2019台灣胸腔暨重症加護醫學會年會

THE ROLE OF EPIGENETICS IN HUMAN IMMUNE RESPONSES AGAINST *MYCOBACTERIUM TUBERCULOSIS* INFECTION

表觀遺傳變異在人類抵抗結核菌的免疫反應中所扮演的角色

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日期：2019年12月07日(星期六)上午09:20~10:00
地點：高雄展覽館 3F 301 B

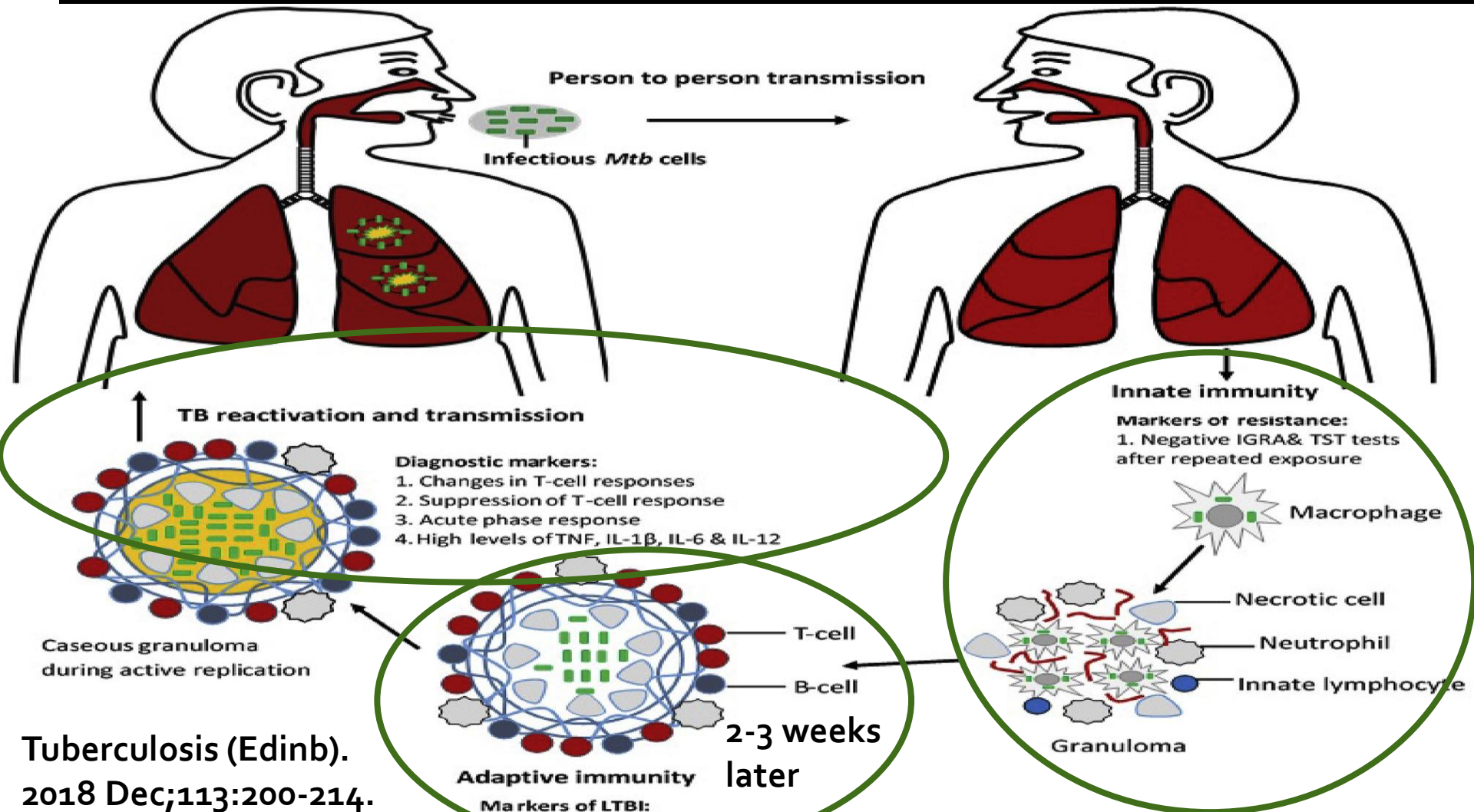
長庚 高雄

I have No commercial interest.

Contents

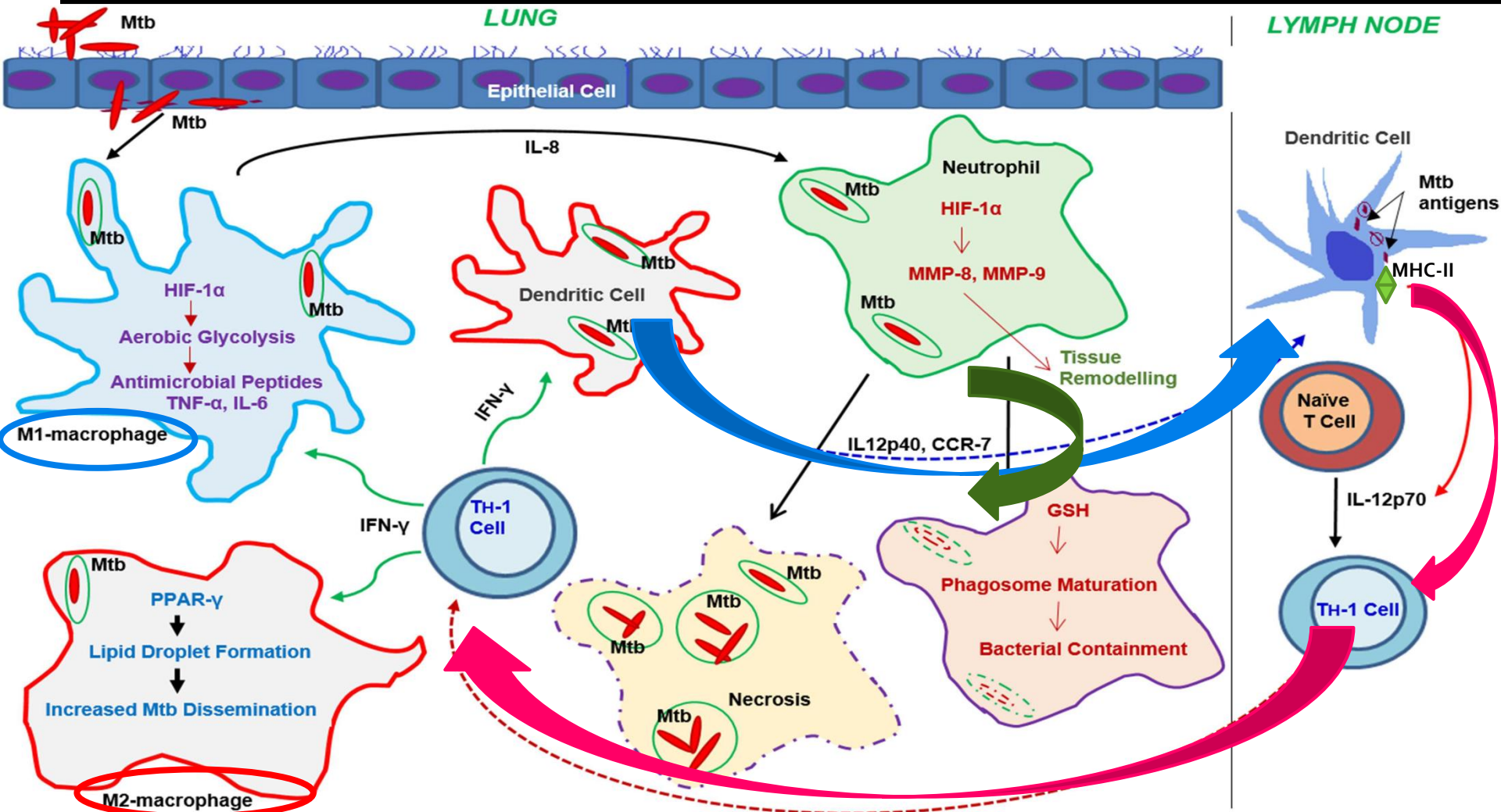
- Epigenetics-mediated immune responses in patients with active TB disease and macrophage infected with *M.TB* in vitro
  - Aberrant DNA methylation: global/gene-specific
  - Histone modification patterns and histone modifying enzymes
  - Non-coding RNA: microRNA
- Clinical application
  - Epigenetic predictors for BCG responders
  - Host-directed immunotherapy
  - Future perspectives

Mycobacterium tuberculosis (MTB) elicits the host innate immune response by recruiting macrophages/neutrophil, followed by adaptive immune response, mainly comprised of T-cells.



Tuberculosis (Edinb). 2018 Dec;113:200-214.

Dendritic cell (DCs) infected with Mtb migrate to the draining lymph nodes, driving Th-1 cell differentiation. The activated Th-1 cells migrate back to the lungs, producing IFN-γ and TNF-α, which activate macrophages leading to bacterial clearance. Front. Mol. Biosci.2019; 6:105.



Which factors play a major role in determining TB susceptibility or resistance?

- *Mycobacterium TB*:
  - rpf genes (rpfA-E), cAMP receptor proteins, Clp protease gene regulator (Rv2745c;clgR), DosR regulon,
  - Drug-Resistant genes: rpoB, katG , inhA, rrs, tlyA, gyrA or gyrB, atpE gene, Rv3547
- Comorbidities: HIV-TB co-infection, Diabetes, Malnutrition, Smoking and alcohol
- Environmental factors: drugs, toxin, diet, exercise, emotion, stress
- Host Genetic and Epigenetic factors: immune competence

Tuberculosis (Edinb). 2018 Dec;113:200-214.

As in several other human diseases, the role of common genetic variants in pulmonary TB seems to be scarce.

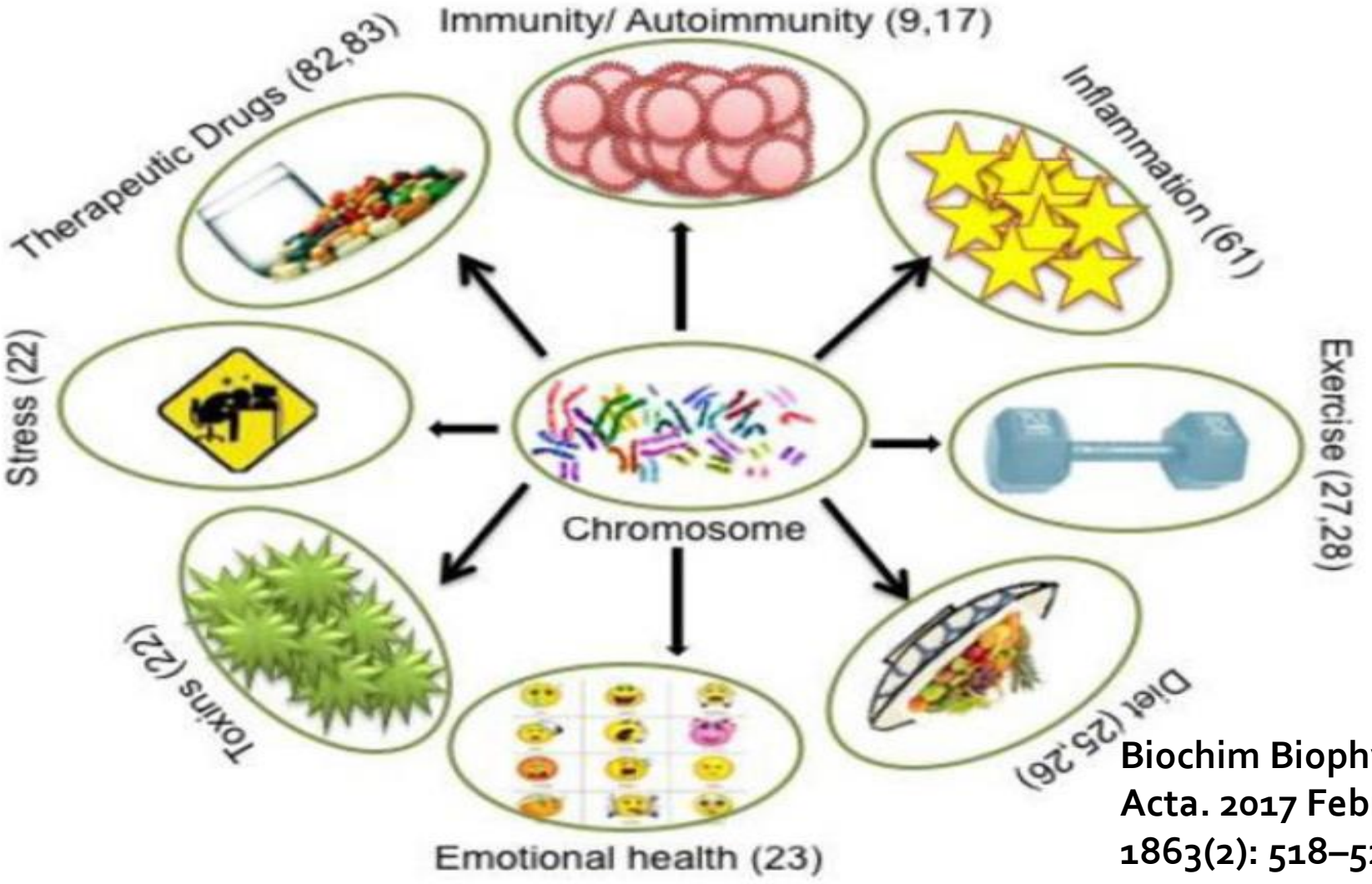
Lancet Infect Dis 2018;18: e64–75

- Most patients with latent TB infections (90–95%) never develop clinical disease.
- In household studies, 30–50% of contacts with heavy short term exposure do not become infected.
- Twins study under comparable environmental exposure and social conditions
  - Higher concordance of TB in monozygotic than in dizygotic
  - Heritability of TST response at 71% and IGRA response at 39%
- Independent gene association studies: an absence of consistency and replication
- GWAS: Common variants might have a little effect on individual predisposition to adult pulmonary TB, at least when considered as a single homogeneous phenotype.

A real challenge is to associate candidate genetic variants with a biologically plausible mechanism that explains the epidemiological data for TB in which only 10% of the infected individuals will develop active TB.

Meta-Analysis	Odds Ratio	Number of studies	Risk of active TB	Race - dependent
mannose-binding lectin gene (MBL2)	0.42-2.7	22	Decreased or increased	Africans, Americans, Europeans
vitamin D receptor (VDR)	0.87-0.92	54	Decreased	South Asians, Caucasians
Interleukin-10/17/18 (IL-10/17/18)	0.53-1.37/0.64-1.36/1.17-1.43	28/11/8	Decreased or increased	Asians, Caucasians.
Toll-like receptor (TLR) 1/2/6	0.61-5.82	16	Increased or decreased	Asians, Europeans, Africans, American Hispanics.
TNF-α		31	Increased	East Asians
IFN-γ/IFNGR1	1.51-1.56/1.24	19/6	Increased	Africans
HLA-Class II: DRB1/DQB1/DQA1	0.5-2.27	11	Decreased or increased	Caucasians

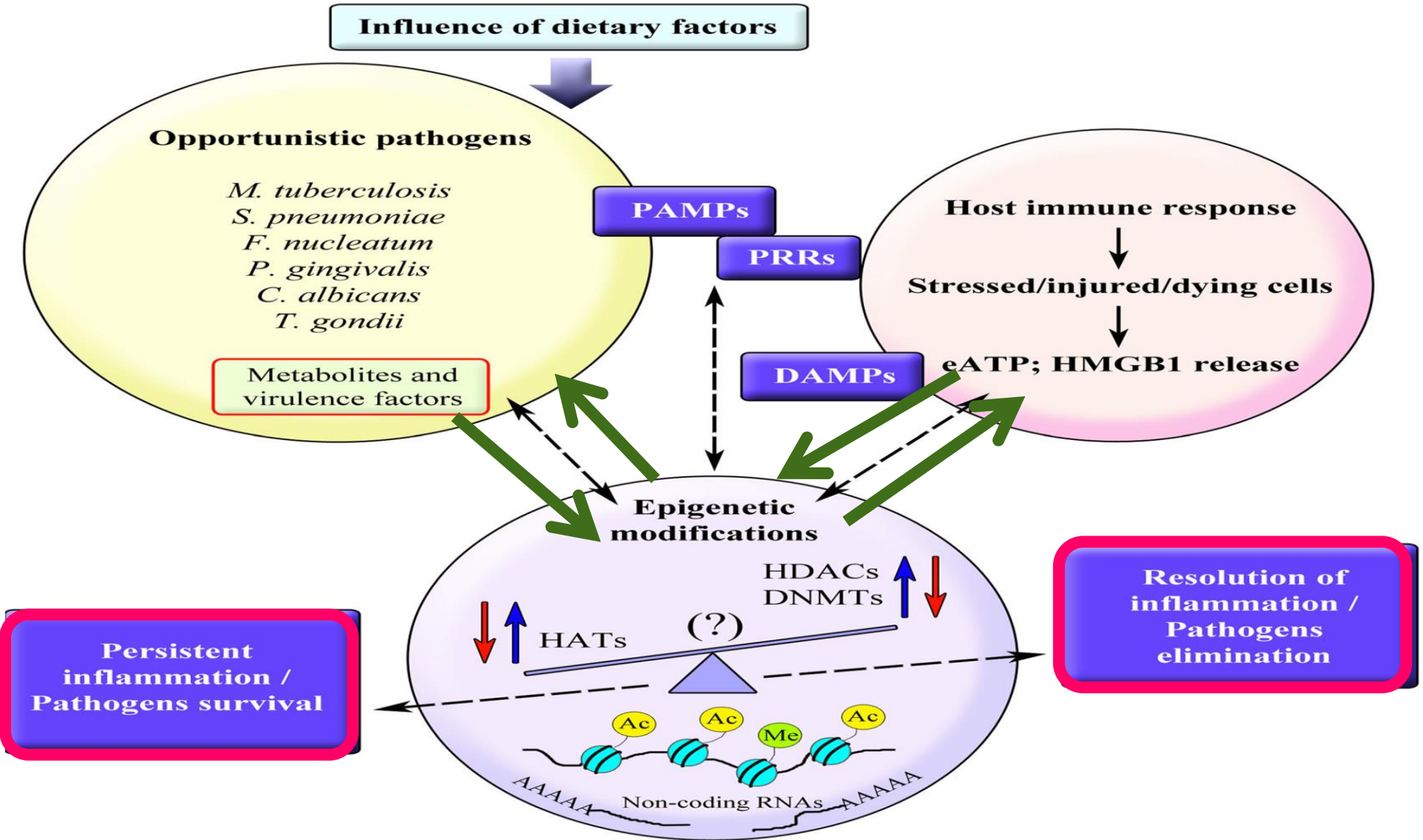
Environmental factors and epigenetic modulation in humans: Various sources presents in the environment regulates epigenetic parameters on humans.



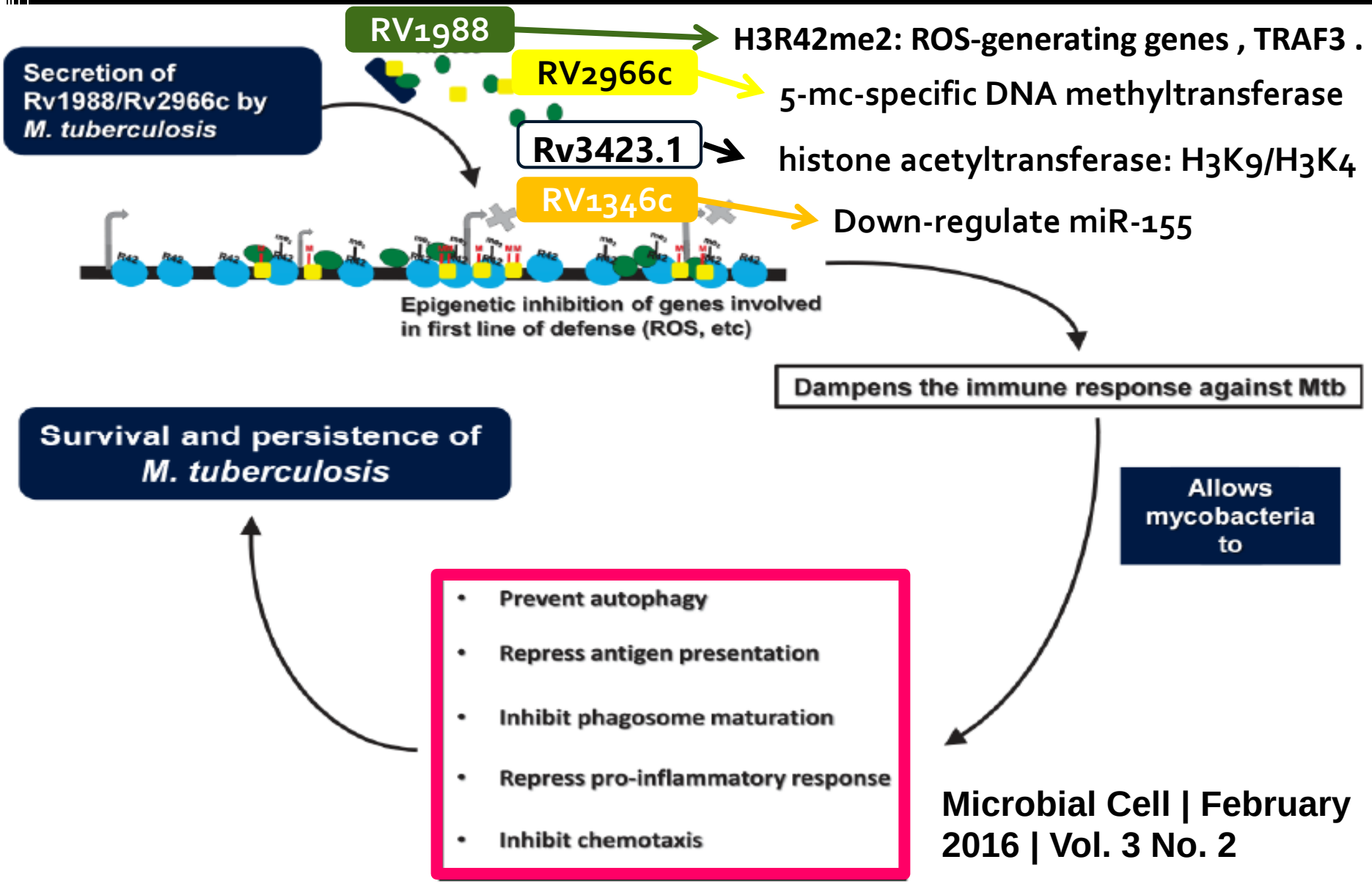
Biochim Biophys Acta. 2017 February ; 1863(2): 518–528.

Bi-directional relationship between epigenetic modifications induced by colonizing pathogens / the host immune response.

Pathogens and Disease, 74, 2016, ftw082



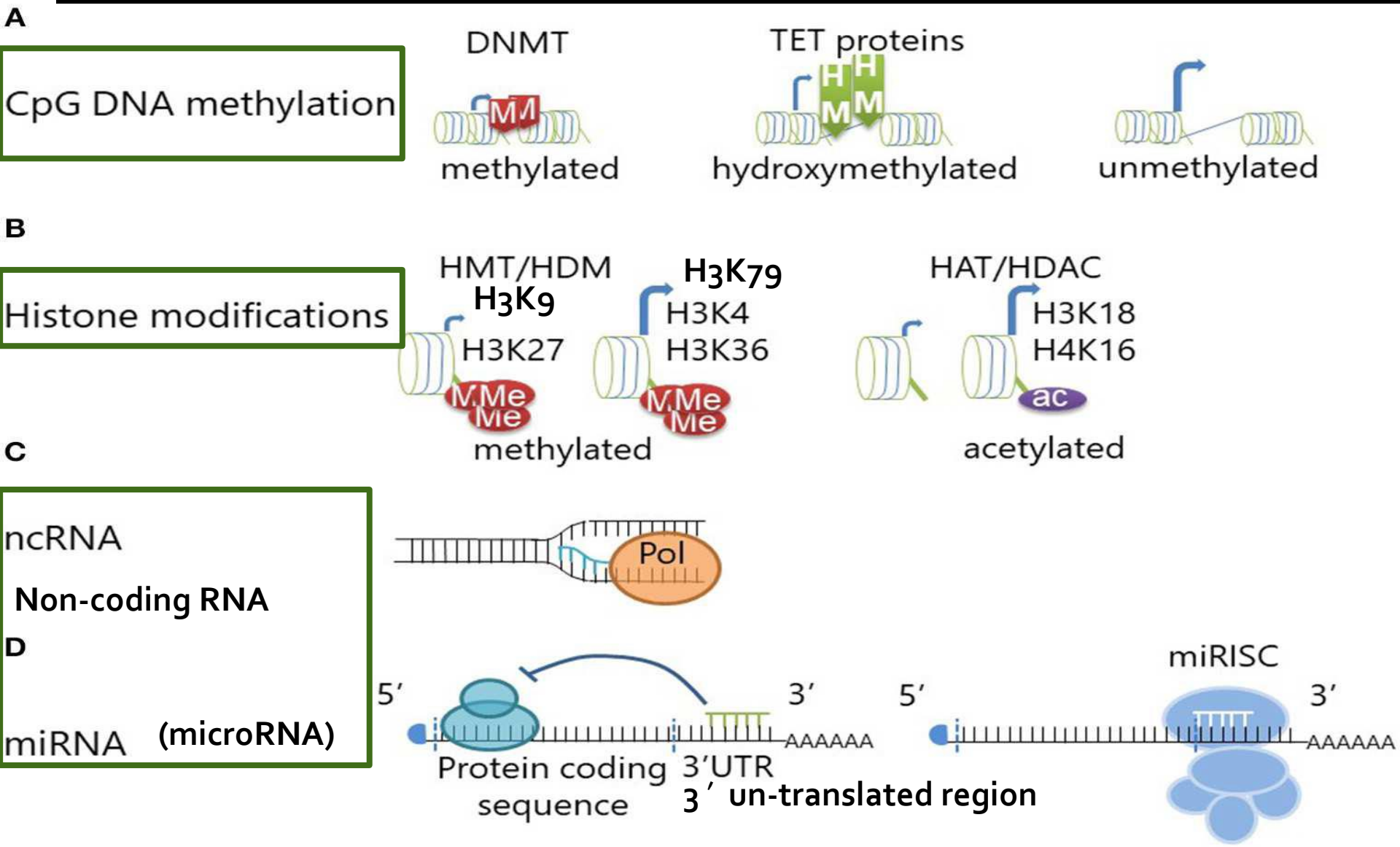
M. TB uses Rv1988/Rv2966c/Rv3423.1/RV1346c to hijack the host transcriptional machinery.



