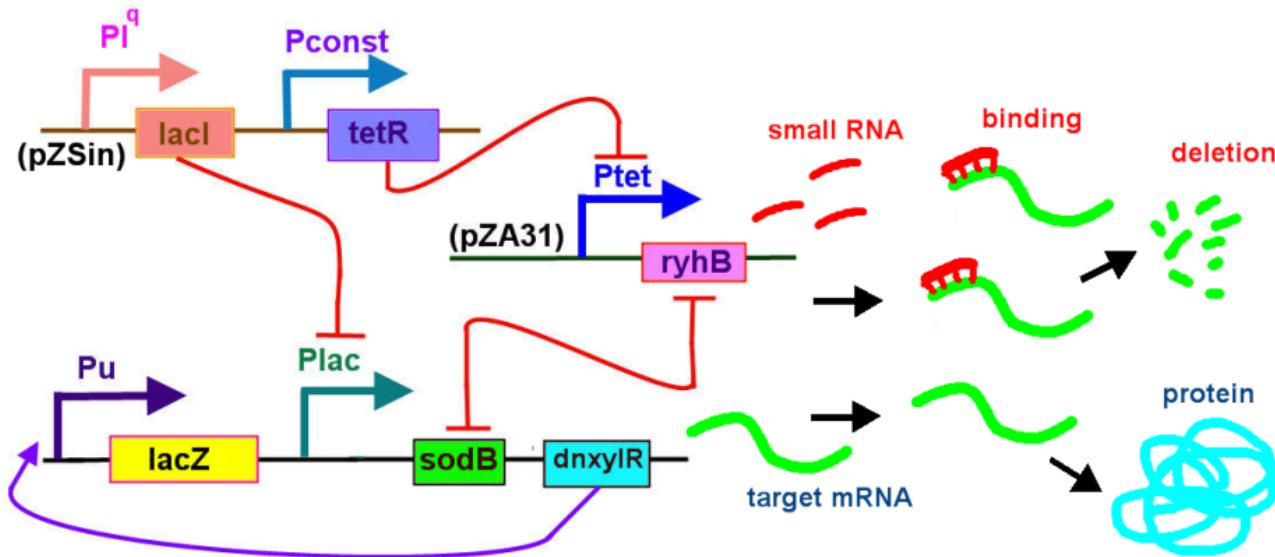


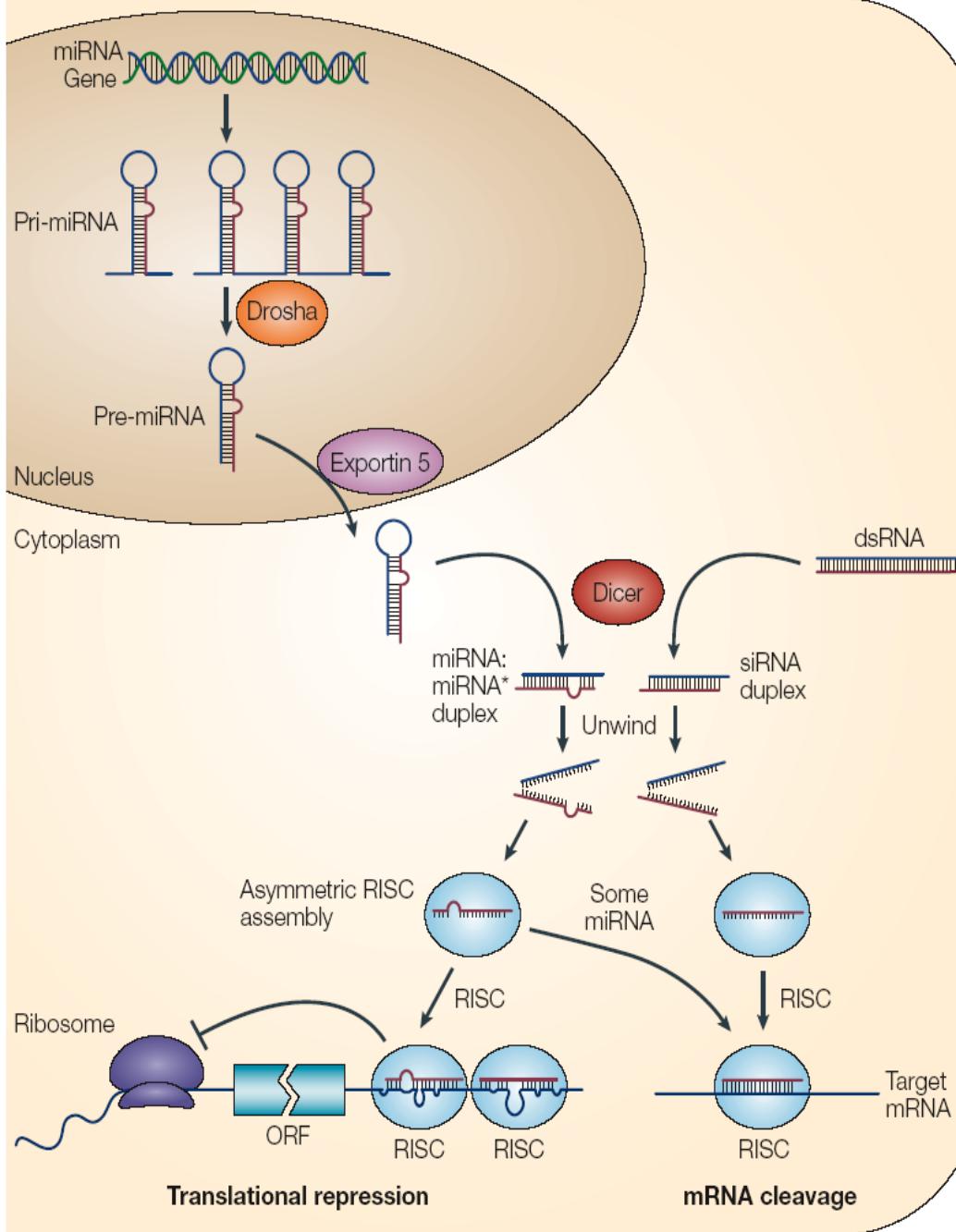
The analysis of regulatory networking with non-coding RNA



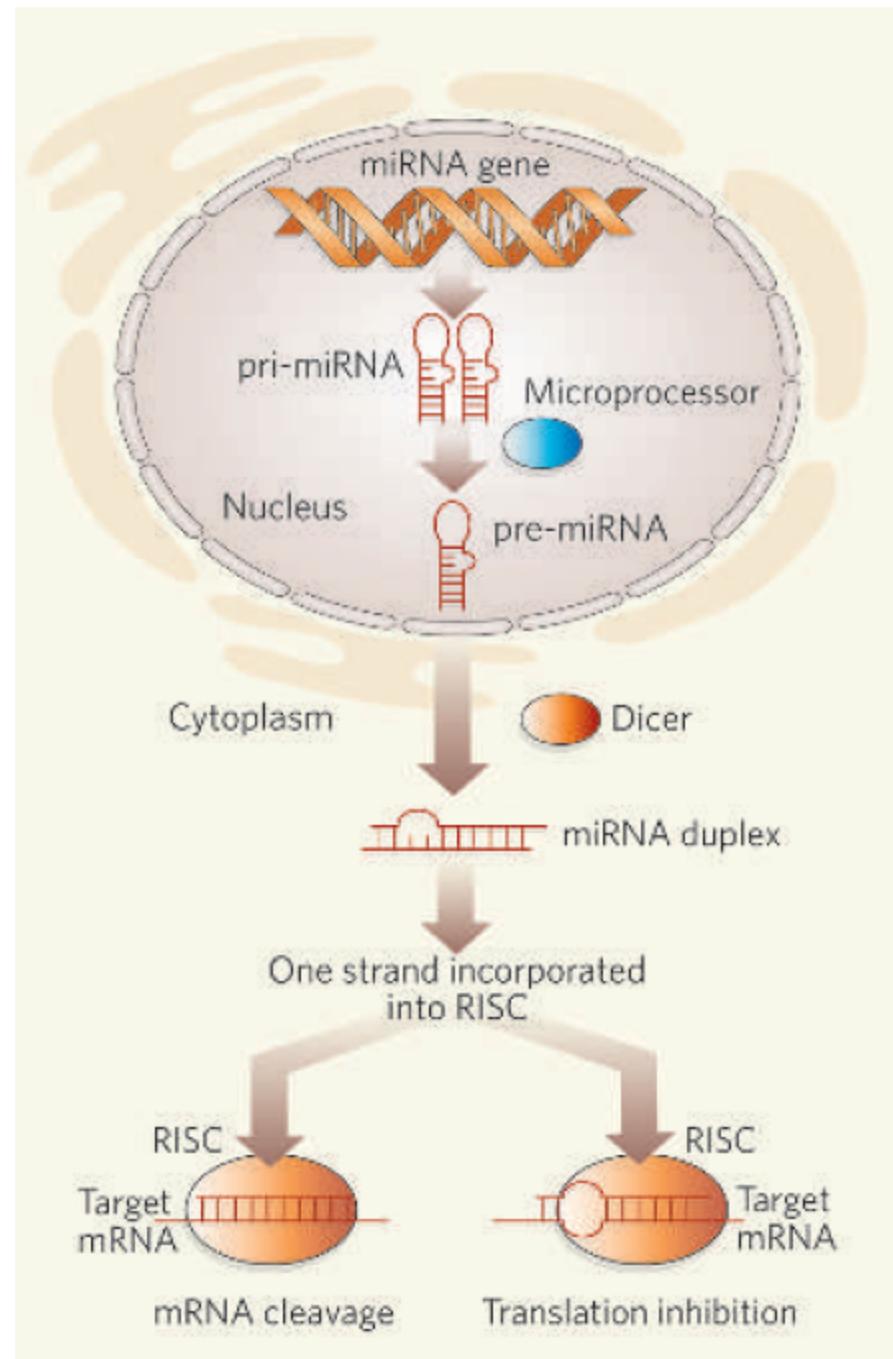
Dr. Po-Han Lee (李柏翰)/phlee@narlabs.org.tw

Biogenesis

- RNase III enzymes
 - Drosha (nucleus)
 - Dicer (cytoplasm)
- Both enzymes involved in the generation of siRNA
- RISC = RNA-induced silencing complex (contains miRNPs and Argonaute family proteins)
- RISC = Degradation/Silencing?



- ↑ Match with target (degree of complementarity)
= ↑ Prob. of degradation
- Possibly each miRNA may target multiple genes
- miRNA = or ≠ siRNA ?
 - Biochemically indistinguishable
 - Single vs double-stranded
 - Repression vs degradation?
- Post-Transcriptional Gene Silencing (PTGS) versus Translational Inhibition



Genetic Regulation (protein/small RNA level)

Negative regulation —Protein (repressor) inhibits transcription (Ex. LacI, TetR protein). Inducer— binds to repressor, alters form, reduces affinity for target, allows expression of gene. Sometimes, small molecule required for repressor activity.

