The Myc oncogene

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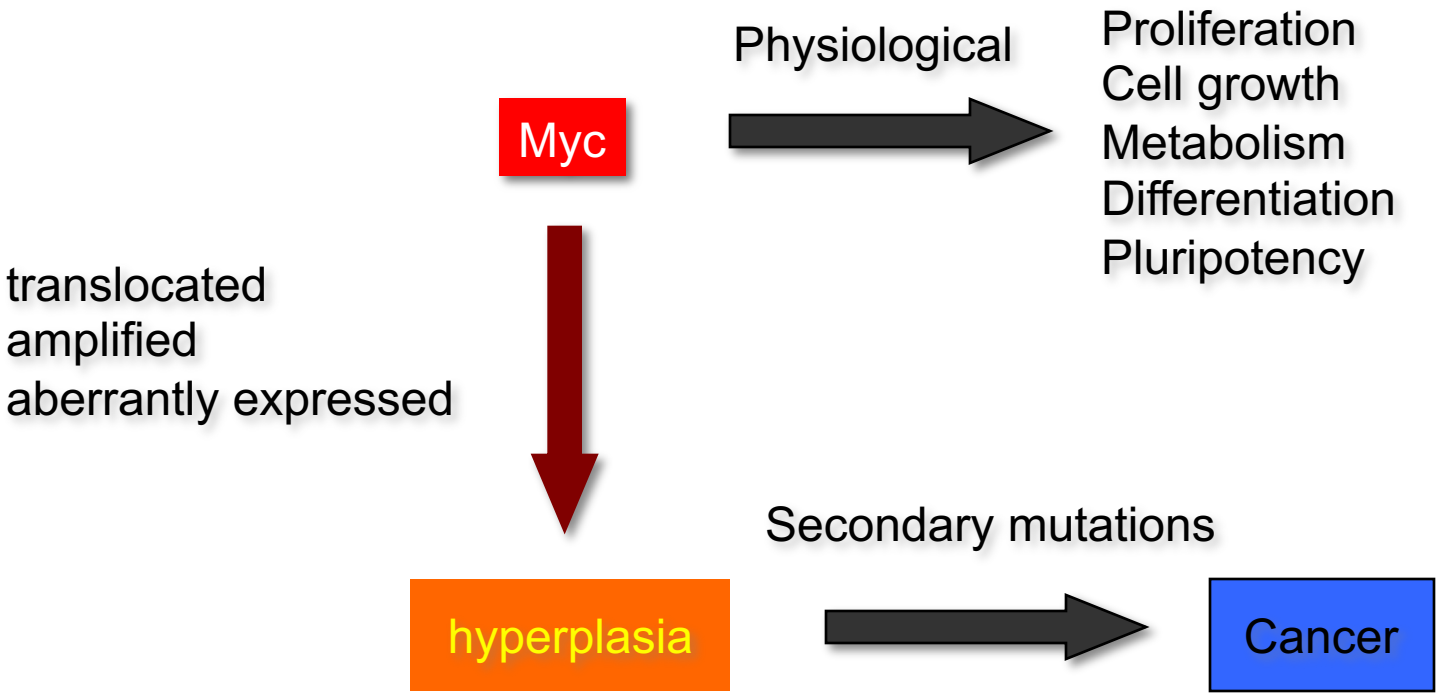
The topic: the c-Myc (proto)-oncogene

Key points:

- What are the cellular processes altered by the Myc oncogene
- Cooperating mutations in cancer
- Oncogene induced tumor suppression

- How we can exploit cellular and animal models to study oncogenes (and why this is so important)

The c-Myc (proto)-oncogene



Identification of c-Myc as a viral oncogene (v-gag-myc, MC29: myelocytomatosis virus)

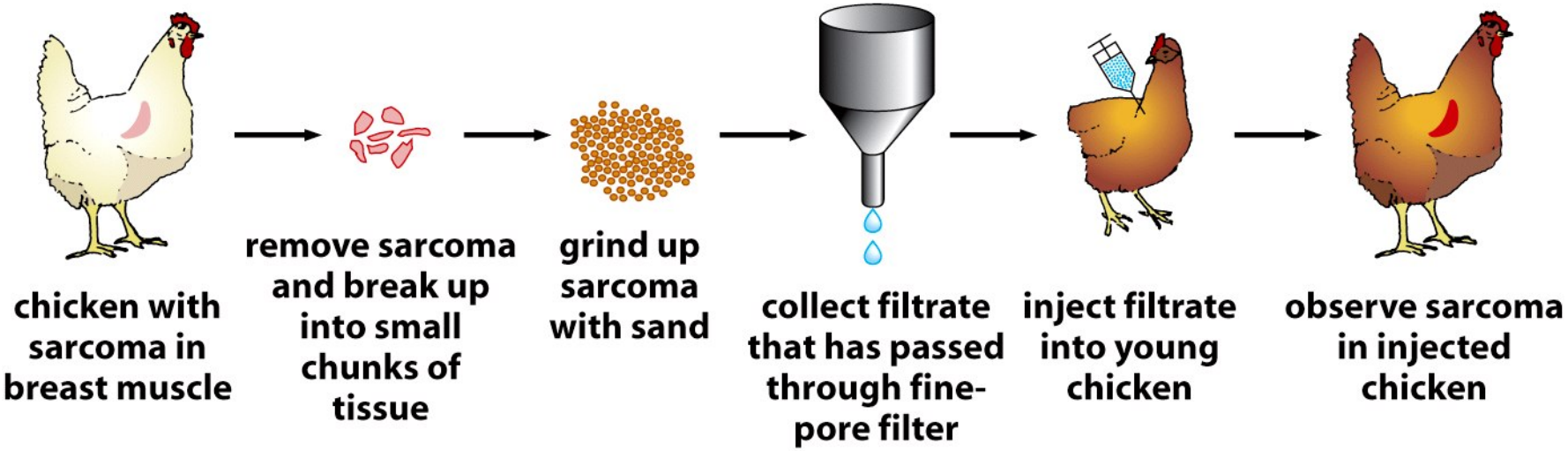


Figure 3.2 *The Biology of Cancer* (© Garland Science 2007)

Myc was also independently identified when Studying slow tumorigenic viruses like the ALV

(ALV: avian leukosis virus and murine leukemia virus):
-slow induction of tumors (months)
-no oncogene carried/integrated in viral genome

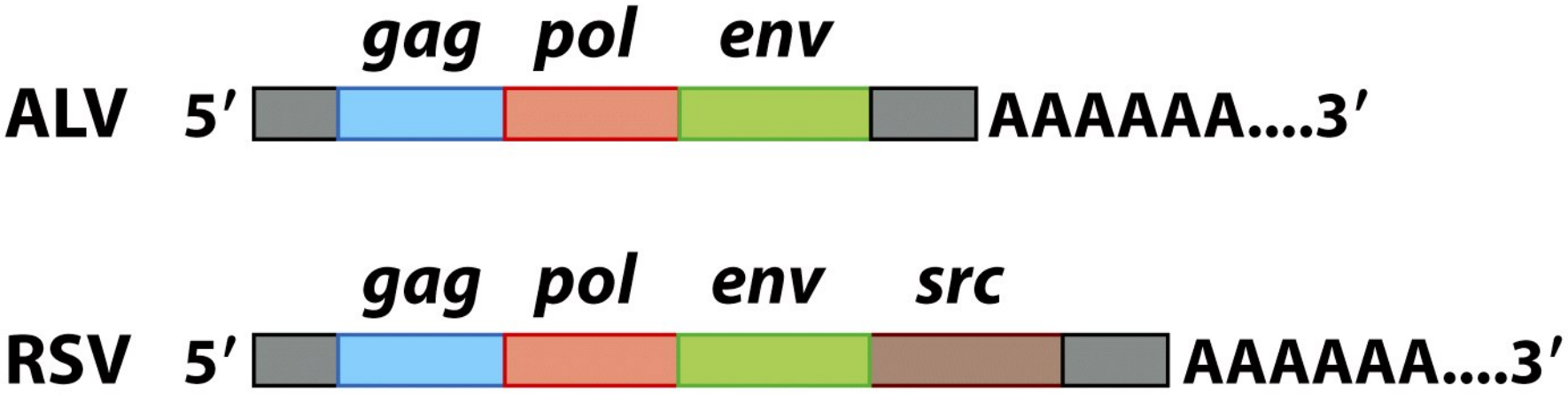
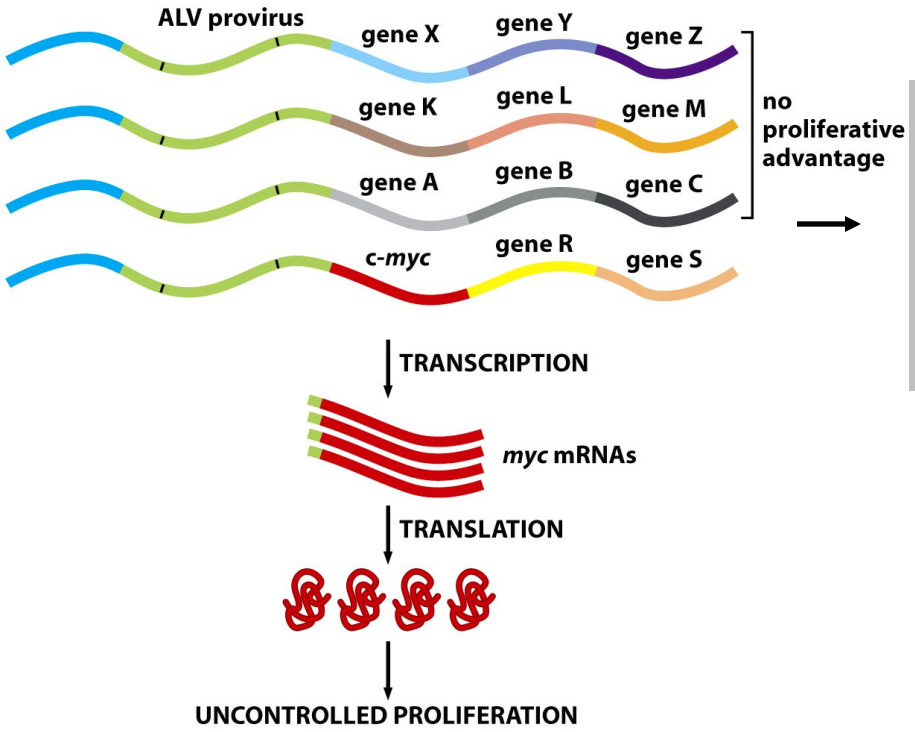


Figure 3.19 *The Biology of Cancer* (© Garland Science 2007)

Analysis of the genomic integration of ALV



Random integration in the genome of the host, yet ”non-random” insertion sites were found in the tumor genome: preference for the c-Myc gene (insertional mutagenesis)