Microbial Cell | February 2016 | Vol. 3 No. 2

Epigenetics refers to the regulation of gene expression not caused by underlying changes in DNA sequence. Heritable and Reversible.

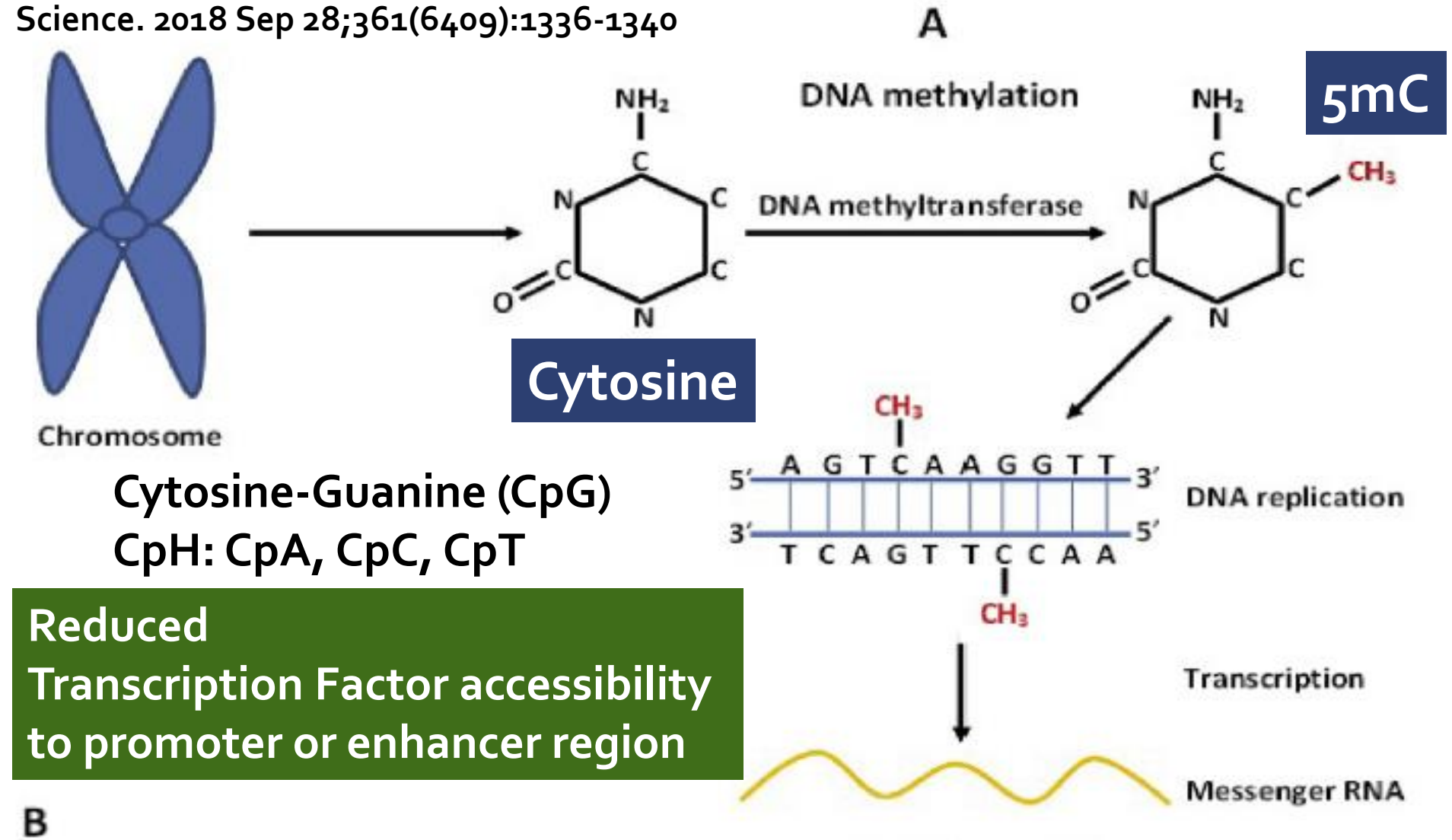
Frontiers in Immunology July 2019 | Volume 10 | Article



Aberrant DNA methylation
of
the immune-related genes
in active TB disease or
in response to *M.tb* infection

Methylation of DNA promoter or enhancer regions generally results in transcriptional silencing or repression.

Science. 2018 Sep 28;361(6409):1336-1340

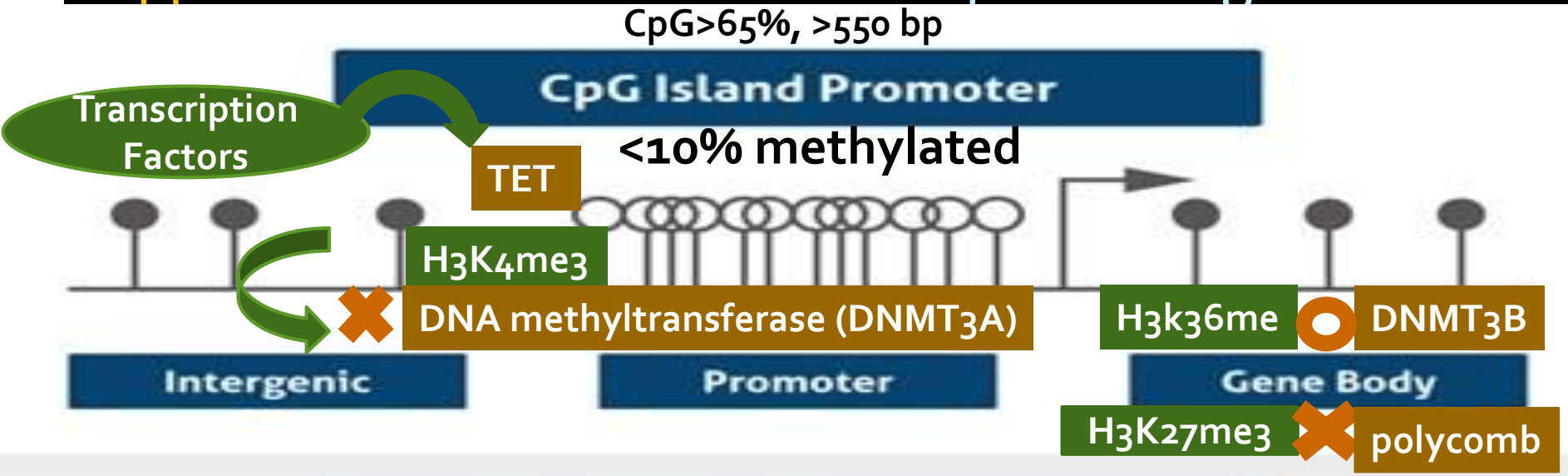


Cytosine-Guanine (CpG)

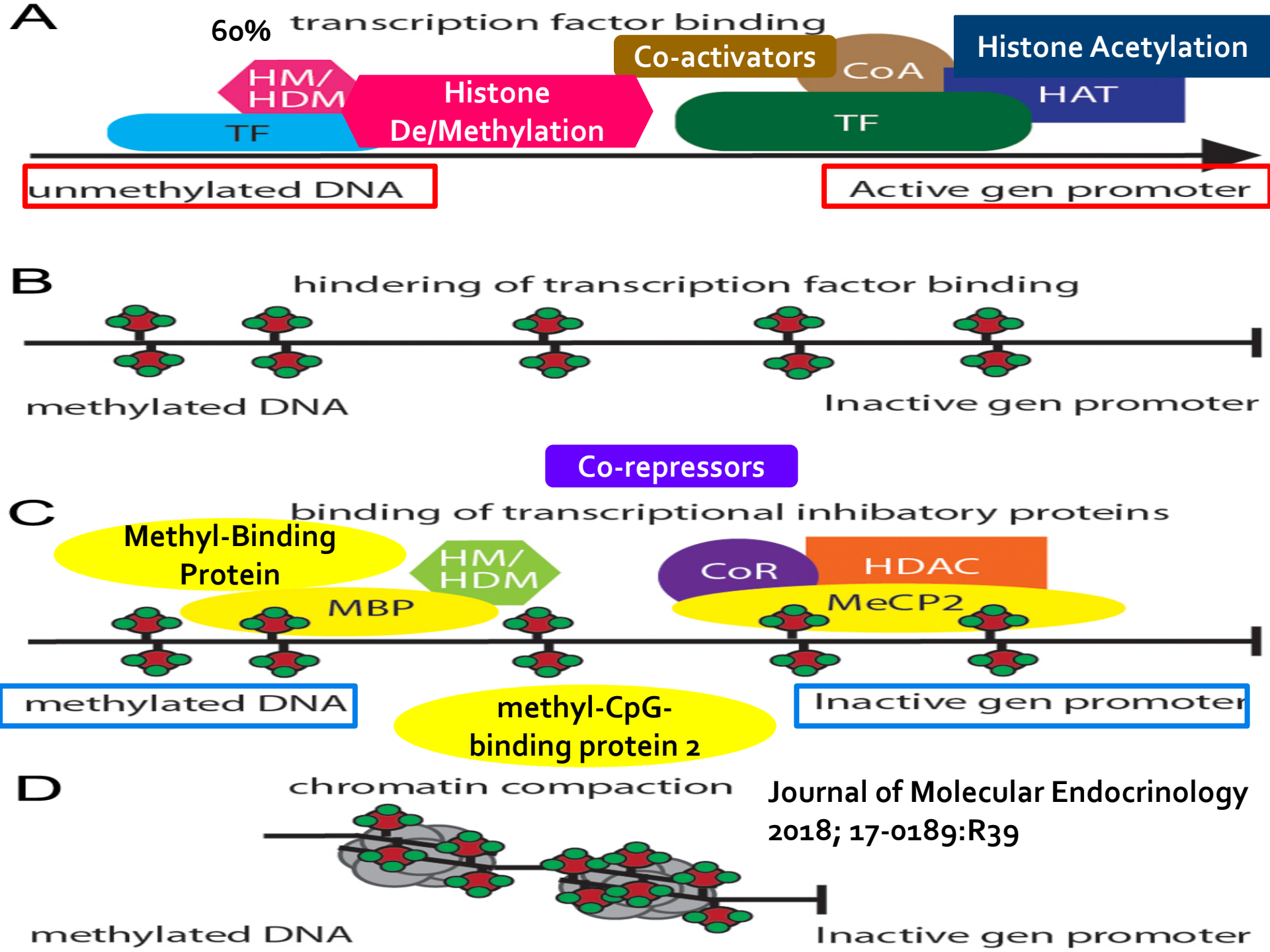
CpH: CpA, CpC, CpT

Reduced
Transcription Factor accessibility
to promoter or enhancer region

The depletion of 5mC at active regulatory is essential for active transcription regulation, while intragenic 5mC performs fine-tuning and supportive roles in transcription regulation.



Science. 2018 Sep 28;361(6409):1336-1340



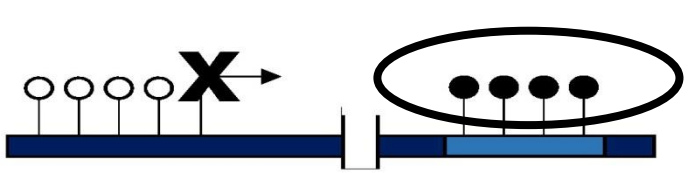
Journal of Molecular Endocrinology
2018; 17-0189:R39

Dynamically methylated CpGs cluster into over 1 million tissue specific differentially methylated regions (DMRs), which are distal to TSS and overlap with enhancers.

Enhancers a short (50–1500 bp) region

Methylated

Enhancers are commonly marked by levels of 5mC ranging from 10% to 50%

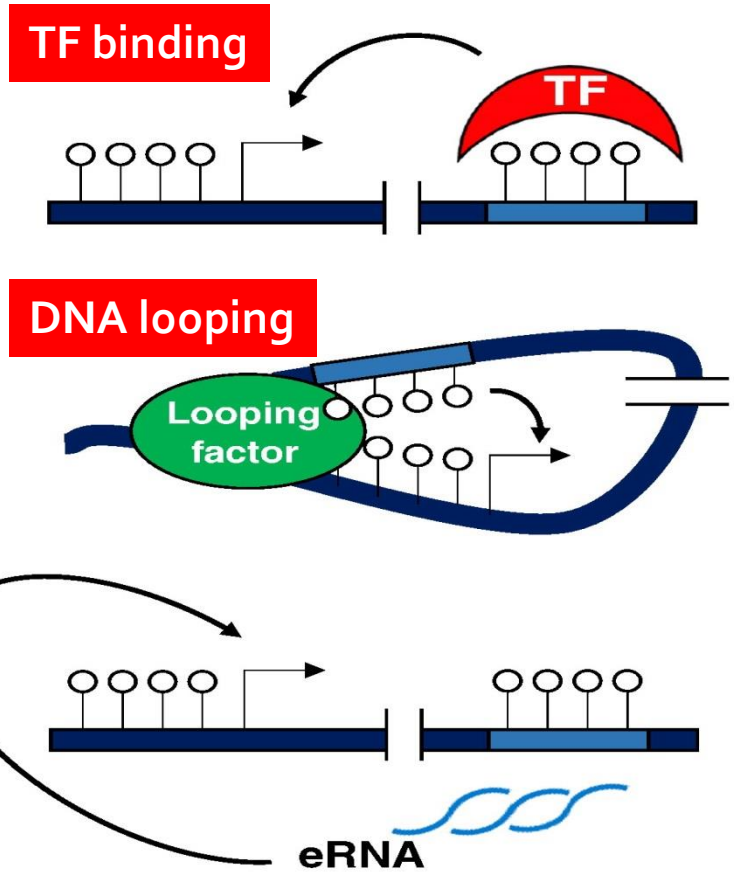


tissue specific

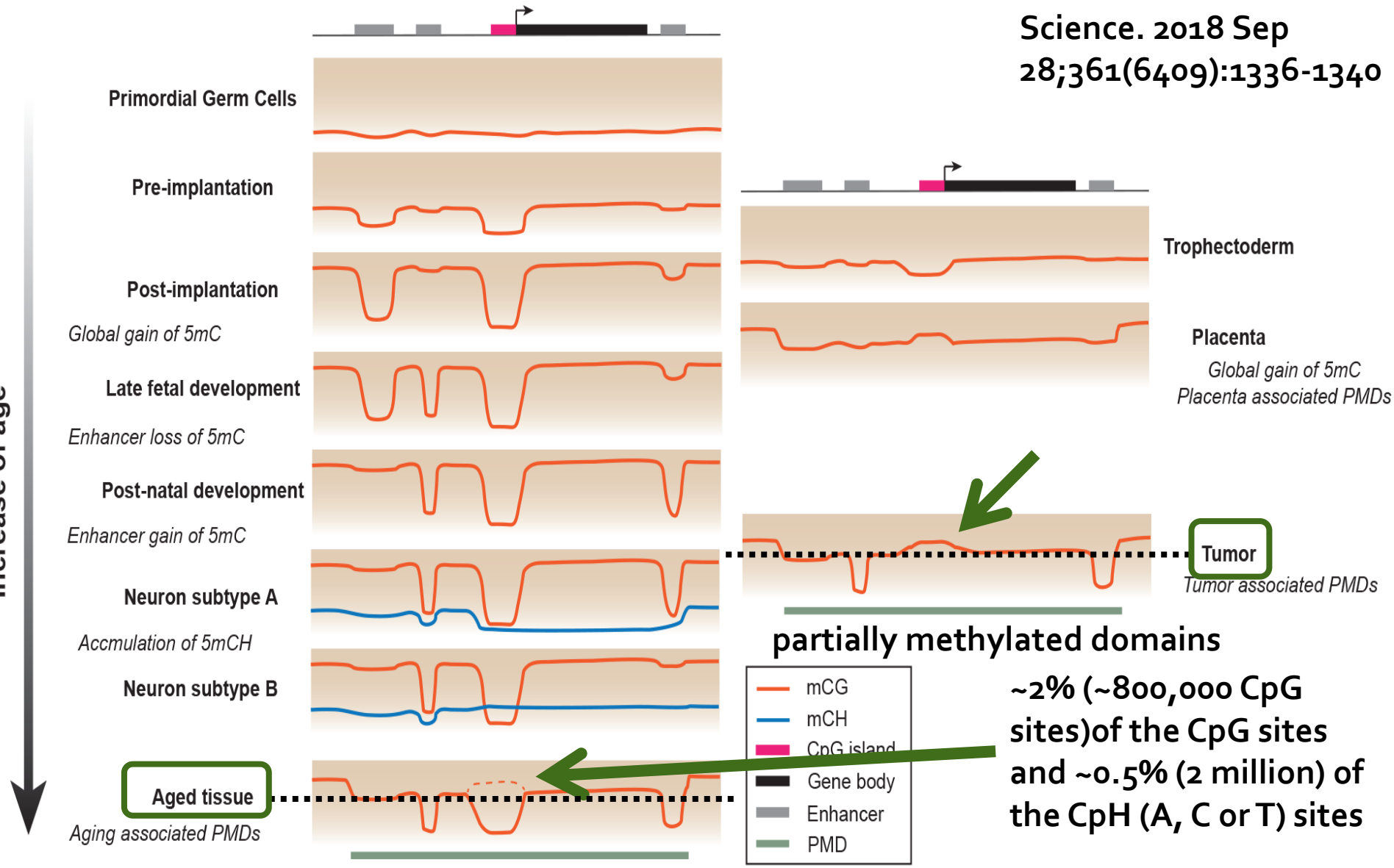
15%-21% of CpGs

Biochimica et Biophysica Acta 1829 (2013) 1161–1174

Unmethylated



Both Aged tissues and Tumor methylomes have global hypomethylation and localized hypermethylation over specific promoter regions.



Science. 2018 Sep 28;361(6409):1336-1340

partially methylated domains

~2% (~800,000 CpG sites)of the CpG sites and ~0.5% (2 million) of the CpH (A, C or T) sites

The global reduced methylation content can be caused by down-regulated expression of DNMTs or insufficient supply of folic acid in elderly subjects.

Frontiers in Genetics 2019 | Volume 10 | Article 107

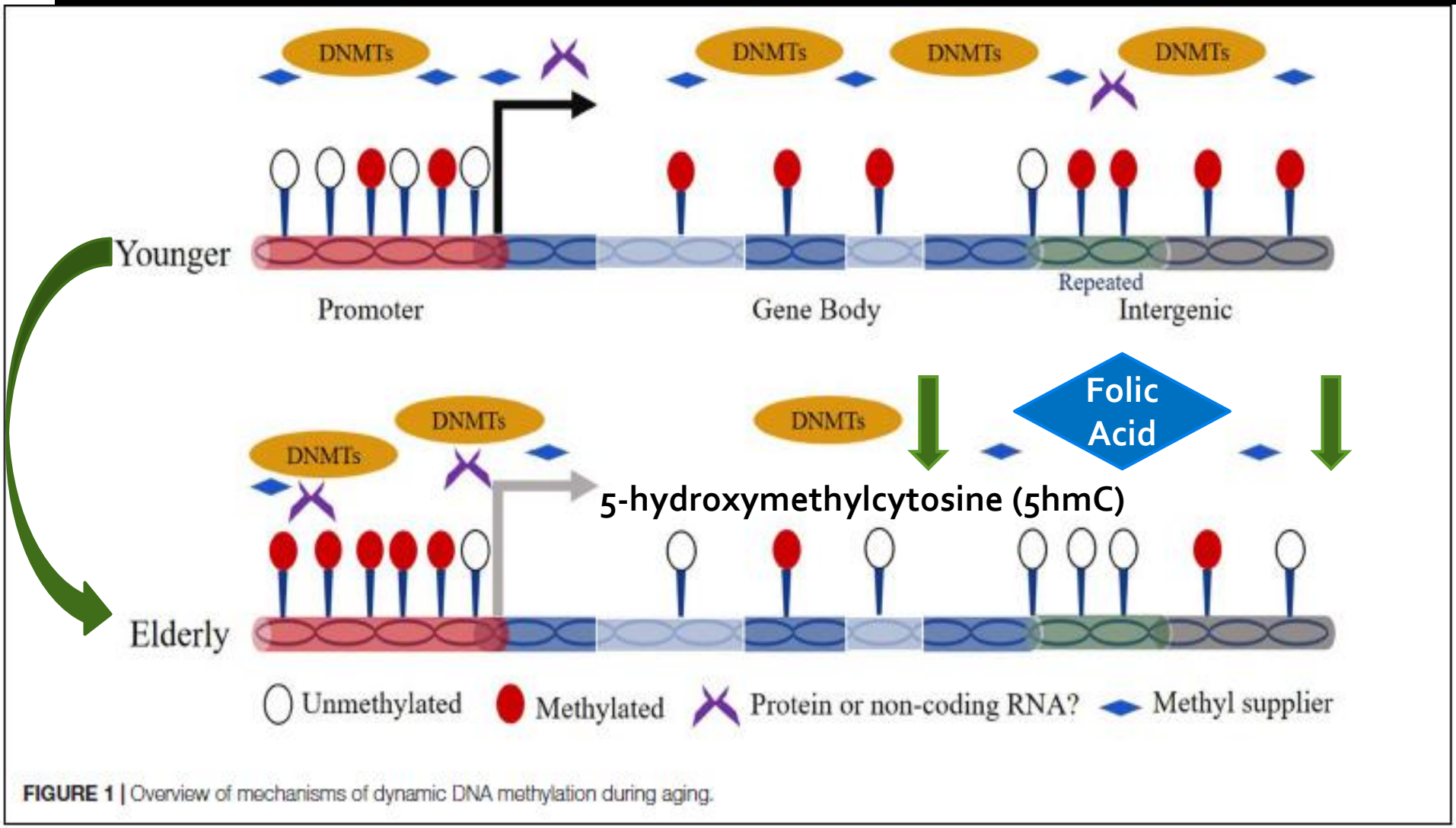
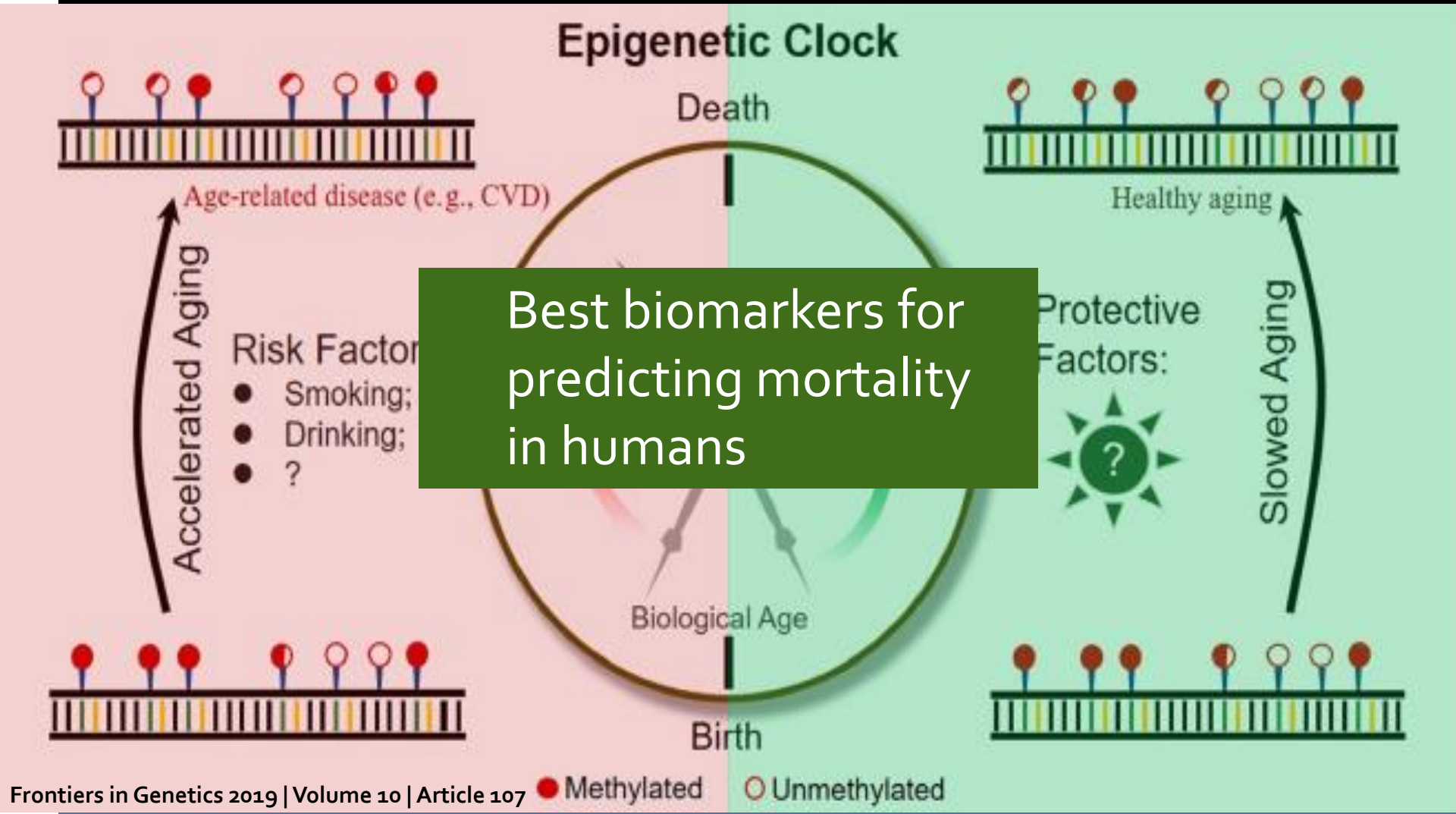


FIGURE 1 | Overview of mechanisms of dynamic DNA methylation during aging.

