EPIGENETICS IN DEVELOPMENT AND CANCER

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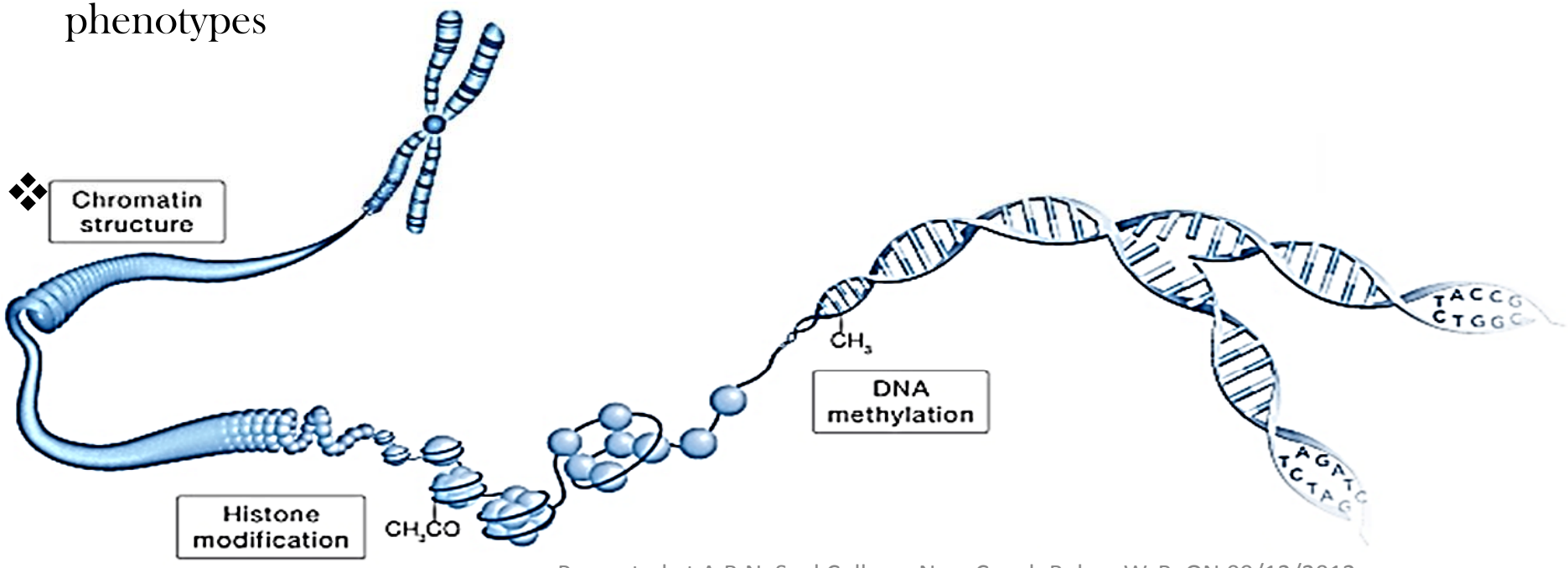
EPIGENETICS – AN INTRODUCTION

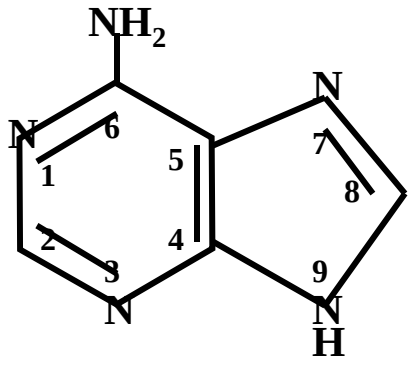
- Epigenetics is the study of mitotically and/or meiotically heritable changes in gene function without changes in the underlying DNA sequence.
- There is no change in the underlying genetic program instead, non-genetic factors cause the organism's genes to express themselves differently resulting in differential phenotypes



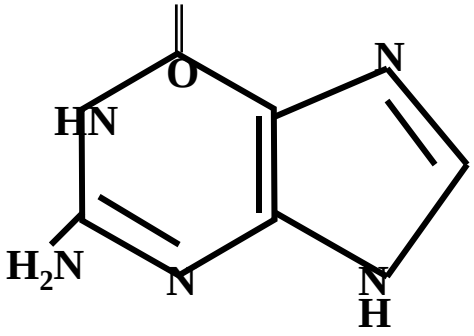
History of The Science

* Epigenesis : A theoretical aspects of developmental biology - The Strategy of Genes by C. H. Waddington (during 1930s to 1960s)
* Discovery of the molecular component, 5-methyl cytosine, in the 1940 s after serendipitous finding of epi-cytosine by R. D. Hotchkiss [J. Biol. Chem. 1948].
* CpG islands: features and distribution in the genomes of vertebrates. G. Bernardi, A Bird in 1980s-1990s
* First cloning of mammalian DNMT-by Bestor et.al. J.Mol.Biol 1988.
* Importance of DNMT1 in embryonic development in 1990 s.
* How do they interact with DNA? How do the other proteins interact?

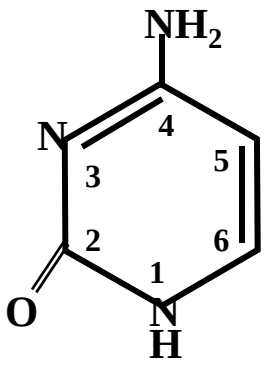
The building blocks of DNA



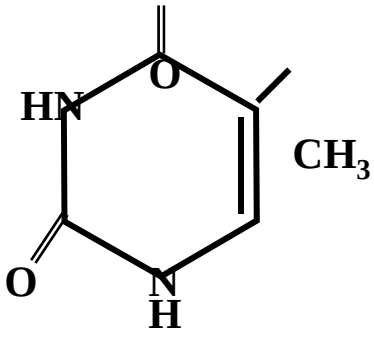
Adenine (A)



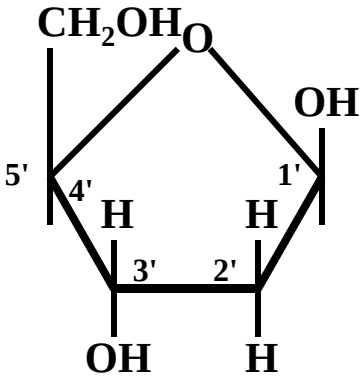
Guanine (G)



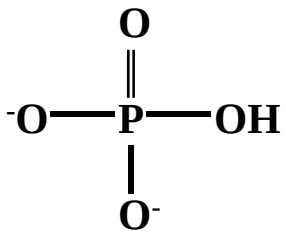
Cytosine (C)



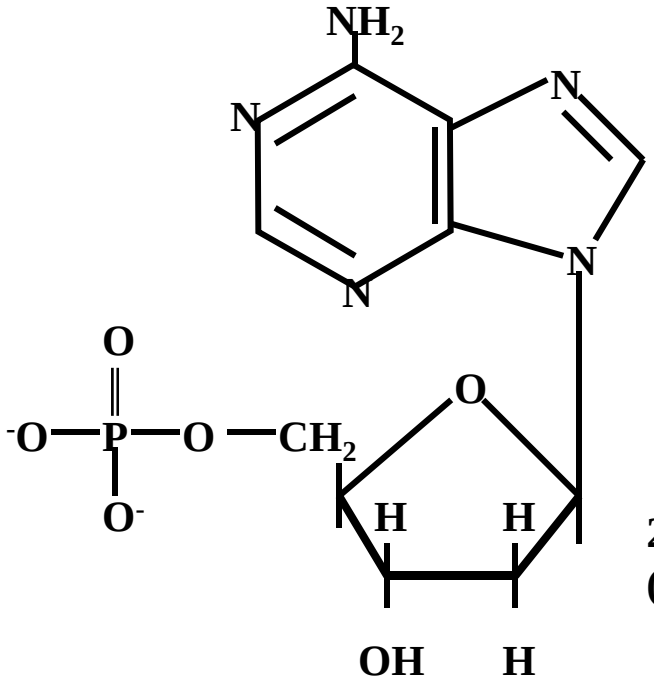
Thymine (T)



β-D-2-deoxyribose

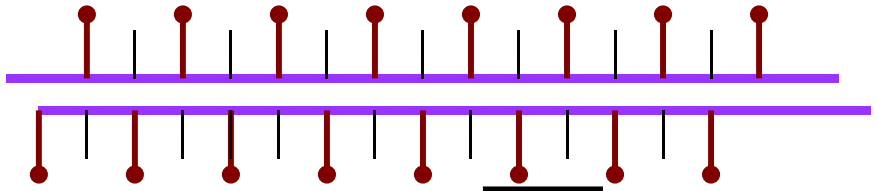


Phosphate



2-deoxy-AMP
(dAMP)

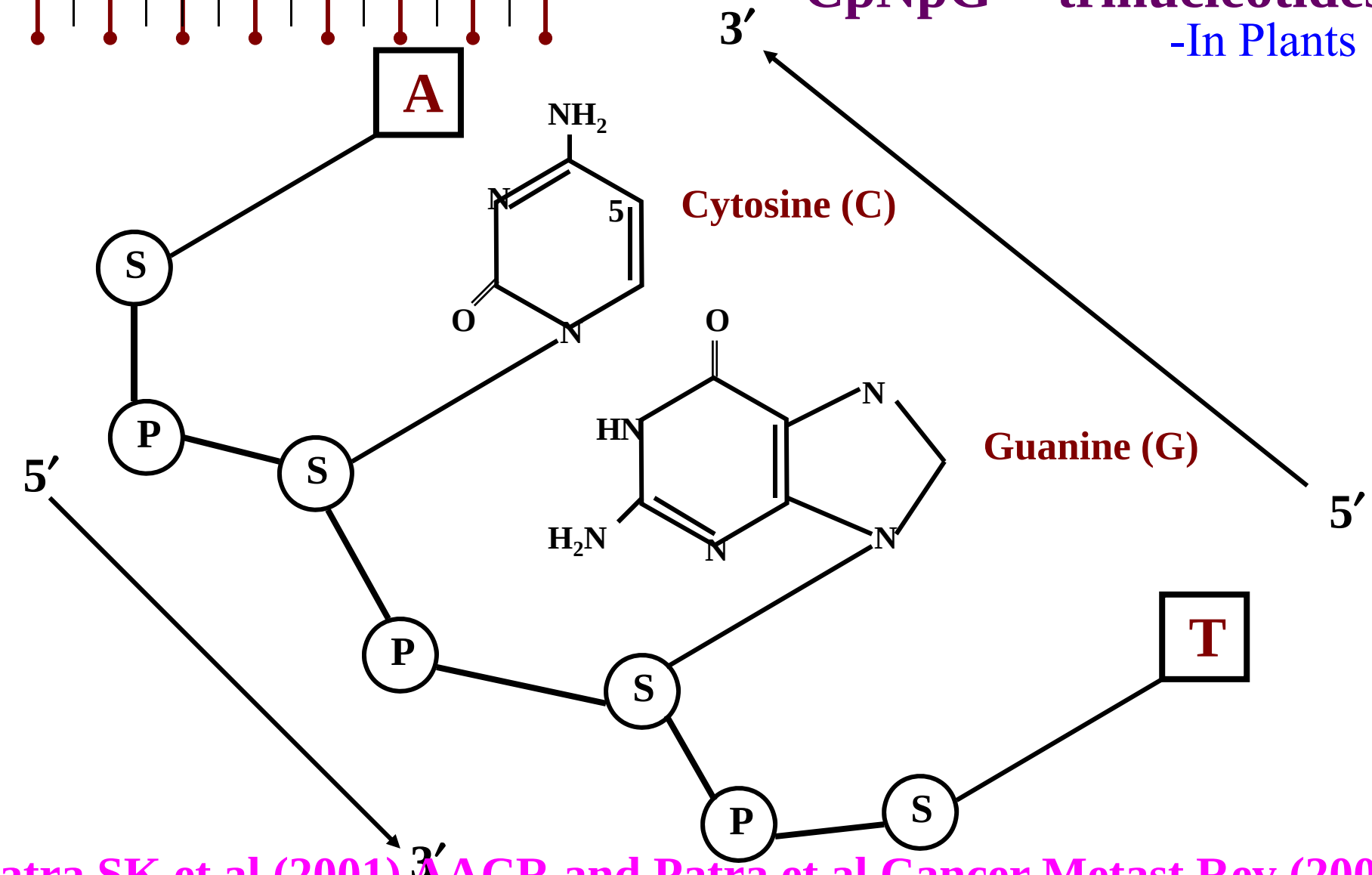
The –CpG- dinucleotides of DNA



-In bacteria and animals

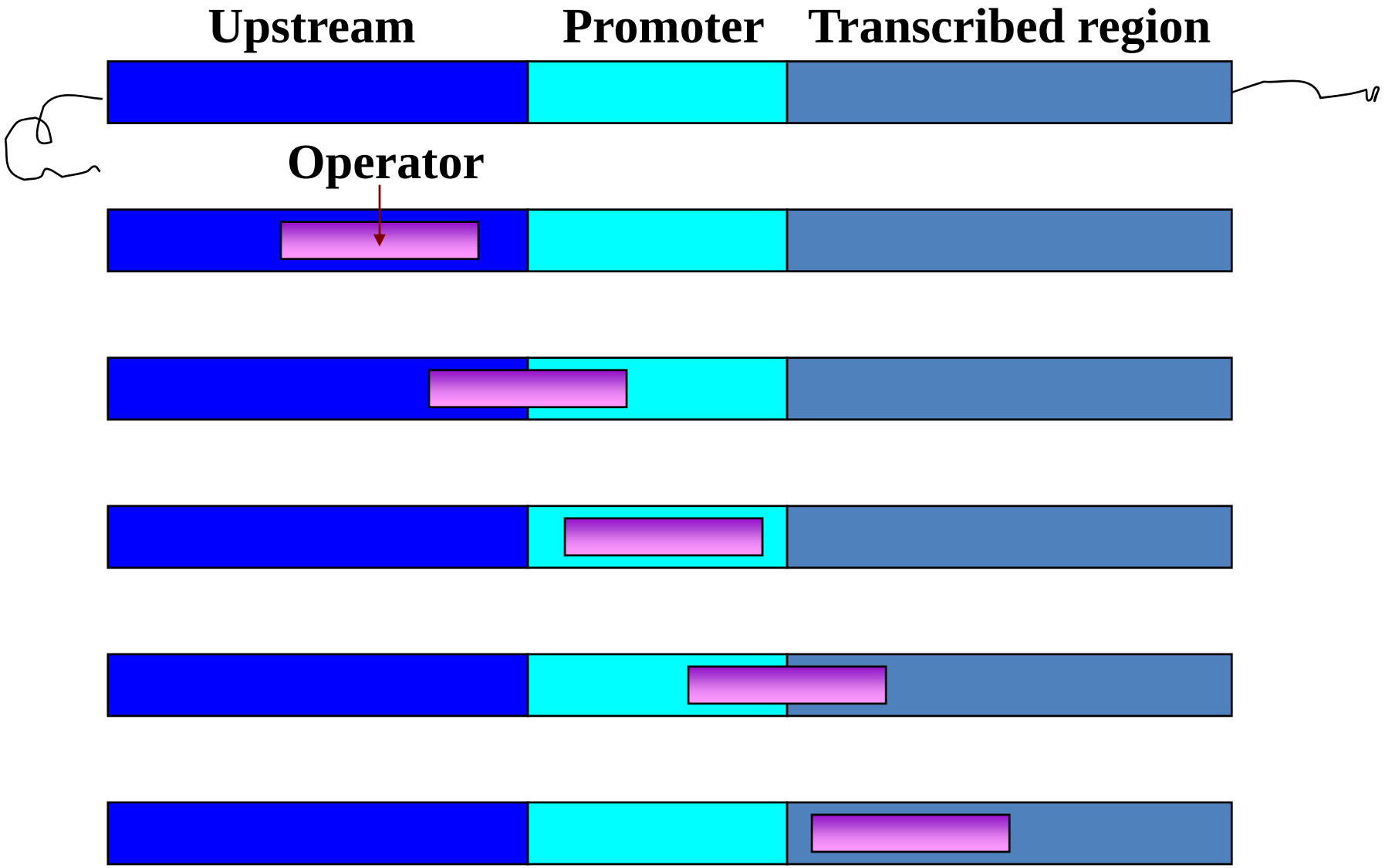
– CpNpG – trinucleotides

-In Plants

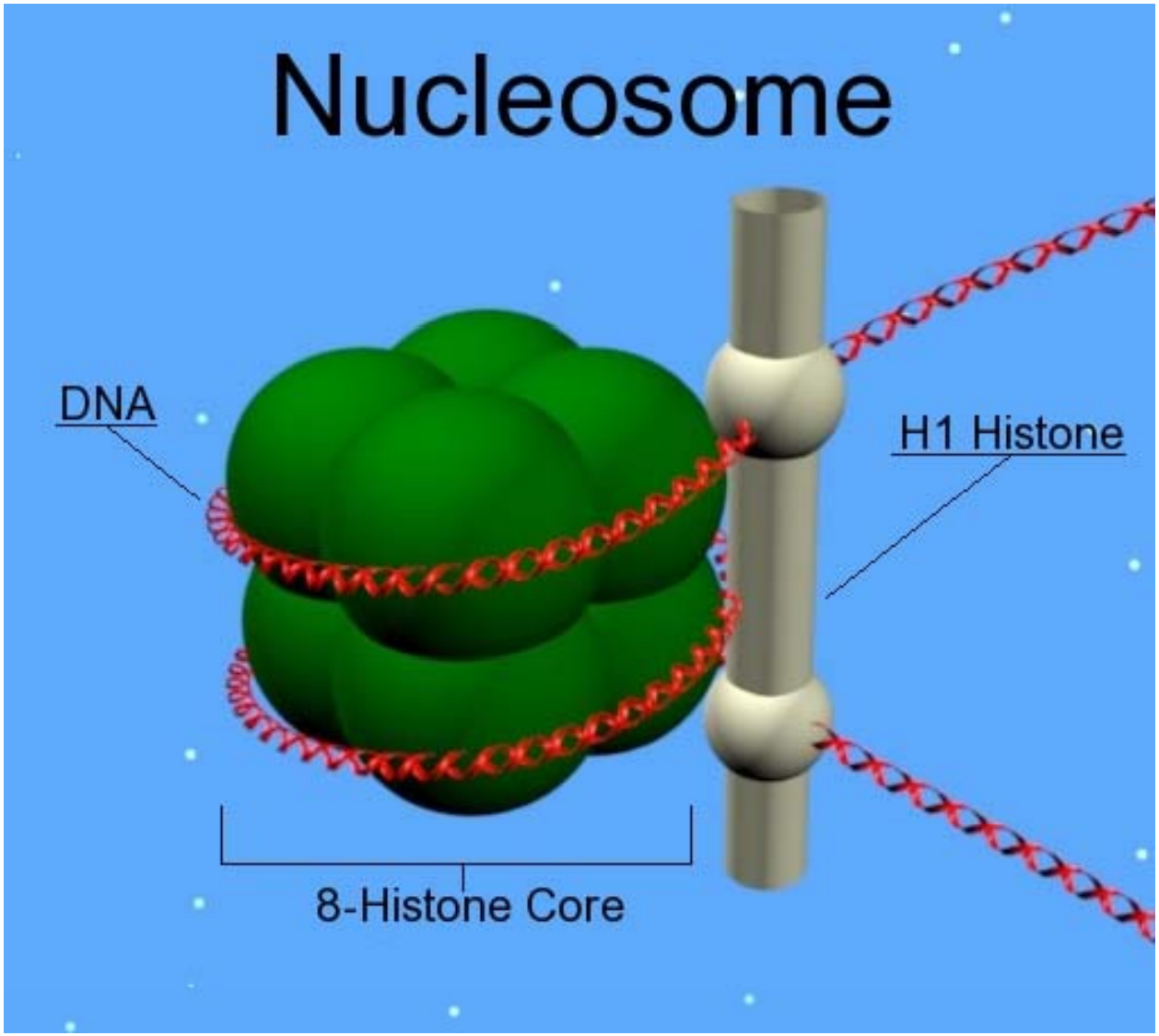


Patra SK et al (2001) AACR and Patra et al Cancer Metast Rev (2008)

