Importance of cellular organelles in controlling the miRNA-mediated gene expression in mammalian cells

Suvendra Nath Bhattacharyya

CSIR- INDIAN INSTITUTE OF CHEMICAL BIOLOGY

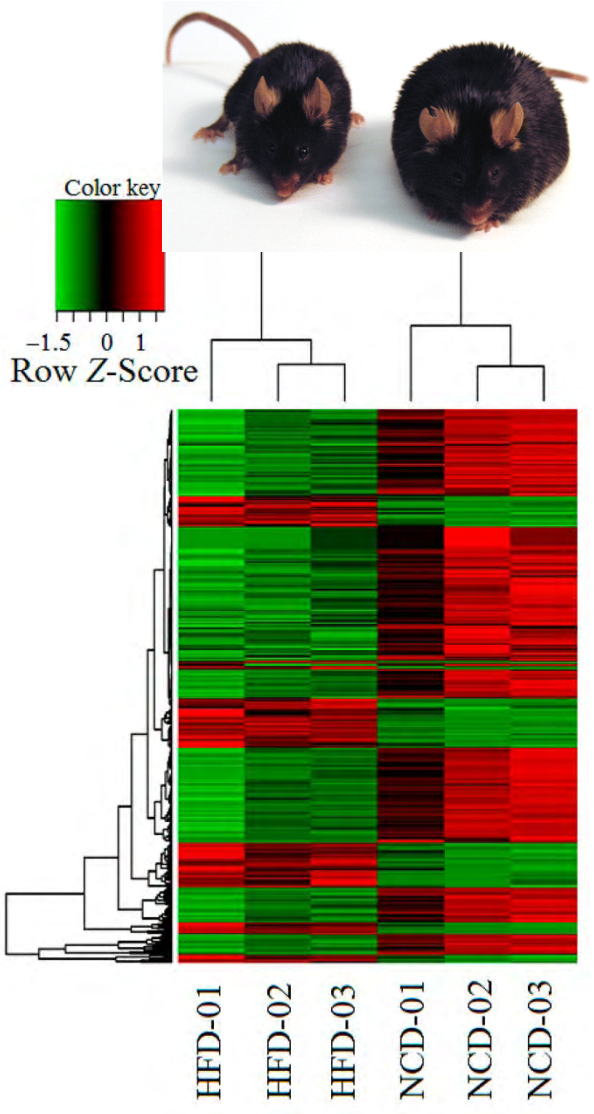
सी एस आई आर - भारतीय रासायनिक जीवविज्ञान संस्थान

সি এস আই আর - ভারতীয় রাসায়নিক জীববিজ্ঞান সংস্থান

84th Annual Meeting of IASc, BHU, Varanasi

Gene Expression and Modified Central Dogma

Altered Profile of
Gene Expression
normal vs high fat diet animal livers



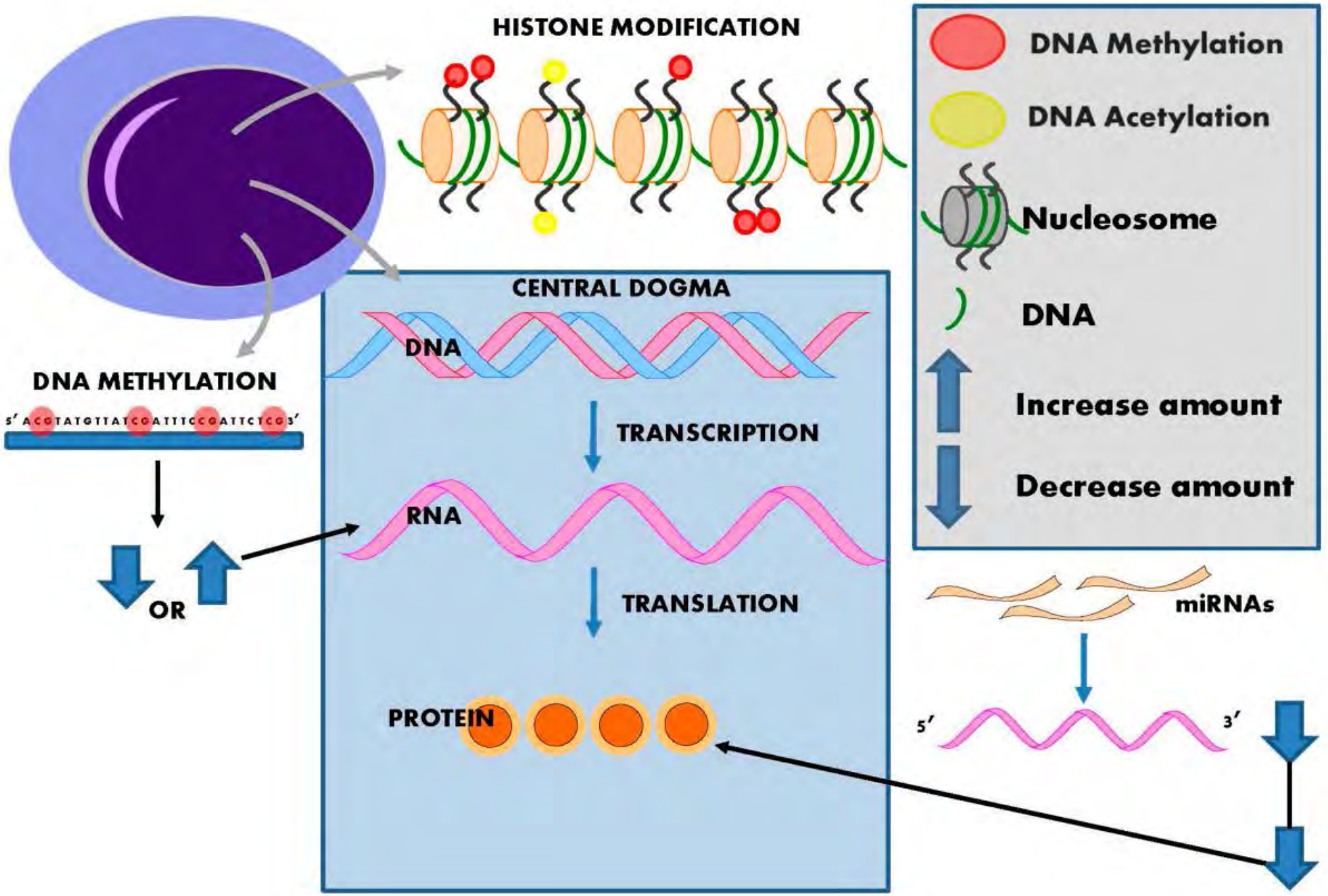
Gene expression pattern is tissue specific

Altered Gene expression under disease condition

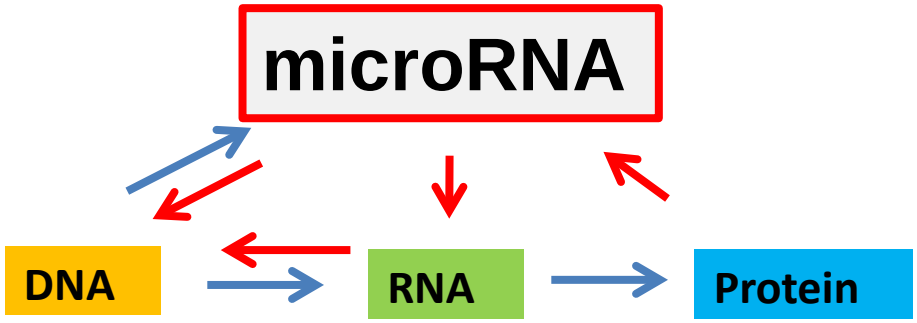
How the gene expression is getting regulated?

Int. J. Mol. Sci. **2014**, *15*(9), 14967-14983

Epigenetics: Non-DNA Sequence Driven Inheritance



Gene Expression : Modified Central Dogma



Career Path: RNA forever

Indian Institute of Chemical Biology
a unit of C.S.I.R.

1998-2003

PhD Student
(CSIR)

Mechanism of Mitochondrial tRNA Import

FMI
Friedrich Miescher Institute for Biomedical Research

Post-doctoral
Researcher

2004-2008

Mechanism microRNA-mediated Gene Regulation

2008-

Principal Scientist
Professor

AcSIR

Mechanism of miRNA activity Regulation in mammalian cells

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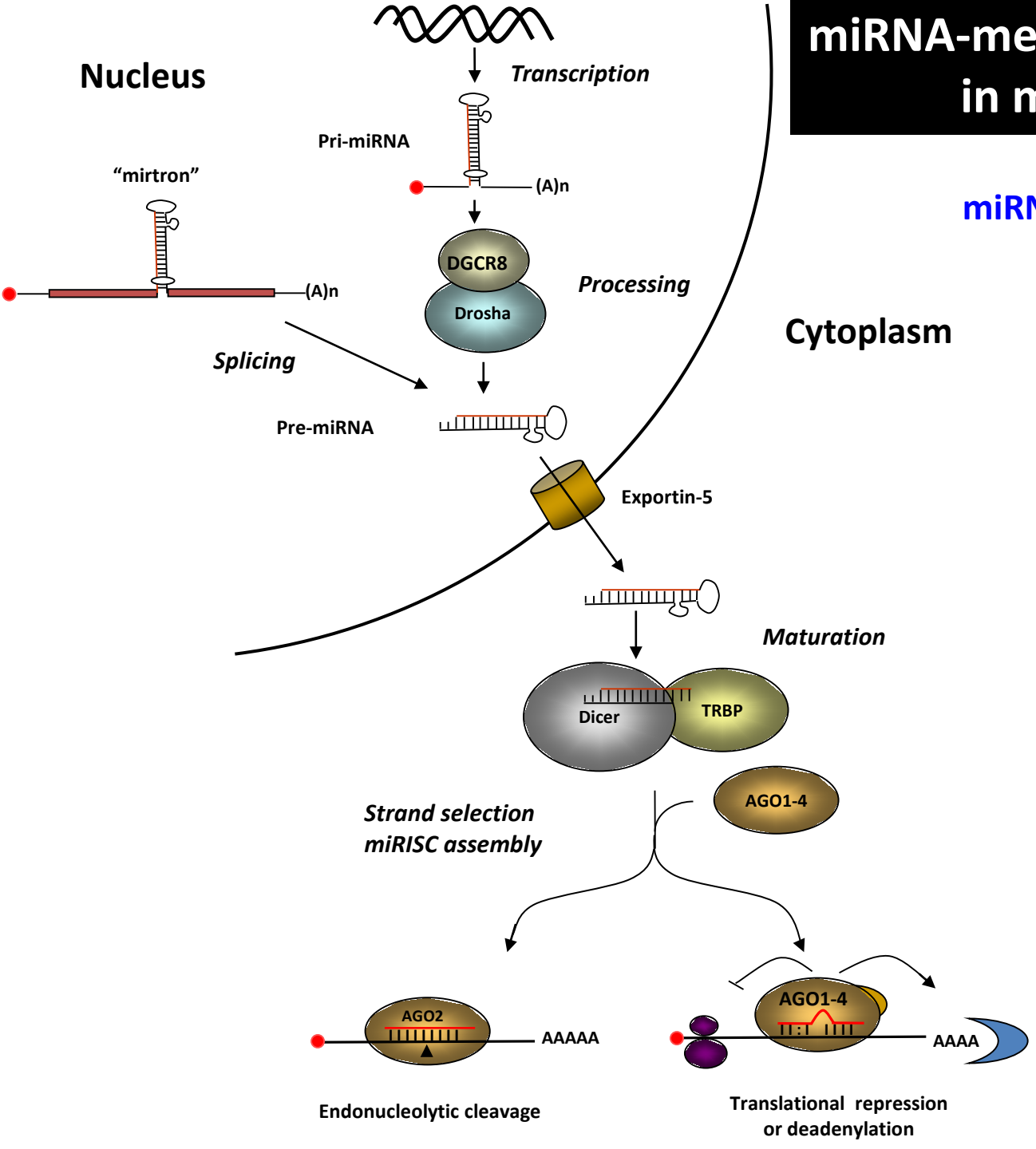
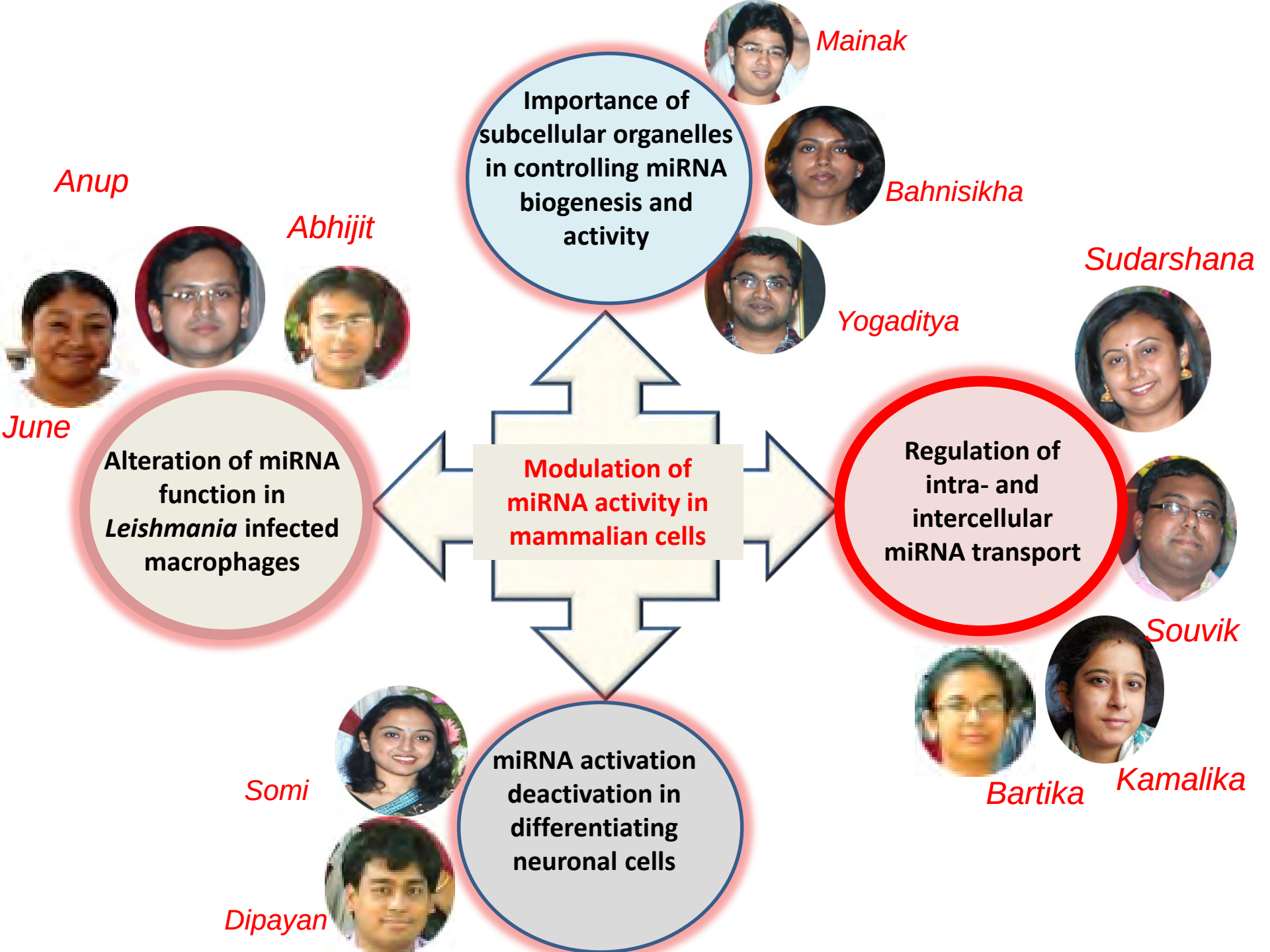
miRNA-mediated gene repression in mammalian cells



miRNA: 22 nt long regulatory RNA

- **>1000 miRNAs may operate in humans**
- **~50% of human genes are predicted to be miRNA regulated**
- **Regulate development, differentiation, apoptosis, …**
- **Expression disregulated in human diseases**
- **Development- and tissue-specific expression (brain, liver, muscle, …)**

What Factors Controls microRNA Activities in Mammalian Cells?


