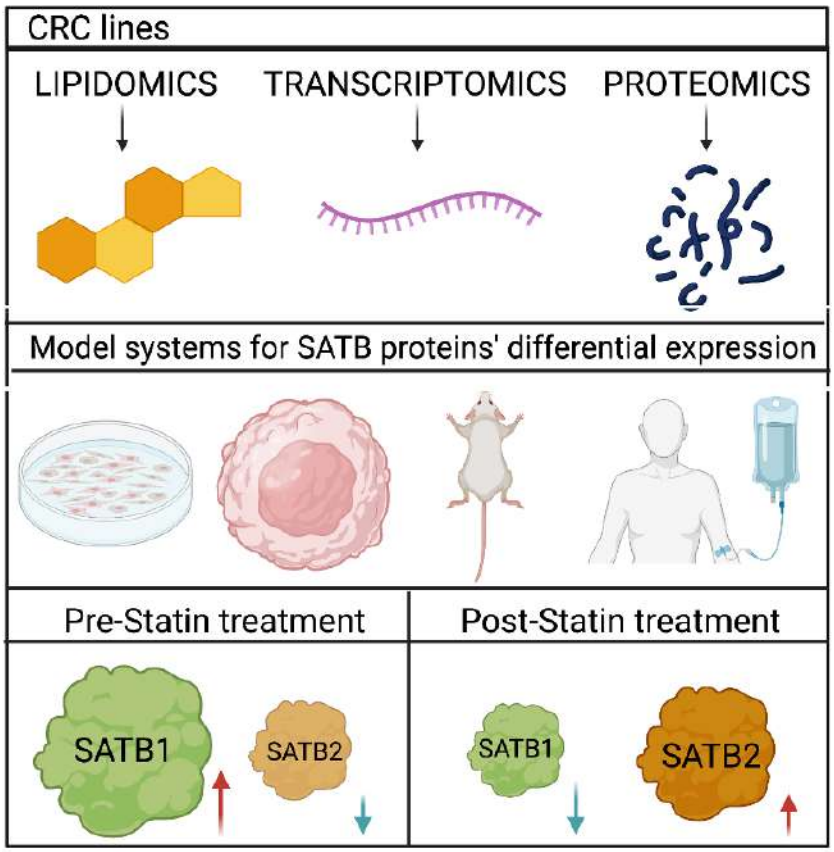
Excellence in Research: Sanjeev Galande (2018 to 2024)

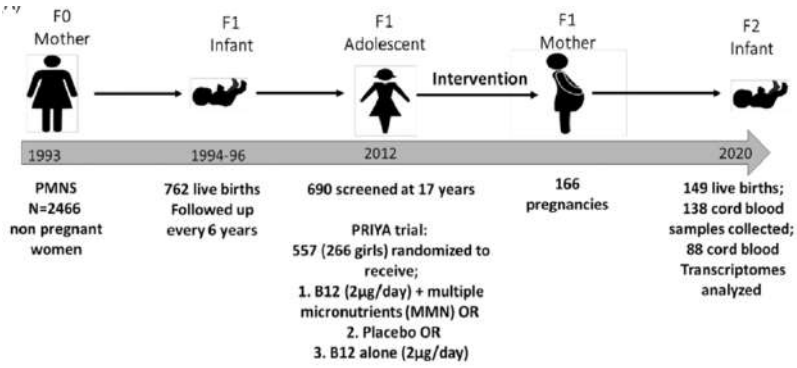
The following are a few examples of recent outcomes of the collaborative projects from Galande group with a translational impact:

Statins attenuate Wnt/β-catenin signaling by targeting SATB family proteins in colorectal cancer: Repurposing of statins for anti-tumor therapeutics.

