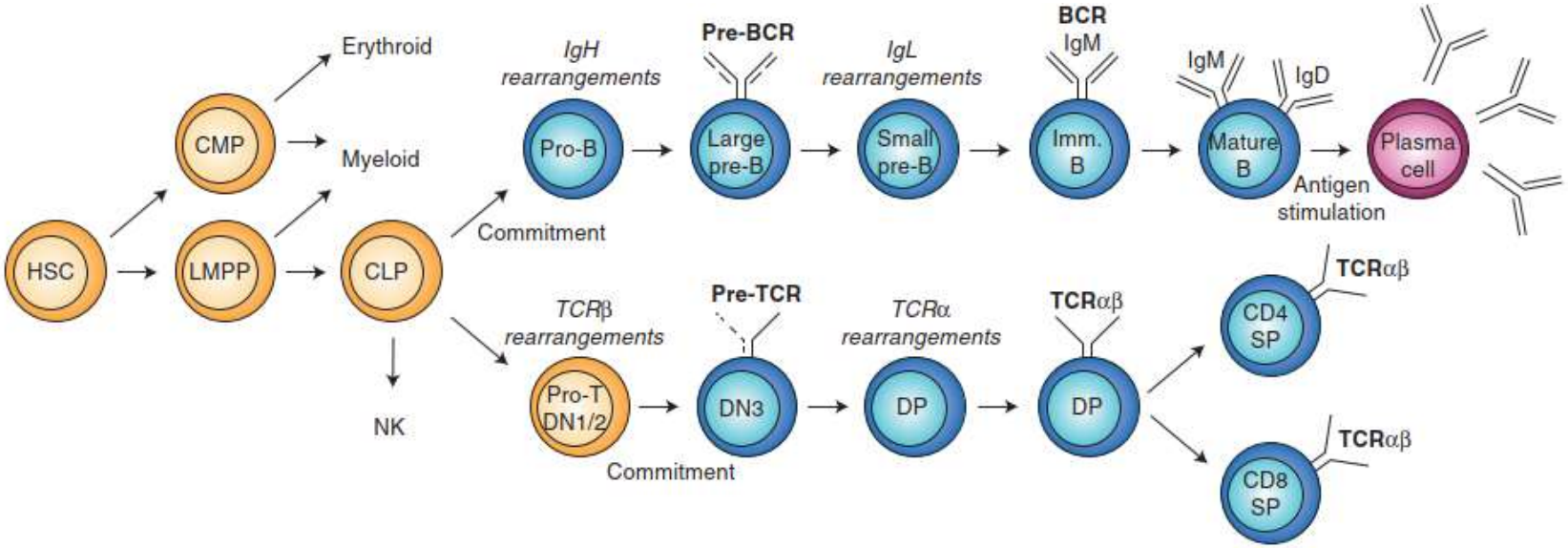
Chapter 29 - Epigenetic Control of Immunity

BRIAN WILEY

Hematopoiesis



HSC: Hematopoietic Stem Cell, LMMP: lymphoid-primed multipotent progenitors, CMP: common myeloid progenitor, CLP: common lymphoid progenitor

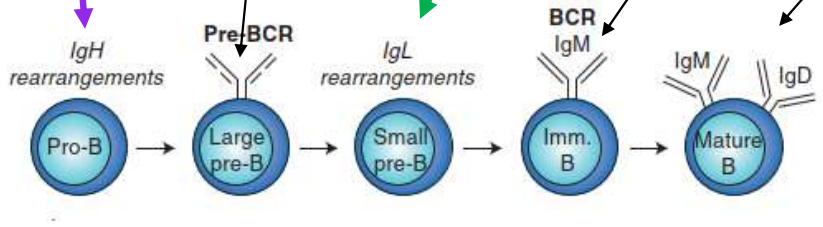
B-cell Development

Stages in B Cell Development							
	stem cell	**early pro-B cell**	**late pro-B cell**	**large pre-B cell**	**small pre-B cell**	**immature B cell**	**mature B cell**
H chain genes	germline	D-J joining	V-DJ joining	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
L chain genes	germline	germline	germline	germline	V-J joining	VJ rearranged	VJ rearranged
Surface Ig	none	none	none	μ chain in pre-B receptor	μ chain in cytoplasm and on surface	membrane IgM	membrane IgM and IgD
RAG, TdT expression	no	yes	yes	no	yes	yes	no
Surrogate L chain expression	no	yes	yes	yes	no	no	no
Ig αβ expression	no	yes	yes	yes	yes	yes	yes

Decker [2]

IgH gene expresses Igμ protein at Pre-BCR

Pre-BCR expression induces proliferative expansion and downregulation of recombinase-activating genes (*RAG1* and *RAG2*)[1]

