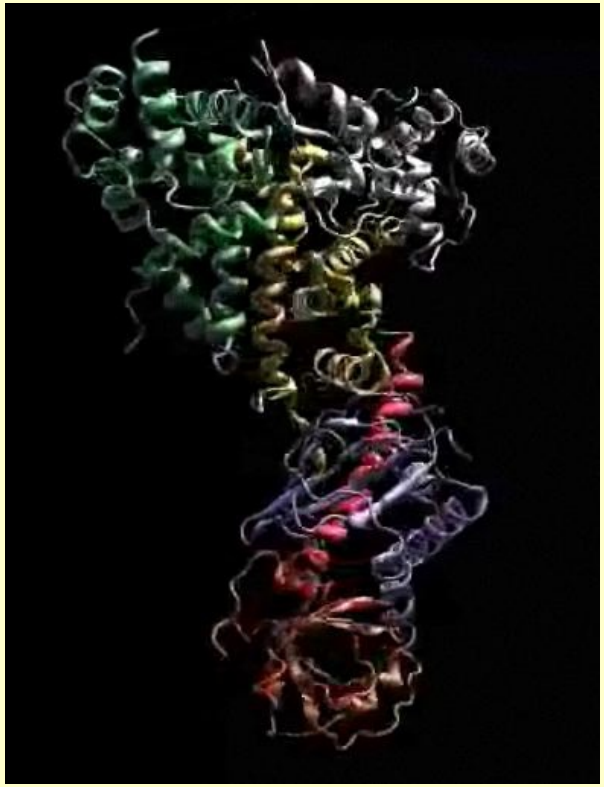
Genomics & Medicine

http://biochem118.stanford.edu/

miRNA Regulatory Networks



Doug Brutlag, Professor Emeritus of
Biochemistry & Medicine
Stanford University School of Medicine

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Gene Regulatory Mechanisms

- Transcriptional Mechanisms
  - Type of promoters & RNA polymerases
  - Control of Transcription
  - Transcription Factors and TFBS
- RNA processing
  - 5′ Capping & 3′ poly-adenylation
  - RNA degradation rates
  - Splicing and Alternative Splicing
- Translational Mechanisms
  - Micro RNAs (miRNAs) control translation & degradation of mRNAs
  - Silencer RNAs (siRNAs or RNAi) catalyze degradation of mRNA
- Epigenetic Mechanisms
  - Chromatin remodeling in active and inactive regions
  - Histone acetylation, phosphorylation and methylation
  - DNA methylation in inactive regions

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The ENCODE Project

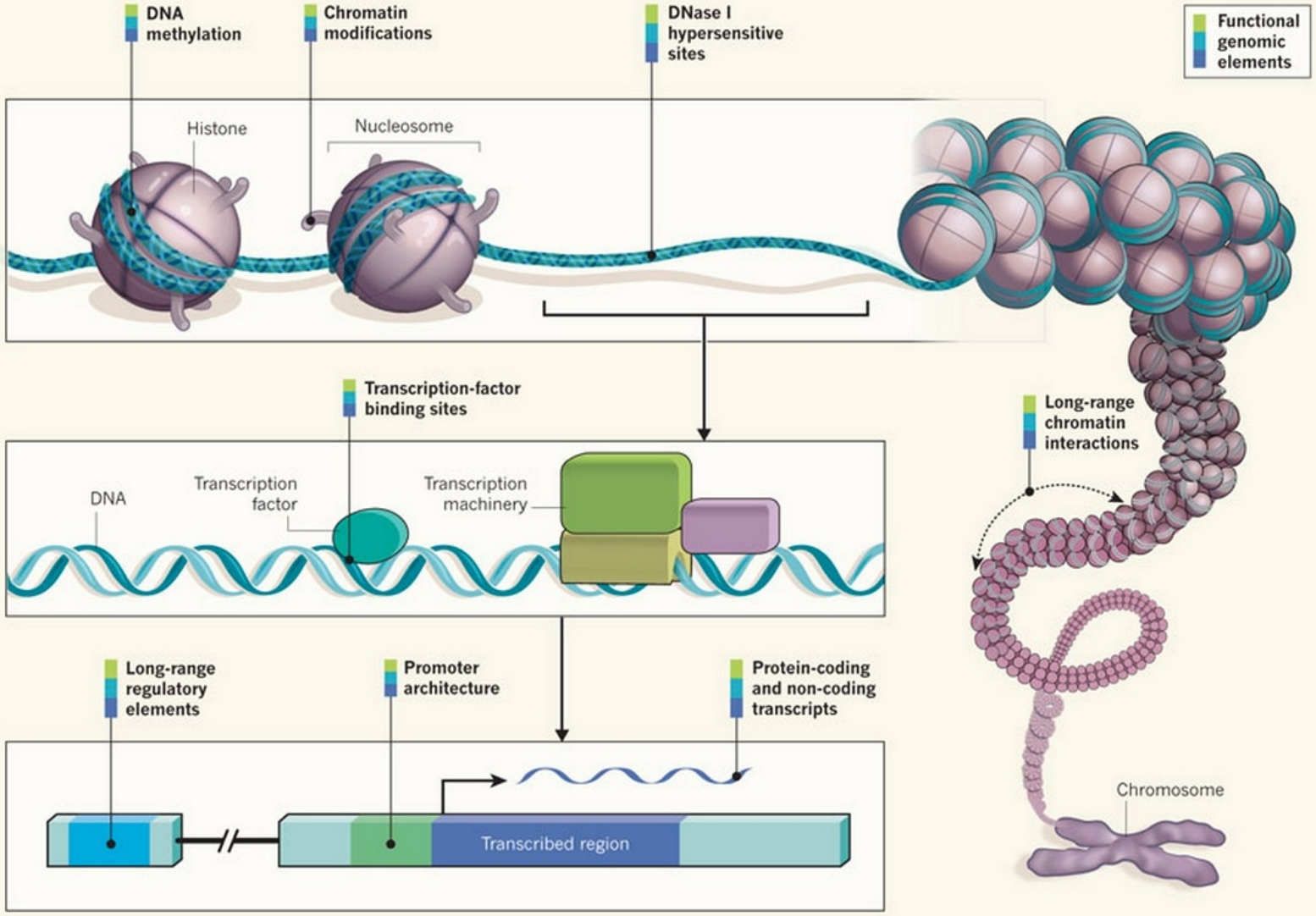
http://www.genome.gov/encode/

- The ENCODE Project searches the human genome for:
  - Transcribed regions
  - Conserved regions
  - Transcription factor genes
  - Transcription factor and other protein binding sites
  - DNA methylated regions
  - Regions with "open" chromatin
  - lncRNA coding regions
  - Regions associated with modified histones (Chip-CHIP)
  - Regions associated with disease (GWAS)
- So far these functional or conserved regions comprise 80% of the human genome.

http://www.nature.com/news/encode-the-human-encyclopaedia-1.11312 © Doug Brutlag 2015

ENCODE Explained

http://www.nature.com/nature/journal/v489/n7414/full/489052a.html



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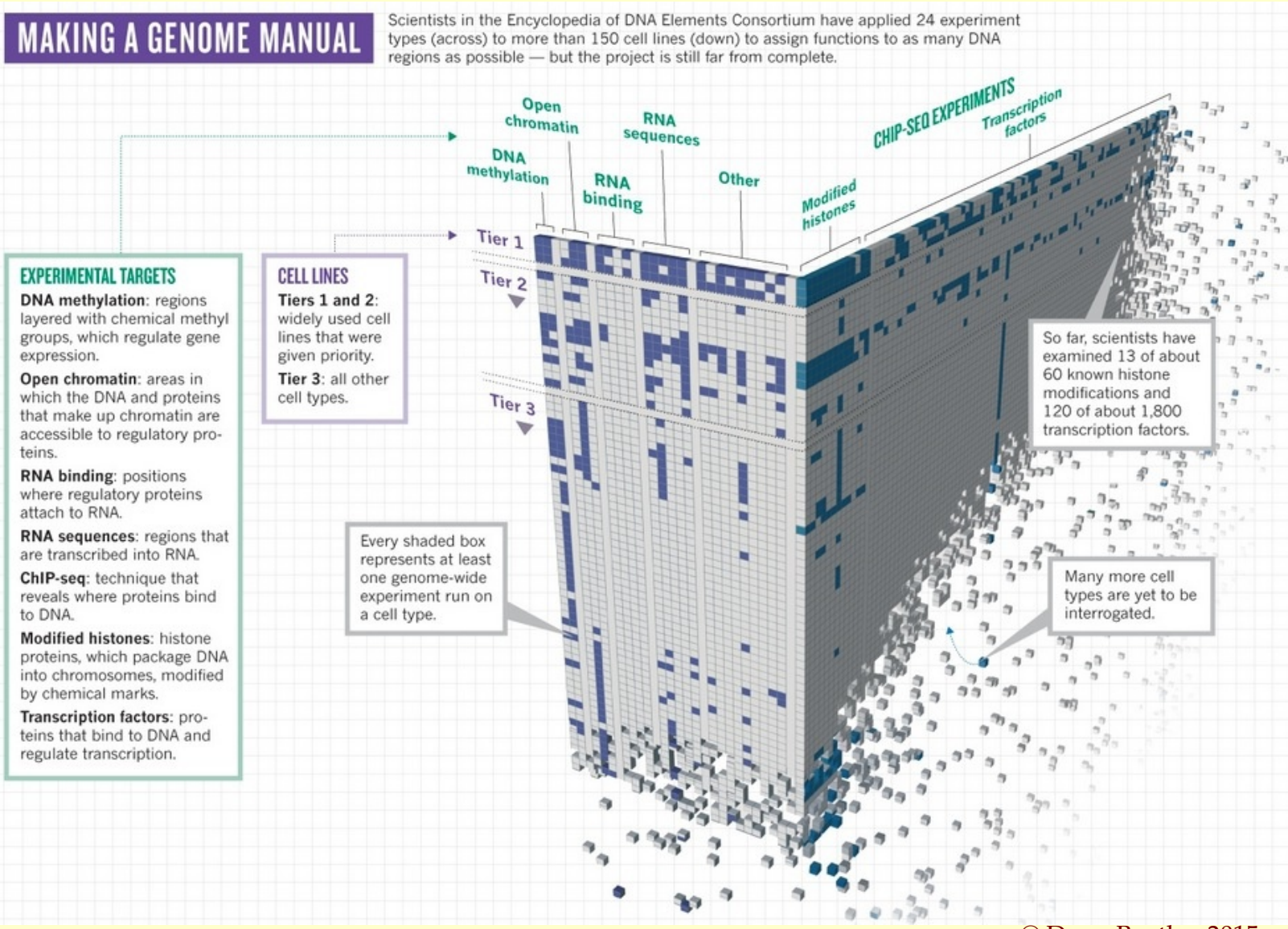
Making a Genome Manual

http://www.nature.com/news/encode-the-human-encyclopaedia-1.11312#/manual



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Epigenome Roadmap

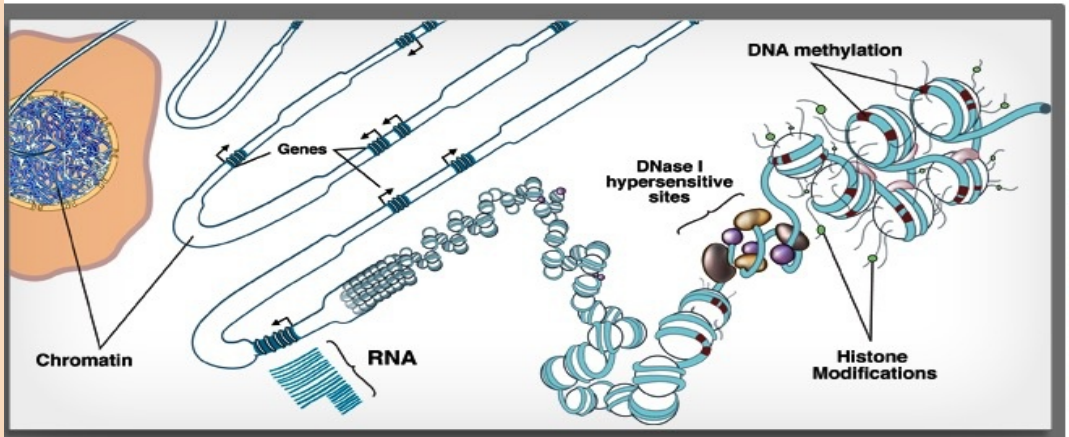
http://www.roadmapepigenomics.org/

ROADMAP epigenomics PROJECT

Search: GO

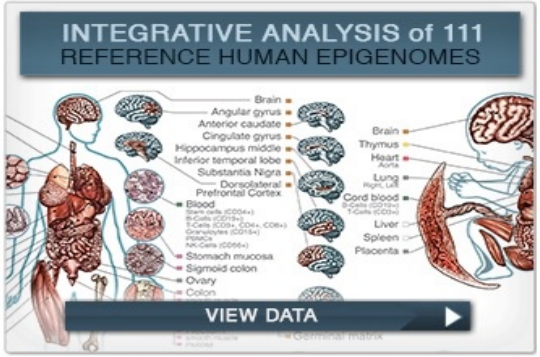
HOME | PARTICIPANTS | BROWSE DATA | PROTOCOLS | COMPLETE EPIGENOMES | TOOLS | PUBLICATIONS