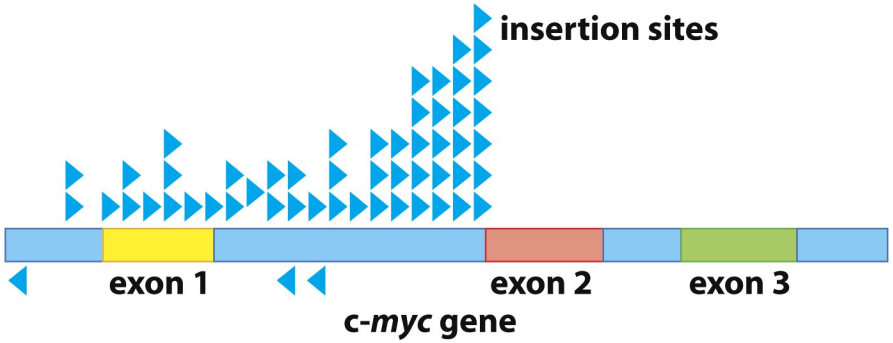
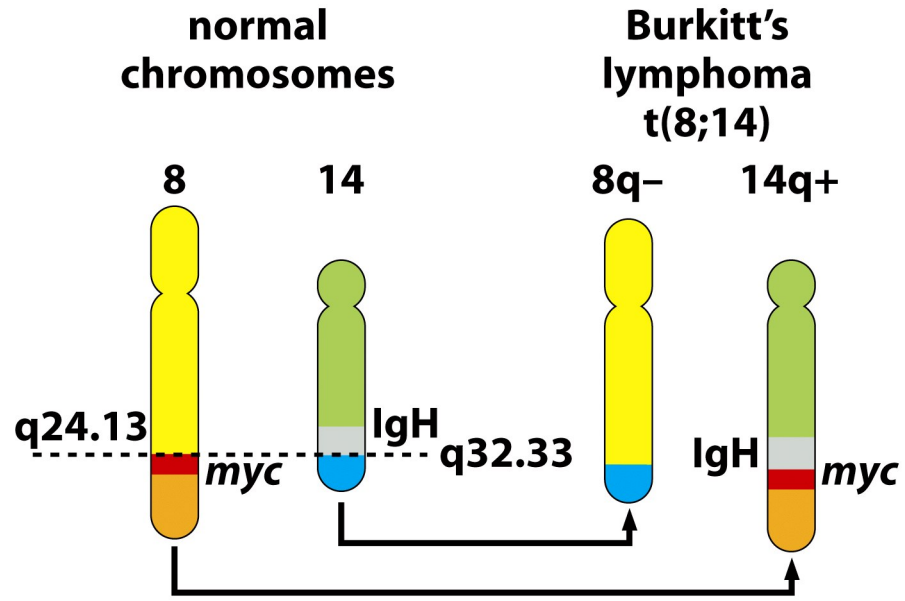
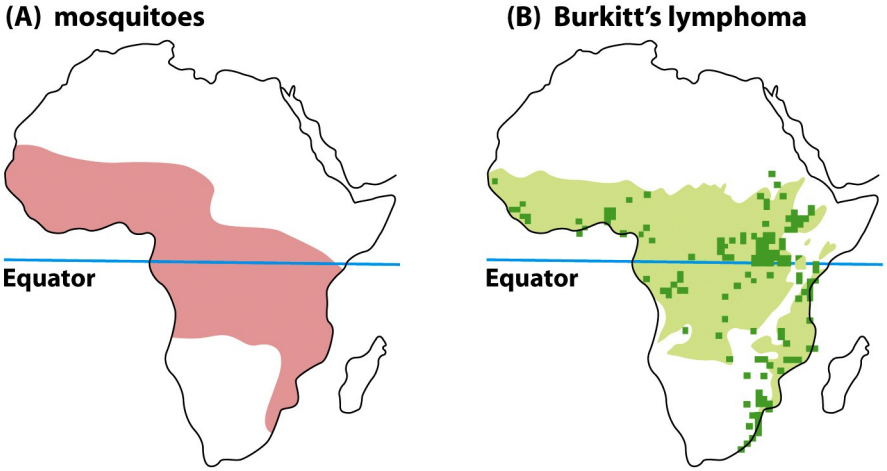


Figure 3.23b *The Biology of Cancer* (© Garland Science 2007)

c-MYC was rediscoverd as translocated in Lymphomas





Blood tumors are characterized by balanced reciprocal chromosomal translocations.

Key aspects of translocations found in leukemias and lymphomas:

1.Recurrent (in every tumor cell, in many different tumors of the same type)

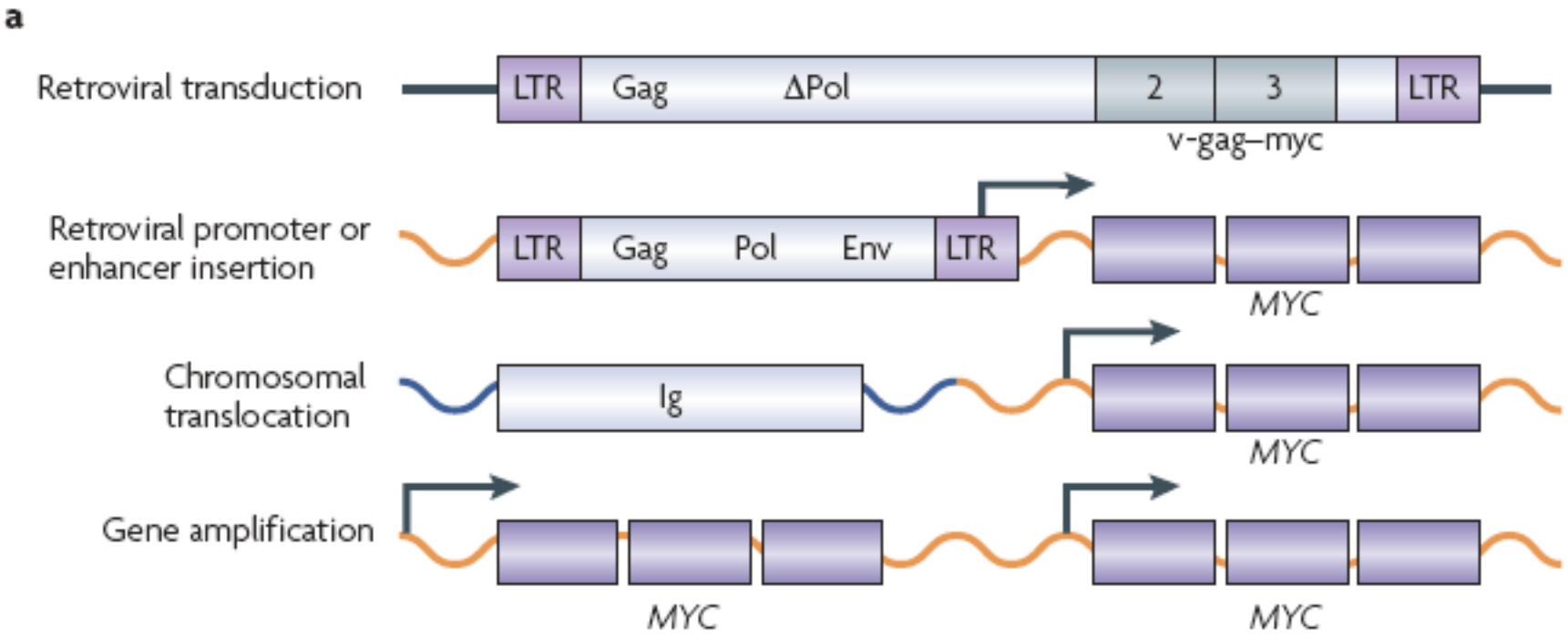
2. Balanced (no loss of genetic material)

3. Reciprocal (2 chromosomes are involved)

4. The Oncogene is found at the break-point

Figure 4.13a *The Biology of Cancer* (© Garland Science 2007)

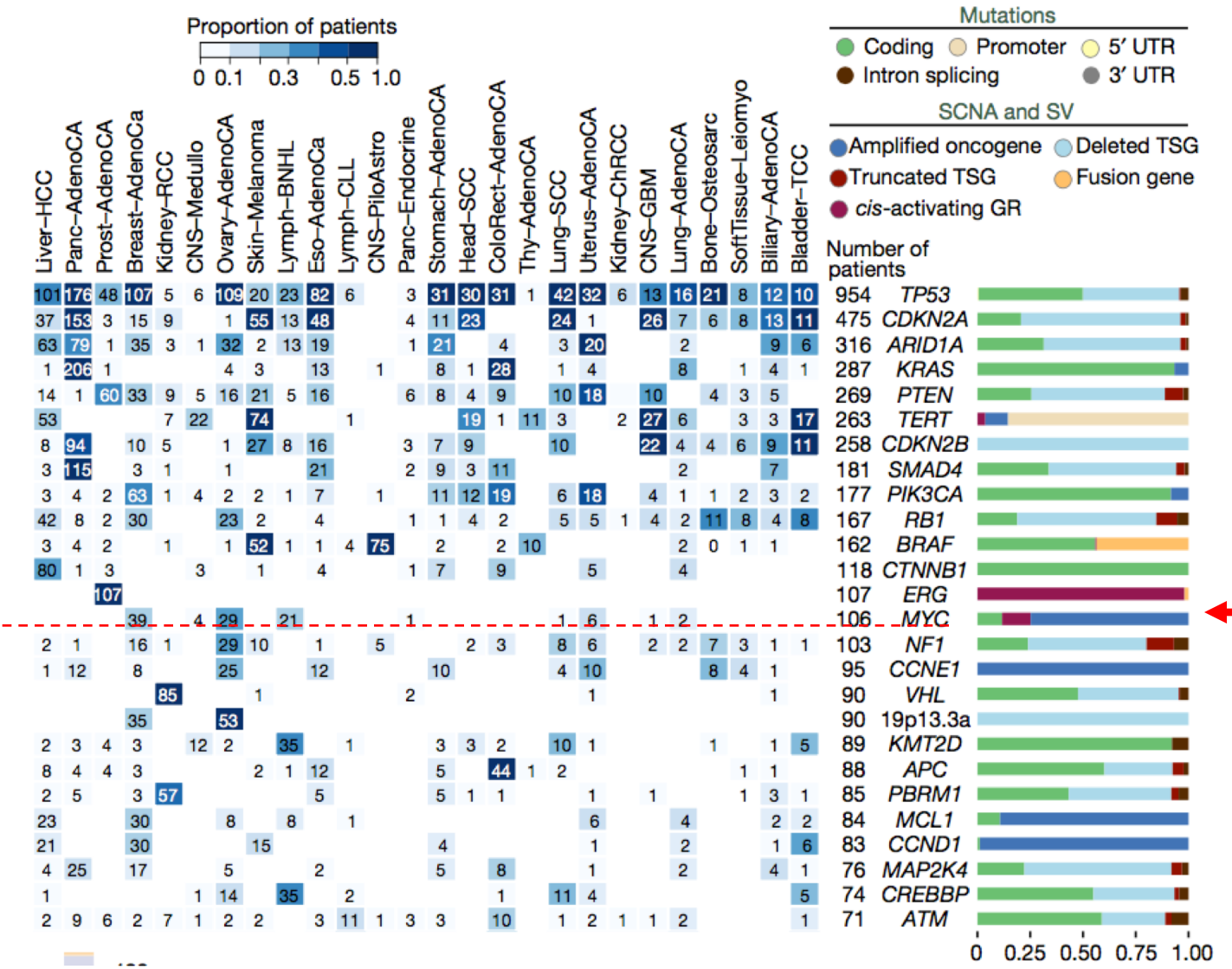
Several ways of making an oncogene out of Myc