Mainak
Importance of subcellular organelles in controlling miRNA biogenesis and activity
Bahnisikha
Anup
Abhijit
Yogaditya
Sudarshana
June
Alteration of miRNA function in *Leishmania* infected macrophages
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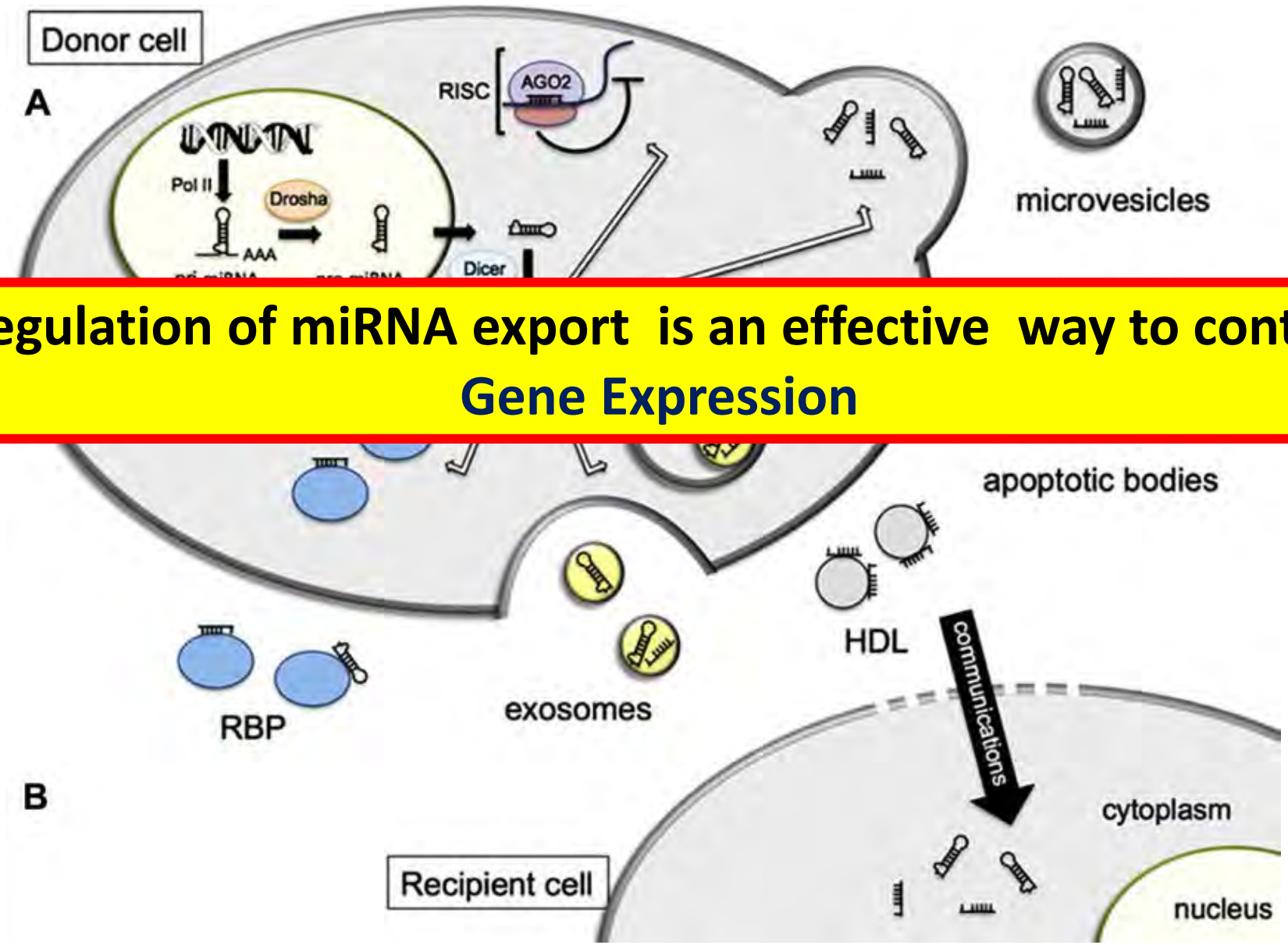
miRNA activation deactivation in differentiating neuronal cells
Bartika
Kamalika
Dipayan

Intercellular transfer of miRNA via Extracellular Vesicles : another way of controlling cellular levels of miRNAs



Regulation of miRNA export is an effective way to control Gene Expression

Regulation of exosomal export of miRNAs

miRNA and anti miRNA signals reciprocate between Normal and Cancer cells: tug of war for proliferation and growth arrest

- Neighbouring cells can transfer miR-122 to arrest growth of hepatoma cells in co-culture.
- IGF1 secreted by HepG2 reduces activity and expression of miR-122 in donor cells.
- Target and donor cells reciprocally regulate each others' growth, proliferation and senescence.

7170–7185 Nucleic Acids Research, 2014, Vol. 42, No. 11 Published online 09 May 2014
doi: 10.1093/nar/gku346

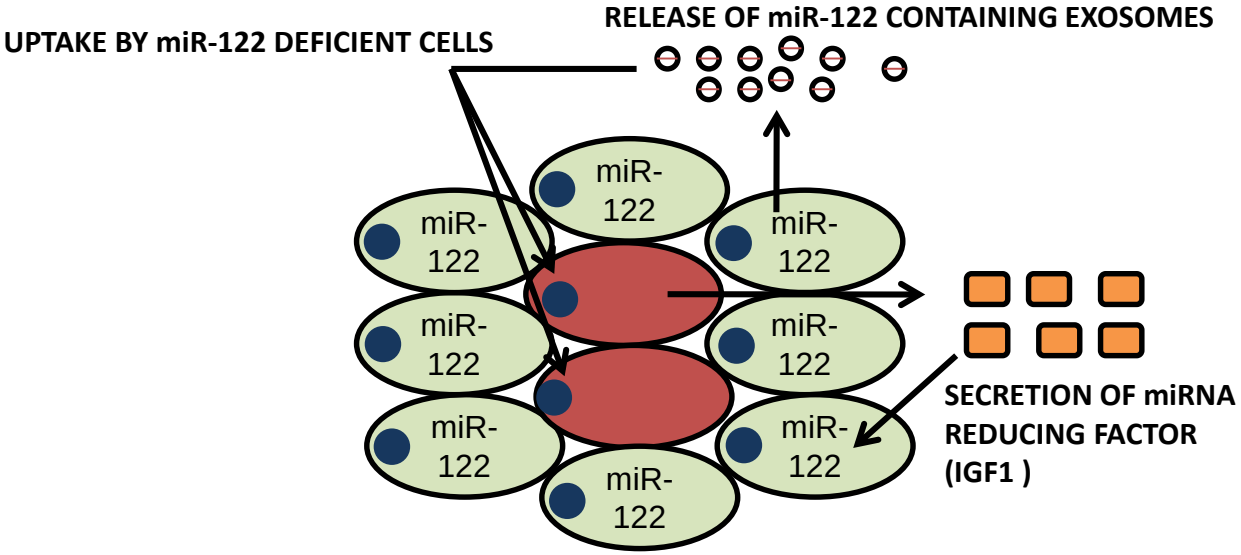
Insulin-like growth factor-1 prevents miR-122 production in neighbouring cells to curtail its intercellular transfer to ensure proliferation of human hepatoma cells

Sudarshana Basu and Suvendra N. Bhattacharyya*

RNA Biology Research Laboratory, Molecular and Human Genetics Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India

Received July 1, 2013; Revised April 02, 2014; Accepted April 11, 2014

Sudarshana



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MOLECULAR BIOLOGY OF THE CELL

MBoC | ARTICLE

Polysome arrest restricts miRNA turnover by preventing exosomal export of miRNA in growth-retarded mammalian cells

Souvik Ghosh, Mainak Bose, Anirban Ray, and Suvendra N. Bhattacharyya
RNA Biology Research Laboratory, Molecular and Human Genetics Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India

Souvik

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You are here: Home Frontier Science Awardees Articles Cell density regulates turnover of microRNAs

Cell density regulates turnover of microRNAs

Mature microRNAs (miRNAs) are stabilized in growth-retarded mammalian cells owing to increased sequestration with poly ribosomes (polysome) which results in increased levels of functionally inactive miRNPs. Polysomal arrest also leads to reduced export of these miRNAs via exosomes thereby restricting turnover of these regulatory molecules.

HFSP Career Development Award holder Suvendra Bhattacharyya and colleagues
authored on Tue, 19 May 2015

Awardees' articles

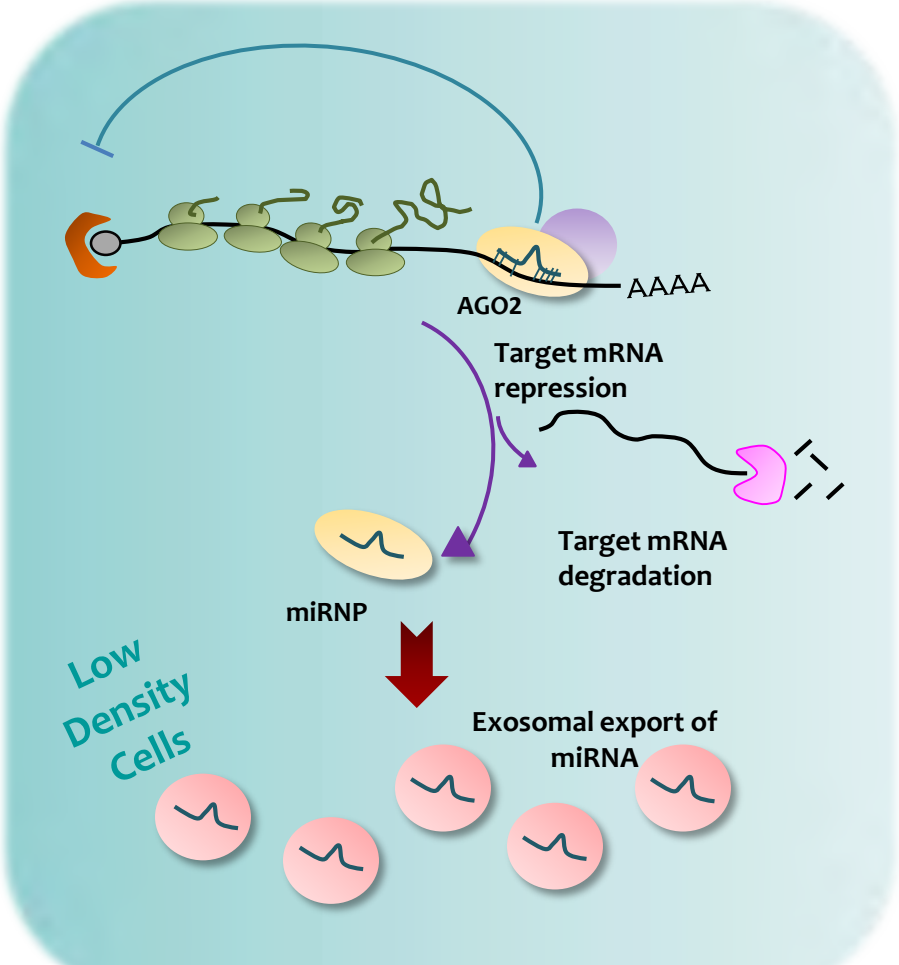
Cell density regulates turnover of microRNAs
Mature microRNAs (miRNAs) are stabilized in...

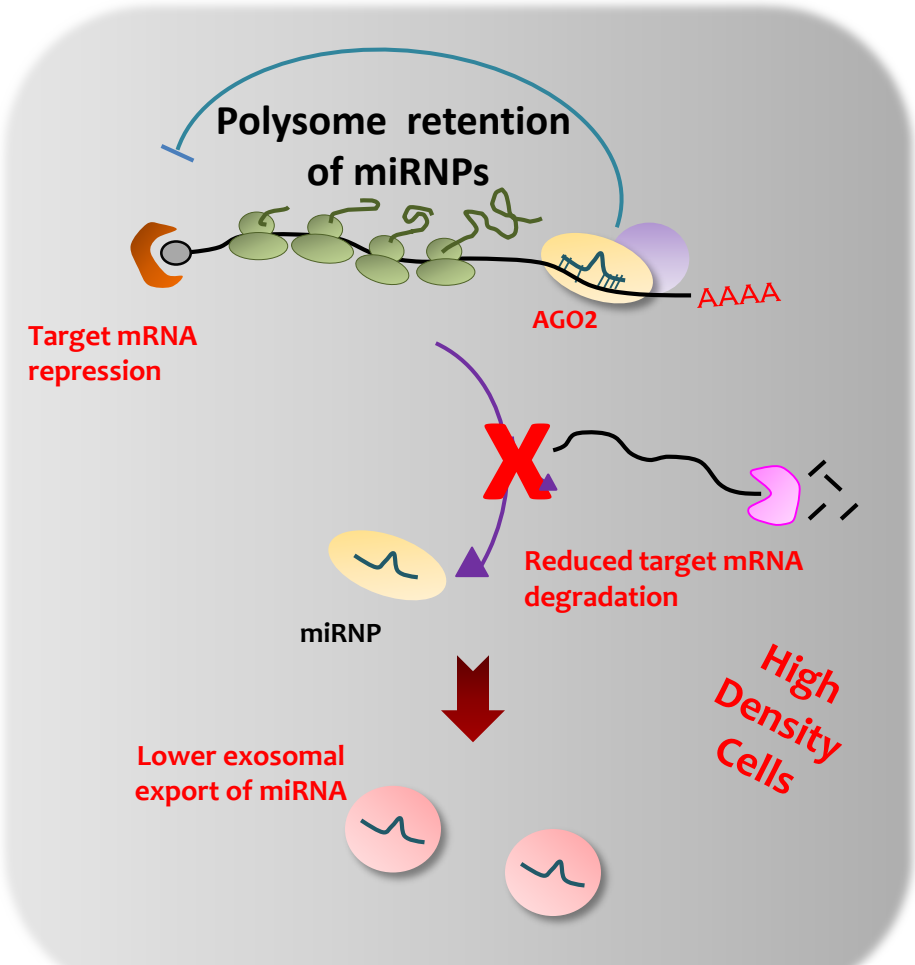
How do proteins tie a knot?
Polypeptide chains, like yarn and headset wires,...