OVERVIEW | PROJECT DATA | MAPPING CENTERS | PROTOCOLS & STANDARDS | PUBLICATIONS



NIH Roadmap Epigenomics Mapping Consortium

The NIH Roadmap Epigenomics Mapping Consortium was launched with the goal of producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research. The Consortium leverages experimental pipelines built around next-generation sequencing technologies to map DNA methylation, histone modifications, chromatin accessibility and small RNA transcripts in stem cells and primary ex vivo tissues selected to represent the normal counterparts of tissues and organ systems frequently involved in human disease. The Consortium expects to deliver a collection of normal epigenomes that will provide a framework or reference for comparison and integration within a broad array of future studies. The Consortium also aims to close the gap between data generation and its public dissemination by rapid release of raw sequence data, profiles of epigenomics features and higher-level integrated maps to the scientific community. The Consortium is also committed to the development, standardization and dissemination of protocols, reagents and analytical tools to enable the research community to utilize, integrate and expand upon this body of data.



VIEW/DOWNLOAD QUICK LINKS

Genome Browsers

- http://genomebrowser.wustl.edu/
- http://epigenomegateway.wustl.edu/

Data Repositories

- NCBI Epigenomics Gateway
- Epigenome Atlas

NEWS

18 FEB — NIH-supported researchers map epigenome of more than 100 tissue and cell types

4 SEP — IHEC data portal online

http://www.nature.com/nature/journal/v518/n7539/full/nature14248.html

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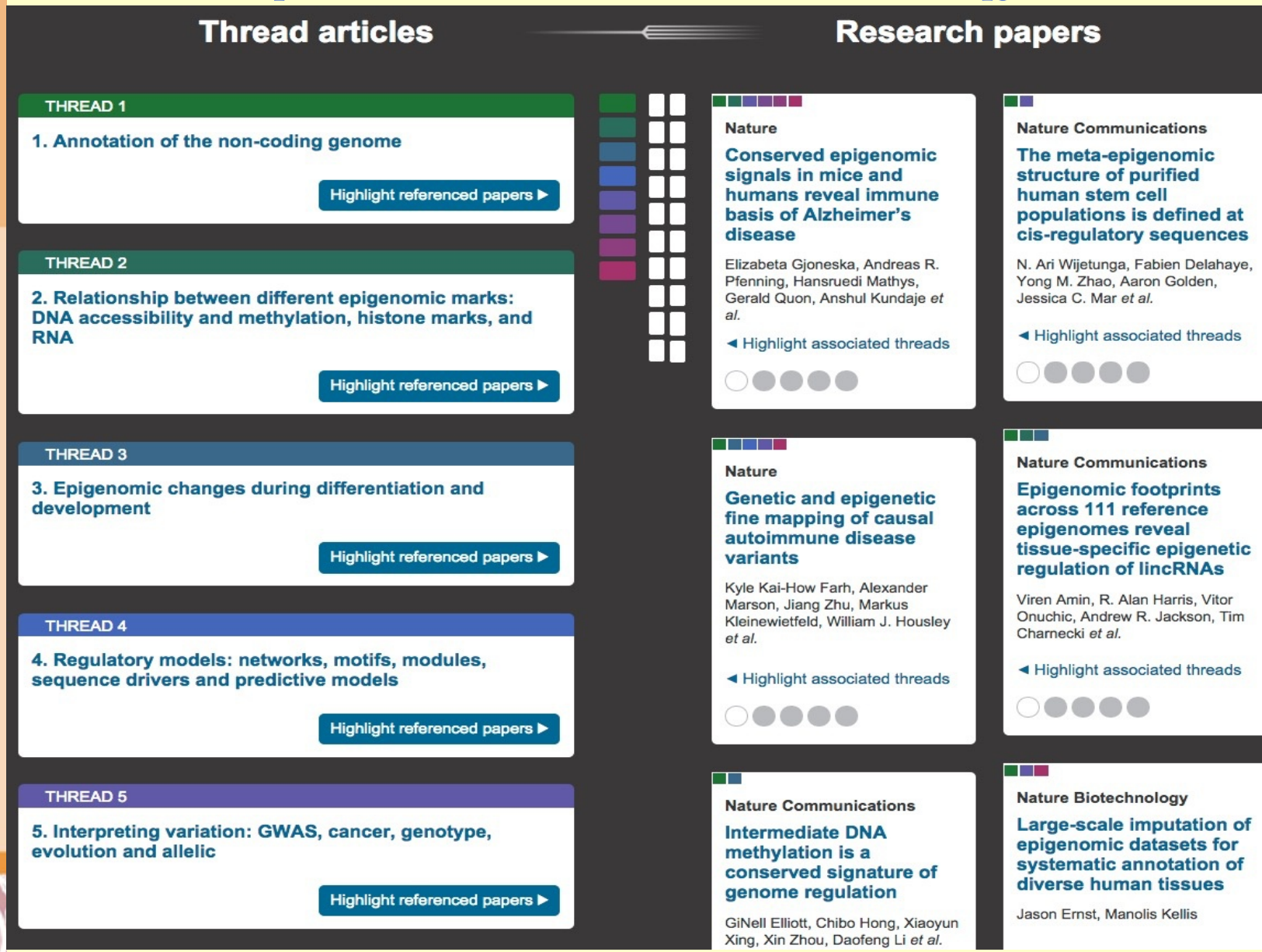
Epigenome Roadmap

http://www.nature.com/collections/vbqgtr



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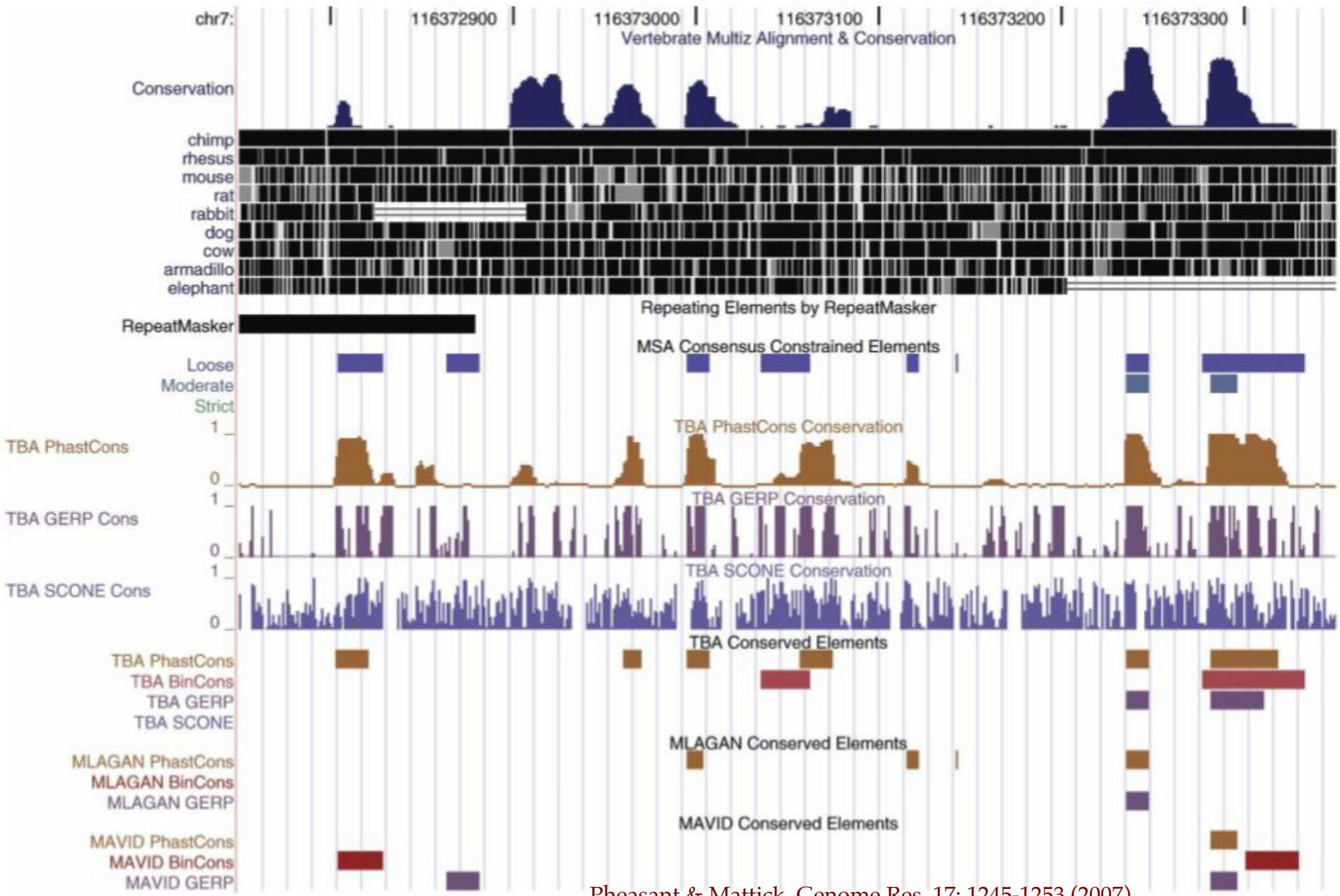
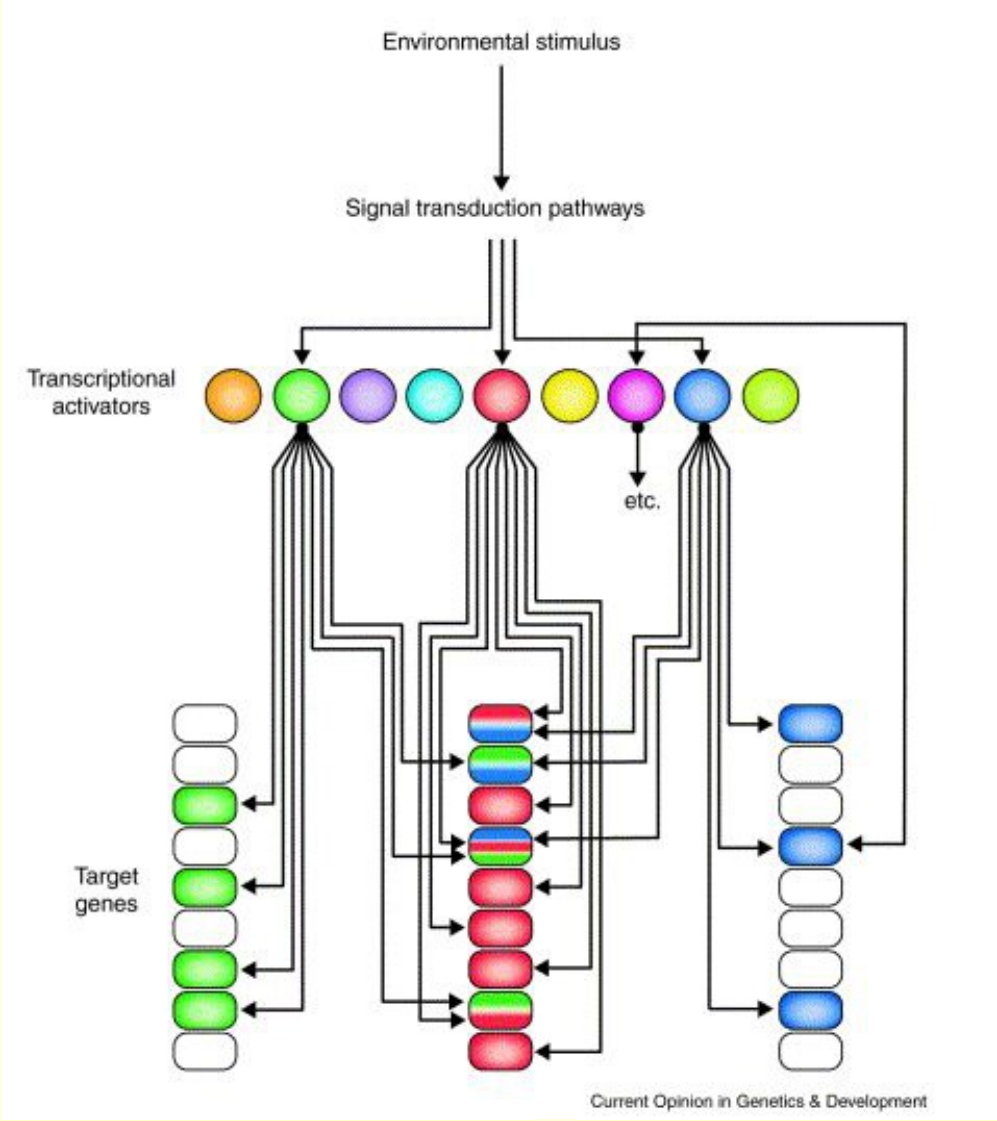
Conserved Regions in CFTR Intron