A linear reverse relationship between biological age and DNA methylation (3-513 CpG sites) of blood cells and various human tissues with an average accuracy of 3-5 years

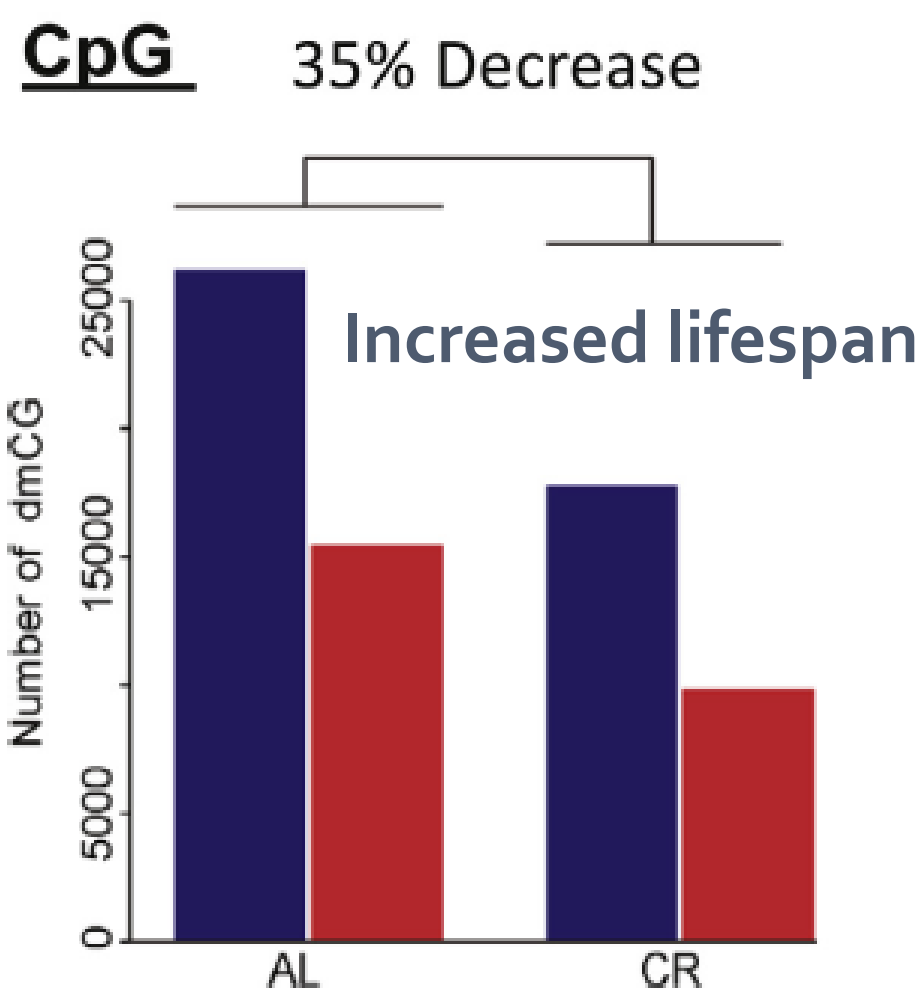


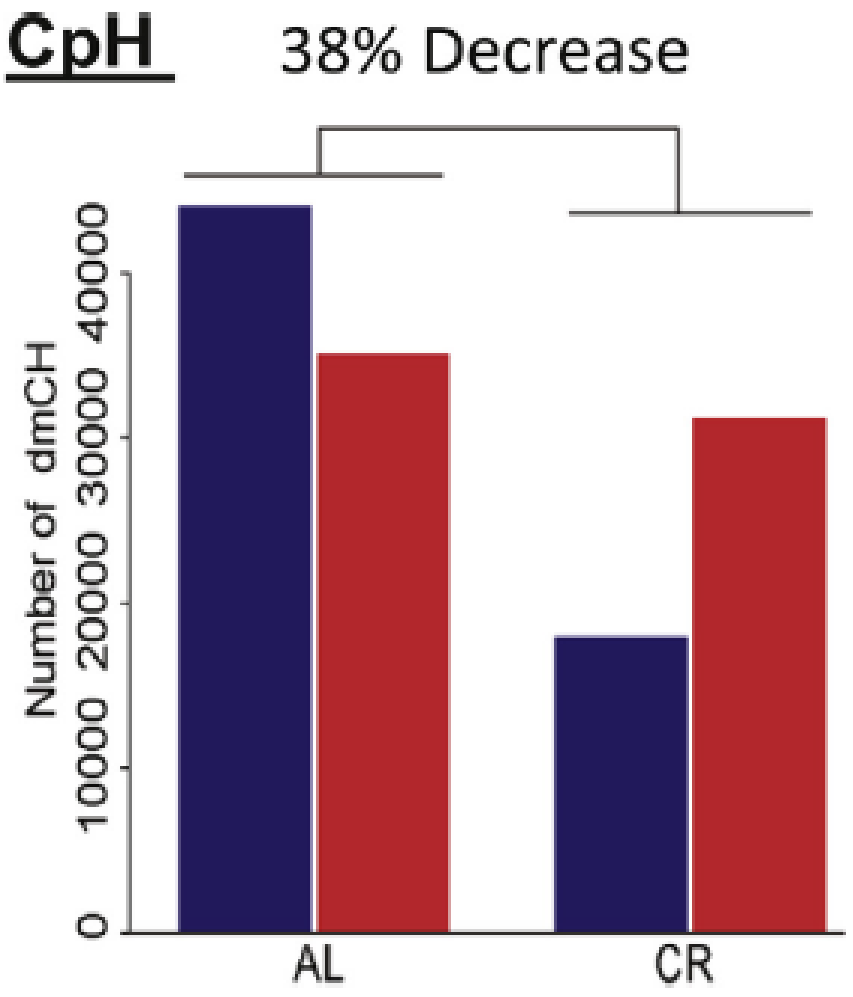
Frontiers in Genetics 2019 | Volume 10 | Article 107

Caloric restriction of 30% extends lifespan of a small primate model by 50% through augmenting DNMT and HDAC1/SIRT1 activity.

DNA Hypermethylation and histone hypoacetylation of $p16^{INK4a}$ gene

Pharmacology & Therapeutics 195 (2019) 172–185





DNA methylation state during innate and adaptive immune cell development, differentiation, and function.

Translational Research 2019; 204:118

Immune cell	DNA methylation-mediated changes
macrophage	myeloid over lymphoid differentiation, monocyte-to-macrophage differentiation, polarization
CD4+ T lymphocytes	differentiation into distinct phenotypes
CD8+ T lymphocytes	cytolytic function , de-differentiates into memory
B cell	Differentiation, activation and plasma cell differentiation.
Regulatory T lymphocytes	Differentiation, suppressive capability, Th2-biased subset

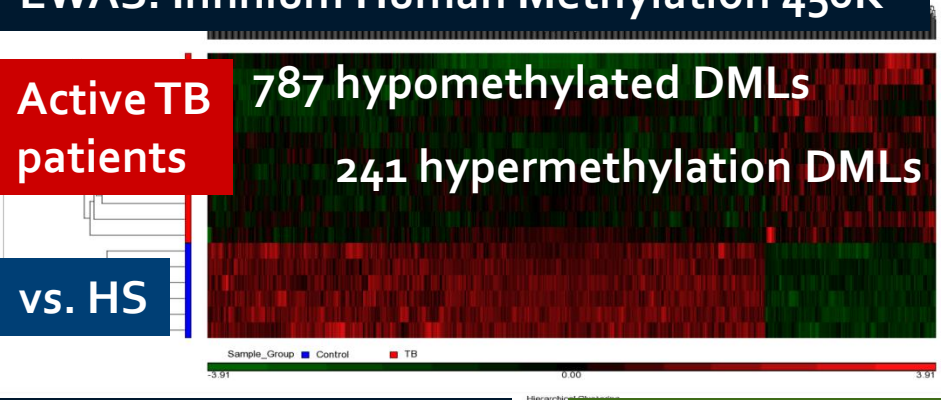
Association between gene-specific aberrant DNA methylation and active TB disease

Gene Name	Region	DNA MET change	Gene or protein change	Model	Clinical Outcome
Vitamin D receptor	Gene body (Exon, 3'UTR)	Hyper-Me (9 CpG sites)	Decreased GE *of AKT, GSK3β ,FOXO1*	Active TB patients vs. HS	
Toll-like receptor 2	promoter	Hyper-Me (5 CpG sites)	Depressed TLR2	Active TB patients vs. HS	Drug-resistant TB
IL 18 receptor 1	promoter	Hyper-Me (1 methyl-SNP)	Depressed IL18R1	Active TB patients vs. HS	
CYP27A1	promoter	Hypo-Me	Decreased 1,25-dihydroxy vitamin D	Active TB patients vs. HS	

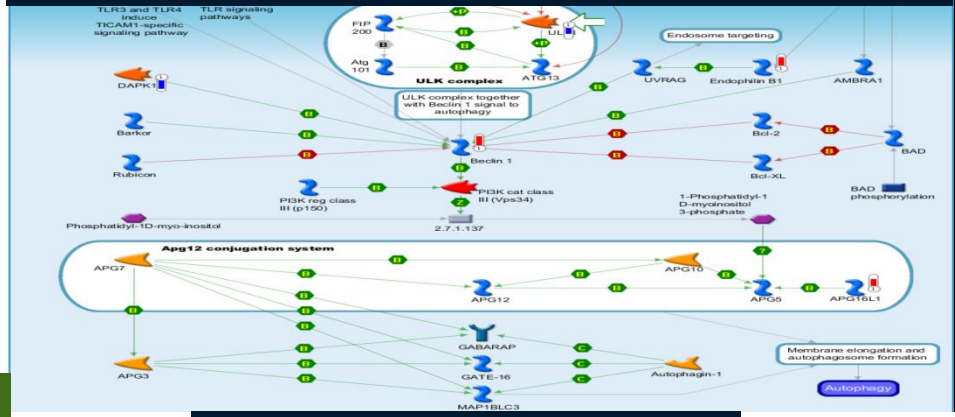
Hum Immunol. 2011 ; 72(3): 262
J Infect. 2014;69(6):546-57
PLoS One. 2014 ;9(10):e110734
Thorac Dis 2017;9(11):4353-4357

Whole genome DNA methylation analysis of active pulmonary TB disease identifies novel epigenotypes: *PARP9/miR505/RASGRP4/GNG12* Met and clinical phenotypes (under review at "J. Infection")

EWAS: Infinium Human Methylation 450K

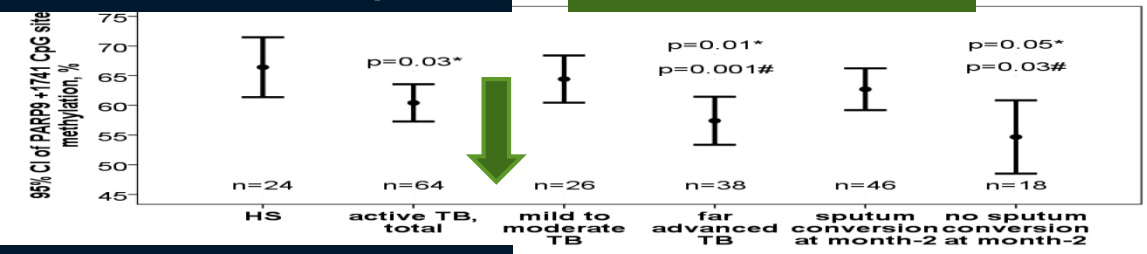


Autophagy-related gene signaling pathway

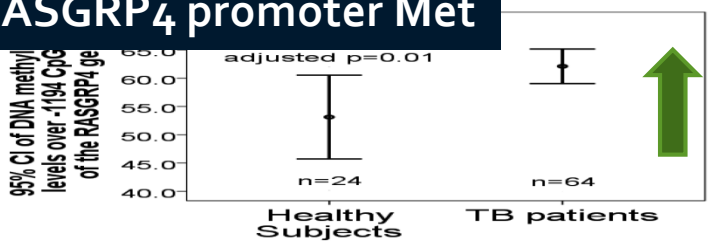


PARP9 Gene Body Met

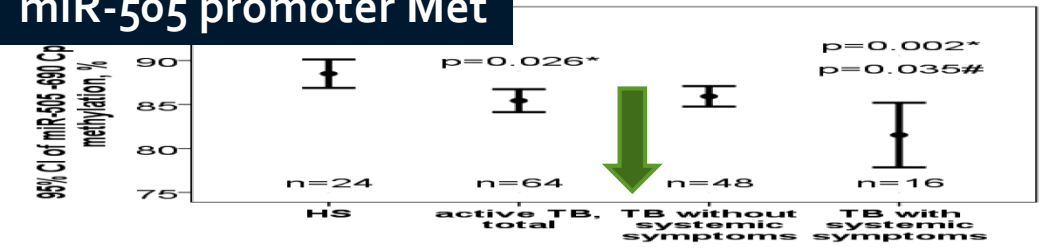
Validation Cohort



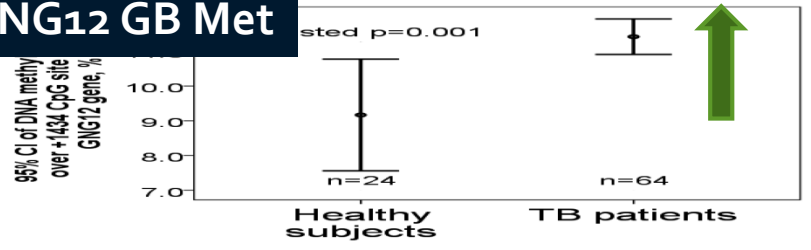
RASGRP4 promoter Met



miR-505 promoter Met



GNG12 GB Met



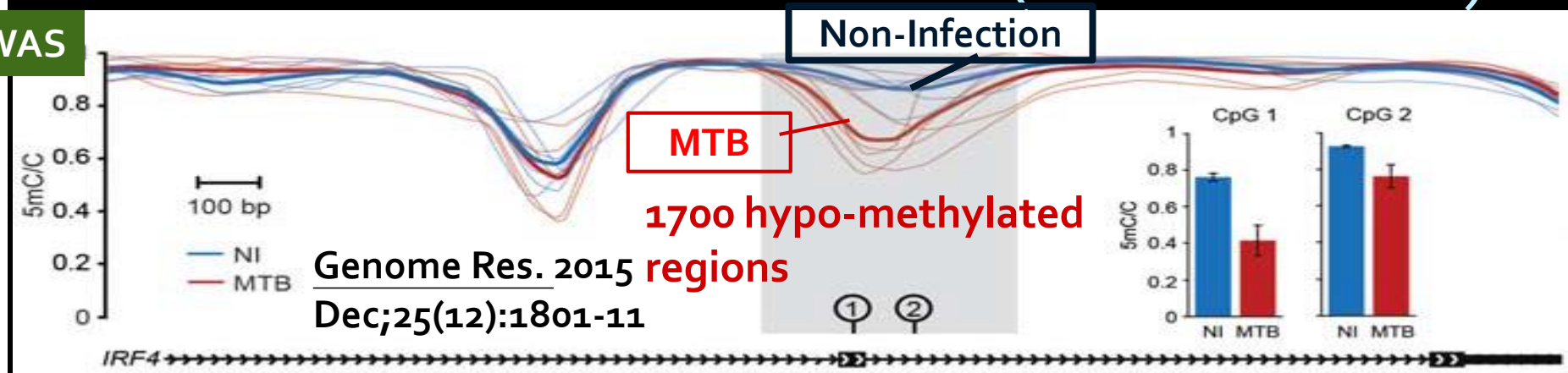
Gene-specific aberrant DNA methylation in response to *M.TB* infection in vitro or in vivo

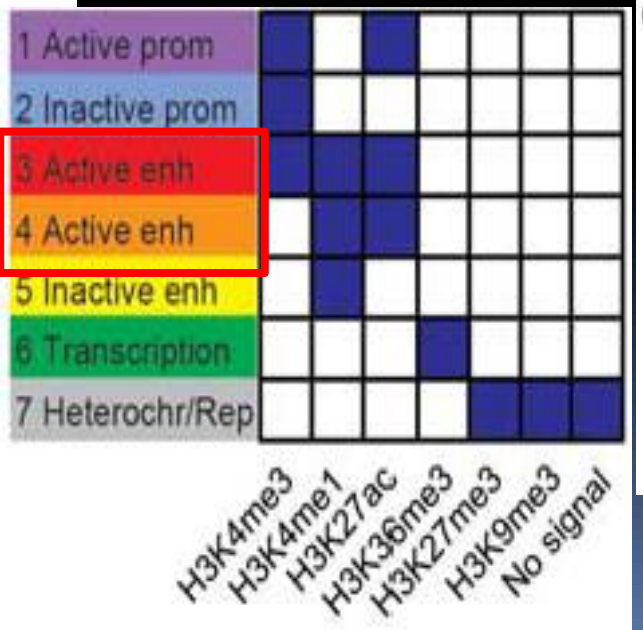
Gene Name	Region	DNA MET	GE	Model	Functional Outcome
IL6R	promoter	Hyper-Me		Beijing/Wild MTB-infected THP-1	
NLRP3	promoter	De-methylation	NLRP3 activation	Mtb H37Rv-infected THP-1	Increased inflammatory cytokines
CD82/KAI1	promoter	De-methylation	RUNX1-binding induced CD82 activation	MTB-infected THP-1 /BMDMs/Mice	Decreased inflammatory cytokines/ phagosome maturation, enhanced MTB survival

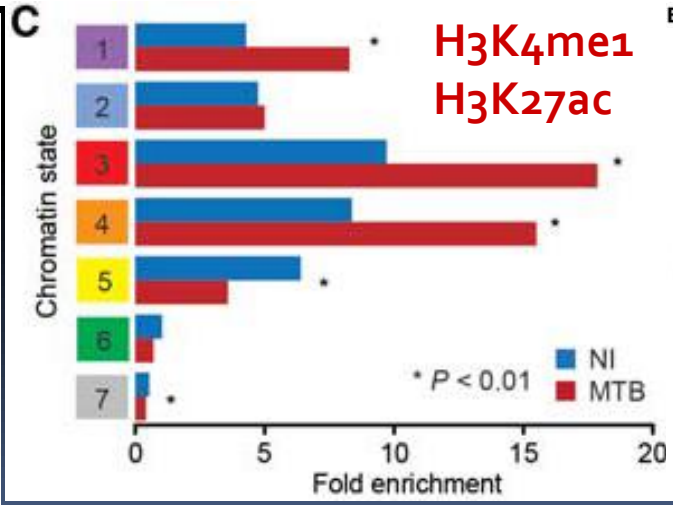
Biomed Res Int. 2016;2016:4323281. Experimental & Molecular Medicine (2018) 50:62
Tuberculosis (Edinb). 2016;98:139-48.

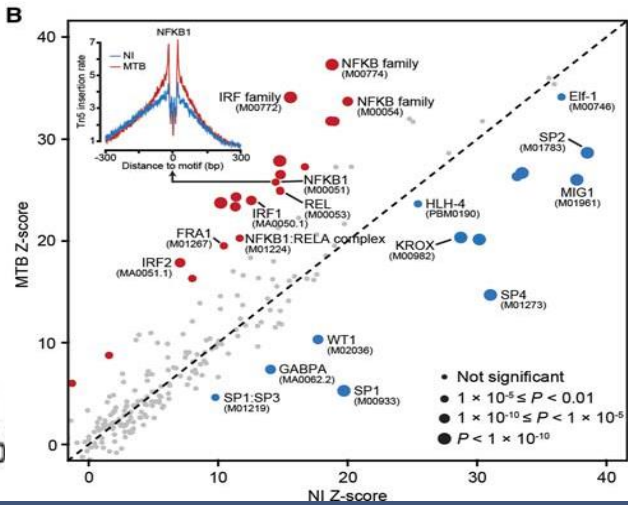
M.TB infection of human dendritic cells (DCs) is associated with rapid and active de-methylation at thousands of loci, mostly localized to distal enhancers (3.5 KB to TSS).

