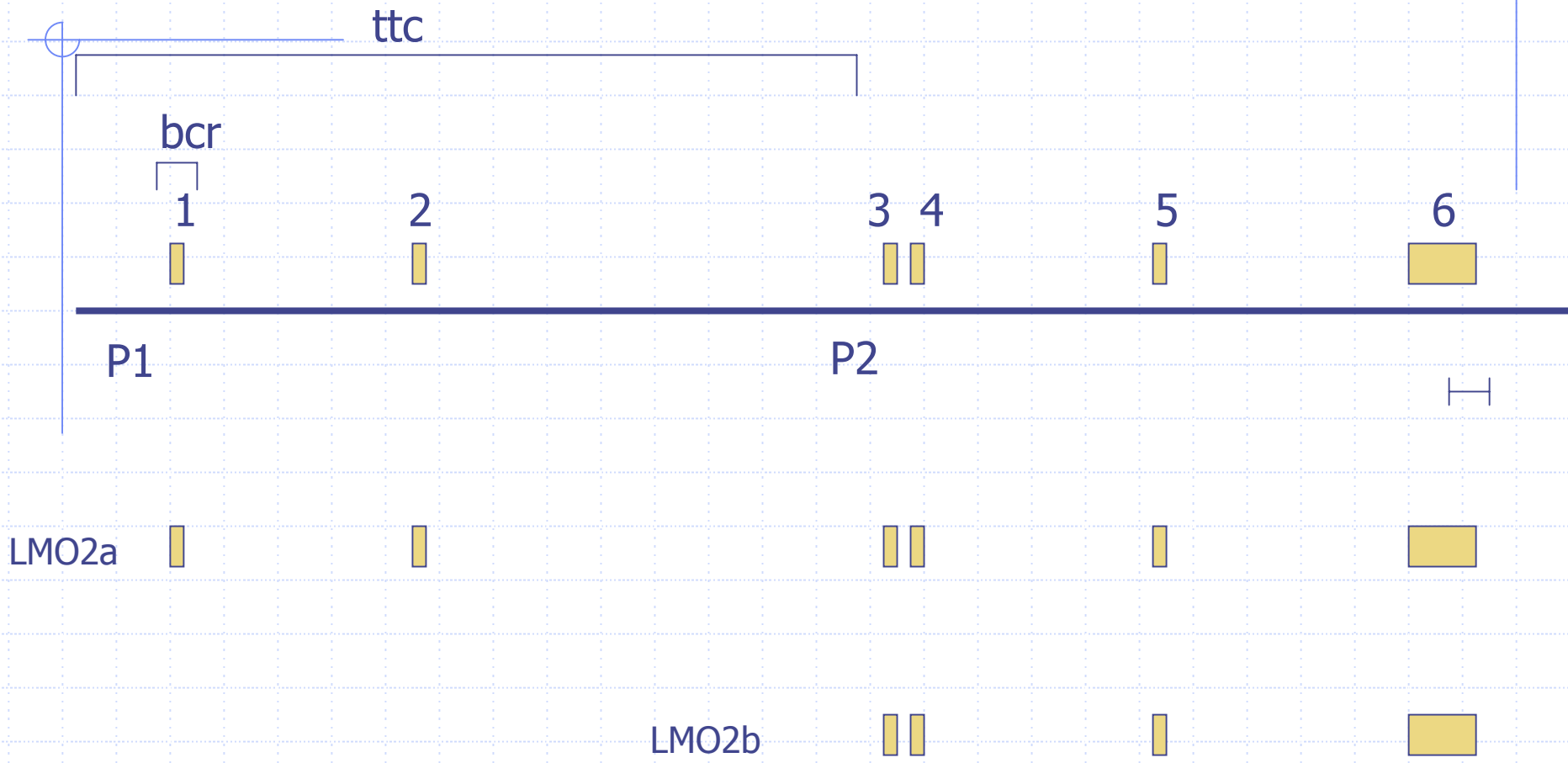
from Ferrando, et al., Cancer Cell 1:75-87, 2002



Clinical features of T-ALL with t(11;14)