Positive regulation —Activator protein increases transcription rate. Generally bound to a smaller signal molecule. (Ex. XylR protein activates Pu promoter).

sRNA-mediated gene silencing -(sRNA) are small (50-250 nucleotide) non-coding RNA molecules produced by bacteria; they are highly structured and contain several stem-loops. sRNAs can either bind to protein targets, and modify the function of the bound protein, or bind to mRNA targets and regulate gene expression. (Ex. RyhB and sodB)

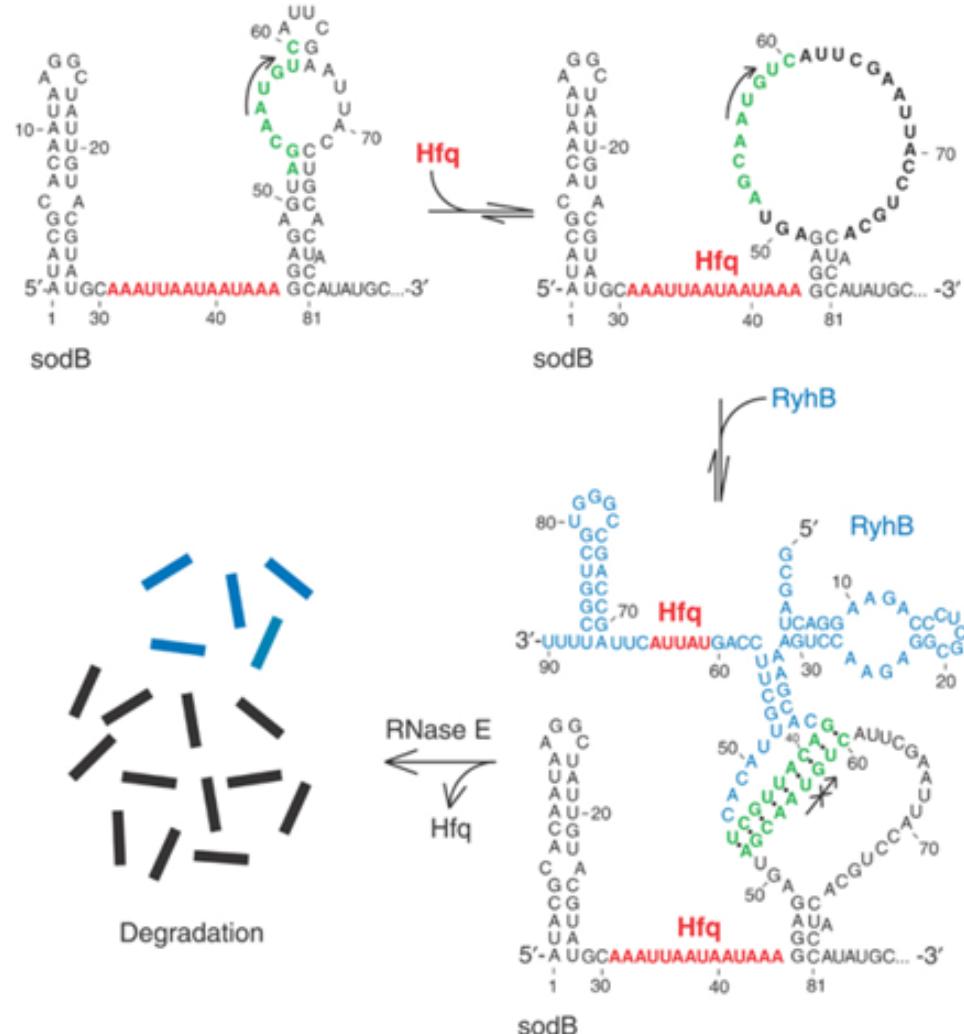
The small RNA RyhB and its sodB

The small RNA RyhB and its sodB (iron superoxide dismutase) mRNA target in E. coli.

RyhB binding blocked the translation initiation codon of sodB and triggered the degradation of both RyhB and sodB mRNA.

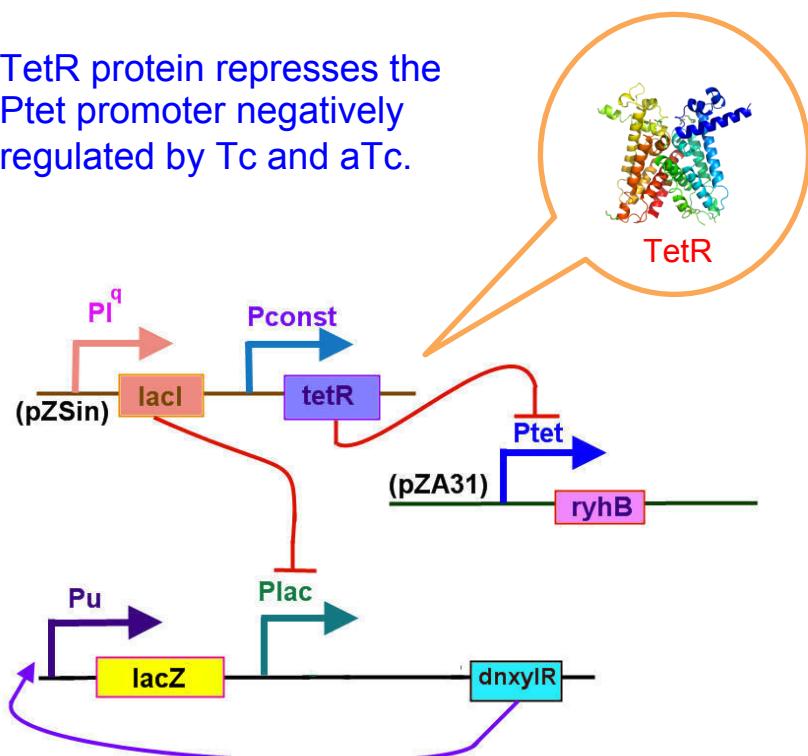
RyhB RNA is a 90 nucleotide non-coding RNA regulates a set of iron-storage and iron-using proteins when iron is limiting.

Negatively regulated by the ferric uptake repressor protein, Fur (Ferric uptake regulator).



Application on sodB-rhyB circuit

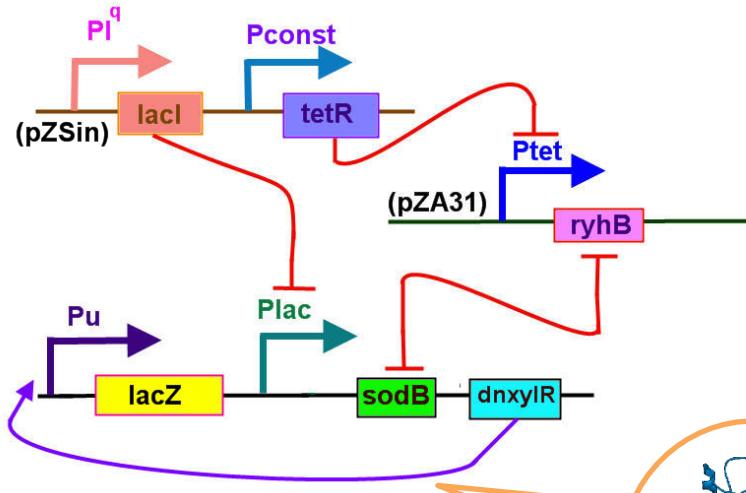
TetR protein represses the Ptet promoter negatively regulated by Tc and aTc.



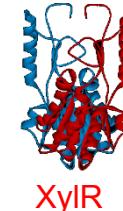
strain PZA31-ryhB: MGΔIYB chs Km:Pu-lacZ /

ϕ Ap: Plac-dnxylR /

pZA31 Ptet ryhB/pZSin PI^q lacI P_{const}tetR



Truncated XylR protein activates the Pu promoter with positive regulation.



strain PZA31-sodB-ryhB: MGΔIYB chs Km:Pu-lacZ /

ϕ Ap: Plac-sodB-dnxylR /

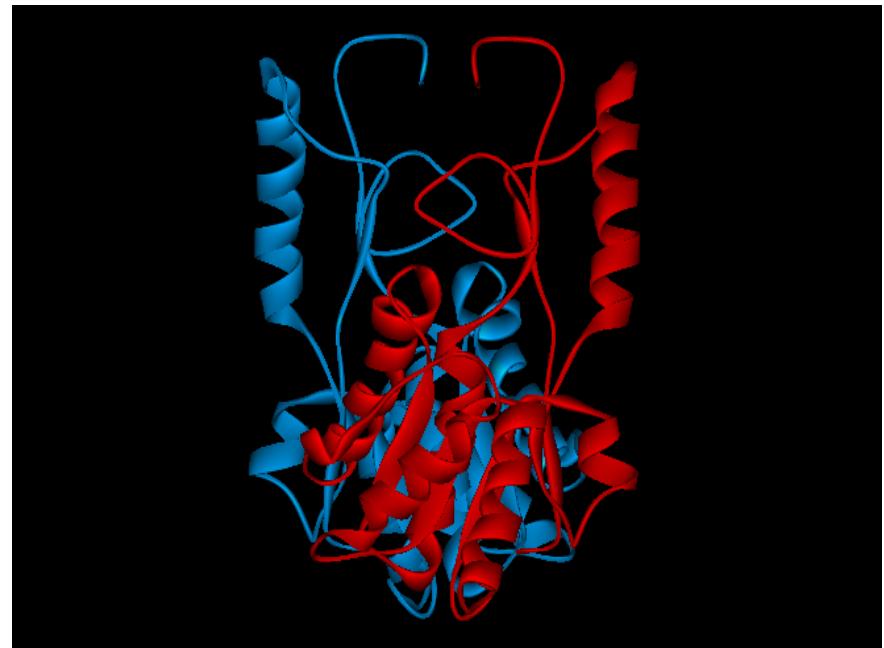
pZA31 Ptet ryhB/pZSin PI^q lacI P_{const}tetR