EWAS









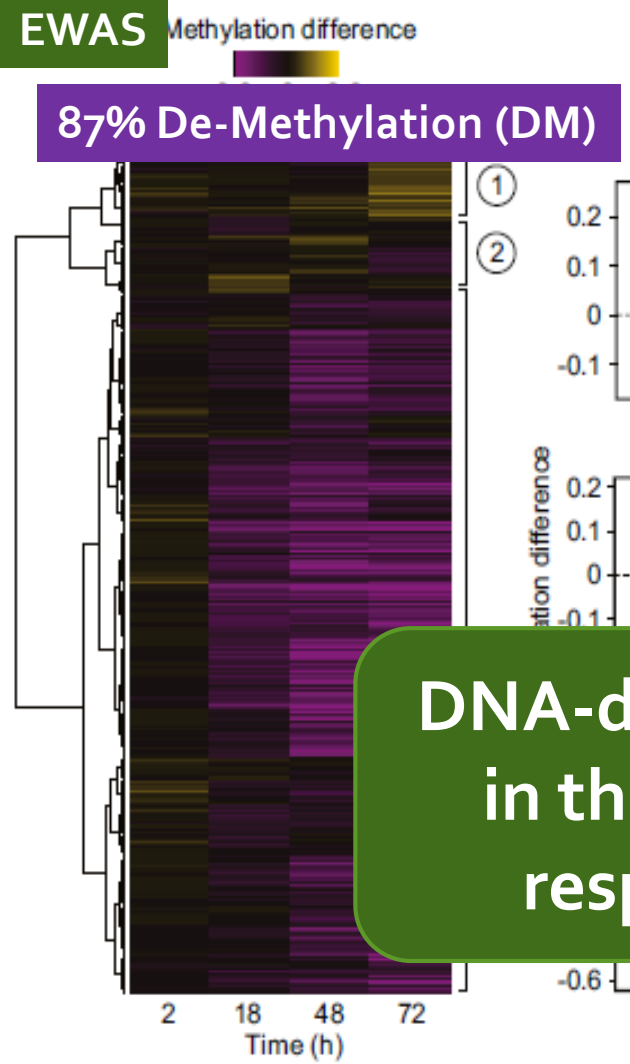
Gain of histone activation marks
Increased chromatin accessibility

27 TF increased binding:
NF-κB/Rel/IRF

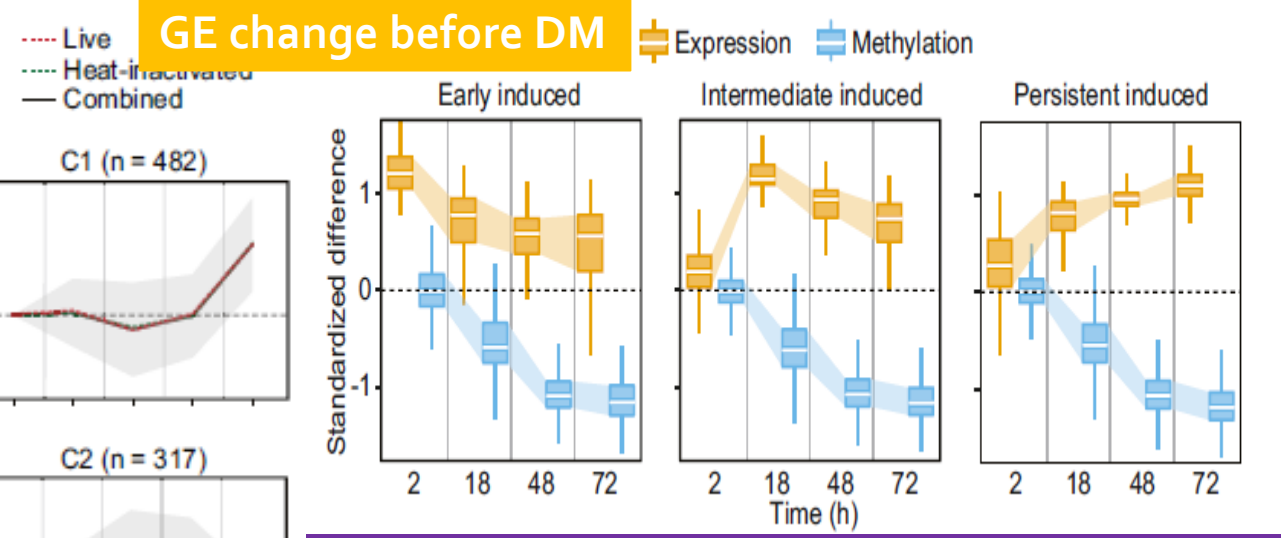
Dendritic Cell immune gene expression changes to *M.tb* infection is followed by active de-methylation of 5371 CpG sites over distal enhancers, mediated by TF binding. PNAS 2019; 116:6938

EWAS

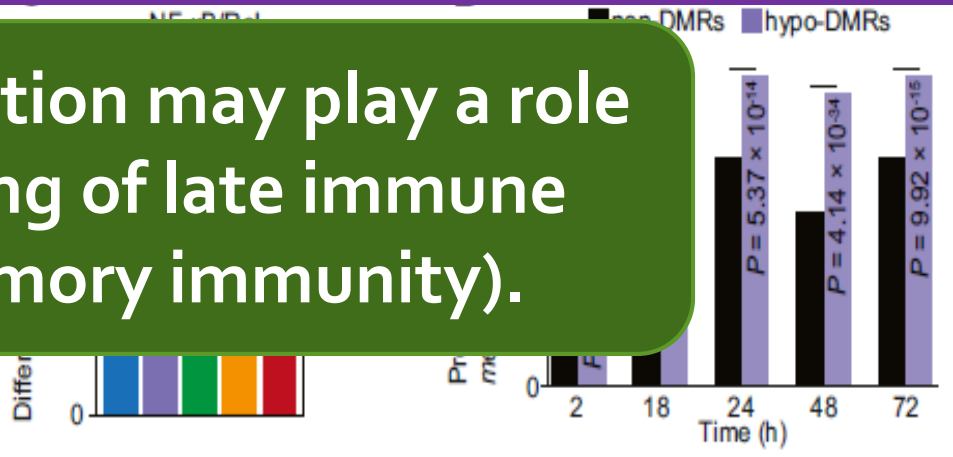
87% De-Methylation (DM)



GE change before DM



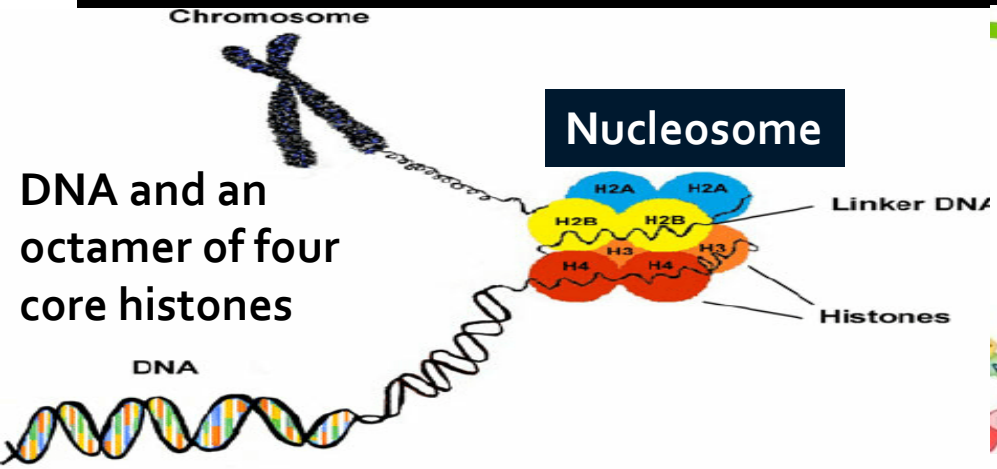
enriched for binding of immune-related TFs

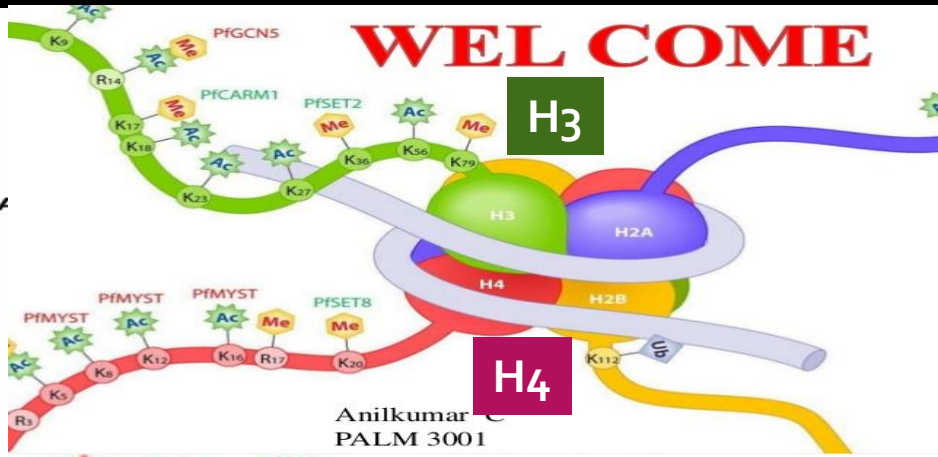


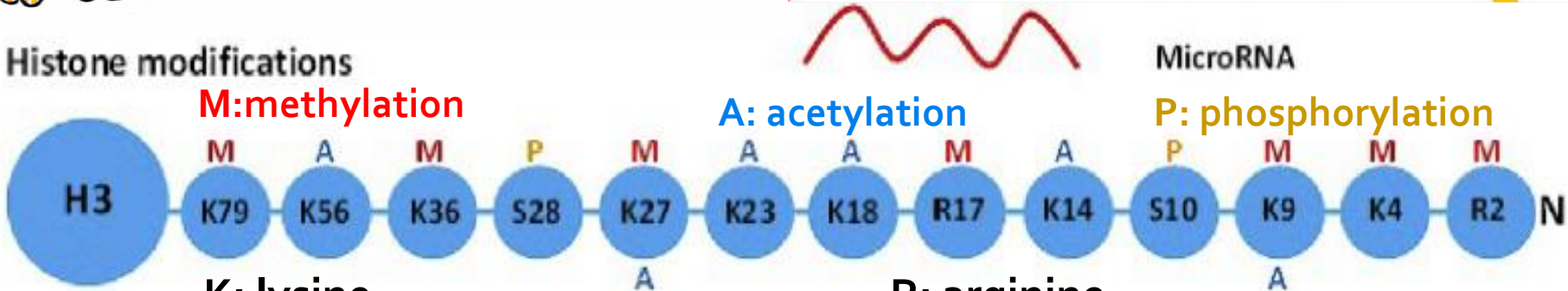
DNA-de-methylation may play a role in the fine tuning of late immune response (memory immunity).

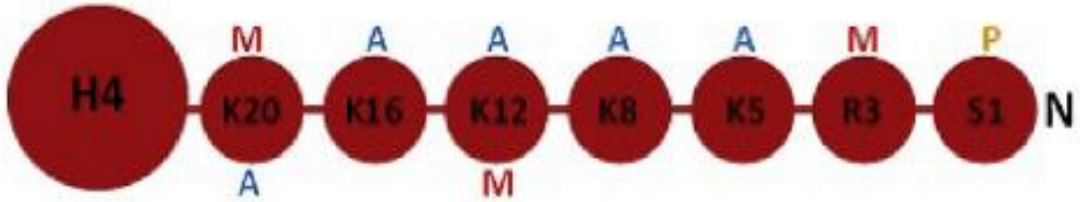
Aberrant histone modifications and histone modifying enzymes in active TB disease or in response to *M.TB* infection

Amino-terminal tails of the core histones (H3, H4) can be posttranslational covalent modified by addition of methyl (red), acetyl (blue) or phosphoryl moiety (orange).





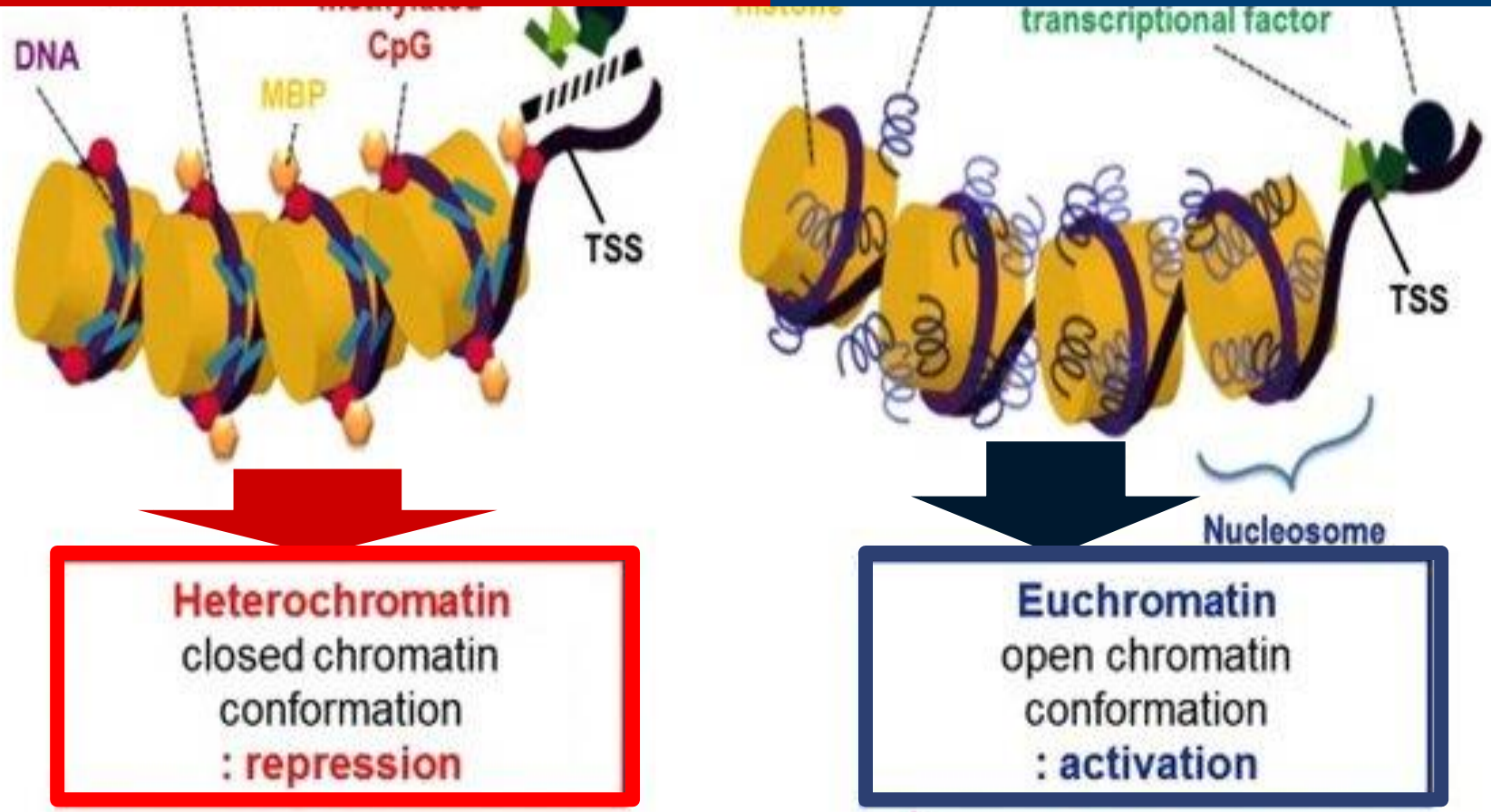




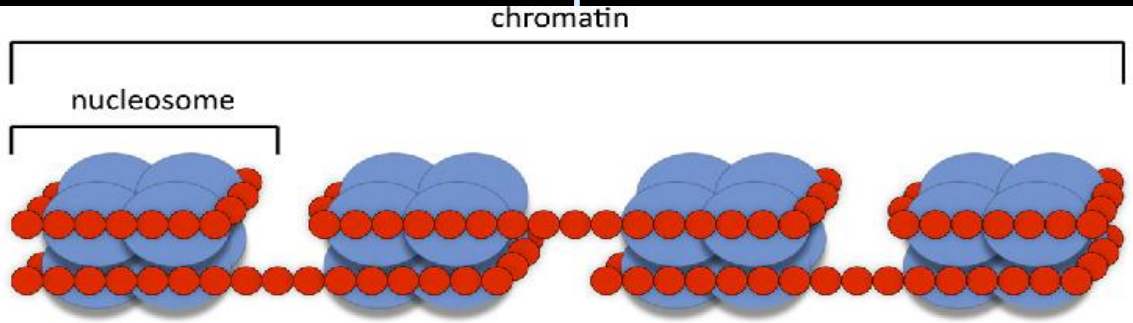
Tuberculosis (Edinb). 2018 Dec;113:200-214.

Low Histone Acetylation
High levels of Methylation over H3K9, H3K27, H4K20

High Histone Acetylation
High levels of Methylation over H3K4, H3K36, H3K79,



Histone acetylation exposes promoter and coding regions to transcriptional regulators, including RNA polymerase (Pol II) and the various isoforms of the basal transcription factors (TFIIs), which results in transcriptional activation.

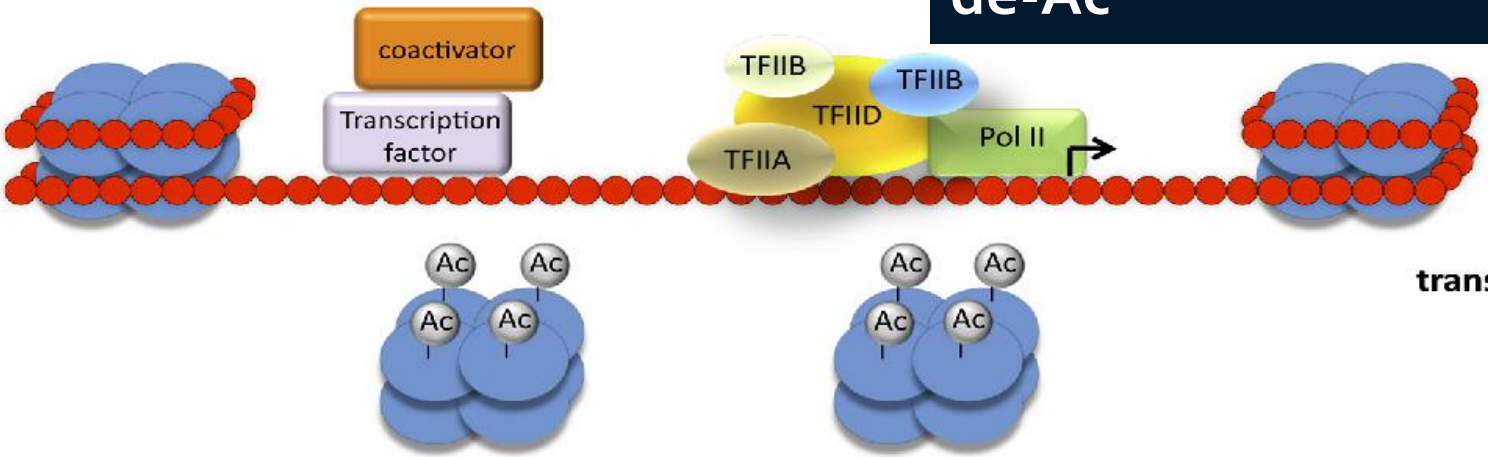


transcriptional repression

chromatin de-compaction, active transcription

Histone Acetylation (Ac)

Histone Acetyl-Transferase (HAT) : Ac
Histone De-Acetylase (HDAC): de-Ac

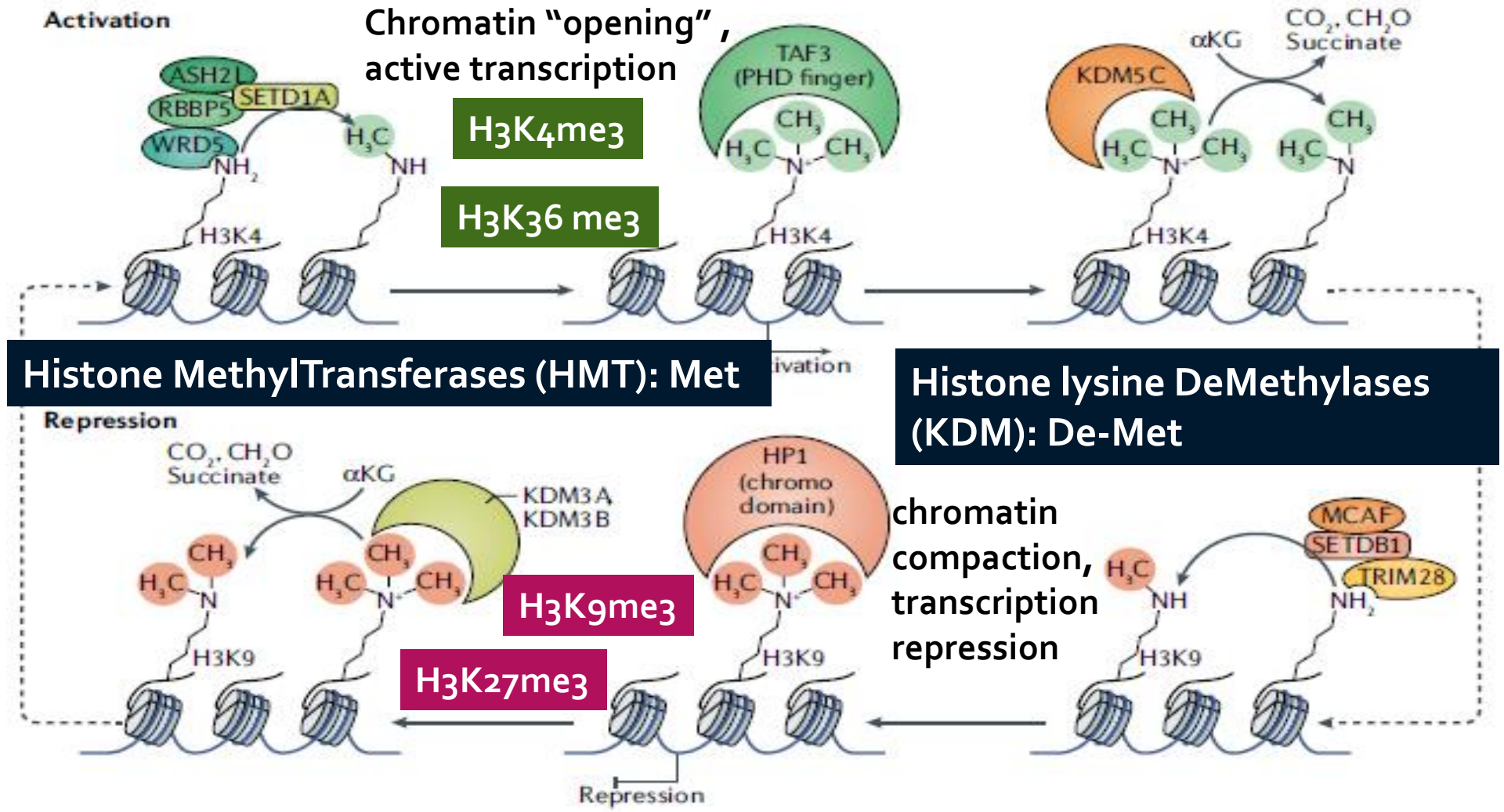


transcriptional activation

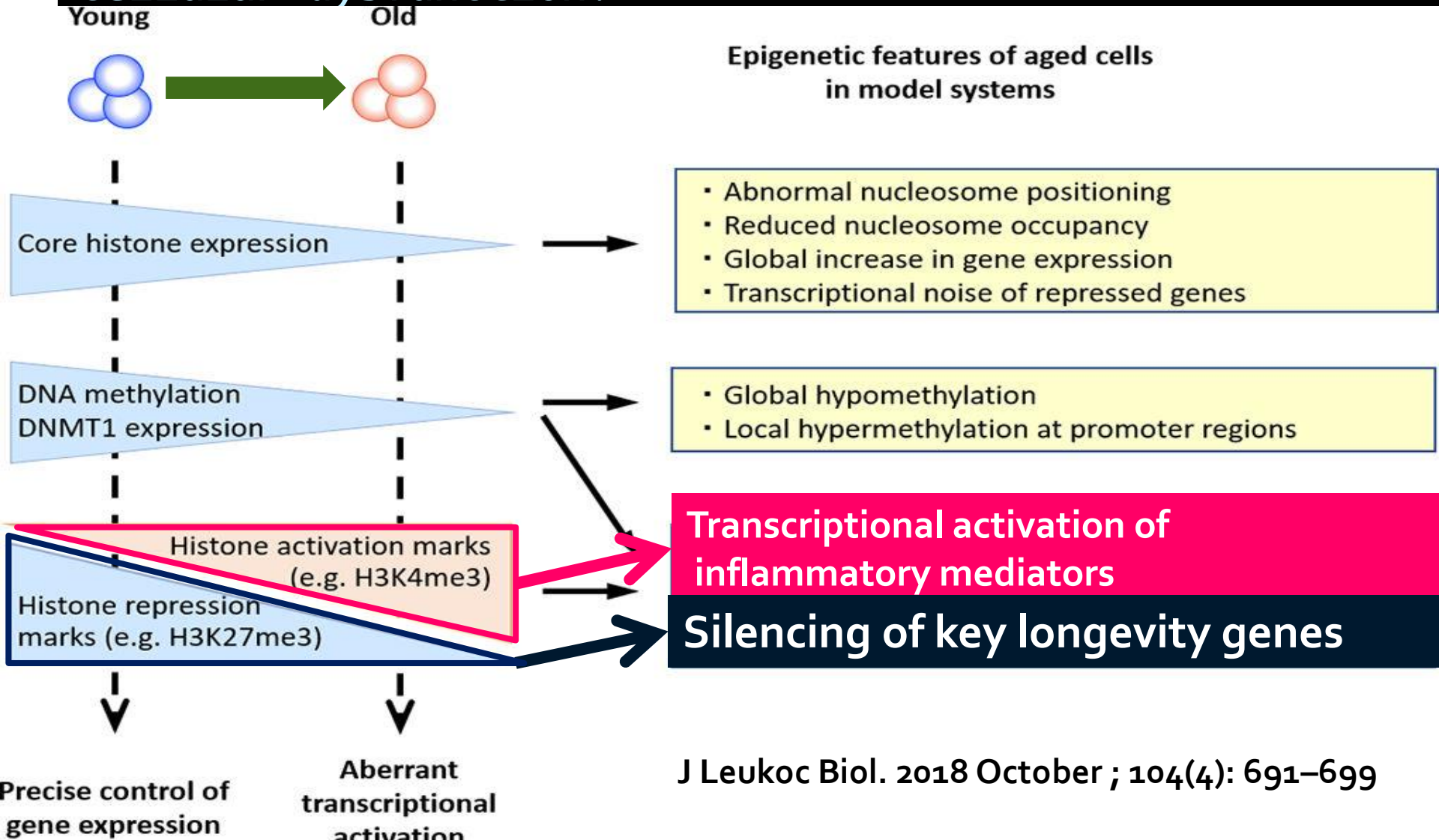
Gene-activating H3 Lys4 tri-methyl (H3K4me3)/H3K36me3/H3K79 mark at the promoters of various genes.

Gene-repressive states established by the deposition at the promoters of H3K9me3/H3K27me3.

Nature Reviews | MolECulaR CEll BioloGy 20 | 2019 | 625



Changes in chromatin structure due to altered histone expression, histone modifications and DNA methylation occur with aging and contribute to cellular dysfunction.



J Leukoc Biol. 2018 October ; 104(4): 691–699

Association between histone modification/modifying enzymes and active TB

Histone	Attribute	Change	Model	Mechanism	Clinical Outcome
H3K14 Ac	Global	Hypo-acetylation (Ac)	PBMCs from Active TB patients vs. HS	TNF-α /IL12B promoter-specific H3K14 hypo-Ac	lower one-year survival
H3K27 Me	Global	Hyper-methylation (me2/me3)	PBMCs from Active TB patients vs. HS		bacterial burden, symptom
HDAC1	Non-specific de-Ac	Increased	PBMCs from Active TB patients vs. HS		Reversed with anti-TB Treatment
KDM6B	H3K27me3 De-Me	Decreased	PBMCs from Active TB patients vs. HS		Reversed with anti-TB Treatment

Am J Transl Res. 2017 Apr 15;9(4):1943-1955.