- 8/103 consecutive childhood T-ALL patients
- Typical T-ALL features--male, adolescents, hyperleukocytosis, mediastinal mass, high Hgb, high LDH, CNS involvement
- CD4+/CD8+
- Most patients do not have additional karyotypic abnormalities, but 3/17 in one series had chrom 6 deletions [del(6)(q21q25), del(6)(q21), del(6)(q24)
- Not associated with B-lineage ALL

LMO2 genomic structure



*ORF contained within exons 4,5,and 6

T-cell ALL

Translocation	Genes involved	Protein domain	Frequency
t(1;14)(p33;q11)	*TCRδ; SCL(TAL1, TCL5)*	bHLH	25%* (α/β)
t(7;19)(q35;p13)	*TCRβ, LYL1*	bHLH	<1%
t(8;14)(q24;q11)	*TCRα, cMYC*	bHLH	2-5%
t(11;14)(p15;q11)	*TCRδ, LMO1*	LIM	1-2%
t(11;14)(p13;q11)	*TCRα/δ; LMO2*	LIM	5-10%
t(7;9)(q34;q34)	*TCRβ,TAN-1*	*notch* homolog	5%
t(10;14)(q24;q11)	*TCRα, HOX11*	Homeobox	5-10%
t(5;14)(q35;q32)	*HOX11L2, CTIP2*	Homeobox	20%**
t(11;19)(q23;p13.3)	*MLL(ALL1, HRX), ENL*	*trithorax* homolog	2%
t(10;11)(p13;q14)	*CALM, AF10*		10% (γ/δ)

*t(1;14); 2-5%; SIL-SCL; 15-25%
**based on a single small series

LMO1/2 T-ALL mouse models

- Lck-LMO1 tg mice – 20% T-ALL, mean 11 mos (McGuire et al, MCB 12:4186)
- MTH1-LMO2 tg mice – 11% T-ALL, mean 51 wks (Neale et al., Blood 86:3060)
- TCRB-LMO1 tg mice – 9% mean 12 mos (Fisch et al., Oncogene 7:2389)
- CD2-LMO2 tg mice – 72% mean 10 mos (Larson et al., Oncogene 9:3675)
- In general, mice presented with lethargy, diffuse lymphadenopathy, and tachypnea. Necropsy showed thymic effacement with lymphoblasts and widespread organ involvement.
- TCRB gene analysis showed monoclonal TCRB gene rearrangement. Immunophenotypes predominantly CD3+/4+/8+, both alpha/beta and gamma/delta; late onset disease in CD2-LMO2 mice was CD3+/B220+ with massive spenomegaly.
- Incomplete penetrance and late onset suggest need for cooperating events.

Interaction between LMO1 / 2 and SCL

- *LMO1 / 2* bind *SCL in vitro*.
- A subset of T-ALL patients have both an *LMO1* or *LMO2* translocation and a *SIL/SCL* rearrangement.
- A subset of T-ALL patients have activated both *LMO1* or 2 and *SCL*.
- *MSH2* KO mice develop T-cell leukemia/lymphoma; most of these express *SCL* and *LMO2*.
- 5/6 lck-LMO1 tg mice that developed T-ALL had activated the endogenous mouse SCL.

LMO1/2 T-ALL mouse models (cont.)

- Incomplete penetrance and late onset suggest need for cooperating events.
- Lck-LMO1 + SIL-SCL mice – 95% T-ALL at mean age 4 mos (Aplan et al., EMBO J 16:2408 , Chervinsky et al., MCB 19:5025).
- TCRB-LMO1 mice infected with MoMuLV developed leukemia 1 mos earlier than MoMuLV controls (4.5 v 5.5 mos)