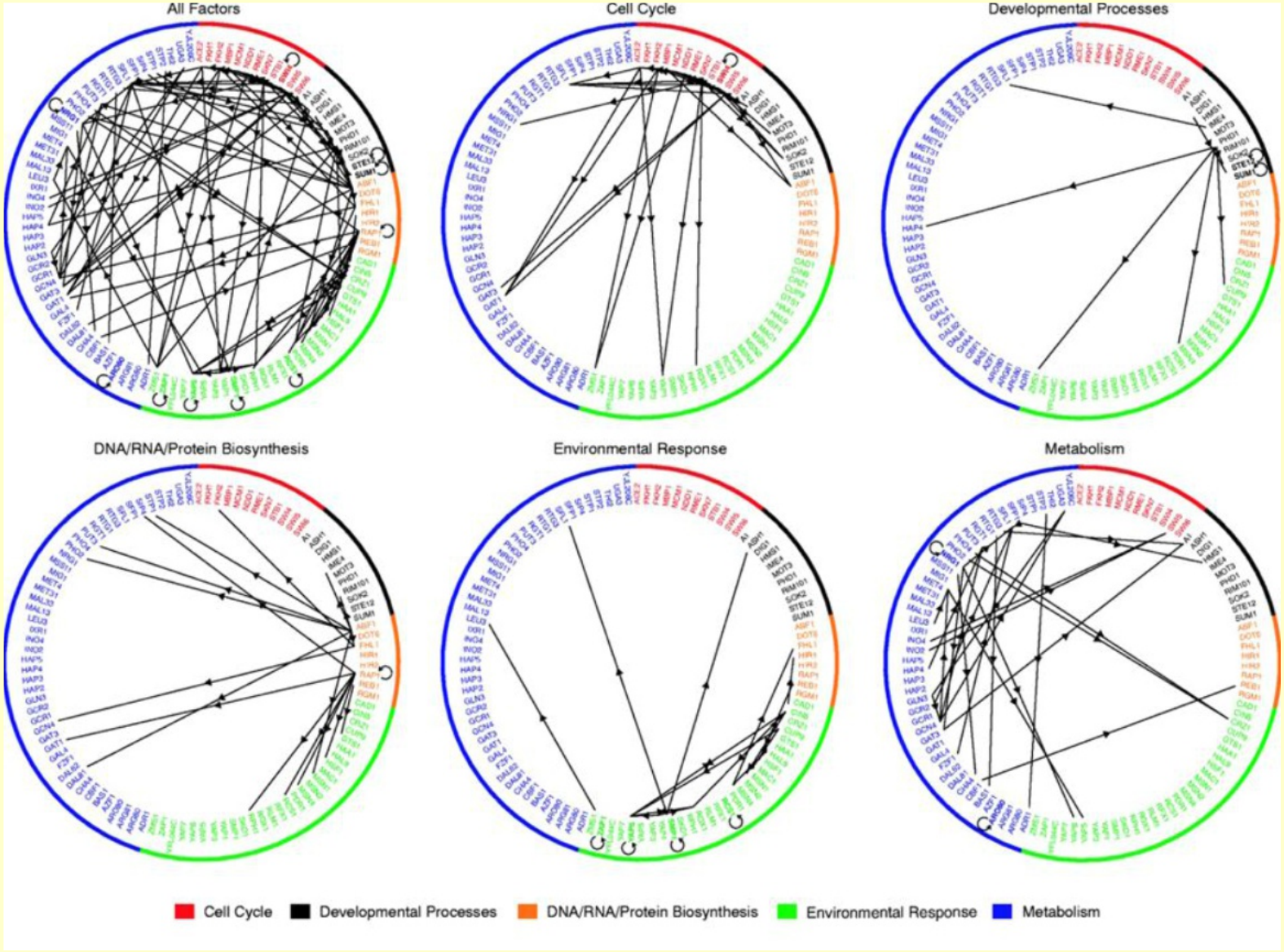
Pheasant & Mattick, Genome Res. 17: 1245-1253 (2007)

Gene Expression Regulatory Network



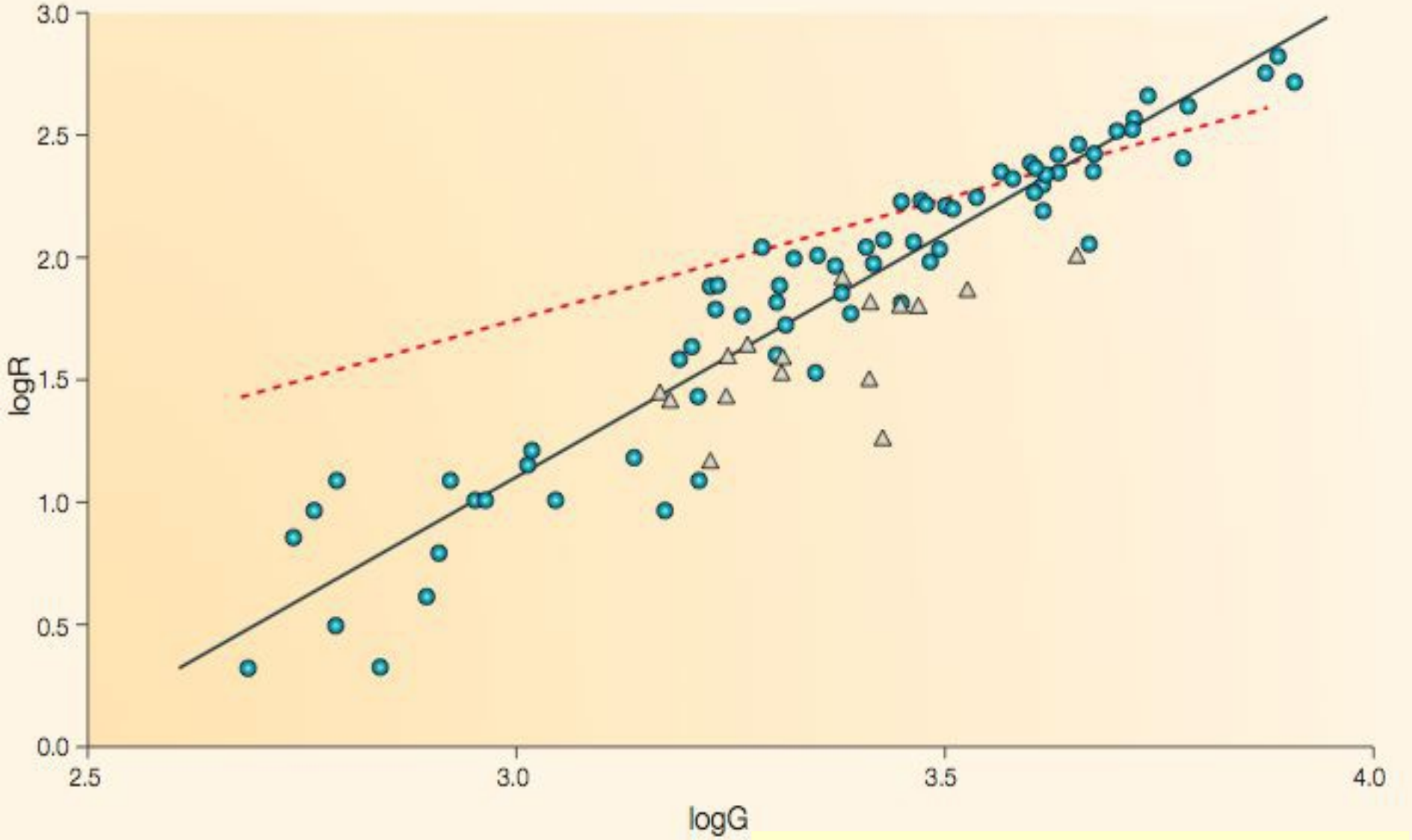
Current Opinion in Genetics & Development

Yeast Regulatory Motifs



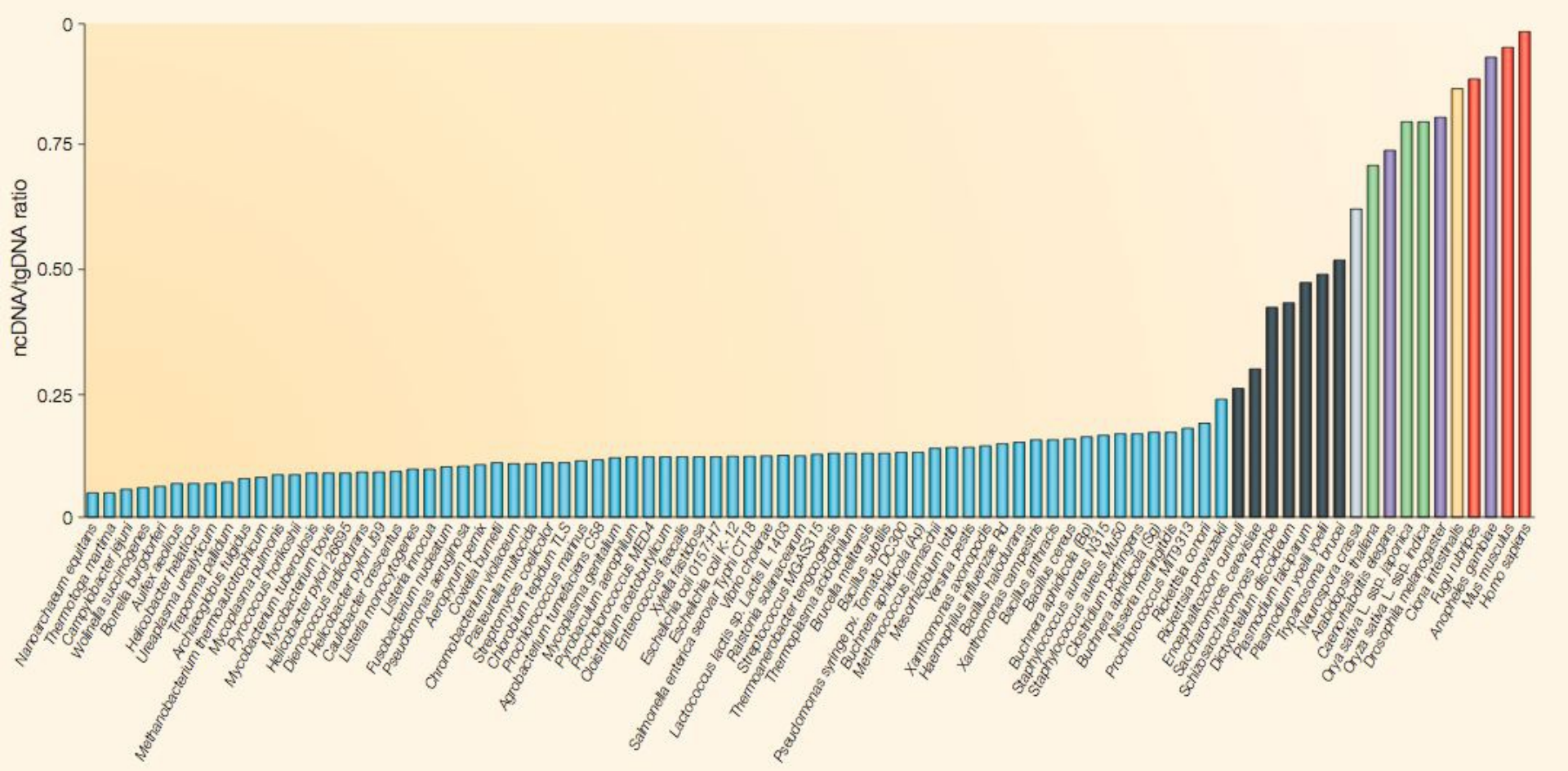
Lee et al. Science 298, 799-804 (2002)