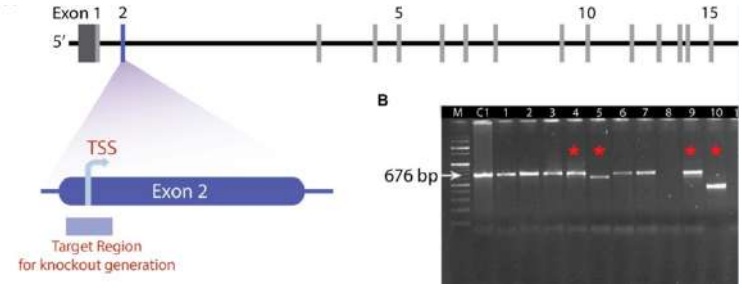
Colorectal cancer is the second leading cause of cancer-related deaths worldwide, highlighting the need for improved treatments and advanced molecular research. A recent therapeutic approach focuses on repurposing drugs to target dysregulated pathways involved in tumorigenesis. Among these, statins, commonly known for lowering cholesterol, have attracted attention for their potential anti-cancer properties. Here, we provide direct evidence for the same by assessing the impact of statin treatment on lipid, transcript, and protein levels. Our findings reveal that statins specifically target key components of the Wnt/β-catenin pathway, a major factor in adenoma formation, including the SATB (Special AT-rich Binding protein) family proteins. While SATB1 is recognized as a regulator of tumorigenesis, particularly under Wnt signaling, SATB2 appears to exert an opposing role. We demonstrate that statin treatment reciprocally alters the expression pattern of these proteins. Furthermore, a human clinical trial evaluating statins as an anti-cancer therapy supports the hypothesis that differential expression of SATB proteins is crucial in tumorigenic outcomes. In conclusion, this modulation by statin treatment suggests promising new therapeutic avenues through drug repurposing (Tripathi et al, 2024).

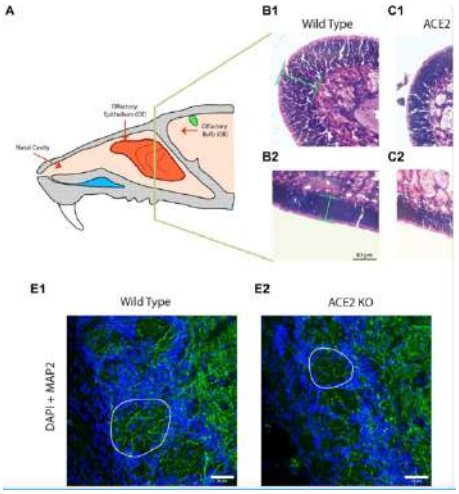


A multigenerational trial revealed multimicronutrient intervention epigenetically affects cell cycle dynamics - Implications for the malnourished Indian population (Khare et al., J DOHAD 2023): In the Pune Maternal Nutrition Study (Collaboration with the Diabetes Research Unit, KEM Hospital Pune), vitamin B12 deficiency was seen in 65% of pregnant women, folate deficiency was rare. Maternal total homocysteine concentrations were inversely associated with offspring birthweight, and low vitamin B12 and high folate concentrations predicted higher offspring adiposity and insulin resistance. These findings guided a nested preconceptional randomised controlled trial 'Pune Rural Intervention in Young Adolescents' (PRIYA). The interventions included: (1) vitamin B12 + multi-micronutrients as per the United Nations International Multiple Micronutrient Antenatal Preparation, and proteins (B12 + MMN), (2) vitamin B12 (B12 alone), and (3) placebo. Intervention improved maternal pre-conceptional and in-pregnancy micronutrient nutrition. Gene expression analysis in cord blood mononuclear cells in 88 pregnancies revealed 75 differentially expressed genes between the B12 + MMN and placebo groups. The enriched biological processes included G2/M phase transition, chromosome segregation, and nuclear division. Enriched pathways included, mitotic spindle checkpoint and DNA damage response while enriched human phenotypes were sloping forehead and decreased head circumference. Fructose-bisphosphatase 2 (FBP2) and Cell Division Cycle Associated 2 (CDCA2) genes were under-expressed in the B12 alone group. The latter, involved in chromosome segregation was under-expressed in both intervention groups. Based on the role of B-complex vitamins in the synthesis of nucleotides and S-adenosyl methionine, and the roles of vitamins A and D on gene expression, we propose that the multimicronutrient intervention epigenetically affected cell cycle dynamics. Neonates in the B12 + MMN group had the highest ponderal index. Follow-up studies will reveal if the intervention and the altered biological processes influence offspring diabesity.