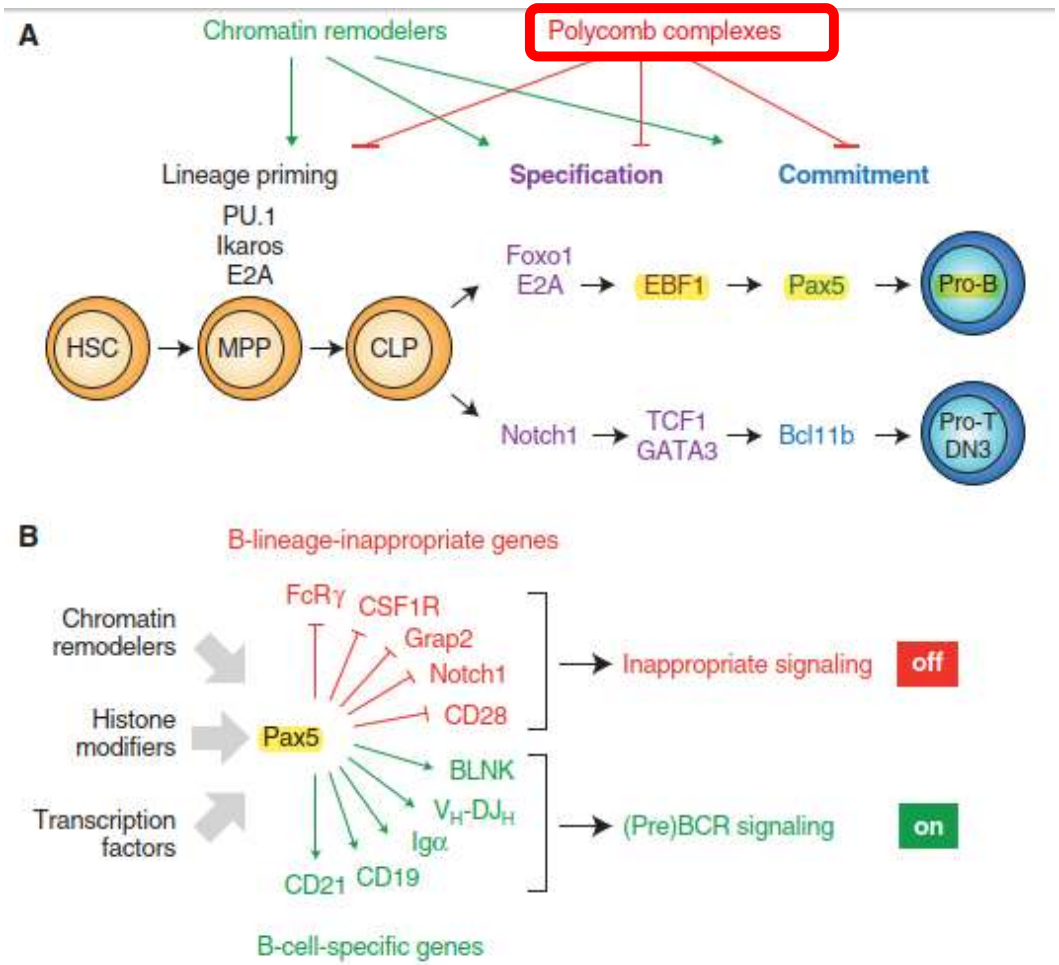


http://www2.nau.edu/~fpm/immunology/Exams/Bcelldevelopment-401.html

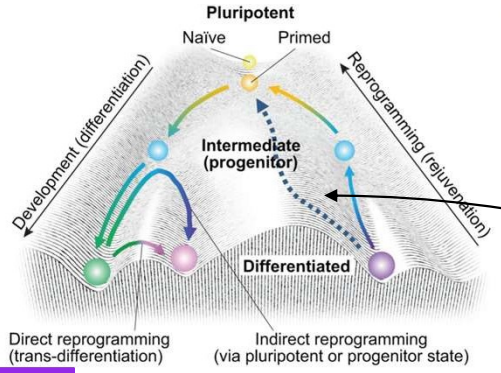
PRC2 H3K27me3 & PRC1 H2AK119ub1

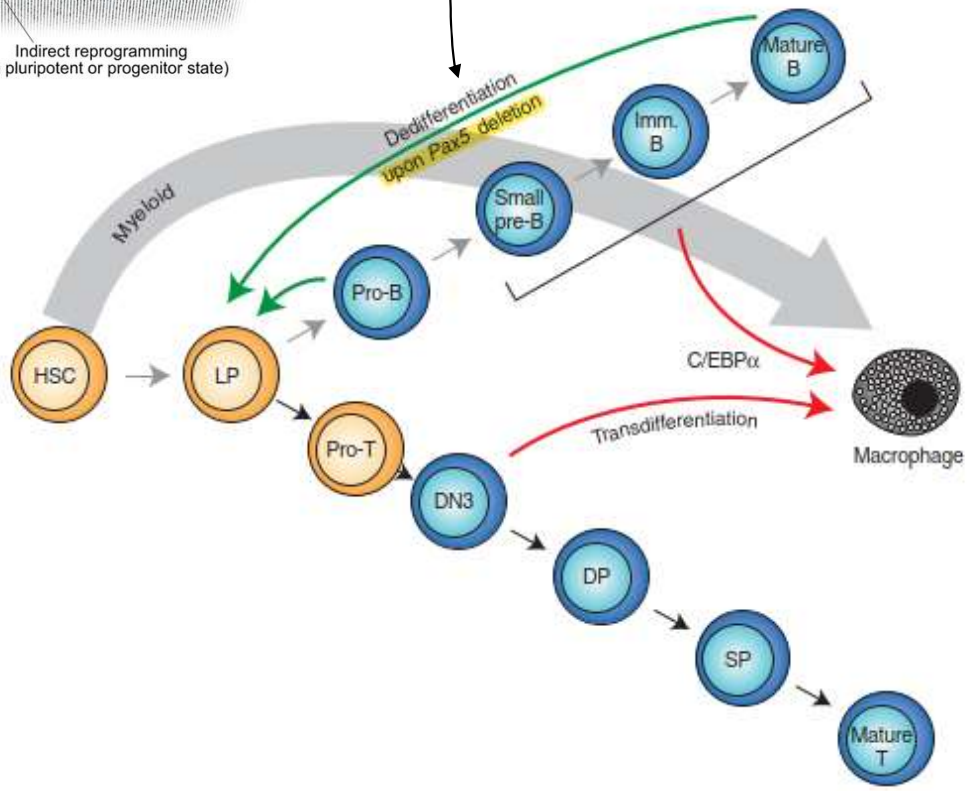
silence EBF1 and Pax5

"In conclusion, our study unequivocally demonstrates that Pax5 does not function as a master regulator of B cell development, as its ectopic expression is unable to divert the HSC and erythro-myeloid progenitors B cell pathway" - Souabni, et al. (2002) [3]



Interplay between Pax5 and Myc in B-lymphomagenesis





Study involving myc and PAX5:
https://grantome.com/grant/NIH/R01-CA102709-04
http://pathology.med.upenn.edu/department/people/505/andrei-thomas-tikhonenko

Plasticity-
mature B cells seem to lose their B-cell identity on Pax5 loss because they down-regulate B-cell-specific genes and reactivate lineage-inappropriate genes.

Loss of Pax5 allows mature B cells from peripheral lymphoid organs to dedifferentiate in vivo back to early uncommitted progenitors that migrate to the bone marrow

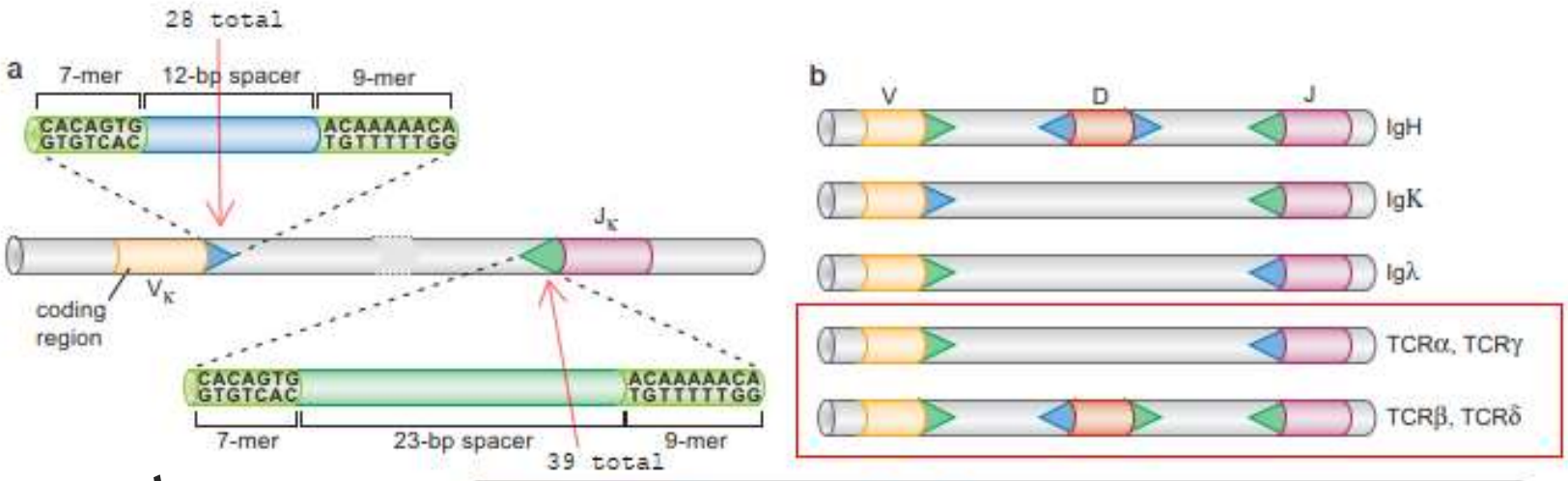


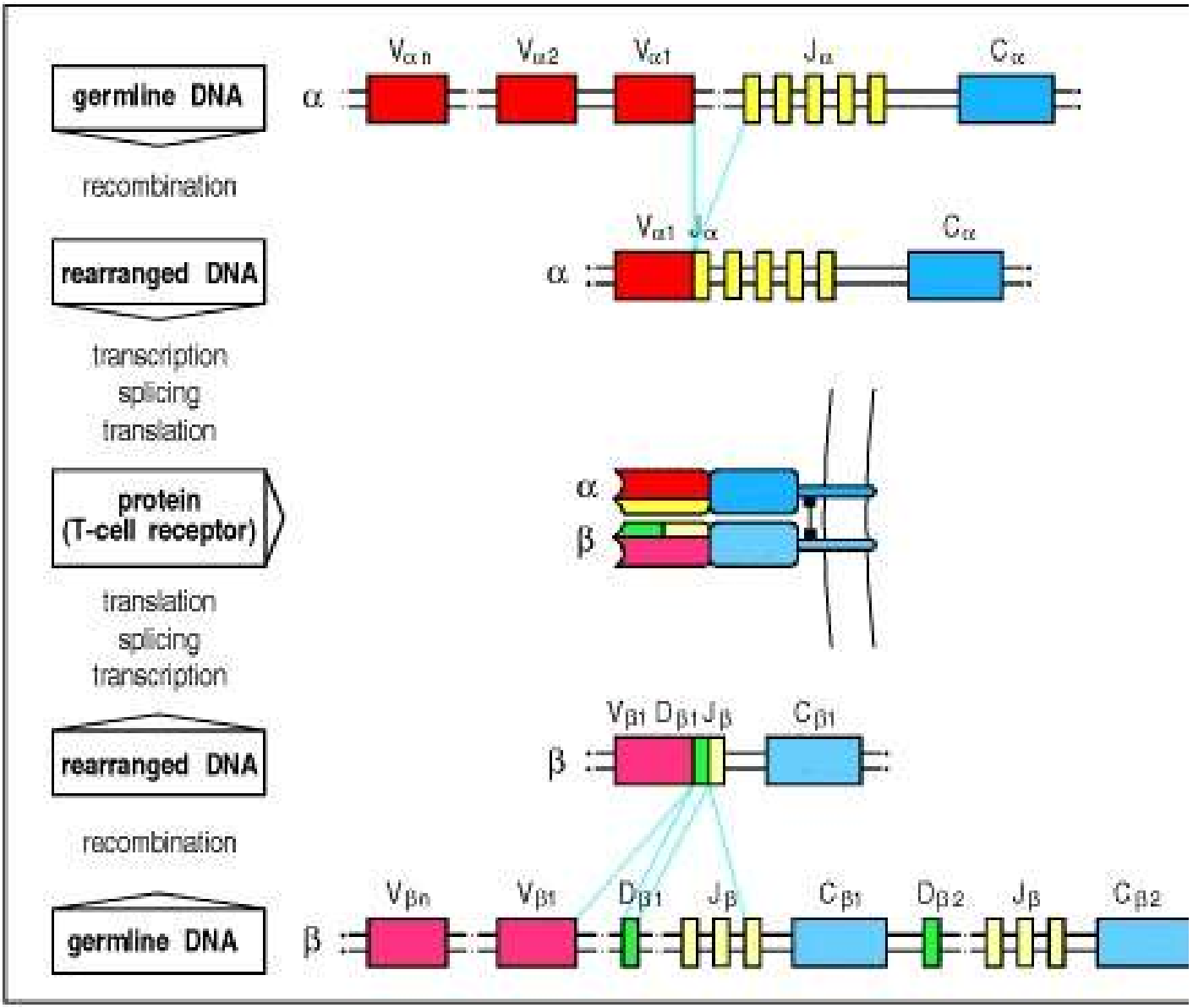
FIGURE 12-35 Recombination signal sequences recognized in V(D)J recombination. (a) Close-up of the two types of recombination signal sequences (RSSs). (Blue) The 12-bp spacer; (green) the 23-bp spacer; (light green) conserved 7-mer and 9-mer sequence elements, shared by both types of sequences. The nucleotide sequence in the spacer region is not important. The length, however, is critical. (b) Examples of RSS arrangements in the genetic regions encoding antibodies (Ig genes) and T-cell receptor proteins (TCR genes). (a, Adapted, with permission, from Bushman F. 2002. *Lateral DNA transfer*, p. 346, Fig. 11.5. © Cold Spring Harbor Laboratory Press.)

REVIEW

Watson, p. 418 [4]

Recombination always occurs between a pair of recombination signal sequences in which one partner has the 12-bp (Blue) "spacer" and the other partner has the 23-bp (Light Green) "spacer."