Prokaryotic Regulatory Genes Grow as the Square of the Total Number of Genes



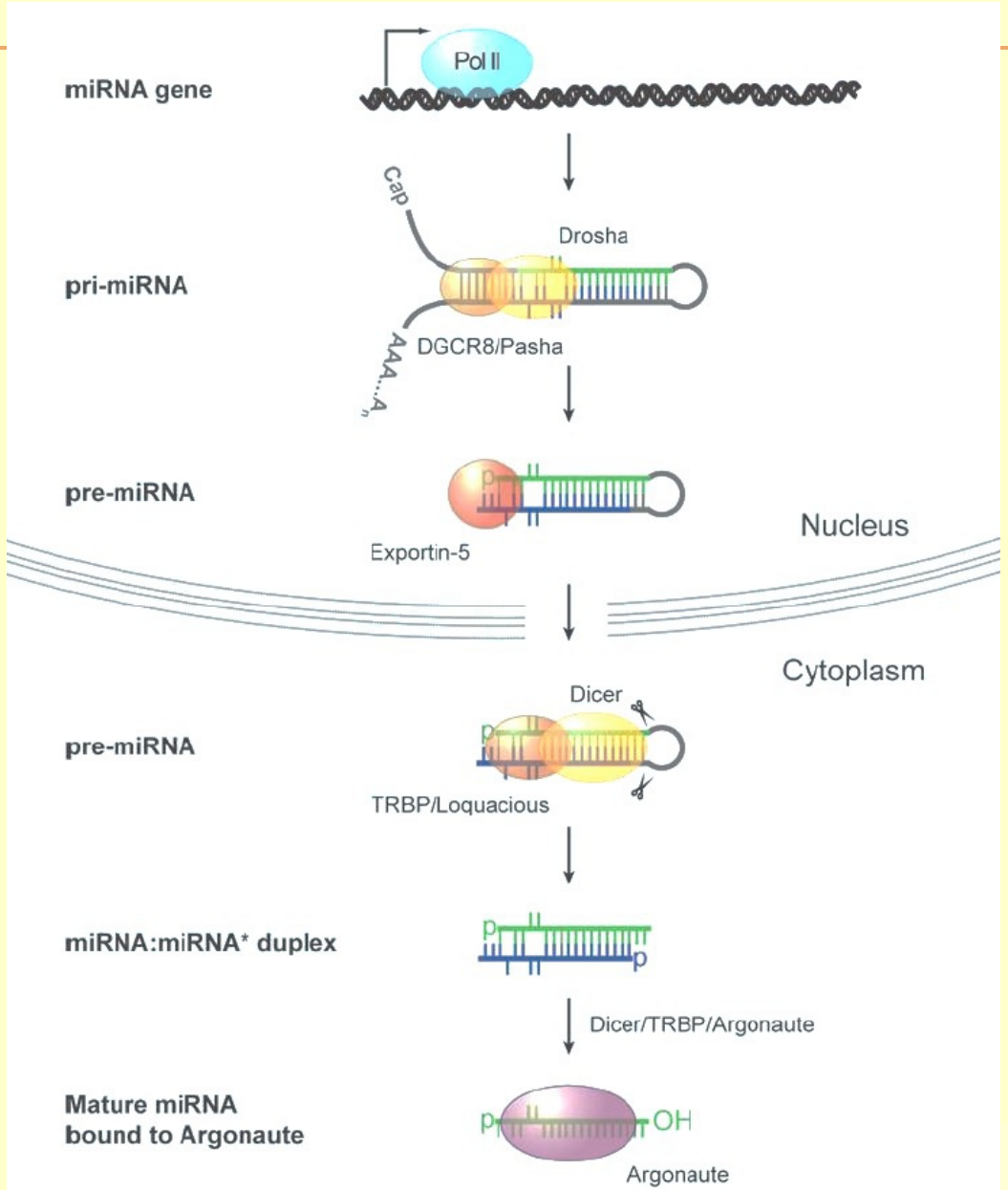
Mattick (2004) Nature Reviews Genetics 5: 316-323.

Fraction Non-Protein-Coding DNA During Evolution



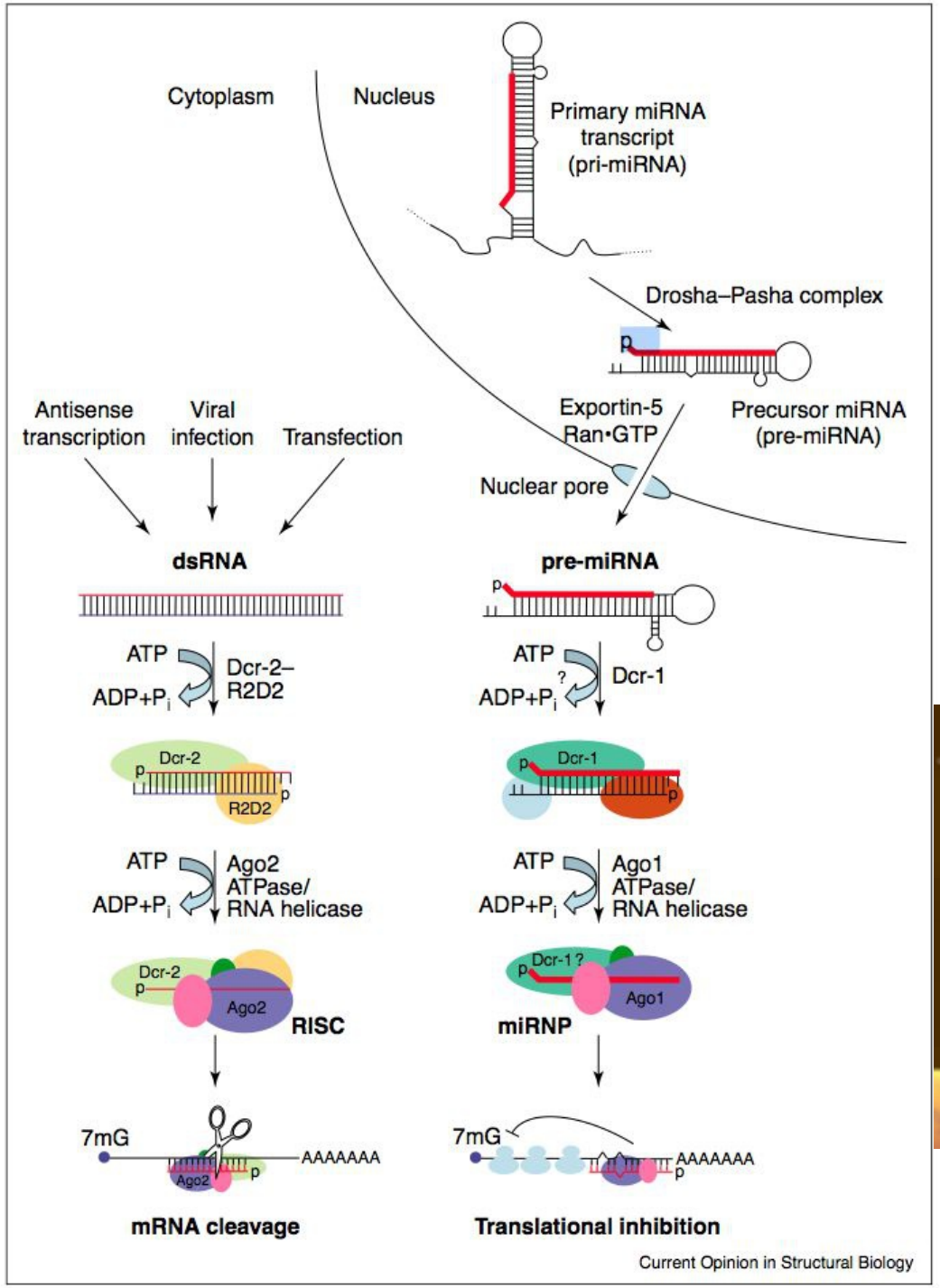
Mattick (2004) Nature Reviews Genetics 5: 316-323.

microRNA Biogenesis



Annu. Rev. Cell Dev. Biol. 2007.23:175-205

siRNA mediated degradation of mRNA & miRNA mediated inhibition of mRNA translation