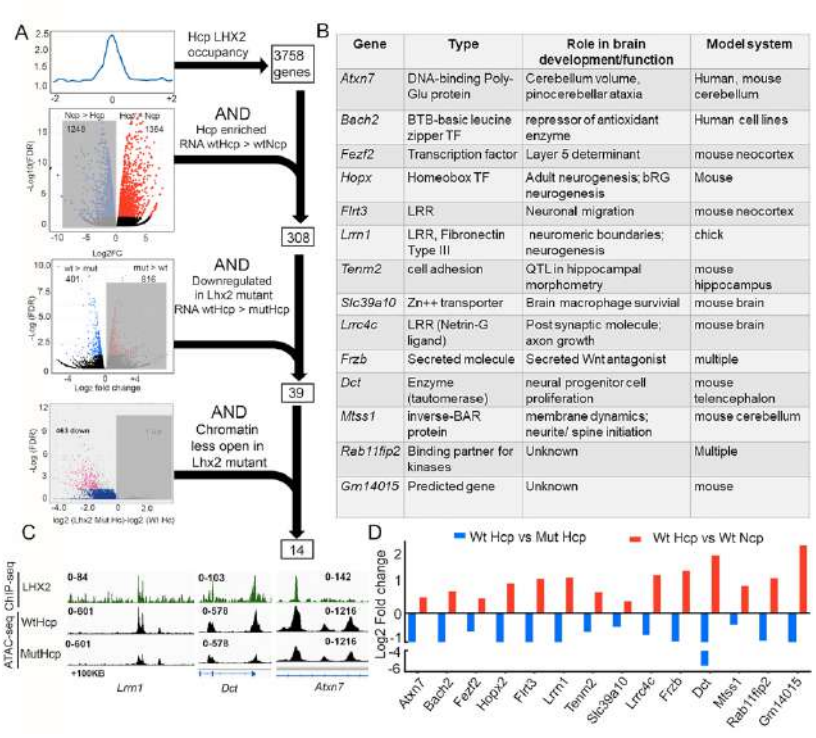
Knockout of angiotensin converting enzyme-2 receptor leads to morphological aberrations in rodent olfactory centers and dysfunctions associated with sense of smell (Mahajan et al., Front Neurosci 2023): ACE2 KO mice were generated using CRISPR-Cas9 based genome editing tools. This was my contribution during the pandemic, we worked hard to generate an animal model system that was not available in India to study COVID Pathogenesis. Neuronal morphological characterization and behavioral phenotyping in mouse models help dissecting neural mechanisms of brain disorders. Olfactory dysfunctions and other cognitive problems were widely reported in asymptomatic carriers and symptomatic patients infected with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This led us to generate the knockout mouse model for Angiotensin Converting Enzyme-2 (ACE2) receptor, one of the molecular factors mediating SARS-CoV-2 entry to the central nervous system, using CRISPRCas9-based genome editing tools.





Reduced thickness of olfactory sensory neuron layer, and a decrease in the cross-sectional area of glomeruli were observed in these mice. ACE2 KO mice exhibited slower learning of odor discriminations at the threshold levels and novel odor identification impairments ACE2 KO mice failed to memorize the pheromonal locations while trained on a multimodal task, implying the aberrations of neural circuits involved in higher cognitive functions. ACE2 KO mice exhibited slower learning of odor discriminations at the threshold levels and novel odor identification impairments. Further, ACE2 KO mice failed to memorize the pheromonal locations while trained on a multimodal task implying the aberrations of neural circuits involved in higher cognitive functions. Our results thus provide the morphological basis for the sensory and cognitive disabilities caused by the deletion of ACE2 receptors and offer a potential experimental approach to study the neural circuit mechanisms of cognitive impairments observed in long COVID.

Regulation of chromatin accessibility and gene expression in the developing hippocampal primordium by LHX2 (Suresh et al., PLoS Genetics 2023): In the developing mouse brain, co-workers from Galande and Tole lab examined the hippocampal primordium (Hcp) and the adjacent neocortical primordium (Ncp) at embryonic day (E)12.5, when both structures predominantly contain progenitors and also contain newborn postmitotic neurons. We found that the Hcp displays strikingly distinct gene expression and chromatin accessibility from the Ncp. The Hcp chromatin is more accessible at over 14,000 loci compared with the Ncp, while the Ncp chromatin is more accessible at only 70 loci. We examined the transcription factor (TF) binding motifs on the DARs and compared these with TFs expressed in both tissues. TF LHX2 emerged as the top candidate that met these conditions. Loss of *Lhx2* selectively affected the accessibility of chromatin in the Hcp but not the Ncp. The majority