Altered expressions of Histone modifying enzymes in response to *M.tb* infection in vitro

Histone modifying enzymes	Attribute	Regulation	Model	Mechanism	Functional Outcome
HDAC1	Non-specific de-Ac	Up	MTB H37Rv infection	Decreased H3Ac over the IL-12B promoter	Increased survival of intracellular MTB
HDAC 6	Non-specific de-Ac	Up	Mtb H37Ra infection in mice/THP-1 cells	Increased IL-10 expression	Increased MTB growth
JMJD3 (KDM6B)	H3K27me3 (repressive) De-Me	Up	MTB H37Rv infected macrophage of mice	Augmenting NOTCH1-PI3K-mTOR-NF-κB signaling	Foamy Macrophage, M2 polarization
SET8	H4K20 monomethylase	Up	Mtb-infected macrophages	Induce NQO1-TRXR1	M2 polarization

Tuberculosis (Edinb). 2018 Jan;108:118-123. PLoS Pathog. 2016 Aug 17;12(8):e1005814. J Infect Dis. 2017 Aug 15;216(4):477-488.

Altered Histone modification patterns in response to M.tb infection in vitro

Histone modification	Change	Model	Mechanism	Functional Outcome
H3 and H4	Hyper-Ac	Mtb-infected macrophages /epithelial cells	Increased RNA Pol II binding to MMP-1 promoter	increased MMP-1 secretion
H3K9Me (repressive)	Hyper-Me	Mtb-infected macrophages	down-regulated the expression of CIITA/MHC-II	inhibits antigen presentation
H3K4Me (active mark)	Hypo-Me/Ac	ESAT-6 – stimulated macrophage	Inhibit class II transactivator (CIITA)	Inhibit MHC II
H3R42Me (repressive)	Hyper-Me	H37Rv Mtb-infected THP1	Rv1988 repress NOX1, NOX4, NOS2, TNFAIP2, and lincRNA, ENSG00000250584	Increased MTB survival

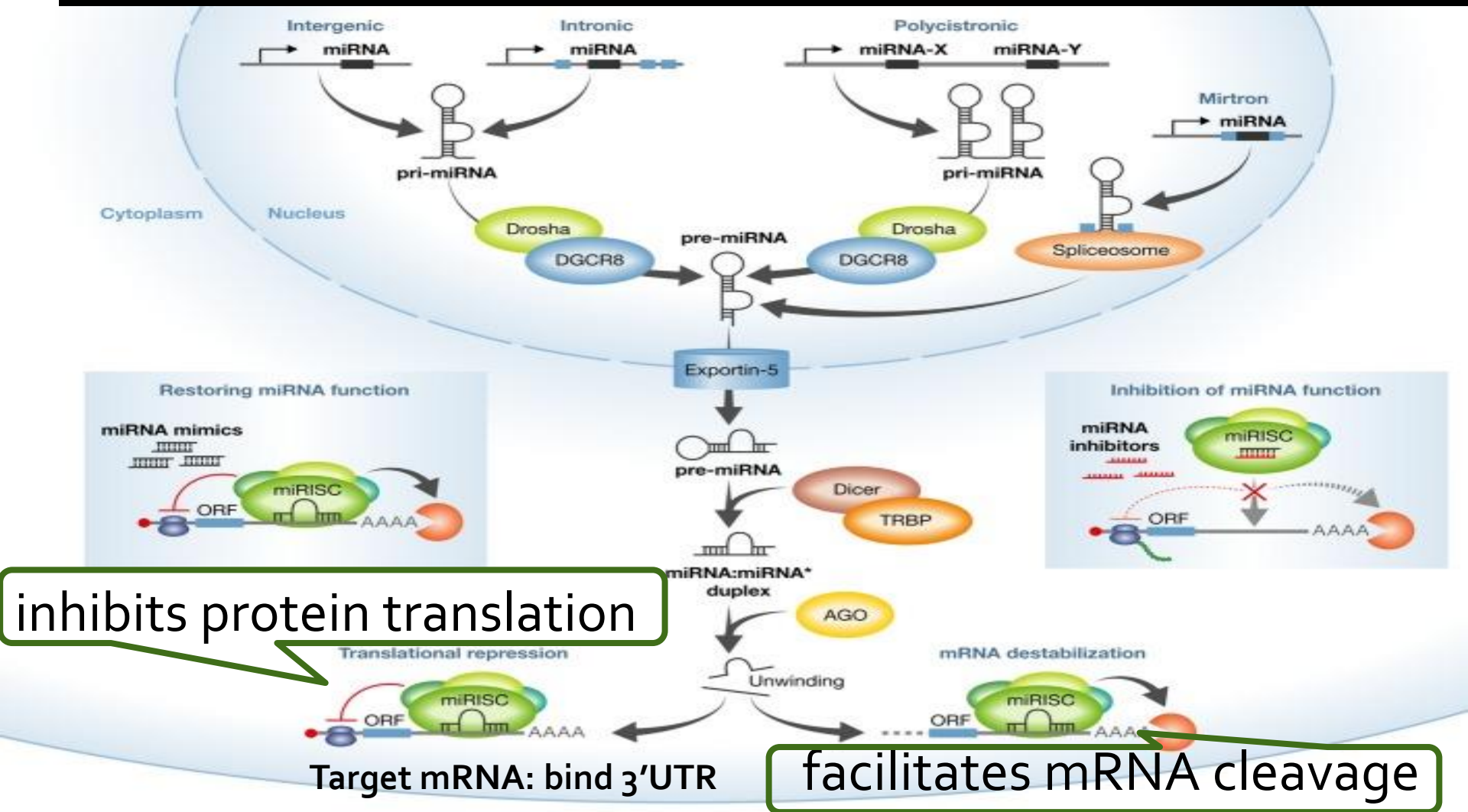
Adv Protein Chem Struct Biol. 2017;106:71

Front Immunol. 2017 May 24;8:602.

J Biol Chem. 2017 ;292(17):6855-6868.

Altered microRNA (miR) expressions
in active TB disease or
in response to *M.TB* infection

MicroRNAs (miRNAs) are small non-coding ssRNAs, ~22 nucleotides in length, are produced by two RNase III proteins (Drosha/Dicer), regulate up to 60% of the protein-encoding genome.



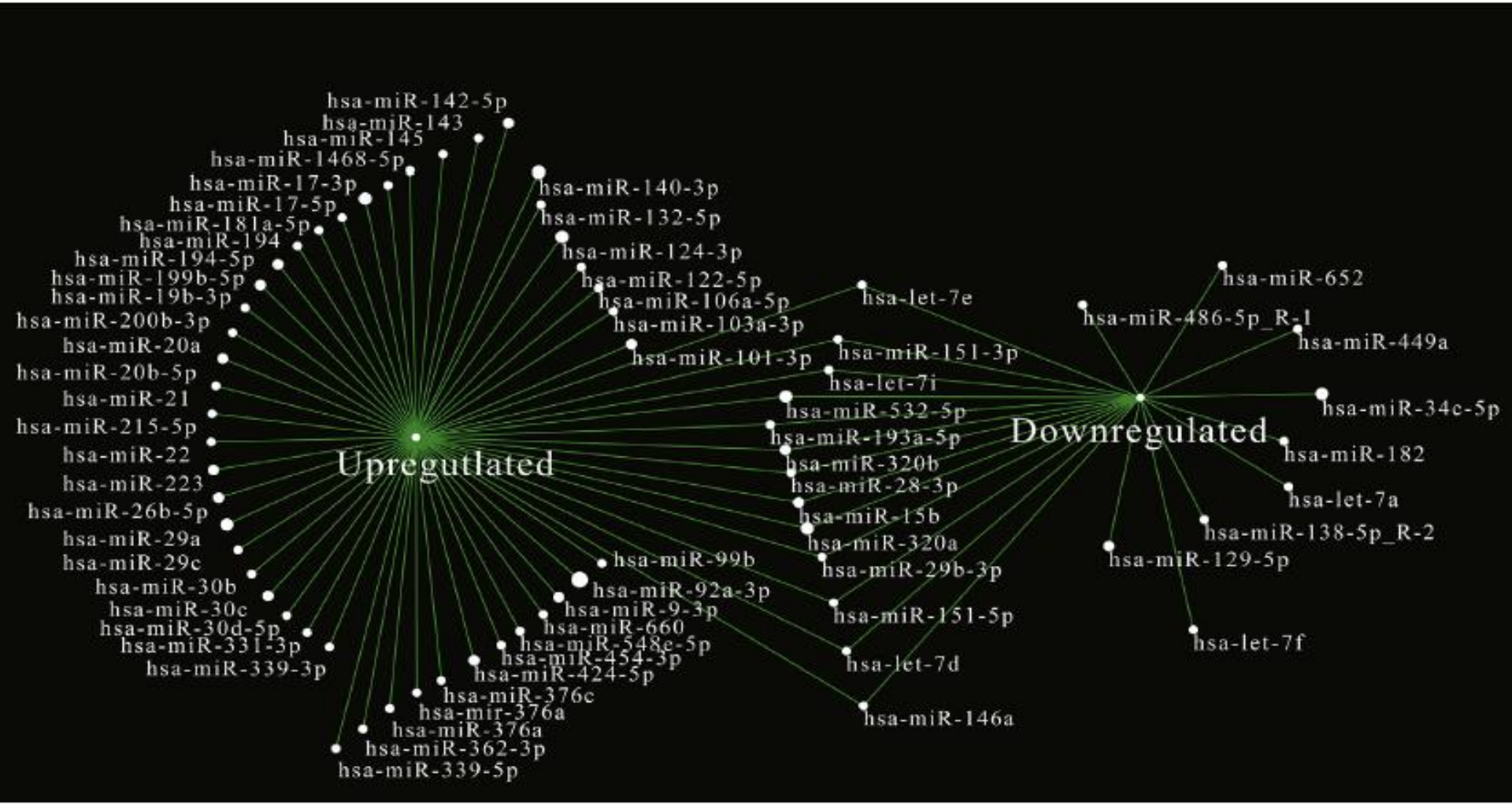
inhibits protein translation

Target mRNA: bind 3'UTR

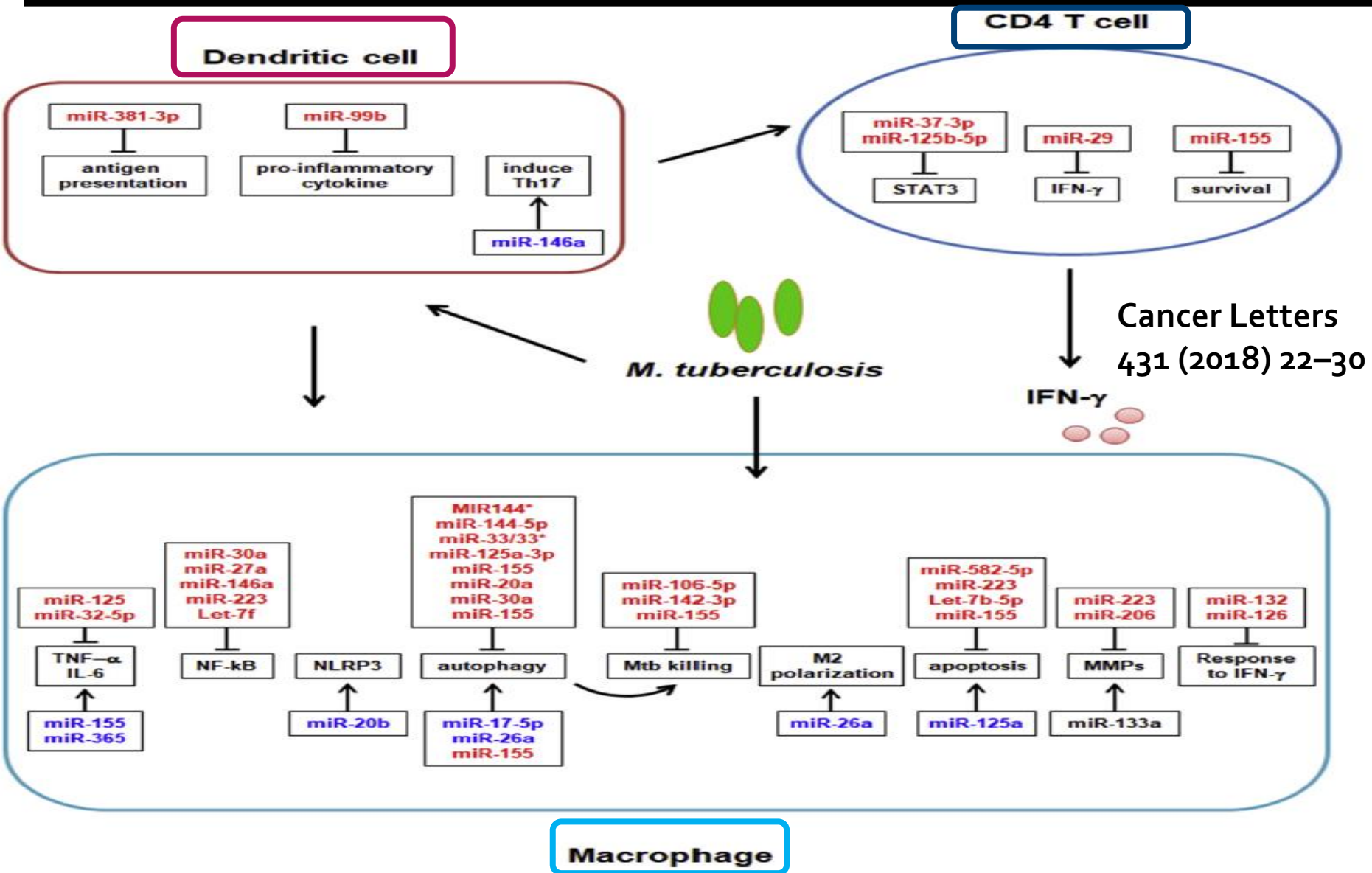
facilitates mRNA cleavage

53 miRNA differentially expressed in TB p'ts vs. HS.
Only miR-21 showed an overlap in up / down regulation.
Only 8 of these miRNA were identified in 2 or more studies: miR-20b, 21–5p, 22–3p, 26a, 29a-5p, 29c-3p, 378a-3p, 155.

Tuberculosis 118 (2019) 101860



miRNAs either promotes or inhibits important pathways and cellular responses in macrophages, dendritic cells and CD4+ T cells against *M.tb*.



List of immune-suppressive miRNAs in TB

MirRNA	Mechanism of action	Final effect
miR-21	Overexpression of IL-10 mRNA and Down-regulation of IL-12 mRNA	Suppression of immune-response against TB
miR-29	Degradation of IFN-Ɣ	Intercellular growth of tubercle bacillus within macrophages
miR-99b	Decline expression of TNF-α	Suppression of immune-response against TB
miR-125b	Blockade TNF-α mRNA	Suppression of immune-response against TB
miR-27b	Suppression of NF-kB signaling pathway	Suppression of immune-response against TB and intercellular growth of MTB
miR-1178	Attenuation of TLR-4 expression and inhibition of pro-inflammatory cytokines	Suppression of immune-response against TB

List of immune-effective miRNAs in TB

MirRNA	Mechanism of action	Final effect
miR-155	Stability of TNF-α mRNA and activation of MAPKs signaling pathway	Efficient immune-response against MTB, provoke phagocytosis and elimination of MTB
miR-424	Dysregulation of NFI-A78	Macrophage maturation and differentiation
miR-223	Targeted Mef2c	Granulocytes production and stimulation pro-inflammatory response
miR27a	Blocking IRAK4 signaling pathway	Increase of pro-inflammatory cytokines such as IL-β, IL-6 and IFN-γ
miR-20b	targeting the NLRP3/caspase-1/IL-1β pathways	Induce inflammation process
miR-582-5p	Decline monocytes apoptosis via down-regulating FOXO1	Promotion of anti-tuberculosis immune response

Adv Biomed Res. 2019; 8: 3

BCG-induced trained innate immunity through epigenetic mechanisms

How dose the BCG vaccine induce specific and non-specific immunity? What factors influence the immune responses induced by BCG?

Front Immunol. 2019 Jun 11;10:1317

- The BCG vaccine has been used since 1921 to prevent TB and is considered to be the world's most widely used vaccine.
- Specific effect of BCG : about 60% efficacy
  - good protection against disseminated and pulmonary TB disease in young children
  - variable efficacy against pulmonary TB in adults when given later
  - lasting for up to 15 years in the United Kingdom, 30–40 years in Norway, and even as long as 50–60 years in Alaska.
- Non-specific effects of BCG : about 25% efficacy
  - beneficial effects on all-cause mortality in infants (low birth weight) in West Africa.
  - There was no difference between outcomes in normal birth weight infants or premature infants in this European setting.

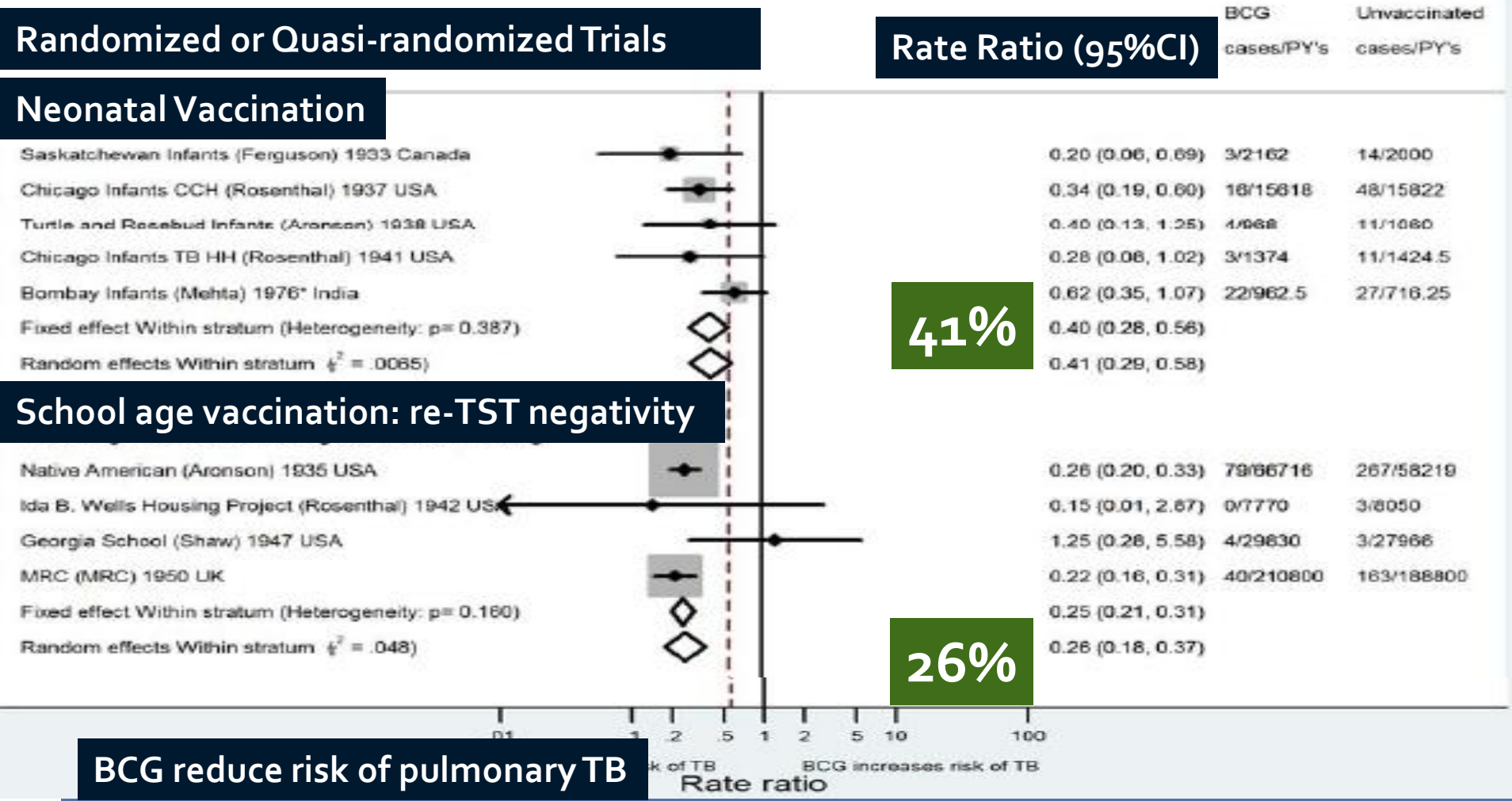
BCG Protection against active pulmonary TB appeared greatest in children stringently TB tested, (rate ratio [RR], 0.26; 95% confidence interval [CI], .18-.37), or infants (RR, 0.41; 95% CI, .29-.58).

Clinical infectious diseases 2014, 58 (4). pp. 470-80

Randomized or Quasi-randomized Trials

Rate Ratio (95%CI)

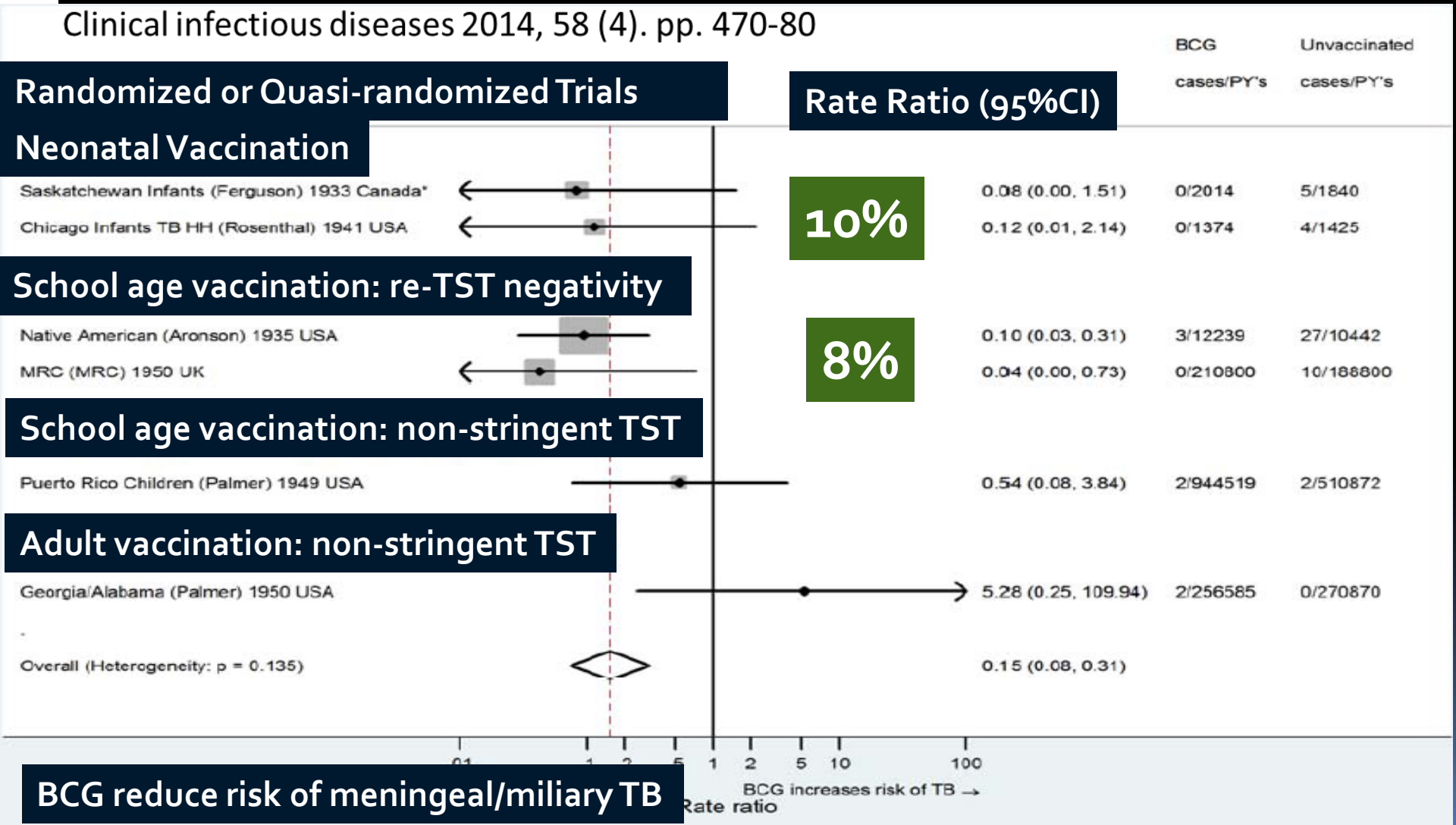
Neonatal Vaccination



BCG reduce risk of pulmonary TB

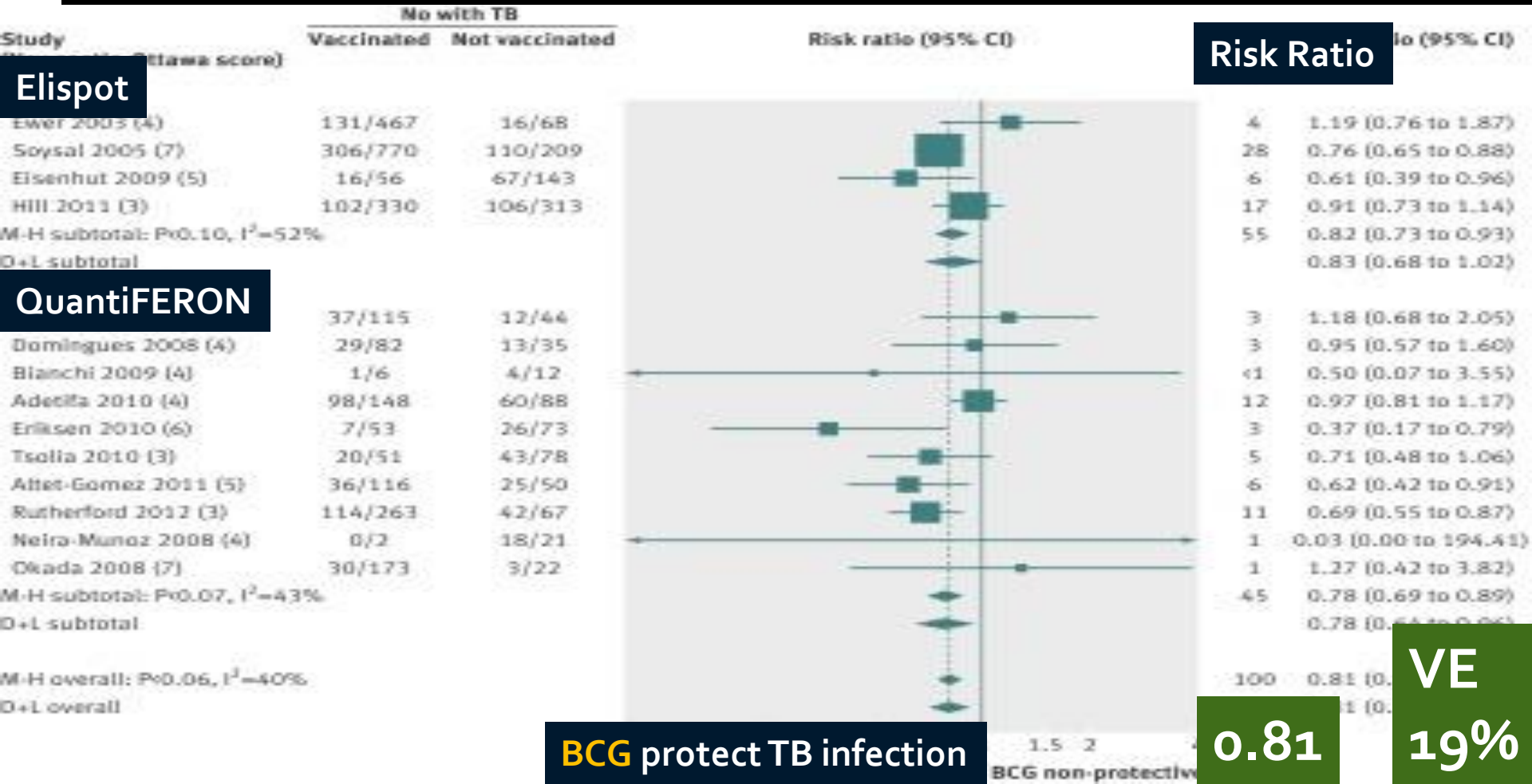
BCG Protection against meningeal and miliary TB was also high in infants (RR, 0.1; 95% CI, .01-.77) and children stringently tuberculin tested (RR, 0.08; 95% CI, .03-.25).