Filipowicz, Curr. Op. Structural Biolog

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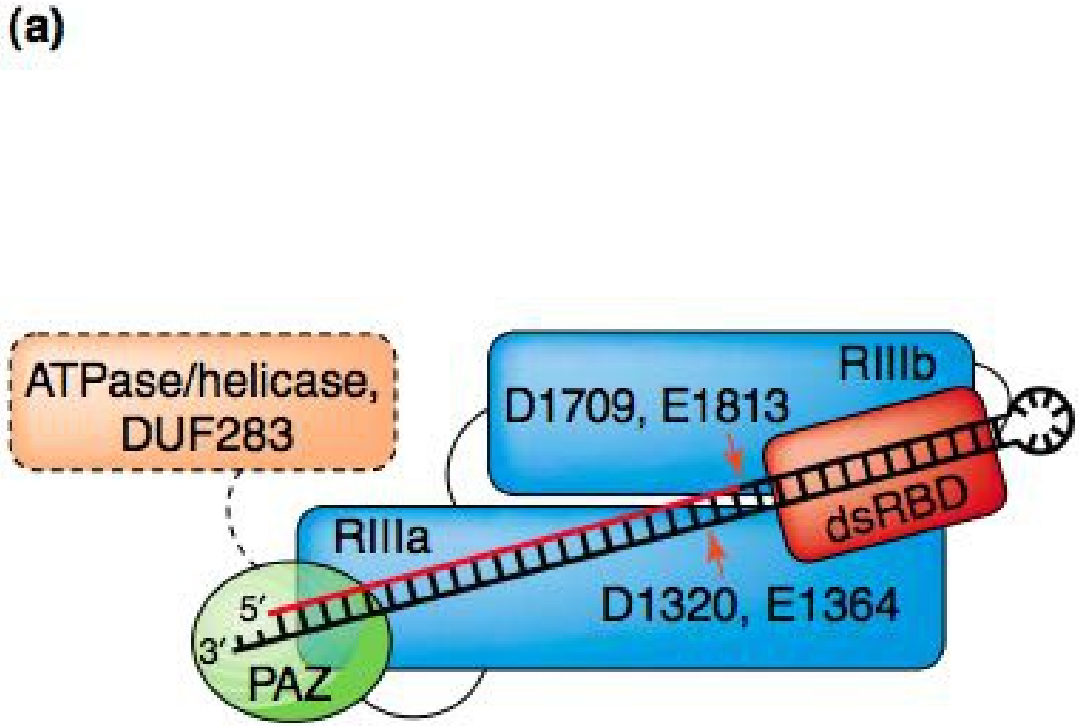
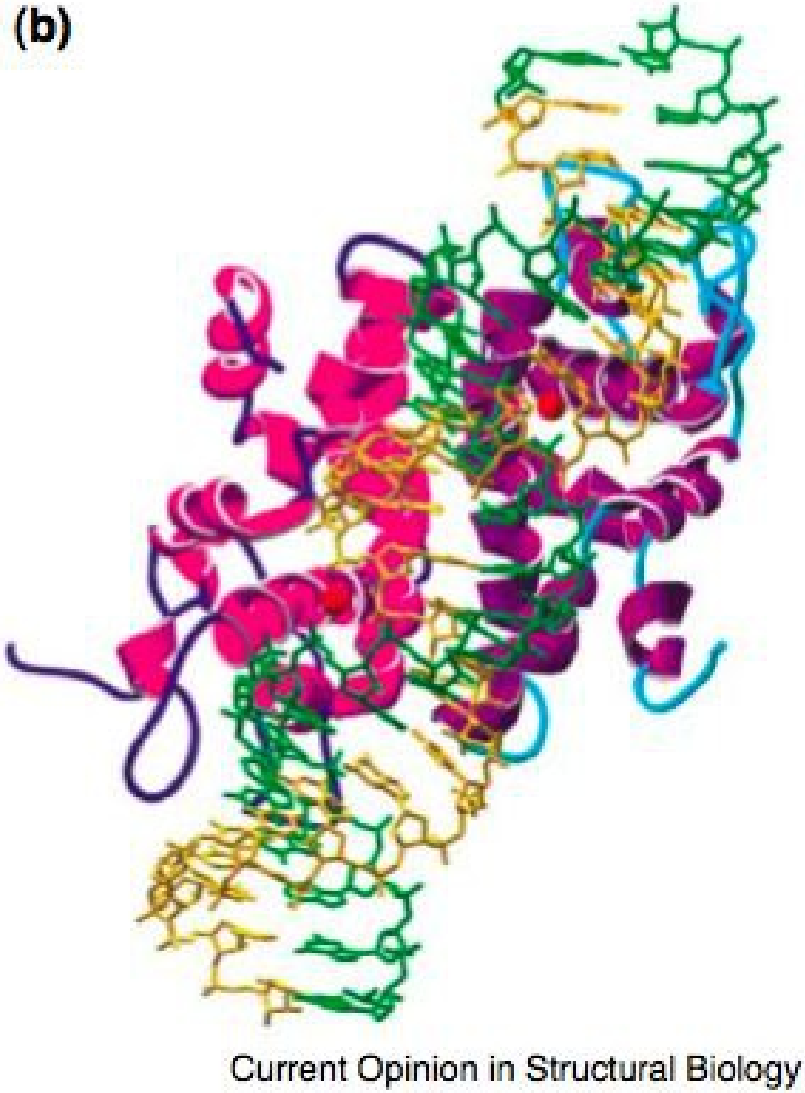
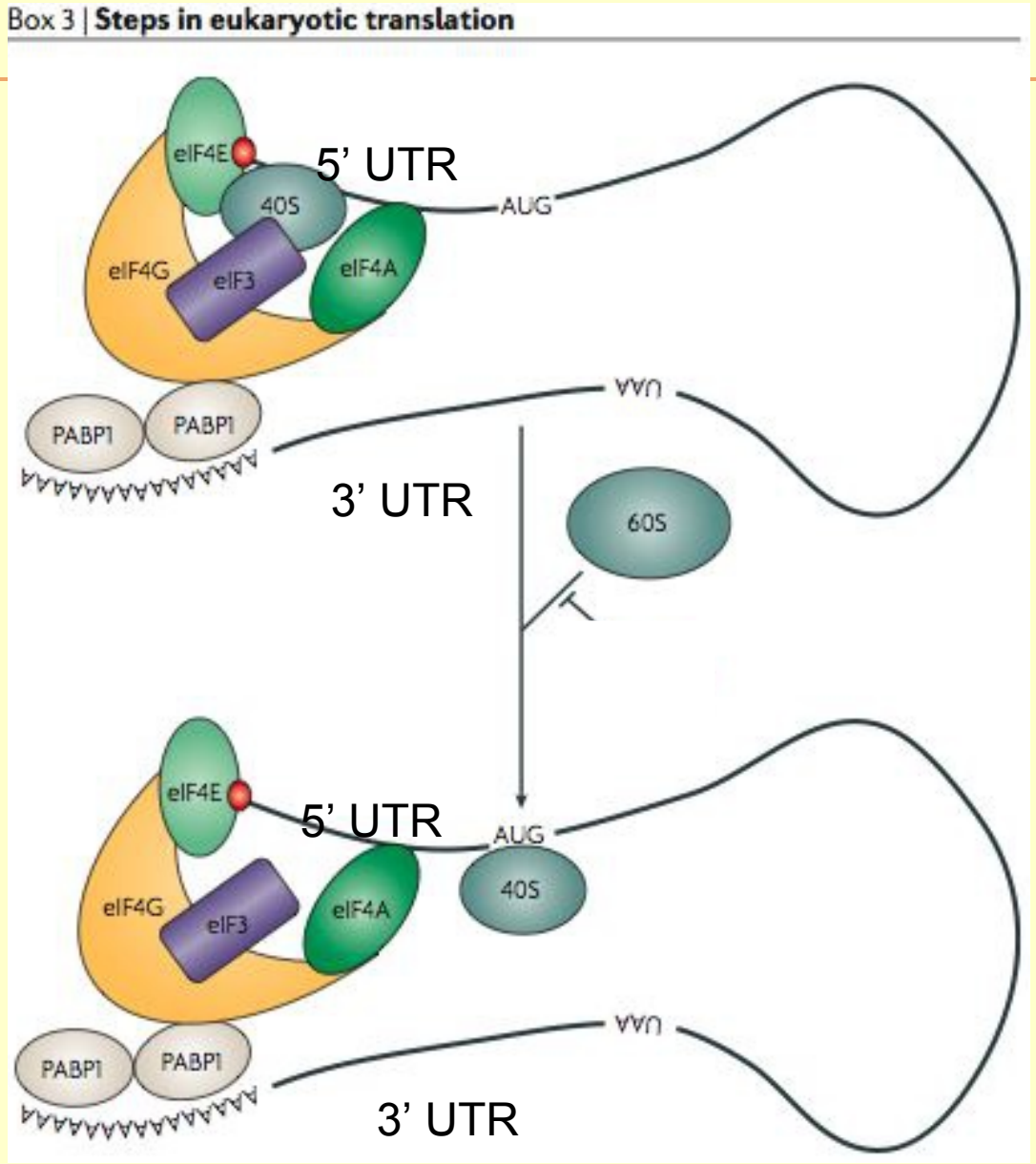
Dicer Structure & Function





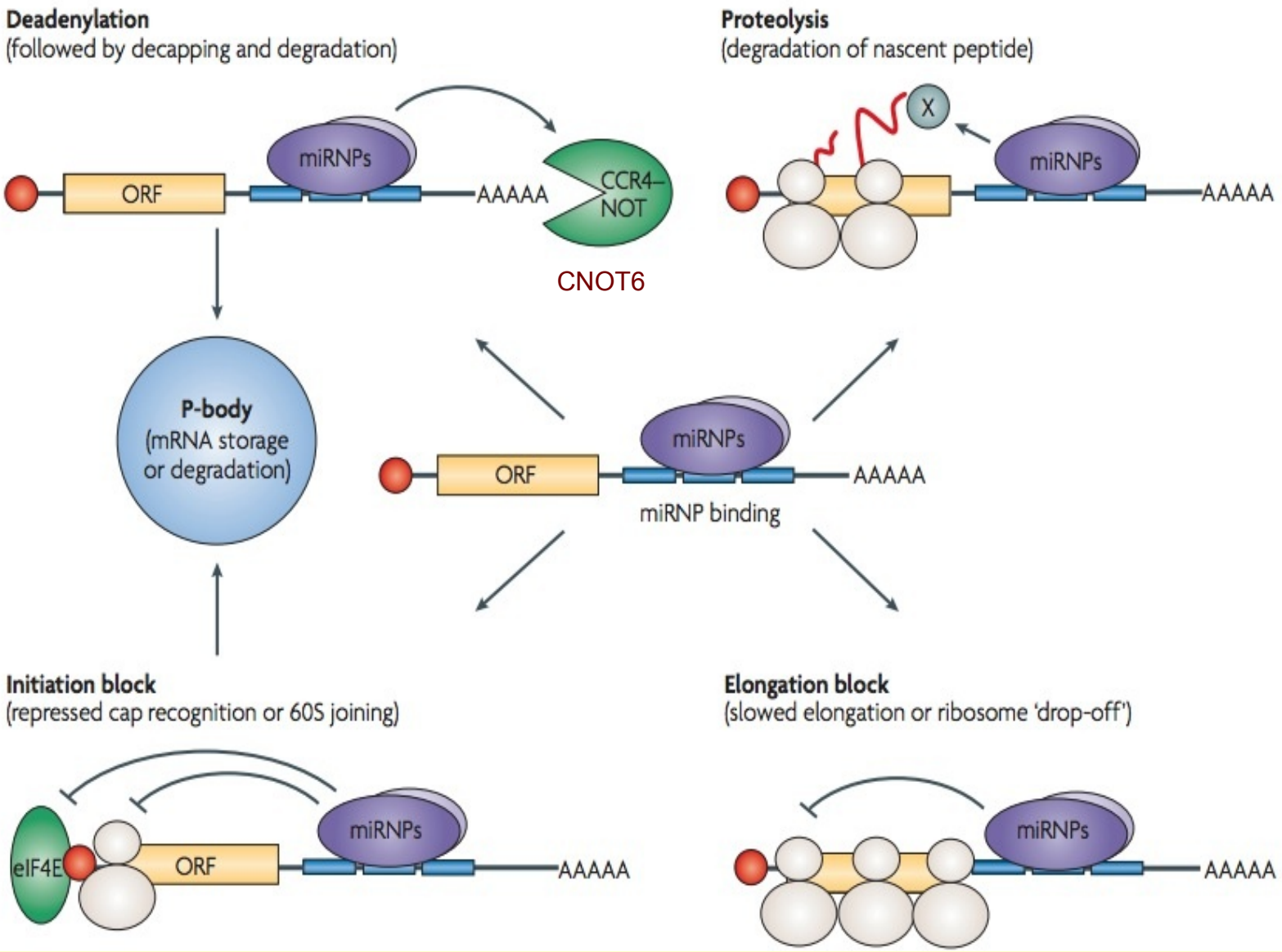
Filipowicz, Curr. Op. Structural Biology 15: 331-341 (2005)

Initiation of Translation



Filipowicz (2008) Nature Review Genetics 9:102-112.

Mechanisms of Translational Regulation by miRNP Complexes



Filipowicz (2008) Nature Review Genetics 9:102-112.