"These pairs of recombination signal sequences are organized as inverted repeats flanking the DNA segments that are destined to be joined". (Watson 2014)



T-Cell Receptors Recombination Similar to B-cell

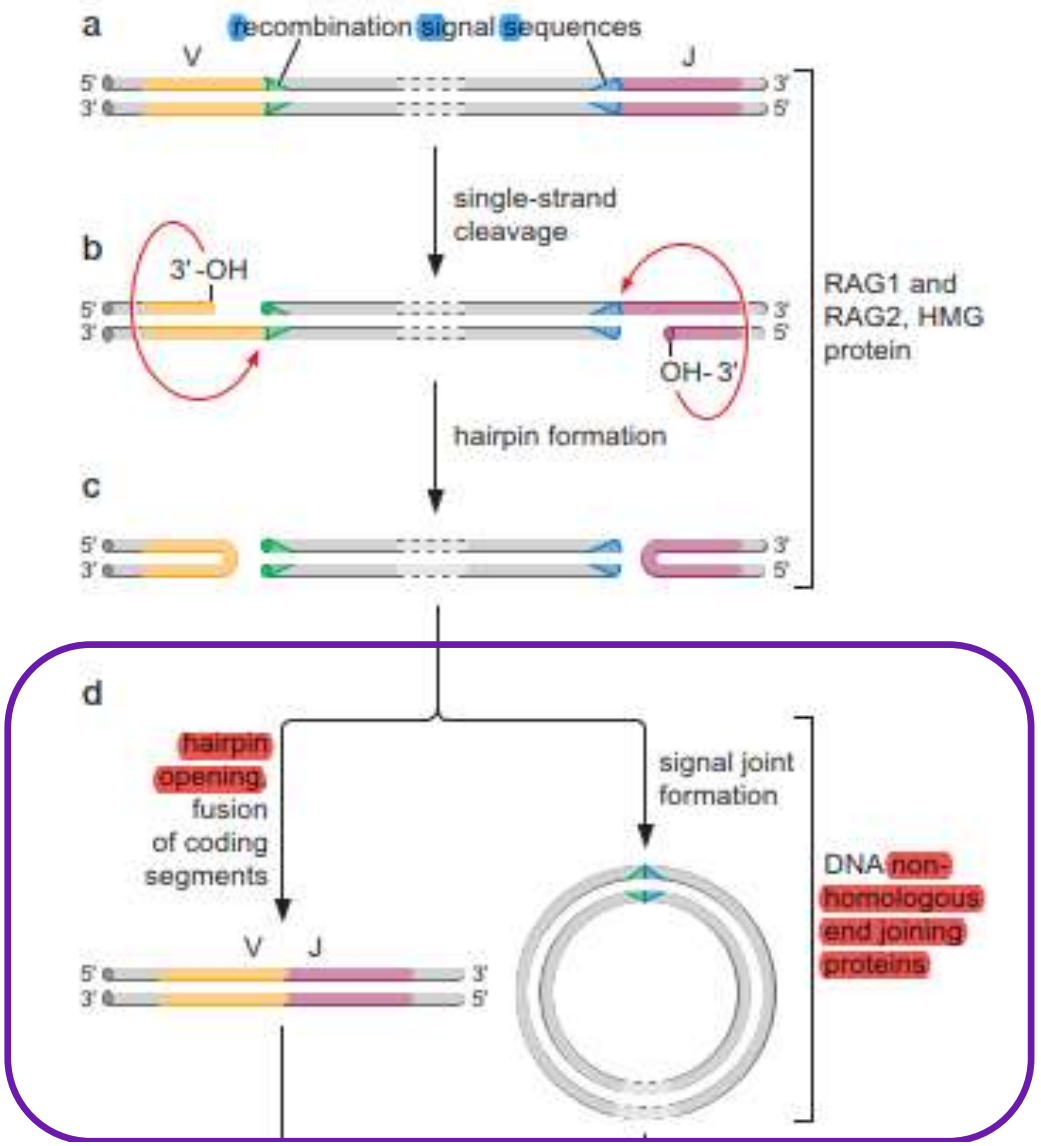
The TCRα locus, like those for the immunoglobulin light chains, contains V and J gene segments (V_α and J_α).

The TCRβ locus, like that for the immunoglobulin heavy-chain, contains D gene segments in addition to V_β and J_β gene segments.[1]

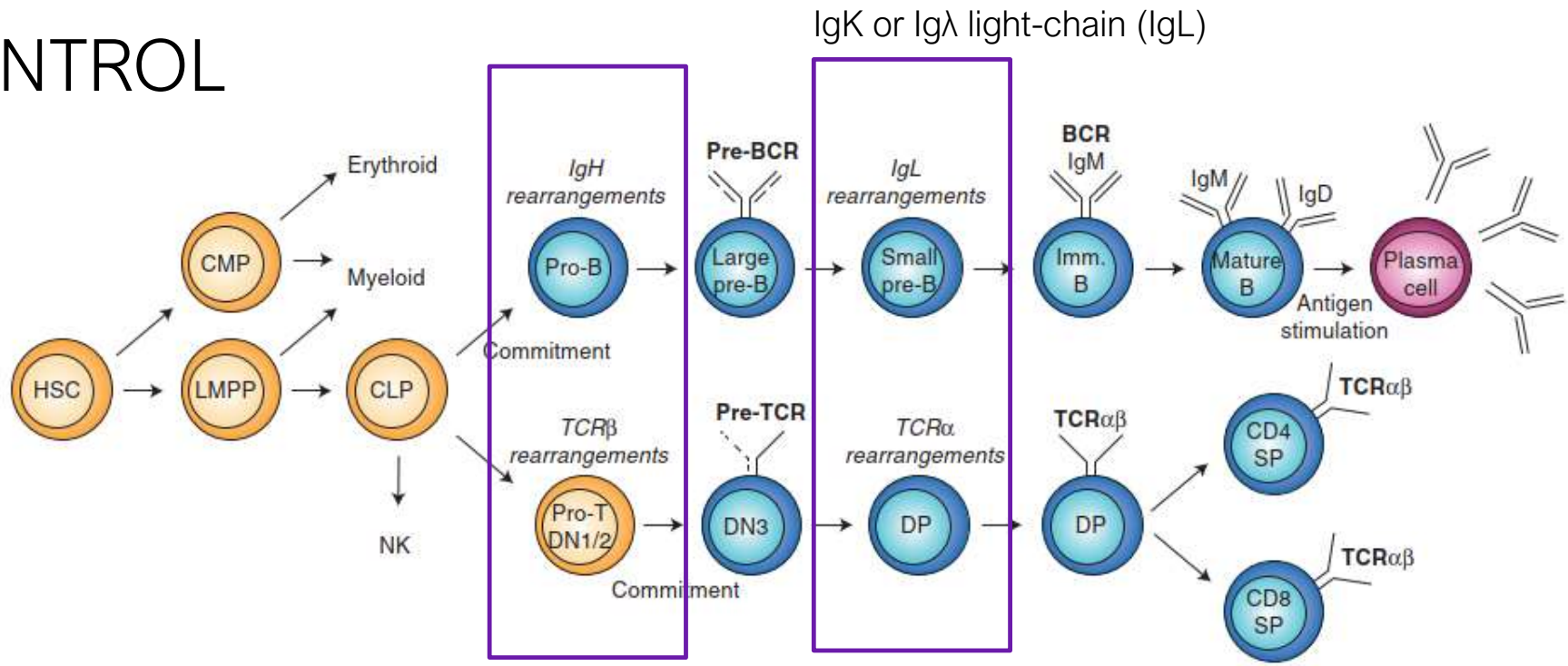
Janeway, C.A. (2001) [5]

REVIEW

Non-Homologous End Joining after hairpin opening.



CONTROL



V(D)J recombination is tightly controlled in a lineage- and stage-specific manner.

Within the B-lymphoid lineage, the IgH locus is rearranged in pro-B cells before recombination of IgK and Igλ genes in pre-B cells, whereas TCRβ and TCRα genes are rearranged in pro-T (DN) and DP thymocytes, respectively (Fig. 1)

Murine –

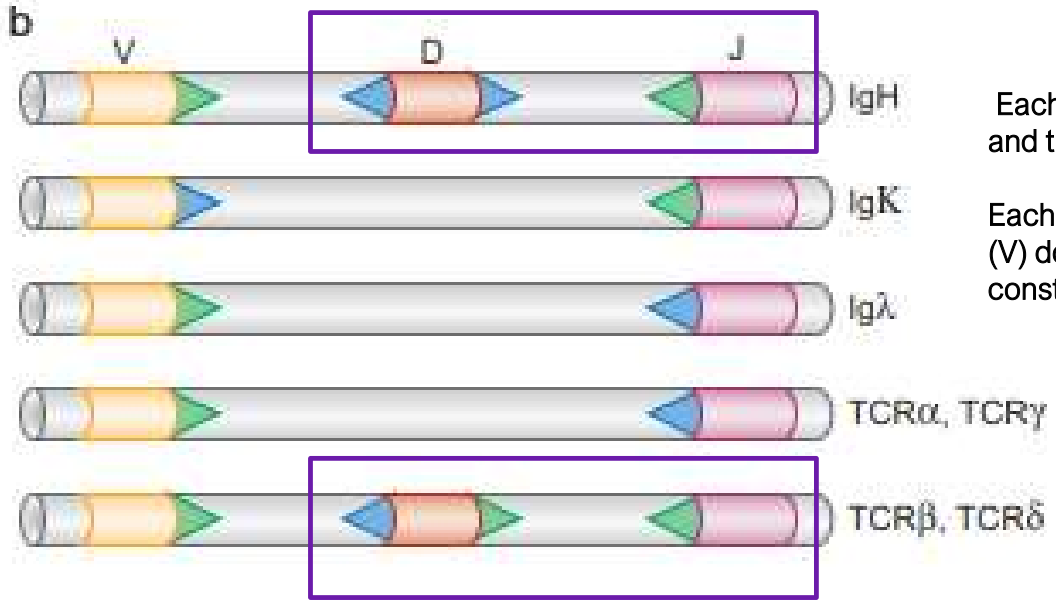
V_H: 100-200

D_H: 12-16

J_H: 4

Control mechanisms must therefore exist to shield all V genes from RAG-mediated cleavage during D-J recombination and to facilitate rearrangement of only one out of 100 V genes

Process of antigen receptor generation entirely depends on accurate regulation of the accessibility of RSSs for the RAG1/2 recombinase



Each H chain has a V domain and three to four C domains.