Promoter-Operator Relative Positions



DNA-Histones (H2A, H2B, H3 and H4) complex



http://upload.wikimedia.org/wikipedia/commons/8/87/Nucleosome.jpg

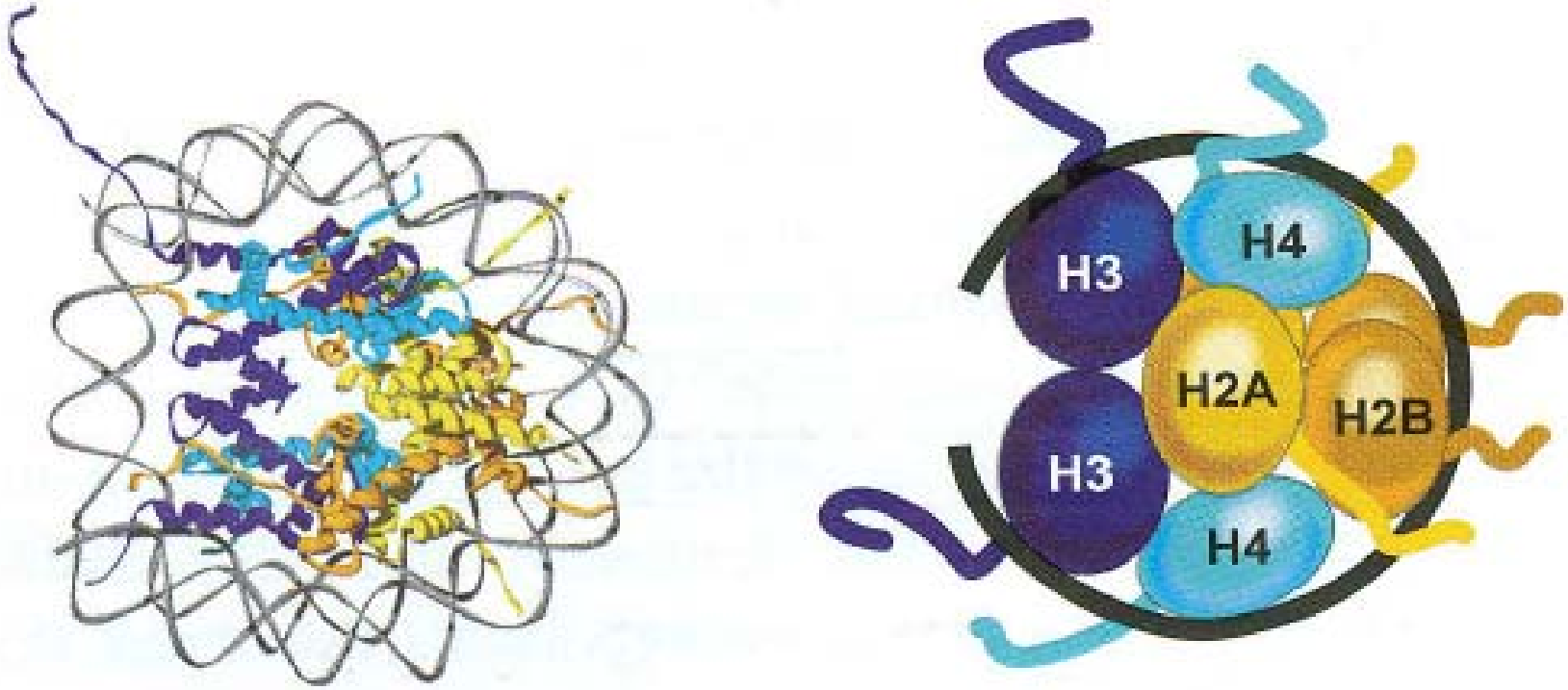
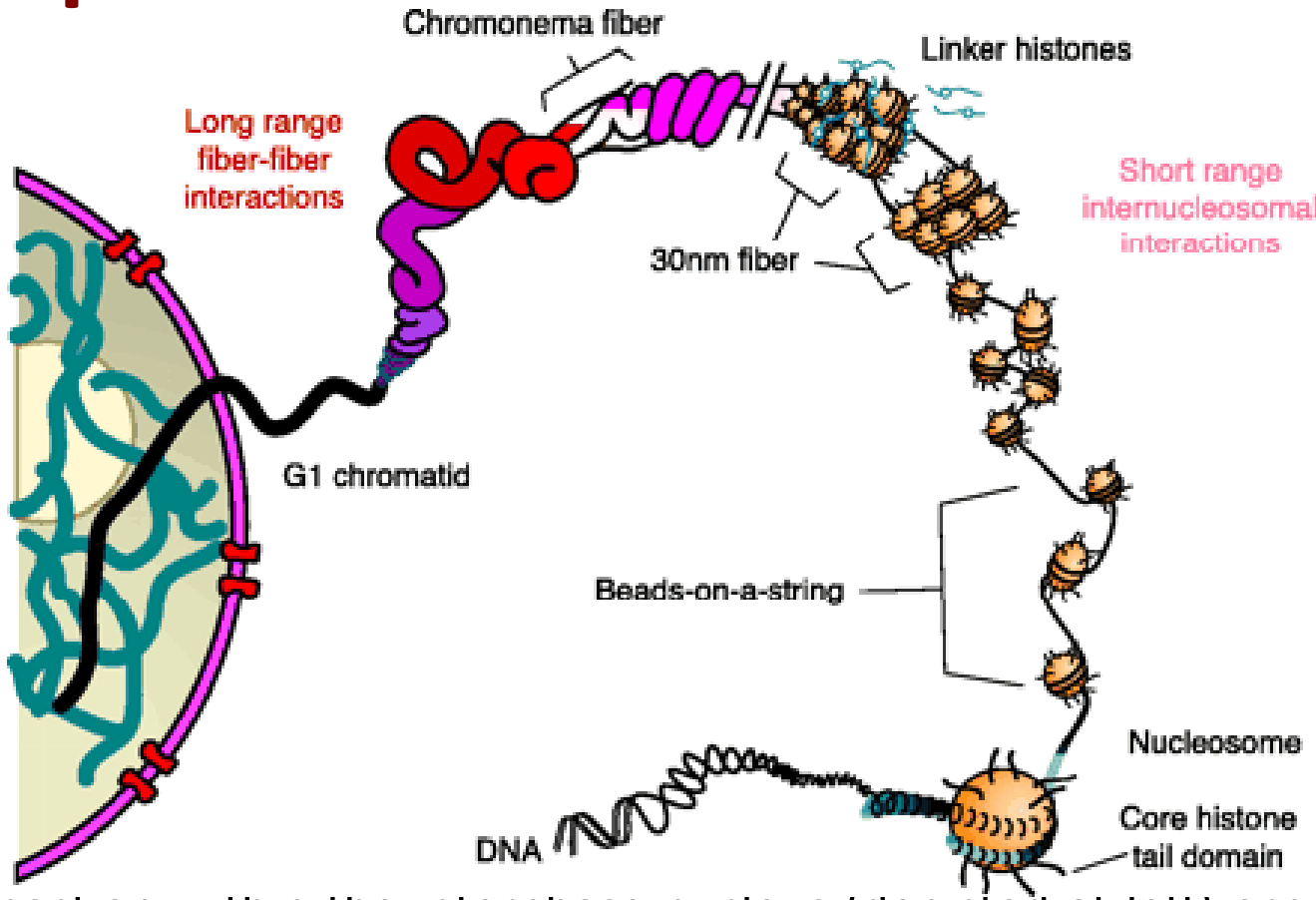


Figure 5. Nucleosome Structure

(*Left*) A 2.8 Å model of a nucleosome. (*Right*) A schematic representation of histone organization within the octamer core around which the DNA (*black line*) is wrapped. Nucleosome formation occurs first through the deposition of an H3/H4 tetramer on the DNA, followed by two sets of H2A/H2B dimers. Unstructured amino-terminal histone tails extrude from the nucleosome core, which consists of structured globular domains of the eight histone proteins.

Multiple levels of chromatin folding

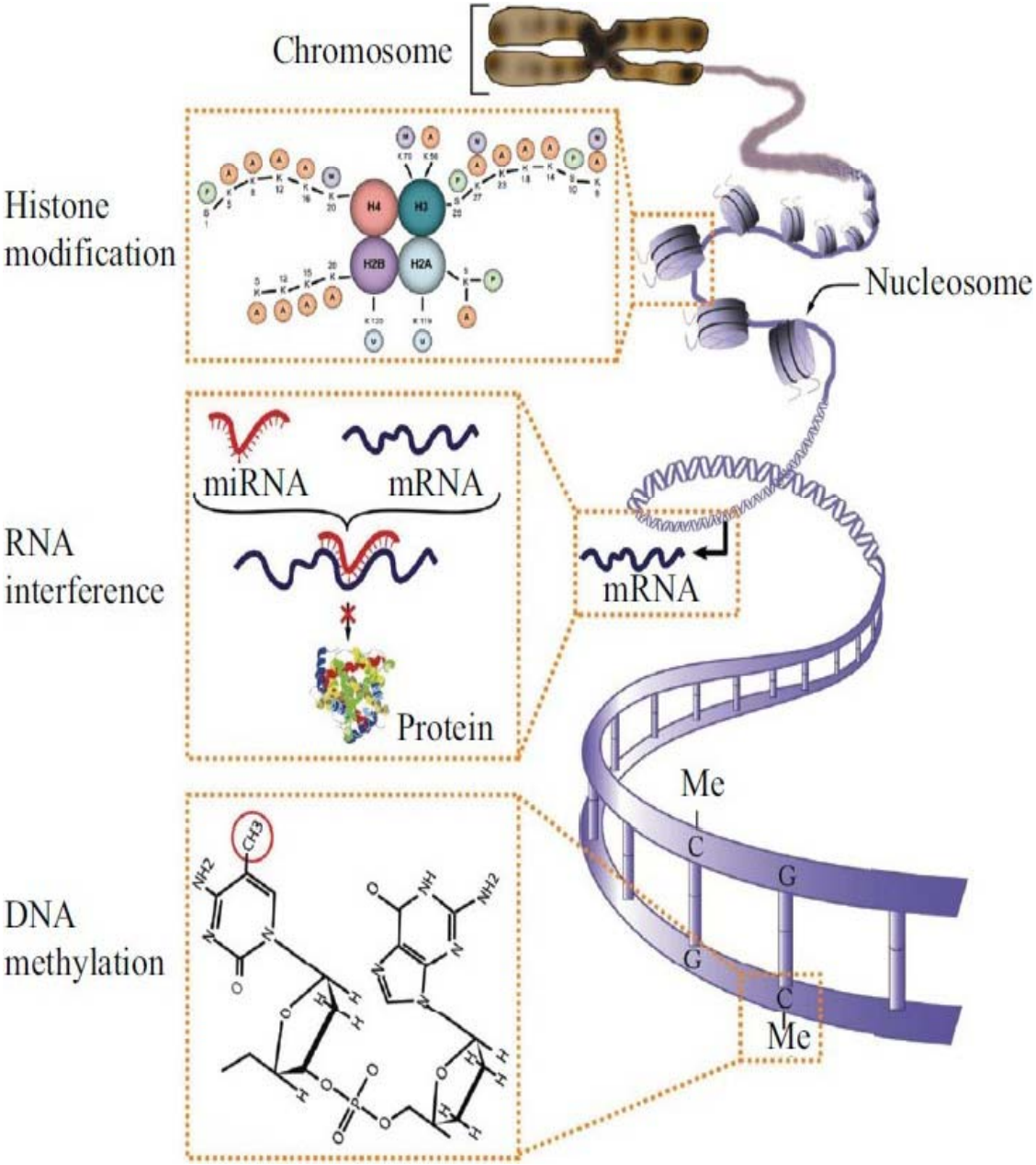


DNA compaction within the interphase nucleus (depicted at left) occurs through a hierarchy of histone-dependent interactions that can be subdivided into primary, secondary, and tertiary levels of structure. Strings of nucleosomes compose the primary structural unit. Formation of 30-nm fibers through histones tail-mediated nucleosome-nucleosome interactions provides a secondary level of compaction, whereas tail-mediated association of individual fibers produces tertiary structures J. C. Hansen, *Annu. Rev. Biophys. Biomol. Struct.* **31**, 361 (2002)

EPIGENETIC MODIFICATIONS

Three major components:

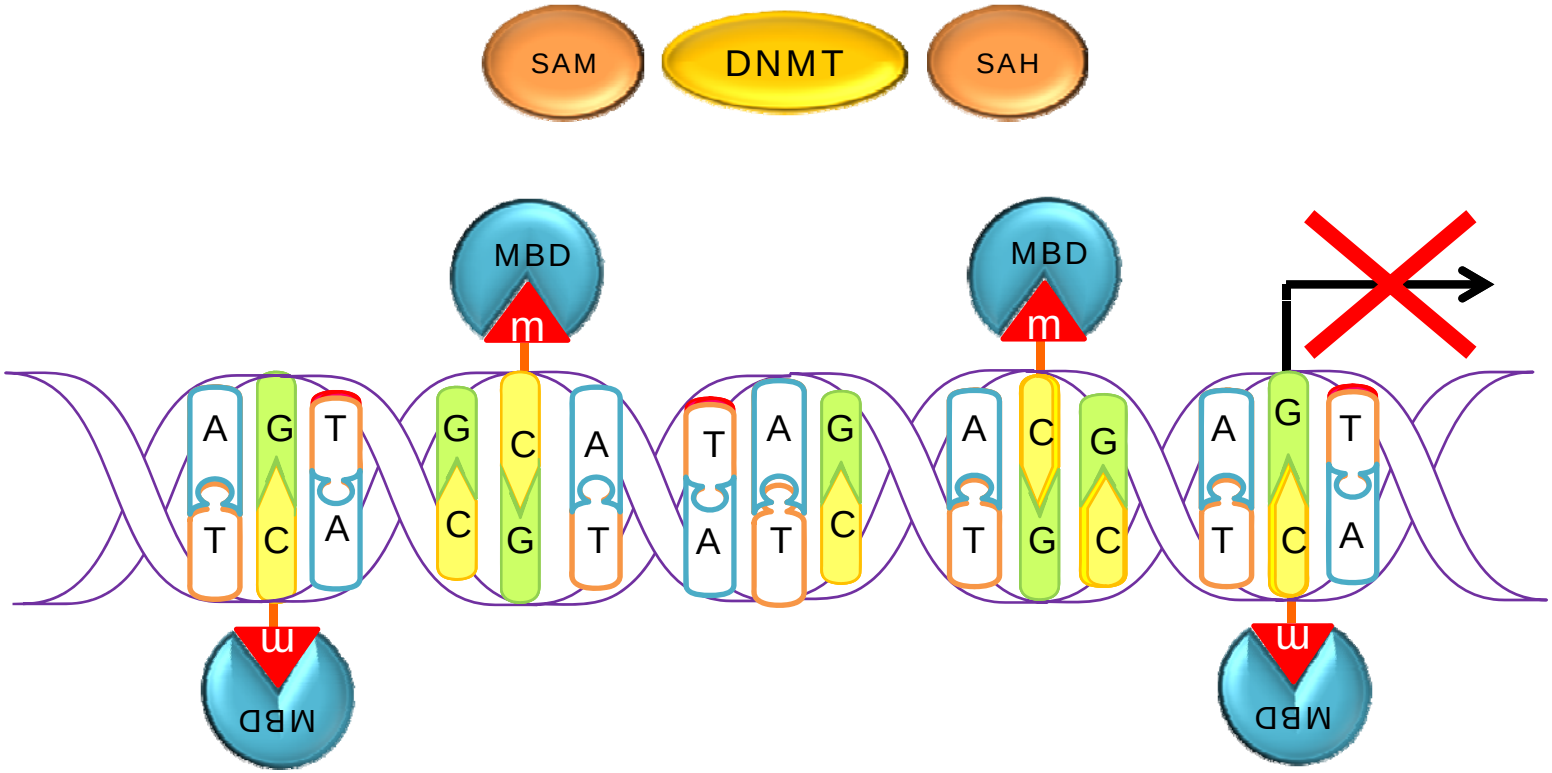
1. DNA methylation
2. Histone modifications
3. RNAi mediated gene silencing.



[Kim, et al., 2011, Pulmonary Circulation]

DNA METHYLATION

Methylation of DNA is a post synthetic process catalyzed by a family of dedicated enzymes known as DNMTs. DNMT1, DNMT3A and DNMT3B methylate the cytosine residue specifically at CpG rich promoter sequences in the presence of cofactor SAM (S-Adenosyl methionine) which donates the $-CH_3$ group and is converted to SAH (S-Adenosyl homocysteine)



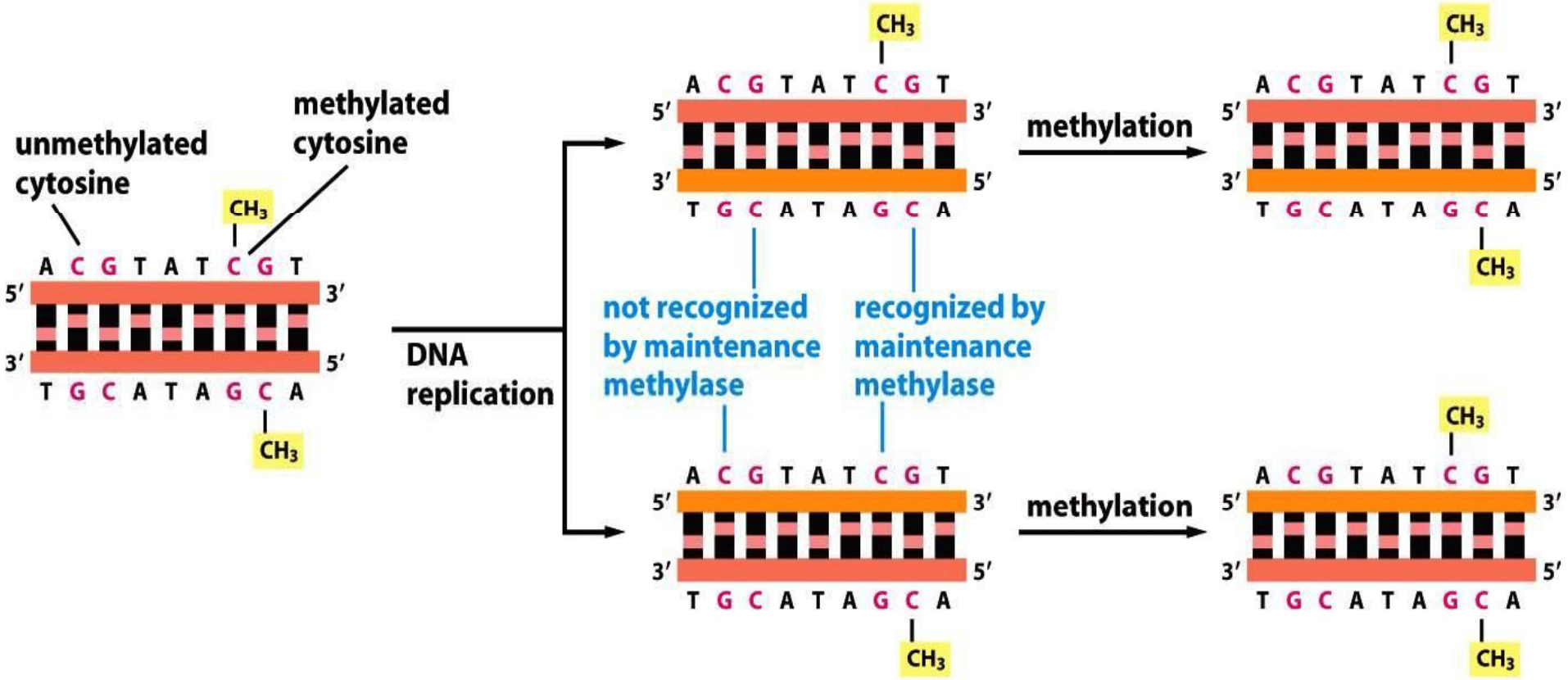
IMPORTANCE OF DNA METHYLATION

Genomic Imprinting	X-Chromosome Inactivation
Tissue-specific Gene Expression	Embryogenesis and development

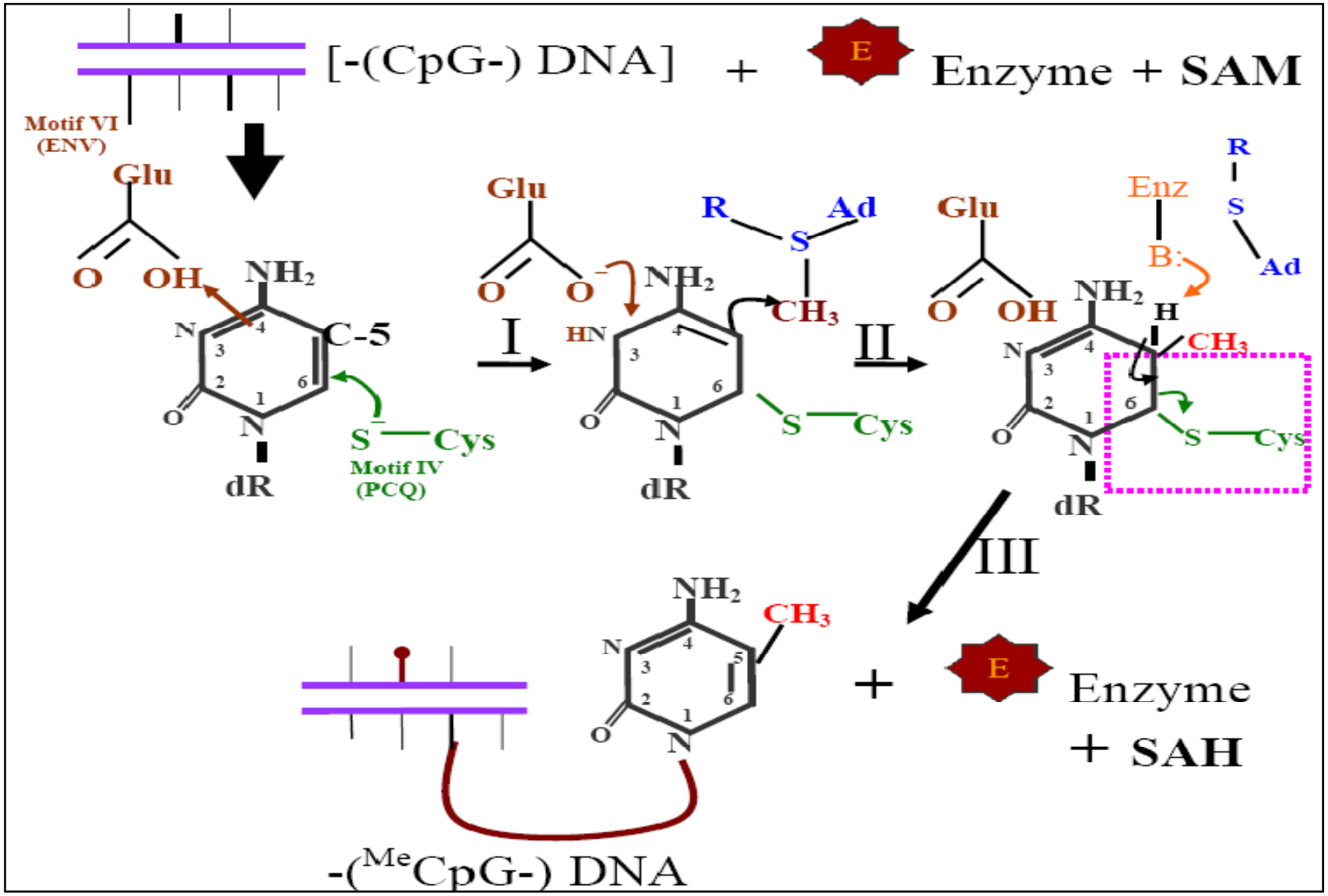
Types of DNA Methylation

De novo methylation – mediated by DNMT3A and DNMT3B.