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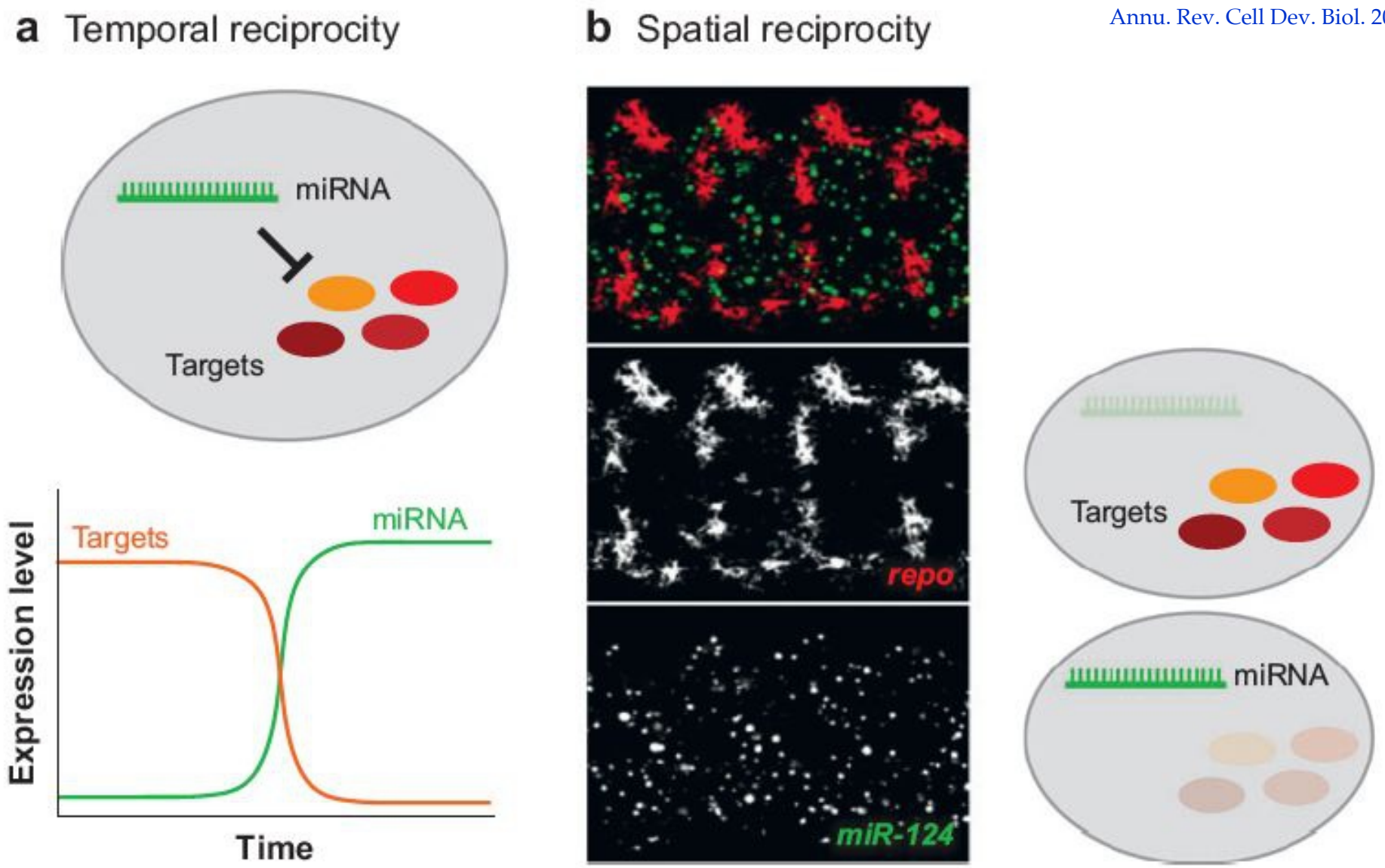
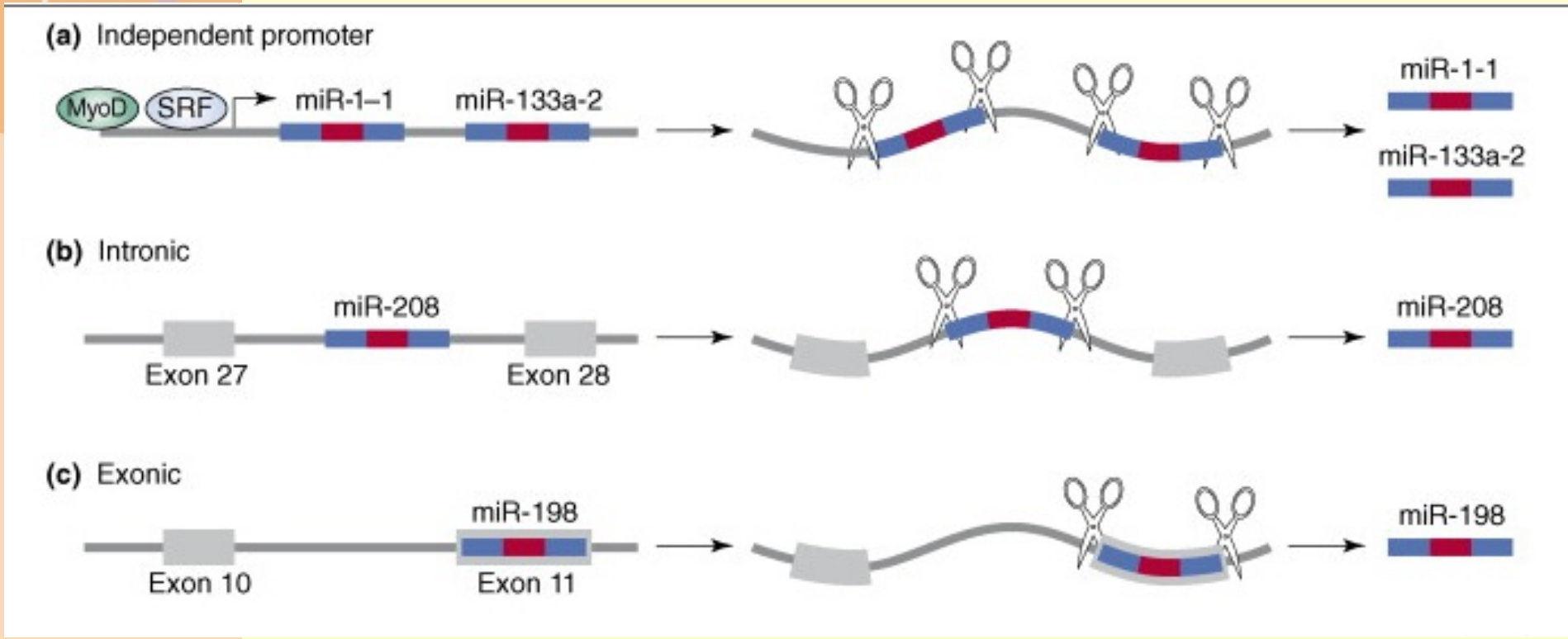
miRNA Expression Results in Temporal and Spatial Reciprocity with Target Expression

Annu. Rev. Cell Dev. Biol. 2007



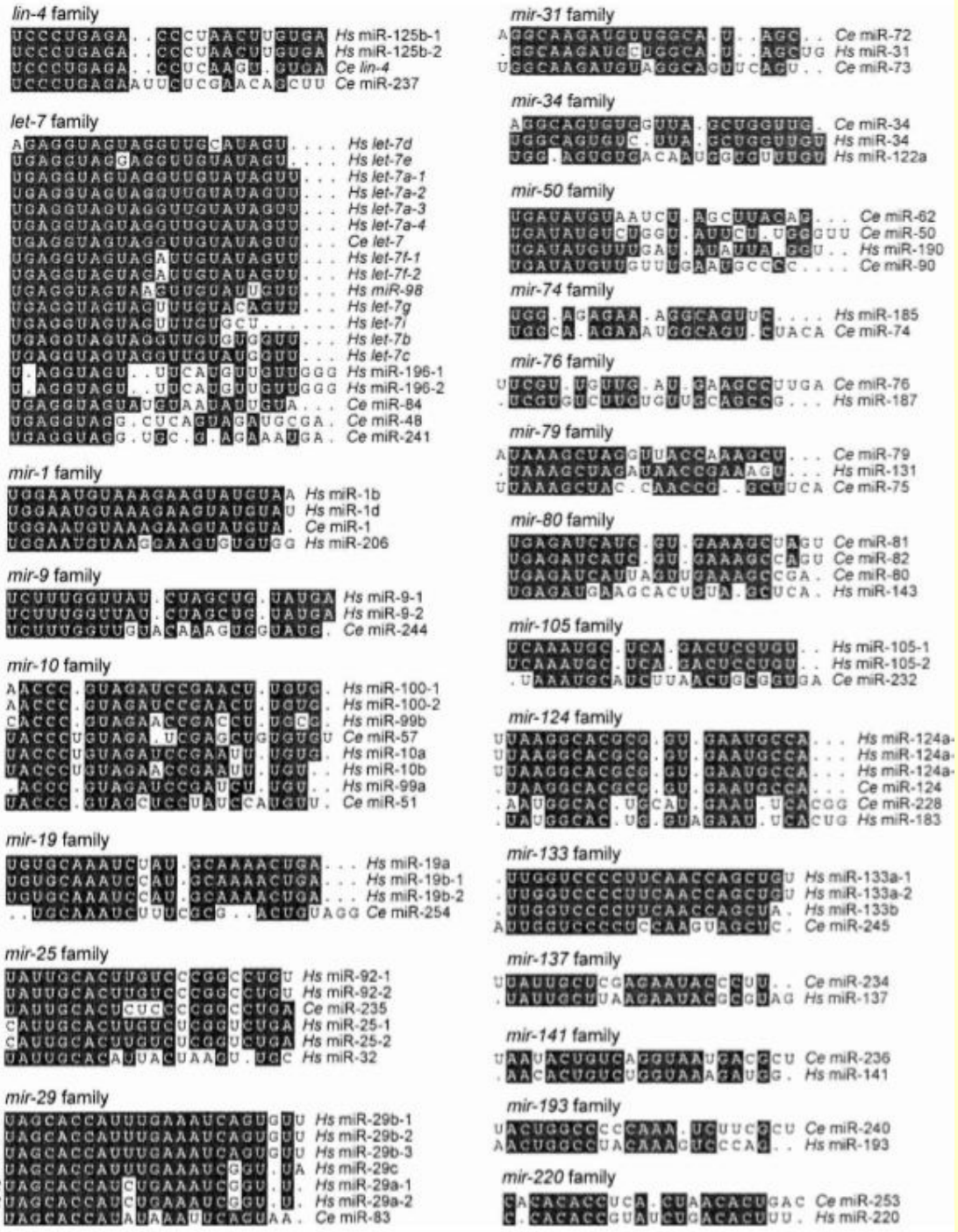
Genomic Organization of miRNA Genes



- Intronic miRNAs often in antisense direction, made from own promoter
- Exonic miRNAs - non-coding (or in alternatively spliced exons)

Zhao Y, Srivastava D, TIBS 32:189,2007

Homology Between *C. elegans* and *Homo sapiens* miRNAs



Lim (2003) Genes & Dev. 17: 991-1

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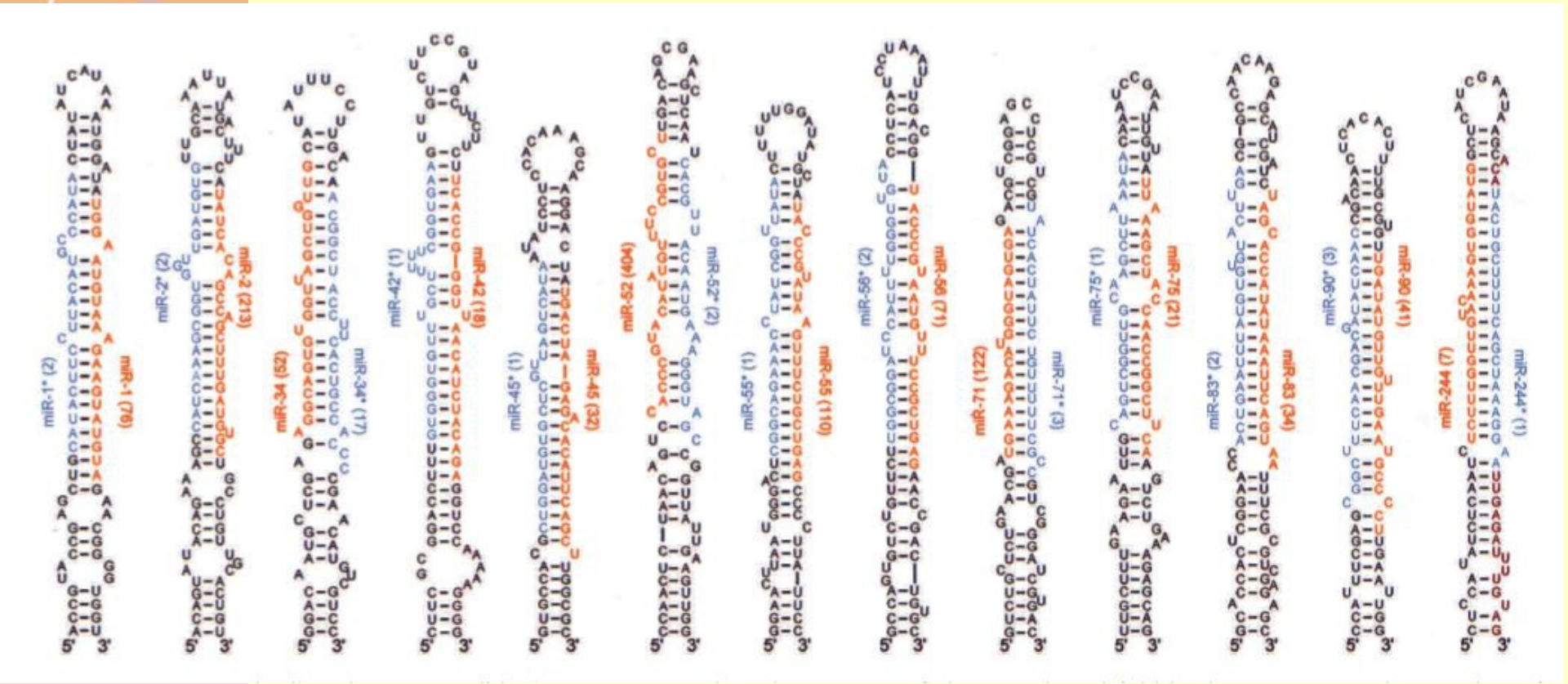
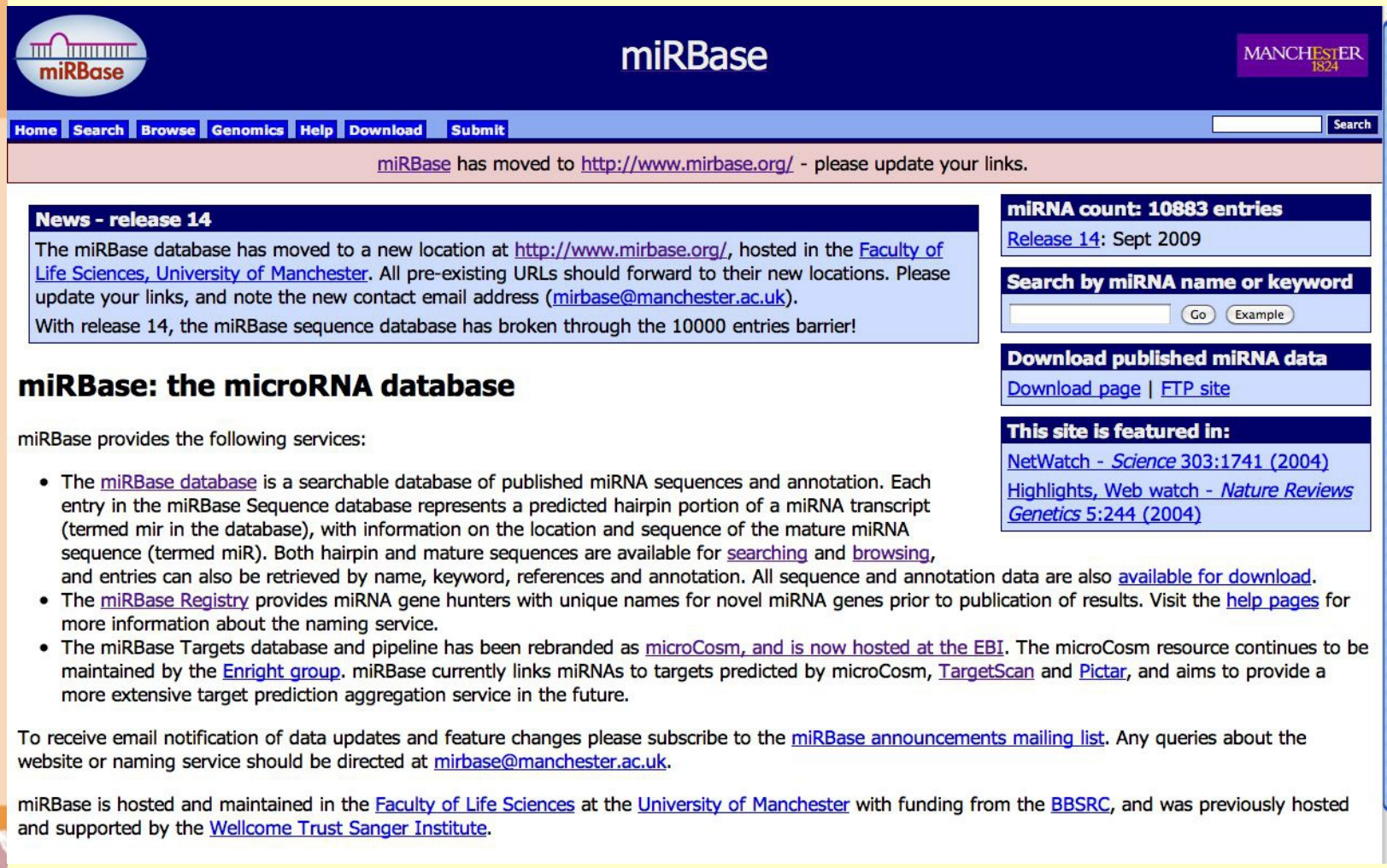
Predicted miRNA Precursors