RNA G-quadruplex structure and function in plants
RNA folds into fascinating structures that govern...

Different stability of miRNAs in growth retarded state

- Increased miRNA levels in slow growing /senescent cells
- increased miRNA levels are due to increased miRNA stability and reduced exosomal export
- increased miRNAs are associated with polysomes.

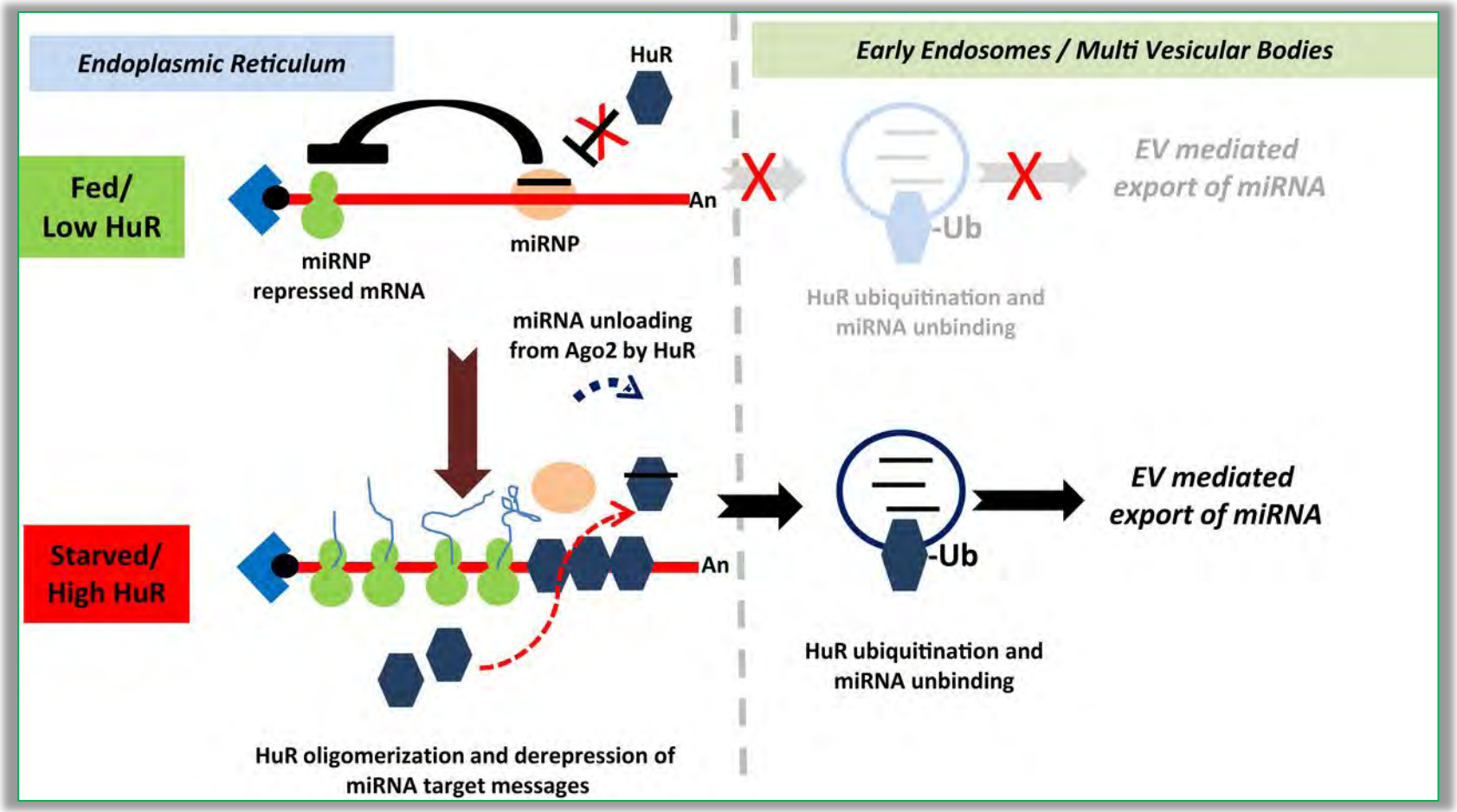




What are the cellular and extracellular factors that control miRNA trafficking between cells?

What is the mechanism of selective export of miRNAs via exosomes?

A possible model of HuR-driven extracellular export of miRNA in human cells



Kamalika Mukherjee

Kamalika Mukherjee et al. EMBO Rep. 2016;17:1184-1203

Published online: July 11, 2016

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

EMBO reports

Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments

This was selected by F-1000 Prime!!!

Shwetha[2], Saumitra Das[2] & Suvendra N Bhattacharyya[1,*]

Abstract

microRNAs (miRNAs), the tiny but stable regulatory RNAs in

The majority of biochemical pathways in humans are miRNA controlled, and human diseases including several forms of cancer are associated with abnormal expression of miRNAs [2–4].

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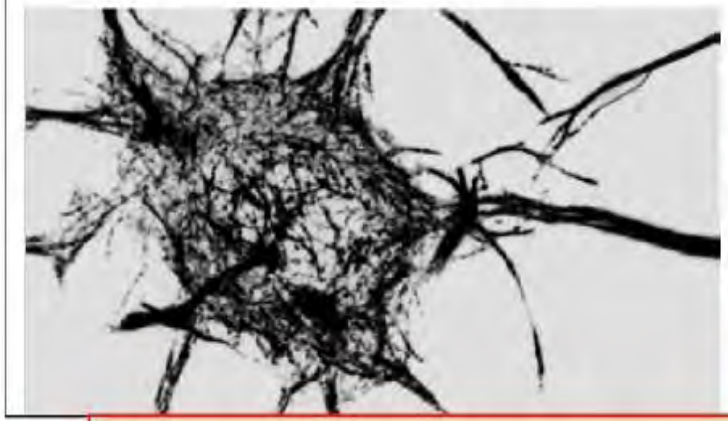


Announcements

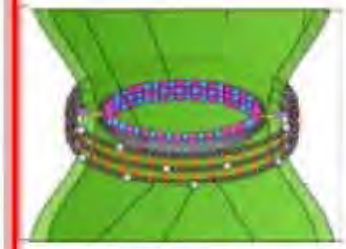
Guidelines for 2017 Young Investigator and Program Grant applications are now available. The website for online applications will...

All announcements

Awardees' Articles

Human liver cells fight stress by shedding off regulatory RNAs

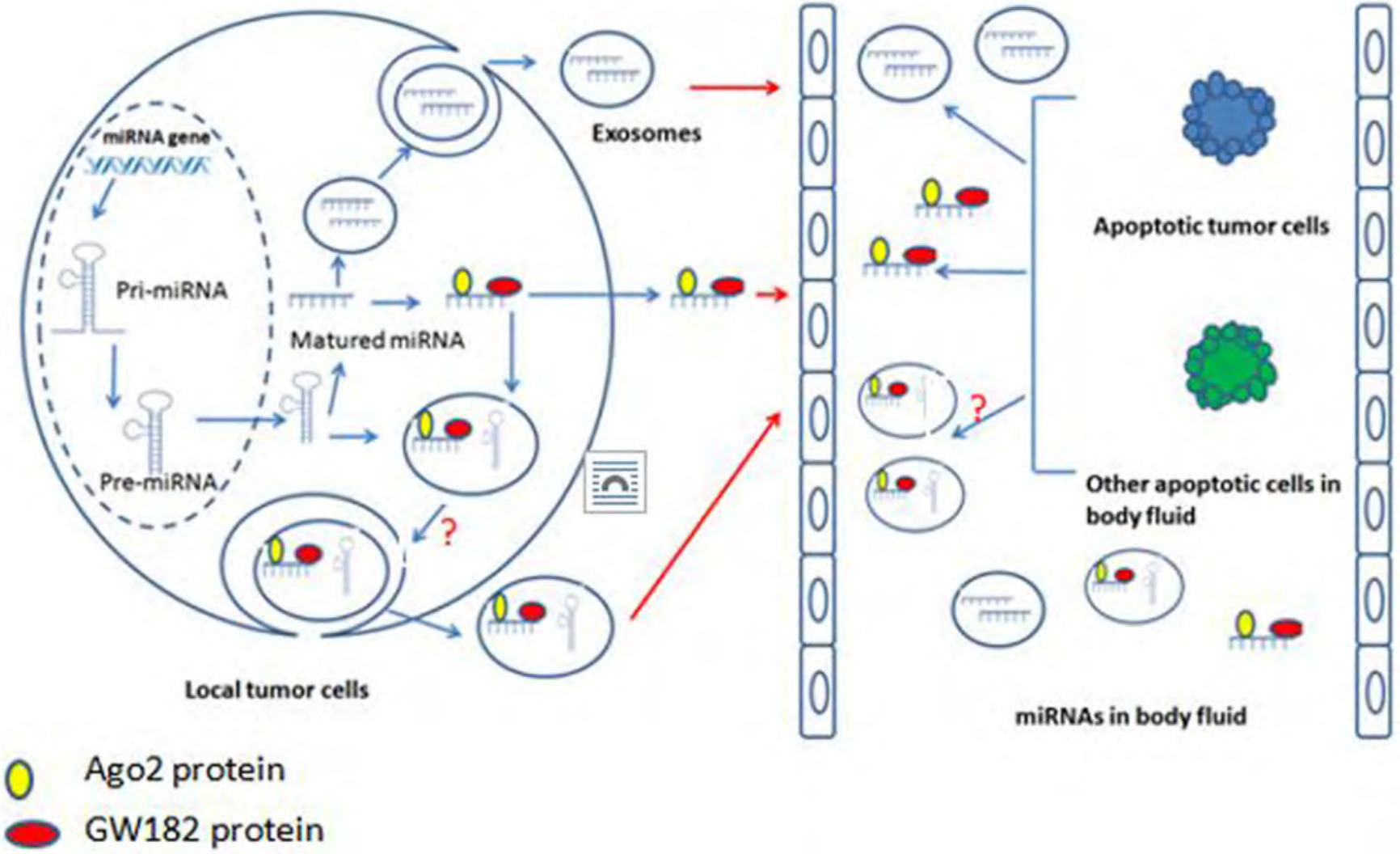
In mammalian cells, gene expression is usually under tight regulation where tiny regulatory RNAs, known as microRNAs, play a...



Reconstitution of the chloroplast FtsZ ring ex vivo

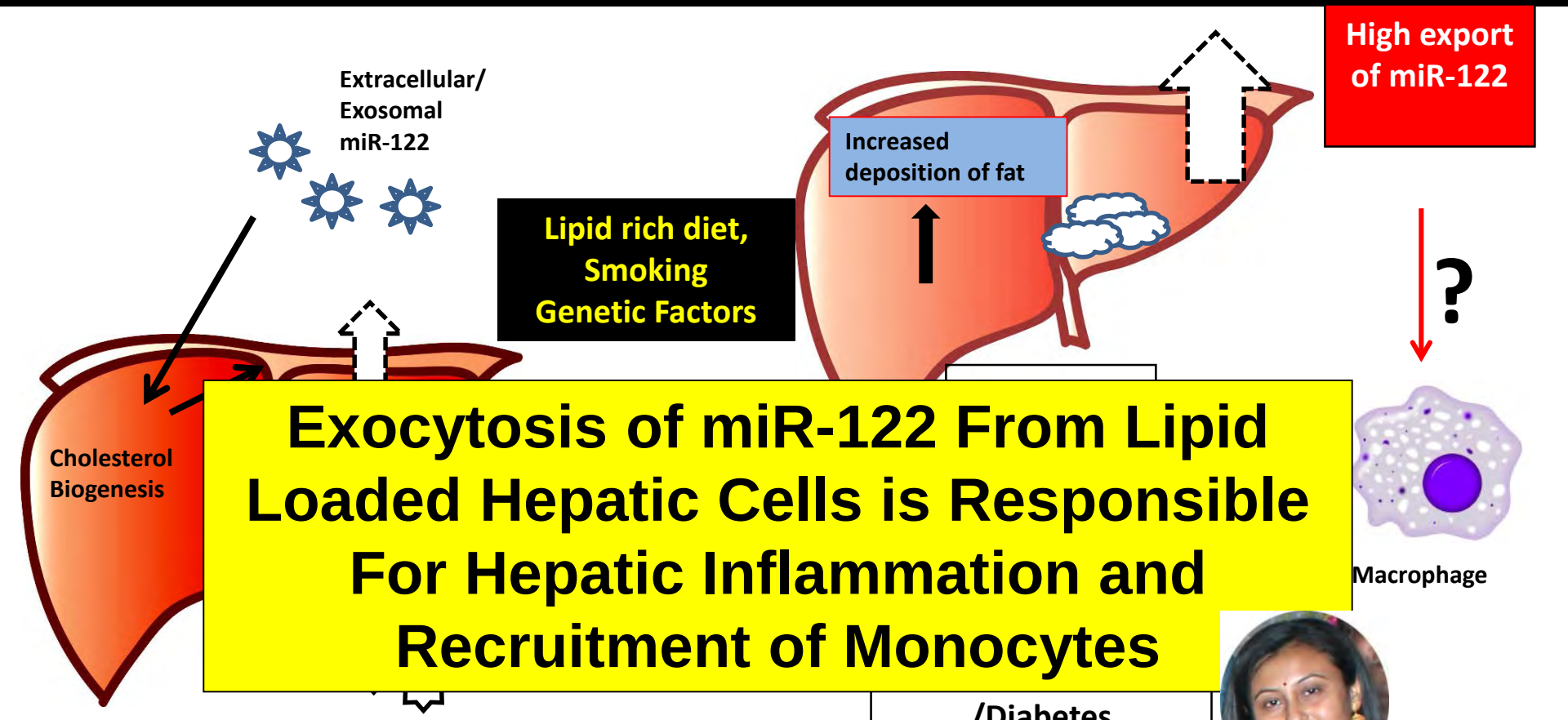
To vacillate is human

Vesicular Hormones: *Inter-organ transfer of signals by exosomal miRNAs*



Ongoing Research Project:

How intercellular transport of miRNA via Exosomes controls lipid metabolism in liver?