Each L chain has a variable (V) domain and an invariant constant (C) domain.

Epigenetic Regulation of RAG

Problem

The simplicity of the V(D)J recombination process at the DNA template level poses logistic problems for the assembly of the different antigen receptors because the RAG proteins are expressed in all immature B and T lymphocytes.

How to Solve

Regulation must be in place to restrict the access of RAG proteins to only specific subsets of all of the recombination substrates.

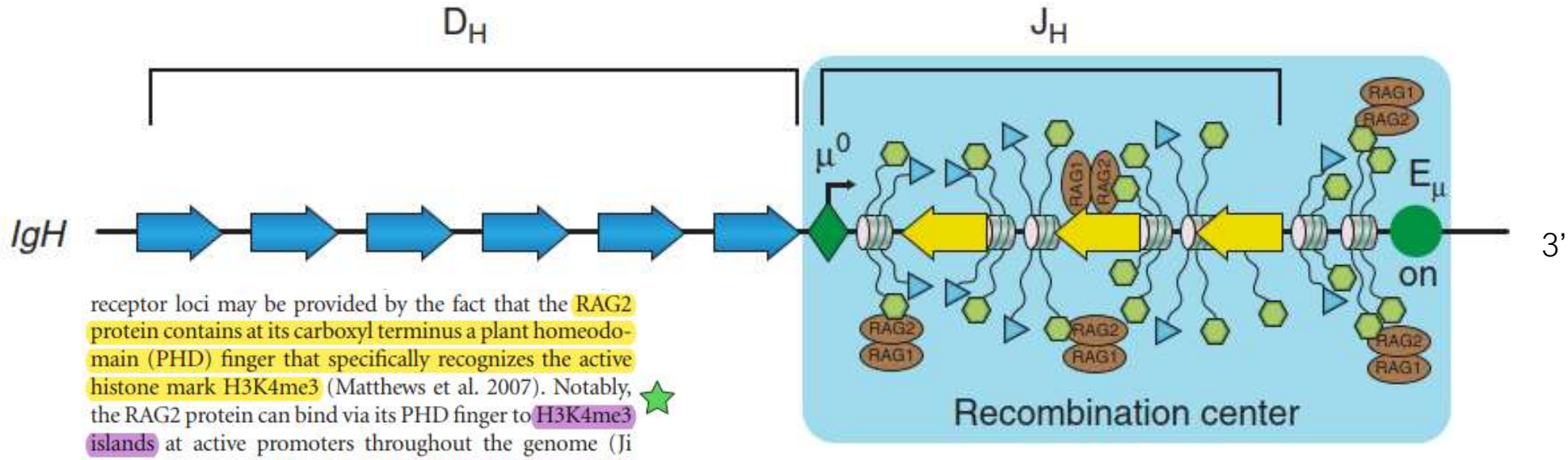
Part of Solution outside of Lymphoid Progenitors

Ig or TCR genes are present inaccessible chromatin where RAG cannot cleave.

Part of Solution in Lymphoid Progenitors

Recombinant RAG proteins added to isolated lymphocyte nuclei can only cleave the Ig or TCR gene that is actively undergoing V(D)J recombination

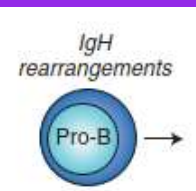
Proximal Domain



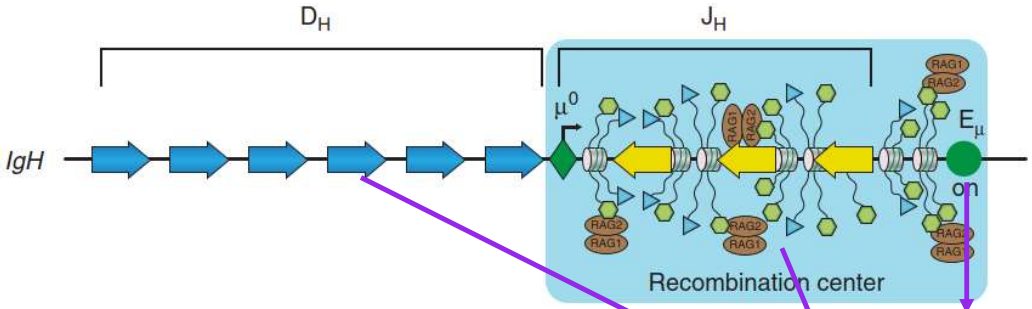
receptor loci may be provided by the fact that the RAG2 protein contains at its carboxyl terminus a plant homeodomain (PHD) finger that specifically recognizes the active histone mark H3K4me3 (Matthews et al. 2007). Notably, the RAG2 protein can bind via its PHD finger to H3K4me3 islands at active promoters throughout the genome (Ji et al. 2010). More importantly, RAG2 also binds to the H3K4me3 island at the J gene segments of the different antigen receptor loci (*IgH*, *Igκ*, *TCRβ*, and *TCRα/δ*) once they become accessible during lymphocyte development

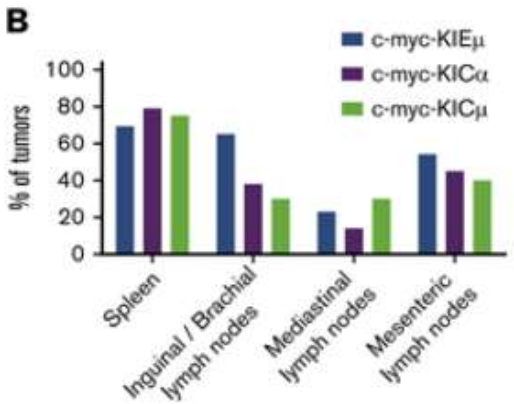
The intronic Eμ enhancer and **adjacent J_H segments** are characterized by the abundant presence of the **three active histone marks H3K4me2, H3K4me3, and H3K9ac** in pro-B cells, where the IgH locus undergoes V(D)J recombination.

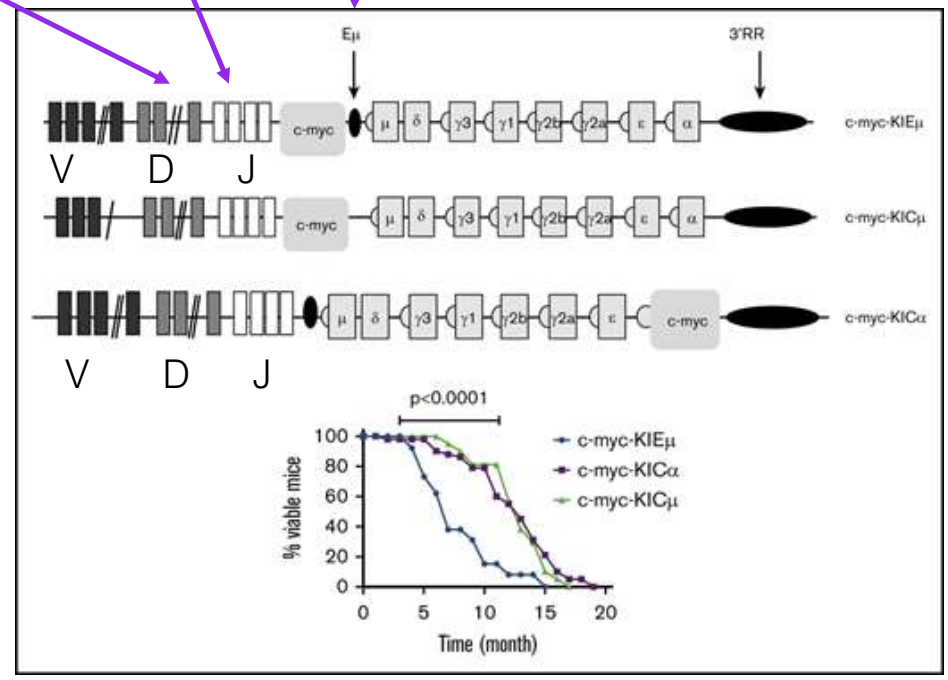
Three histone marks largely absent at V_H genes



Cancer Relation – Burkitt Lymphoma and c-Myc







Why is c-Myc oncogenenic?