Premalignant lesions in LMO2 transgenic mice

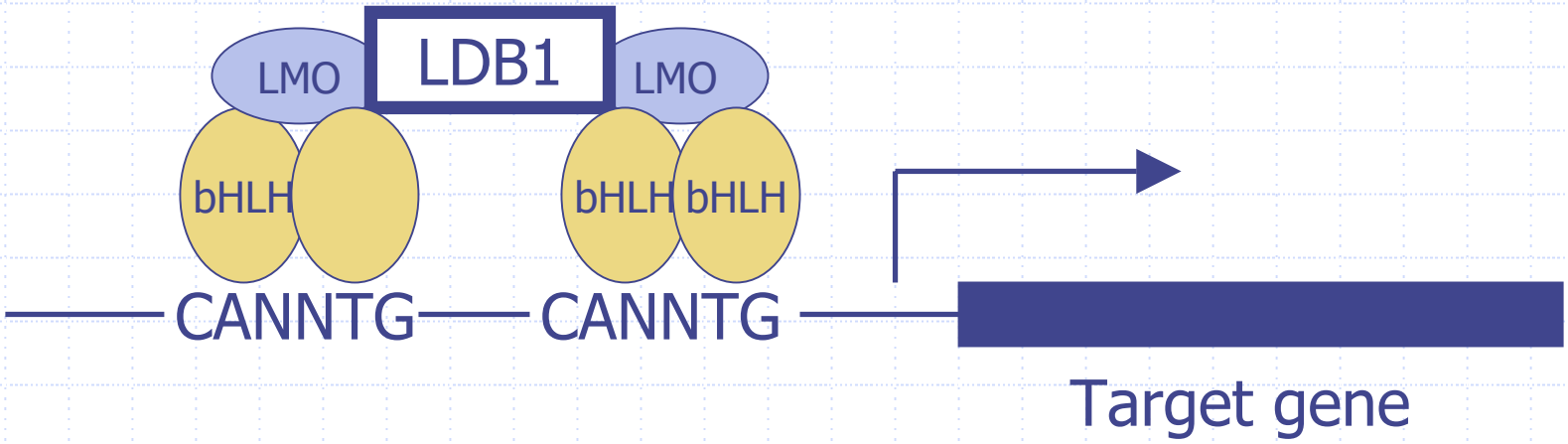
- Increased proportion of CD4-8- thymocytes (10-15%) at 4 mos of age.
- Oligoclonal TCRB gene rearrangements at 6 mos of age.
- These findings are accelerated (occur earlier) in SCL/LMO1 double transgenic mice.

Molecular mechanism(s) of LMO1/2 leukemogenicity

- Gene activation
- Dominant negative
- ? Angiogenesis--LMO2 highly expressed in lung tumor endothelium; LMO2 -/- ES cells form teratocarcinoma, but vessels exclusively of host origin