Maintenance methylation – mediated by DNMT1



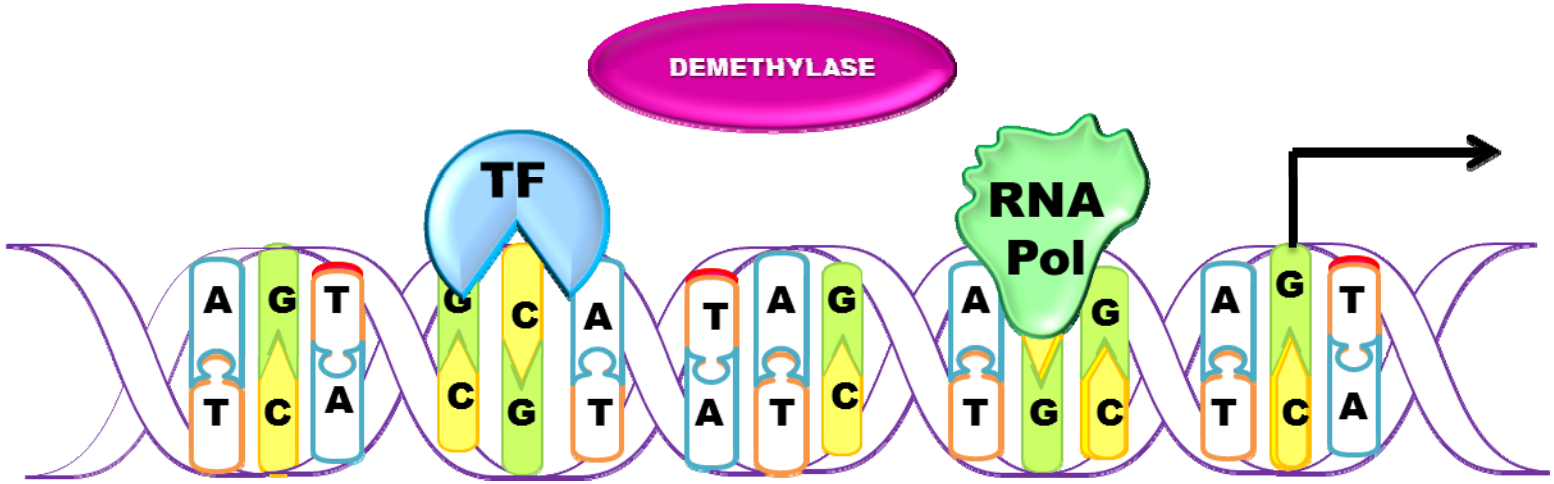
Mechanism of DNA Methylation by DNMT1, 3A, 3B



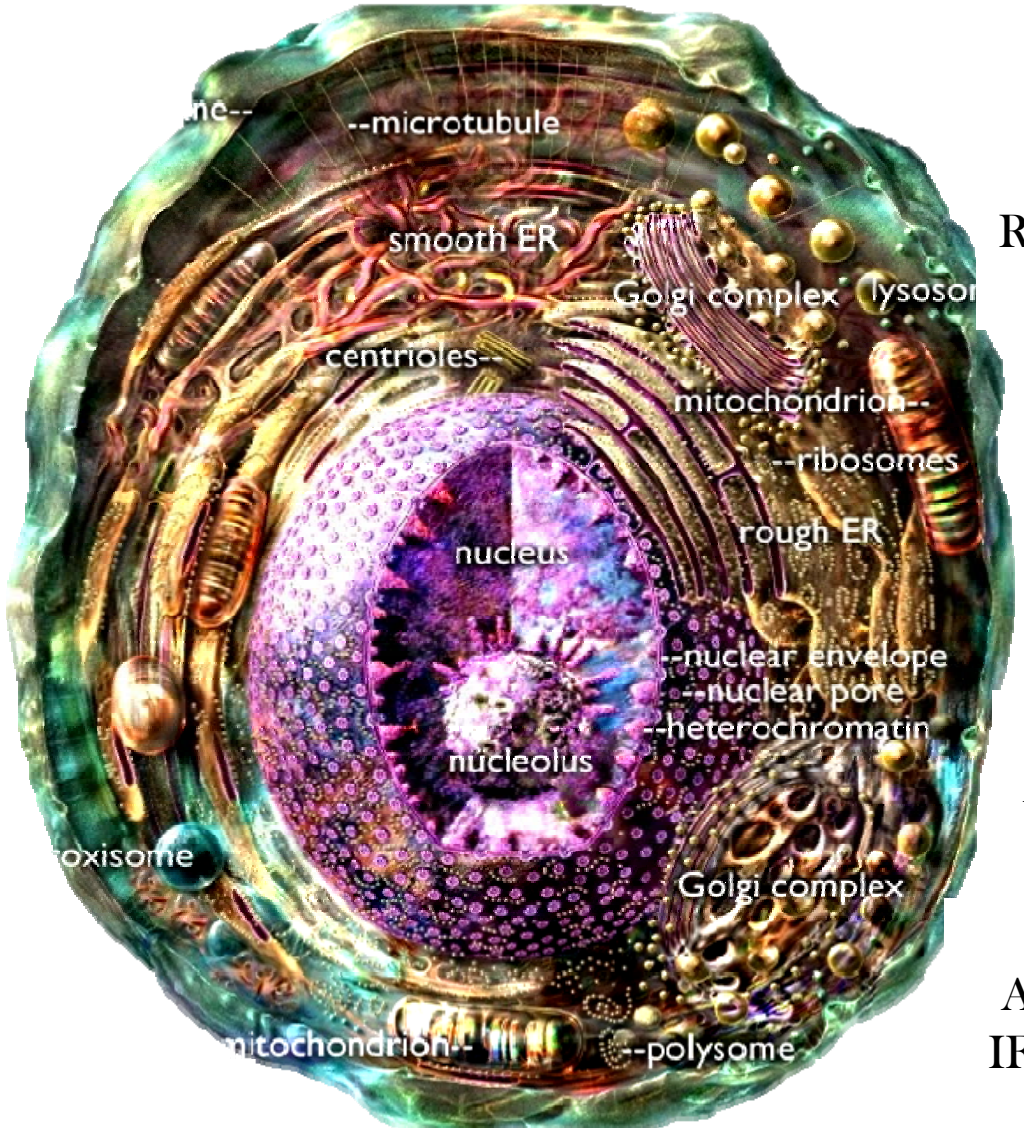
[Patra and Bettuzzi (2008/2009) Biochemistry, Moscow]

DNA DEMETHYLATION

DNA demethylation is the removal of the methyl groups from methylated-cytosine bases (MeC) of DNA and is the earliest observed epigenetic mechanism in mammals.



DNA DEMETHYLATION



• Role During Gametogenesis

Active promoter demethylation of pluripotency regulatory genes Oct 4 restores pluripotency in primordial germ cells

• Role in Early Development

Rapid Demethylation of the male pronucleus in the pre-implantation zygote

• Role During Neurogenesis

Active Demethylation of BDNF and FGF-1 genes are critical for adult neurogenesis.

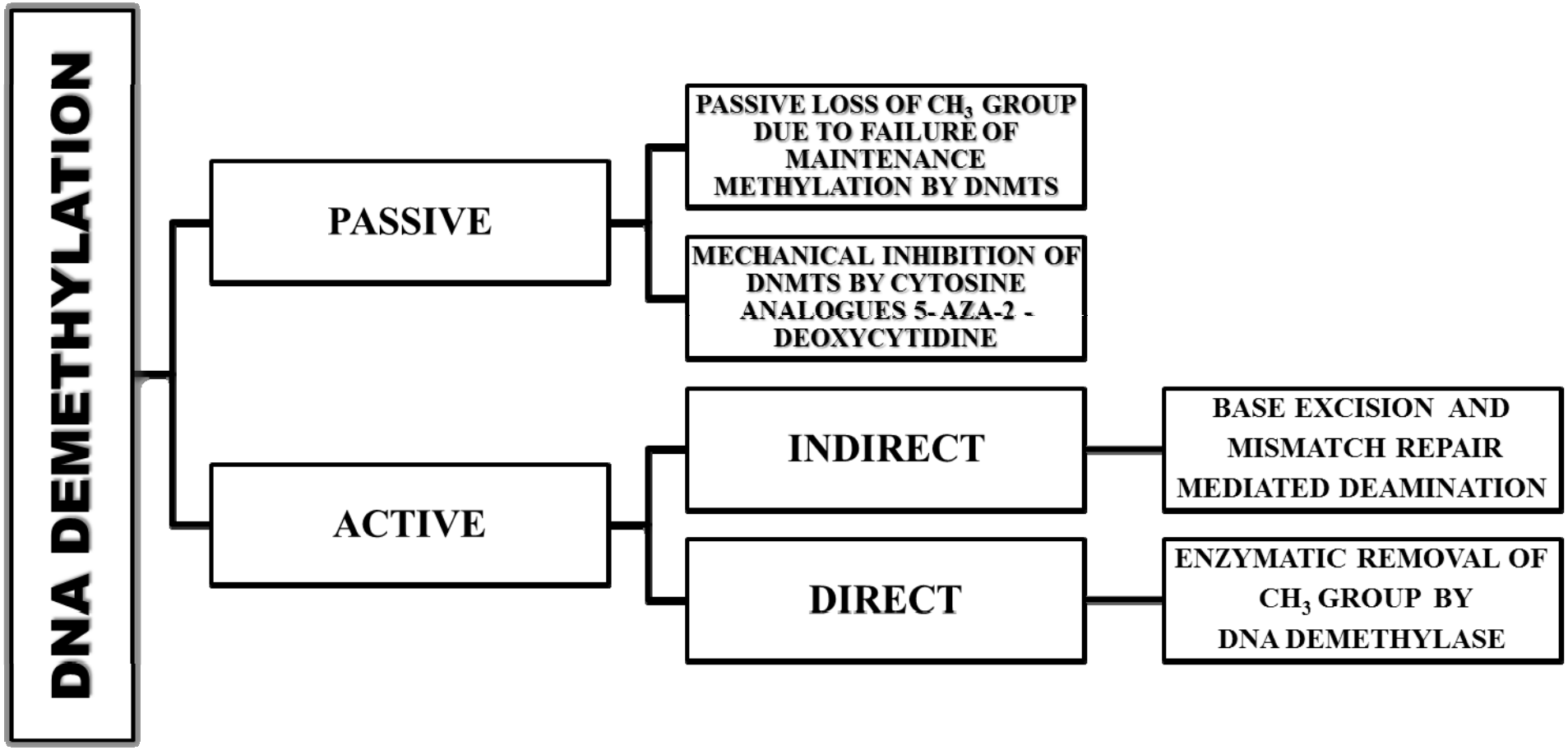
• Role During Memory Formation

Active promoter demethylation is associated with memory-promoting gene- reelin.

• Role in Immune Function

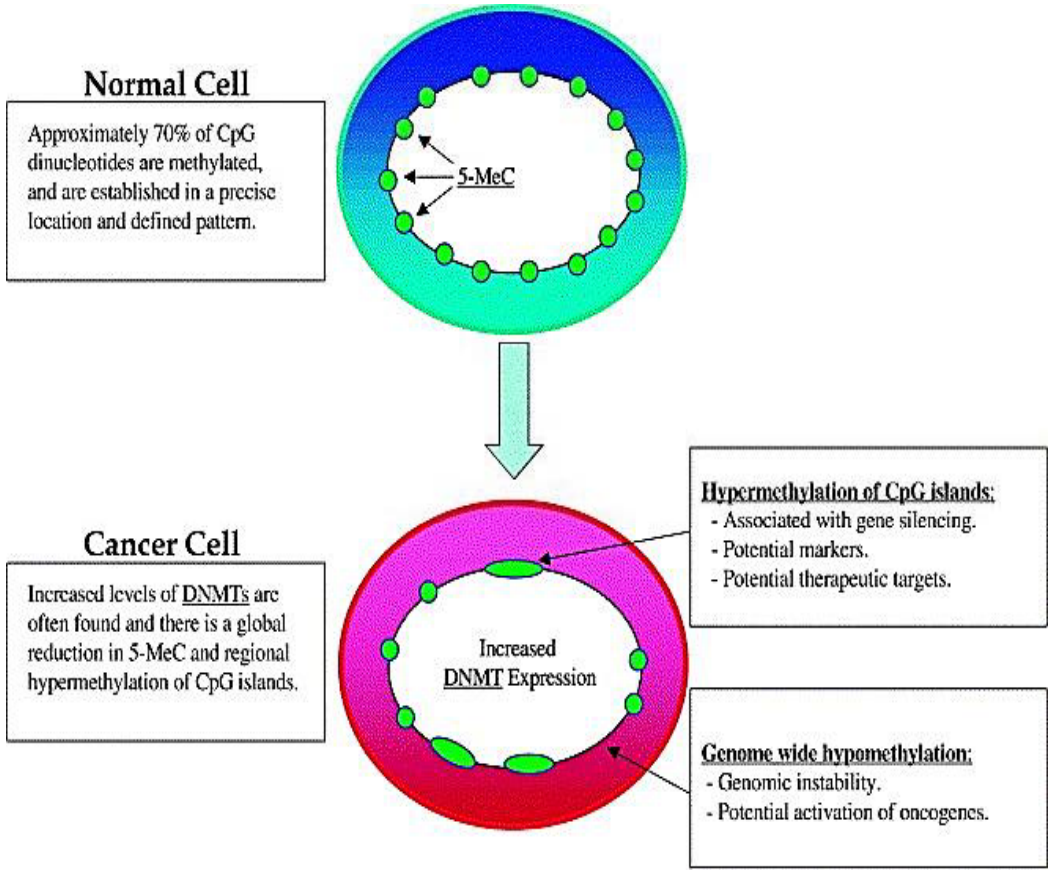
Active promoter demethylation of IL-2 and IFN- γ results in rapid cytokine production in memory CD8 T cells.

Mechanisms of DNA Demethylation



METHYLATION PARADOX IN CANCER

One important epigenetic hallmark of cancer is a paradoxical alternation in the established DNA methylation patterns involving both gene-specific hypermethylation, which suppress tumorigenesis by silencing tumor suppressor genes, and genome-wide global hypomethylation that targets transcriptional activation of oncogenes.



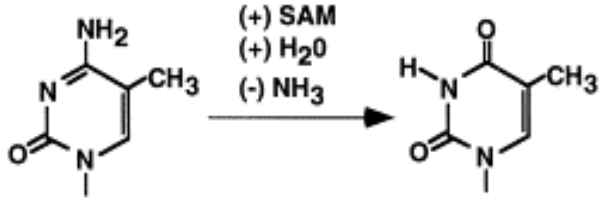
[Melki and Clarke , 2002, Seminars in Cancer Biology]

DNA methylation mediates oncogenesis in a number of ways contributing to many epigenetic hallmarks of cancer.