…..also point mutations

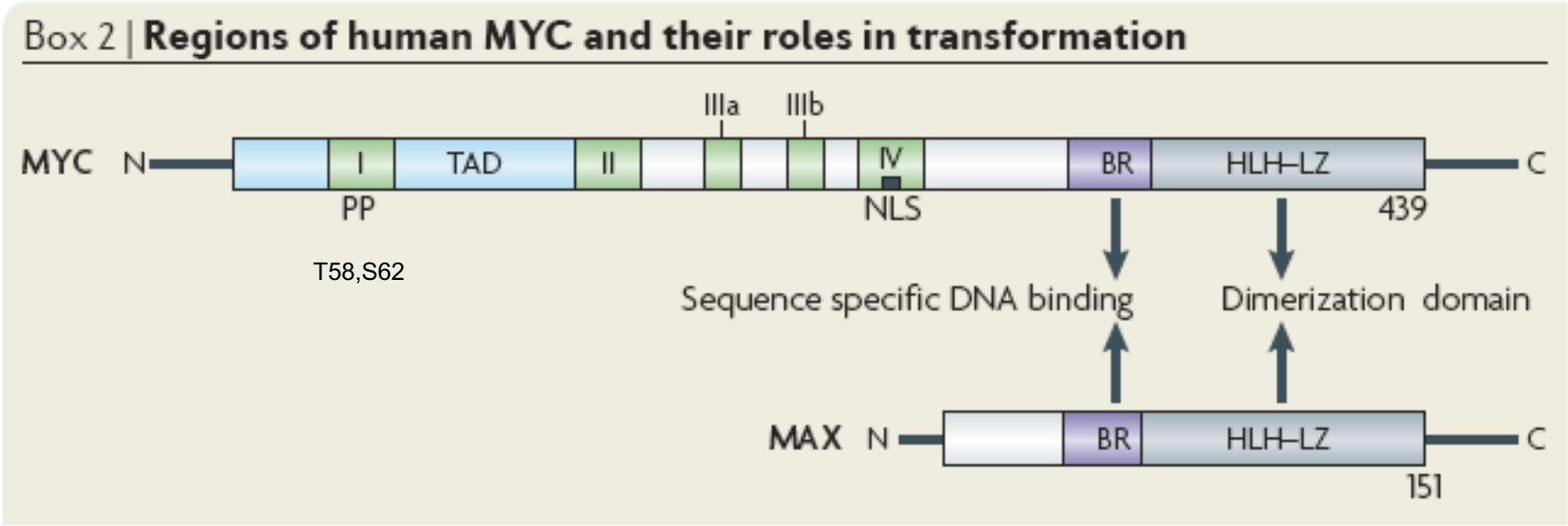
Meyer, N., and Penn, L.Z. (2008). Reflecting on 25 years with MYC. Nat Rev Cancer *8*, 976-990.

Myc is frequently mutated in cancer



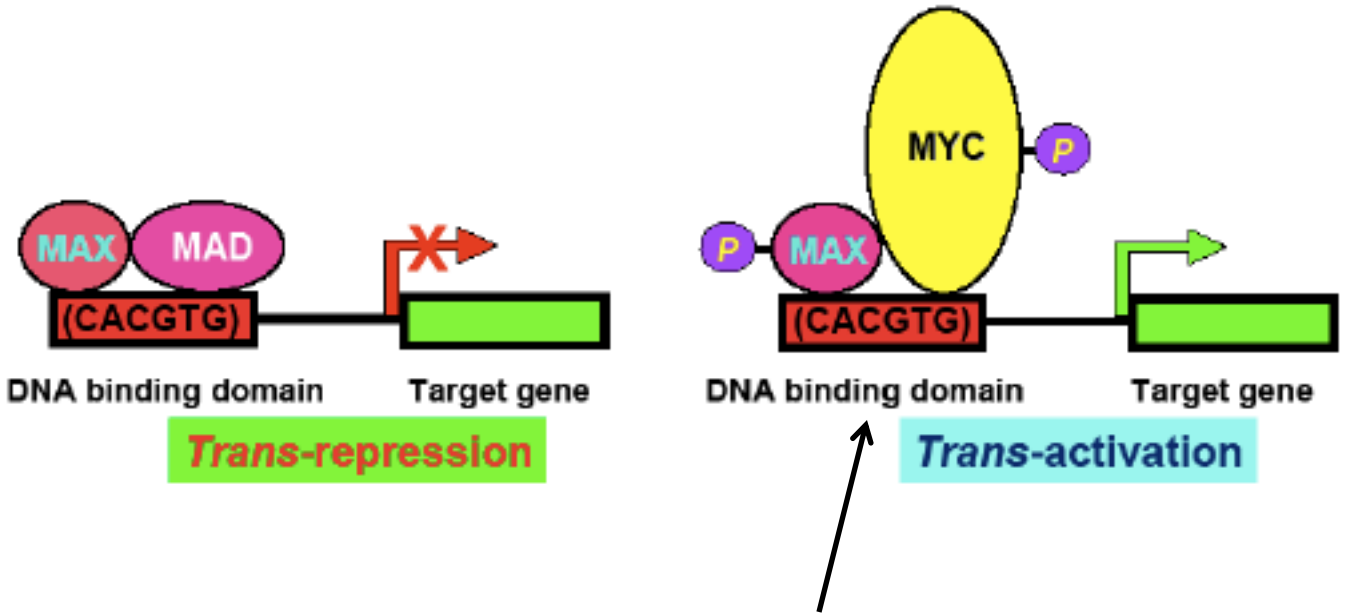
n = 2,583 patients

Myc is an heterodimeric transcription factor



MYC homology box is a region that is highly conserved between MYC, MYCN and MYCL1, unless otherwise stated[95,113].

The Myc-Max-Mad network



E-BOX: sequence recognized by Myc

NB: Myc can also act as a repressor

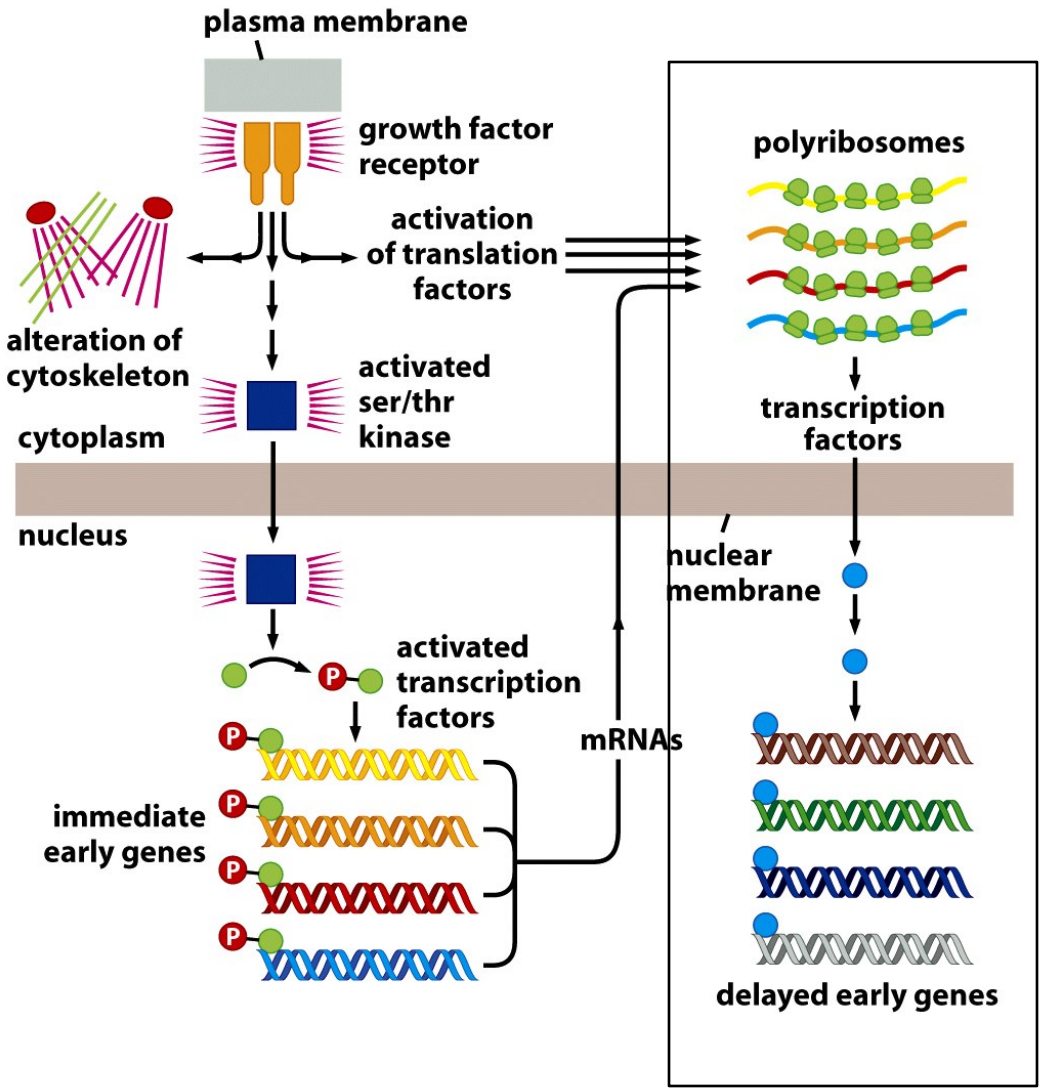
Myc binds to and regulates a large number of genes (thousands)

Table 1 | **MYC-regulated activities and gene targets associated with transformation**

Functional class	Description of function	Examples of responsible genes*
Cell cycle	MYC–ER activation drives quiescent cells to enter and transit through the cell cycle; primary cells from conditional knockout mice arrest in the absence of MYC expression	Cyclin D2, CDK4 (induced); p21, p15, GADD45 (repressed)
Differentiation	Deregulated MYC blocks differentiation of many cell systems; MYC accelerates epidermal differentiation	CEBP (repressed)
Cell growth, metabolism and protein synthesis	MYC expression levels are associated with body size owing to regulation of cell size and cell number	Lactate dehydrogenase, CAD, ODC, ribosomal proteins, EIF4E, EIF2A (induced)
Cell adhesion and migration	MYC drives tumorigenesis in part by allowing for anchorage-independent growth	N-cadherin, integrins (both repressed)
Angiogenesis	MYC induces angiogenesis in a wide range of tissues	IL1β, miR-17–92 microRNA cluster (induced), thrombospondin (repressed)
ROS, DNA breaks and chromosomal instability	MYC can contribute to instability, trigger telomere aggregation and increase ROS production	MAD2, TOP1, BUBR1, cyclin B1, MT-MCI
Stem cell self-renewal and/or differentiation	Ectopic MYC can potentiate induced pluripotent stem cells; MYC can control the balance between stem cell self-renewal and differentiation	To be determined, potentially genes associated with cell cycle, immortalization, adhesion and migration
Transformation	MYC can drive focus formation and anchorage-indepenent growth *in vitro* and full tumorigenesis *in vivo*; MYC is often deregulated in primary human cancers	Multiple targets are thought to contribute to transformation

This information is adapted from Dang[138]. *For further information see MYC Cancer Gene. CDK, cyclin-dependent kinase; CEBP, CCAAT/enhancer-binding protein; EIF, eukaryotic translation initiation factor; ER, oestrogen receptor; IL1β, interleukin 1β; MT-MCI, MYC target in myeloid cells 1; ROS, reactive oxygen species; TOP1, topoisomerase 1.