Exocytosis of miR-122 From Lipid Loaded Hepatic Cells is Responsible For Hepatic Inflammation and Recruitment of Monocytes

/Diabetes

↓

Hepatosteatosis

↓

Hepatocellular Carcinoma

Sudarshana

Homeostasis of Lipid metabolism

Funded by:

Swarnajayanti Fellowship

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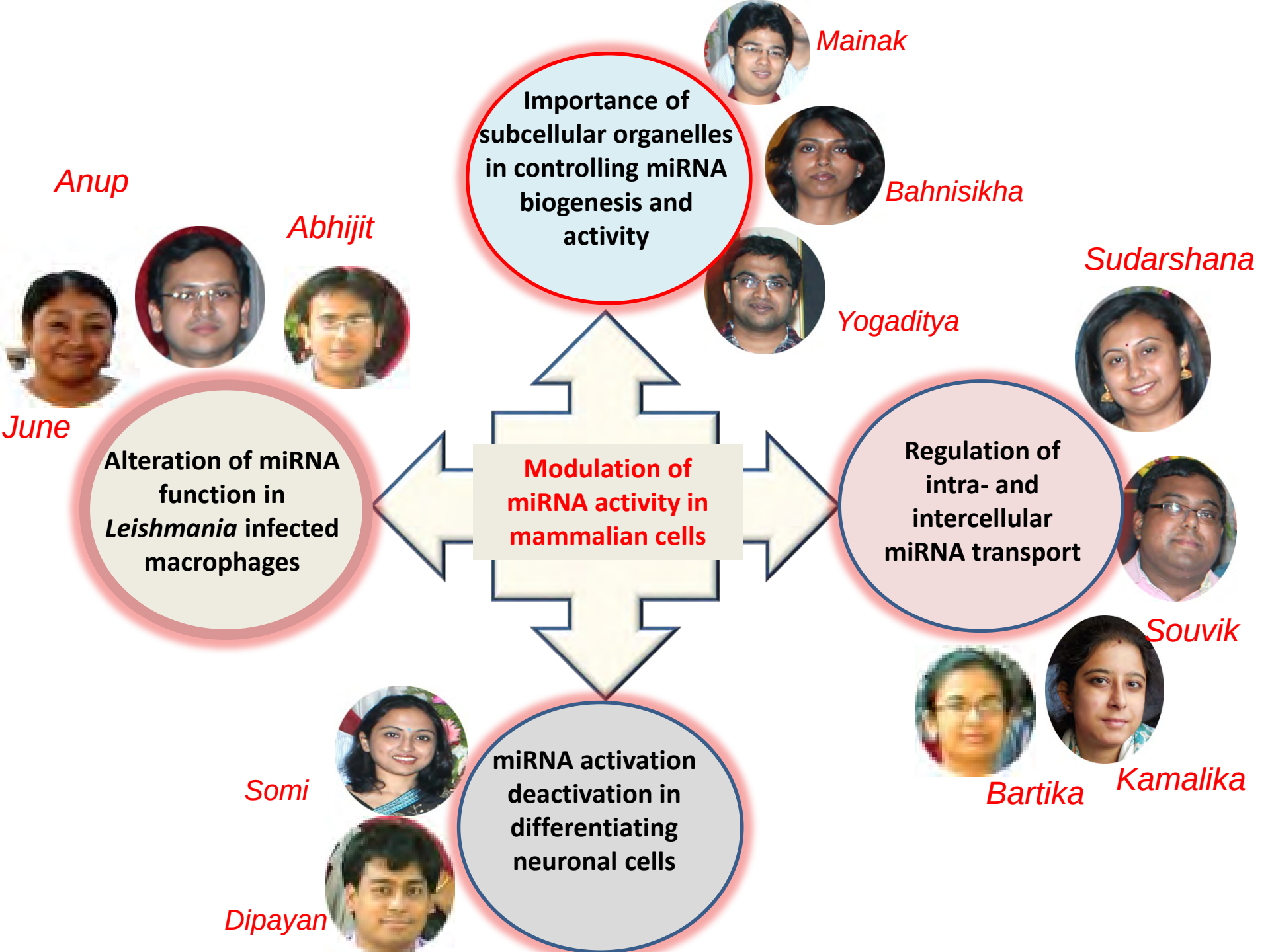
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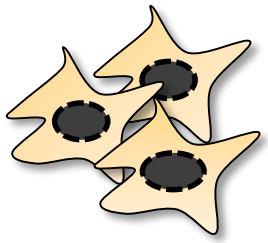
Kamalika
Dipayan

Extensive research over the last decade has convincingly established how miRNAs regulate the fate of target mRNAs. However, the effect of target mRNA on miRNA biology is largely unexplored.

REGULATION OF miRNA ACTIVITY BY TARGET mRNA

Elevation Of miRNA Levels In Presence Of Target mRNA

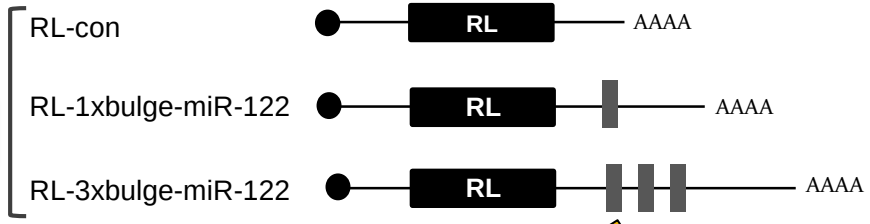


HEK293
(do not express trace amounts of miR-122)

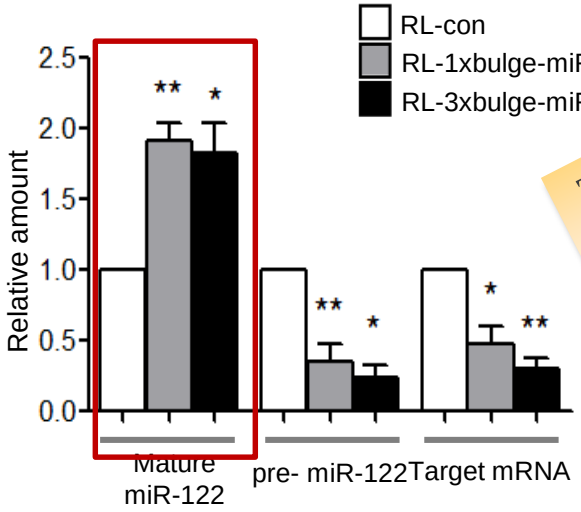
exogenously expressing

miR-122

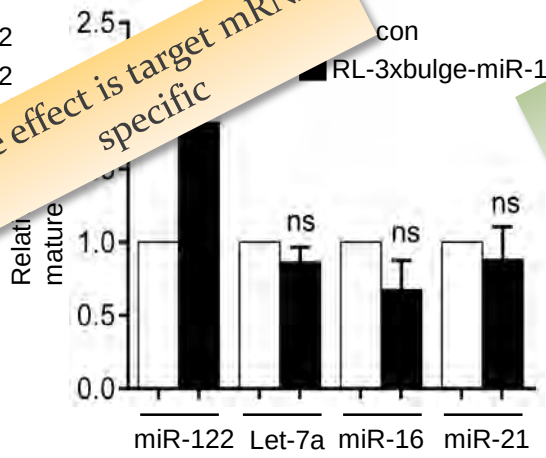
Target mRNA



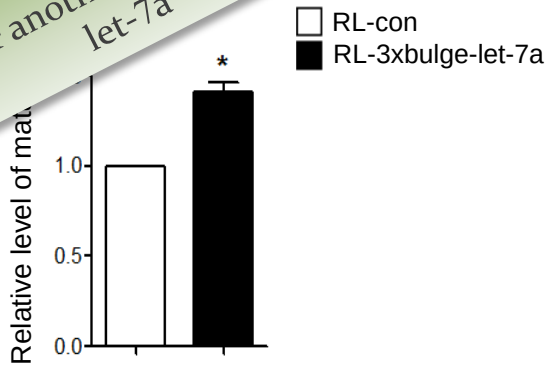

```
                A A C
                A   A
5'    AUACUAUU    CACACUCCA 3'   mRNA
      |:||:|||    |||||||||
3'GUUUGUGGUAACA GUGUGAGGU 5' miR-122
```



The effect is target mRNA specific



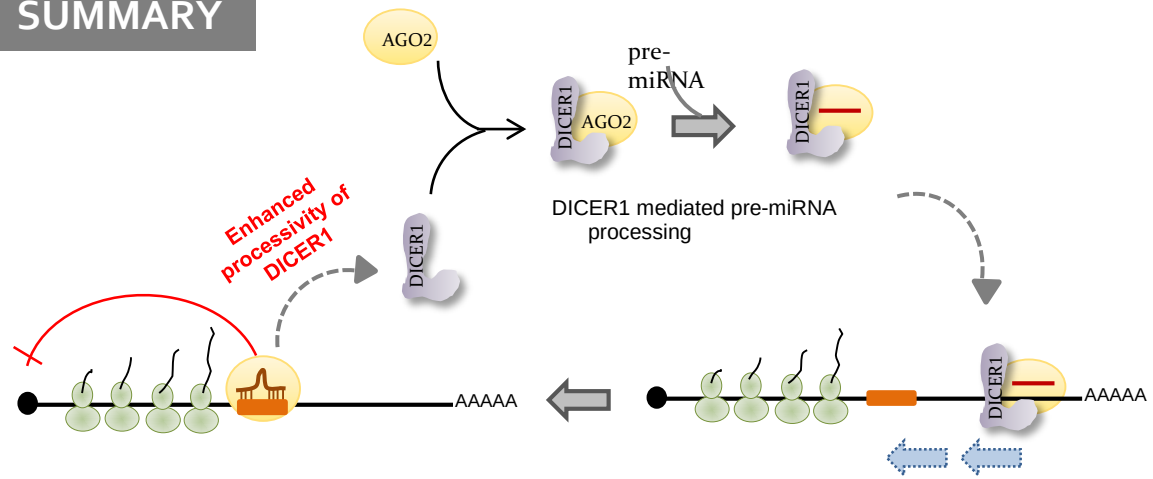
For another miRNA – let-7a



Target Driven Biogenesis of miRNAs in Mammalian Cells

Mainak

SUMMARY



1. miRISC hybridization with target site
2. DICER1 dissociates from AGO2
3. Translation repression by miRISC

1. Assembly of miRLC
2. Scanning of miRISC-DICER1 complex along 3'UTR

Key Findings

- **Presence of target mRNA enhances miRNA biogenesis**

- **Increased activity of Ago2 associated Dicer1 causes enhanced miRNA production**

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nature COMMUNICATIONS

ARTICLE

Received 14 Dec 2015 | Accepted 10 Jun 2016 | Published 22 Jul 2016

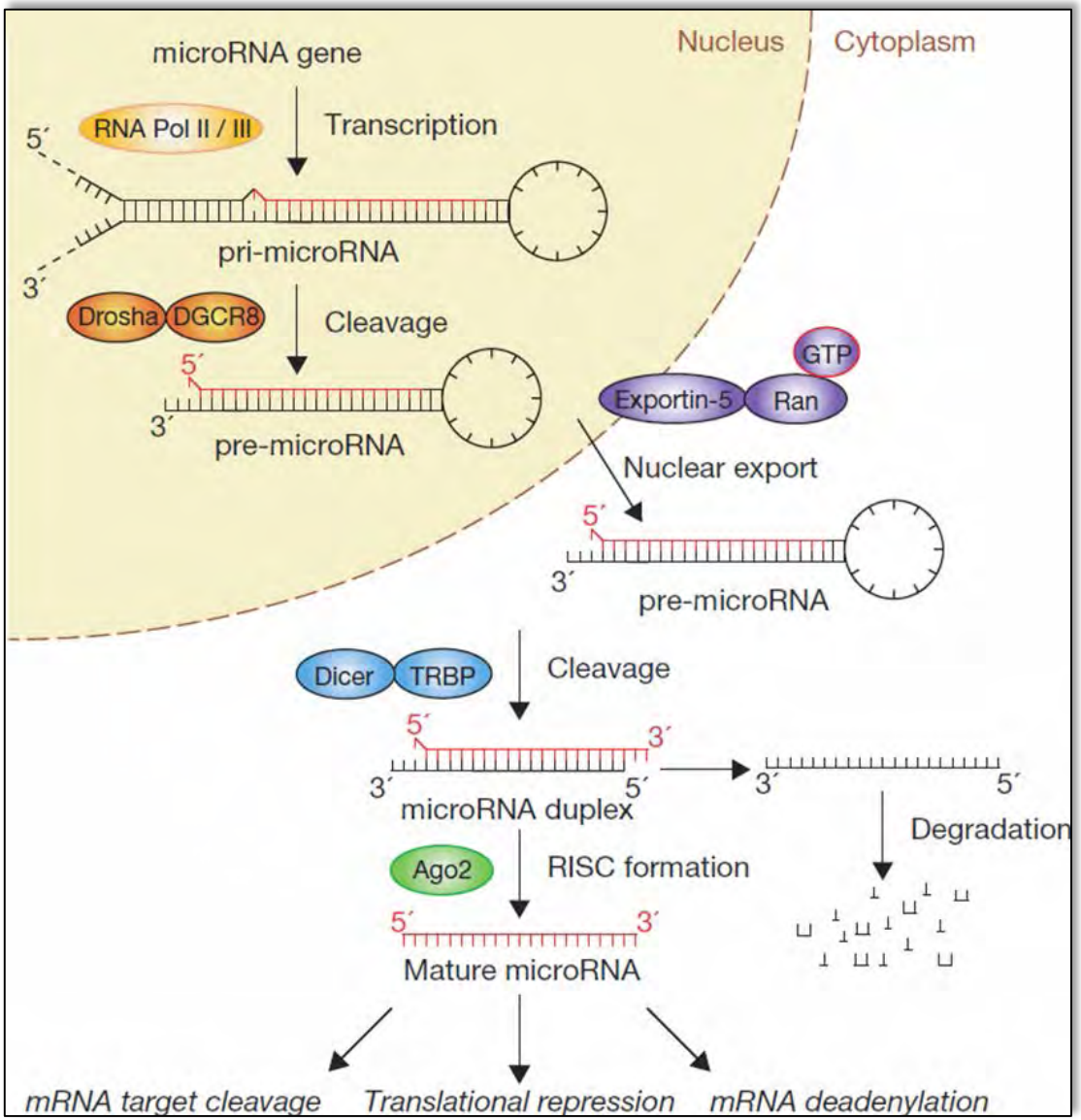
DOI: 10.1038/ncomms12200 OPEN

Target-dependent biogenesis of cognate microRNAs in human cells

Mainak Bose[1] & Suvendra N. Bhattacharyya[1]

Extensive research has established how miRNAs regulate target mRNAs by translation repression and/or endonucleolytic degradation in metazoans. However, information related to the effect of target mRNA on biogenesis and stability of corresponding miRNAs in animals is limited. Here we report regulated biogenesis of cognate miRNAs by their target mRNAs. Enhanced pre-miRNA processing by AGO-associated DICER1 contributes to this increased miRNP formation. The processed miRNAs are loaded onto AGO2 to form functionally competent miRISCs both *in vivo* and also in a cell-free *in vitro* system. Thus, we identify an additional layer of posttranscriptional regulation that helps the cell to maintain requisite levels of mature forms of respective miRNAs by modulating their processing in a target-dependent manner, a process happening for miR-122 during stress reversal in human hepatic cells.