Possible Contribution of DNA Methylation to Cancer

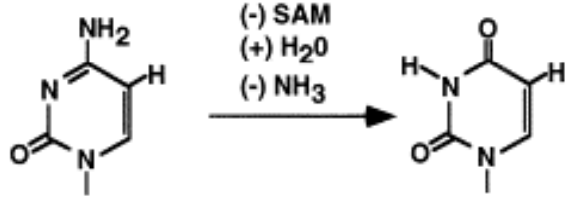
A. Mutagenic Effects

1. Spontaneous deamination of 5-mCyt → T



5-mCyt T

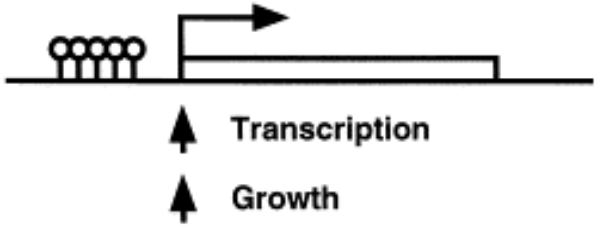
2. Enzyme-mediated deamination of C → U



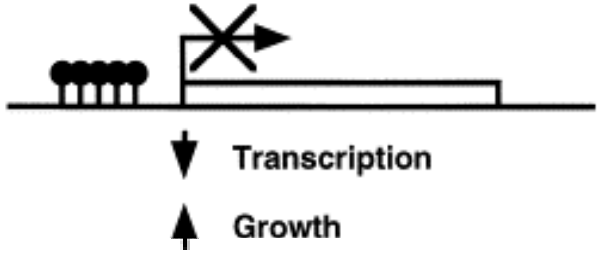
C U

B. Epigenetic Effects

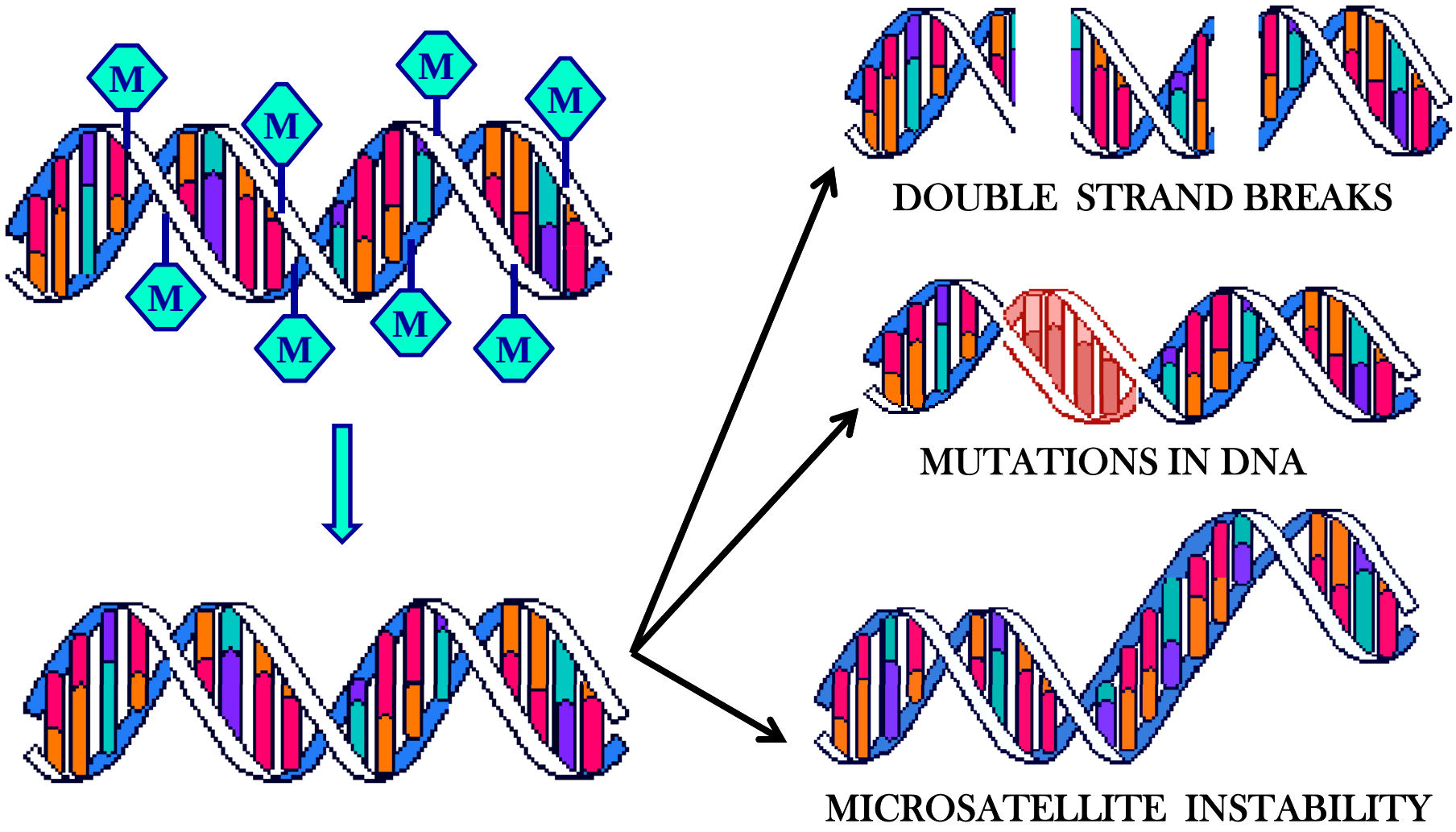
1. Hypomethylation of proto-oncogenes



2. Hypermethylation of tumor suppressor genes



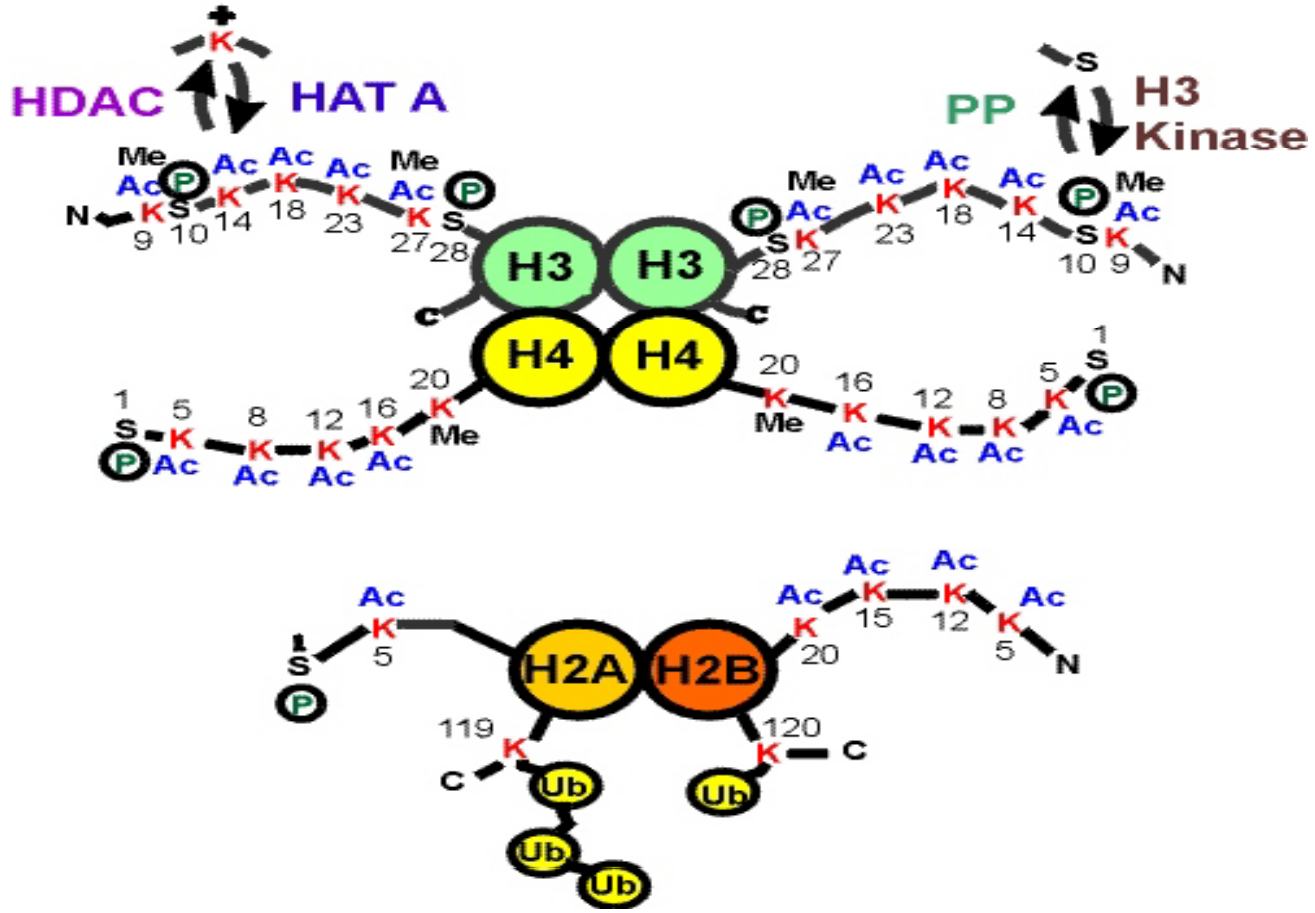
HOW IS DNA DEMETHYLATION RESPONSIBLE FOR CANCER ?



[Wilson et al., 2007, Biochimica et Biophysica Acta]

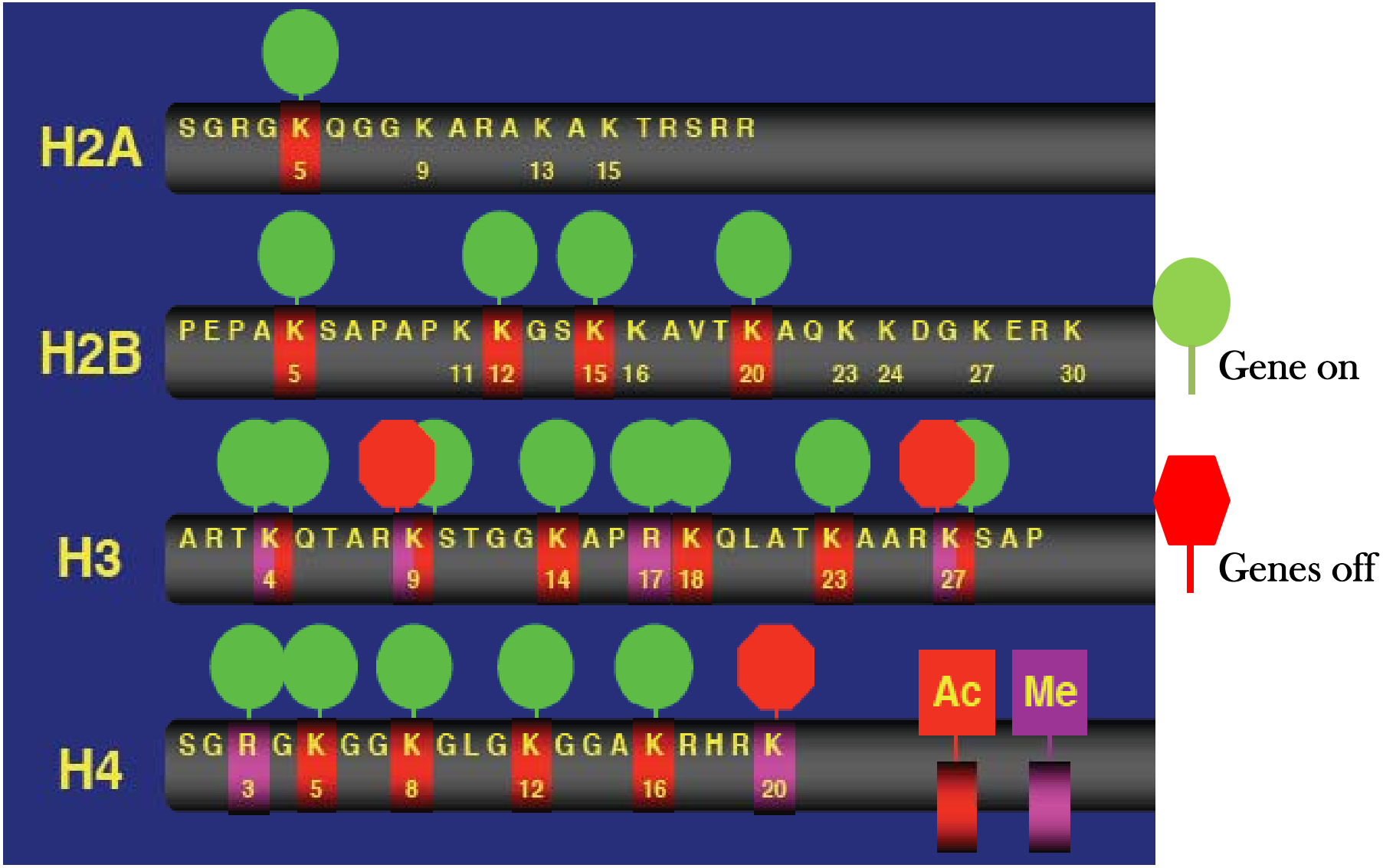
HISTONE MODIFICATIONS

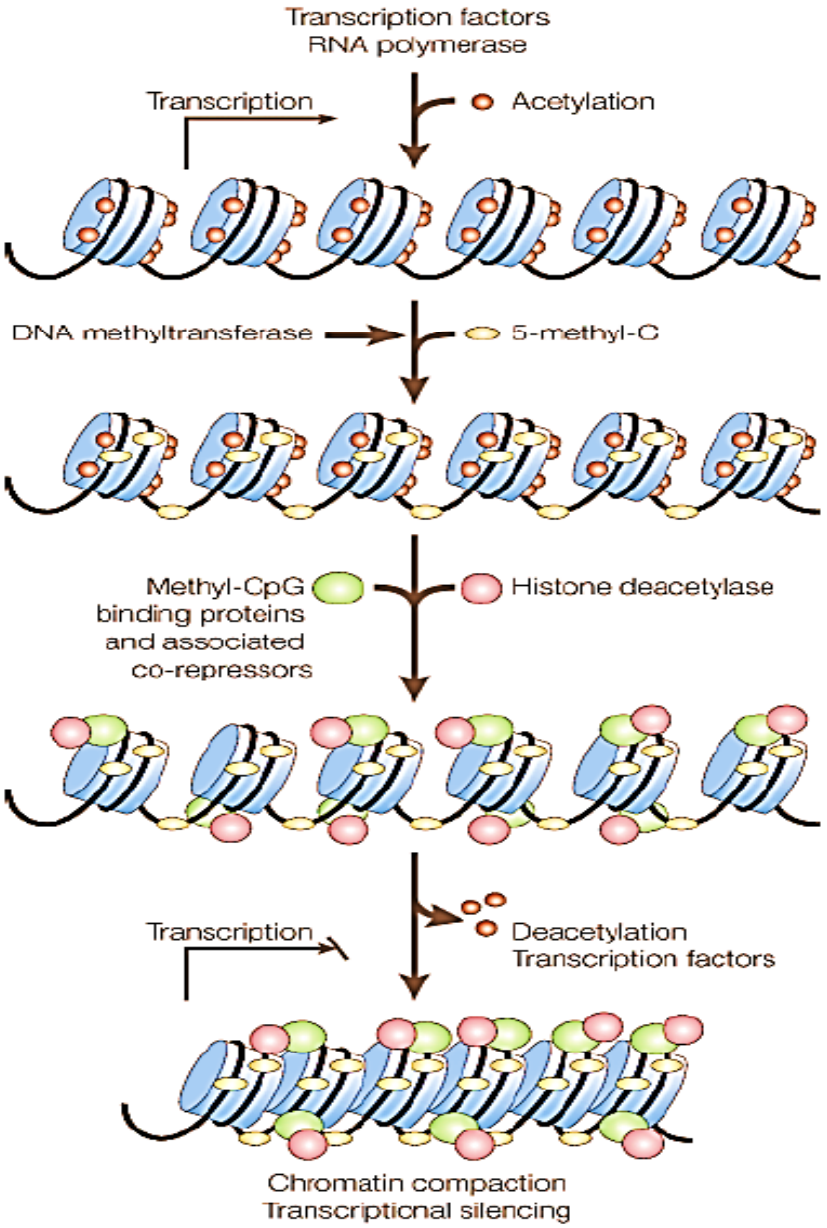
Post-translational Covalent Modifications in the N-terminal tails of Histone Proteins regulate the transcriptional state of the genome via chromatin structure resulting in differential expression of genes.



HISTONE MODIFICATIONS AND HISTONE MODIFIYING ENZYMES

HOW HISTONE MODIFICATIONS AFFECT GENE EXPRESSION





[Robertson and Wolfe, 2000, Nature review Genetics]

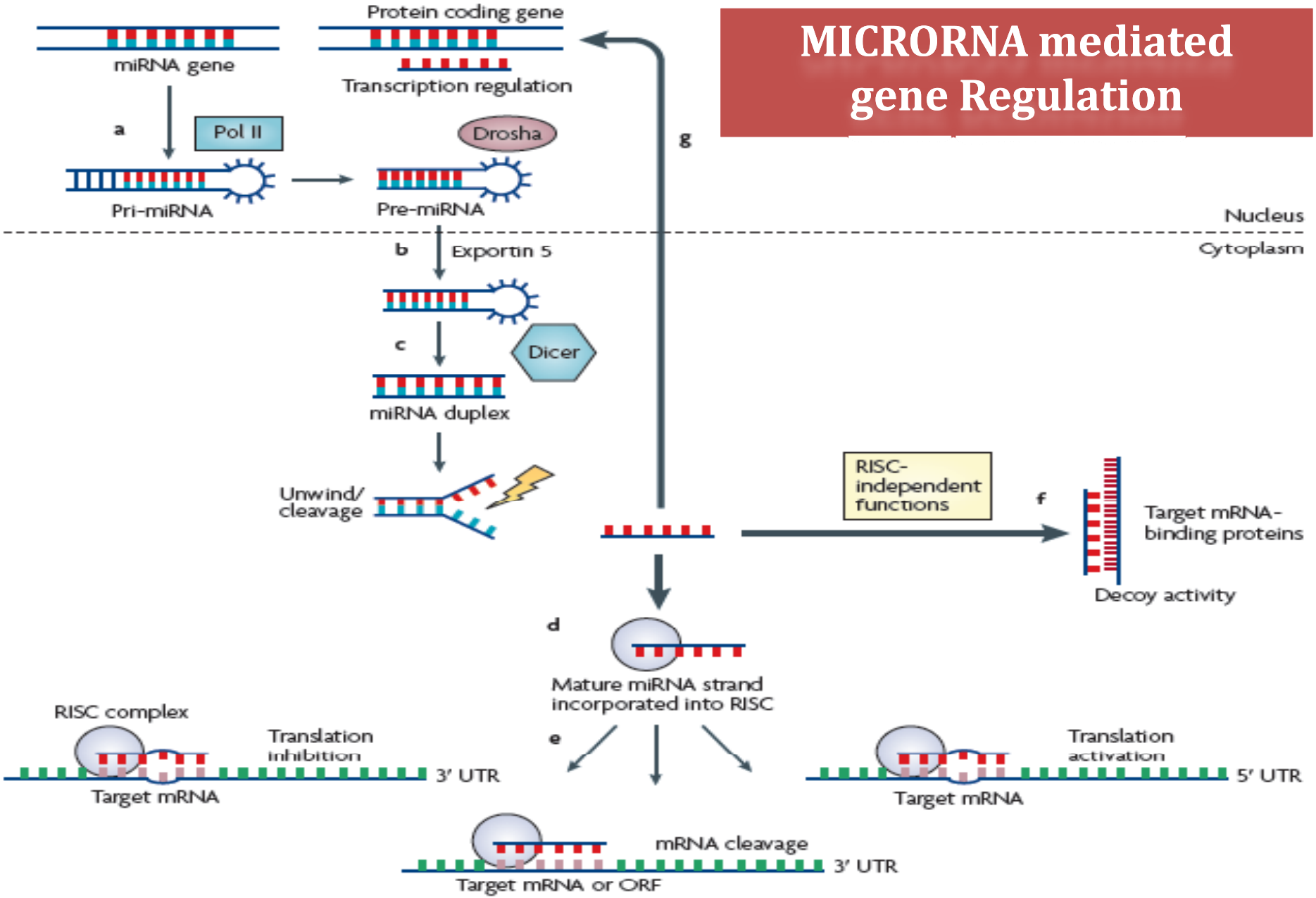
HISTONE MODIFICATIONS AFFECT GENE EXPRESSION AND TRANSCRIPTIONAL STATE

RNAi MEDIATED GENE SILENCING

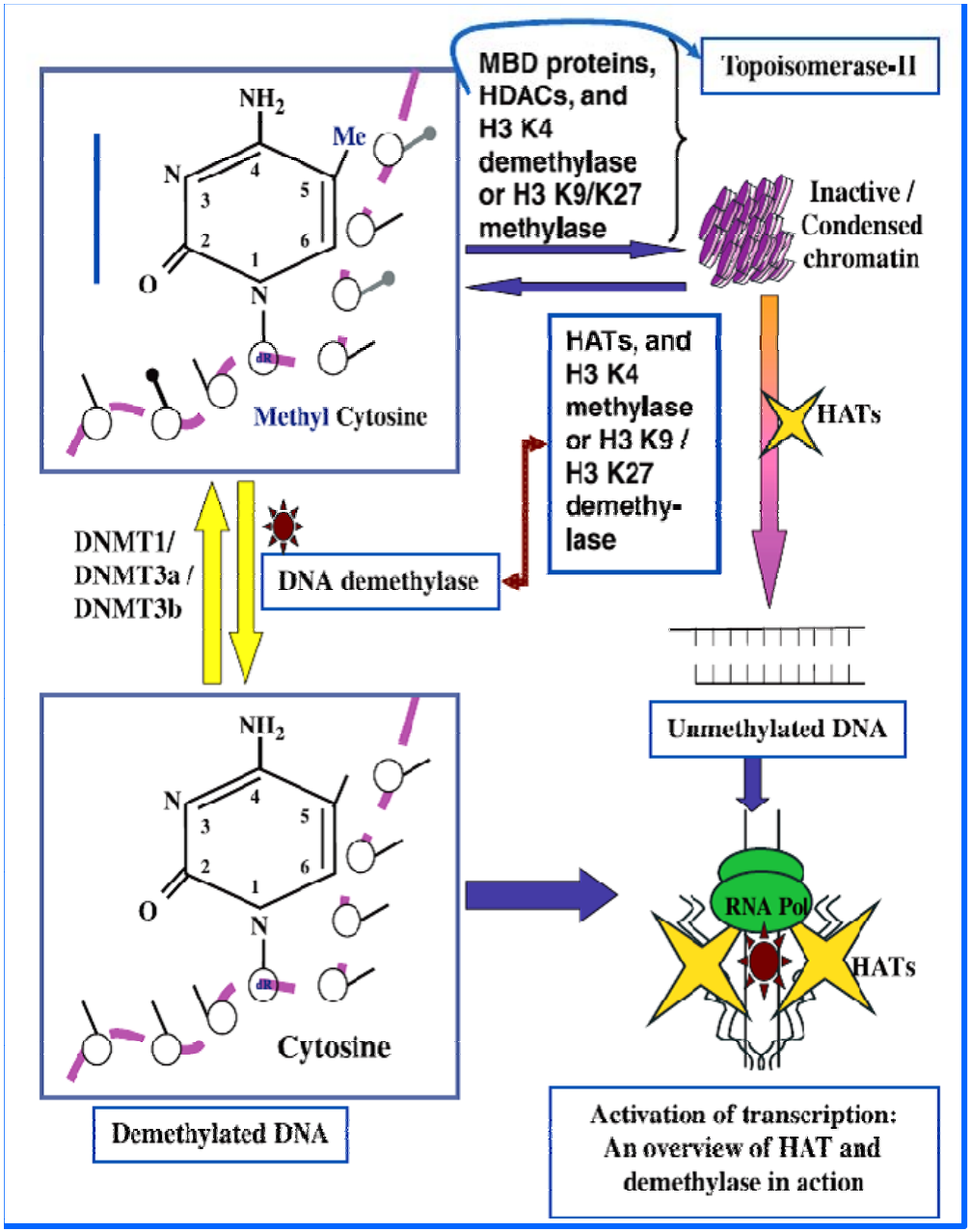
- MicroRNAs are a group of small non coding RNAs of about 19–25 nucleotides (nt) in length that are integral elements in the post-transcriptional regulation of gene expression

- miRNA plays important role in different biological functions, including developmental pattern formation, embryogenesis, differentiation, organogenesis, growth control and cell death

MICRORNA mediated gene Regulation



Yoo, et al., 2011, Nature



[Patra and Syzf, 2008, FEBS journal]

EPIGENETICS AND CANCER RESEARCH LABORATORY

BIOCHEMISTRY AND MOLECULAR BIOLOGY GROUP

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

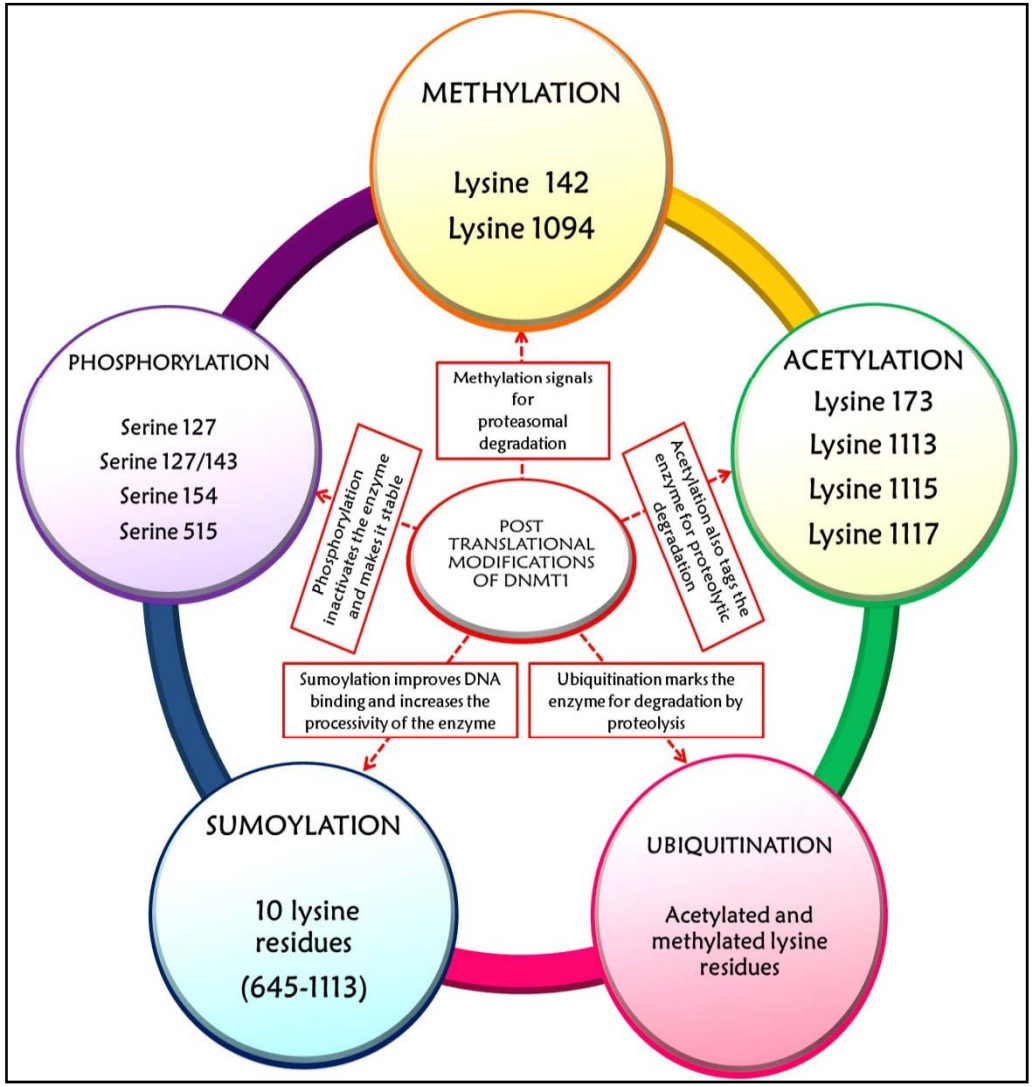
Research in my laboratory is mainly focused on the fundamental problems of epigenetics such as:

- Reversible methylation modifications of DNA and regulation of transcription.
- Reversible Acetylation and Methylation Modifications of Histone 3 at Lysine 4 and 9 residues (H3K4 and H3K9).
- MicroRNA mediated gene silencing and correlation with the other epigenetic modulators.
- Elucidation of the molecular signaling networks that co-ordinate the above epigenetic modifications and their dynamicity.
- Impact of gene-environment interaction in modulating the methylation status on histones as well as many developmental genes such as OCT4, SOX2, KLf4.