Myc is an immediate early gene



Experimentally blocked by Cycloheximide (inhibits translation)

Figure 6.3 *The Biology of Cancer* (© Garland Science 2007)

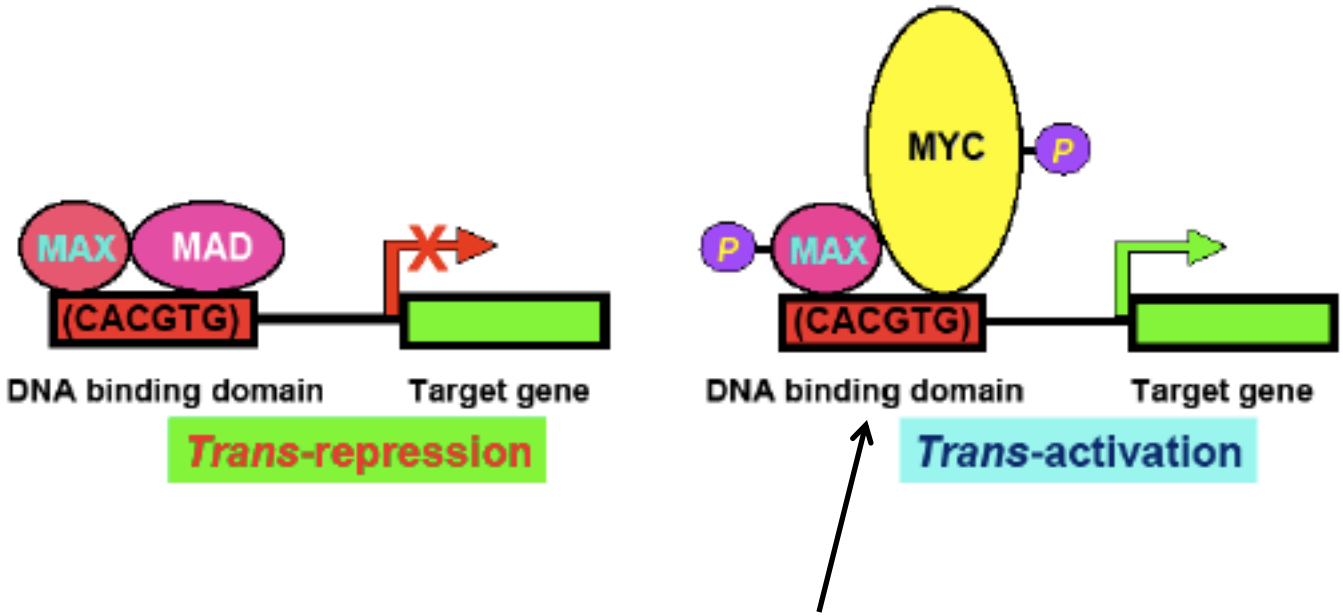
Table 6.1 A sampling of immediate early genes[a]

Name of gene	Location of gene product	Function of gene product
fos[b]	nucleus	component of AP-1 TF
junB	nucleus	component of AP-1 TF
egr-1	nucleus	zinc finger TF
nur77	nucleus	related to steroid receptors
Srf-1[c]	nucleus	TF
myc	nucleus	bHLH TF
β-*actin*	cytoplasm	cytoskeleton
γ-*actin*	cytoplasm	cytoskeleton
tropomyosin	cytoplasm	cytoskeleton
fibronectin	extracellular	extracellular matrix
glucose transporter	plasma membrane	glucose import
JE	extracellular	cytokine
KC	extracellular	cytokine

[a]The total number of distinct immediate early genes is variously estimated to be between 50 and 100.
[b]Expression of a group of fos-related genes is also induced as IEGs. These include *fosB*, *fra-1*, and *fra-2*.
[c]Srf is a TF that binds to the promoters of other immediate early genes such as *fos*, *fosB*, *junB*, *egr-1* and *egr-2*, *nur77*, and cytoskeletal genes such as actins and myosins.

Adapted in part from H.R. Herschman, *Annu. Rev. Biochem.* 60:381–319, 1991; and from B.H. Cochran, in R. Grzanna and R. Brown (eds.), Activation of Immediate Early Genes by Drugs of Abuse. Rockville, MD: National Institutes of Health, 1993, pp. 3–24.

The Myc-Max-Mad network



E-BOX: sequence recognized by Myc

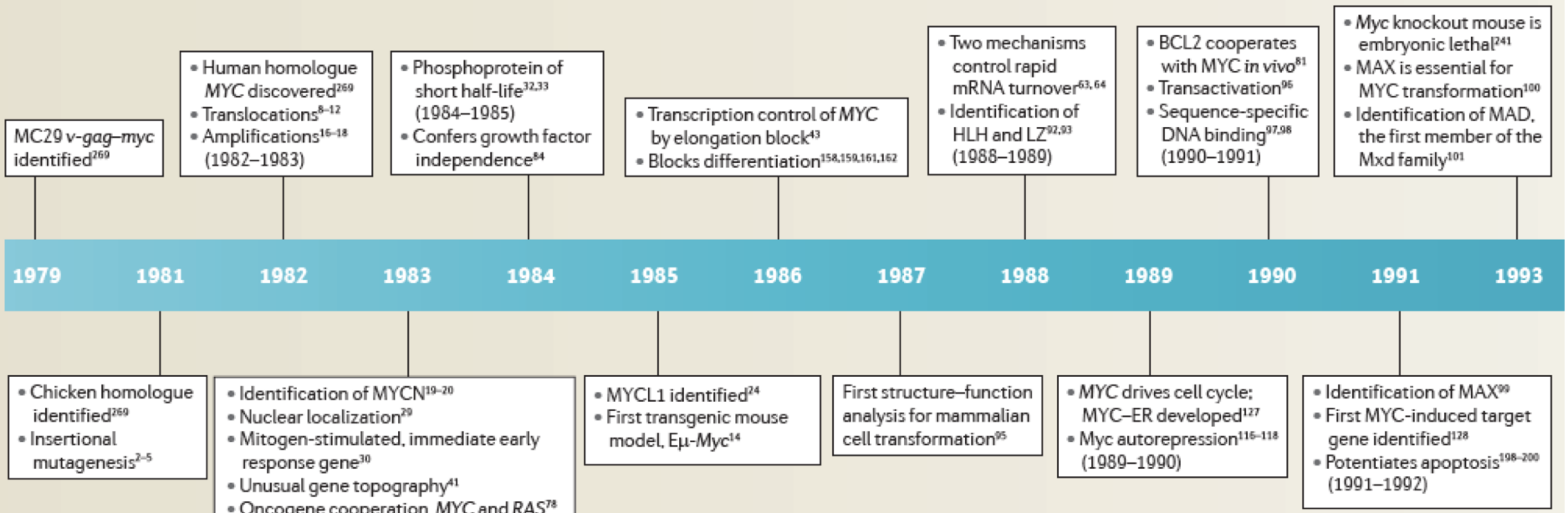
NB: Myc can also act as a repressor

MYC — TIMELINE

Reflecting on 25 years with MYC

Natalie Meyer and Linda Z. Penn

Timeline | **MYC research and cancer**



APC, adenomatous polyposis coli; HLH, helix–loop–helix; LZ, leucine zipper; TRRAP, transactivation/transformation-associated protein.

The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice

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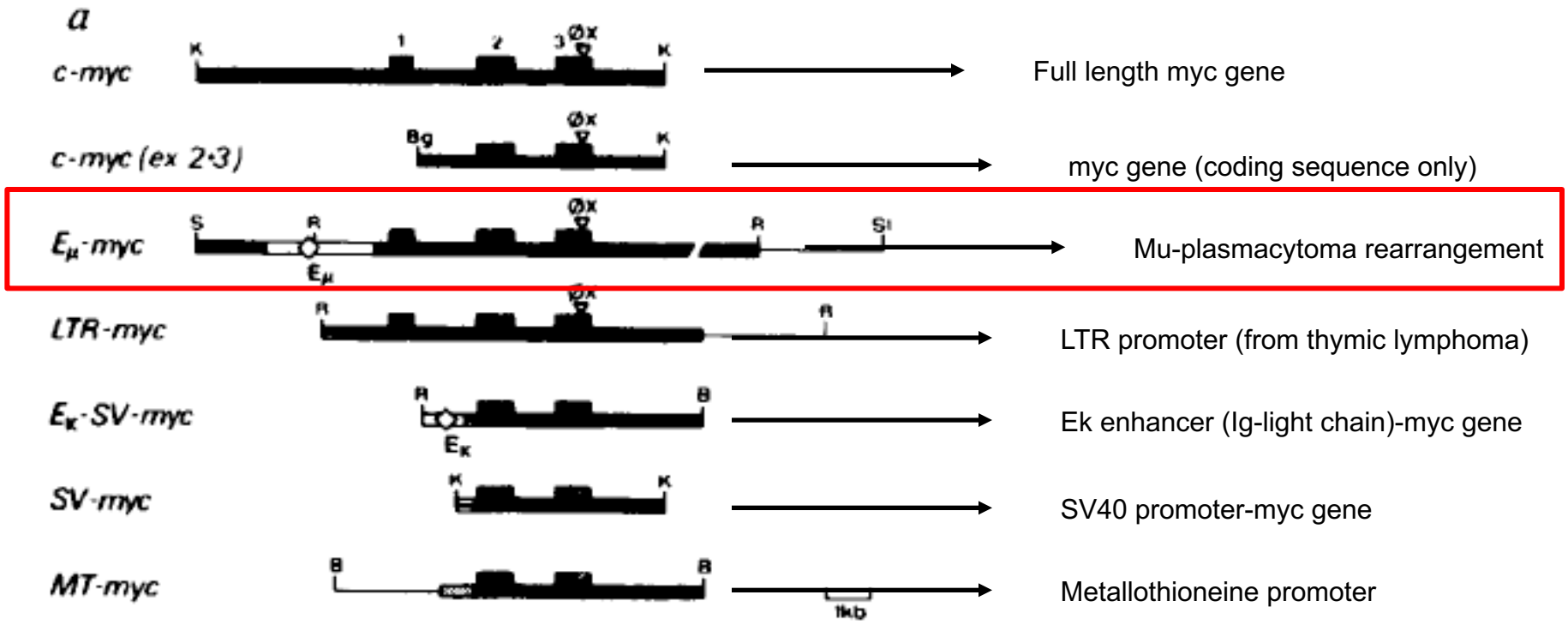
† School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
‡ Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA

How we can devise a mouse model carring a deregulated oncogene

What we can learn from such a model

Design of different transgenic mice

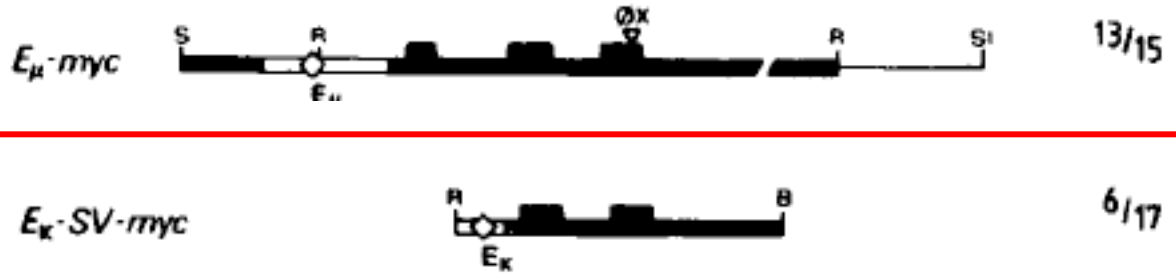


Key Questions

- Will mice develop tumors?
- What kind of tumors?