More sodB-ryhB → more deletion → less lacZ

Application on TetR and XylR protein



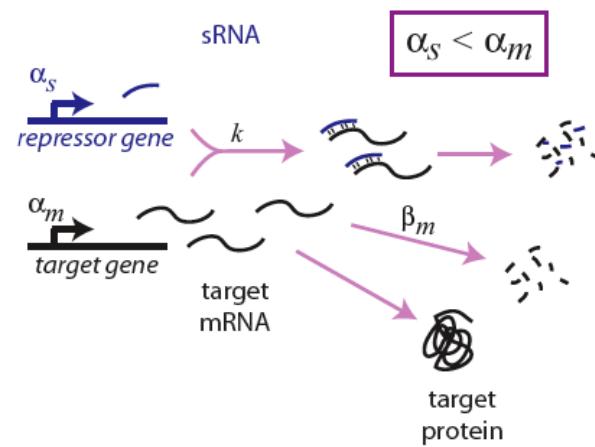
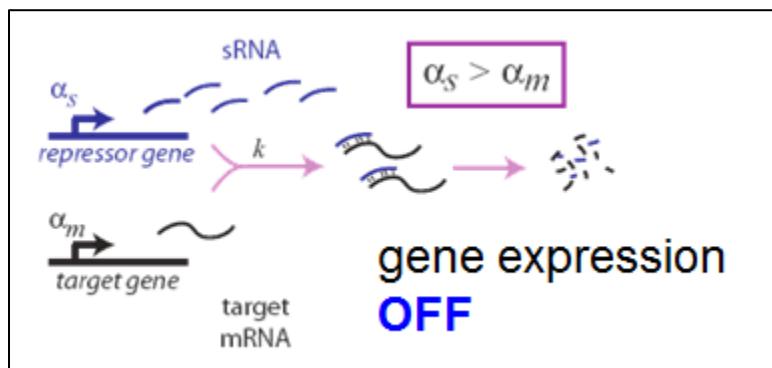
TetR protein represses the P_{tet} promoter
negatively regulated by Tc and aTc



XylR protein activates the P_u promoter
positive regulation

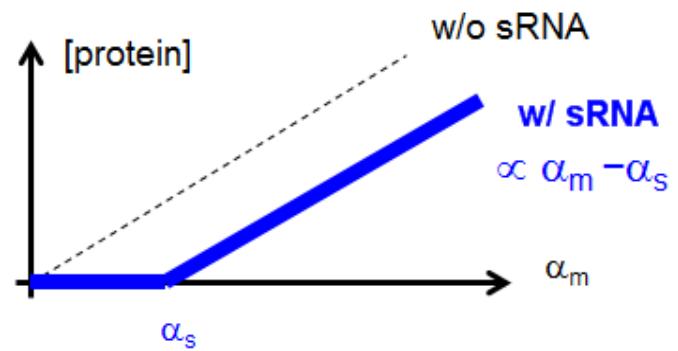
small RNA regulation

Escherichia. Coli K12 MG1655: there are some non-coding small RNAs, like ffs、micF、rnpB、spf、ssrS、dicF、oxyS、dsrA、csrB、sokB、gcvB、rprA、rttB、rhyB and etc. . E. Levine try to use small RNA rhyB and target mRNA sodB as the genetic regulation switch



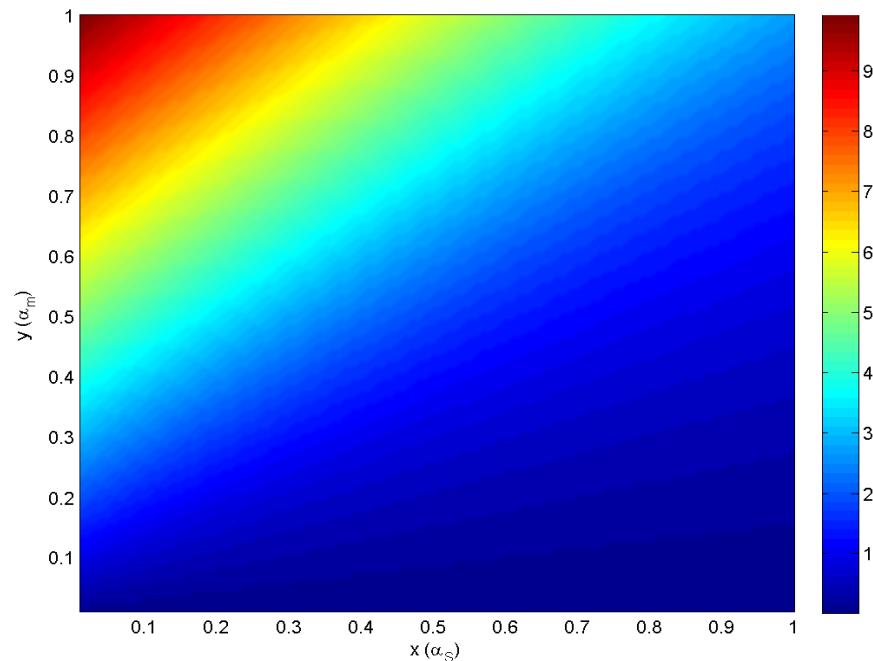
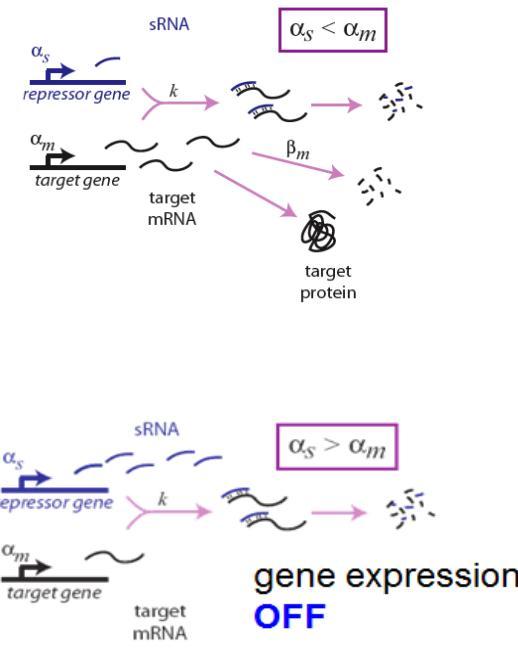
Mean field theory: a dynamic model

$$\frac{dS}{dt} = \alpha_s - \beta_s m - K \cdot m \cdot S$$
$$\frac{dm}{dt} = \alpha_m - \beta_m m - K \cdot m \cdot S$$



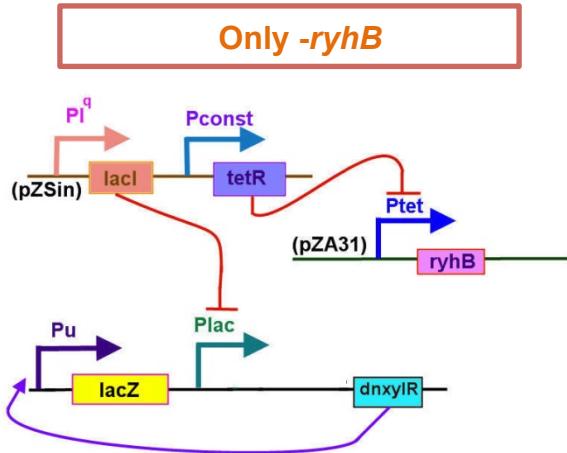
α_P : Protein 生成率 , α_m : Transcription rate of target mRNA ,
 α_s : Transcription rate of small RNA , K : Binding rate of small RNA:mRNA complex ,
 β_P : Protein 降解率 , β_m : Decay rate of free mRNA , β_s : Decay rate of free small RNA ,
 n_S : Hill 函式 S 調控因子 , n_P : Hill 函式 調控因子

The parameter space for α_m and α_s



α_m : Transcription rate of target mRNA vs α_s : Transcription rate of small RNA
Expression Result: mRNA concentration [nM]

The Synthetic Regulatory Circuit with Small RNA



TetR protein represses the Ptet promoter negatively regulated by Tc and aTc; LacI represses Plac promoter and inducer as IPTG

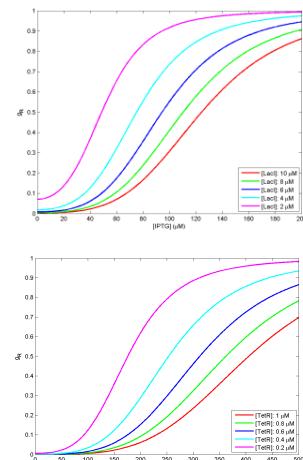
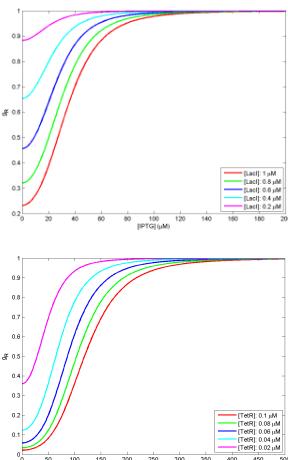
$$K_{\text{plac-Laci}} = 0.55 \mu\text{M}$$

$$K_{\text{Laci-IPTG}} = 30 \mu\text{M}$$

$$K_{\text{pTet-TetR}} = 50 \text{ nM}$$

$$K_{\text{pTetR-aTc}} = 15 \text{ nM}$$

Regulation function



Truncated XylR protein activates the Pu promoter with positive regulation.

a kinetic model

$$d[m \downarrow B]/dt = \alpha \downarrow mB \cdot g \downarrow R1 ([R \downarrow 1]/K \downarrow R1, [I \downarrow 1]) - \beta \downarrow mB \cdot [m \downarrow B] - K[m \downarrow B][m \downarrow SR]$$

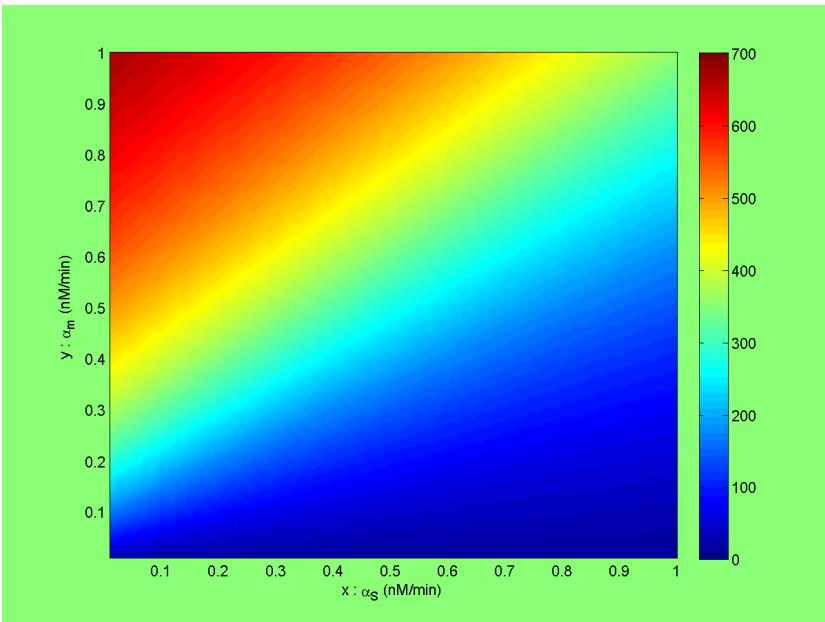
$$d[m \downarrow SR]/dt = \alpha \downarrow mSR \cdot g \downarrow R2 ([R \downarrow 2]/K \downarrow R2, [I \downarrow 2]) - \beta \downarrow mSR \cdot [m \downarrow SR] - K[m \downarrow B][m \downarrow SR]$$

$$d[SR]/dt = \alpha \downarrow SR \cdot [m \downarrow SR] - \beta \downarrow SR \cdot [SR]$$