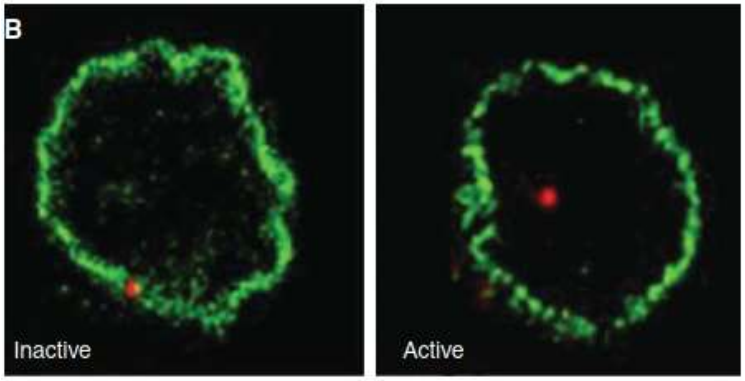
In 80% of cases, the translocation involves the IgH locus

Two potent enhancers E_u and 3' Regulatory Region

https://ashpublications.org/bloodadvances/article/4/1/28/430038/E-and-3-RR-transcriptional-enhancers-of-the-IgH

Chromatin-Mediated Function Control

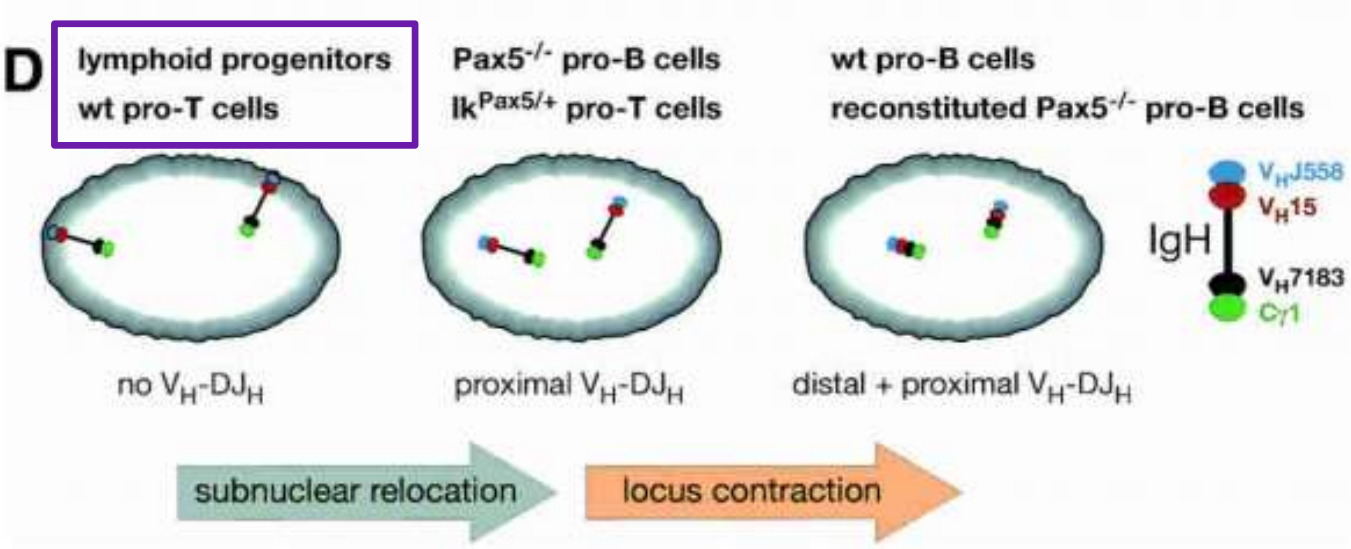
- In nonlymphoid cells, the Ig and TCR genes are present in inaccessible chromatin.
- In non-B cells and early lymphoid progenitors, the two IgH alleles are present in heterochromatic regions at the nuclear periphery and are only relocated to central euchromatic domains during pro-B cell development when V_H-DJ_H recombination takes place [7].
  - Remember from Chapter 19?



Left: EL-4 T cells
Right: 38B9 B cells

Kosak et al. (2002) [7]

Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene



http://genesdev.cshlp.org/content/18/4/411.full.pdf

"The IgH locus is thereby anchored via distal V_H genes at the nuclear periphery and is oriented with the proximal IgH domain toward the center of the nucleus, facilitating DH-JH rearrangements in lymphoid progenitors" – Martin Fuxa, Abdallah Souabni 2004, Vienna Biocenter [8]

Reconstitution of Pax5 expression in Pax5−/− pro-B cells induced large-scale contraction (Using retroviral vectors)

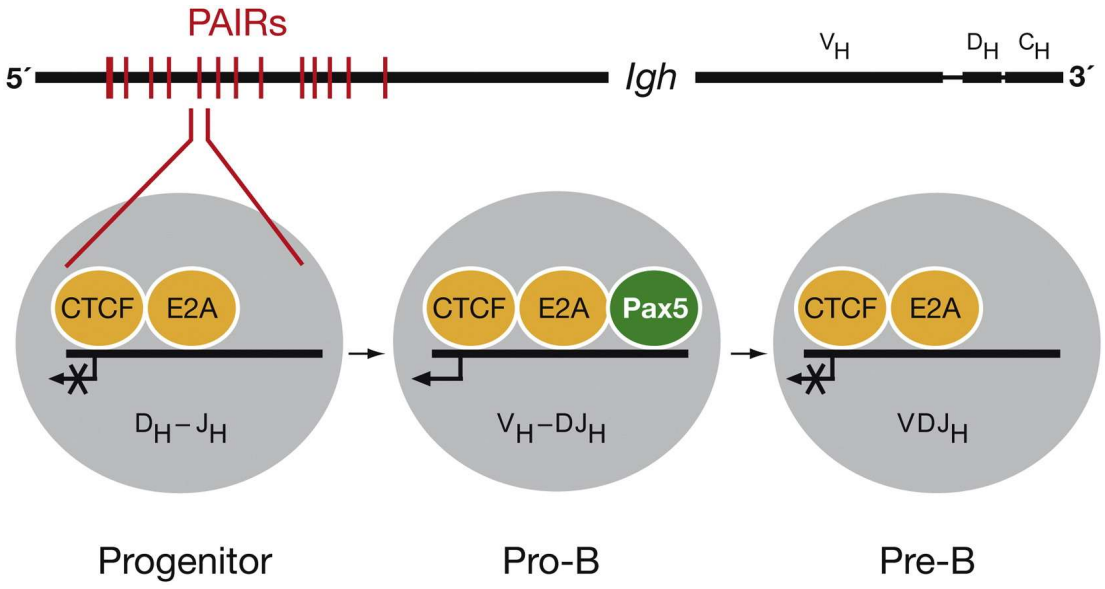
Chromatin-Mediated Function

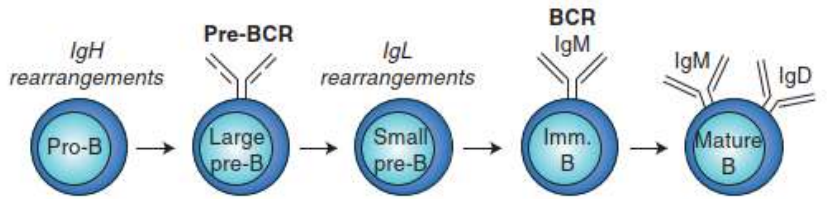
- Antisense intergenic transcription throughout the V_H gene cluster is known to precede V_H-DJ_H rearrangements at the IgH locus in pro-B cells, suggesting that these long antisense transcripts may direct chromatin remodeling of the V_H gene domain
- V_H genes do not show the active chromatin signature characteristic of expressed genes but must be accessible at the chromatin level in pro-B cells

CTCF!

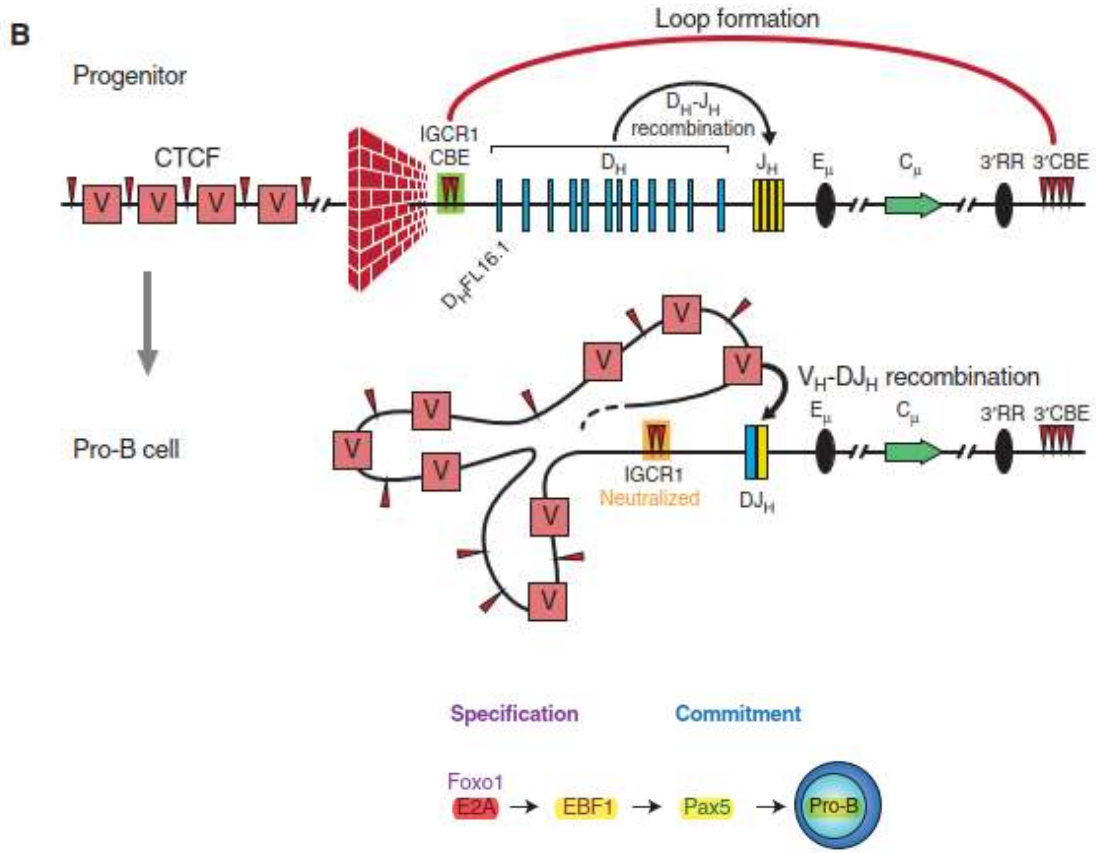
Final project spoiler alert?? 😉

The distal V_H gene cluster contains Pax5-dependent regulatory elements (PAIR)
Pax5 induces active chromatin at PAIR elements
PAIR elements display Pax5-dependent and pro-B cell-specific antisense transcription
Pax5 binds to PAIR elements only in pro-B cells in contrast to CTCF and E2A [9]