Clinical infectious diseases 2014, 58 (4). pp. 470-80

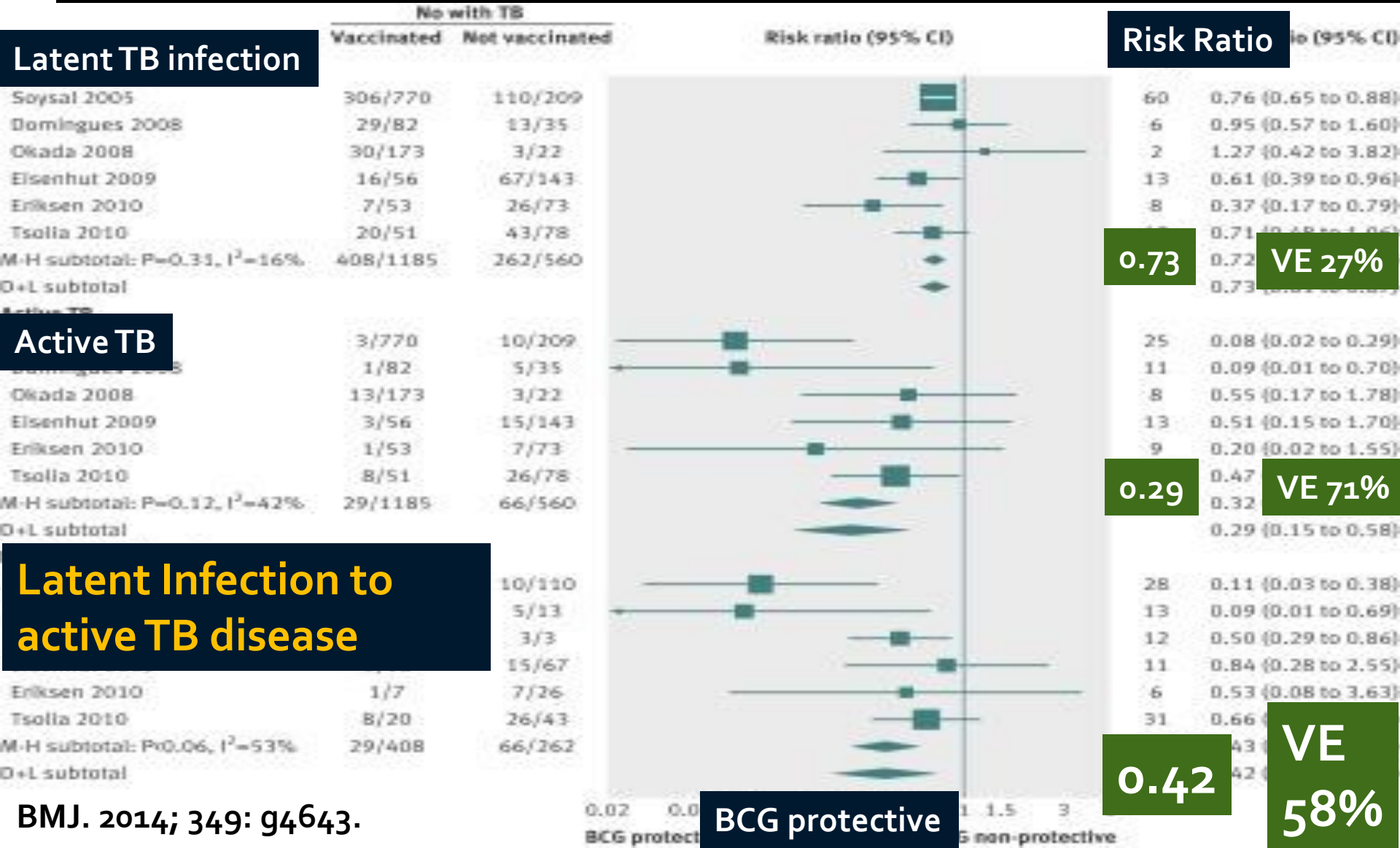


BCG reduce risk of meningeal/miliary TB

Primary analysis with 14 studies (n=3855) estimated an overall risk ratio of 0.81 (95% confidence interval 0.71 to 0.92), indicating BCG 19% protective efficacy against latent TB infection among vaccinated children. BMJ. 2014;



Subgroup analysis of six studies (n=1745) that reported the number of people who progressed to active tuberculosis disease during screen.



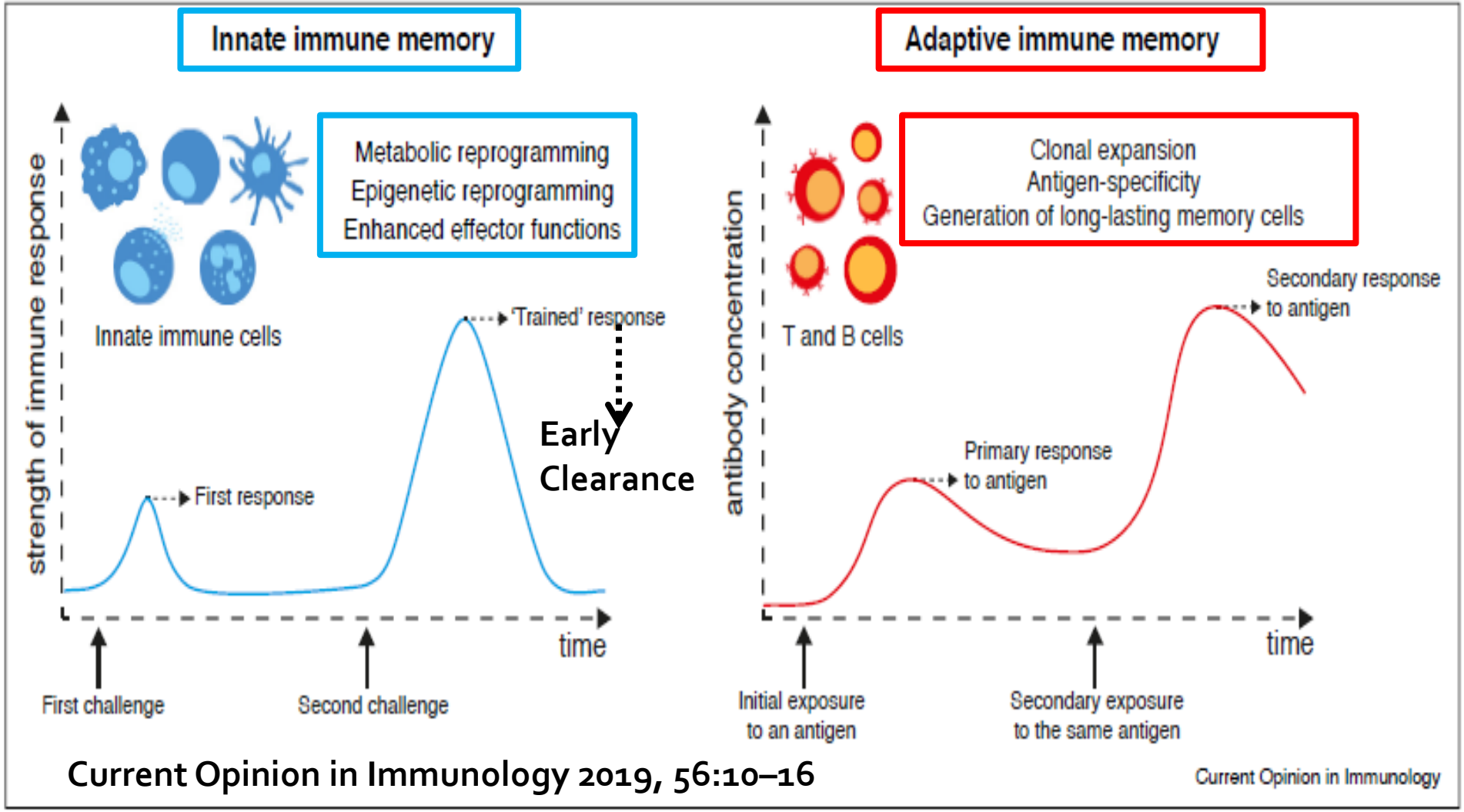
BMJ. 2014; 349: g4643.

Non-Specific Effect: SIX RCTs showed that BCG reduced mortality from diseases other than TB by 25% (95% CI 6% to 41%).

Table 2 Controlled trials of the effect of BCG on mortality from causes other than tuberculosis among children in the USA and the UK (reported in papers published between 1948 and 1961)

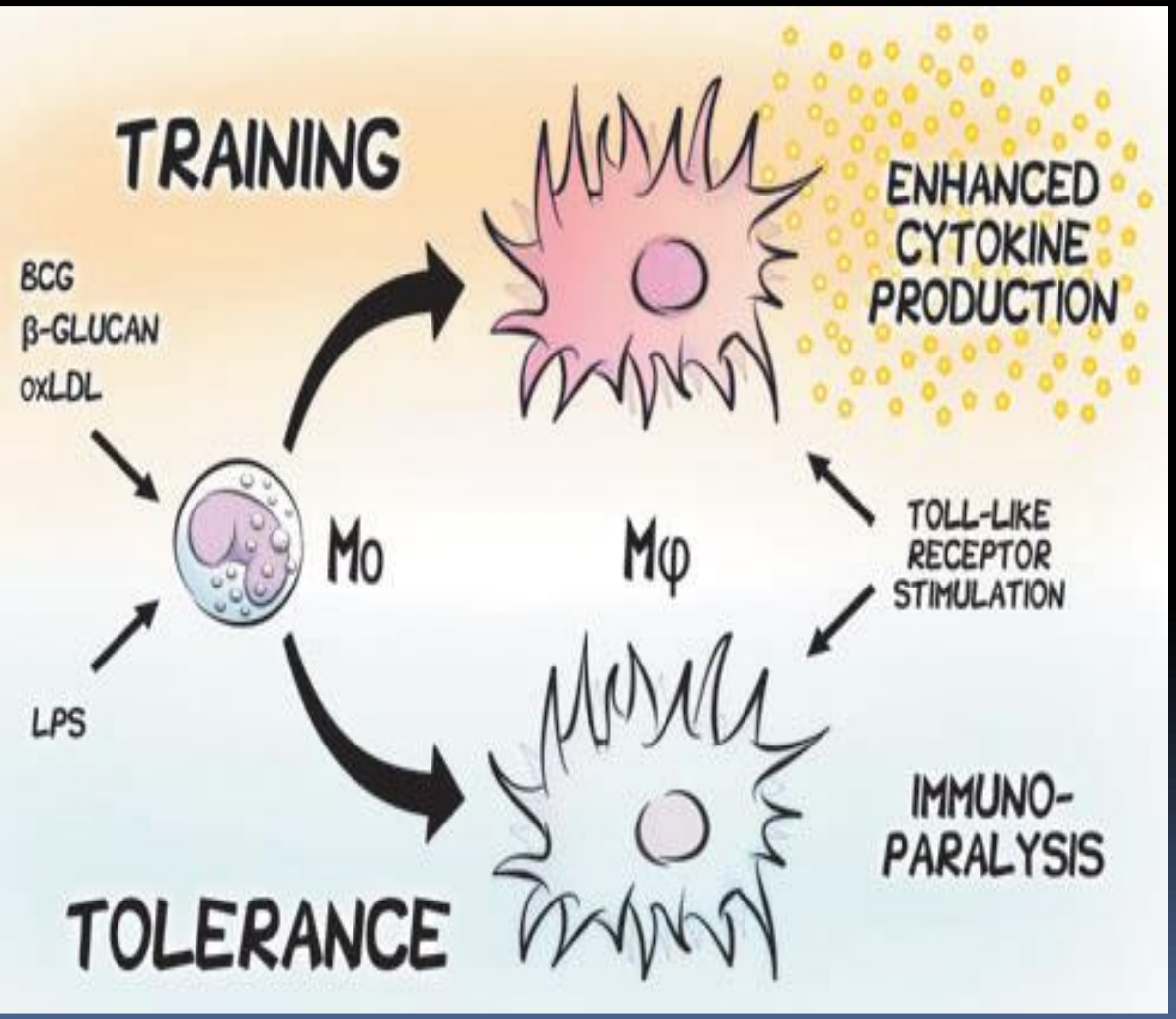
Study	Age followed	Allocation	*BCG*	No BCG	Reduction in mortality (95% CI)*
USA[45]	0–13 years	Alternate	N=231: 3/1261 (2.4‰)	N=220: 3/1320 (2.3‰)	−4% (−682% to 86%)†
USA[46]	0–15 years	Random	N=306: 49/2013.5 (24.3‰)	N=303: 51/1839.2 (27.7‰)	12% (−33% to 42%)†
USA[47]	0–20 years	Alternate	N=1551: 49/16406 (3.0‰)	N=1457: 56/15207 (3.7‰)	19% (−21% to 46%)
UK[48]	14–21 years	Odd/even	N=6700: 7/6700 (1.0‰)‡ §	N=6500: 10/6500 (1.5‰)‡	32% (−98% to 78%)†
USA[49]	0–16 years	Alternate	N=566: 14/566 (24.7‰)‡	N=528: 25/528 (47.3‰)‡	48% (−4% to 75%)
UK[48]	14–21 years	Odd/even	N=14100: 8/14100 (0.6‰)‡	N=16/13200 (1.2‰)‡	53% (−12% to 83%)†
Total					25% (6% to 41%)¶
Twins[35]	0–17 months	Twins	1/5 (89)=0.20 (0.02–1.68)**	DTP: 22/3 (164)=7.3 (2–38)**	p<0.001

Innate immune cells are able to undergo long-term adaptation and acquire enhanced capability to respond to certain stimuli, termed innate immune memory or trained immunity.



Current Opinion in Immunology 2019, 56:10–16

Trained immunity: Monocyte (Mo) memories of past encounters with microbial/nonmicrobial products can elicit vastly different responses to future exposures on differentiation to macrophages (Mu).



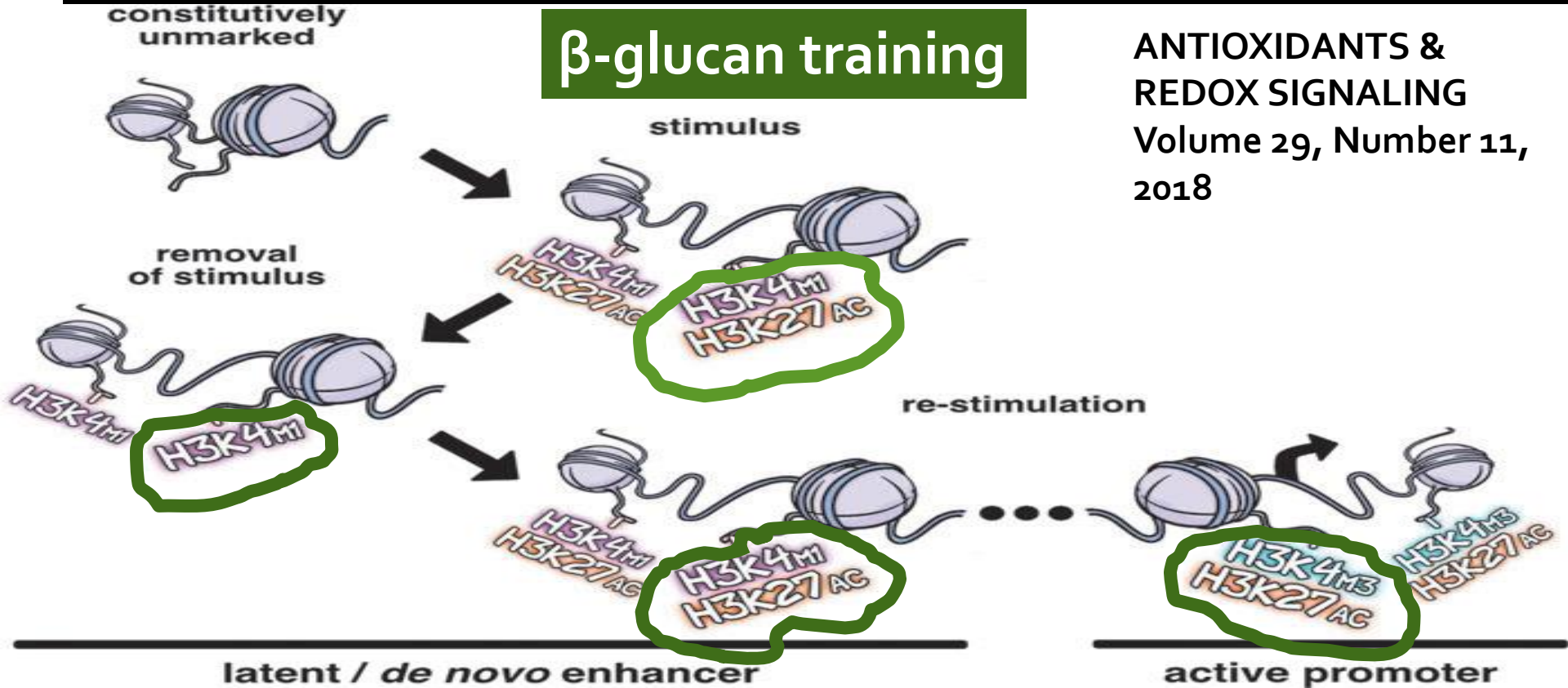
- Trained immunity:
  - induced by BCG, b-glucan, or oxLDL,
  - enhanced nonspecific response to subsequent infections
  - enhancing the inflammatory and antimicrobial properties of innate immune cells.
- Immune tolerance:
  - primary stimulation with LPS induces a persistent refractory state,
  - markedly reduced capacity to respond to re-stimulation.

ANTIOXIDANTS & REDOX SIGNALING Volume 29, Number 11, 2018

Latent enhancers prime a transcriptional memory in macrophages via Epigenetic change.

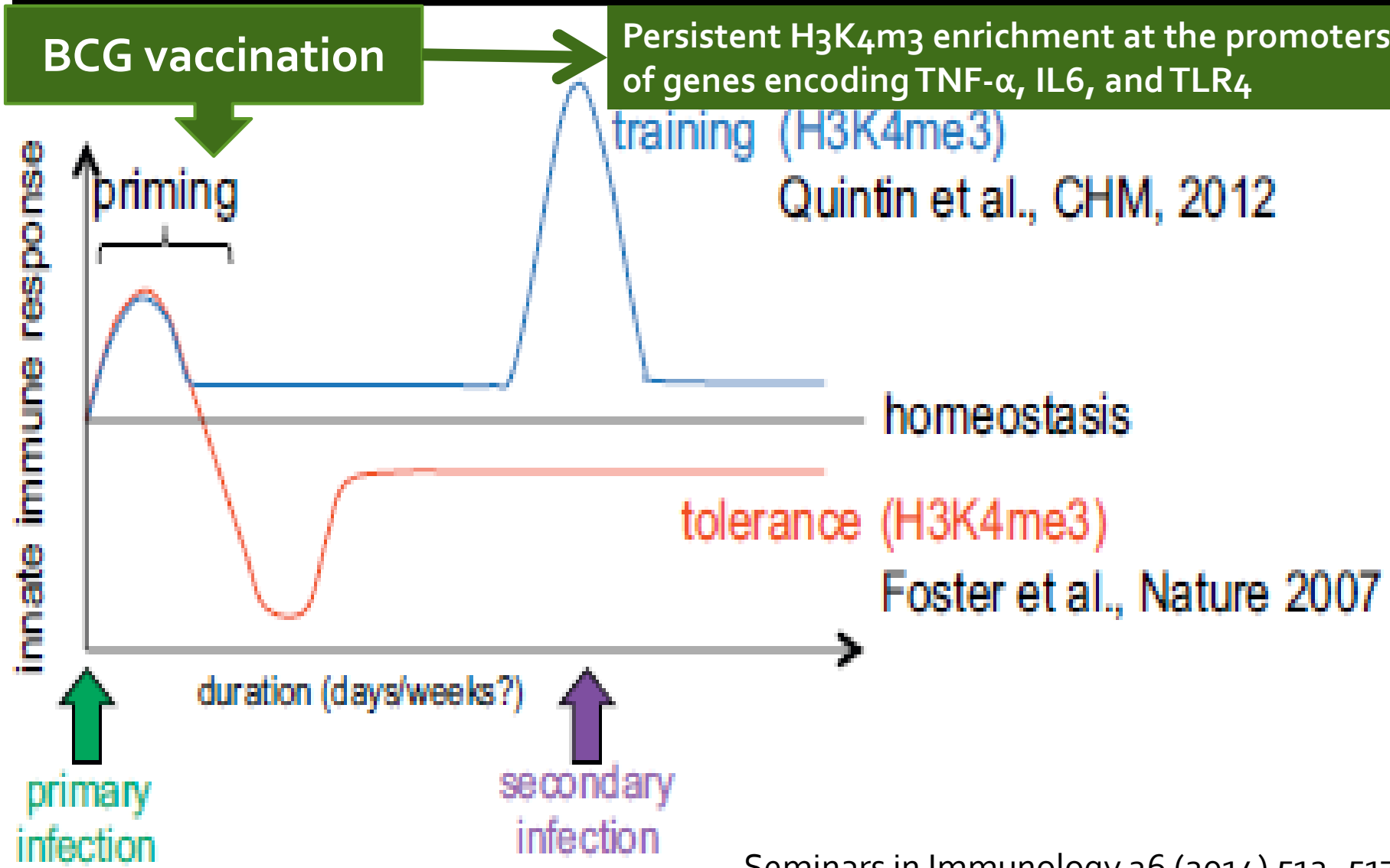
Constitutively unmarked distal regulatory elements acquire epigenetic features of enhancers (open chromatin: H3K4m1 and H3K27ac) in response to specific stimuli.

On removal of the activating stimulus, regions that retain the H3K4m1 enrichment mediate a faster and more robust response to re-stimulation.