μ and κ enhancer: lymphoid tumors

Tumor incidence



Conclusion 1: the Eμ-enhancer/c-Myc transgene is oncogenic

Eμ-Myc pathology

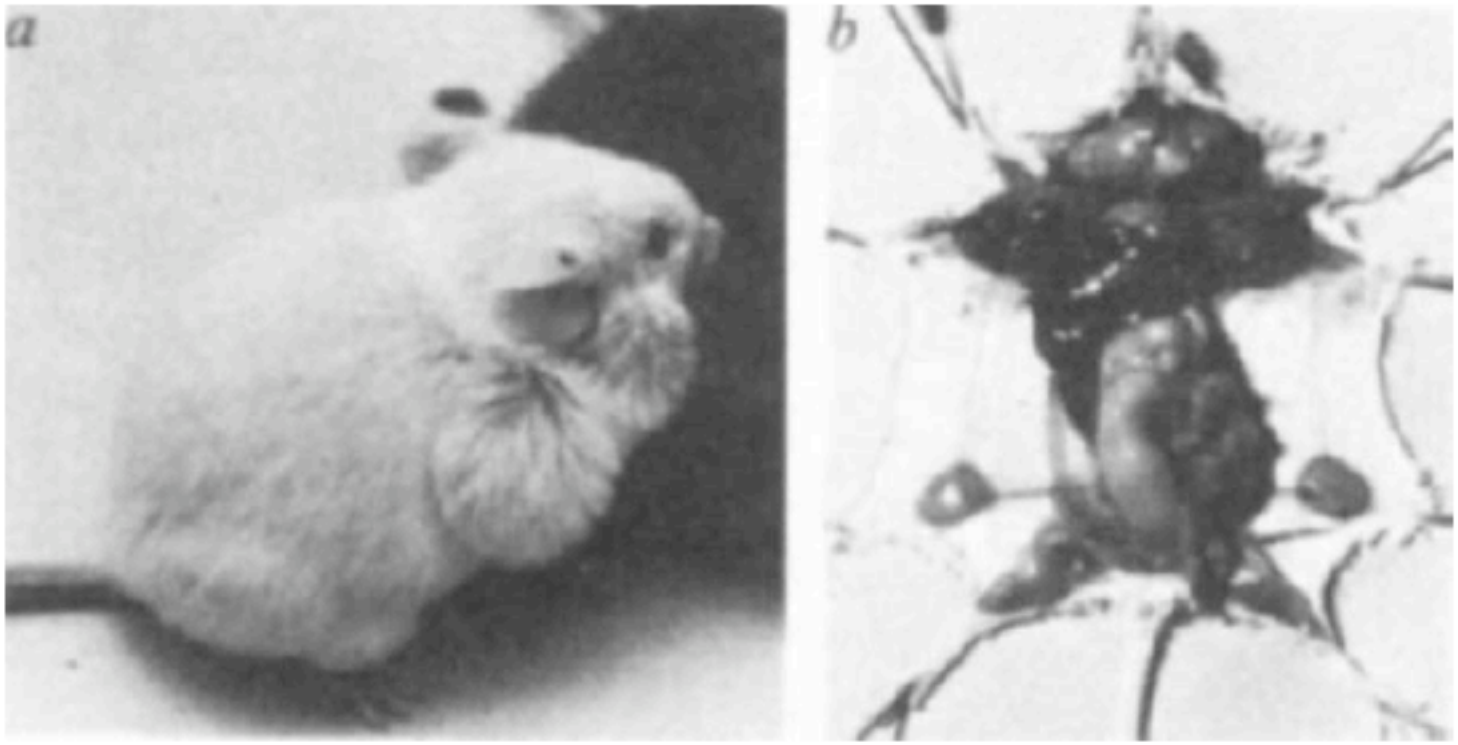


Fig. 2 *a*, Fourth-generation E_μ-*myc* mouse, 11 weeks old, with swellings due to grossly enlarged lymph nodes. *b*, Interior of same mouse, displaying gross bilateral enlargement of (from the top) cervical, axillary, brachial, mesenteric and inguinal lymph nodes.

Lymph node map

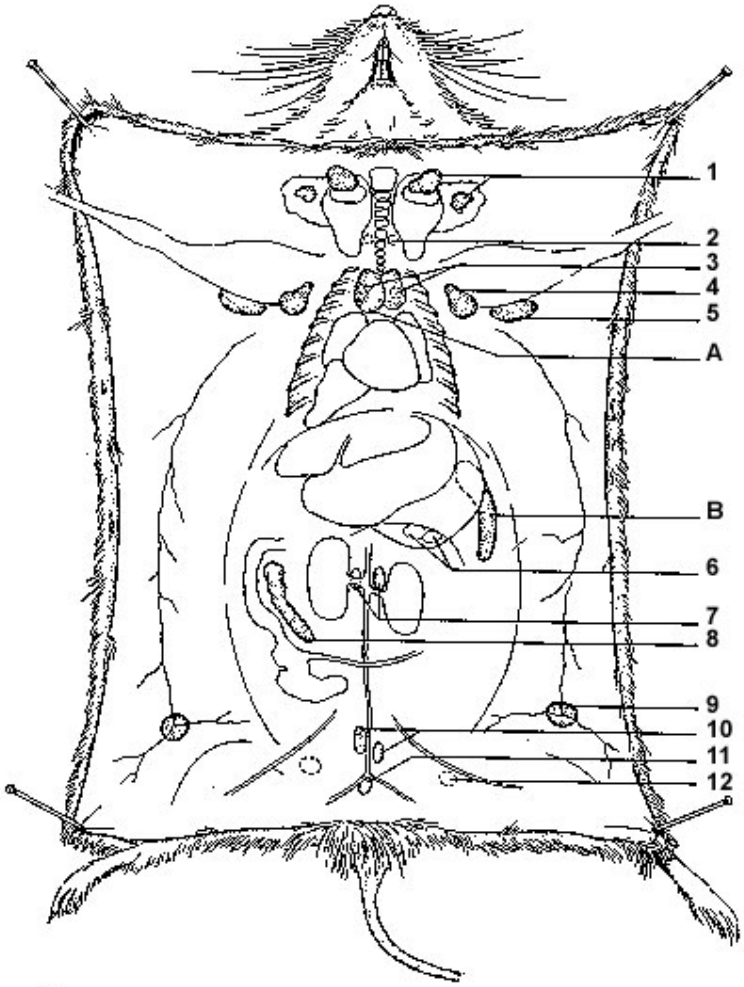


Diagram Key

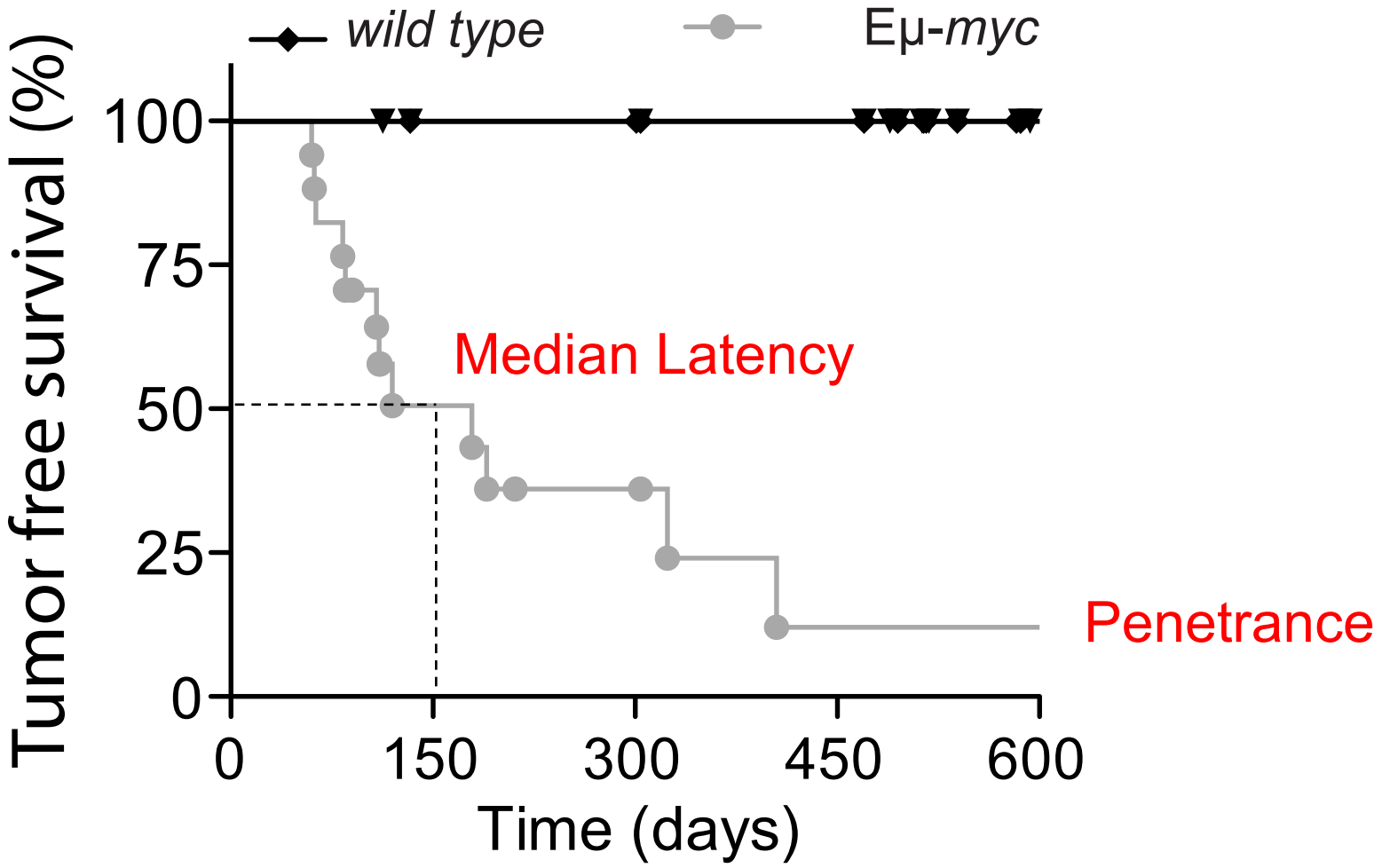
1. Superficial cervical nodes
2. Deep cervical nodes
3. Mediastenial nodes
4. Axillary node
5. Brachial node