miRBase

http://www.mirbase.org/



miRBase

MANCHESTER 1824

Home | Search | Browse | Genomics | Help | Download | Submit

Search

miRBase has moved to http://www.mirbase.org/ - please update your links.

News - release 14

The miRBase database has moved to a new location at http://www.mirbase.org/, hosted in the Faculty of Life Sciences, University of Manchester. All pre-existing URLs should forward to their new locations. Please update your links, and note the new contact email address (mirbase@manchester.ac.uk).

With release 14, the miRBase sequence database has broken through the 10000 entries barrier!

miRNA count: 10883 entries

Release 14: Sept 2009

Search by miRNA name or keyword

Go | Example

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

miRBase provides the following services:

- The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.
- The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.
- The miRBase Targets database and pipeline has been rebranded as microCosm, and is now hosted at the EBI. The microCosm resource continues to be maintained by the Enright group. miRBase currently links miRNAs to targets predicted by microCosm, TargetScan and Pictar, and aims to provide a more extensive target prediction aggregation service in the future.

To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.

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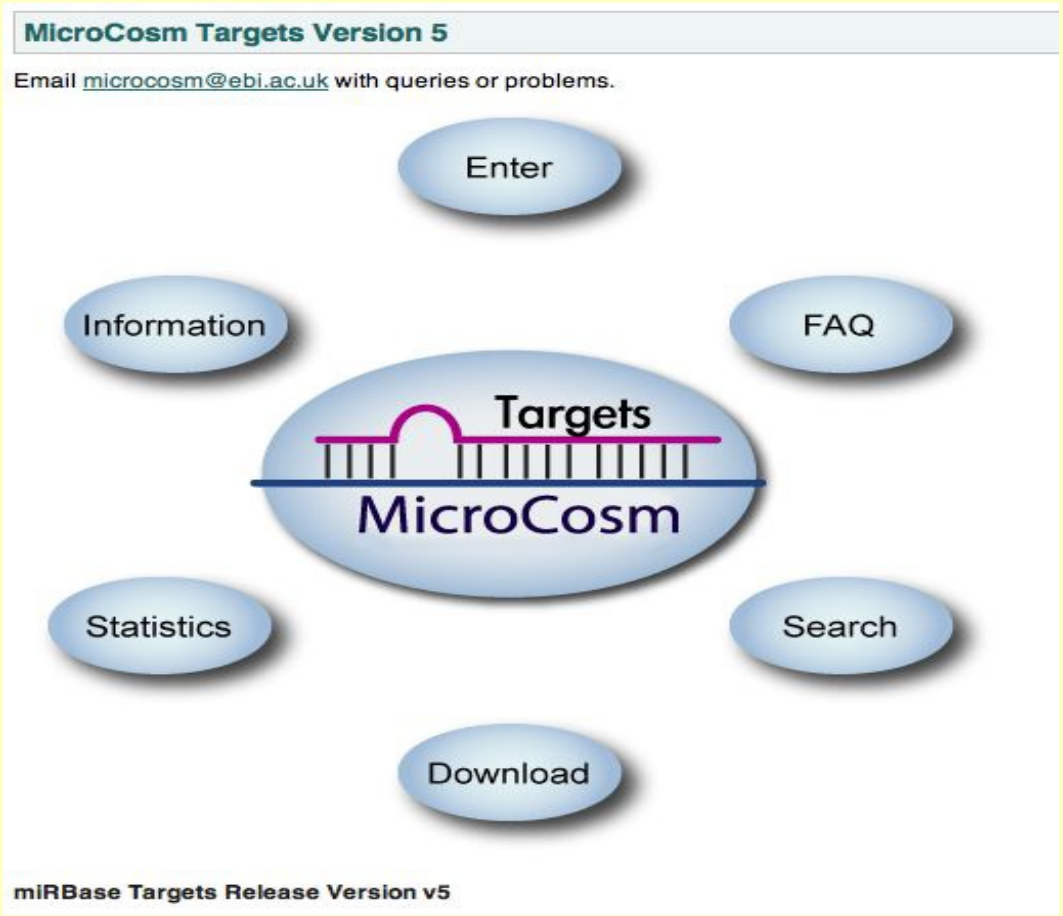
miRBase::MicroCosm miRNA Targets

http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/#



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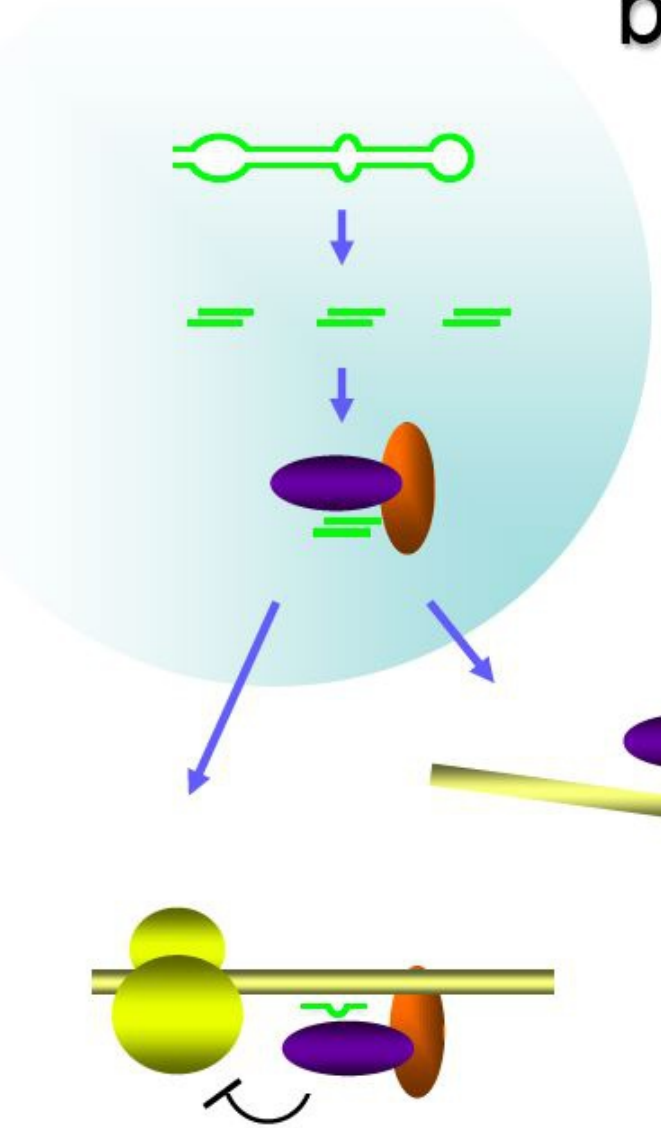
Human miRNAs (March 4, 2014)

- Total number of miRNAs known 24,521
- Number human miRNAs identified 2,652
- Number of human mRNA targets 34,788

- miRNAs can have multiple targets
- Target mRNAs can have multiple miRNA binding sites

miRBase @ http://www.mirbase.org/
MicroCosm @http://www.ebi.ac.uk/enright-srv/microcosm

Thousands of microRNAs act in multiple biological events



Developmental timing

Differentiation

Aging

Apoptosis

Metabolism

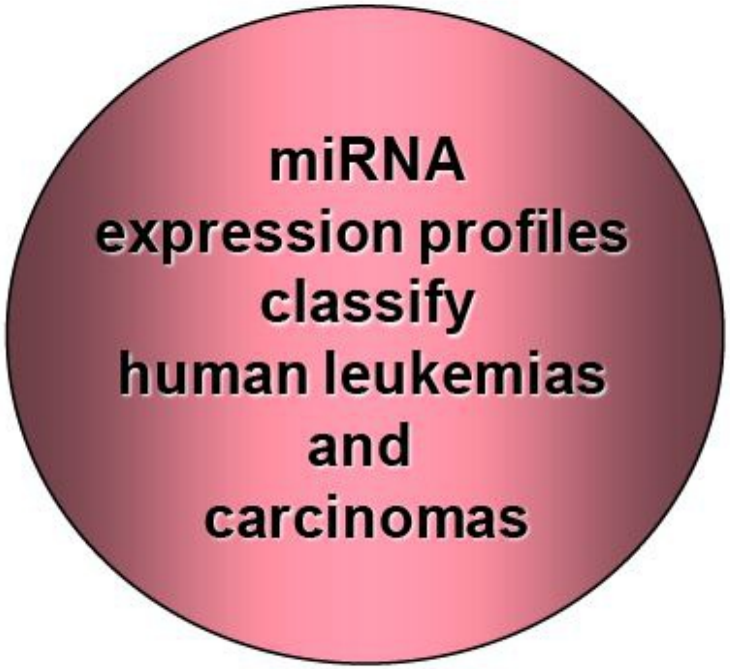
Cancer

…

Cell fate/differentiation, Cell cycle…

© Frank Slack frank.slack@yale.edu

ALTERATIONS OF MICRORNAS ARE FOUND IN EVERY TYPE OF HUMAN CANCER



(Calin et al, PNAS 2002; Lu et al, Nature, 2005; Volinia & Calin et al, PNAS 2006; Landgraf et al, Cell 2007)

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miRNAs Involved in Human Cancer

(Croce Nat Rev Genet. 2009 Oct;10(10):704-14.)