Gene	Type	Role in brain development/function	Model system
Atxn7	DNA-binding Poly-Glu protein	Cerebellum volume, pinocerebellar ataxia	Human, mouse cerebellum
Bach2	BTB-basic leucine zipper TF	repressor of antioxidant enzyme	Human cell lines
Fezf2	Transcription factor	Layer 5 determinant	mouse neocortex
Hopx	Homeobox TF	Adult neurogenesis; bRG neurogenesis	Mouse
Flrt3	LRR	Neuronal migration	mouse neocortex
Lrrn1	LRR, Fibronectin Type III	neuromeric boundaries; neurogenesis	chick
Tenm2	cell adhesion	QTL in hippocampal morphometry	mouse hippocampus
Slc39a10	Zn++ transporter	Brain macrophage survivial	mouse brain
Lrrc4c	LRR (Netrin-G ligand)	Post synaptic molecule; axon growth	mouse brain
Frzb	Secreted molecule	Secreted Wnt antagonist	multiple
Dct	Enzyme (tautomerase)	neural progenitor cell proliferation	mouse telencephalon
Mfss1	inverse-BAR protein	membrane dynamics; neurite/ spine initiation	mouse cerebellum
Rab11fip2	Binding partner for kinases	Unknown	Multiple
Gm14015	Predicted gene	Unknown	mouse

of the DARs that displayed increased accessibility upon loss of Lhx2 were also occupied by Lhx2. Transcriptomically, the Hcp displayed dysregulation of pathways related to DNA conformation, recombination, repair, replication, and organization. Comparing LHX2 occupancy with differentially expressed genes (DEGs) upon loss of Lhx2, we identified 4 key pathways dysregulated in the Ncp and Hcp: Wnt/ Hippo signaling, axon guidance, and pluripotency. Sequential filtering of the ChIP-seq, RNA-seq, and ATAC-seq data identified 14 genes that are controlled by *Lhx2* in the Hcp in terms of chromatin accessibility and mRNA expression. Some are known players in Ncp development, but none have thus far been examined in the Hcp. Our Hcp versus Ncp comparisons of chromatin accessibility, gene expression, and dysregulation upon loss of Lhx2 offer a means to arrive at a mechanistic understanding of the range of distinct phenotypes in these structures when Lhx2 is lost at different stages of development.

The following are a few examples of outcomes of the projects from Galande group with a high impact:

SATB (Special AT-rich binding protein) family proteins have emerged as key regulators that integrate higher-order chromatin organization with the regulation of gene expression. The Galande lab has been working towards elucidating the specific roles of SATB1 and SATB2, two closely related family members during embryogenesis, T cell function, and cancer progression.

To understand the molecular circuitry deployed by Satb2 to achieve early cell fate specification leading to primordial organ formation our lab developed an experimental model by generating mutants for Satb2 in zebrafish. Transcriptome profiling of Satb2 mutants revealed the global deregulation of genes involved in NC specification and migration. Furthermore, the epigenetic landscape of zebrafish embryos revealed that maternal Satb2 prevents premature transcription of zygotic genes by influencing the interplay between the pluripotency factors, while zygotic Satb2 activates transcription of the same group of genes during neural crest development and organogenesis. Interestingly, our study highlights the switch between functions of SATB2 from a repressor of transcription during ZGA to an activator of a special subset of neural crest progenitor cells during organogenesis in a biphasic and bimodal manner (Pradhan et al. 2021). On the other hand, we present evidence that in mammalian cells, SATB1 interacts with Dishevelled, an upstream component of the Wnt/Wg pathway. Conversely, ectopic expression of full-length human SATB1 but not that of its N- or C-terminal domains in the eye imaginal discs and salivary glands of third instar Drosophila larvae increased the expression of Wnt/Wg pathway antagonists and suppressed phenotypes associated with activated Wnt/Wg pathway. Collectively, these findings indicate that regulation of Wnt/Wg pathway by SATB1 is context-dependent manner (Ramanujam et al. 2021).

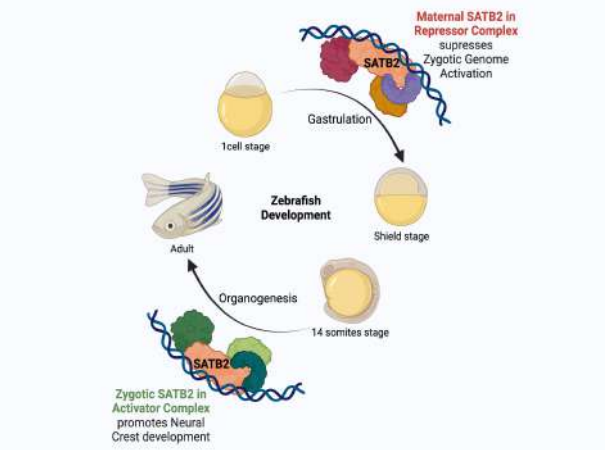


Figure 3: Schematic summary highlighting differential functions of Satb2 during various stages of zebrafish embryogenesis.