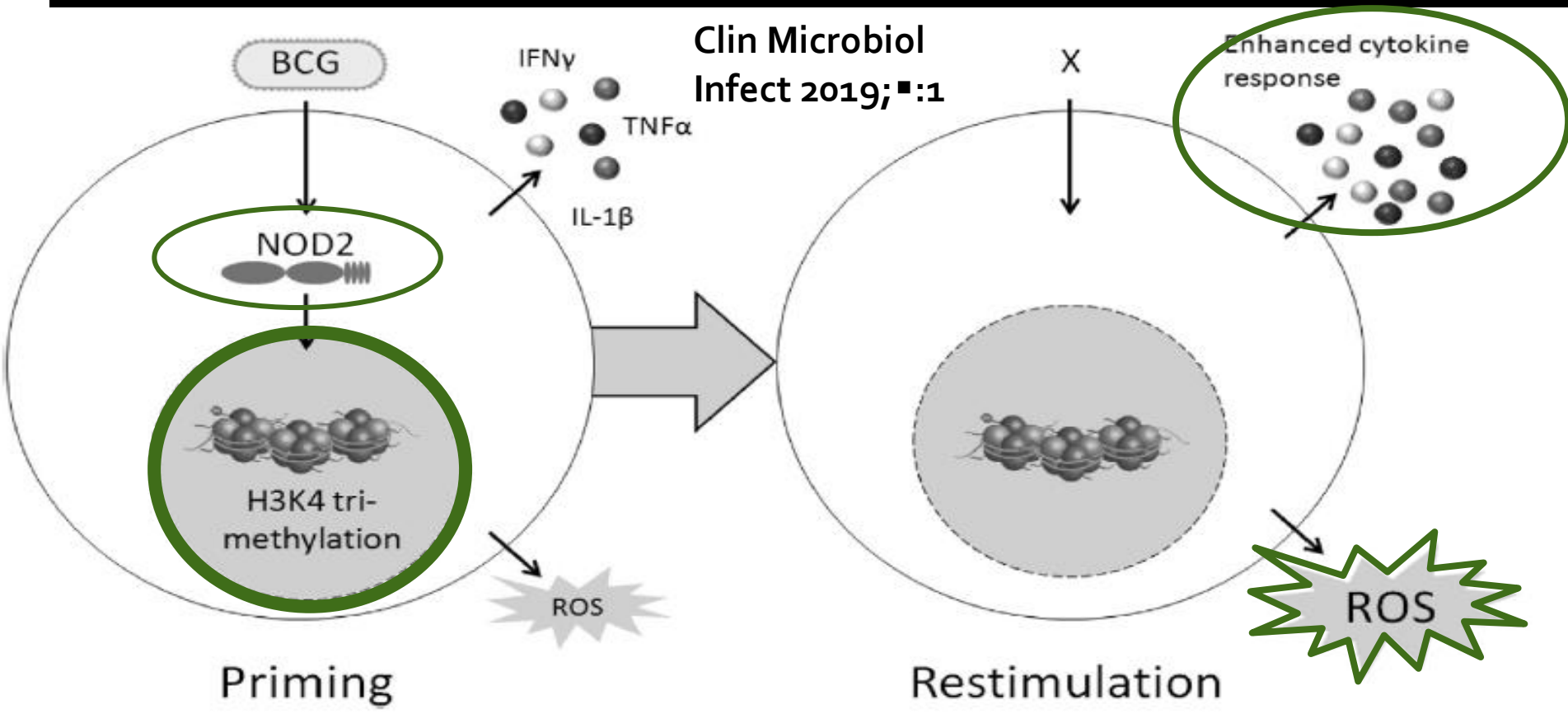
ANTIOXIDANTS & REDOX SIGNALING
Volume 29, Number 11, 2018

BCG vaccination induces trained immunity and this effect is beneficial both for protection against TB, as well as unrelated non-mycobacterial infections.

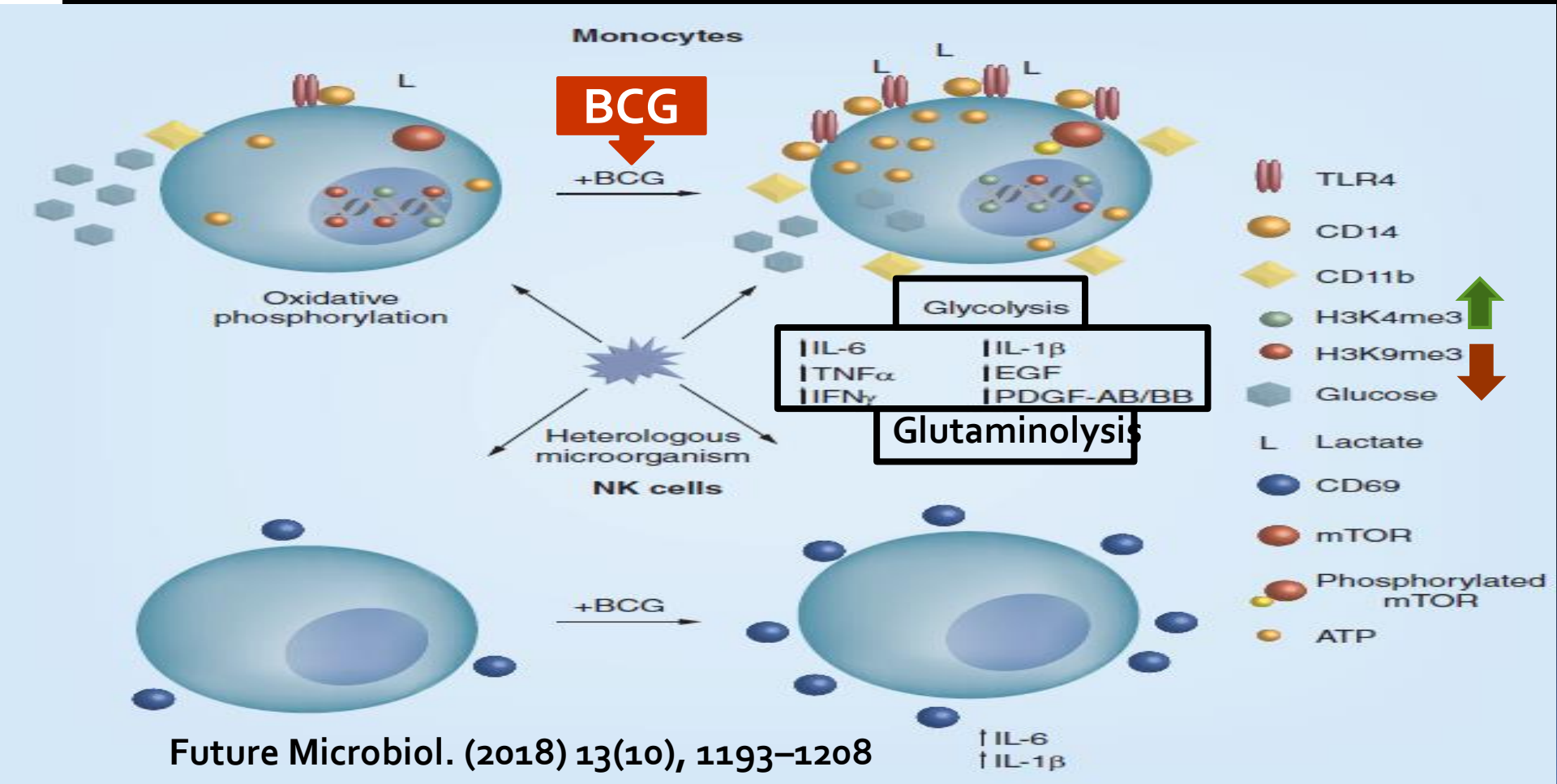


Seminars in Immunology 26 (2014) 512–517

After BCG is taken up by the monocyte, it is recognized by the NOD2 receptor, which upon activation induces epigenetic and metabolic reprogramming of the cell (H3K4me3), leading to an enhanced, non-specific response to a subsequent infection through an increased production of cytokines and reactive oxygen species

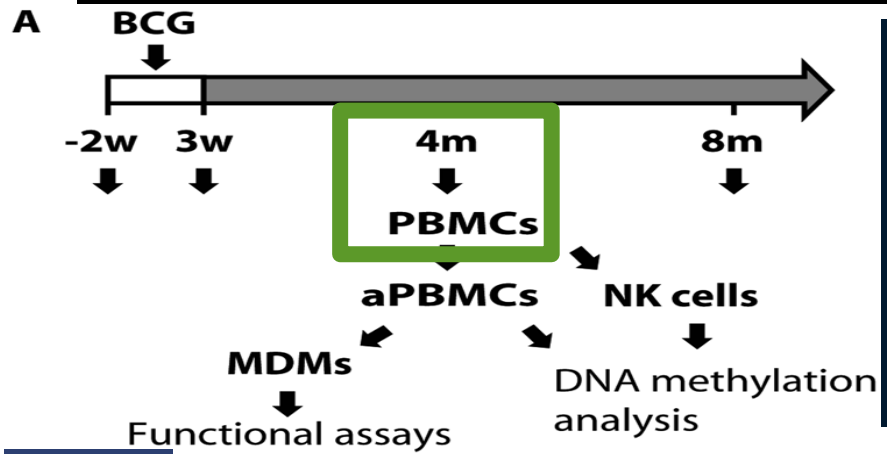


BCG training-induced increased frequency of permissive H3K4me3 and reduced presence of inhibitory H3K9me3 at the promoters of cytokine, receptor and metabolic pathway component encoding genes.



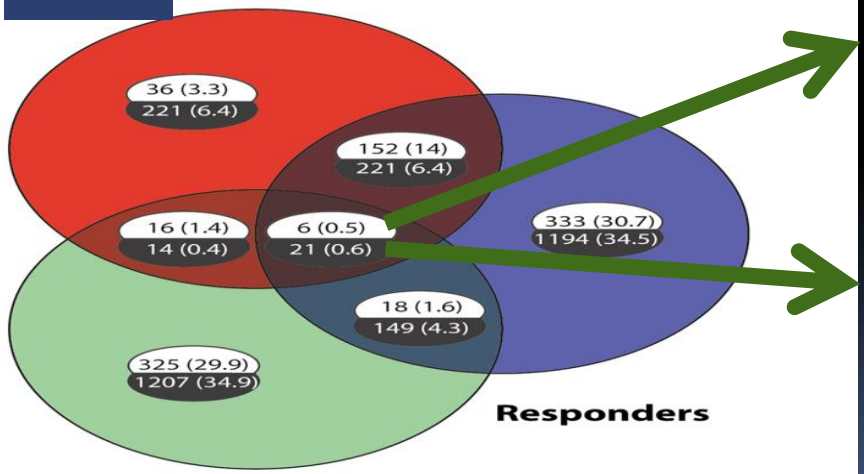
Future Microbiol. (2018) 13(10), 1193–1208

Anti-mycobacterial activity correlates with altered DNA methylation pattern in immune cells from BCG-vaccinated subjects.



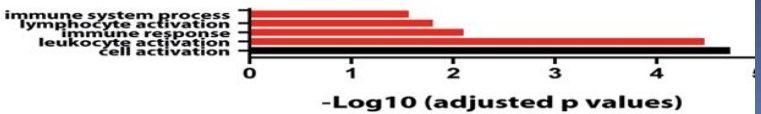
4 **responders** vs. 4 non-responders: enhanced anti-mycobacterial activity in Mtb-infected **macrophages** and increased **IL-1β** production

EWAS



6 Promoters displaying persistent hypo-Me at all time points (3 weeks, 4 months, 8 months): IFN-γ, RASAL1, GIMAP7, ADCY3, ATXN1, DIABLO

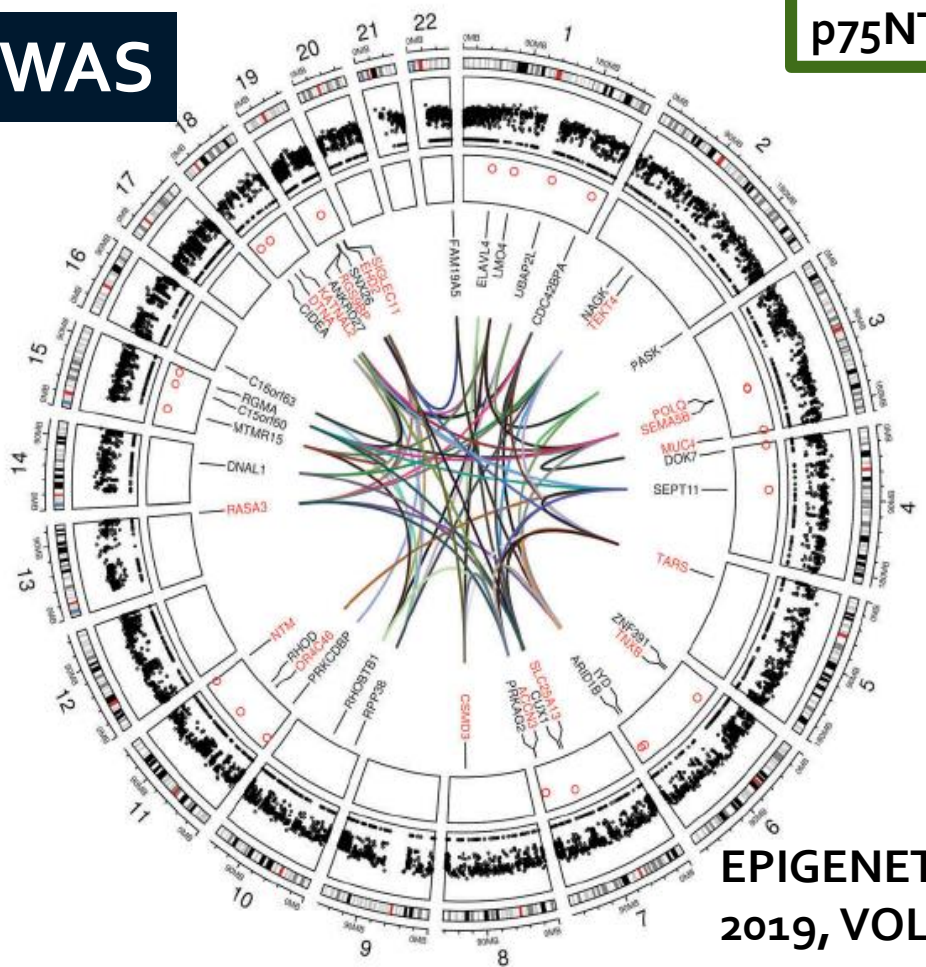
21 Promoters with persistent hyper-Me: TLR6, SRD5A2, SOX5, GNG7, SBNO2, SULT1C , NFKBIE, TRIM2, GPR84, SPATS2, CD59, ATXN1, NCOR2, ADARB1, LOC404266, SRGAP3, PIWIL2, SPG20, TSPAN4, CSGALNACT1



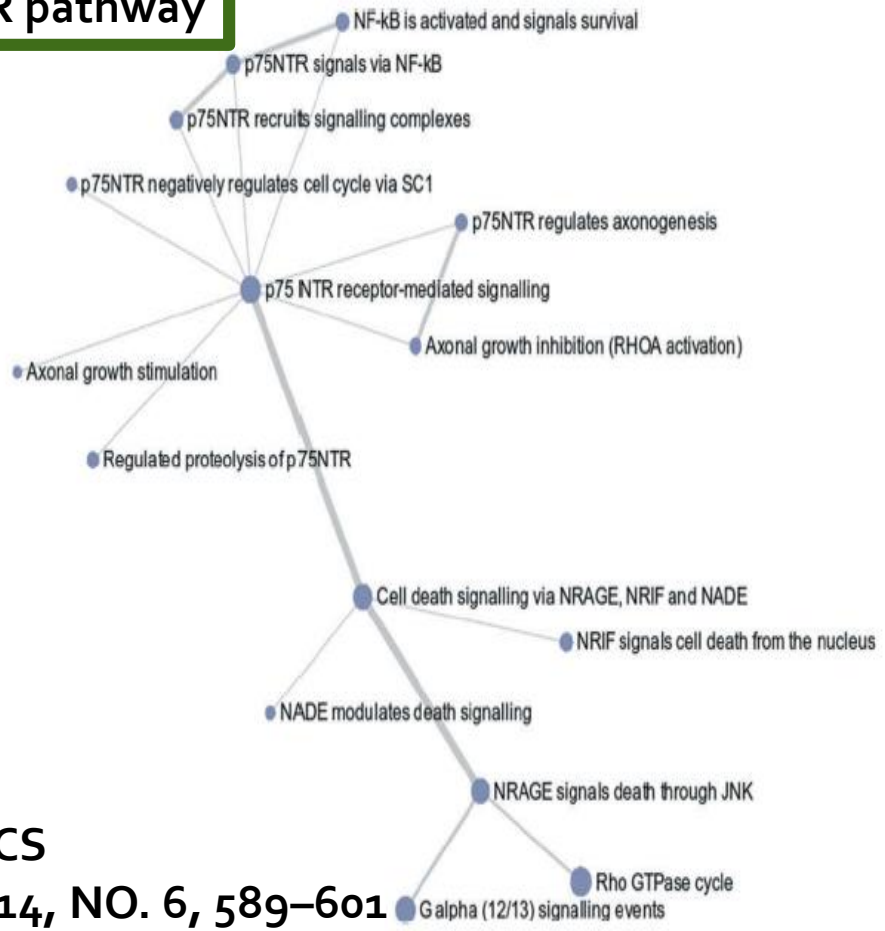
ScIeNtIfIc REPOrtS 2017 | 7: 12305

43 differentially methylated genes in PBMCs isolated from responders vs. non-responders at the time point before BCG vaccination--enriched for actin-modulating pathways predicting differences in phagocytosis.

EWAS



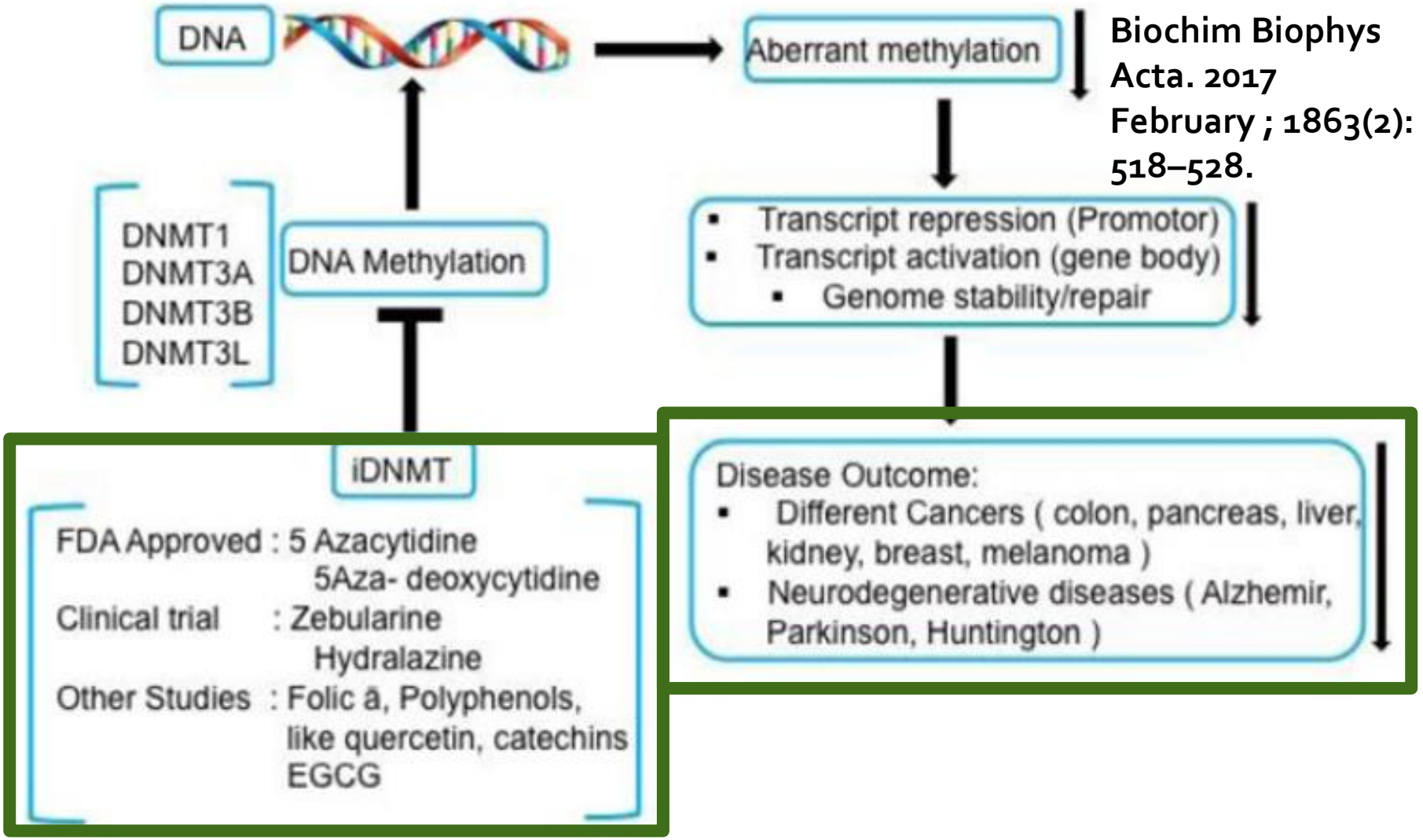
p75NTR pathway



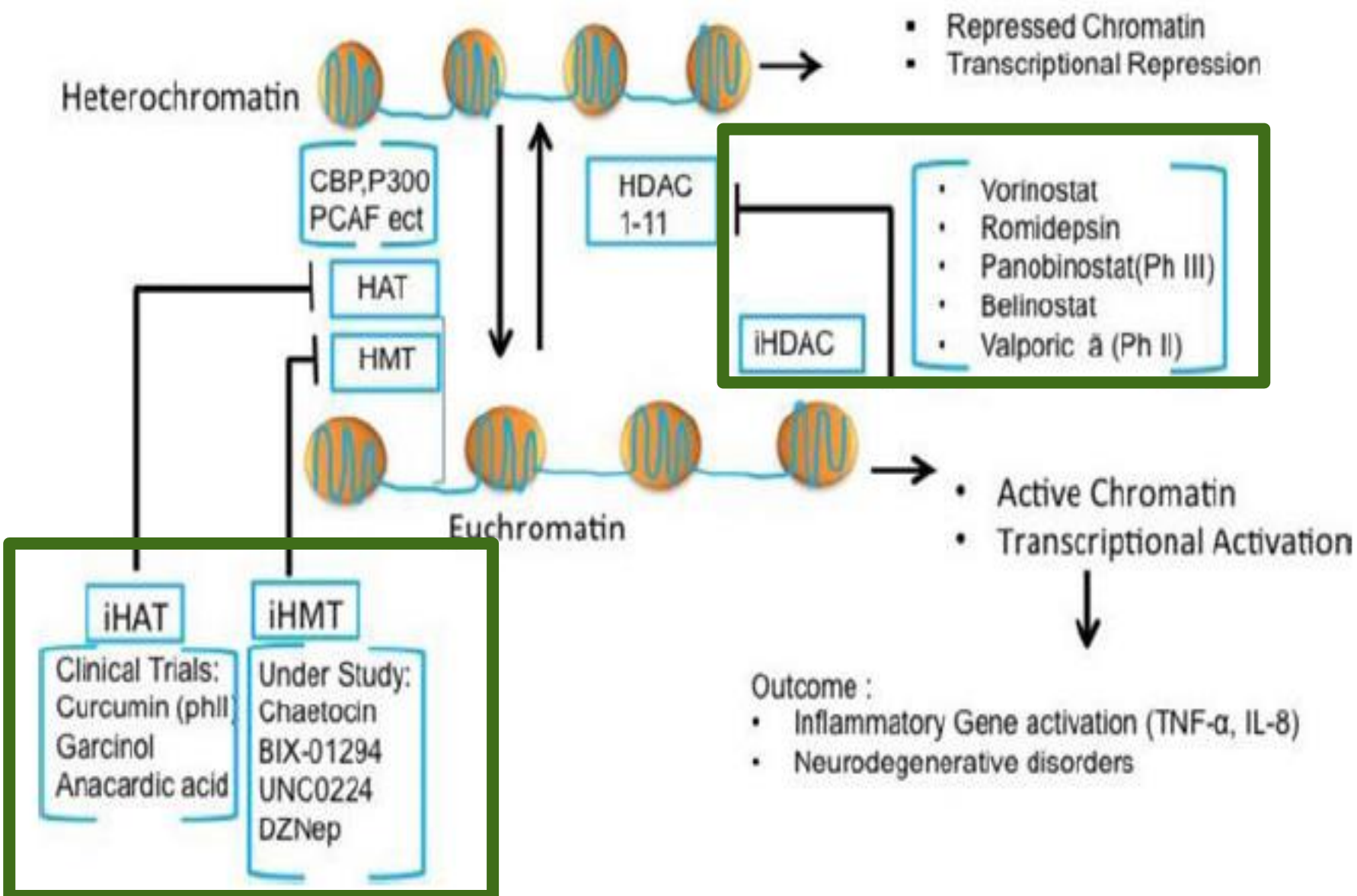
EPIGENETICS
2019, VOL. 14, NO. 6, 589–601

Future perspectives: Epigenetic targets for Host-Directed immunotherapy?

Several molecules have been screened for altering the DNA methylation status associated with the disease outcome and are currently in different phases of clinical trial.