Regulation of DNMT1 and the various checkpoints

We focus on the numerous intrinsic and extrinsic factors that co-ordinate to form an extremely complex and interconnected network for regulating the stability and activity of DNMT1 and also the DNA methylation machinery. A comprehensive knowledge regarding the functional intricacy and enzymatic activity of DNMT1 as well as its potential restraining points will help in designing novel mechanistic based drugs targeting DNMT1 for effective cancer therapy in future.

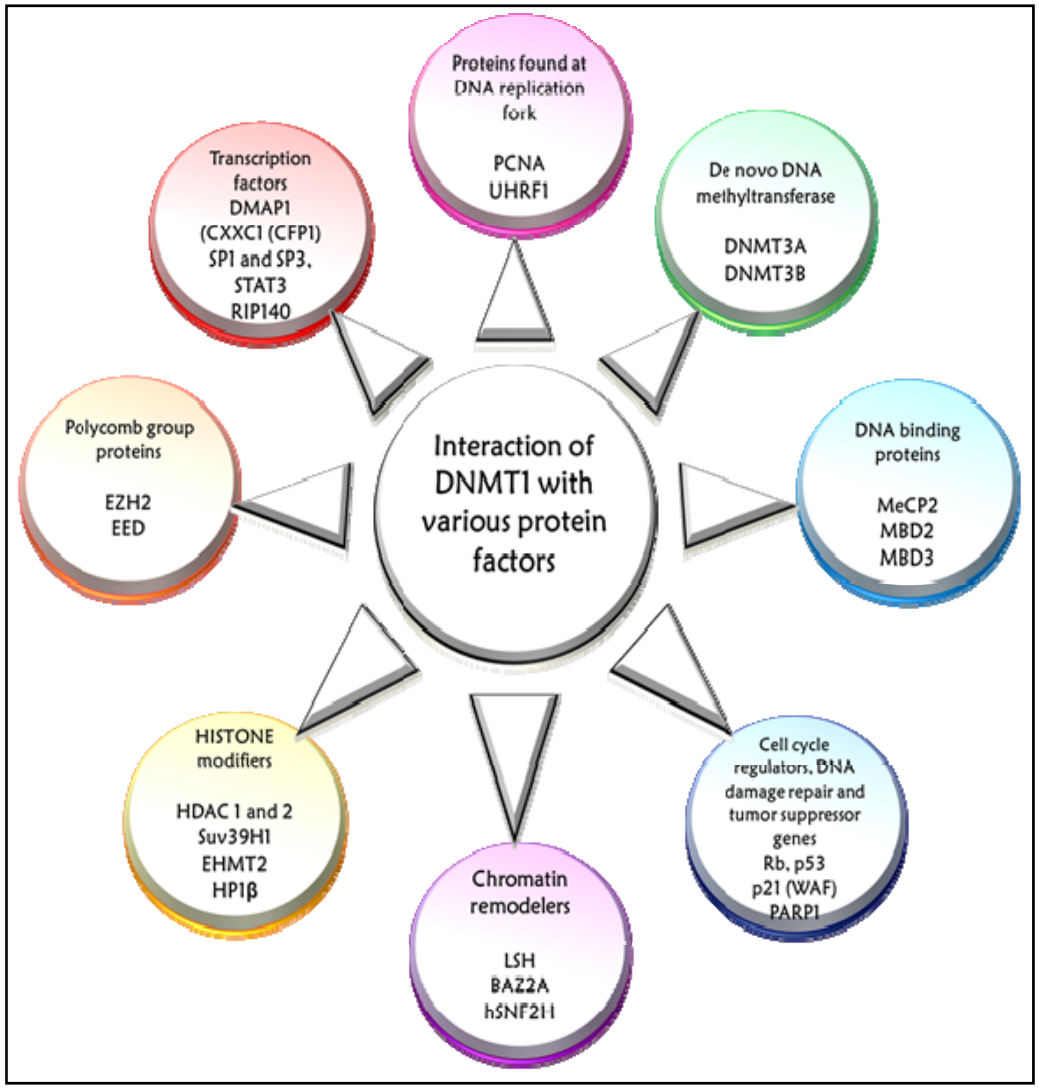
VARIOUS POST-TRANSLATIONAL MODIFICATIONS THAT AFFECT THE STABILITY AND ACTIVITY OF DNMT1



[Kar et al., 2012, Epigenetics]

All of these post-translational regulatory operations are interlinked to maintain a rigid control over the stability, abundance and activity of DNMT1 in a cell-cycle dependent manner.

VARIOUS INTERACTIONS OF DNMT1

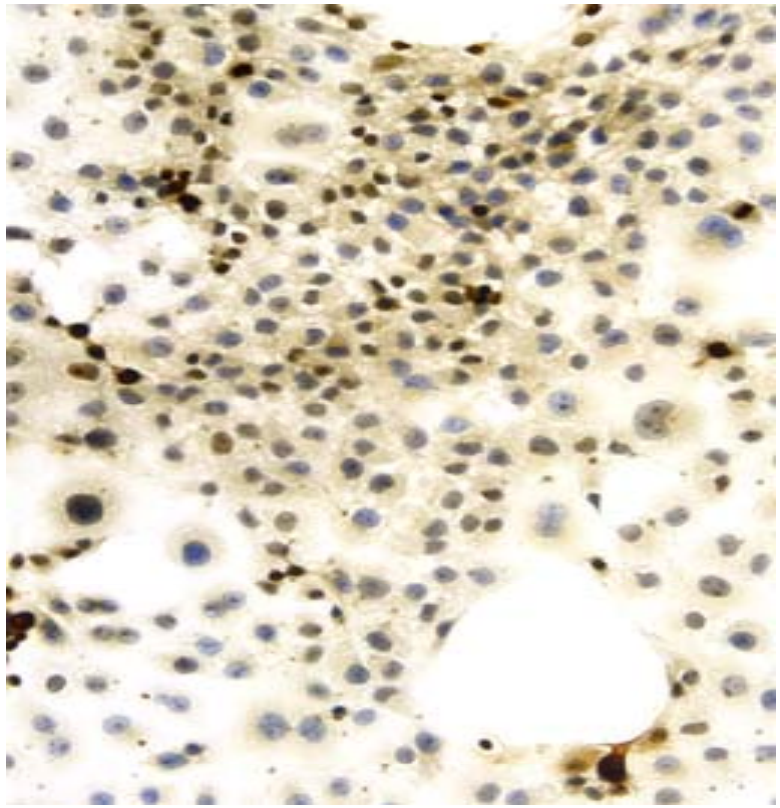


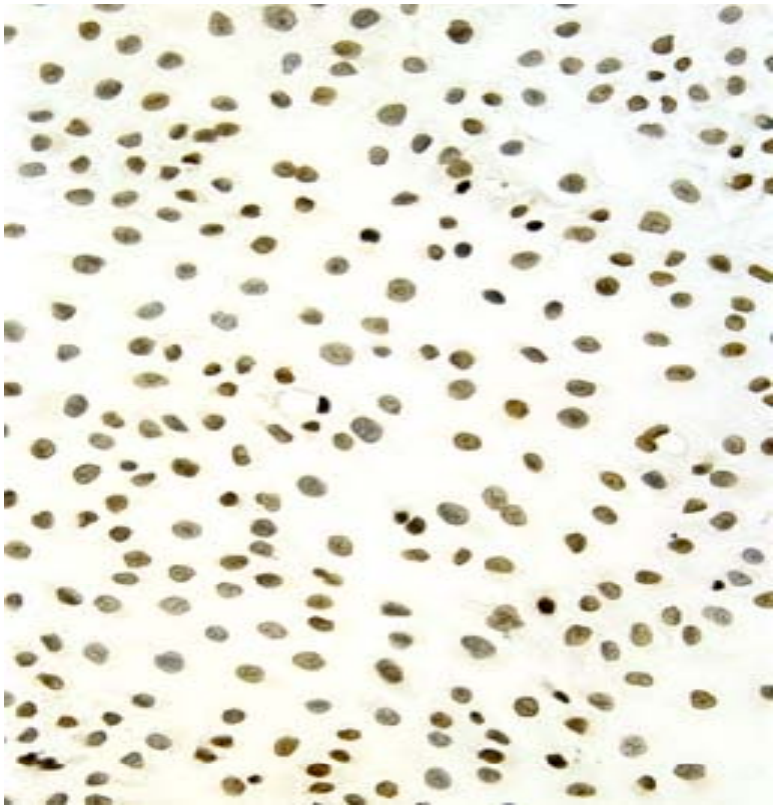
[Kar et al., 2012, Epigenetics]

DNMT1 works in concert with:

- proteins found at DNA replication forks – PCNA.
- proteins participating in chromatin re-organization DNMT3A, DNMT3B, HDAC1, HDAC2, MeCp2, MBD2, MBD3 and UHRF1 and polycomb proteins.
- proteins associated with cell cycle regulation or response to DNA damage and tumor suppressors – p21 (WAF), Rb protein, p53 protein, PARP1.
- A number of transcription factors and regulators involved in DNA methylation inheritance.

Expression of DNMT1 in normal and cancer cell lines





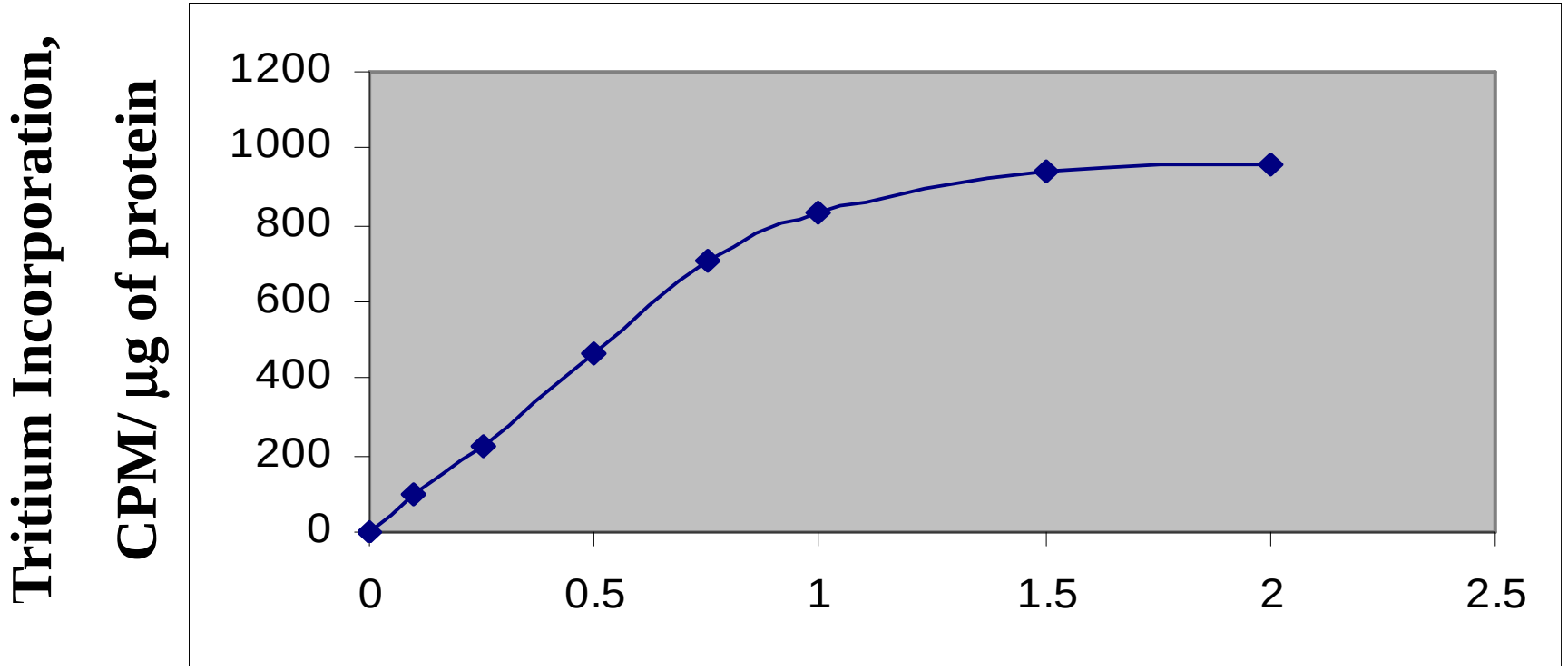
BPH1 **Du145**

[Patra, et al., 2002. Mol Carcinogenesis]

DNMTase activity assay:

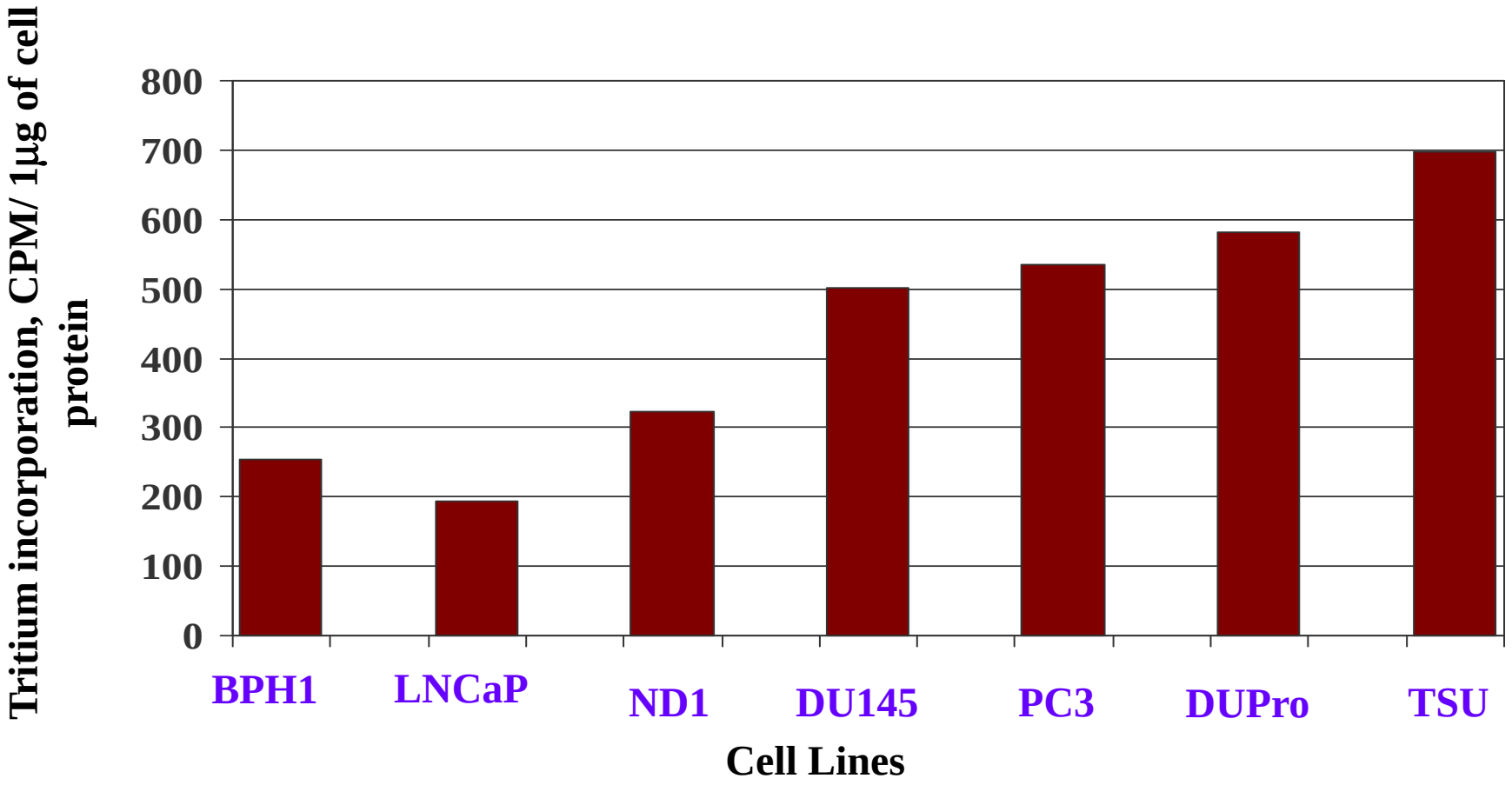
Optimum substrate concentration

Substrate: Poly [-dI-dC-dI-dC-dI-dC-] duplex, CH_3- donor is SAM



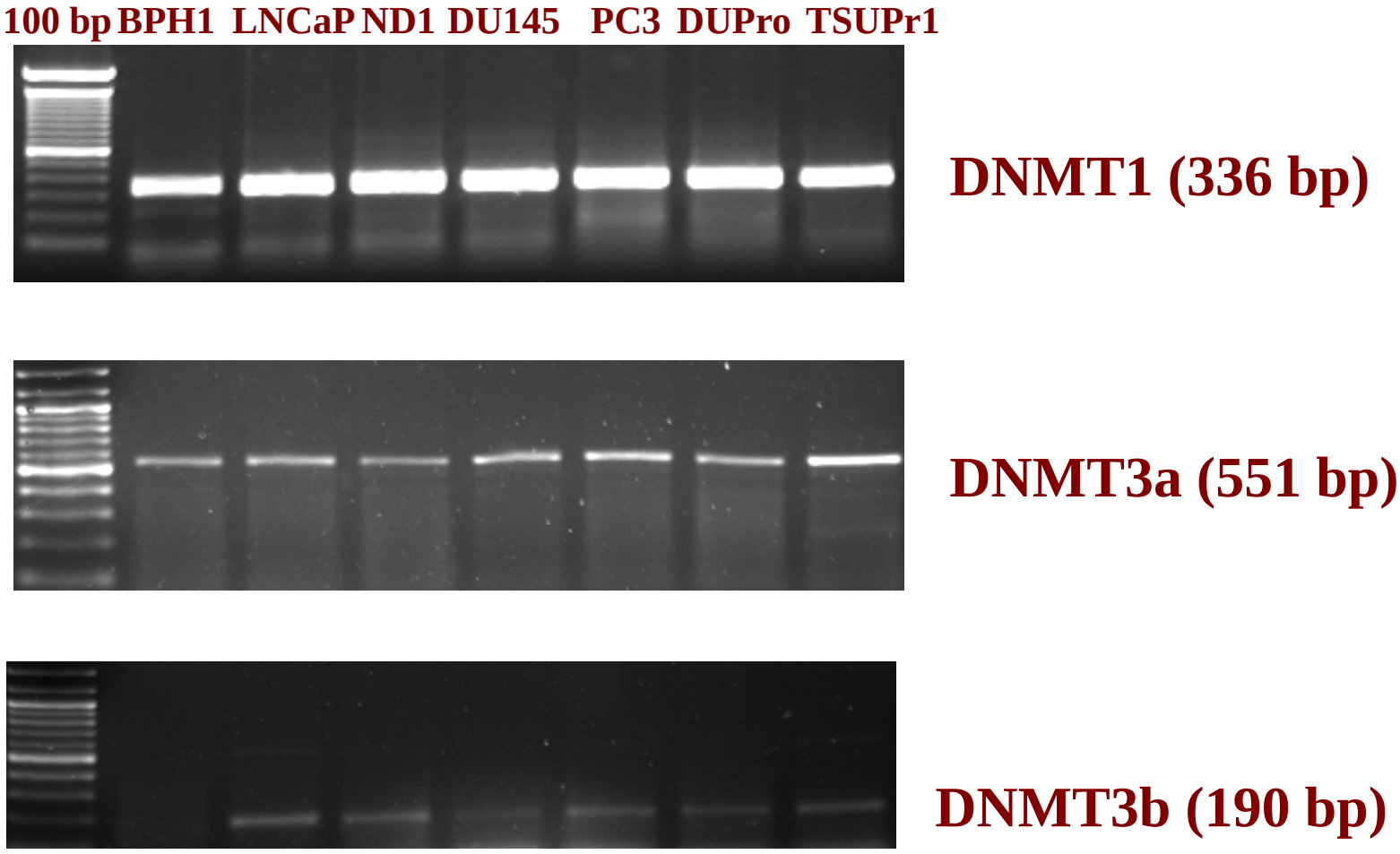
[Patra, et al., 2002. Mol Carcinogenesis]