SATB1 expression profile coincides with T lineage commitment and upregulation of SATB1 correlates with positive selection of thymocytes (Gottimukkala et al. 2016). SATB1 is expressed in a lineage-specific manner in CD4+ T-cells. Analysis of RNA-seq data revealed multiple transcription start sites at the upstream regulatory region of *SATB1*. We further demonstrated that *SATB1* gene is expressed via al ternative promoters during T-helper (Th) cell differentiation.The proximal promoter "P1" is used more by the naïve and activated CD4+ T-cells whereas the middle "P2" and the distal "P3" promoters are used at a significantly higher level by polarized T-helper cells.

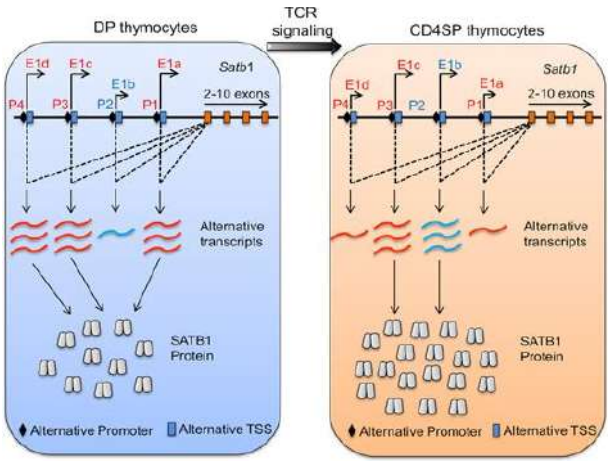


Figure 4: A schematic model depicting TCR signal-mediated Satb1 alternative promoter switch in developing thymocytes

Thus, the promoter switch might play a crucial role in fine-tuning of SATB1 protein expression in a cell type-specific manner (Khare et al. 2019). In our study to understand the mechanism(s) regulating the expression of SATB1 during T-cell development, four alternative promoters of *Satb1* in mouse thymocytes were predicted by ChIP-seq for H3K4me1/3 and their transcript variants were characterized. The discrepancy between the expression levels of SATB1 mRNA and protein in developing thymocytes was explainable by the differential translatability of *Satb1* transcript variants. The selective expression of a combination of *Satb1* transcript variants during T-cell development plays a crucial role toward the regulation of SATB1 protein levels (Patta et al. 2020). From a disease perspective Gamma delta (γδ) T cell, especially the Vγ9Vδ2 subtype, have been implicated in cancer therapy. we demonstrate the effect of the activation of TCR signaling by phosphoantigens or anti-CD3 on the transcriptional status of Vγ9Vδ2 T cells along with IL2 stimulation. We further show that the blockade of Notch signaling antagonistically affects this activation. Signaling via SATB1, an early TCR-responsive chromatin organizer, is also affected by Notch inhibition (Madhok et al. 2021).

SATB1 has been implicated in the development and progression of multiple cancers including colorectal cancer. we demonstrate that expression of SATB1 is induced upon hyperactivation of Wnt/β-catenin signaling and repressed upon depletion of TCF7L2 (TCF4) and β-catenin. Using several colorectal cancer cell line models and the APC min mutant zebrafish in vivo model, we established that SATB1 is a novel target of Wnt/β-catenin signaling. We show that direct binding of TCF7L2/β-catenin complex on Satb1 promoter is required for the regulation of SATB1 (Mir et al. 2016). In a detailed review, we focus on the epigenetic players which influence the Wnt/β-catenin pathway via modulation of its components and coordinated regulation of the Wnt target genes (Sharma et al. 2021). Further our data provides the biochemical and functional evidences for SP1 as an integral part of the Wnt signaling pathway. A mammalian