A. Thymus

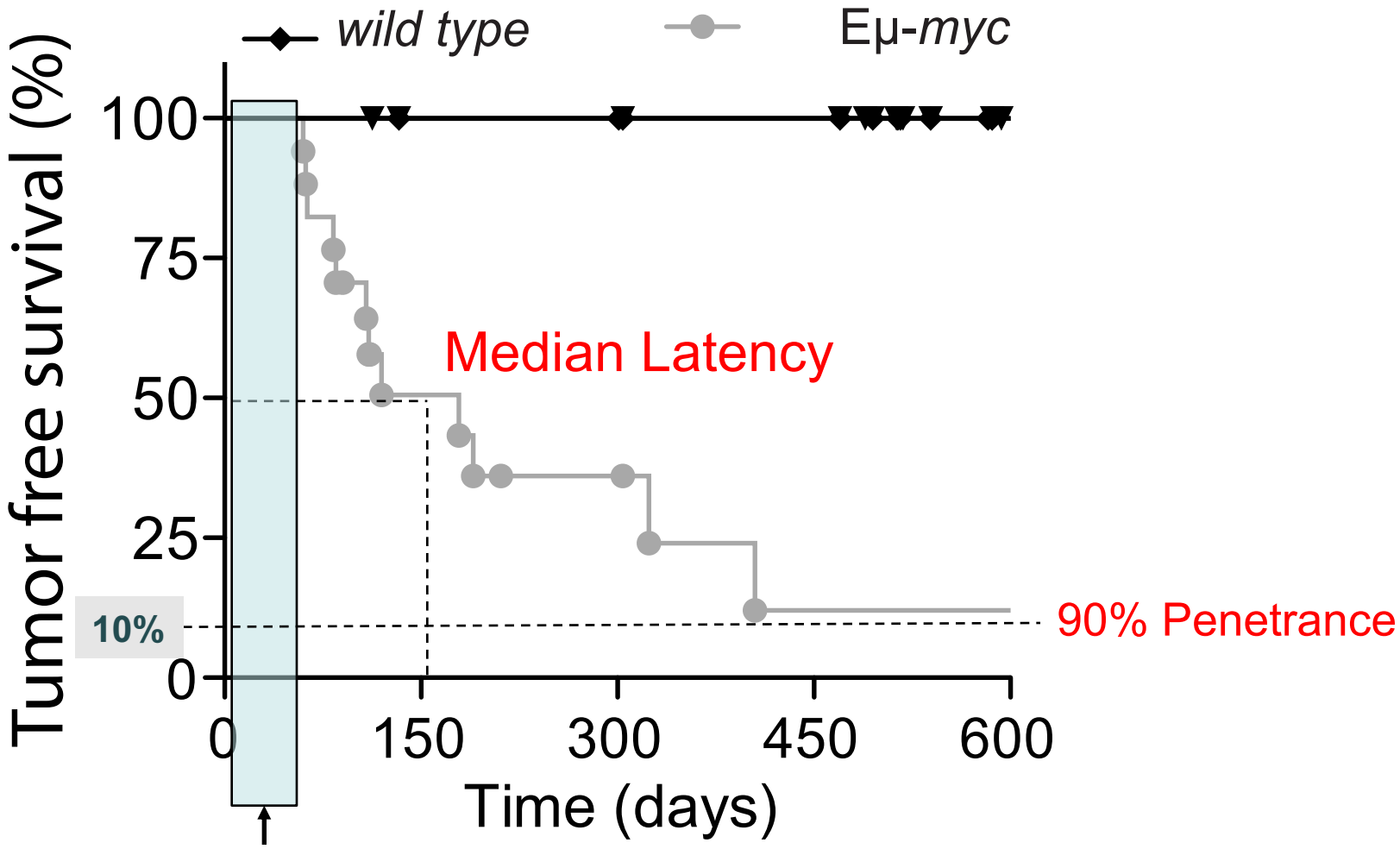
B. Spleen

6. Pancreatic node
7. Renal nodes
8. Mesenteric node
9. Inguinal node
10. Lumbar nodes
11. Sacral Node
12. Sciatic node

Disease free survival (Kaplan Meier survival curve)

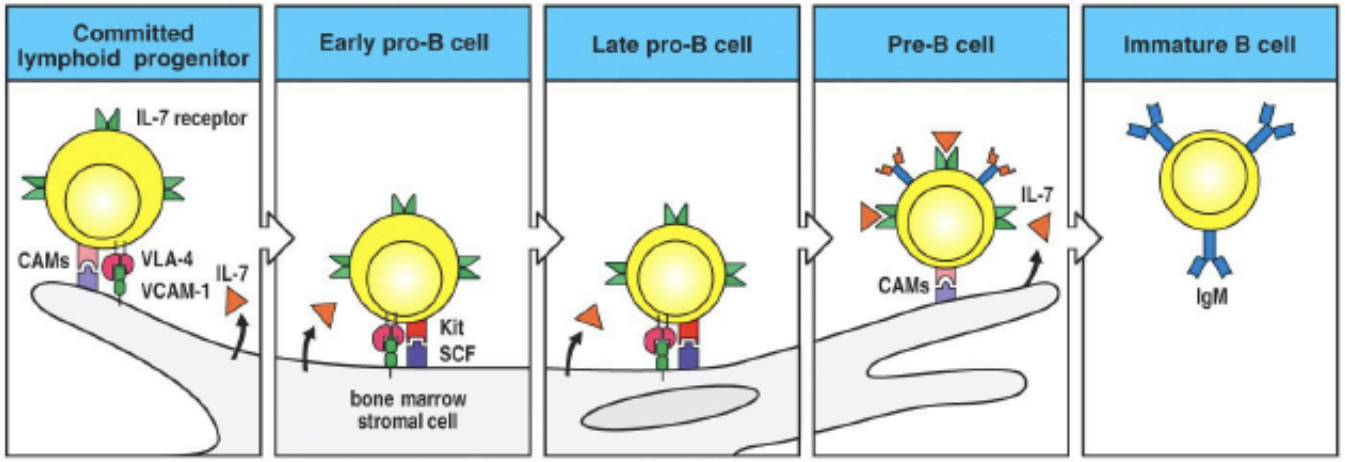


Disease free survival (Kaplan Meier survival curve)

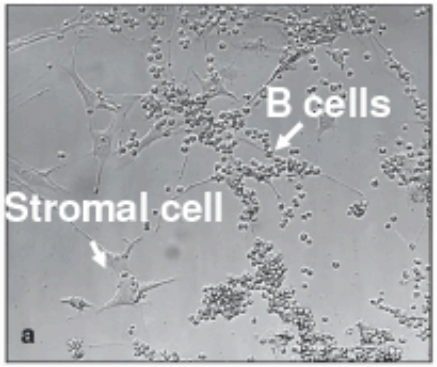


B cell Development in Bone Marrow

Model:



→ Migrate to lymphoid tissues



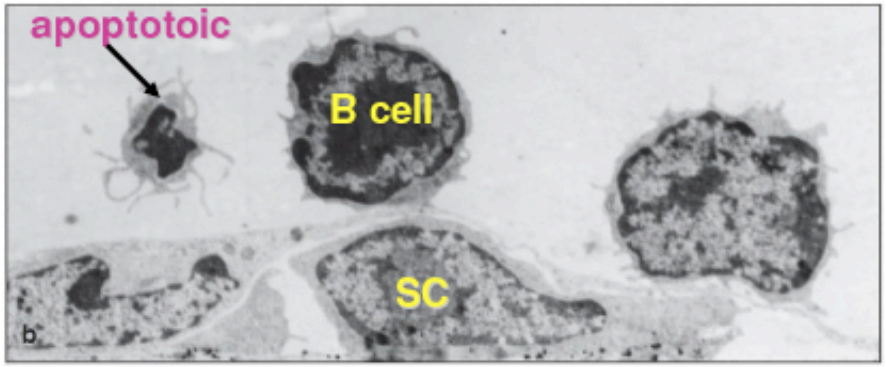


Figure 7-4 Immunobiology, 6/e. (© Garland Science 2005)