DNA methyltransferase activity in normal and cancer cell lines



[Patra, et al., 2002. Mol Carcinogenesis]

Semi-Quantitative for DNA methyltransferases

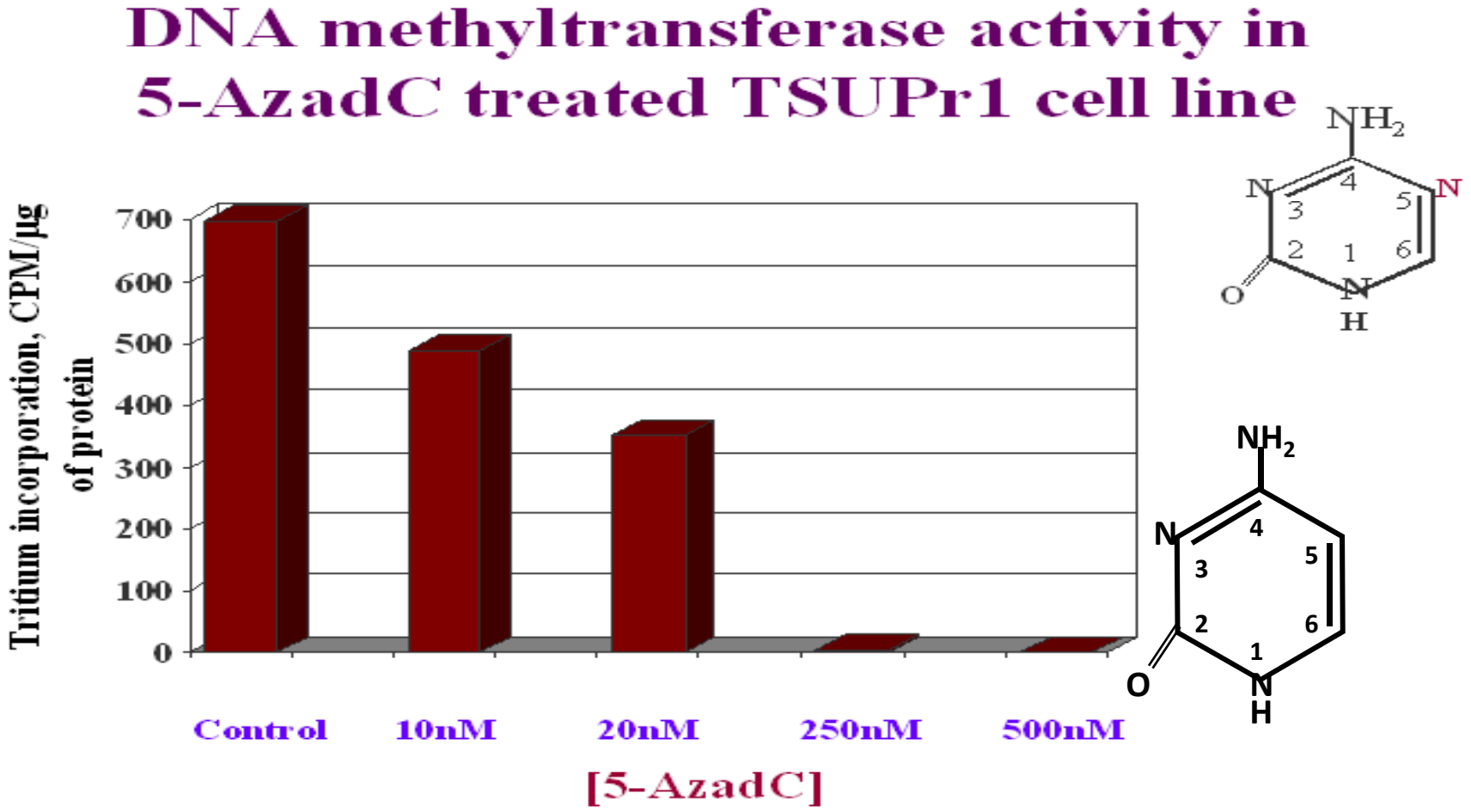


[Patra, et al., 2002. Mol Carcinogenesis]

Demystifying the DNA demethylase enigma and deciphering its mechanism

The quest for a demethylase enzyme (s), the associated cofactors and elucidation of the detailed biochemical reaction is being passionately conducted in our laboratory. The discovery will provide new avenues for therapeutic applications in cancer treatment, in solving the human infertility problems, in addressing the dilemma of epigenetic engineering of induced pluripotent stem cells and the failure of gene therapy. The lab is currently focused in unraveling the active mechanism of DNA demethylation mediated by direct removal of the CH_3 group from the methylated cytosine bases catalyzed by an active DNA demethylase enzyme.

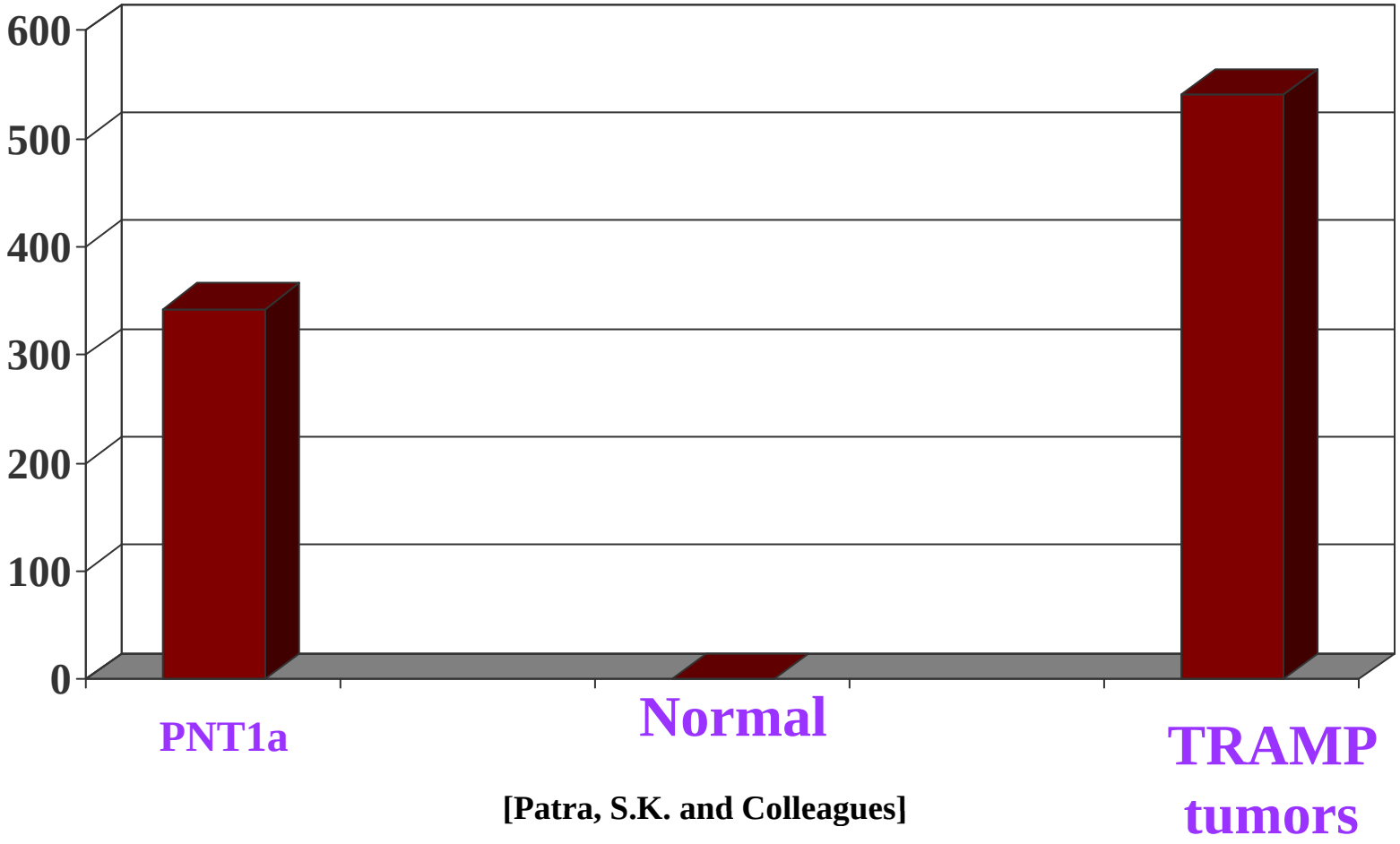
5-Aza-2′-deoxycytidine and Mechanical Inhibition of DNA-methylation



[Patra, S. K. et al. , 2001. Biochem. Biophys. Res. Commn.]

Demethylase activity

Substrate: Methylated DNA, Poly[methyl-CpG-] duplex



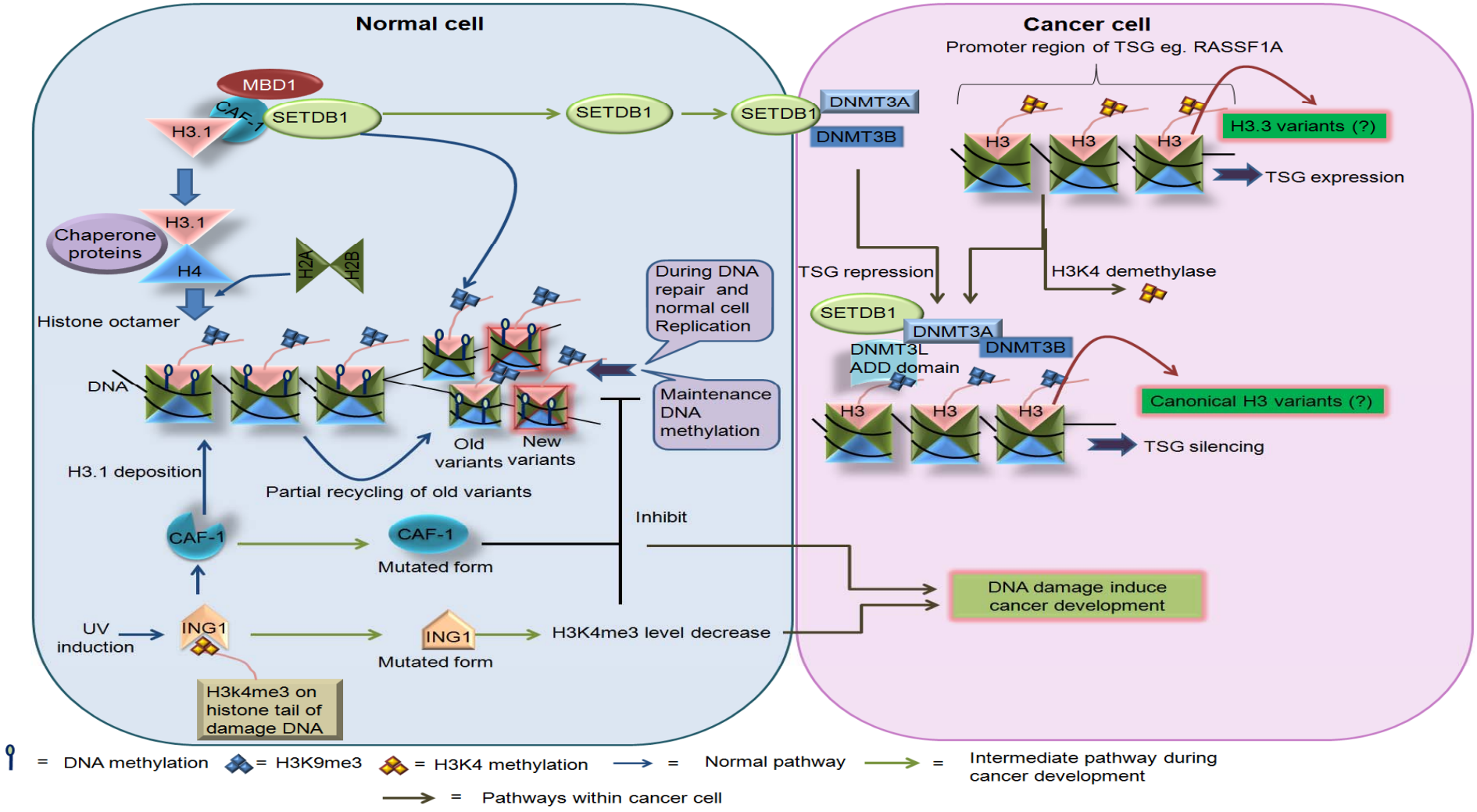
[Patra, S.K. and Colleagues]

Study of Co-Relation between Chromatin Dynamics and Epigenetic Modifications During Cancer Development

Gene expression profile changes during tumorigenesis. A clear picture of the dynamic epigenome and maintenance of epigenetic signatures, the relationship between H3 variant deposition and H3K4 methylation during normal development and especially during cancer development is still behind a shade of mist. The main emphasis of this lab is unraveling the unique epigenetic features of development and tumorigenesis and studying the co-relation among reversible H3K4 methylation (–mono,-di,-tri), H3K9 Acetylation and DNA methylation in normal and malignant tissues.

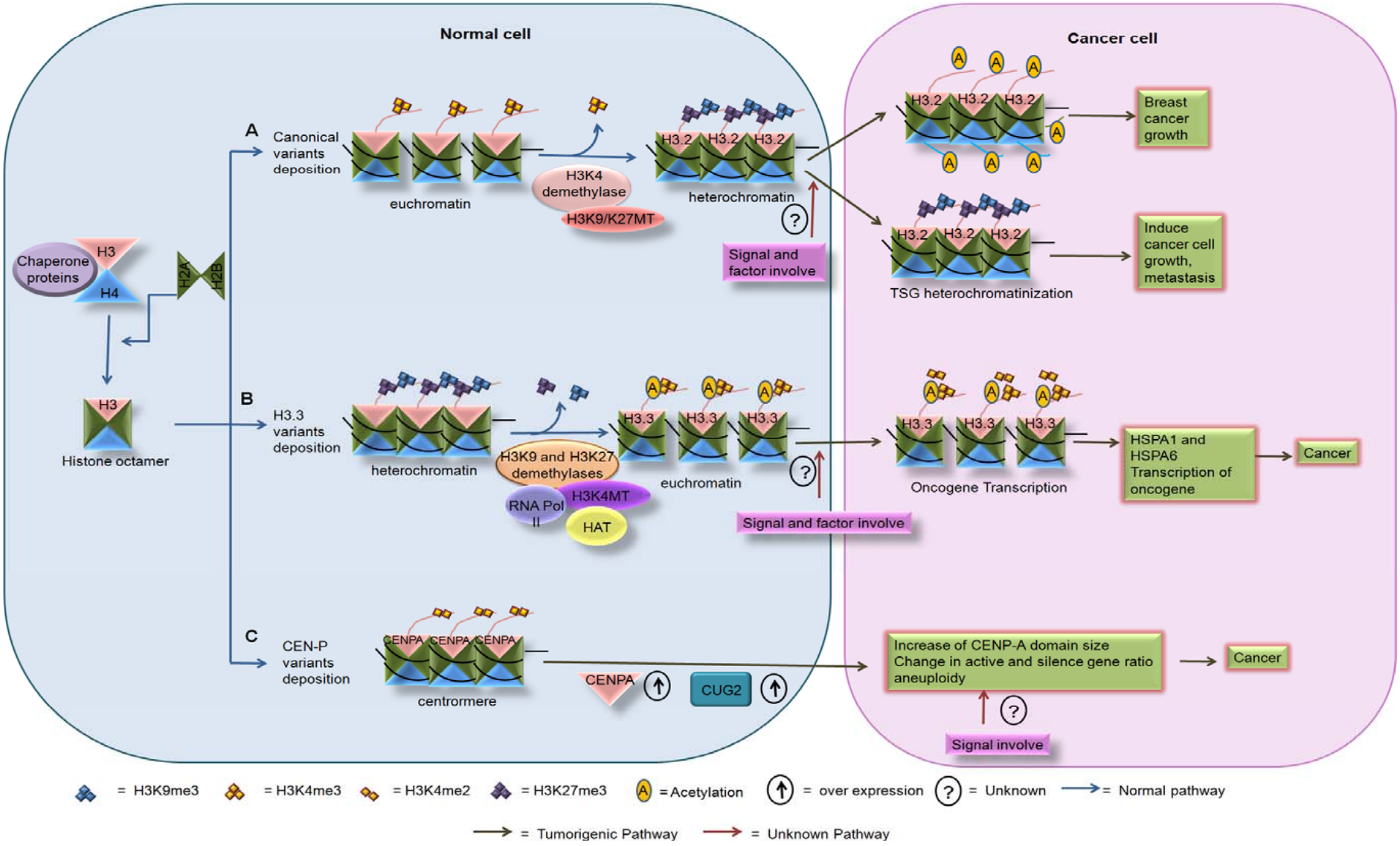
Presented at A.B.N. Seal College, New Cooch Behar, W. B. ON 09/12/2012

ASSOCIATION OF H3.1 AND H3K4 METHYLATION DURING CANCER DEVELOPMENT



[Deb et al., 2012. Communicated]

H3 VARIANTS AND CANCER

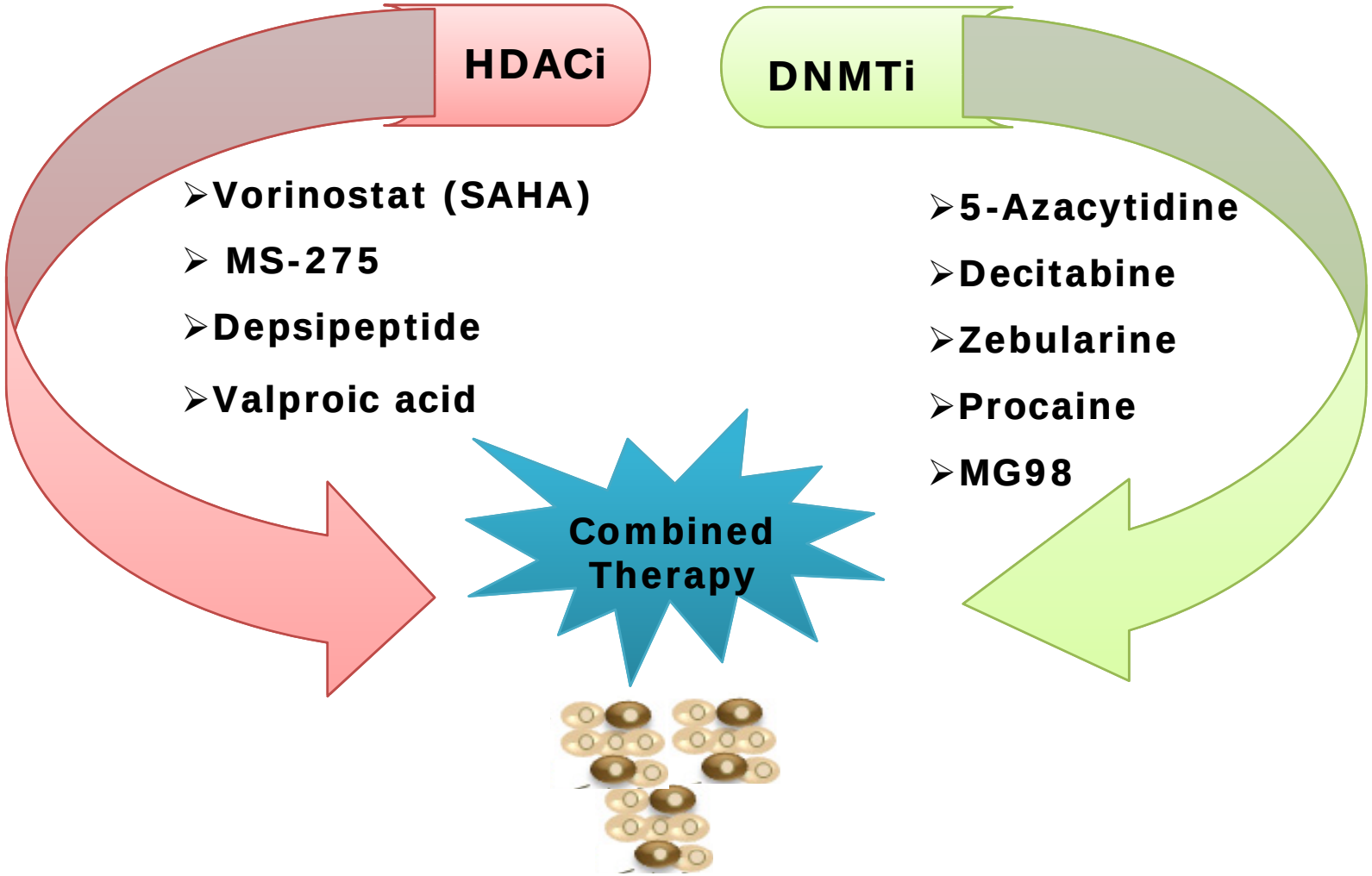


[Deb et al., 2012. Communicated]