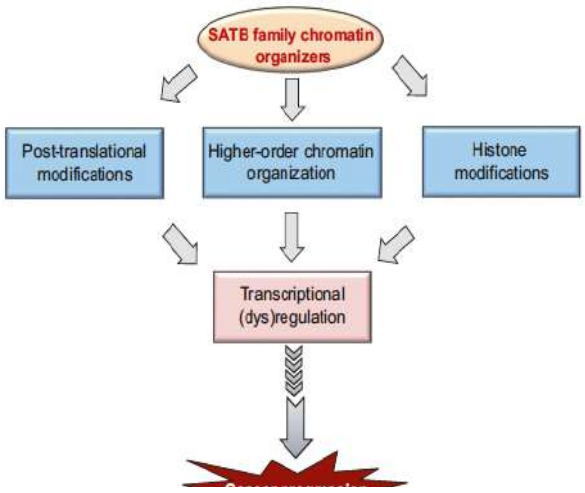


Figure 5:Schematic representation of molecular mechanisms regulated by SATB family chromatin organizers leading to tumorigenesis and cancer progression.

phosphodegron motif in SP1 was identified. GSK3β-mediated phosphorylation and β-TrCP-mediated ubiquitination induce SP1 degradation in the absence of Wnt signaling, while SP1 is directly stabilized by β-catenin when Wnt signaling is on by impeding its interaction with β-TrCP and axin1– components of the destruction complex (Mir et al. 2018). To provide new insights into cancer biology from the chromatin organization perspective, an instructive review including tumor survival data analyses was recently contributed by Galande. This review article highlights the cellular and molecular events governed by SATB1 influencing the structural organization of chromatin and interacting with several co-activators and co-repressors of transcription towards tumor progression. Contrastingly, SATB2 is differentially expressed in an array of cancer types and is involved in tumorigenesis. Patient survival analysis across cancer types correlated with expression of SATB family chromatin organizers, suggesting expression of SATB1 and SATB2 contribute to disease prognosis (Naik and Galande 2019).