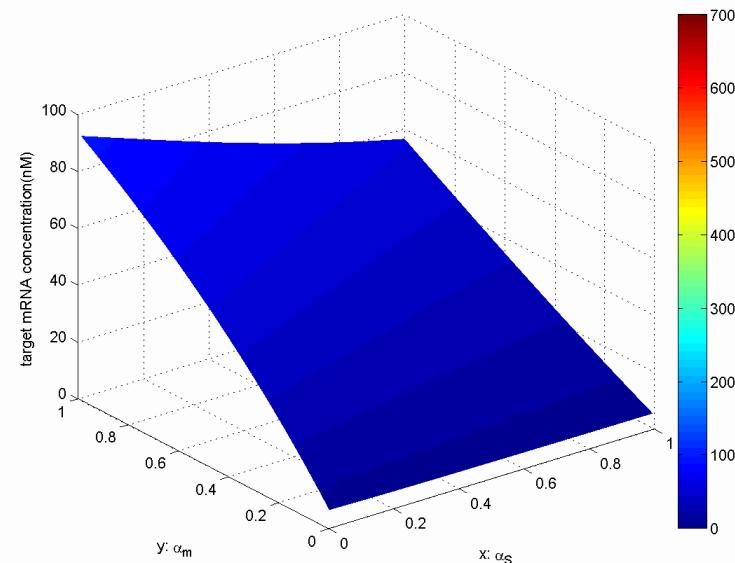
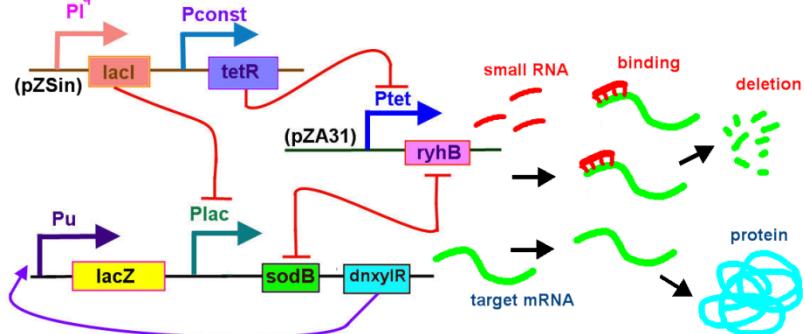
$$d[m \downarrow Z]/dt = \alpha \downarrow mZ \cdot g \downarrow A ([SR]/K \downarrow SR) - \beta \downarrow mZ \cdot [m \downarrow Z]$$

$$g \downarrow R ([R]/K \downarrow R, [I]) = 1/1 + ([R]/K \downarrow R \cdot 1/1 + ([I]/K \downarrow I)^m)^n, \quad g \downarrow A ([SR]/K \downarrow SR) = 1 + \omega [SR]/K \downarrow SR / 1 + [SR]/K \downarrow SR$$

α_m : Transcription rate, β_m : Degradation rate, $[m]$: mRNA, K : Binding rate, $[R]$: Repressor, α_{SR} : Translation rate, g_R : Regulation function, g_A : Activation function, $[I]$: Inducer, $[SR]$: Target protein, K_R : $[R]$ dissociation const., ω : fold const., m or n : Hill const.



The dynamic variation of target m-RNA concentration with the various parameters, finally to be the steady state



#protein-coding genes ≠cellular complexity



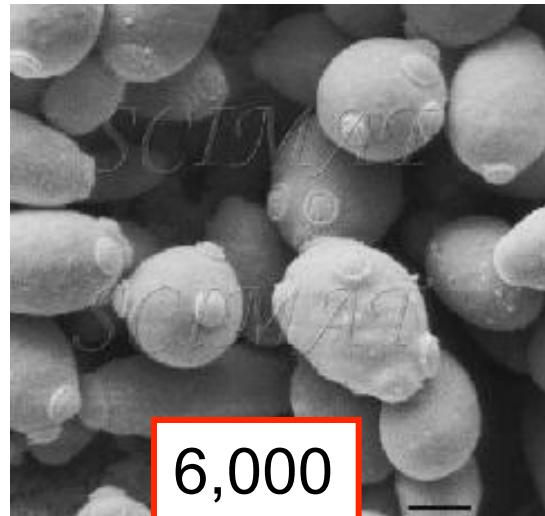
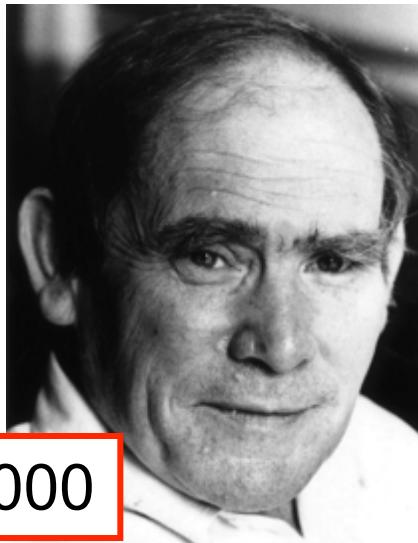
19,000



14,000



~20-25,000



6,000

Long non-coding RNA

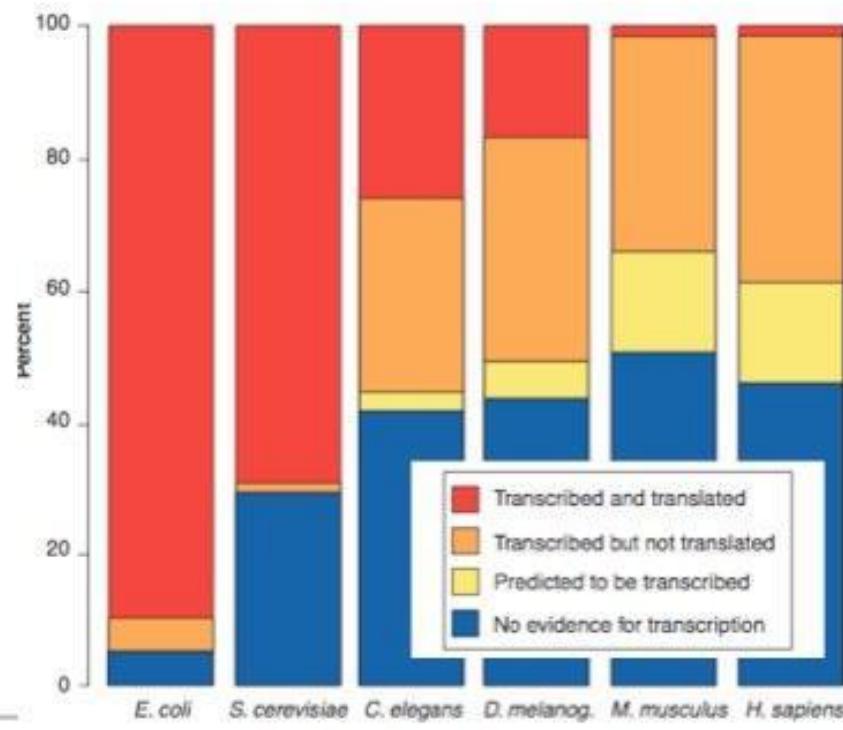
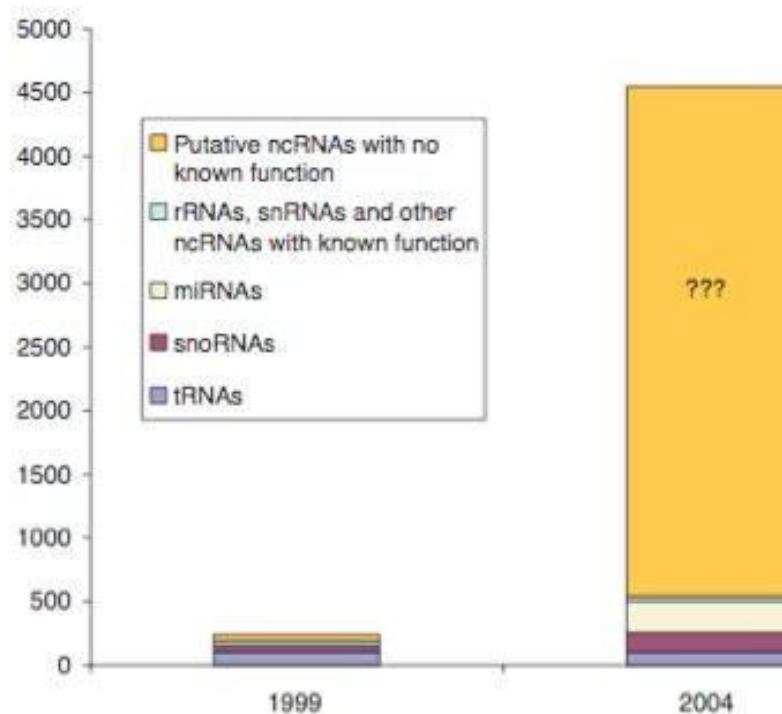
- 80% of the transcription in mammalian genomes is exclusively associated with long non-coding RNAs (lncRNAs)
- >2 (some >100) kb in length, spliced and could contain polyA signals
- No obvious ORF
- Mouse transcriptome (~180,000)
 - ~20,000 protein coding genes
 - ~160,000 lncRNAs

Discovery

1980s-1990s: Individual lncRNAs are discovered through traditional gene mapping approaches, *Xist* and *H19*.

Early 2000s: Development of large scale cDNA sequencing leads to the discovery of a surprising number of lncRNA transcripts.

Mid 2000s: The number of predicted genes in the mammalian genome goes down and the number of detected lncRNA transcripts increases exponentially.