Cell biology of miRNA-mediated repression



- **Where the miRNAs act on their targets?**
- **Does translation repression and degradation occurs simultaneously and where?**

Nat. Cell Biol. **2009**

Polysome association of target mRNA on Endoplasmic Reticulum precedes AGO2 interaction and translation repression

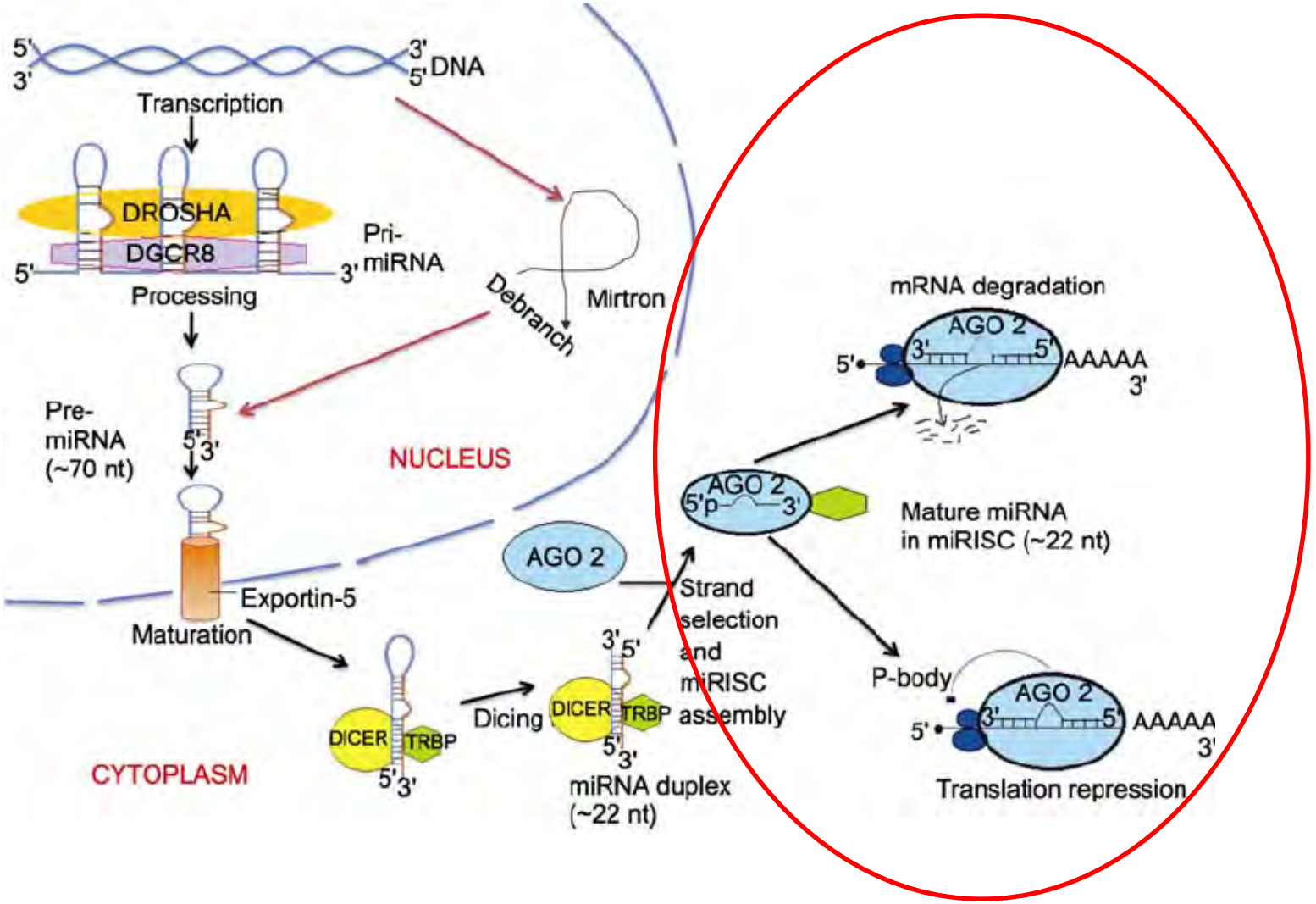
Bahnisikha

Key Findings

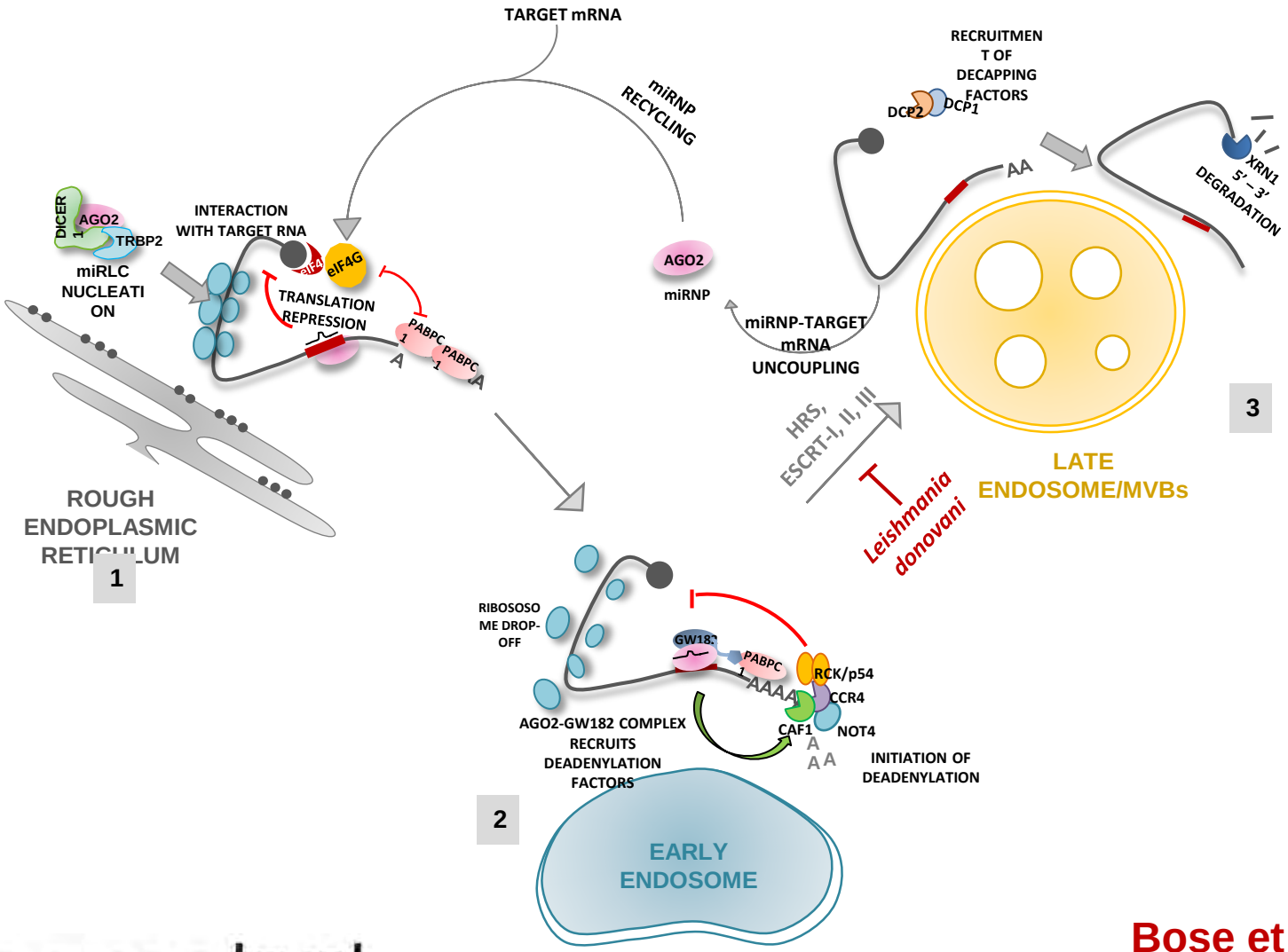
- **miRNPS and target messages are enriched on ER bound polysomes**
- **mRNA get associted with ER bound polysome before interacting Ago2**
- **Ago2 interaction and repression happens on ER**

Barman and Bhattacharyya, *J. Biol. Chem (2015)*

Where does the repression and degradation of target mRNA happen



Spatio-Temporal Uncoupling of MicroRNA-mediated Translational Repression and Target RNA Degradation in Mammalian Cells



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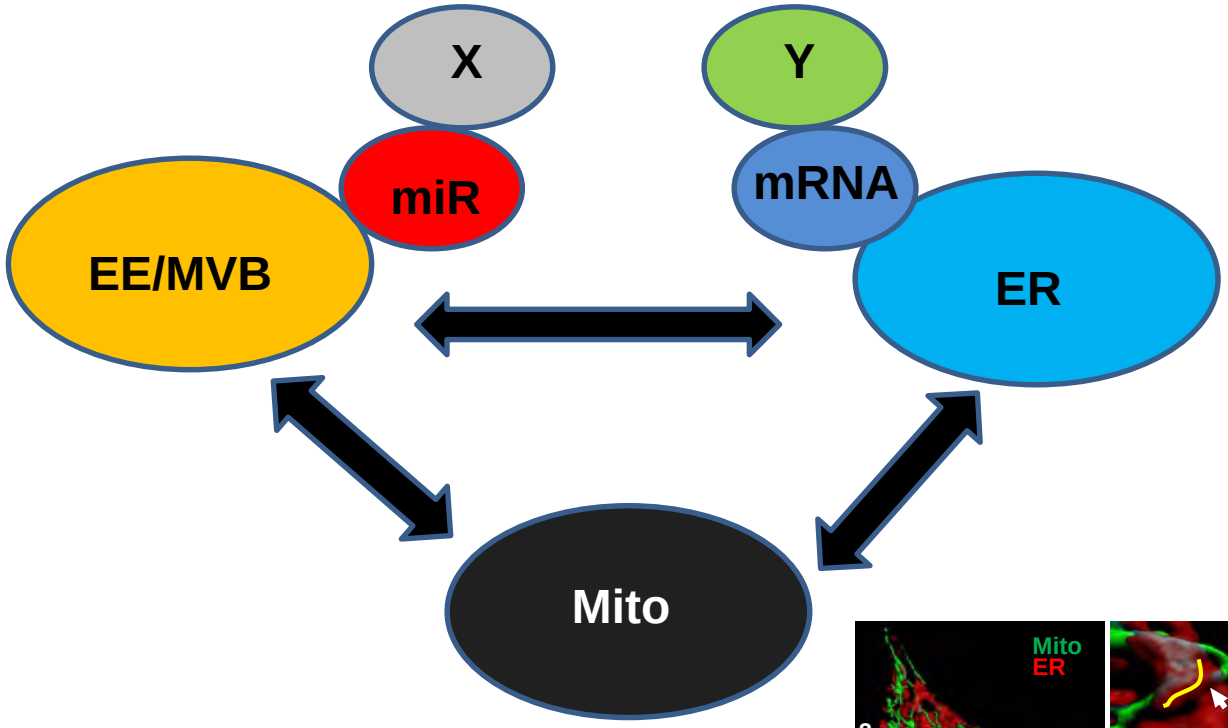
Bose et al, 2016,
Mol. Cell. Biol.

Key questions

- **How organeller dynamics affects the miRNA compartmentalization and activity?**
- **How compartmentalization ensure co-operativity in translation repression process?**

Inter-organelle Interaction Decides Gene Expression



Gene Expression

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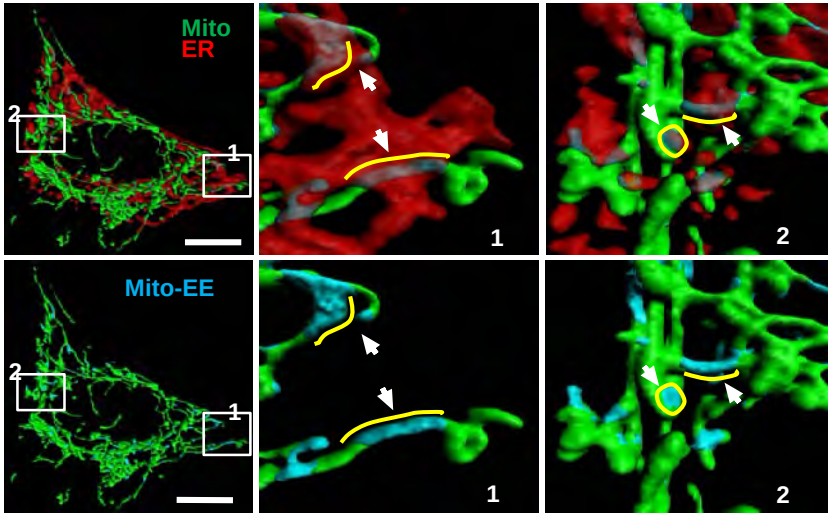
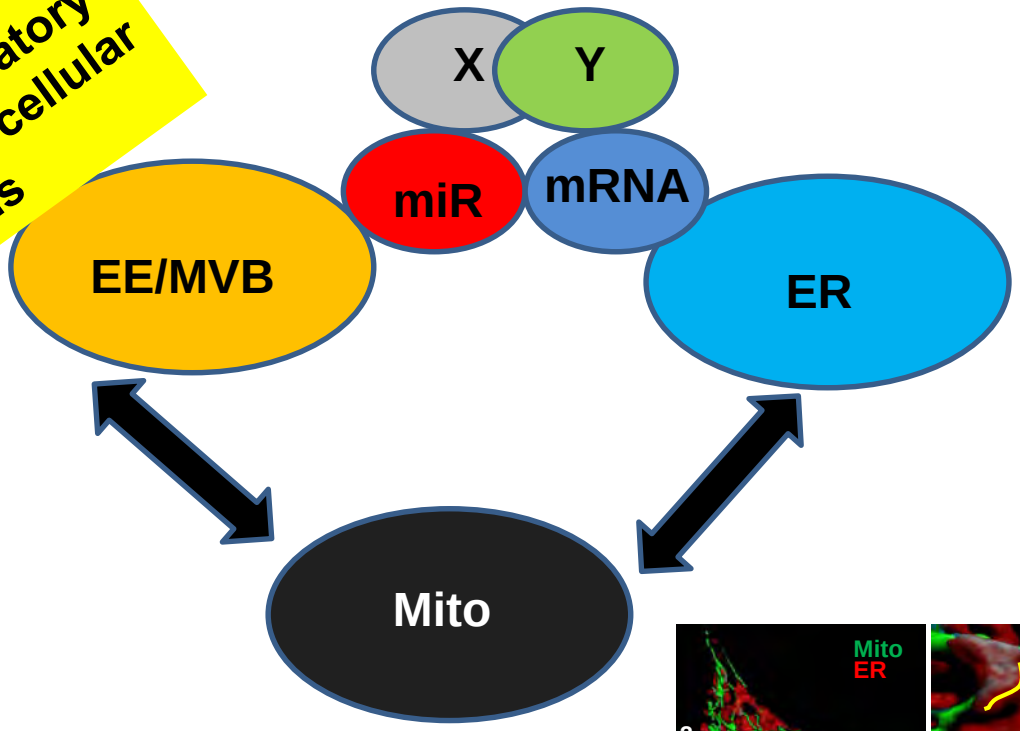
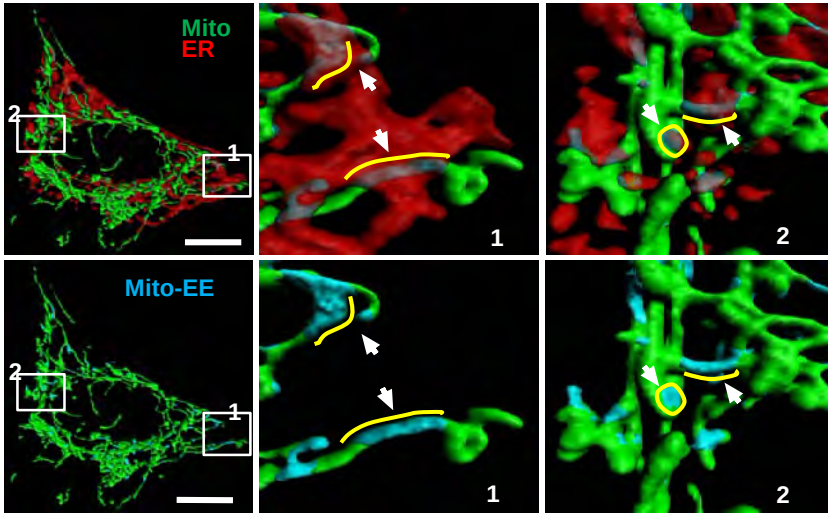