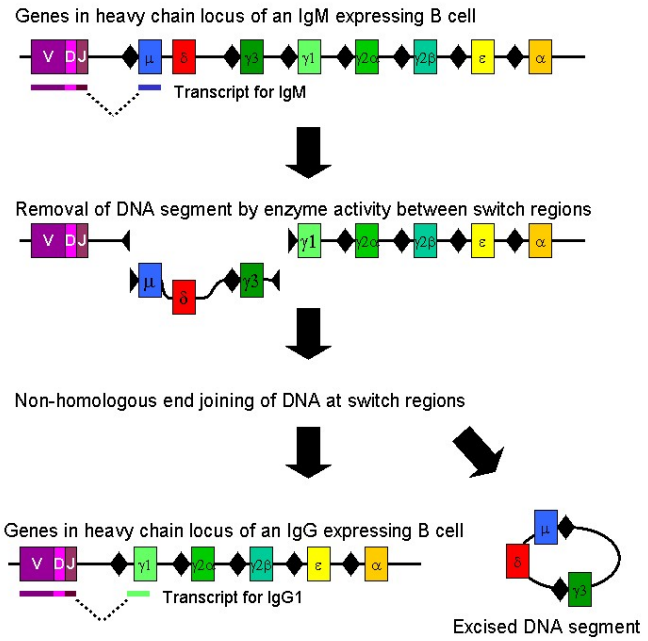


CTCF and Intergenic Control Regions (IGCR)



Class Switch Recombination

Constant regions are as constant as their names make them out to be.



Sources

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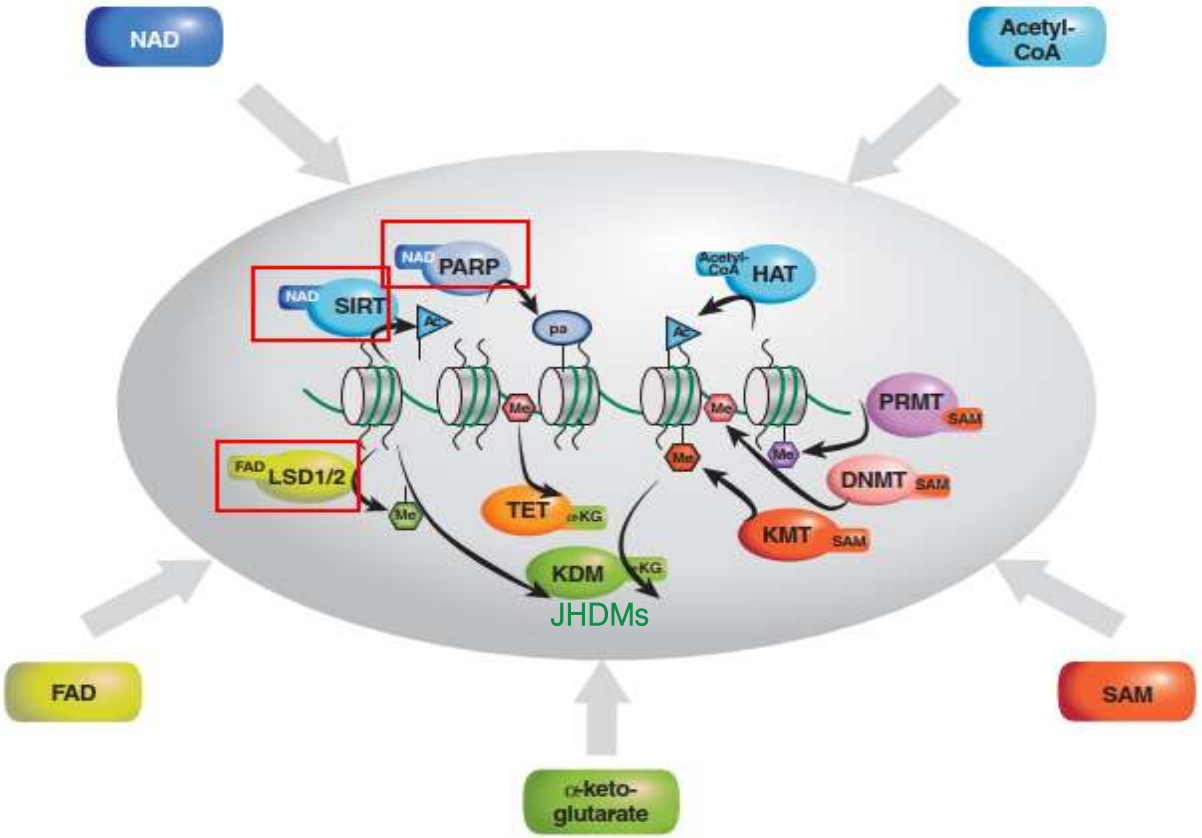
8. Fuxa M, Skok J, Souabni A, Salvagiotto G, Rolda´n E, Busslinger M. 2004. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. Genes Dev 18: 411–422.
9. Ebert A, McManus S, Tagoh H, Medvedovic J, Salvagiotto G, Novatchkova M, Tamir I, Sommer A, Jaritz M, Busslinger M. 2011. The distal VH gene cluster of the Igh locus contains distinct regulatory elements with Pax5 transcription factor-dependent activity in pro-B cells. Immunity 34: 175–187.

Chapter 30 – Metabolic Signaling to Chromatin

NOT DONE YET!?!


NAD
Acetyl-CoA
NAD
PARP
NAD
SIRT
Ac
pa
Acetyl-CoA
HAT
Ac
Me
Me
Me
Me
PRMT
SAM
FAD
LSD1/2
Me
TET
αKG
DNMT
SAM
KMT
SAM
KDM
αKG
JHDMs
FAD
SAM
α-keto-glutarate

Acetyl-CoA

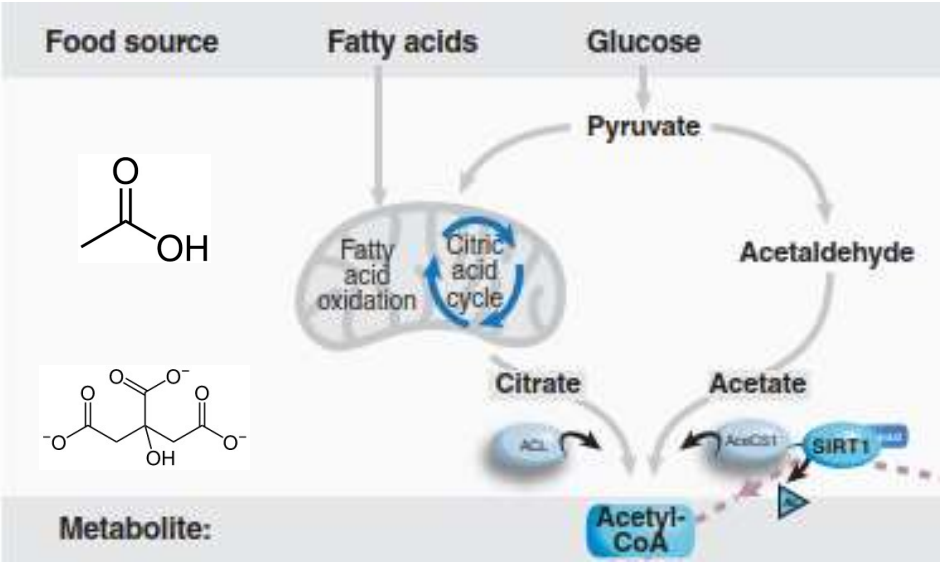
Knew:

Mitochondrial Pool – PDC and Fatty Acid Oxidation

Did not know:

Nuclear/Cytosolic Pool – 2 Enzymes:

- acetyl-CoA synthetase 1 (AceCS1) (Acetate)
- ATP-citrate lyase (ACL) (Citrate from TCA Cycle)



Interestingly HDAC Class 1/2 can produce Acetate

Cycle: 1) HDAC deacetylate 2) Acetate used by AceCS1 to synthesize acetyl-CoA 3) acetyl-CoA used to acetylate Histones

Acetyl-CoA

Loss of AceS1 or ACL leads to reduction in global histone acetylation

- Supplementing acetate for AceCS1 can overcome loss of ACL
- Both influence chromatin remodeling.
- AceCS1 is acetylated – event controlled in cyclic manner by SIRT1

SIRT1 can increase histone acetylation??????? Indirectly???

- Acetylated form Lys-661 of AceCS1 is inactive
- SIRT1 deacetylation of Lys-661 in AceCS1 activates

- acetyl-CoA synthetase 1 (**AceCS1**) (Acetate)
- ATP-citrate lyase (**ACL**) (Citrate from TCA Cycle)

CONNECTION TIME!!