Catagorization

- "housekeeping" (tRNA rRNA, RNaseP) vs. Regulatory (H19, Xist)
- "high abundance" (Xist, NEAT1) vs. "low abundance" (CCND1)
- trans-acting vs cis-acting
- loci of origin; sense, antisense, bidirectional, intergenic, totally intronic, partially intronic

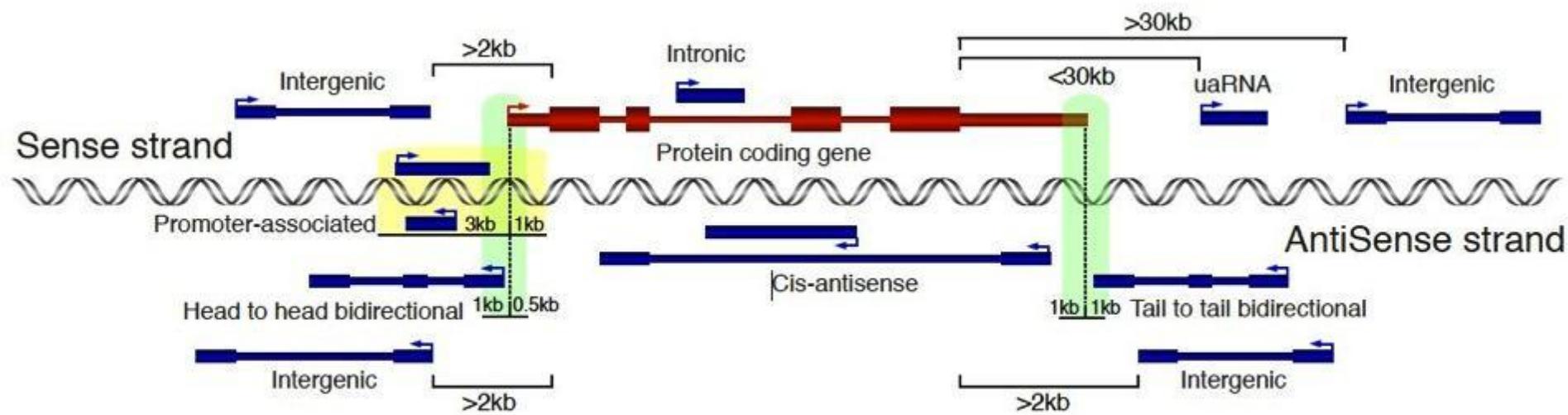
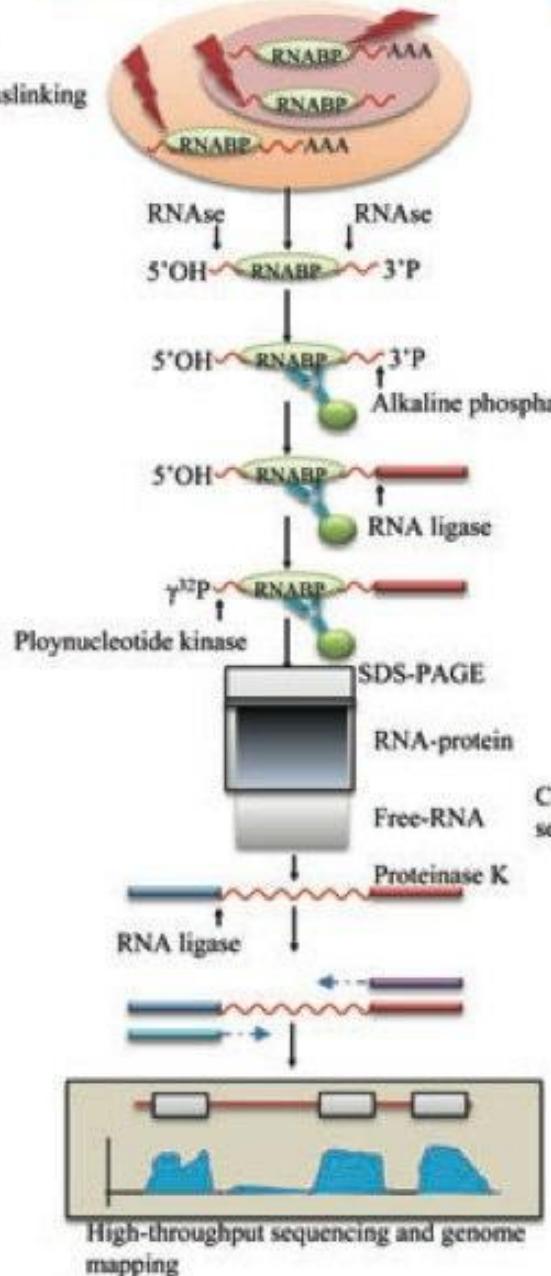


Figure S5: Classification of lncRNAs by genomic context.

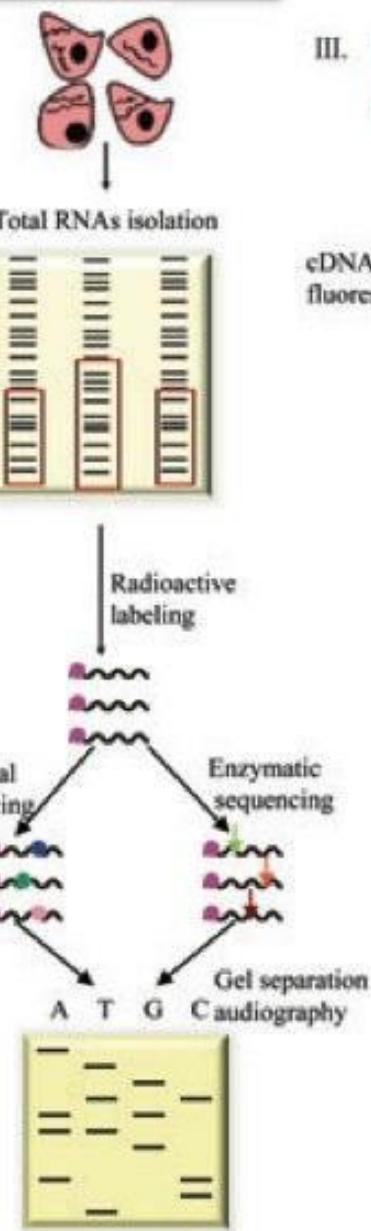
I.

CLIP and HITS-CLIP

UV
Crosslinking

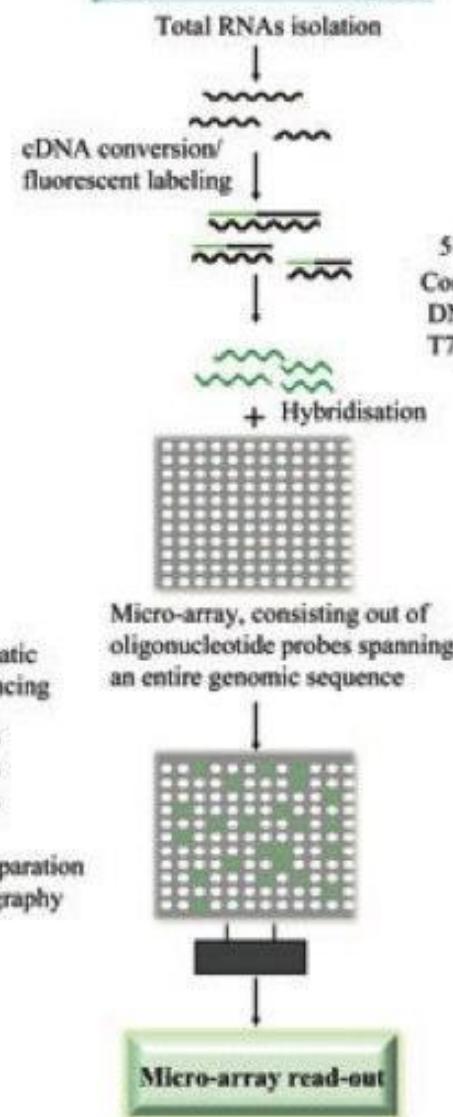
II.

RNA deep-sequencing



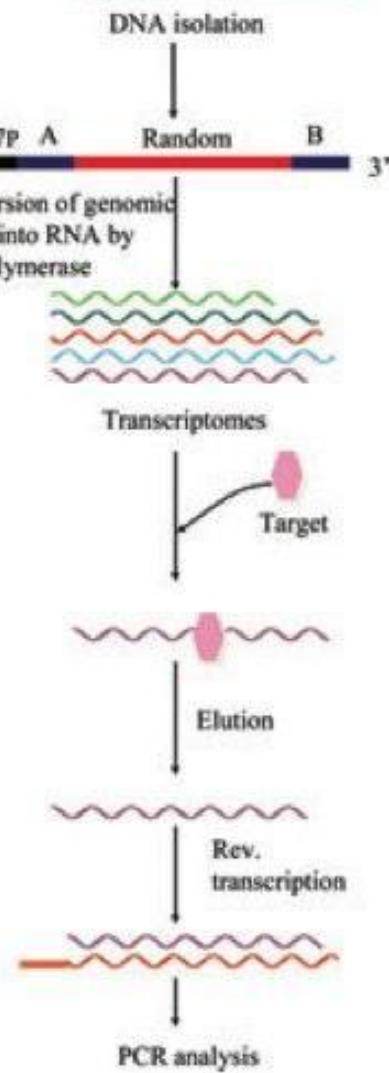
III.

Micro-array analysis



IV.

Genomic SELEX



Li X. Long Noncoding RNAs: Insights from Biological Features and Functions to Diseases. Med Res Rev. 2012

Mining Affymetrix microarray data for long non-coding RNAs: altered expression in the nucleus accumbens of heroin abusers

Sharon K. Michelhaugh,^{*,†} Leonard Lipovich,^{†,‡} Jason Blythe,[†] Hui Jia,[†] Gregory Kapatos^{*,†} and Michael J. Bannon^{*}

ncFANs

non-coding RNA Function ANnotation Server

| Home | **Server** | Download

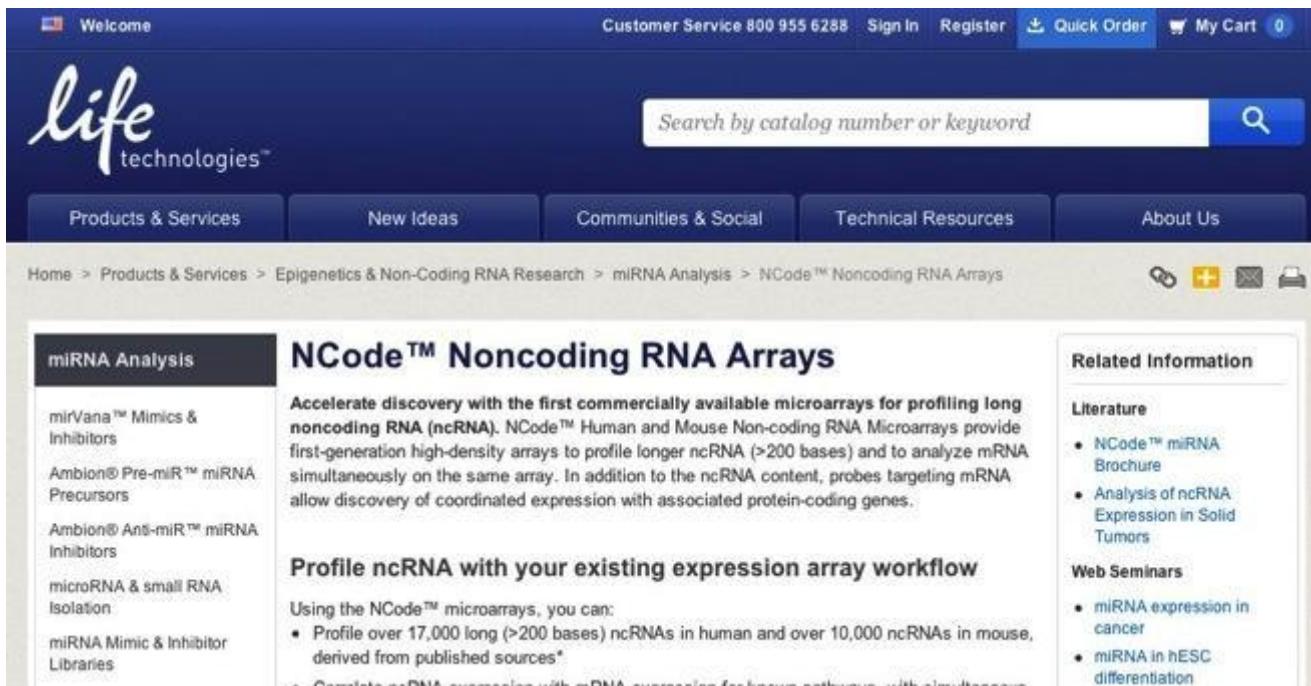
eBioMed » ncFANs

<http://www.ebiomed.org/ncFANs/>

[Home](#) » [Microarrays](#)

LncRNA Array Service

Long non-coding RNAs (LncRNAs) are evolutionarily conserved, longer than 200 nt, non-coding RNA molecules found in eukaryotes. Arraystar provides a full range of LncRNA microarray profiling services, from sample preparation to in-depth data analysis. Our step by step quality controls are designed to ensure you get the most reliable results. All you need to do is to submit your samples and we will complete the entire project for you.