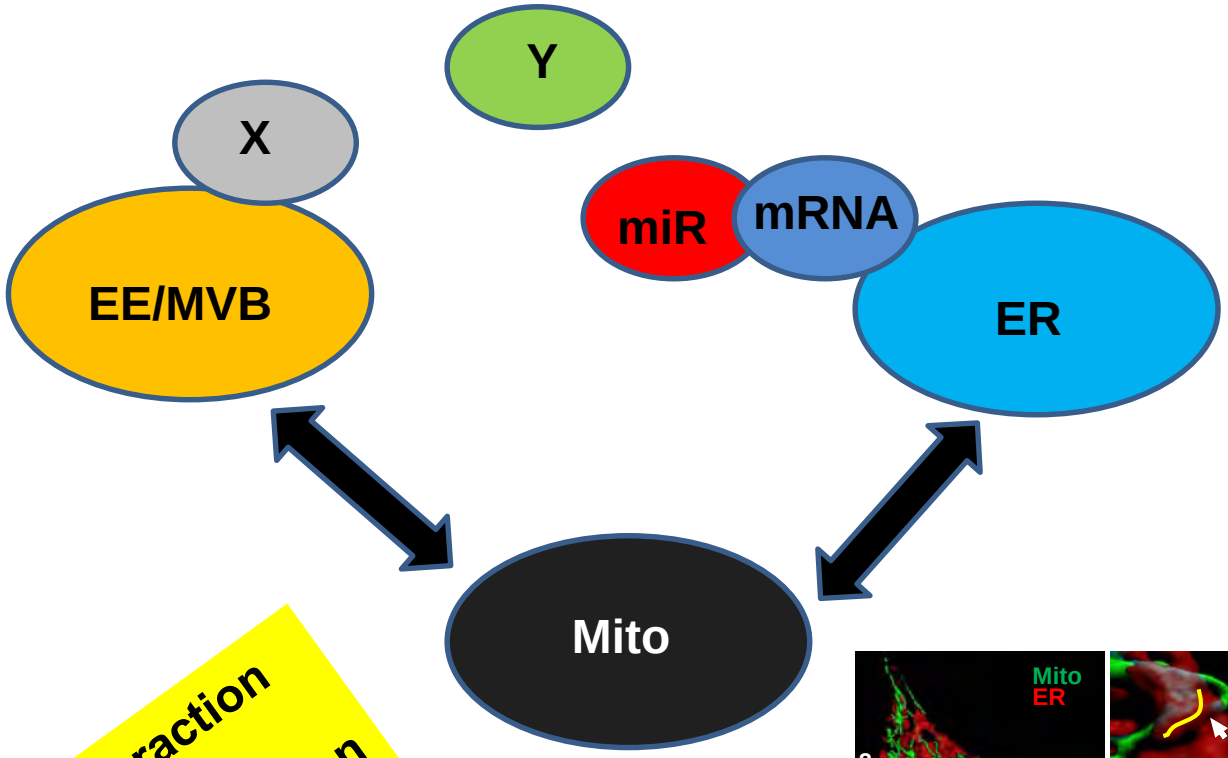
Inter-organelle Interaction Decide Gene Expression

Organeller interaction
Is essential for
exchange of regulatory
factors between cellular
domains



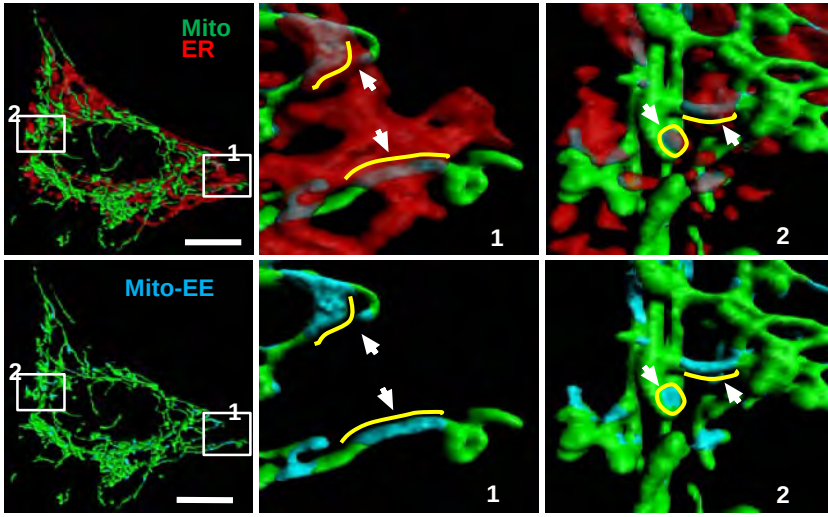


Inter-organelle Interaction Decide Gene Expression



Gene Expression

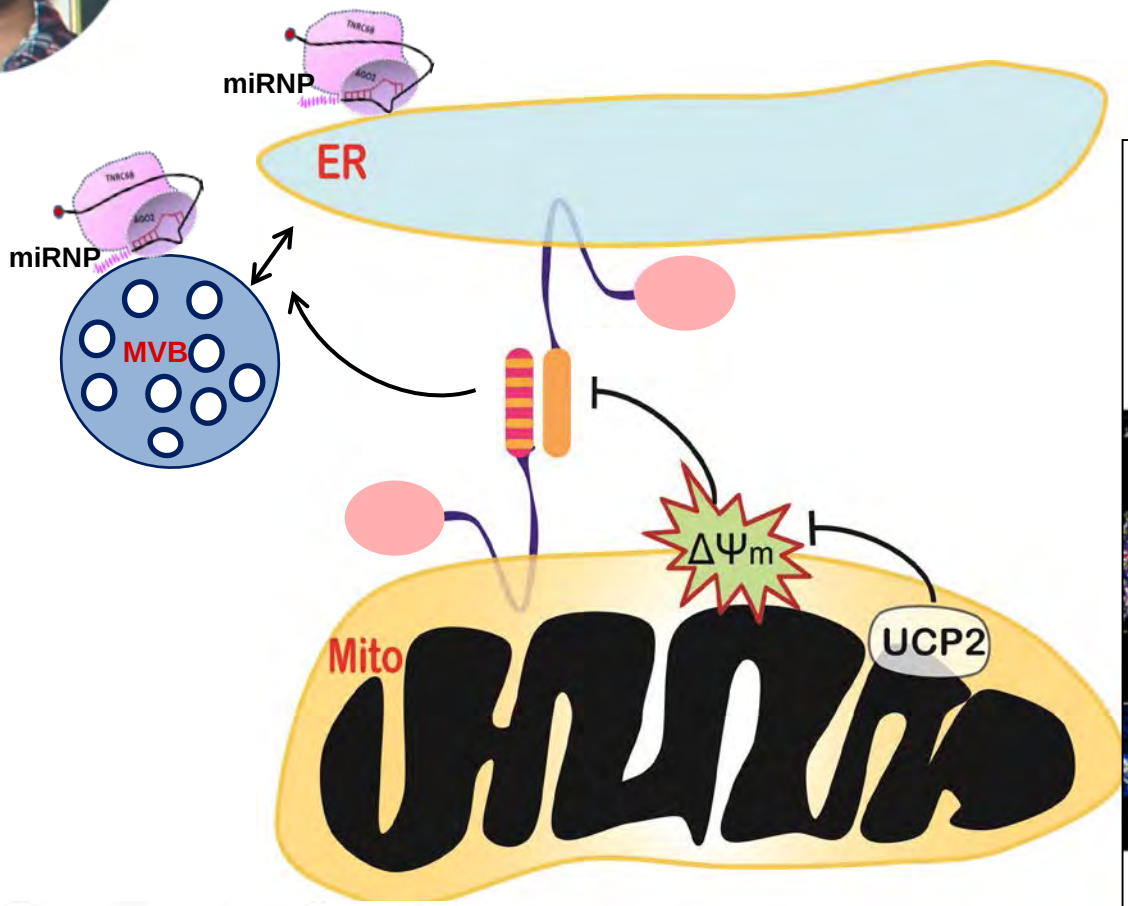
Organeller interaction is essential for Effective gene regulation

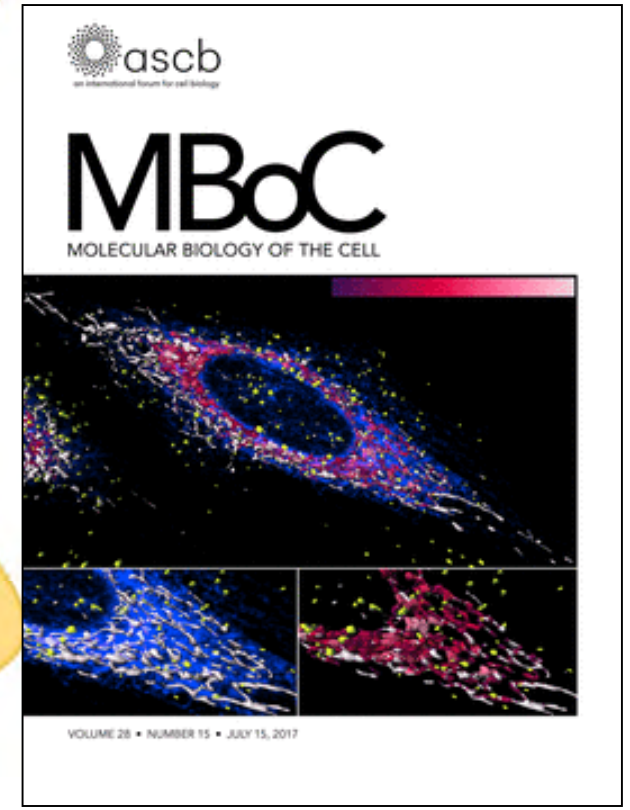


Leishmania donovani restricts mitochondrial dynamics to enhance miRNP stability and target RNA repression in host macrophages

Yogaditya

Yogaditya Chakrabarty and Suvendra N. Bhattacharyya*
RNA Biology Research Laboratories, Molecular Genetics Division, CSIR–Indian Institute of Chemical Biology, Kolkata 700032, India





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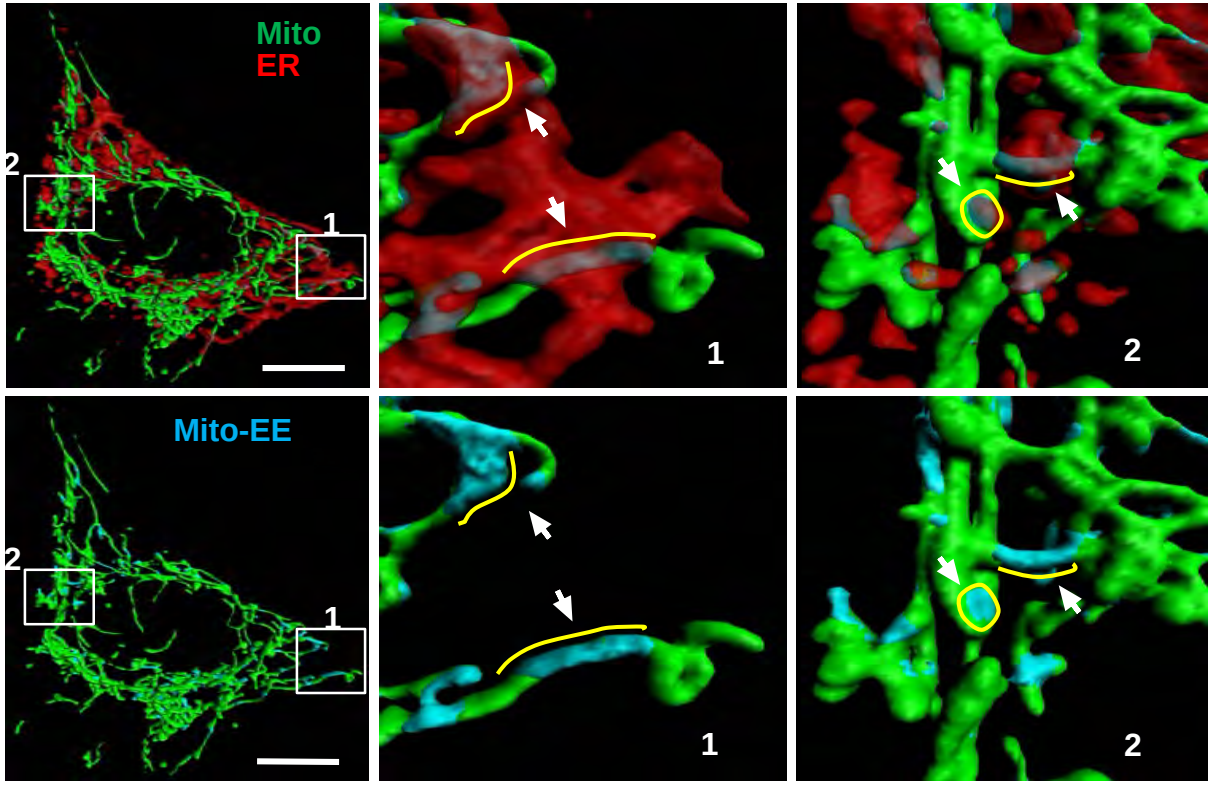
The Unknown:



Department of Sciences & Technology
Government of India

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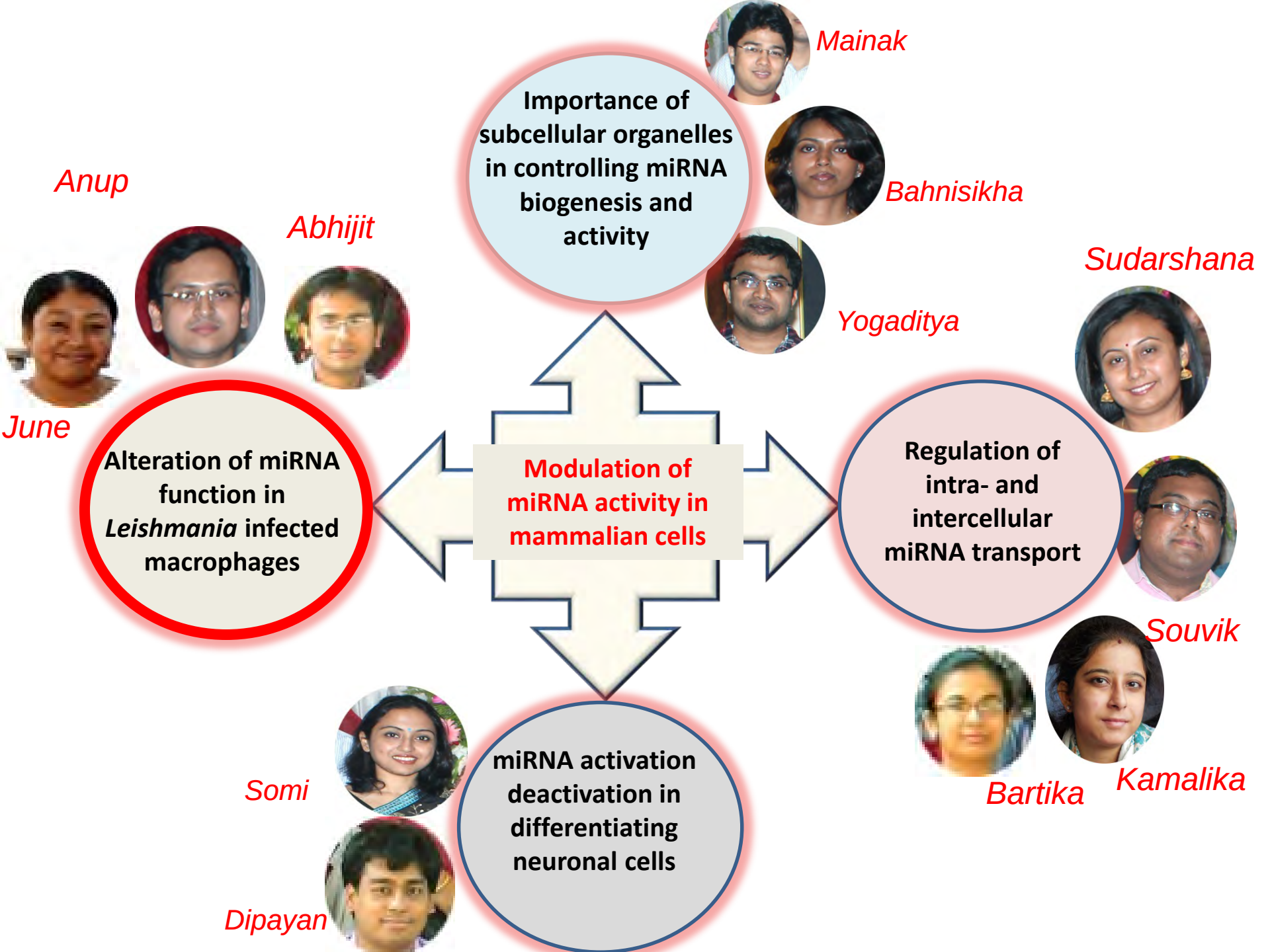
Compartmentalization mechanism of post-transcriptional gene regulatory machineries in animal cells and its connection disease process. How, Why and Where?


Mainak
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Bahnisikha
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Souvik
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miRNA activation deactivation in differentiating neuronal cells
Bartika
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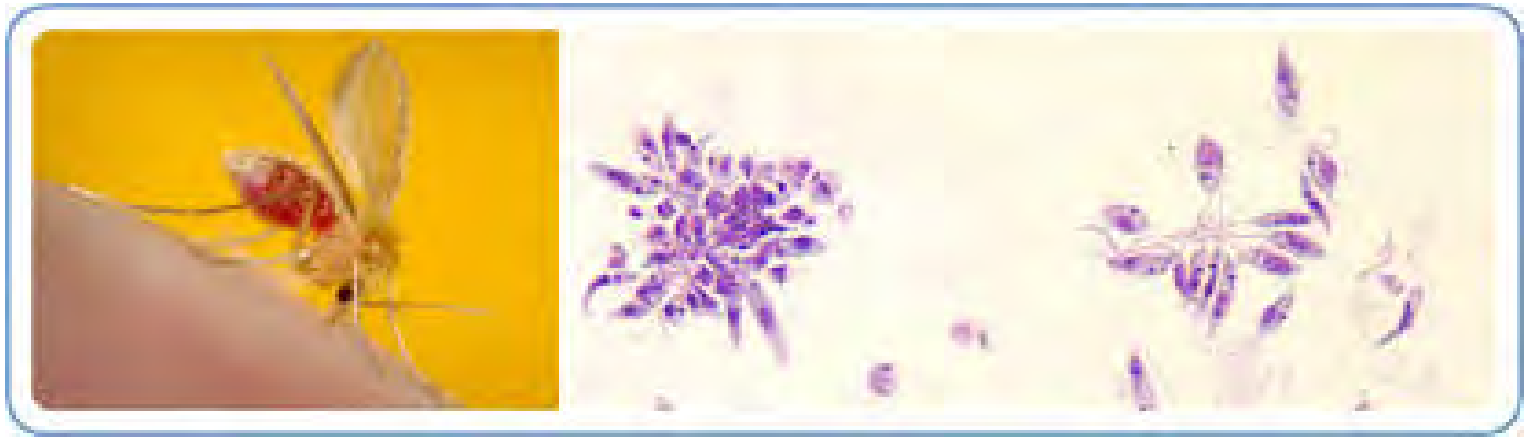
Dipayan

What is the role of miRNAs in modulation of host-pathogen interaction?

Use of *Leishmania*-macrophage interaction as a working model



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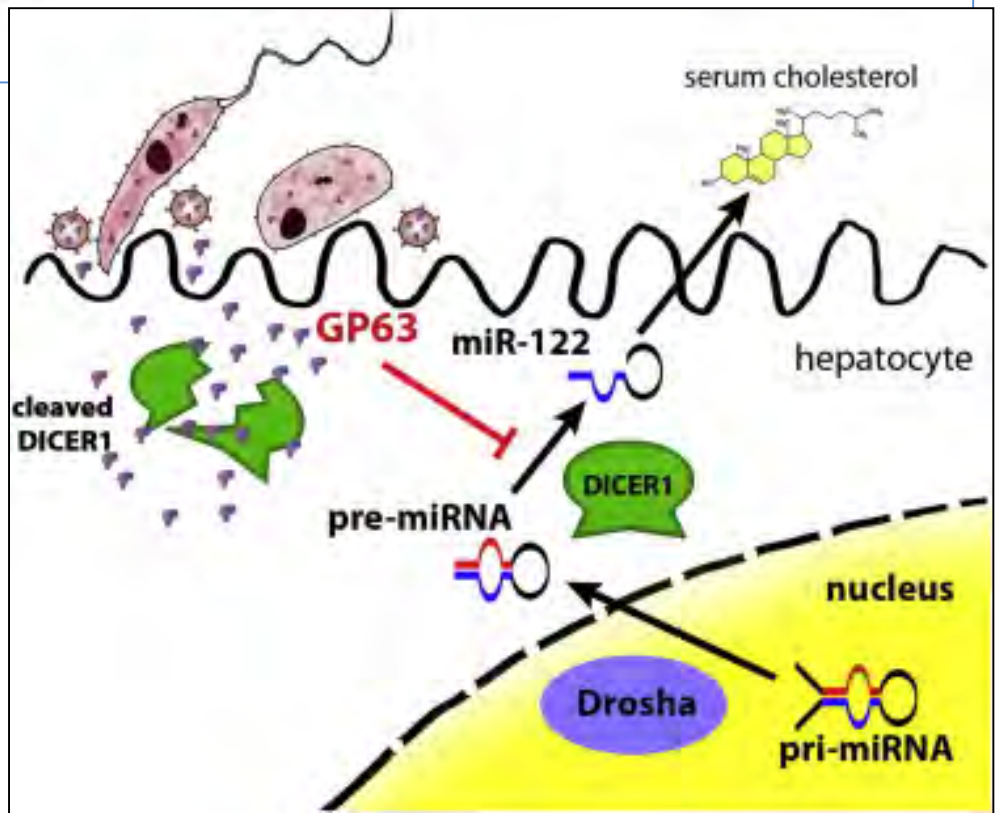
Cell Host & Microbe
Article

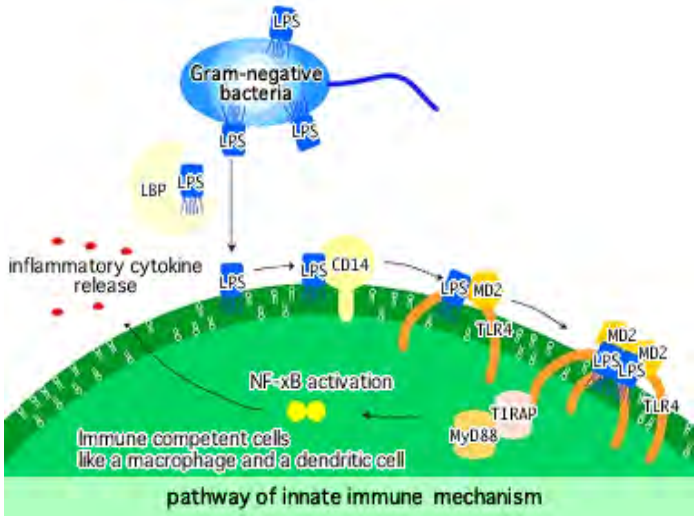
Leishmania donovani Targets Dicer1 to Downregulate miR-122, Lower Serum Cholesterol, and Facilitate Murine Liver Infection

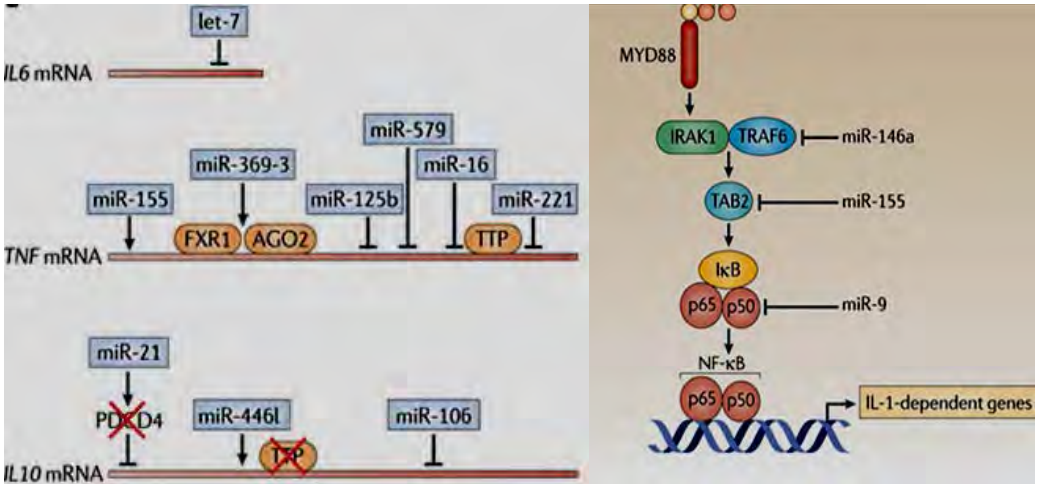
June Ghosh,[1,2] Mainak Bose,[1] Syamal Roy,[2] and Suvendra N. Bhattacharyya[1,*]
[1]RNA Biology Research Laboratory, Molecular and Human Genetics Division
[2]Infectious Diseases and Immunology Division
CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India
*Correspondence: sb@csiriicb.in
http://dx.doi.org/10.1016/j.chom.2013.02.005



How proinflammatory /anti-inflammatory response of macrophage is regulated by miRNAs?

miRNA and Proinflammatory Response





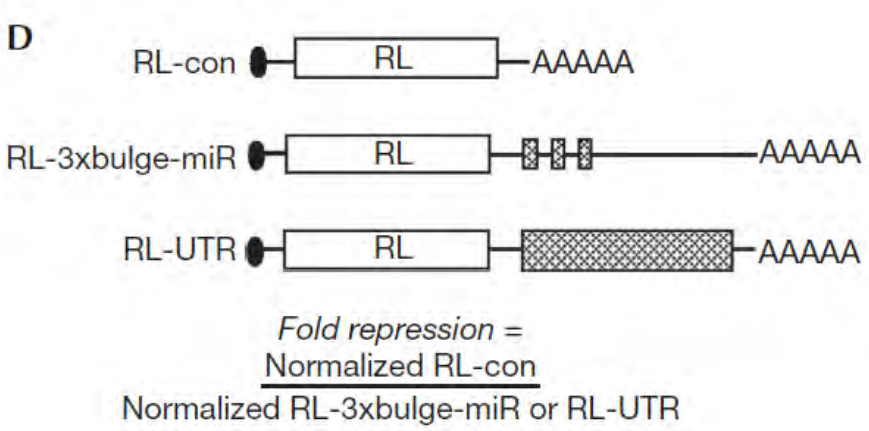
Luke A. O'neill *et. al* Nat Rev Immunnol, 2011