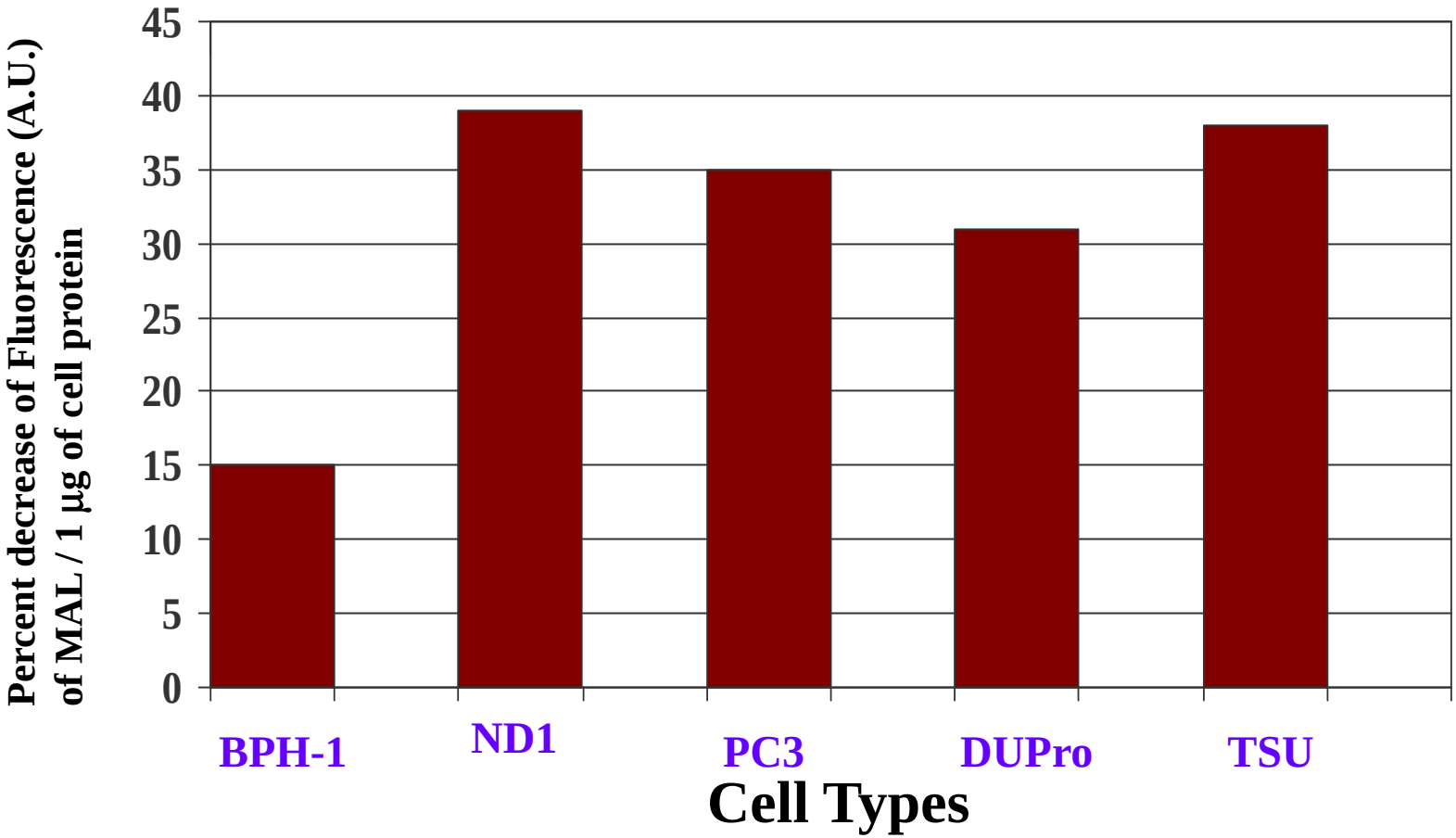
Epigenetic Causes And Consequences With Histone Deacetylases And D NA Methyltransferase In Human Cancers

HDACs and DNMTs are two potent repressors of tumor suppressor genes. Recently, a great deal of research interest has been focused for restoration of acetylation/deacetylation balance and methylation/demethylation balance by using HDAC and DNMT inhibitors respectively. The lab is involved in developing specific inhibitors which may include synthetic drugs, phytochemicals, various elements spreading heterochromatinization in the genome such as microRNA, CLR 4 proteins etc., to achieve gene re-expression of tumor suppressor genes as an effective cancer therapy.

HDACi AND DNMTi: ANTI-CANCER AGENTS



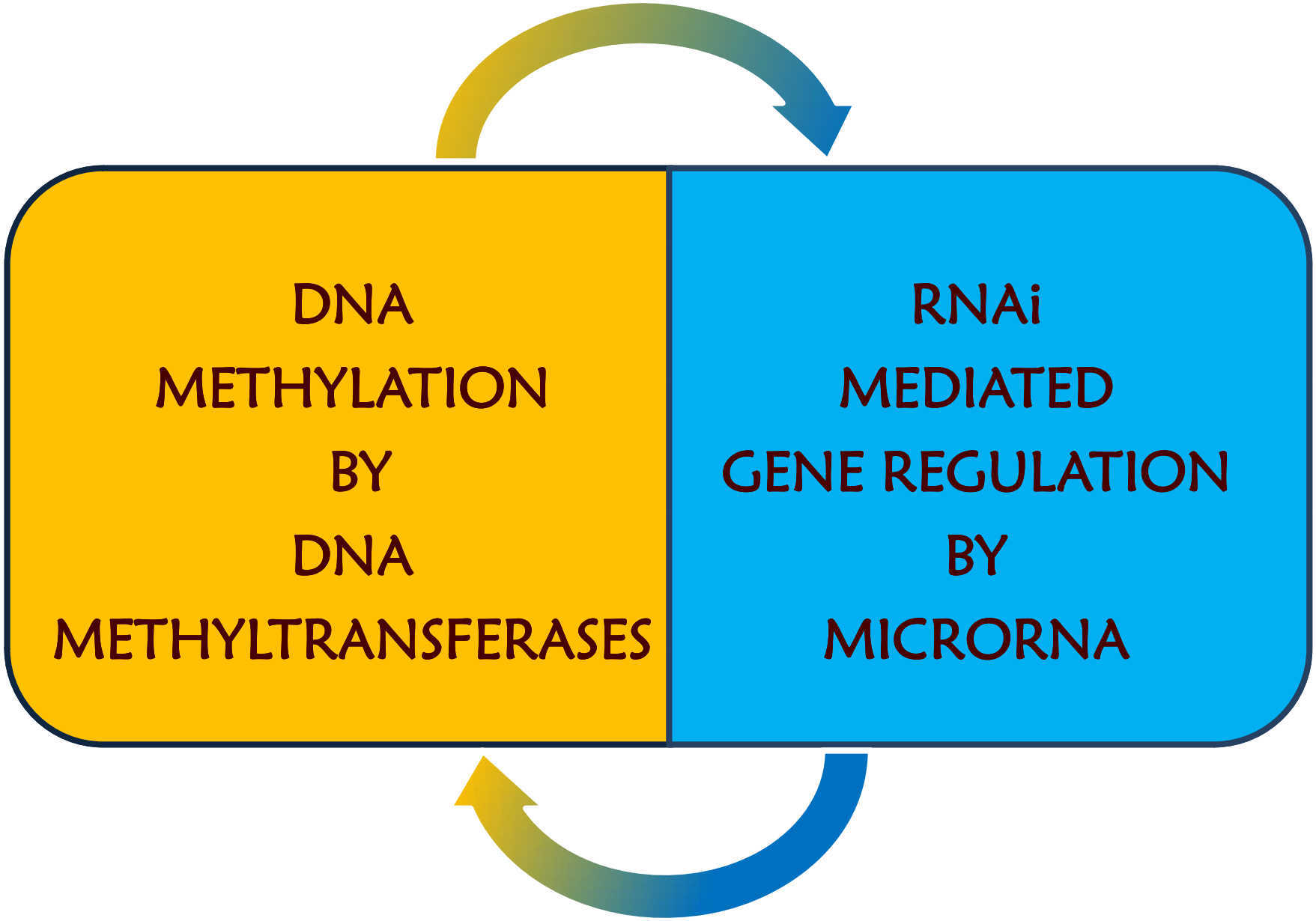
Histone deacetylase activity in prostate cells



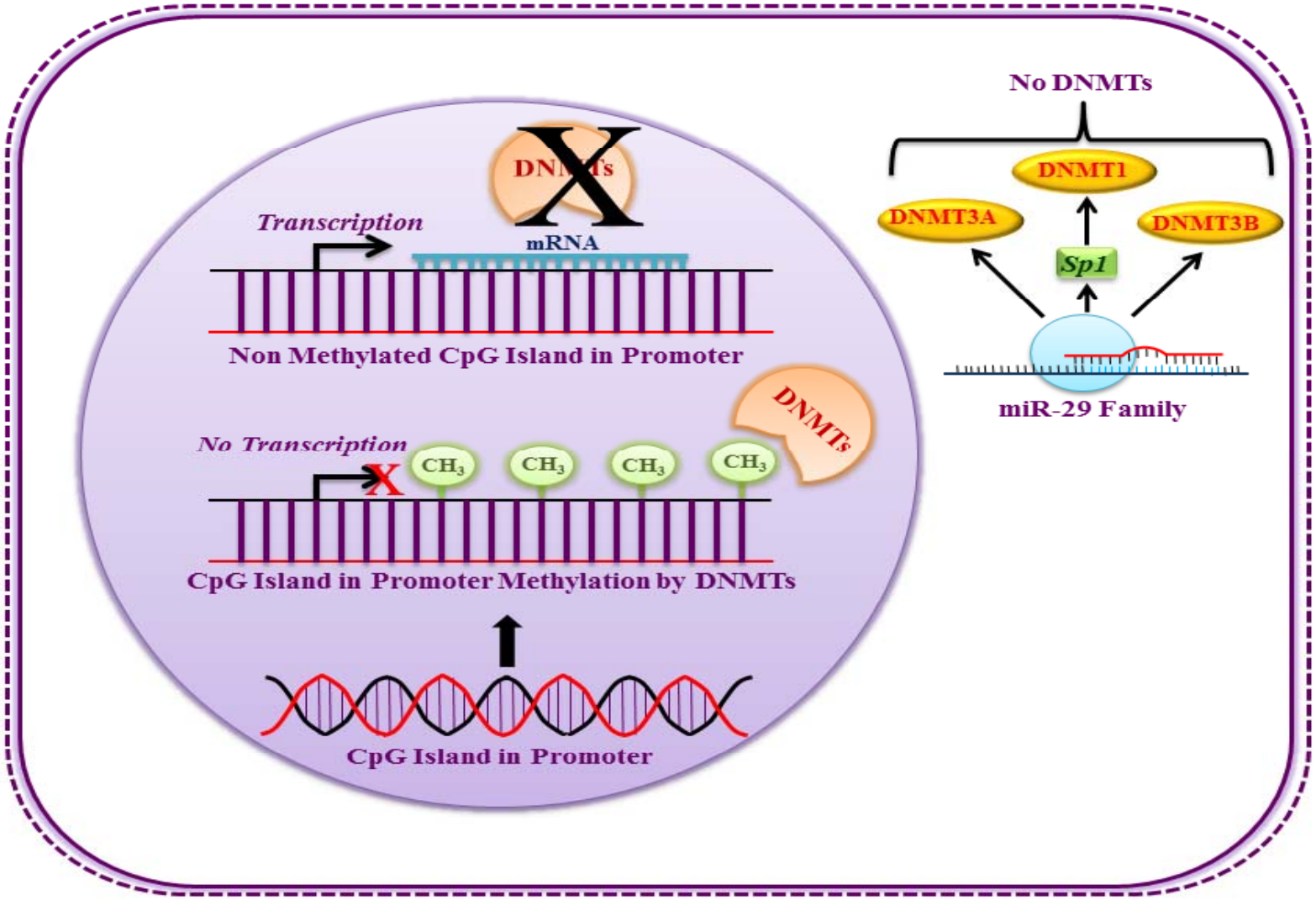
[Patra, et al., 2001, AACR; Patra, et al., 2001, Biochem. Biophys. Res. Commn]

Regulation of epigenetic machineries via micro-RNA mediated gene control

MicroRNAs are now considered as important biomarker in cancer diagnosis and prognosis. Research in this lab is mainly employed in identifying the microRNAs that target DNMT and Histone modifying enzymes, their effects on DNMTs and Histone modifying enzymes in cancer and creating the methylation profile of those miRNA genes. The lab is aiming for new avenues in the form of novel miRNA based drugs that target the various epigenetic manipulations for effective cancer therapeutics.


DNA METHYLATION BY DNA METHYLTRANSFERASES
RNAi MEDIATED GENE REGULATION BY MICRORNA

miRNA regulates DNA Methyltransferase Expression



[Sengupta et al., 2012, Communicated to Cancer Letters]

Computational Approach to Epigenomics Study

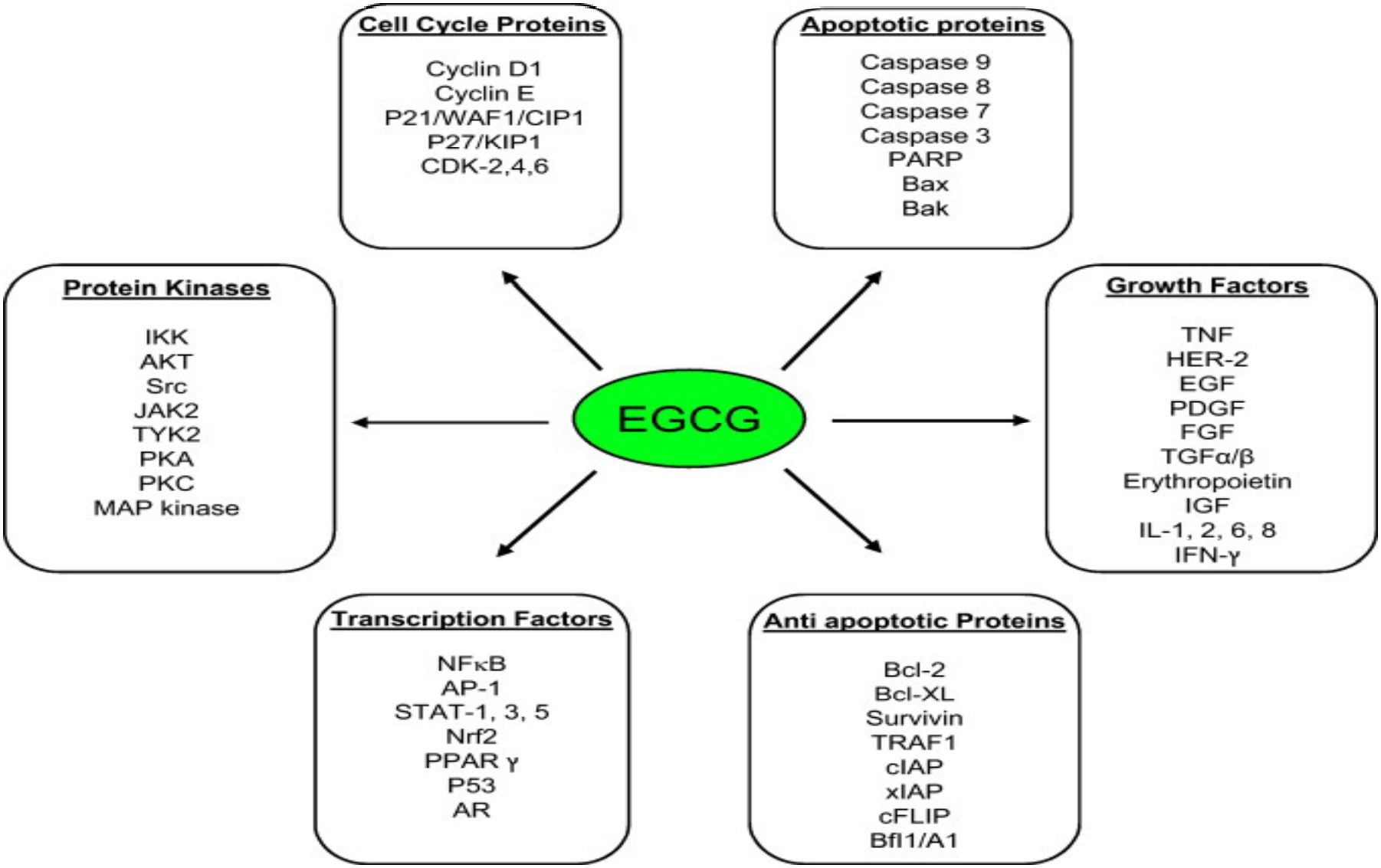
EPIGENOFORMATICS

An *in silico* approach via computational epigenomics combines traditional genomics with computer science, mathematics, chemistry, biochemistry and proteomics for the large-scale analysis of heritable changes in phenotype, gene function or gene expression that are not dependent on gene sequence. This field offers exciting and novel opportunities to further our understanding of transcriptional regulation, nuclear organization, development and disease. The lab employs bioinformatics tools in directing the selection of key experiments, formulating new testable hypotheses through detailed analysis of complex genomic information and studying of molecular dynamics.

Bioactive Molecules in Modulation of Epigenetic Marks

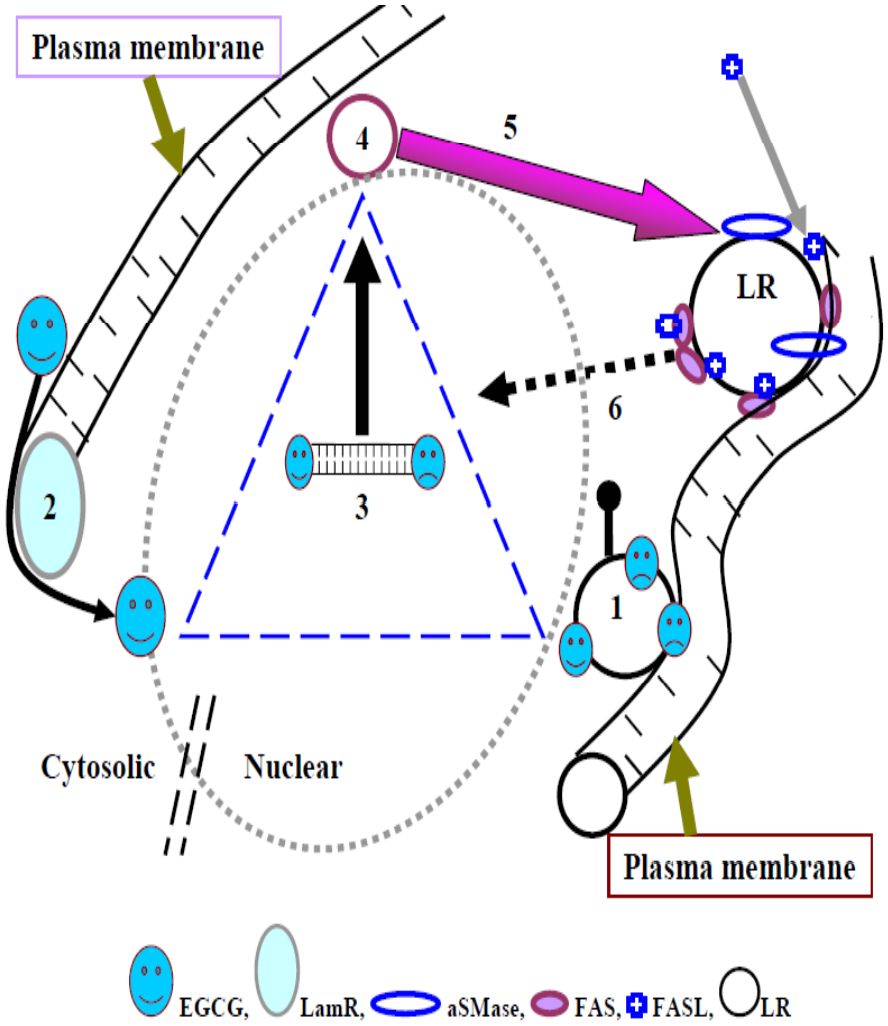
Epigenetic marks (epimutations) are more readily reversible in contrast to genetic defects, hence chemopreventive bioactive molecules are currently evaluated for their ability to reverse adverse epigenetic marks in cancer cells. The chemopreventive effects of these molecules on specific epigenetic alternations may provide unique & novel chemopreventive strategies to attenuate tumorigenic progression, prevent metastasis or sensitize for drug sensitivity. the lab is working towards developing therapeutic strategies by the development of various bioactive molecules like (5-azacytidine, EGCG, SAHA, TSA) that target the activity of DNMTs & HDACs in order to treat various disease including human cancer.