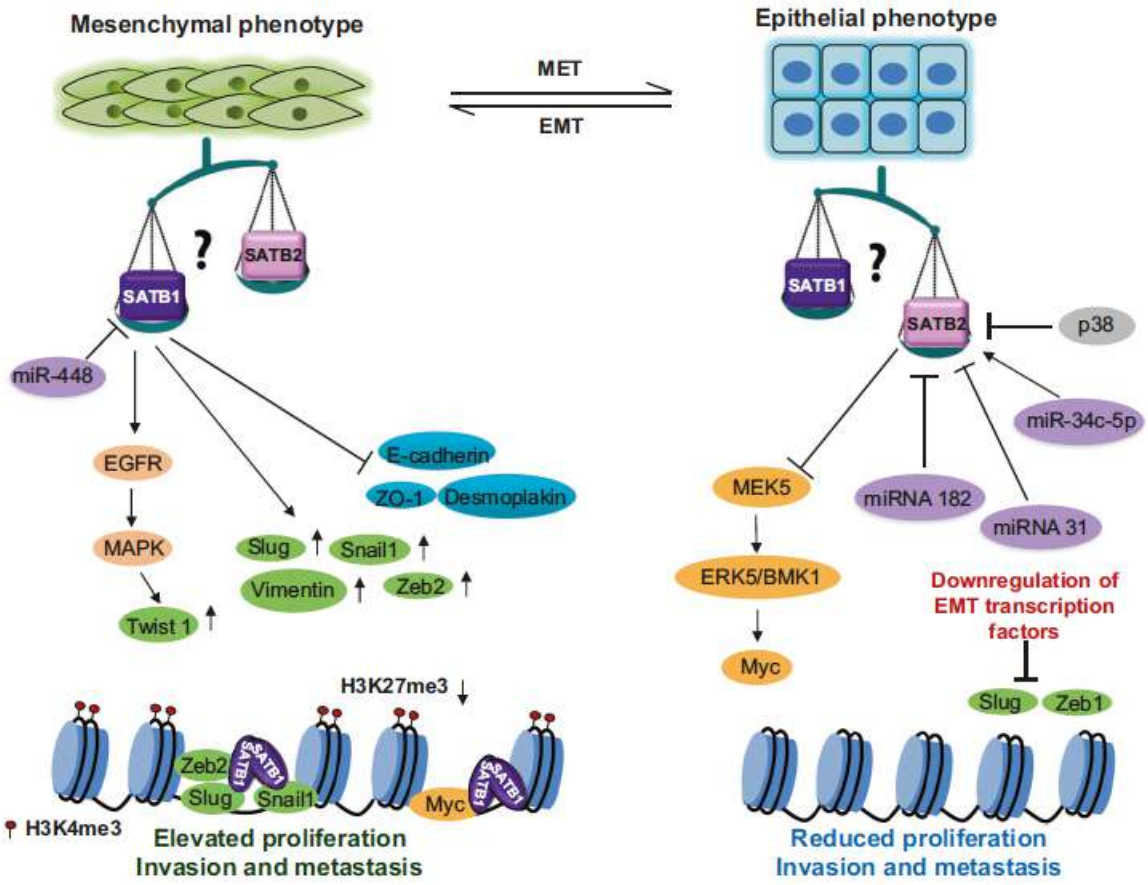


Figure 6: Schematic representation of dynamic balance between expression patterns of SATB family chromatin organizers as regulators of EMT-MET cellular states during metastasis.

Based on the above studies, Dr. Galande has filed a patent (in multiple countries) for cancer prognosis and it is under advanced stages of review. Indian patent is already granted in March 2024.

In continuation with cis regulation of chromatin organization and function, a cis regulatory element (cRE) within exon 1 of human XIST was identified and functionally characterized. In the initiation phase, pluripotency factors bind to this cRE and keep *XIST* repressed, while the same is enriched for CTCF in the maintenance phase, activating *XIST* transcription. A CRISPR-dCas9-KRAB-based interference approach targeting the cRE corroborated the significance of its manifesting transcriptional regulation of *XIST* in a CTCF- and YY1-dependent manner (Shah et al. 2021). Further, Galande lab also reported the generation and utility of a reporter system that enables simultaneous scoring of transcriptional activity in opposite directions. We demonstrated the unique distribution of histone modification marks that correlate robustly with the transcription status of genes regulated by bidirectional promoters. These findings strongly imply that the occurrence of these marks might signal the transcription machinery to drive the maturation of antisense transcription from the bidirectional promoters (Jangid et al. 2018).

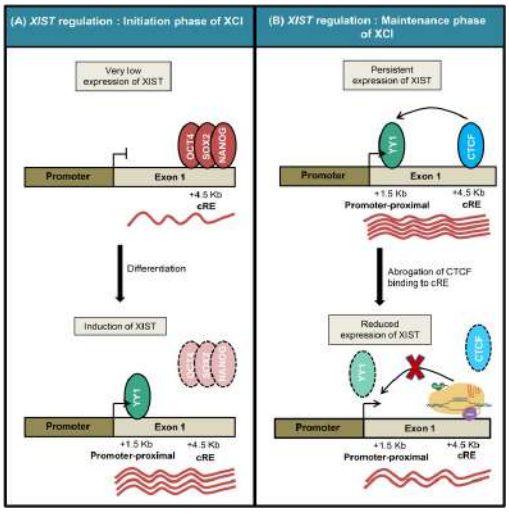


Figure 7:A model illustrating the role of CTCF-bound cRE in dictating the transcription from XIST promoter.

References:

- Jangid, R.K. *et al.* (2018) 'Bidirectional promoters exhibit characteristic chromatin modification signature associated with transcription elongation in both sense and antisense directions', *BMC Genomics*, 19(1), p. 313.
- Khare, S.P. *et al.* (2019) 'NF-κB Signaling and IL-4 Signaling Regulate SATB1 Expression via Alternative Promoter Usage During Th2 Differentiation', *Frontiers in Immunology*, 10, p. 667.
- Madhok, A. *et al.* (2021) 'Transcriptome Signature of Vγ9Vδ2 T Cells Treated With Phosphoantigens and Notch Inhibitor Reveals Interplay Between TCR and Notch Signaling Pathways', *Frontiers in Immunology*, 12, p. 660361.
- Mir, R. *et al.* (2016) 'Wnt/β-catenin signaling regulated SATB1 promotes colorectal cancer tumorigenesis and progression', *Oncogene*, 35(13), pp. 1679–1691.
- Mir, R. *et al.* (2018) 'Regulation of Transcription Factor SP1 by the β-Catenin Destruction Complex Modulates Wnt Response', *Molecular and Cellular Biology*, 38(22). doi:10.1128/MCB.00188-18.
- Naik, R. and Galande, S. (2019) 'SATB family chromatin organizers as master regulators of tumor progression', *Oncogene*, 38(12), pp. 1989–2004.
- Naik, S. *et al.* (2020) 'Differential tissue stiffness of body column facilitates locomotion of Hydra on solid substrates', *The Journal of Experimental Biology*, 223(Pt 20). doi:10.1242/jeb.232702.
- Patta, I. *et al.* (2020) 'Dynamic regulation of chromatin organizer SATB1 via TCR-induced alternative promoter switch during T-cell development', *Nucleic Acids Research*, 48(11), pp. 5873–5890.
- Pillai, A. *et al.* (2021) 'Epigenetic Regulation in Hydra: Conserved and Divergent Roles', *Frontiers in Cell and Developmental Biology*, 9, p. 663208.