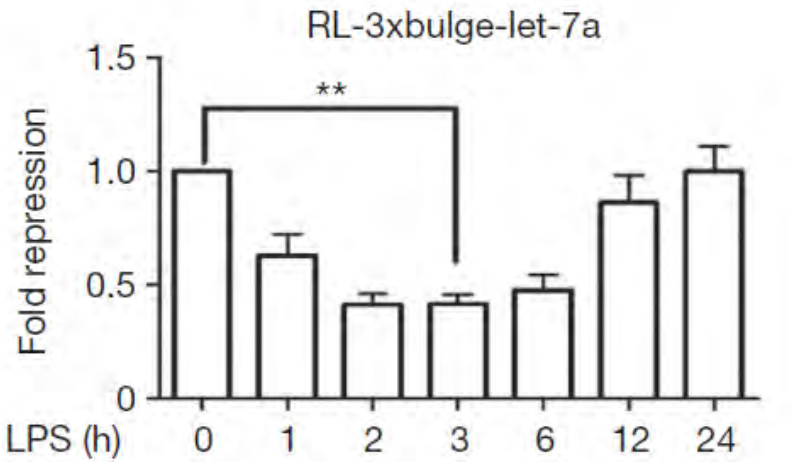
❖ **LPS is a ligand for Toll-Like Receptor 4 (TLR4) and can activate macrophage to induce pro-inflammatory immune response.**

❖ **Most of the cytokines are regulated by different miRNAs**

❖ **But how the cytokine mRNAs become immuned to miRNAs in activated macrophages is unknown!**

LPS induced reversal of miRNA action in activated macrophage

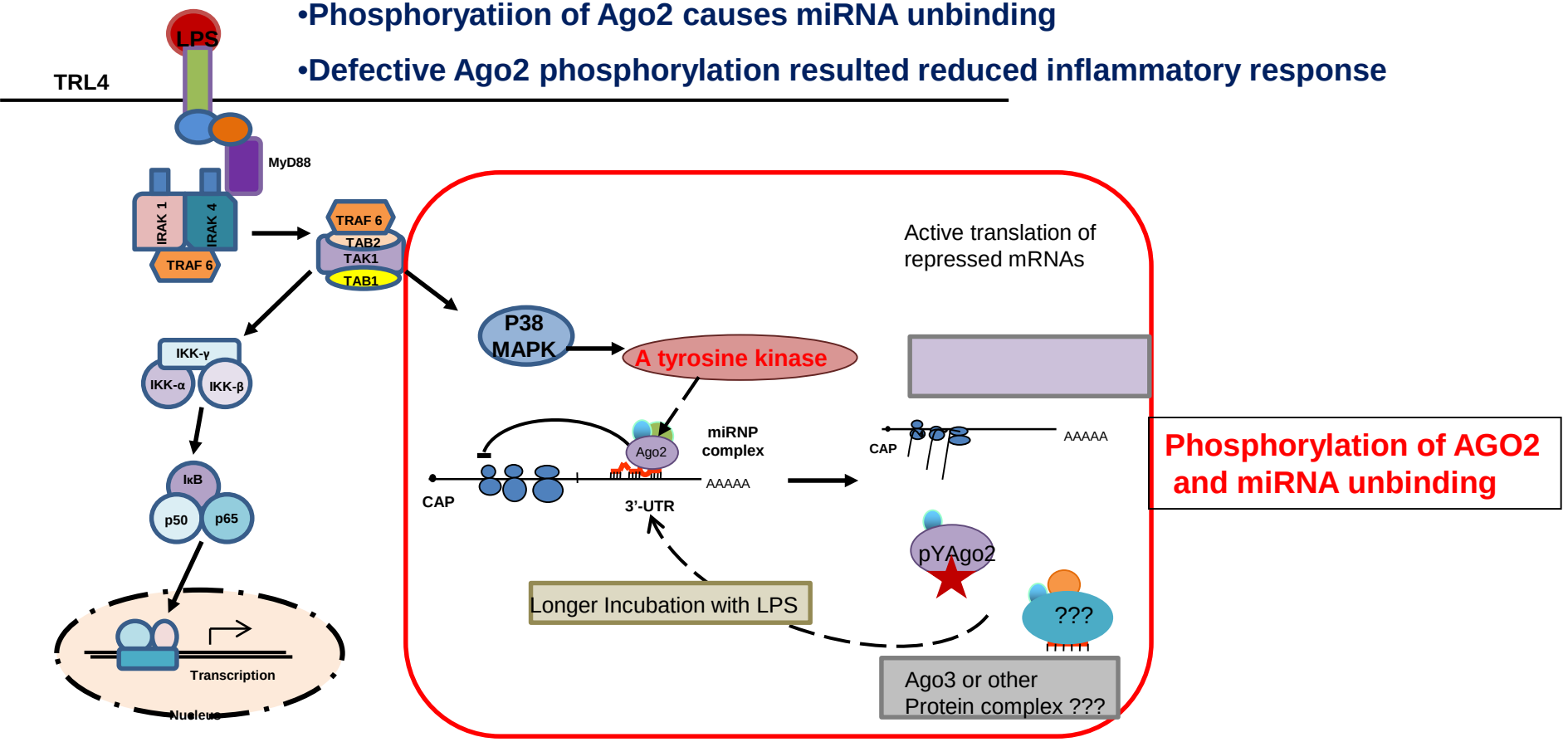




- **With LPS stimulation, reversal of miRNA mediated repression**

Transient Derepression of miRNA mediated repression in activated macrophage

- LPS treatment derepress miRNA activity in macrophage
- Loss of miRNA from Ago2-miRNA complexes resulted derepression
- Phosphoryatiion of Ago2 causes miRNA unbinding
- Defective Ago2 phosphorylation resulted reduced inflammatory response



Phosphorylation of AGO2 and miRNA unbinding


EMBO reports

scientific report

A transient reversal of miRNA-mediated repression controls macrophage activation

Anup Mazumder[1], Mainak Bose[1], Abhijit Chakraborty[2], Saikat Chakrabarti[2] & Suvendra N. Bhattacharyya[1+]
[1]RNA Biology Research Laboratory, Molecular and Human Genetics Division and [2]Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

nature INDIA

doi:10.1038/nindia.2013.151 Published online 18 November 2013

MiRNA as disease therapy target

Researchers have discovered a new mechanism that could makes microRNAs (miRNAs) a therapeutic target to treat diseases such as tuberculosis, leishmaniasis and cancer. They have shown how a temporary and reversible repression mediated by miRNA helps the expression of pro-inflammatory genes and prevent pathogen attacks .

References

1. Mazumder, A. *et al*. A transient reversal of miRNA-mediated repression controls macrophage activation. EMBO Rep. 14, 1008-1016 (2013) | Article | PubMed |

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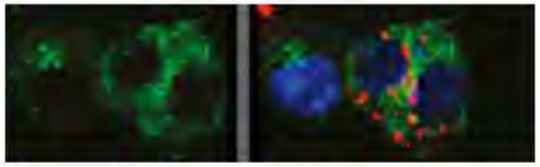
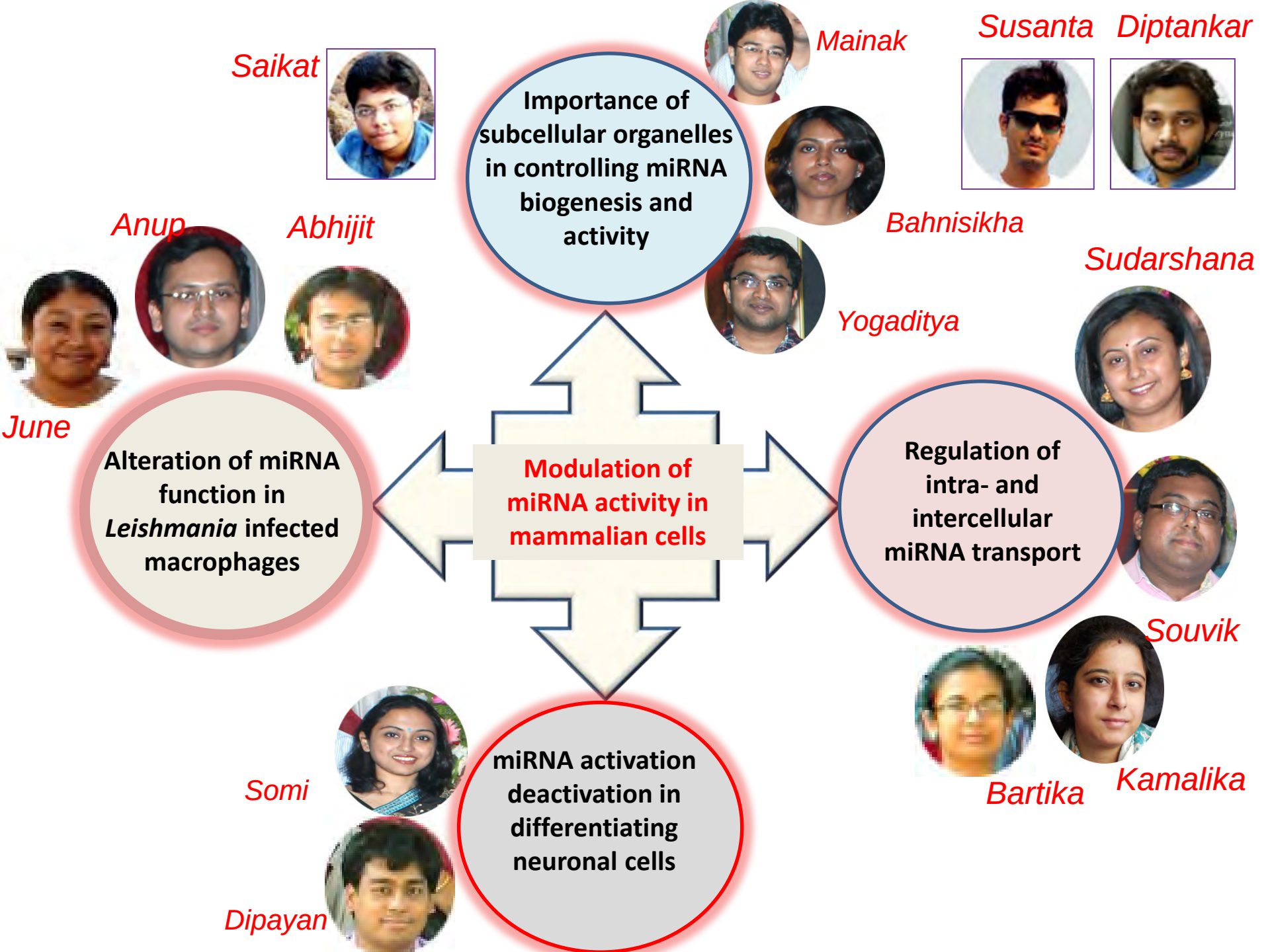


Relief of miRNA repression in macrophage activation

During macrophage activation, cytokine mRNAs are translated despite high levels of counteracting miRNAs. Here, Suvendra Bhattacharyya and colleagues show that phosphorylation of Ago2 impairs its binding to miRNAs and cognate mRNAs, enabling macrophage activation and prevention of pathogen invasion.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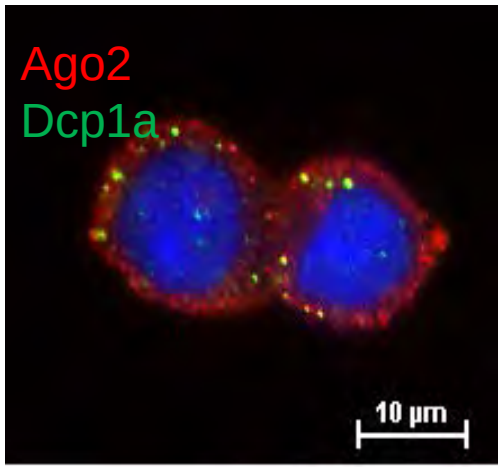
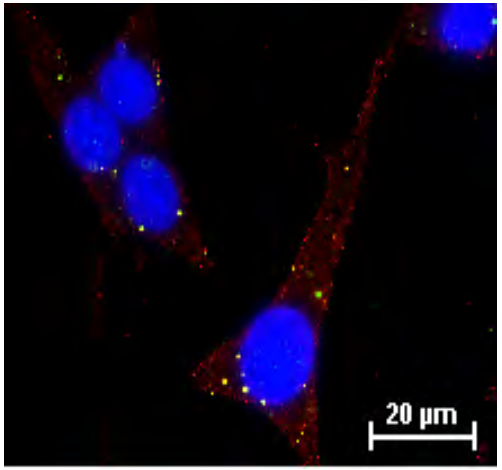
RNA Processing body components inactivate let-7a miRNPs to induce differentiation of sympathetic neurons

Somi





Key Findings

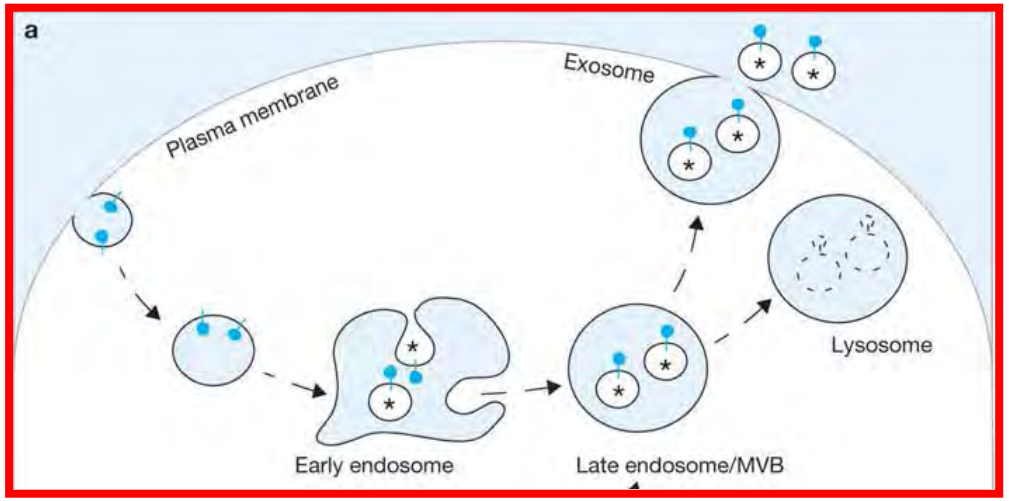
- P-body components are necessary and sufficient for differentiation of PC12 cells and sympathetic neurons
- P-body factors activate p38/MSK1 to phosphorylate Ago2
- Phosphorylation and inactivation of let-7a miRNPs is necessary and sufficient for neuronal differentiation

wellcome trust

Patranabis and Bhattacharyya
Mol. Cell. Biol. 2016
FASEB J 2018

The Unknown:

How the inter- and intracellular exchange of epigenetic signals contributes and maintains gene expression homeostasis in animal tissues



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Department of Sciences & Technology
Government of India

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Sourav Homchoudhury
Sudarshana Basu
Arnab Das

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CSIR "EMPOWER" Research Grant

CSIR network project Grants

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