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Home > Products & Services > Epigenetics & Non-Coding RNA Research > miRNA Analysis > NCode™ Noncoding RNA Arrays

miRNA Analysis

NCode™ Noncoding RNA Arrays

Accelerate discovery with the first commercially available microarrays for profiling long noncoding RNA (ncRNA). NCode™ Human and Mouse Non-coding RNA Microarrays provide first-generation high-density arrays to profile longer ncRNA (>200 bases) and to analyze mRNA simultaneously on the same array. In addition to the ncRNA content, probes targeting mRNA allow discovery of coordinated expression with associated protein-coding genes.

Profile ncRNA with your existing expression array workflow

Using the NCode™ microarrays, you can:

- Profile over 17,000 long (>200 bases) ncRNAs in human and over 10,000 ncRNAs in mouse, derived from published sources*
- *Complete ncRNA expression with mRNA expression for human pathways with clinical relevance

Related Information

Literature

- NCode™ miRNA Brochure
- Analysis of ncRNA Expression in Solid Tumors

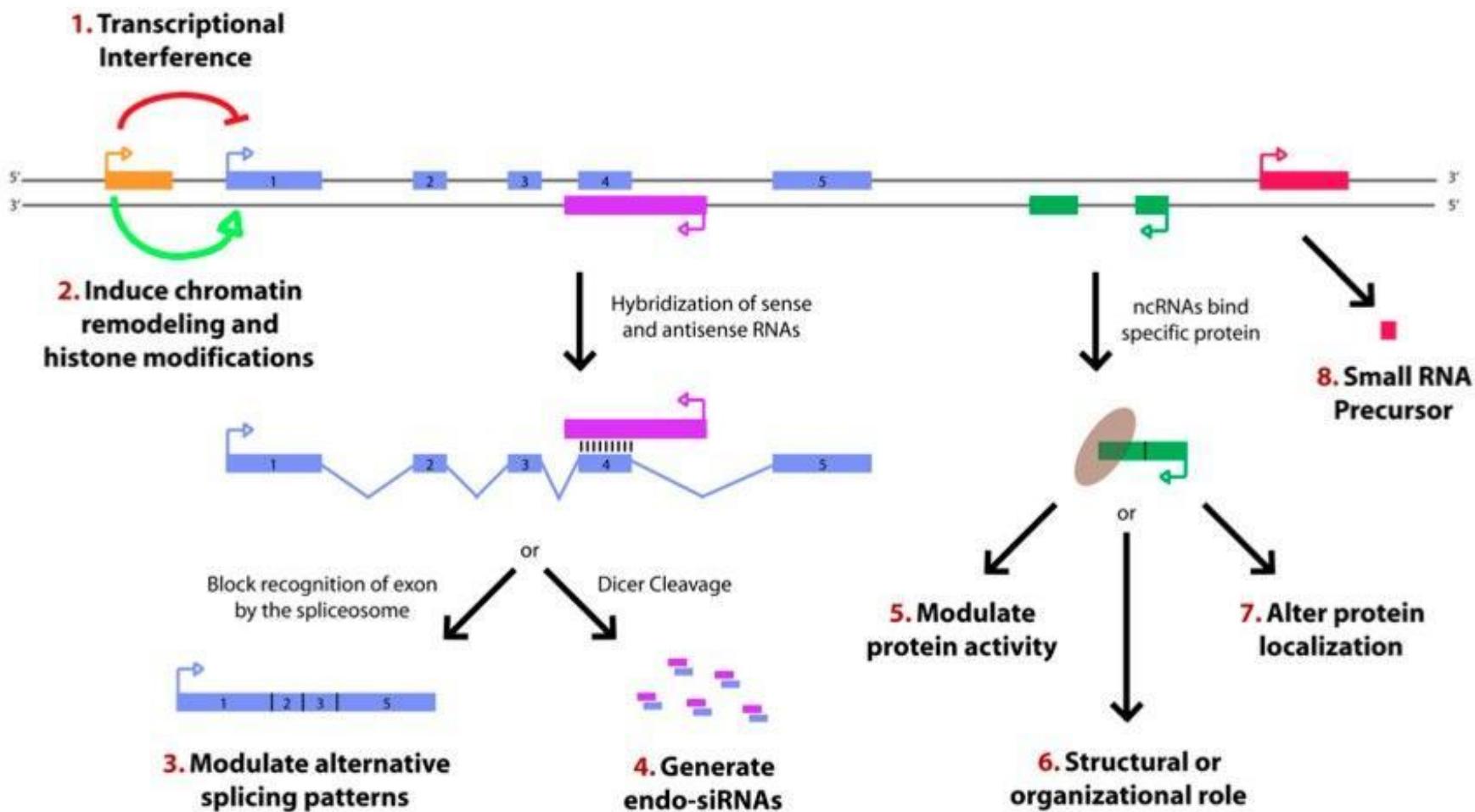
Web Seminars

- miRNA expression in cancer
- miRNA in hESC differentiation

lncRNA Databases

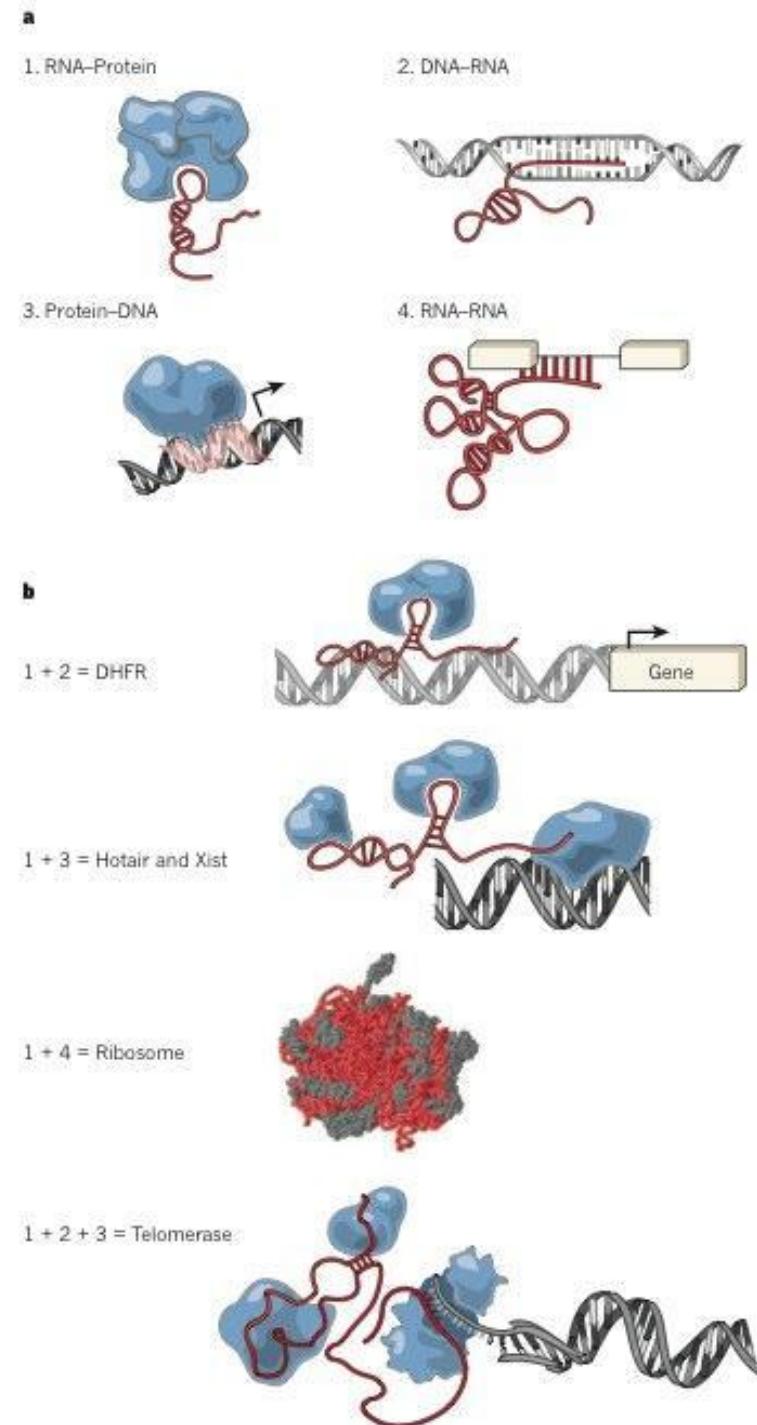
- Lncrna db (<http://lncrnadb.com/>)
- FAMTOM3 (<http://fantom.gsc.riken.jp/4/>)
- NONCODE v3.0
- ncFANS

Potential functions of lncRNA



Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev.* 2009 23(13):1494-504.