- Pradhan, S.J. *et al.* (2021) 'Satb2 acts as a gatekeeper for major developmental transitions during early vertebrate embryogenesis', *Nature communications*, 12(1), p. 6094.
- Ramanujam, P.L. *et al.* (2021) 'Global chromatin organizer SATB1 acts as a context-dependent regulator of the Wnt/Wg target genes', *Scientific Reports*, 11(1), p. 3385.
- Reddy, P.C. *et al.* (2019) 'Molecular signature of an ancient organizer regulated by Wnt/β-catenin signalling during primary body axis patterning in Hydra', *Communications Biology*, 2, p. 434.
- Reddy, P.C. *et al.* (2020) 'Epigenomic landscape of enhancer elements during Hydra head organizer formation', *Epigenetics & Chromatin*, 13(1), p. 43.
- Shah, R. *et al.* (2021) 'A Novel cis Regulatory Element Regulates Human XIST in a CTCF-Dependent Manner', *Molecular and Cellular Biology*, 41(8), p. e0038220.
- Sharma, A., Mir, R. and Galande, S. (2021) 'Epigenetic Regulation of the Wnt/β-Catenin Signaling Pathway in Cancer', *Frontiers in Genetics*, 12, p. 681053.
- Unni, M. *et al.* (2021) 'Identification of Components of the Hippo Pathway in Hydra and Potential Role of YAP in Cell Division and Differentiation', *Frontiers in Genetics*, 12, p. 676182.
- Suresh V. *, Muralidharan, B. *, Pradhan, S. J. *, Bose, M., D'Souza, L., Parichha, A., Reddy, P. C., Galande, S.[#] and Tole, S.[#] 2023. Regulation of chromatin accessibility and gene expression in the developing hippocampal primordium by LIM-HD transcription factor LHX2. *PLoS Genetics* (Online publication).
- Mahajan S., Sen S., Sunil A., Srikanth P., Marathe S.D, Shaw K., Sahare M., Galande S.[#,] and Abraham N.A.[#] 2023. Knockout of ACE2 receptors lead to morphological aberrations in rodent olfactory centers and dysfunctions associated with sense of smell. *Frontiers in Neuroscience*, 17:1180868. doi: 10.3389/fnins.2023.1180868
- Gungi, A., Saha, S., Pal, M. and Galande, S. [#], 2023. H4K20me1 plays a dual role in transcriptional regulation of regeneration and axis patterning in Hydra. *Life Science Alliance*, 6(5).
- Khare, S.P. *, Madhok, A. *, Patta, I., Sukla, K.K., Wagh, V.V., Kunte, P.S., Raut, D., Bhat, D., Kumaran, K., Fall, C. and Tatu, U., Chandak G.R., Yajnik C. S. [#], Galande, S. [#] 2023. Differential expression of genes influencing mitotic processes in cord blood mononuclear cells after a pre-conceptional micronutrient-based randomised controlled trial: Pune Rural Intervention in Young Adolescents (PRIYA). *Journal of Developmental Origins of Health and Disease*, 14: 437-448. doi: 10.1017/S204017442200068X
- Tripathi S., Gupta E., Naik N., Khare K., Mir R., Desai S., Humane S., Yadav S., Bal M., Saklani A., Patil P., Kamat S., and **Galande S.[#]** 2024. Statins attenuate Wnt/β-catenin signaling by targeting SATB family proteins in colorectal cancer. *bioRxiv*. https://www.biorxiv.org/content/10.1101/2024.08.23.609189v1 (Under advanced stage of review in a reputed cancer journal)

*Equal contribution [#]Corresponding author

Certified that I have not received any award for the recent research work (2018-24) summarized above.

Sanjeev Galande