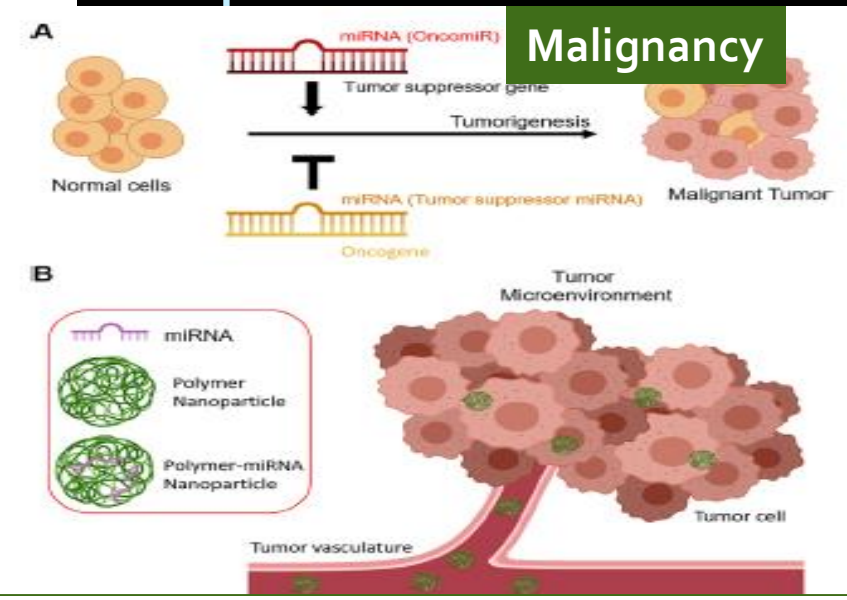


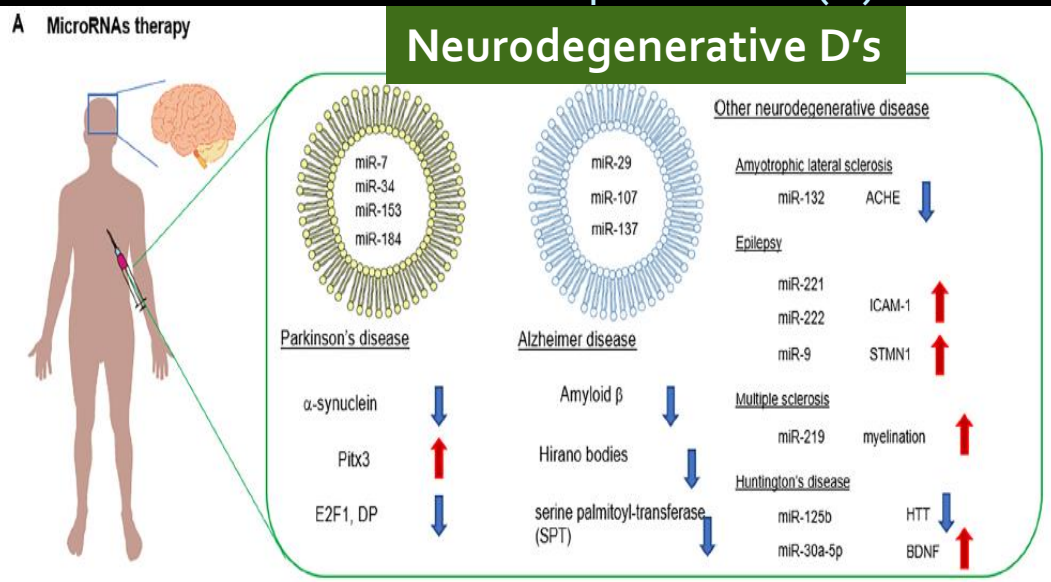
Clinical trials in aberrant histone modifications associated with disease outcome

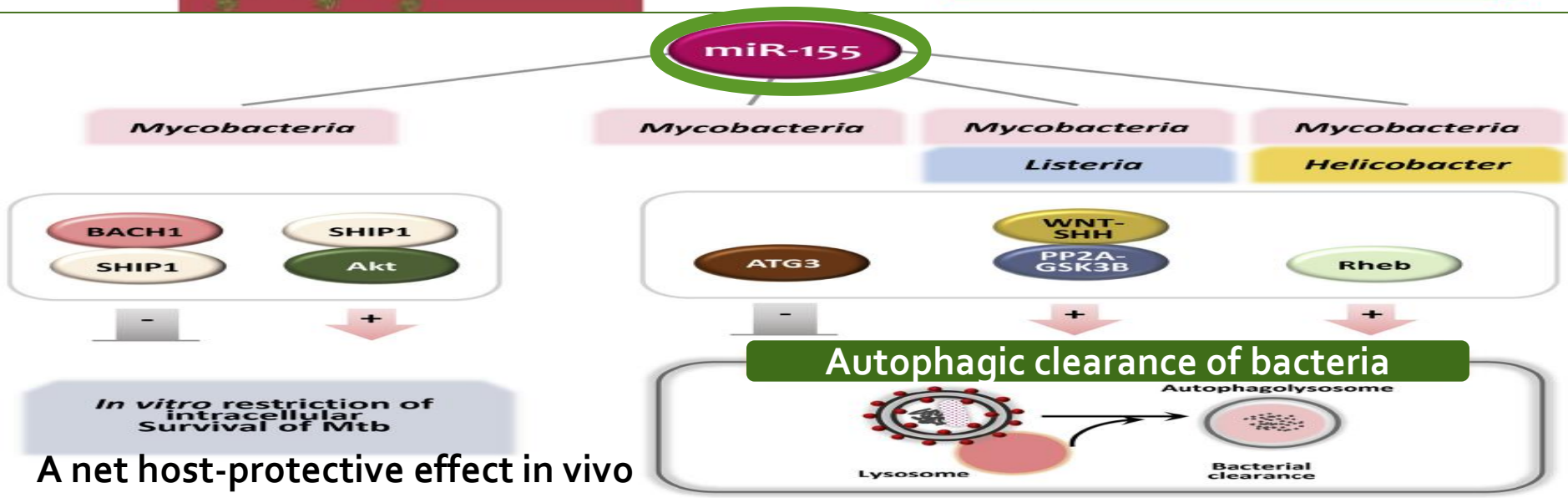


miRNAs may influence the outcome of bacterial infection by regulating autophagy/xenophagy responses in host cells

Semin Cell Dev Biol. 2019 pii: S1084-9521(18)30239-8

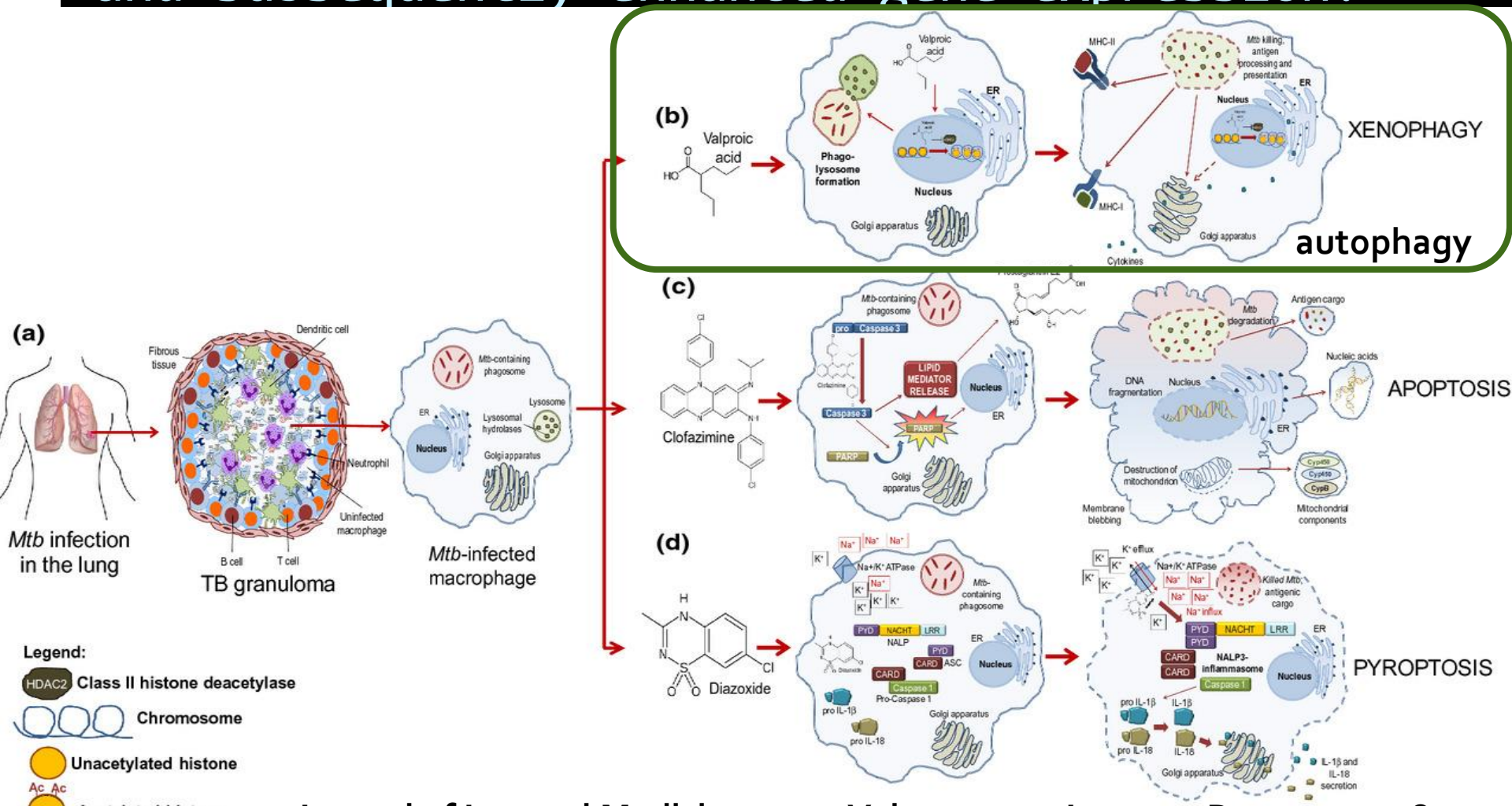






A net host-protective effect in vivo

Host-directed therapies: Xenophagy may be initiated by valproic acid to allow histone acetylation followed by chromosome unwinding and subsequently enhanced gene expression.



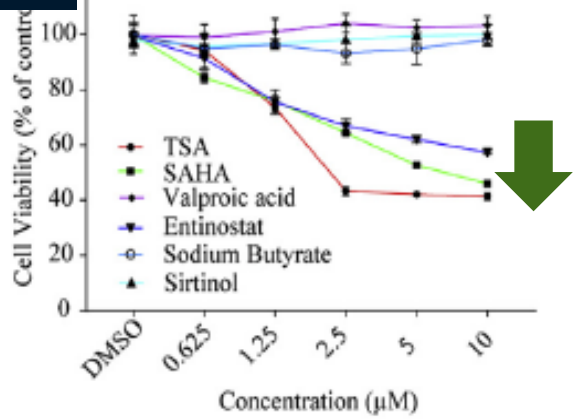
Journal of Internal Medicine 2015, Volume: 277, Issue: 4, Pages: 373-387

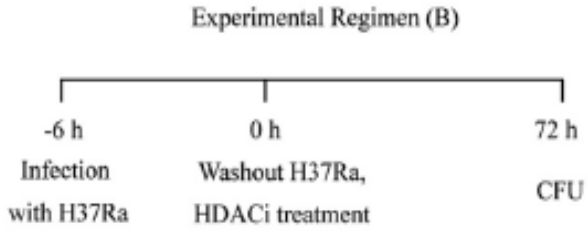
Non-specific Histone de-acetylase inhibitors impair the host immune response (autophagy & ROS) against MTB.

Tuberculosis 118 (2019) 101861

In Vitro

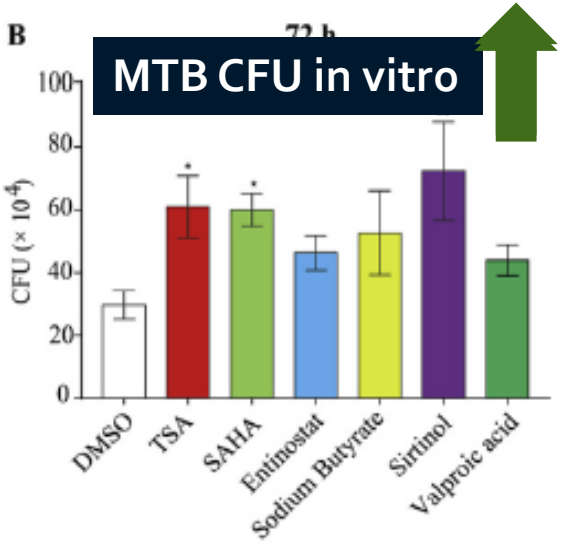
Macrophage viability



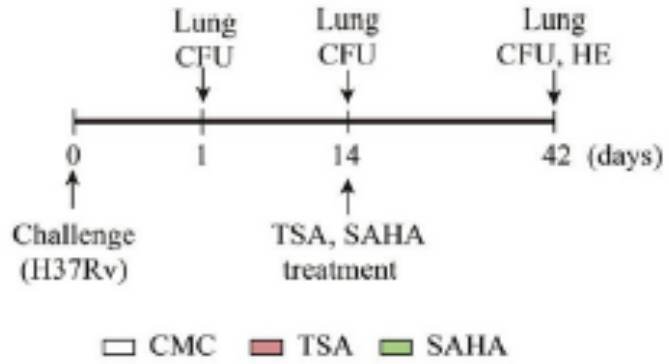


HDAC inhibitors

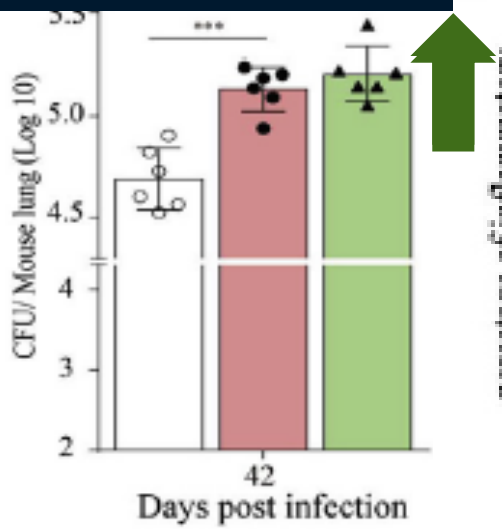
MTB CFU in vitro



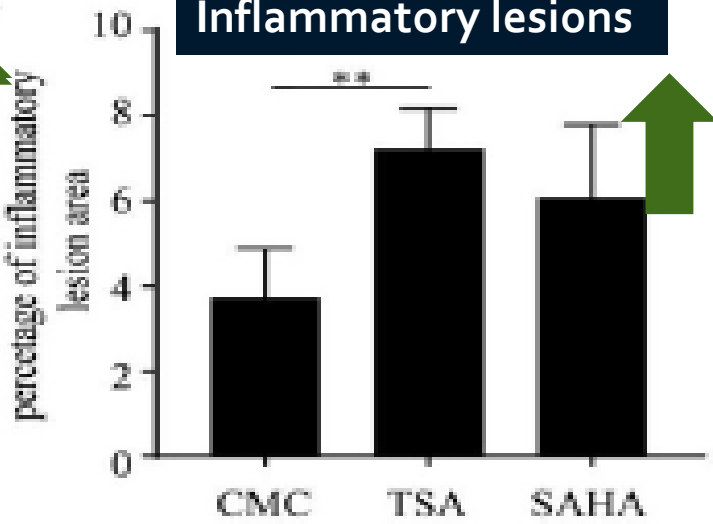
In vivo



MTB CFU in mouse lung



Inflammatory lesions



Gene-Editing: clustered regularly interspaced short palindromic repeat (CRISPR)

Sequence-specific RNAi: microRNA mimic/siRNA

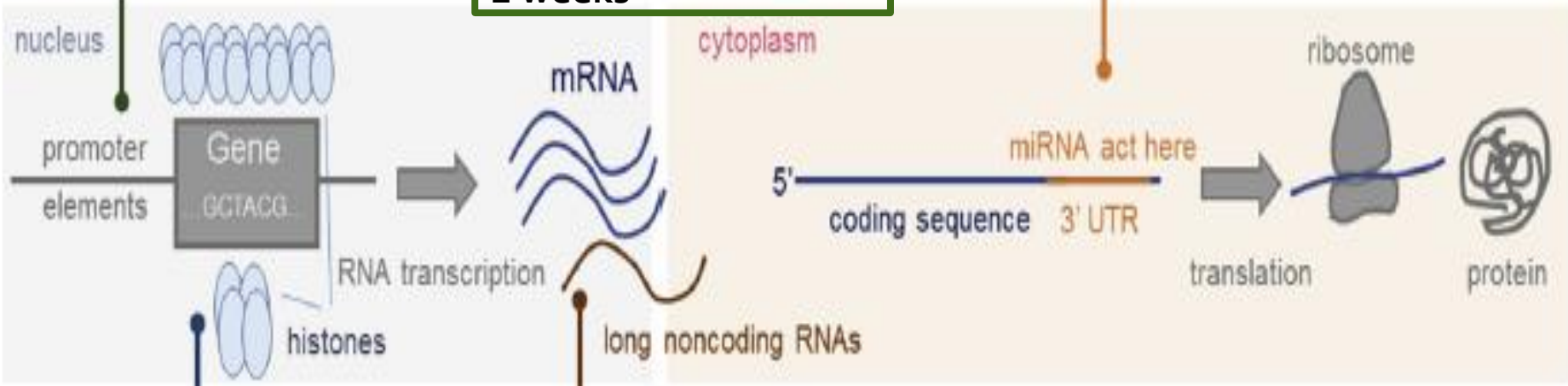
highly selective targeting

DNA methylation
Therapeutic approaches
- DNA methylation inhibitors
- Methionine (dietary)
- CRISPR-based editing

deliver a de-methylating enzyme, 5-azacytidine, to a specific promoter for 2 weeks

Posttranscriptional
Therapeutic approaches
- Oligonucleotides (antagomirs)

miR mimic /siRNA



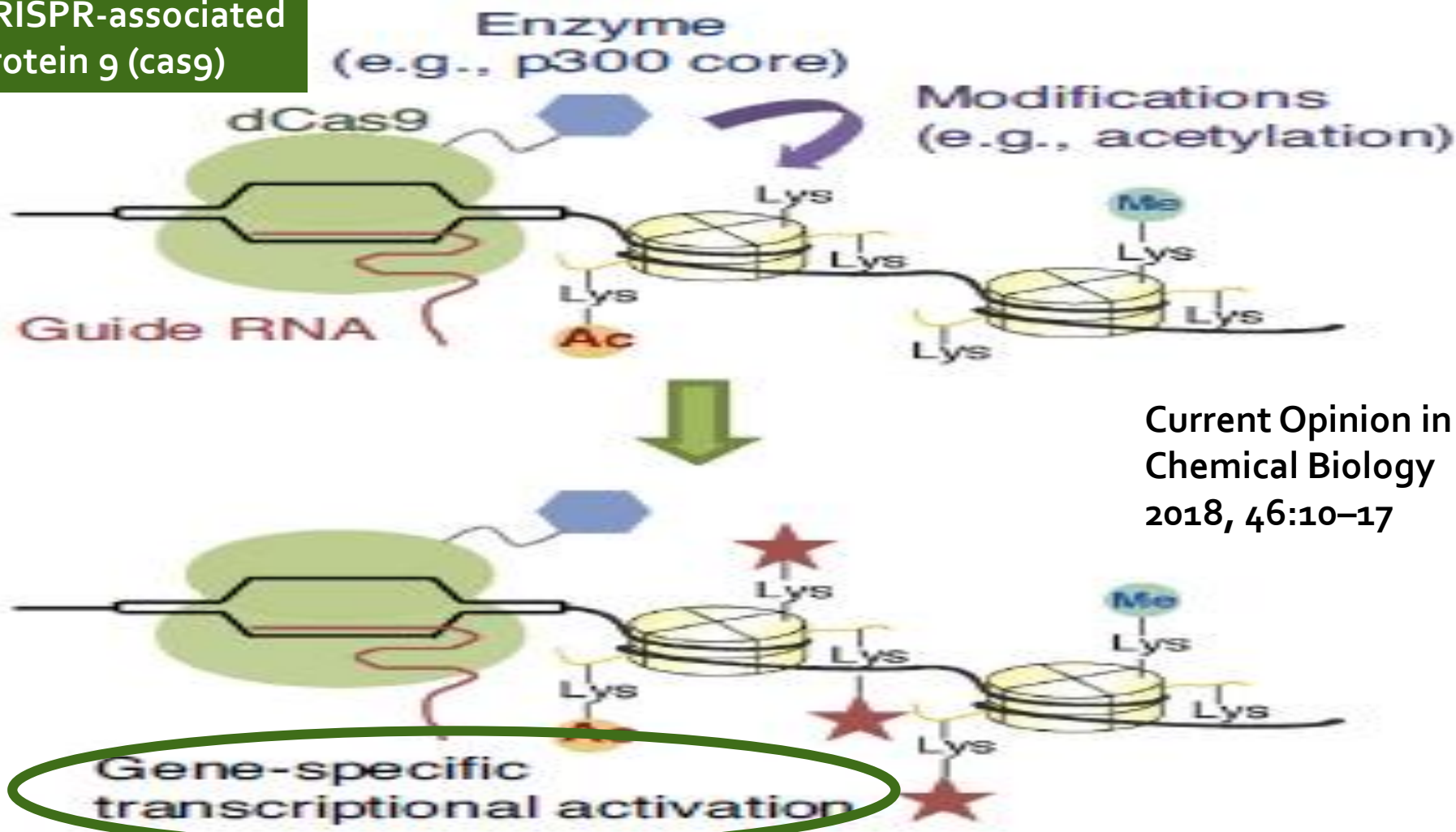
Posttranslational modifications
Therapeutic approaches
- Histone deacetylase inhibitors

Natural antisense transcripts
Therapeutic approaches
- Oligonucleotides (antiNATs)

European j o u r n a l of p a e d i a t r i c neurology 2019

The dCas9-p300 HAT core fusion protein was expressed in cells, which promoted lysine acetylation of histones at a specific genomic locus targeted by the guide RNA sequence.

CRISPR-associated protein 9 (cas9)



Current Opinion in Chemical Biology 2018, 46:10–17

Take Home Message (1)

- *M.TB* uses virulent factors Rv1988/Rv2966c/Rv3423.1/ RV1346c to hijack the host transcriptional machinery through epigenetic regulations.
- Association of active TB disease with
  - **Aberrant DNA Met over VDR, TLR2, IL18R1, CYP27A1 genes**
  - **H3K14 hypo-Ac (TNF-α/IL12-β), HDAC1, KDM6B**
  - **At least 53 differentially expressed miRNAs**
- *M.TB* infection in vitro leads to
  - **de-Met of the NLRB3/CD82 gene promoters/1700 CpGs over distal enhancers**
  - **H3/H4 hyper-Ac, H3K9 hyper-Me, H3R42 hyper-Me, H3K4 hypo-Me/Ac, HDAC1/6, KDM6B, SET8**
  - **Immune-surppressive miRs: 21, 29, 99b, 125b, 27b, 1178**
  - **Immune-effective miRs: 155, 424, 223, 27a, 20b, 582**

Take home message (2)

- Specific and Non-specific effects of BCG vaccine via trained innate immunity (innate immune memory)
  - **$H3K4$ hyper-Me and $H3K9$ hypo-Me at the promoters of cytokine, receptor and metabolic pathway component encoding genes**
  - **BCG Responder: IFN-γ promoter DNA hypo-Me, TLR6 promoter DNA hyper-Me**
- Future perspectives for research:
  - **Epigenetic marks for Early clearance/Progression from LTBI to active TB D's; single cell methylome**
  - **CRISPR-based gene editing to deliver de-methylation agent/histone modifying enzymes to specific genomic locus**
  - **Highly selective antagomiR/miRNA mimic to enhance or surppress specific sequence of target genes**

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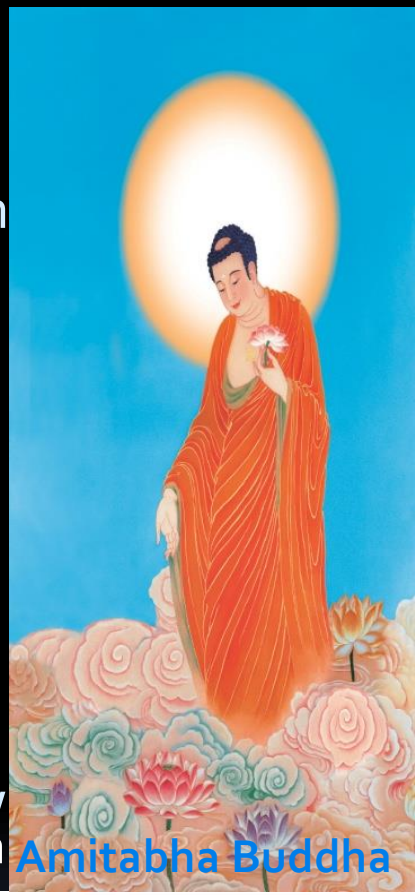

Amitabha Buddha

Thank You for Your Attention