Modular principles of lncRNAs



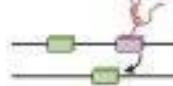
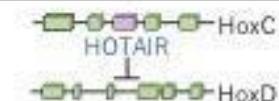
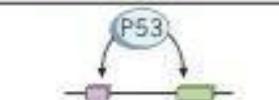
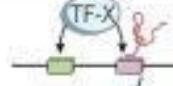
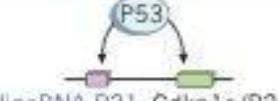
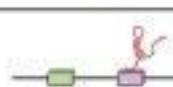
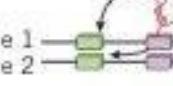
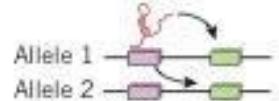
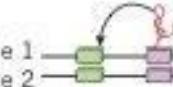
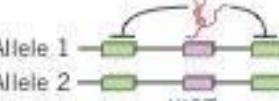
Guttman M, Rinn JL. Modular regulatory principles of large non coding RNAs. *Nature*. 2012;482(7385):339-46

To *cis* or not to *cis*

BOX 1

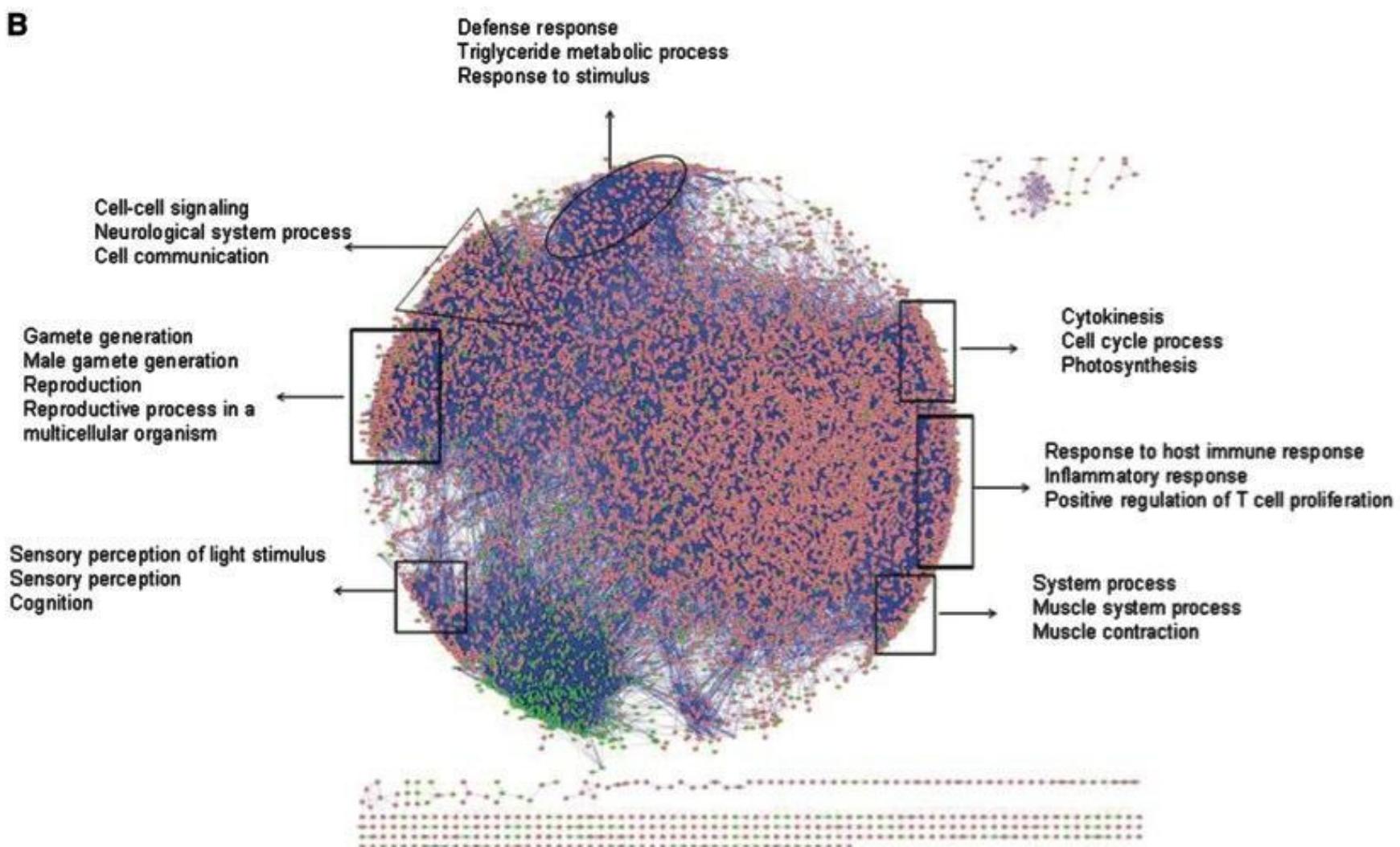
Distinguishing *cis*- from *trans*-regulation

If an ncRNA is a *cis*-regulator, then several observations will be true: (i) the gene-expression levels of a neighbouring gene will be correlated with the RNA expression across all conditions; (ii) loss-of-function of the RNA would affect expression of a neighbouring gene, and (iii) the ncRNA would affect expression of a neighbouring gene on the same allele that it is expressed from. The absence of any of these criteria supports *trans*-regulation. We illustrate this point using five common regulatory models. The figure shows what would be observed using specific computational and experimental methods for each regulatory model. The boxes with a tick indicate observed effects on neighbouring genes for each method, and boxes with a cross indicate no observed effect on neighbouring genes. Known ncRNA examples of each of these regulatory models are shown to the right of the figure.

| | Regulatory model | Expression correlation | Perturbation effect | Allele-specific regulation | Known ncRNA examples |
|-------|--|------------------------|------------------------|----------------------------|--|
| trans |  | ✗ | ✗ | ✗ |   |
| trans |  | ✓ | ✗ | ✗ |  |
| trans |  | ✓ | ✓ | ✗ | Unknown |
| trans |  | ✓ | ✓ | ✗ |  |
| cis |  | ✓ | ✓ | ✓ |  |
| | | ✓ Neighbour affected | ✗ Neighbour unaffected | | |