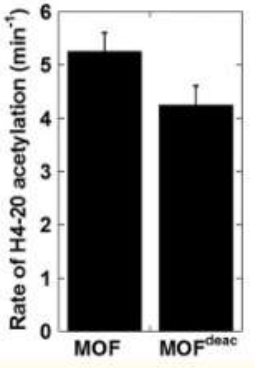
- SIRT1 least specific
- In Yang et al 2012 from Lecture 4 under their Experimental Procedures they used 15 μM Sirt1 and 7.5 mM NAD^+ to deacetylate MOF autoacetylation

Table 2. Sirtuin histone substrates

Sirtuin	Histone substrate
SIRT1	H3K9
	H3K14
	H3K56
	H4K16
	H1K26
SIRT2	H4K16
	H3K56
SIRT3	H4K16
SIRT4	None
SIRT5	None
SIRT6	H3K9
	H3K56
SIRT7	H3K18

"In this study, we were able to produce MOF in the completely deacetylated form by treatment with Sirt1/NAD^+" Yang et al (2012)[1]

"Interestingly, both the autoacetylation activity and the histone acetylation activity of the deacetylated MOF were found to be very close to that of wild-type MOF, ***suggesting that autoacetylation of MOF only marginally modulates the enzymatic activity****." (Yang et al. 2012)*

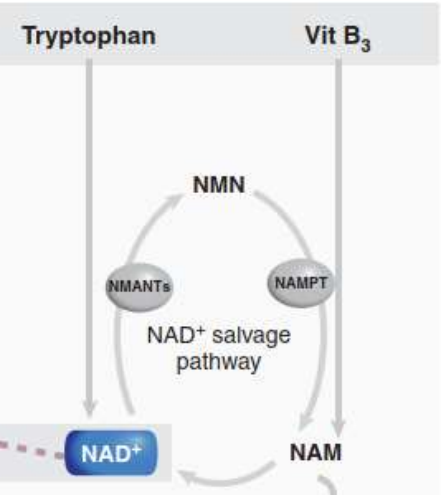


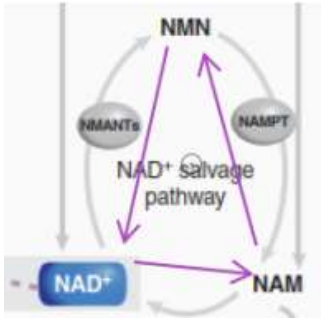
"These data gave strong evidence that acetylation of Lys-274 does not significantly alter MOF activity, and deacetylation at this lysine residue does not abrogate MOF activity."

Fig 4C https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3471714/

NAD+ Deacetylase Sirtuins

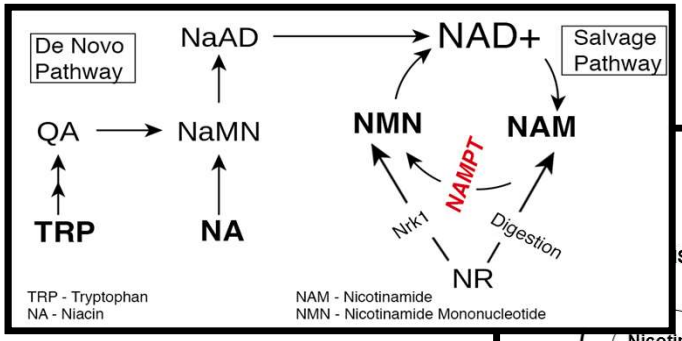
Do they have it backwards?





NAD < NMN < NAM < NAD

NAD > NMN > NAM > NAD



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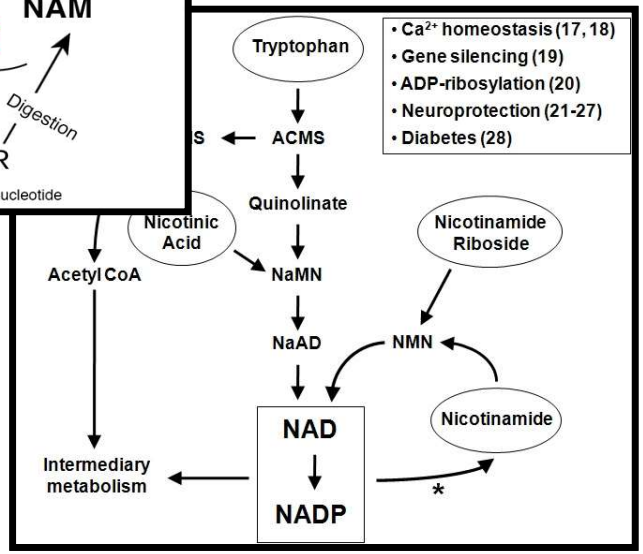


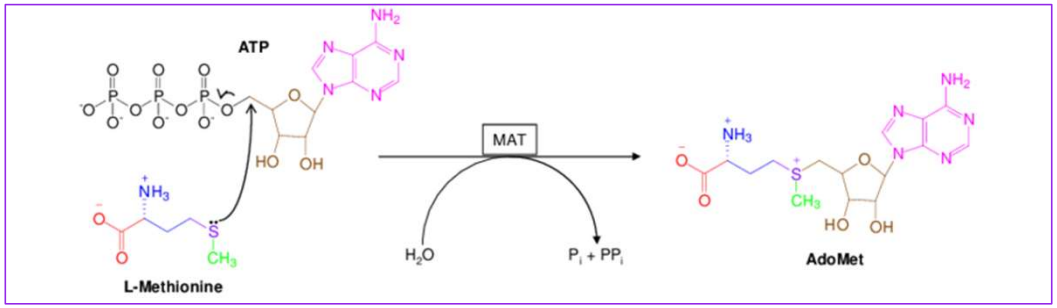
Taken from https://www.fbsserver.org/2008/v13/af/3143/fig1.jpg

NAD+

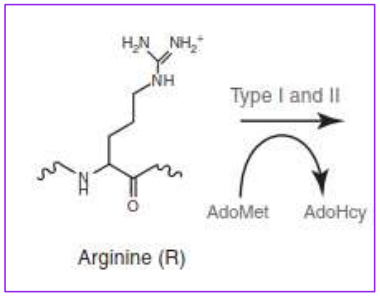
Sirtuins and PARPs

SAM - DNMT/KMT/PRMT

ATP is source or SAM. Low levels of ATP can therefore influence SAM ability.

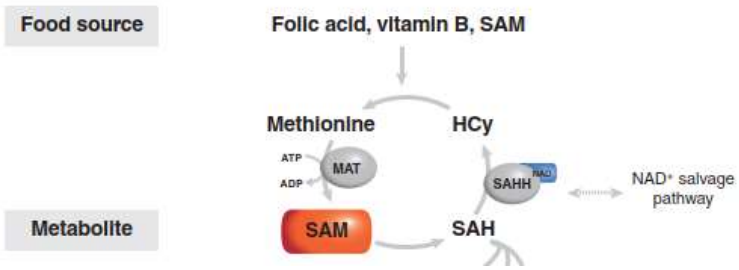


Sufrin et al (2009)

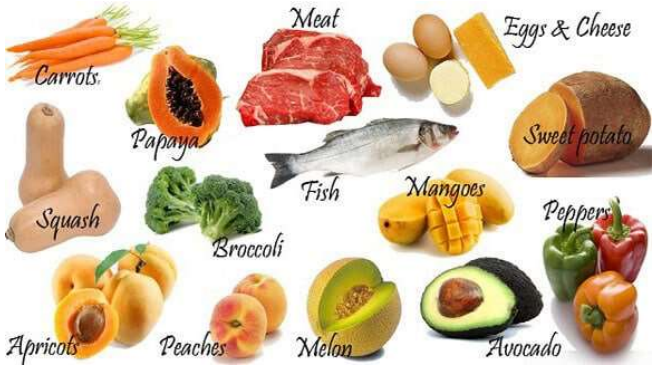


After Methyl group is donated becomes S-adenosyl homocysteine --------| Methyltransferases.

Food Sources



B Vitamins



Foods High in Folate

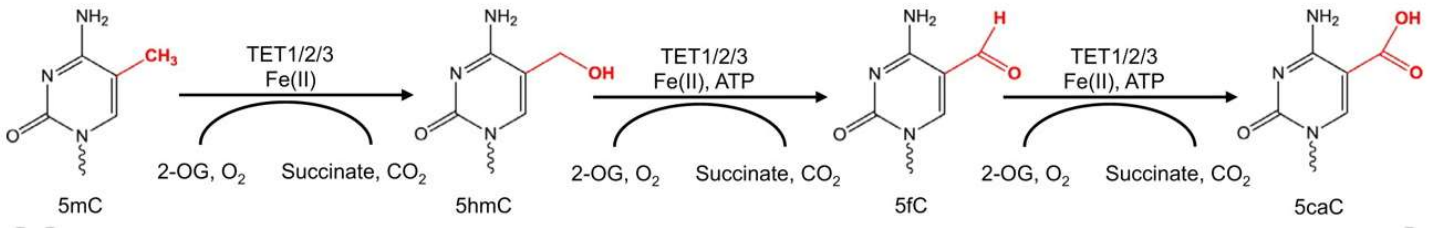
FAD - Demethylase

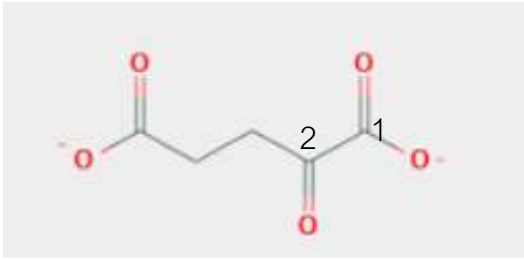
LSD1 and LSD2 use FAD

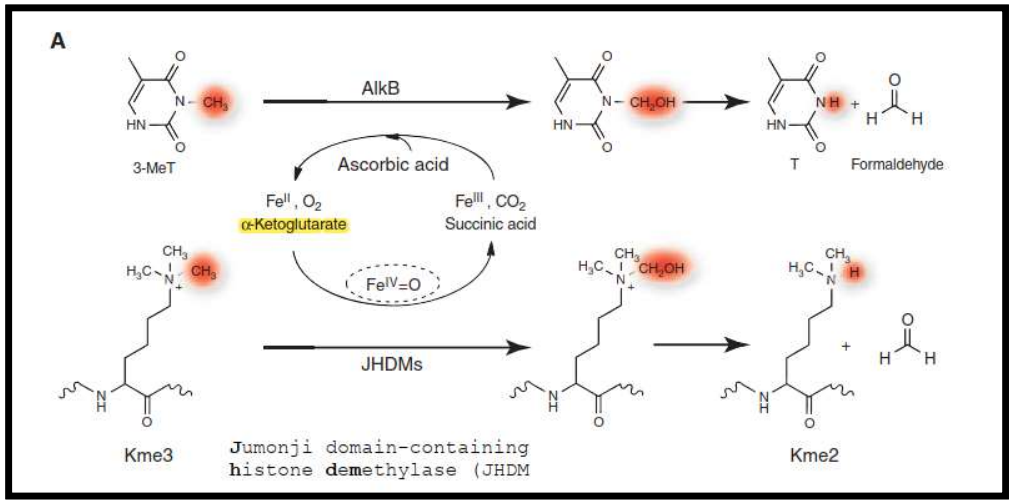
Jumonji Family use Fe(II) and α-ketoglutarate