Light micrograph
Looking down at cells

Light micrograph;
Higher mag; section

Stages of B cell maturation in the bone marrow

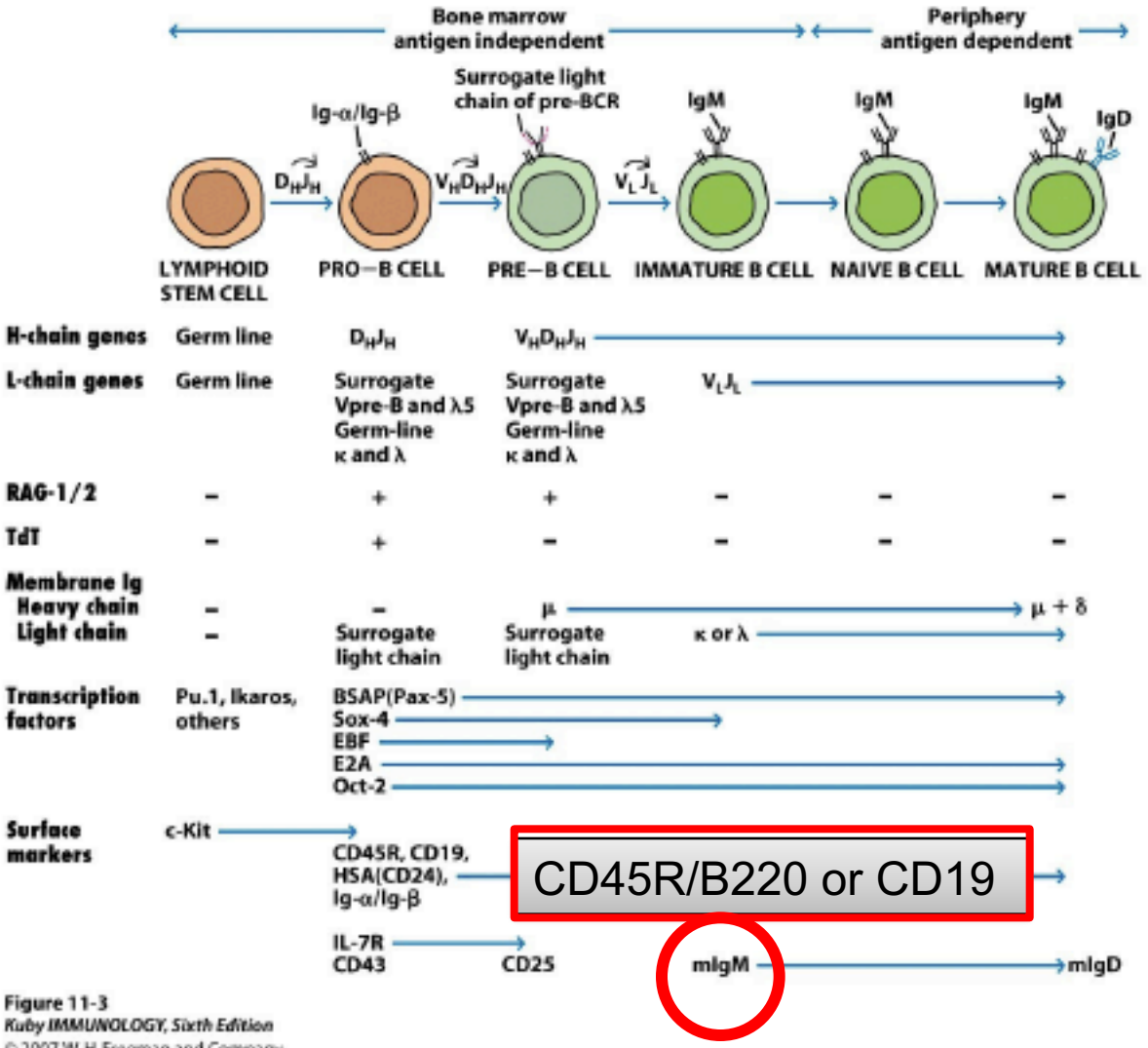
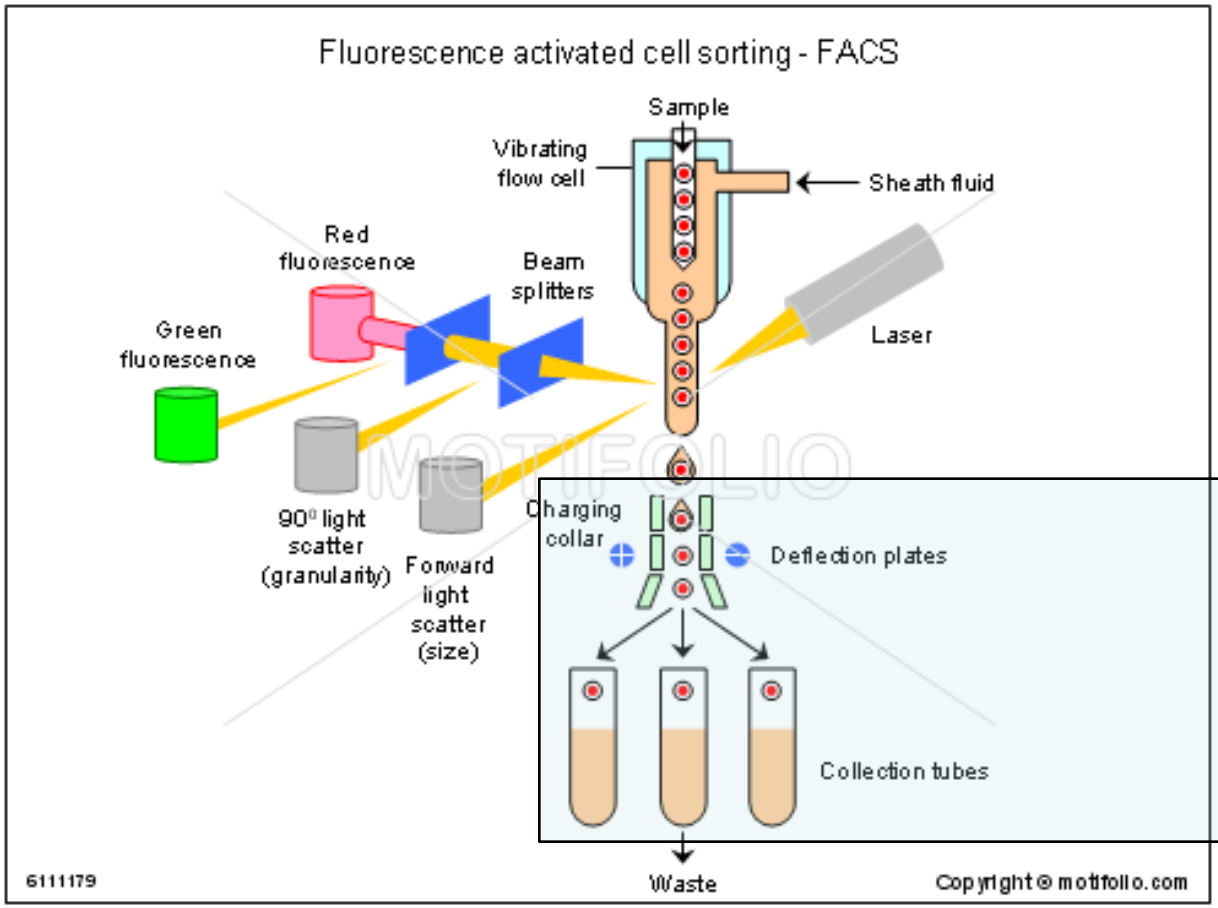


Figure 11-3
Kuby IMMUNOLOGY, Sixth Edition

FACS
(Fluorescent Activated Cell Sorting)



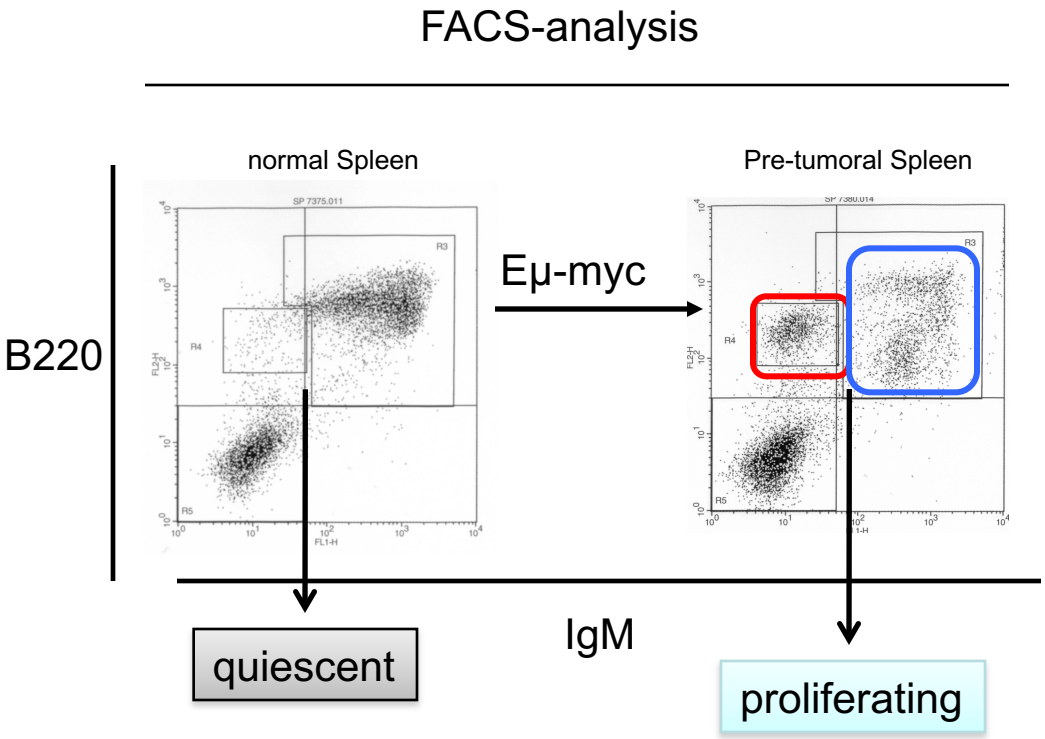
FACS-SORTER

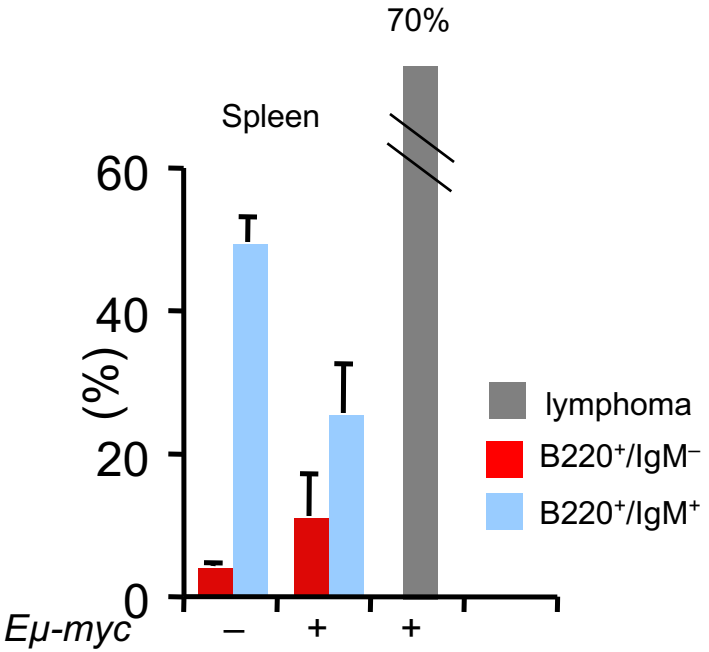
Main chacteristics of the Eμ-myc mouse

Eμ-myc

(B-cell lymphomas)

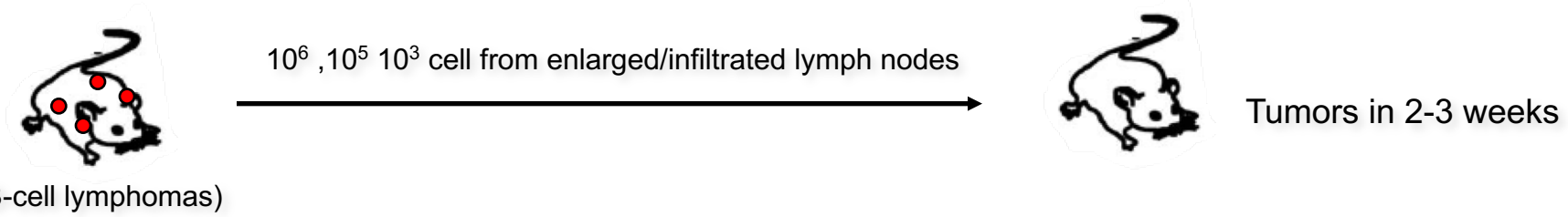
Eμ-myc, two pathological stages:
1.Pre-tumoral phase (proliferation & block of differentiation)
2.Lymphomas (100% penetrance, median onset:150 days)





Are the cells found in "enlarged" lymph nodes, tumor cells?