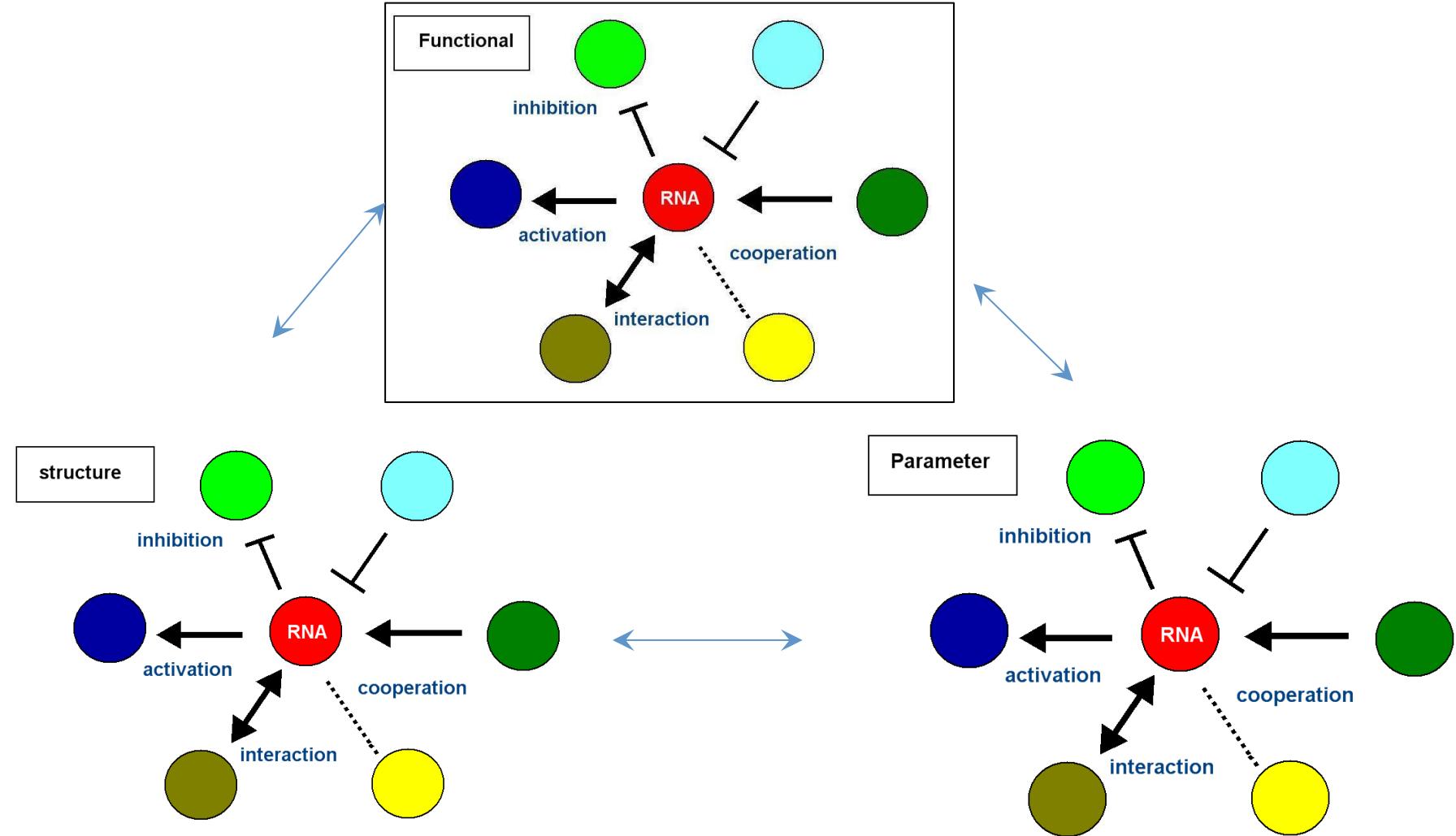
Co-expression network

B

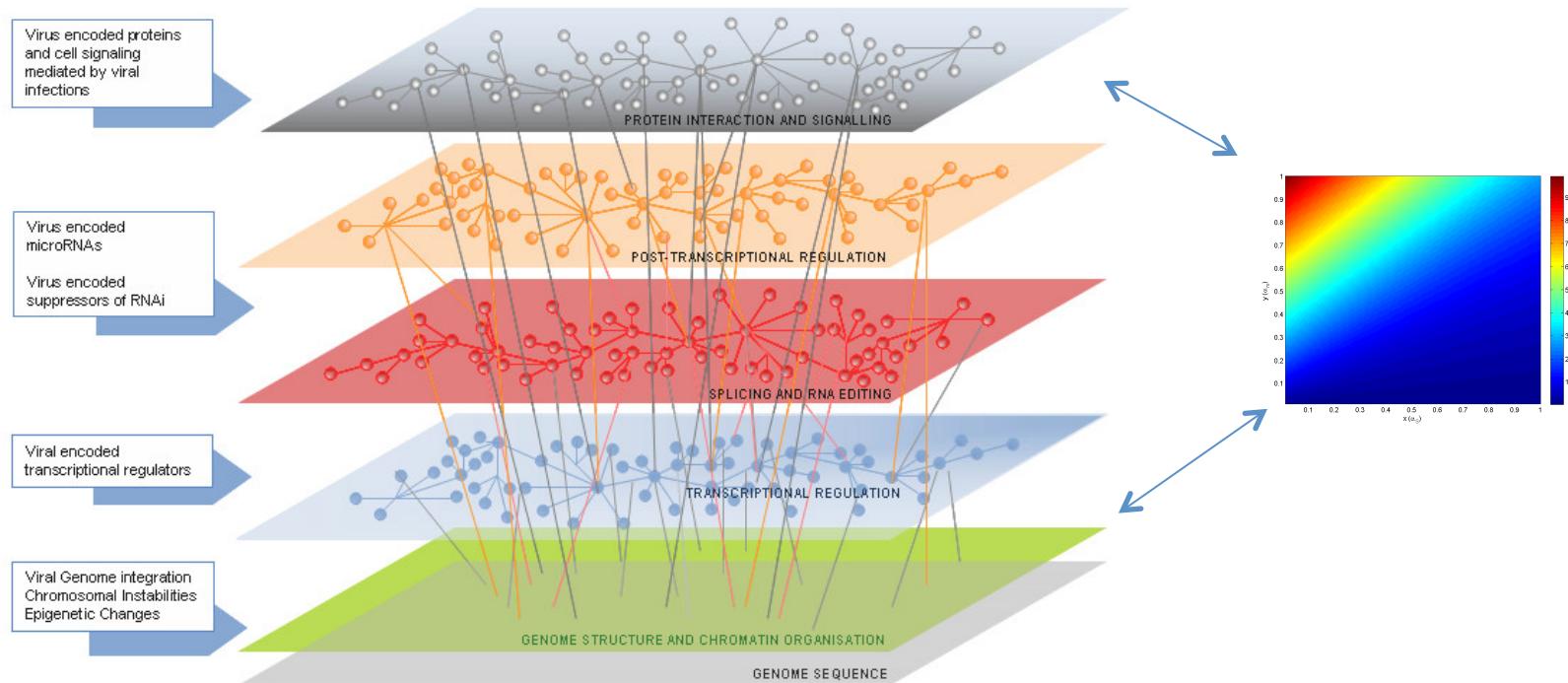


Liao Q., Large-scale prediction of long non-coding RNA functions in a coding-non-coding gene co-expression network. Nucleic Acids Res. 2011 May;39(9):3864-78

關聯式網絡料庫的 node 建立--連結非編碼RNA和IAV感染 (依據 functional, structure, parameter and etc.)



Associated regulation networking



Refer to the bio databases

- 例如EMBL Database, GenBank, IMGT, dbEST, 5S rRNA , SWISS-PROT, PIR (Protein Information Resource database), PDB(Protein data bank), PROSITE (Database of protein domains, families and functional sites), ENZYME(Enzyme nomenclature database), REBASE (The Restriction Enzyme Database), HSSP(Homology-derived secondary structure of proteins database), BLOCKS(Protein Blocks Database), KABAT(Database of Sequences of Proteins of Immunological Interest), InterPro , TMBASE(Database of Membrane Spanning Protein Segments), DICTYDB, EcoGene (E.coli database collection), FLYBASE(A Database of Drosophila Genes & Genomes), MAIZEDB , SGD(Saccharomyces Genome Database), SUBTILIST(a database dedicated to the analysis of the genome of Bacillus subtilis) , WORMPEP (Caenorhabditis Genome database), GCRDB(a G-protein-coupled receptor database), OMIM (Online Mendelian Inheritance in ManTM), RHDP (The Radiation Hybrid Database) , SWISS-2DPAGE(Two-dimensional polyacrylamide gel electrophoresis database) ,

reference

- http://compbio.ucdenver.edu/hunter/cpbs7712/documents/Phang_Lab_Talk2_2012small.pptx
- Medina RA, García-Sastre A. Influenza A viruses: new research developments. *Nat. Rev. Microbiol* 9, 590- 603 (2011)
- E. Levine, Z. Zhang, T. Kuhlman, and T. Hwa, Quantitative Characteristics of Gene Regulation Mediated by small RNA, *PLoS Biol* 5: e229 (2007).
- Carla Winterling, Manuel Koch, Max Koeppel, Fernando Garcia-Alcalde, Alexander Karlas, Thomas F Meyer, Evidence for a crucial role of a host non-coding RNA in influenza A virus replication, *RNA biology*, vol 11, issue 1 (2013)
- Watanabe T, Watanabe S, Kawaoka Y. Cellular networks involved in the influenza virus life cycle. *Cell Host Microbe* 7, 427- 39 (2010)
- Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, et al. Landscape of transcription in human cells. *Nature* 489:101 - 8(2012)

指導資優生科展及論文事蹟

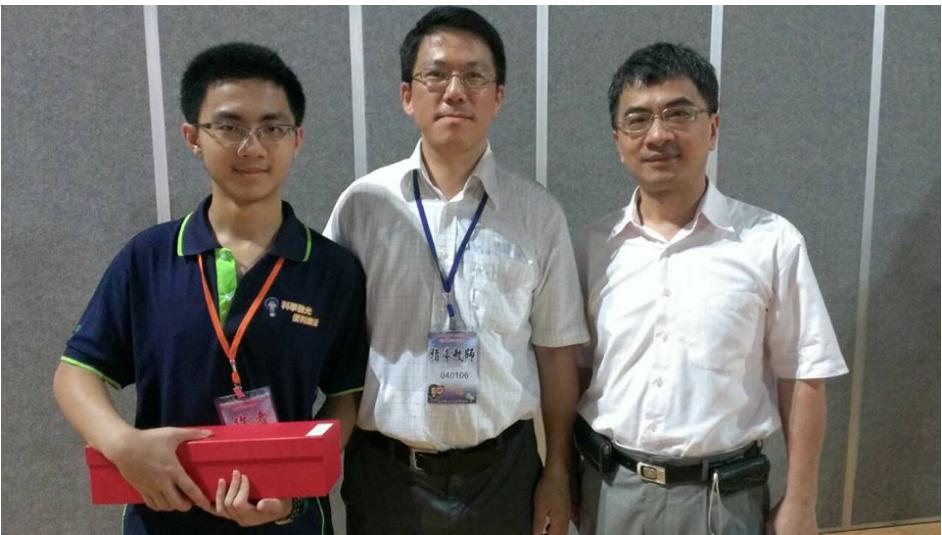
1. 2000年，指導科展作品第40屆應用科學科全國第一名，邏輯映射串流加密器，台北市中正國中，作者：呂任棠、唐維澤、陳毅。
2. 2003～2006年，國立編譯館邀請編寫國中自然科學教科書。
3. 2004年，與陳毅(Chen Yi)發表一篇國際期刊論文，成功推薦陳毅(Chen Yi)進入麻省理工學院就讀，陳毅將於2014年加州理工物理博士畢業，Po-Han Lee, Yi Chen, Soo-Chang Pei and Yih-Yuh Chen, *Evidence of the Correlation Between Positive Lyapunov Exponents and Good Chaotic Random Sequences*, Computer Physics Communications, Volume 160, Issue 3, pp 187-203, (2004).
4. 2005年，指導陳冠儒同學第四屆旺宏科學獎優等獎，鳥音音頻識技術的電子資料庫建立
5. 2011年，第16屆生物物理大會，*The characterization and application of a synthetic autoregulatory circuit* 海報競賽優等獎
6. 指導師大附中學生江政穎發表會議論文，Po-Han Lee (李柏翰), Cheng-Ying Chiang (江政穎) and Wan-Sheng Su (蘇萬生), A study of field enhancement factors of closed single-walled carbon nanotubes enhanced by Cs adsorption, Annual Meeting of The Physical Society of Republic of China, 2012. (Conference paper), 江政穎目前為台大醫學系學生。
7. 指導師大附中學生江政穎發表期刊論文，P. H. Lee, C. Y. Chiang, Y. T. Wang , W. J. Lee, and W. S. Su, Effects of Cs adsorption on the field emission characteristics of closed single-walled carbon nanotubes, Journal of Vacuum Science & Technology B, 31(2), 021802-1 (2013)



2013年國立師附中全國科展第三名代表領獎



2006 Yi Chen 和李柏翰 in UCSD 合作論文



2013年指導國立師附中高瑞璠物理科展全國第三名



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指導資優生 陳冠霖 論文事跡



1. 指導資優生陳冠霖發表國際期刊論文P. H. Lee*, Z. R. Xiao, K. L. Chen, Y. Chen, S. W. Kao and T. S. Chin, *The magnetism of Fe(1-x)CoxB alloys: first principle calculation*, Journal of the Physics B: Condensed Matter, vol. 404 , pp 1989-1992 (2009).
2. P. H. Lee*, T. H. Chen, K. L. Chen, C. Y. Lin, T. W. Wang, B. S. Wu, S. W. Wang and W. S. Su, *The Magnetism of Cr \downarrow 1-x Mn \downarrow x B, Mn \downarrow 1-x Fe \downarrow x B and Fe \downarrow 1-x Co \downarrow x B Alloys: First-Principles Electronic Structure Calculations*, will be submitted (2014).
3. K. L. Chen, T. H. Chen, S. Y. Chou, Y. H. Yang, Y. J. Chang, W. S. Su, P. H. Lee, *Even parasites have parasites: the arms by small RNA*, The 4th Lifestyle and Health International Forum and the 4th Annual Meeting for the Federation of Worldwide Healthy Lifestyle Promotion Association, November 23-24, Taiwan. (Published).
4. Kuan-Ling Chen, Tzu-Han Chen, Shang-Yu Chou, Yung-Hsiang Yang, Yao-Jen Chang, Wan-Sheng Su, Po-Han Lee, *The Nonlinear Analysis of a Synthetic Regulatory Circuit with Small RNA*, The Asian Pacific Organization of Cell Biology (APOCB), February 24-27, Singapore, (2014) (Accepted).

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1. 2006.05.01 ~ 2006.12.31, Project Leader, The Theoretic Calculation and Analysis of Nano-material, Ministry of Education, Taiwan
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科普與科書寫作推廣

1. 並且指導學生錄影，流言追追深獲好評，推廣科學新知不遺餘力，對推廣科普於高中生有極大的影響力。蛋一微波就會爆炸？流言追追追-【流言現場&校園調查局】，李柏翰教師指導，師大附中1233班演出。
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3. 2009~ 2013年，擔任翰林出版公司高中物理科教科書編輯委員。
4. 李柏翰, 基因檢測, Newton牛頓科學雜誌 2月號 第64期, FOCUS閱讀P.10 -11(2013)
5. 李柏翰, 人工自我 調控基因開關設計, Newton牛頓科學雜誌 5月號 第67期, 特集延伸 閱讀P.58 -63(2013)
6. 李柏翰, 審定作者, 新基因體革命, Newton牛頓科學雜誌 8月號 第70期, P.46 -64(2013)