LSD1 enzymatic activity depends on protein kinase Cα (PKCα)-dependent phosphorylation. PKCa responds

α-ketoglutarate (2-oxoglutarate)







https://dev.biologists.org/content/139/11/1895

α-ketoglutarate (2-oxoglutarate)

Connection Time!!!

https://dev.biologists.org/content/139/11/1895

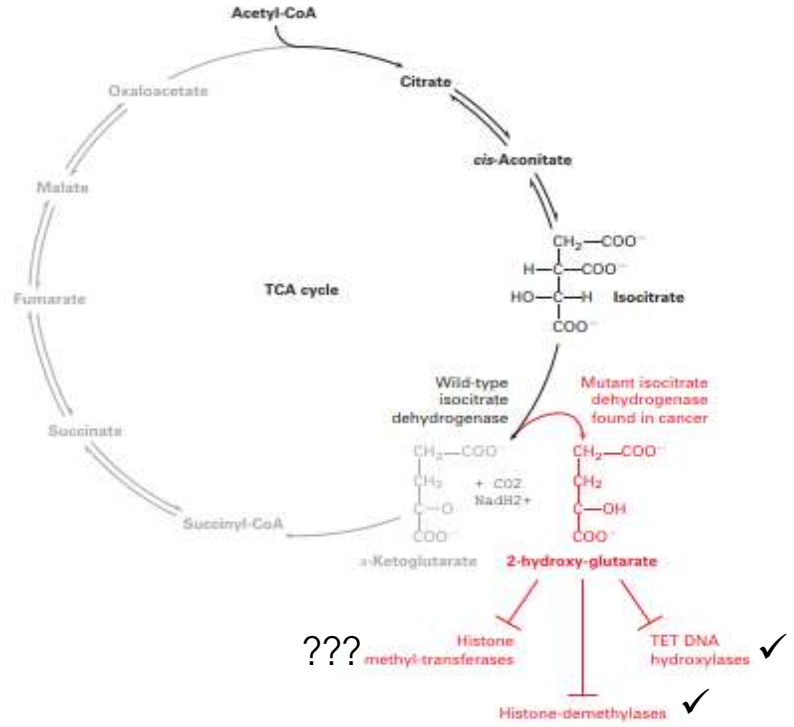
Feinberg – CD113 [2]

by a small population of cells with stem cell proper-ties[72,81,82]. This can be demonstrated by serial grafting of selected tumour-cell populations: for example, only CD113-positive cancer cells (CD113 is a cell-surface marker for self-renewing brain stem cells and early progenitor cells) were able to produce tumours that could be serially grafted into non-SCID mice[82]. In addition, all grades of astrocytoma show stem cell characteristics, whereas early pre-symptomatic lesions reside within a region of the brain, the subventricular zone of the lateral ventricle, that contains neurogenic stem cells[83].

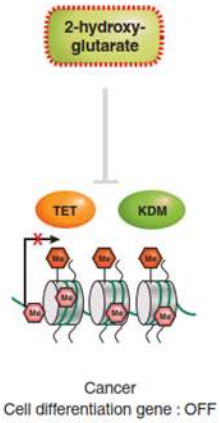
Lodish, Advanced Cell Bio II [3]

Not only do cancer cells rewire their metabolic pathways, but some cancer types produce novel metabolites that play a critical role in the disease. Seventy percent of glioblastomas, oligodendrogliomas, and astrocytomas (all brain cancers) and approximately 25 percent of acute myeloid leukemias harbor mutations in isocitrate dehydrogenase (IDH), a TCA cycle enzyme that converts isocitrate to α-ketoglutarate (Figure 24-4). The IDH mutations found in these cancers cause the enzyme to convert isocitrate into a new metabolite, 2-hydroxyglutarate, which accumulates to levels of up to 5–35 mM in cancer cells! So how does 2-hydroxyglutarate promote tumorigenesis? It inhibits several enzymes that require α-ketoglutarate for their function, including proteins that regulate the methylation state of histones. In this way, 2-hydroxyglutarate alters gene expression. Whether 2-hydroxyglutarate is the only example of a cancer-specific metabolite or whether it is but the first in a new class of *oncometabolites* remains to be seen.

↑2-hydroxy-glutarate ↓ Active DNA Demethylation
↑Promoter DNA CH_3 in Differentiated Associated Genes
↓ Histone Demethylation
↑ Histone Methylation = "repression of the inducible expression of lineage-specific differentiation genes and a block to differentiation" – Lu, et al. (2012) [4]

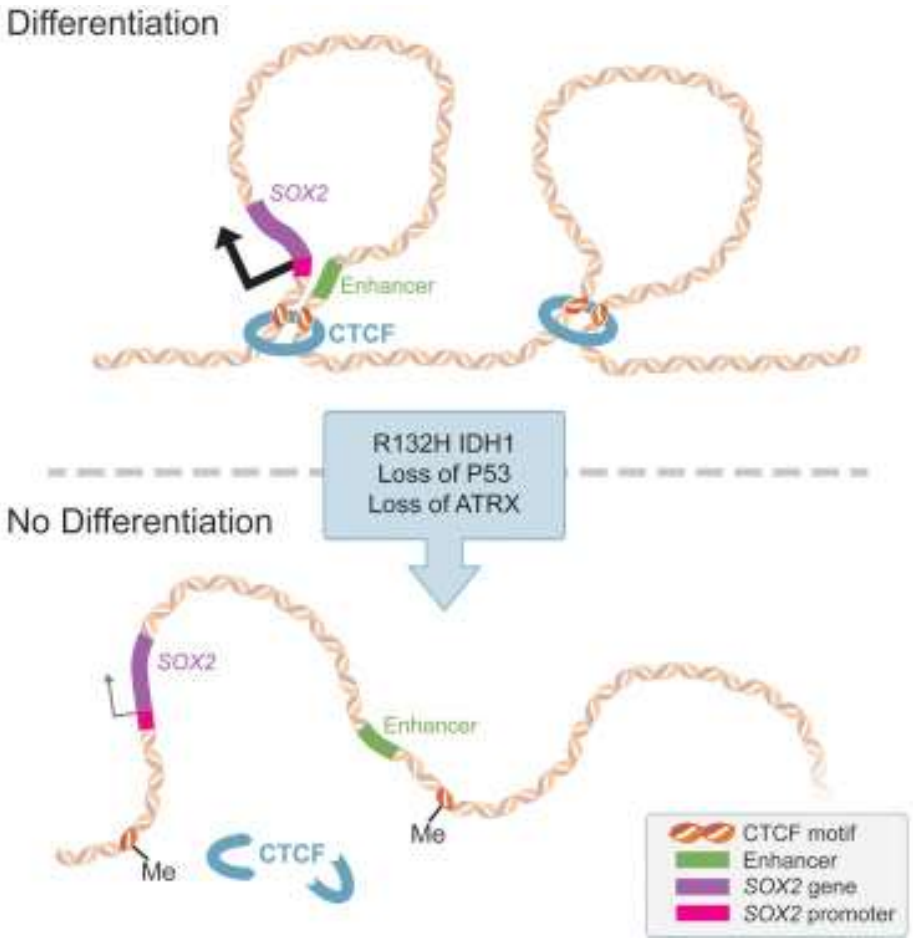


One effect of DNA hypermethylation is blockade of cell Differentiation" – Bledea, et al. (2019) [5]



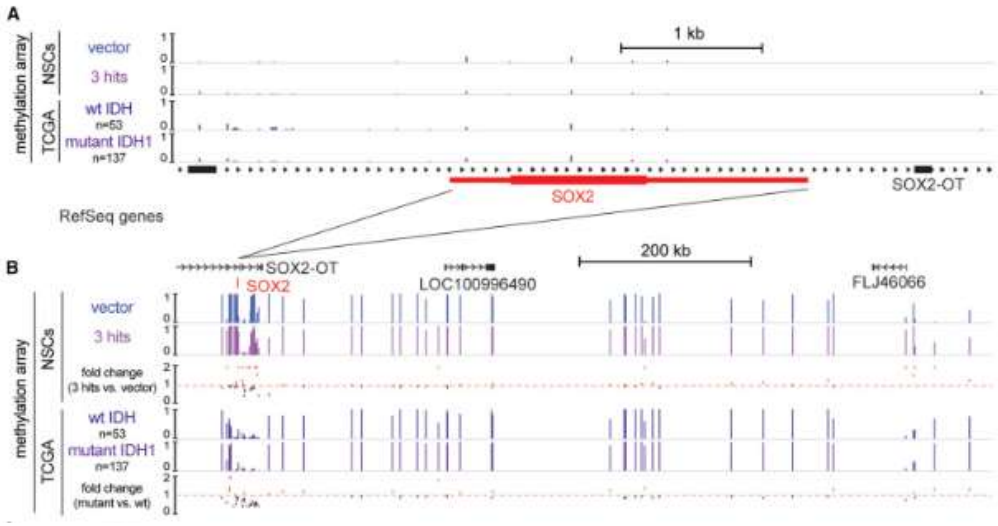
https://www.nature.com/articles/s41598-019-53262-7 [5]

https://www.nature.com/articles/nature10860 [4]



Low-Grade Astrocytoma Mutations in IDH1, P53, and ATRX Cooperate to Block Differentiation of Human Neural Stem Cells via Repression of SOX2 [6]

Illumina 450K Methylation Array



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