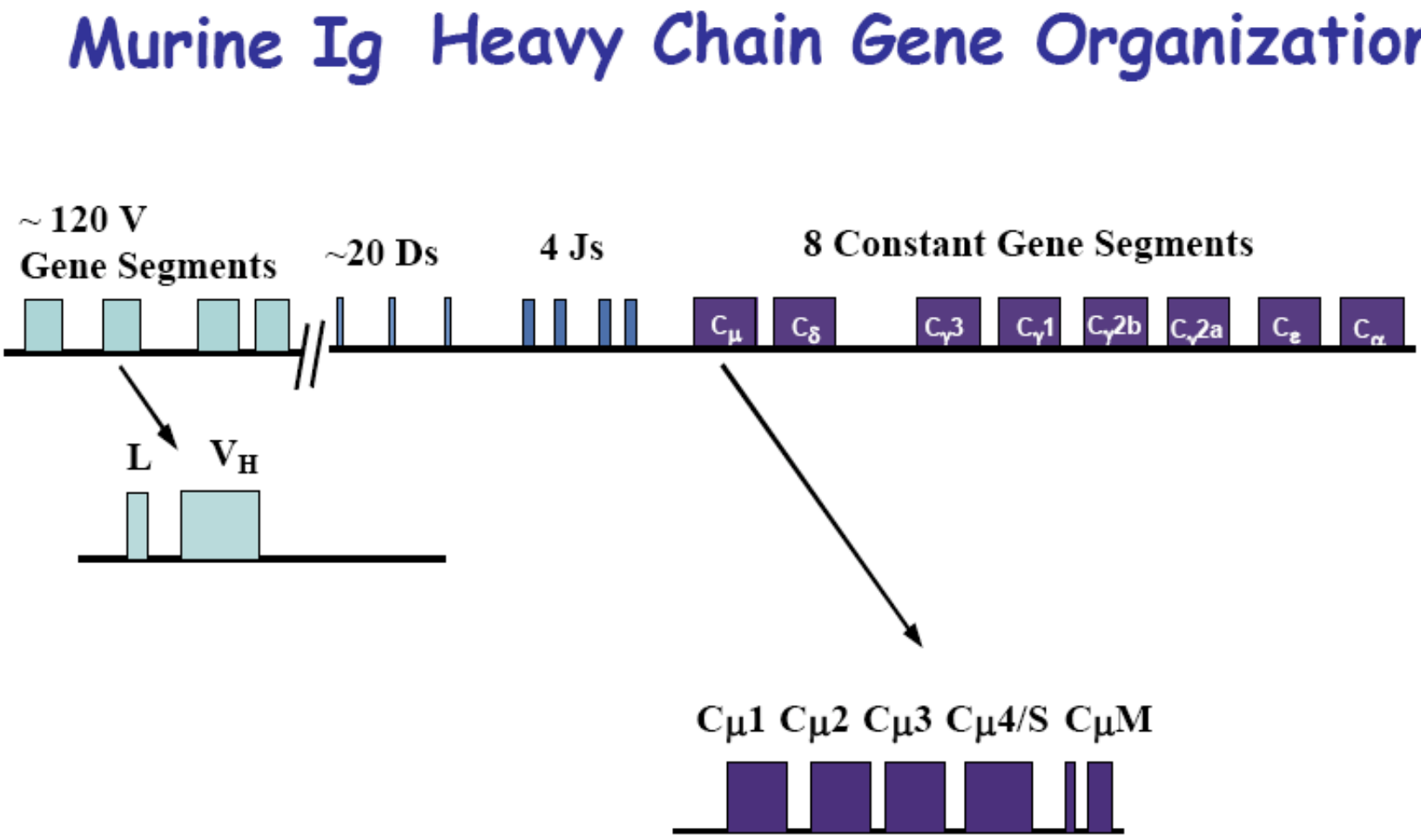
Experiment: transplant of "tumor cells" in healthy recipient mice



Why such a long latency (90-150) days in Eμ-Myc compared to transplanted recipients?

Will all the cells expressing Eμ-Myc become tumor cells?

VDJ recombination to mark clonal growth in tumors



Eμ-Myc tumors are monoclonal or oligoclonal (southern blot)

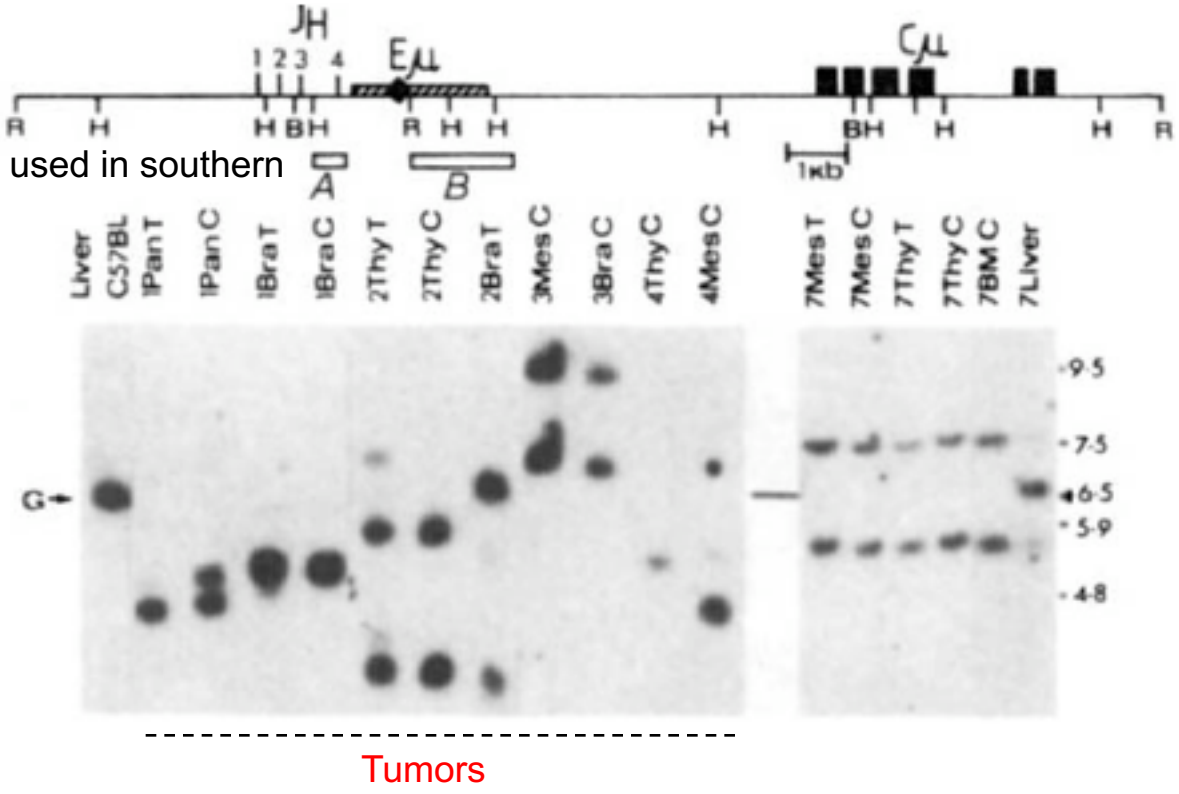


Fig. 3 Rearrangement at the immunoglobulin heavy-chain locus in tumours arising in E_μ-*myc* mice. The diagram indicates the

Negative feedback on endogenous c-Myc (northern blot analysis)

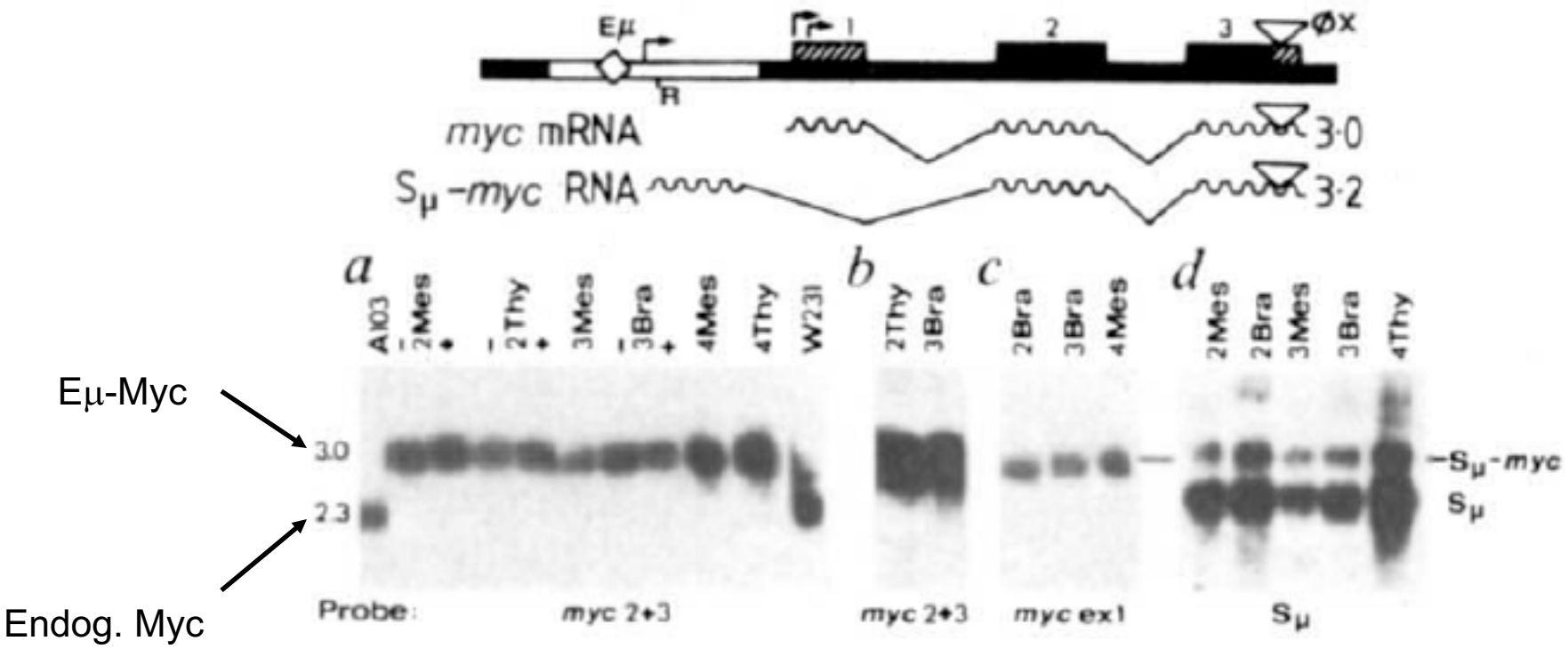


Fig. 5 Transcripts of the *myc* gene in lymphoma cell lines from E_μ-*myc* mice. Proposed transcripts from the E_μ-*myc* insert are

"rearranged" Myc escapes negative feedback loop (negative regulatory regions lost in the rearrangment?)

located *myc* allele is active[45,46,48]. One model to account for such results proposes that the normal *myc* gene is subject to negative feedback control by an excess of the *myc* polypeptide or products induced by it[18,25]. Translocation to the *IgH* locus, or the influence of the E_μ enhancer, frees that c-*myc* gene from this restraint, and the ensuing constitutive *myc* expression silences the unaltered allele(s). An alternative model suggests that

Conclusions

Eμ-Myc transgene is a potent, tissue-specific oncogene

Cancer is a multistep process, i.e. Eμ-Myc has two stages:

1. pretumoral (hyperproliferation/block of differentiation)
2. tumoral

Latency of tumors and mono/oligo clonality suggest "second hit"
(why do we need a second hit?)

Myc deregulation is necessary for tumor development BUT NOT SUFFICIENT

Negative feedback regulation on Myc expression (safety-lock #1)