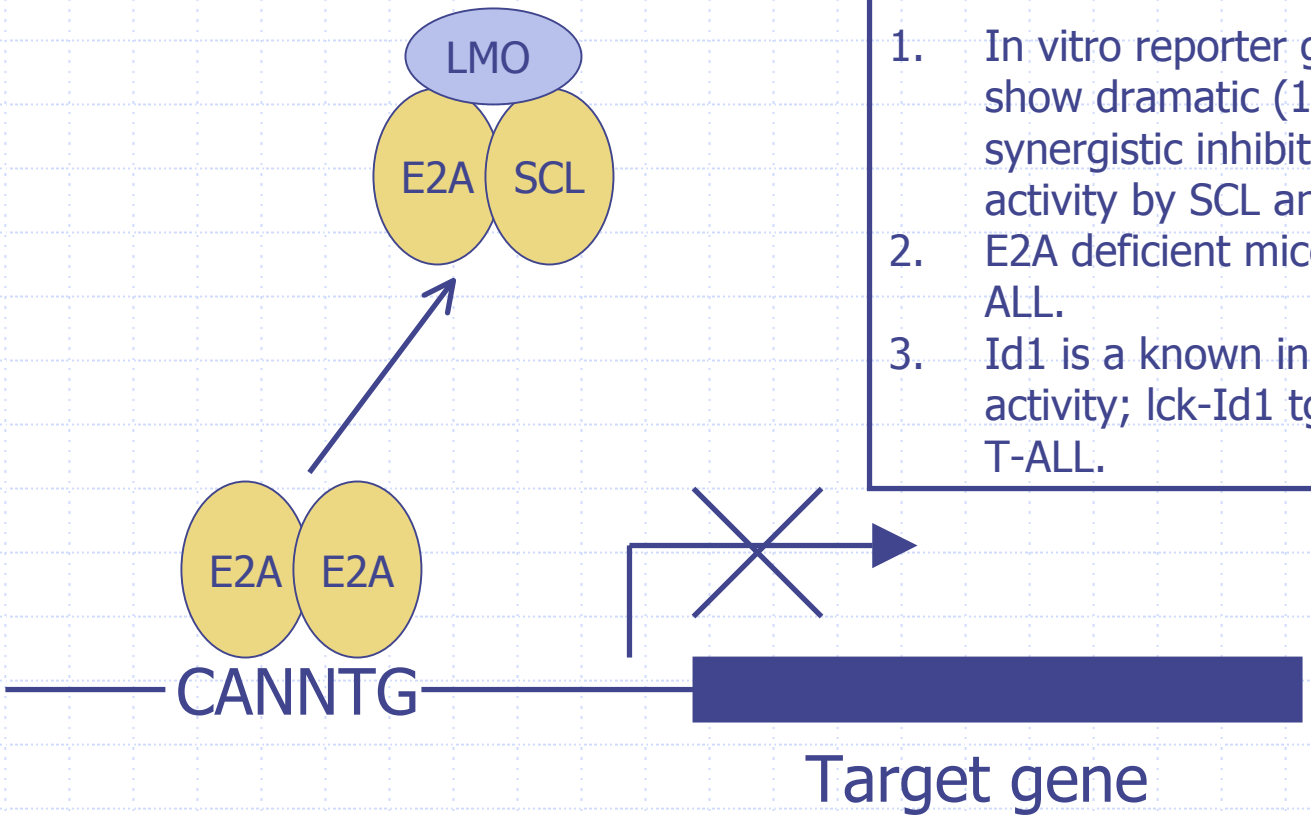
Models for LMO leukemogenicity

A. Abnormal activation complex



Models for LMO leukemogenicity

B. Dominant negative—sequestration of E-proteins



1. In vitro reporter gene assays show dramatic (1000-fold) synergistic inhibition of E2A activity by SCL and LMO1.
2. E2A deficient mice develop T-ALL.
3. Id1 is a known inhibitor of E2A activity; lck-Id1 tg mice develop T-ALL.

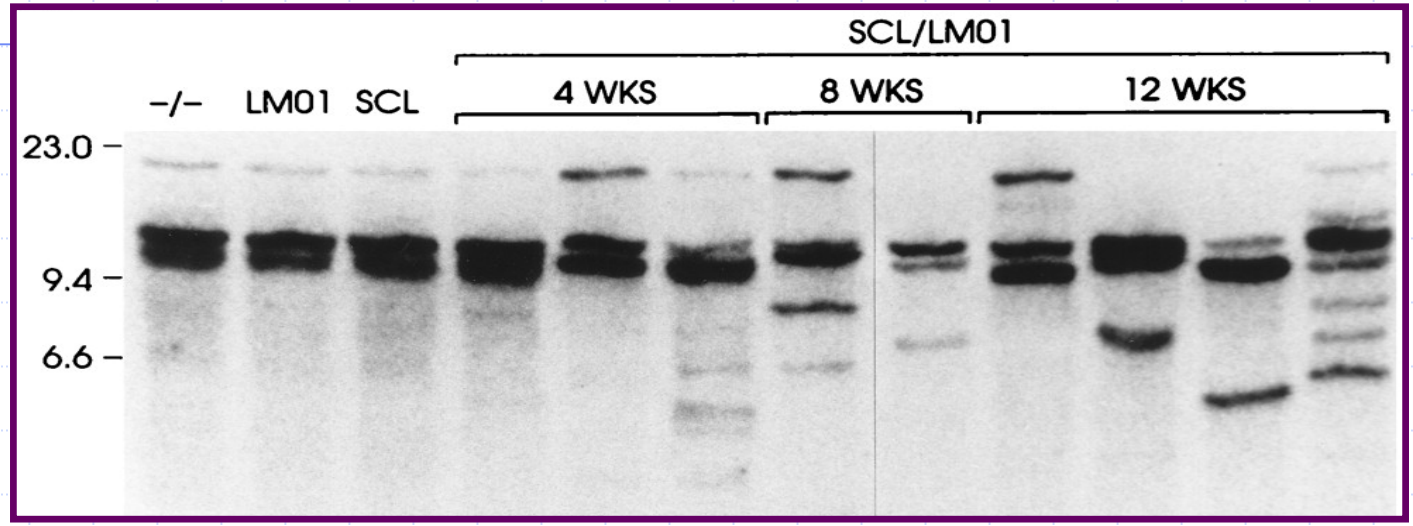
Are LMO1 and LMO2 interchangeable?