ACTION OF EGCG



MOLECULAR TARGETS OF (–)-EPIGALLOCATECHIN-3-GALLATE

SPECIFICITY AND INTERACTION WITH MEMBRANE LIPID RAFTS



[Patra et al., 2008, Journal of physiology and pharmacology]

1. Non-specific binding of EGCG in membrane lipid rafts would destabilize rafts structure and inactivate proliferative MAPK signaling.

2. Uptake of EGCG by LamR would bring EGCG into cytosol and nucleus.

3. EGCG binding to DNA would activate DNA-repair based DNA-demethylation, resulting in gene activation.

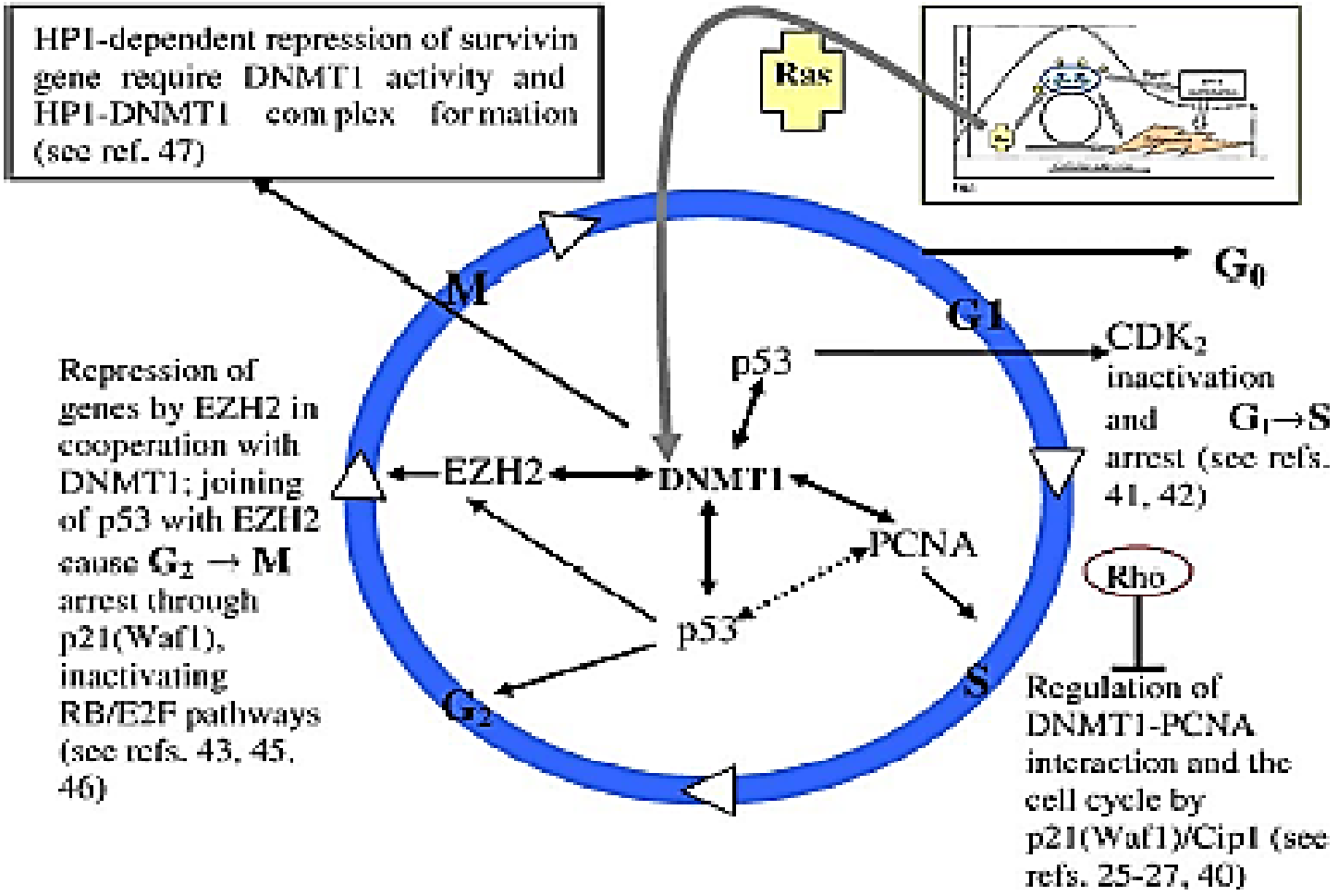
4. Over-expression of tumor suppressor and pro-apoptotic genes (FAS, PAR4 and CLU) will inhibit step 3.

5. aSMase and FAS is now loaded into lipid rafts after post translational modification by Palmitoylation.

Elucidation of the molecular signaling networks that co-ordinate epigenetic modifications and their dynamicity.

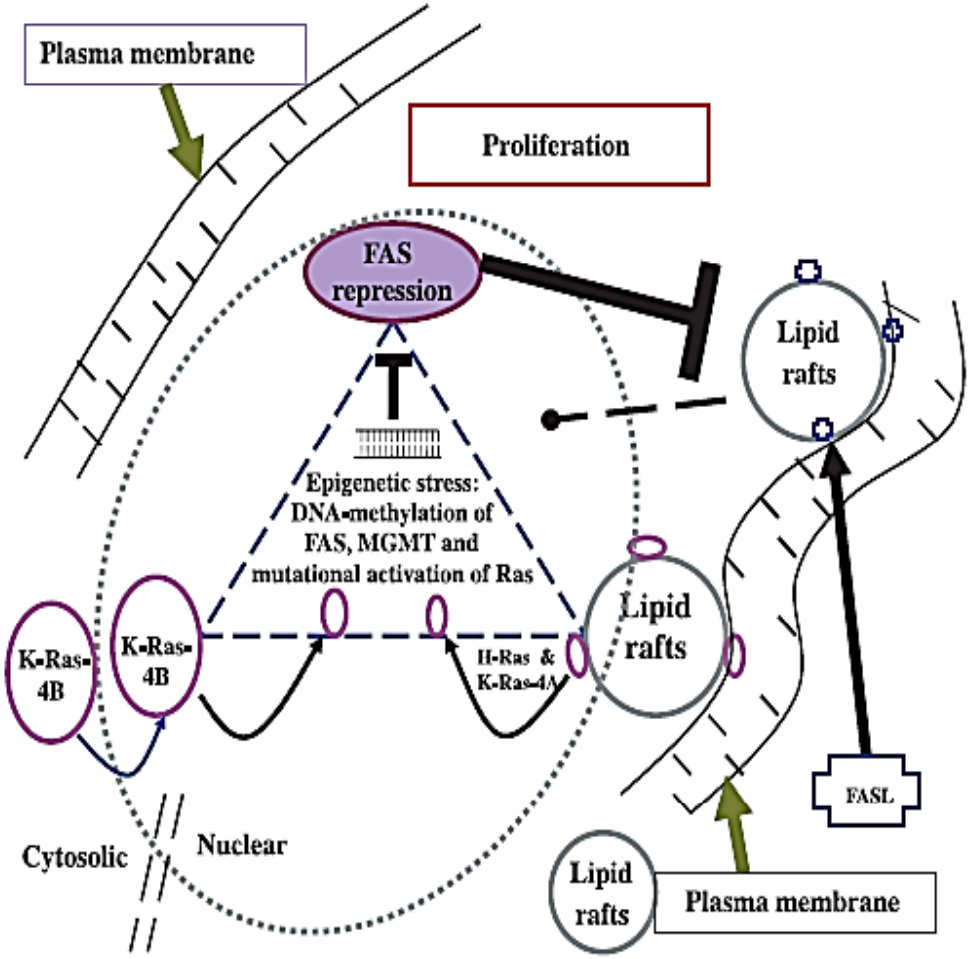
Signaling networks play a crucial role in regulating the transcriptional activity of cells via transmission of signals necessary for initiation of gene expression. The epigenetic regulatory mechanism is also controlled via various signaling molecules which up-regulate of down-regulate the epigenetic modulators according to the state of activity of the cell and the need for that particular modification. Our laboratory investigates the various signaling network involved in epigenetic modulation of the genome via various pathways.

DNMT1 interacting partners in the cell cycle



[Patra, S. K. 2008, Exp. Cell Res.]

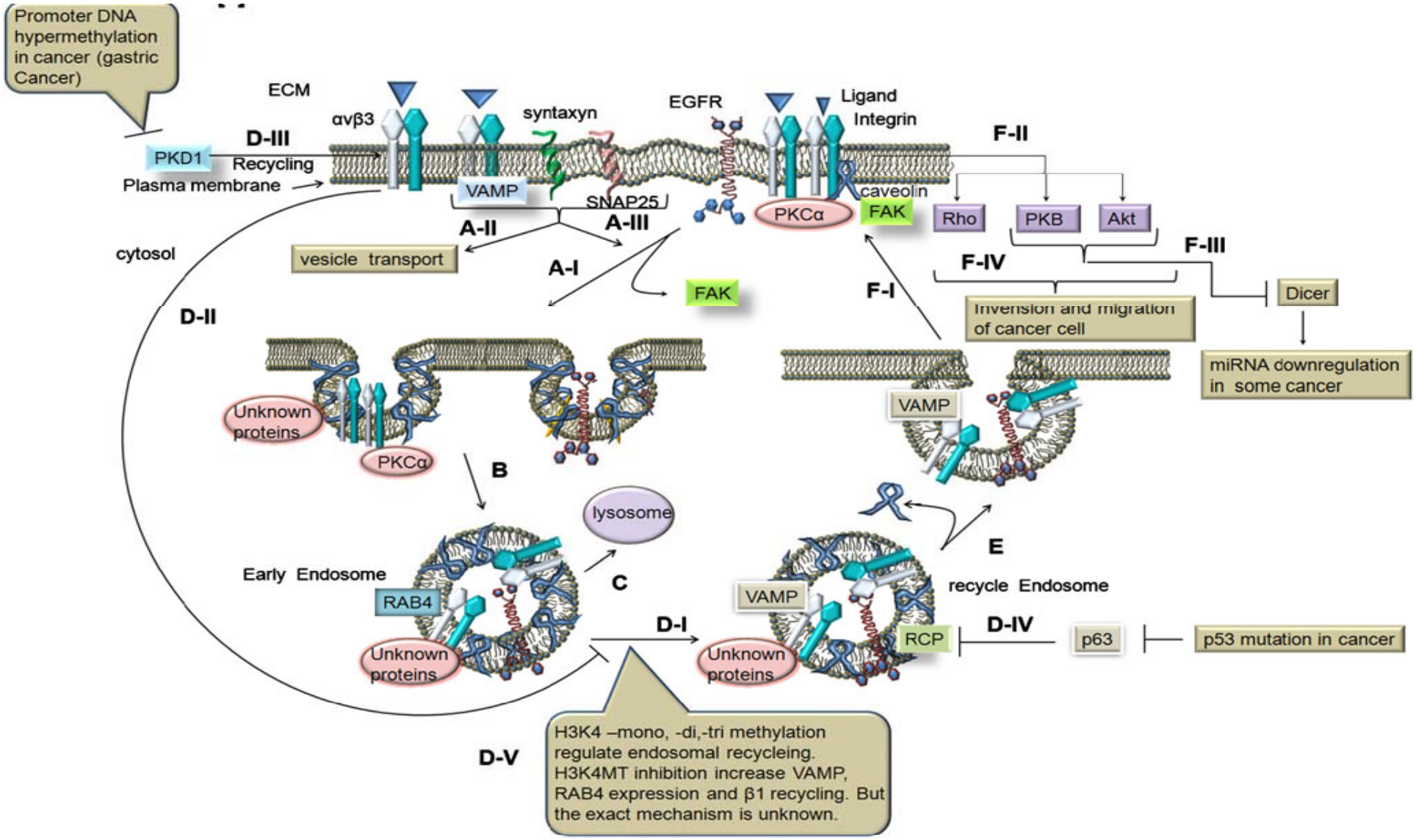
Lipid Raft Facilitated Ras Signaling And Chromatin Modification



[Patra and Syzf, 2008, FEBS journal]

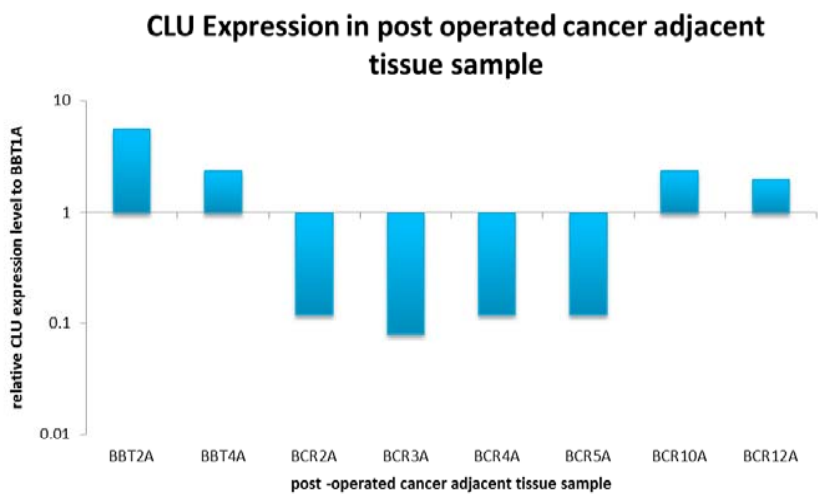
Epigenetic signaling for proliferation or death emanating from the plasma membrane microdomains. Lipid raft-dependent (H-Ras and K-Ras-4A) and independent (K-Ras-4B) signaling. A model scheme proposed for the modulation of transbilayer signaling by clustering of raft protein H-Ras, in which external clustering (antibody or ligand mediated) enhances the association of internal leaflet proteins with the stabilized clusters, promoting either enhancement or inhibition of signaling.

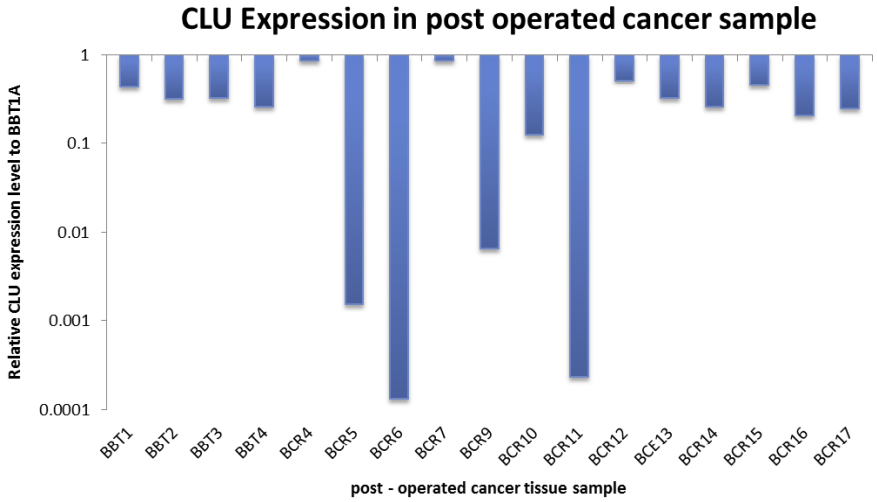
Integrin signaling at the onset of tumorigenesis

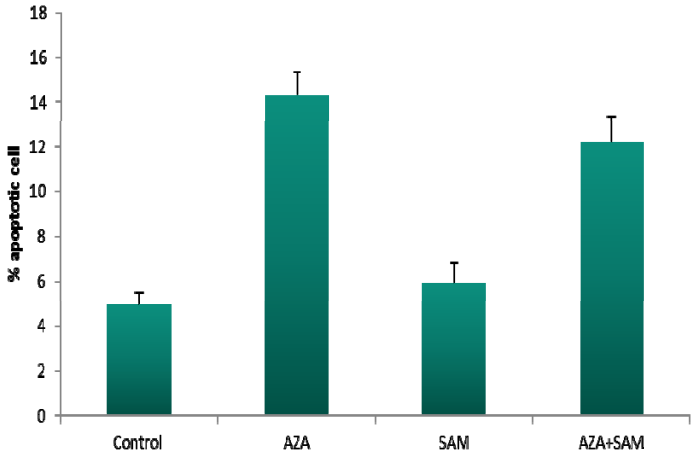


[Deb et al., 2011, Cancer Metastasis Rev]

Clusterin Expression and its Epigenetic Control Switch in Cancer







Cells treated with AZA show increased apoptosis, whereas SAM is seen to have no apparent effect on apoptosis with respect to control. However, conjoint treatment with both SAM and AZA results in higher apoptotic rate.

[Poster presented at 2nd GCGC conference, ACTREC, Mumbai, Nov 19th-20th, 2012]

Recent publications from the laboratory

1. Bhutia SK, Mukhopadhyay S, Sinha N, Das DN, Panda PK, **Patra SK**, Maiti TK, Mandal M, Dent P, Wang X-Y, Das S, Sarkar D, and Fisher, PB (2012) Autophagy: Cancer's Friend or Foe? **Adv Cancer Res.** In Press
2. Kar S, Deb M, Sengupta D, Shilpi A, Parbin S, Torrisani J, Pradhan S and **Patra SK** (2012) An insight into the various regulatory mechanisms modulating Human DNA Methyltransferase 1 stability and function. **Epigenetics, 7:** 994-1007. [I.F. → 4.6]
3. Kar S, Deb M, Sengupta D, Shilpi A, Bhutia SK and **Patra SK** (2012) Intricacies of Hedgehog Signaling Pathways: A perspective in tumorigenesis. **Exp Cell Res,** 318: 1959-1972. doi.org/10.1016/j.yexcr.2012.05.015 [I.F. → 3.580]
4. Deb M, Sengupta D and **Patra SK** (2012) Integrin-Epigenetics: A system with imperative impact on cancer. **Cancer Metast. Rev.** 31: 221–234 [I.F. → **10.573**]
5. Patra A, Deb M, Dahiya R and **Patra SK** (2011) 5-Aza-2'-deoxycytidine stress response and apoptosis in prostate cancer **Clin Epigenet,** 2: 339-348.
6. **Patra SK,** Deb M and Patra A (2011) Molecular Marks for Epigenetic Identification of Developmental and Cancer Stem Cells. **Clin Epigenet,** 2: 27-53.
7. **Patra SK** and Bettuzzi S (2009) Epigenetic DNA-(Cytosine-5-Carbon) Modifications: 5-Aza-2'-Deoxycytidine and DNA-Demethylation. **Biochemistry (Moscow), 74** (6): 613-619. [I.F. → 1.402]
8. **Patra SK,** Patra A, Rizzi F., Silva, A. et al. (2008) Molecular targets of (–)-epigallocatechin-3-gallate (EGCG): specificity and interaction with membrane lipid rafts. **J Physiol Pharmacol,** 59 (Suppl 9):217-235. [I.F. → 4.4]
9. **Patra SK** and Szyf M. (2008) DNA methylation mediated nucleosome dynamics and oncogenic Ras signaling: insights from FAS, FASL and RASSF1A **FEBS J,** 275:5217-5235. [I.F. → 3.05]
10. **Patra SK** (2008) Ras regulation of DNA-methylation and cancer. **Exp Cell Res** 314(6): 1193-1201. [I.F. → 4.148]
11. **Patra SK,** Patra A, Rizi F, Ghosh, T C et al. (2008) Demethylation of (cytosine-5-C-methyl) DNA and regulation of transcription in the epigenetic pathways of cancer development **Cancer Metast. Rev.** 27(2): 315-334. [I.F. → 10.578]
12. **Patra SK** (2008) Dissecting lipid raft facilitated cell signaling pathways in cancer. **Biochim. Biophys. Acta.** 1785:182-206 [I.F. → 12.15]

Foreign Collaborators

1. Dr Jerrome Torrisani, INSERM, Tolouse, France
2. Professor Moshe Szyf, McGill University, Montreal, Canada
3. Professor Faustino Mollinedo, CSIC, University of Salamanca, Spain
4. Professor Saverio Bettuzzi, University of Parma, Italy
5. Dr. Sriharsa Pradhan, New England Biolab., USA

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Postdoctoral Fellow/s:

Madhumita Rakshit

Lab Technician:

Chahat Kauser

The pillars of the laboratory

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Dipta Sengupta
JRF, Institute Fellow

Swayamsiddha Kar
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Sandeep Kumar Rath
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THANKS