- Structurally similar, especially at *lim* domain
- Both LMO1 and LMO2 bind SCL/E2A *in vitro*
- Both leukemogenic when overexpressed in thymus
- Parallels with other gene families
  - L-myc as leukemogenic as c-myc if overexpressed in thymus
  - Hox replacement experiments

- Relevance—is it important to search for LMO1 integrations as well as LMO2 integrations?

SCL/LMO1 Mouse Model

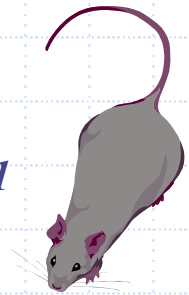
A. Clonal TCRβ gene rearrangements (4-12 wks).



B. Tumorigenicity in nude mice

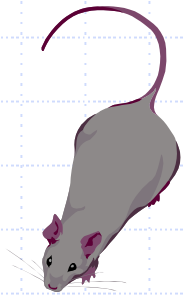
Cells injected	*Route*	*Tumors*	*Latency (wks)*
SCL/LMO1 leukemia	IP	11/11	3
Nl. thymus (12 wks)	IP	0/6	>32
SCL/LMO1 thymus (12 wks)	IP	6/6	3-7
Nl. thymus (8 wks)	IP	0/3	>32
SCL/LMO1 thymus (8 wks)	IP	1/5	19
Nl. thymus (4 wks)	IP	0/2	>32
SCL/LMO1 thymus (4 wks)	IP	0/6	>32

A single clone emerges from an oligoclonal population

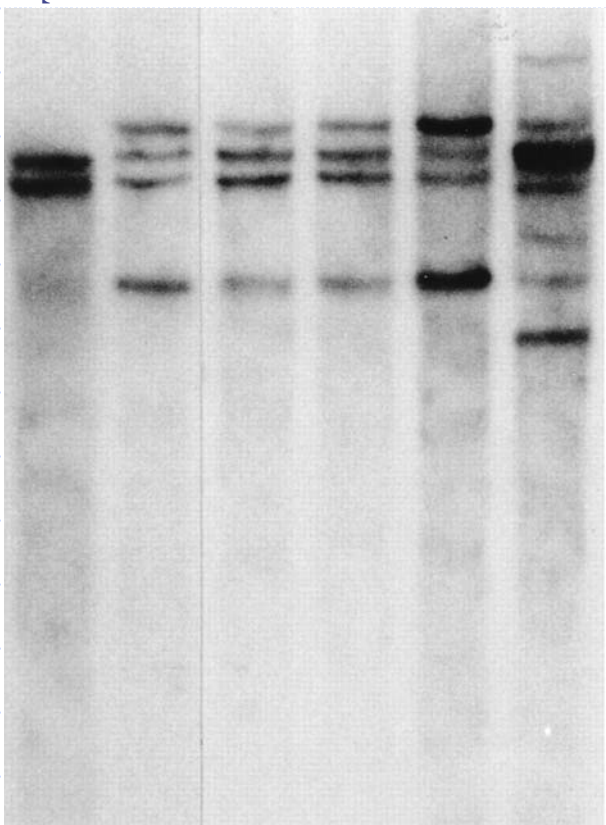


SCL/LMO1 **mouse**

Harvest thymocytes, inject I.P. into *nu/nu*



Harvest tumor cells from 4 distinct locations



Identify clonal TCRβ rearrangements by Southern blot

SCL/LMO1/scid mice

- *Scid* defect—DNAPKcs mutation, impaired ability to complete VDJ recombination, "leaky"
- Onset of malignancy markedly delayed, incomplete penetrance.
- All *SCL/LMO1/scid* tumors have in-frame, clonal, TCRβ gene rearrangments (and express pTα). Rare "leaky" *scid* thymocytes are preferred targets.
- ? Survival signal from TCRβ/pTα?

Are additional events required for LMO2 leukemogenicity?

- Many "leukemic" chromosomal translocations present in clinically healthy individuals.
- Bcr-abl, bcl2-IgH, TEL-AML1 (can be detected in neonatal screening cards 13 years before onset of clinical disease).
- Dδ2-LMO2 fusion were as common as $1/10^6$ thymocytes in one study.
- Suggests that the translocations may be necessary, not sufficient; additional mutations required.
- Are LMO2 activating insertions also not sufficient, but require additional mutations?