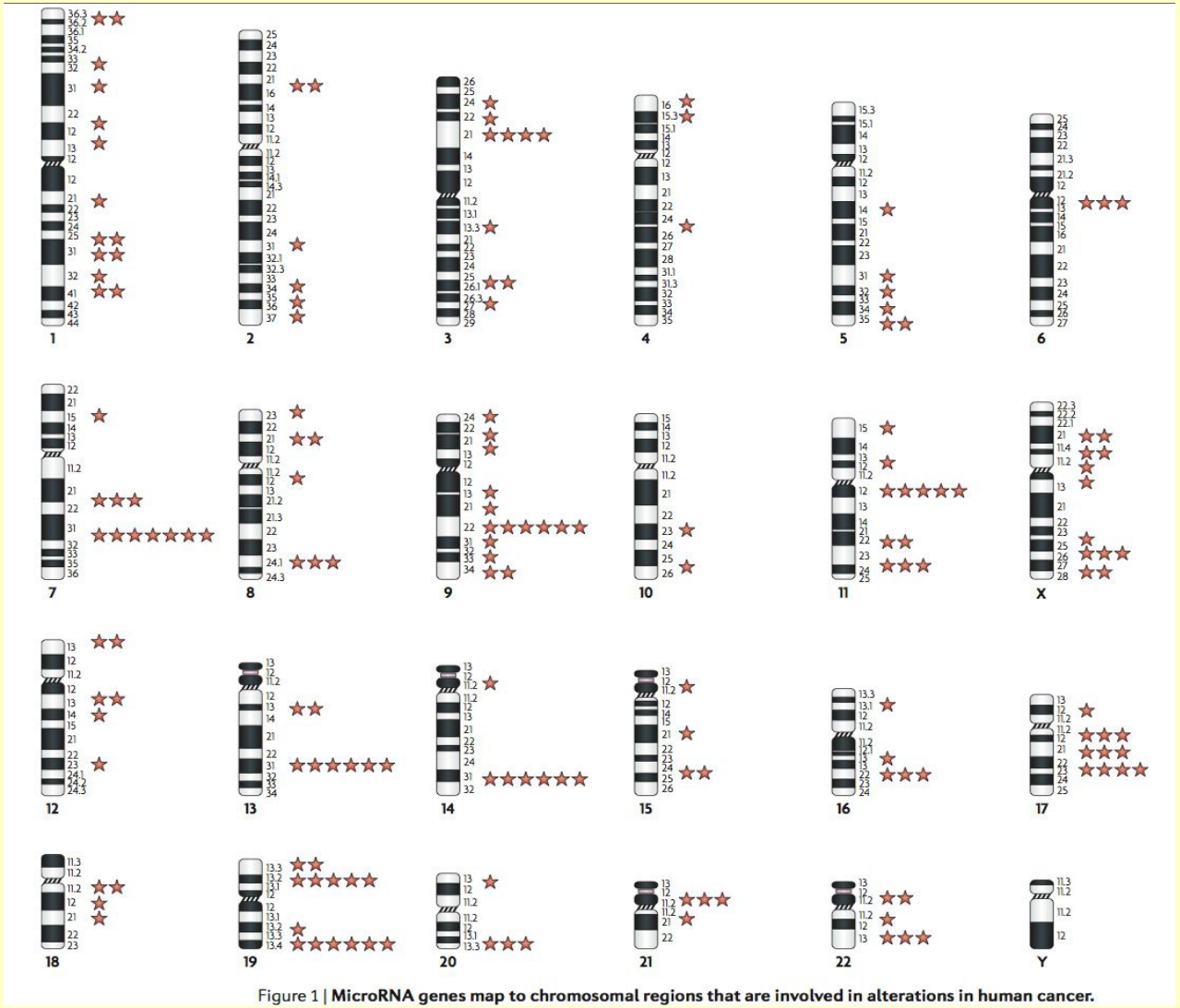
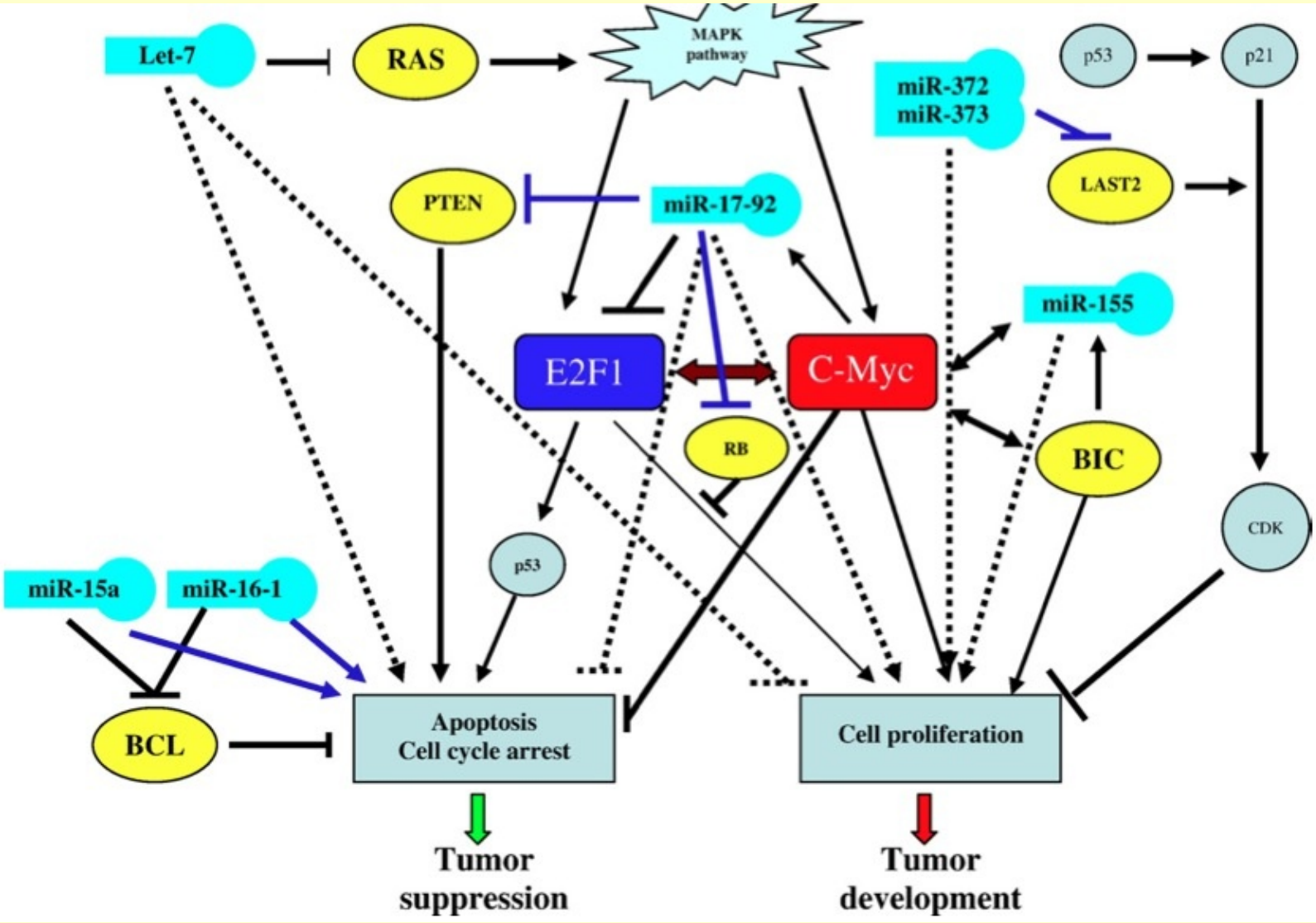


Figure 1 | **MicroRNA genes map to chromosomal regions that are involved in alterations in human cancer.**

Croce Nat Rev Genet. 2009 Oct;10(10):704-14

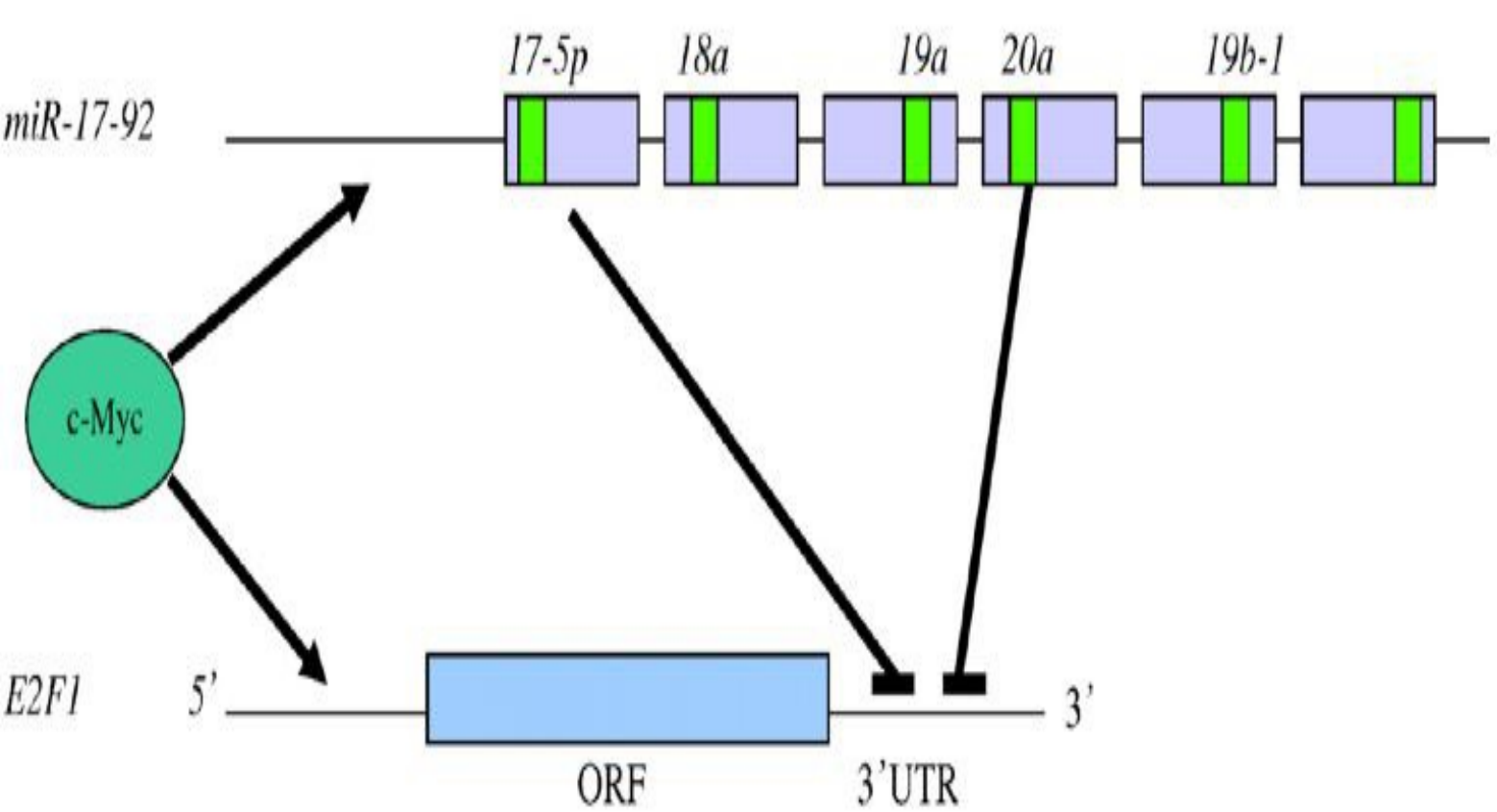
miRNAs as Oncogenes and Tumor Supressors

Zhang et al Dev Biol. 2007 Feb 1;302(1):1-12



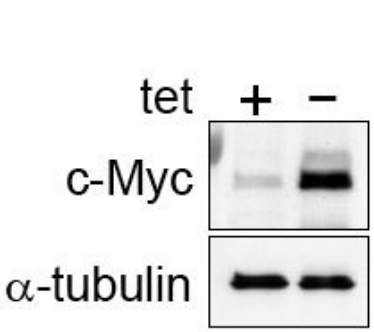
Zhang et al Dev Biol. 2007 Feb 1;302(1):1-12

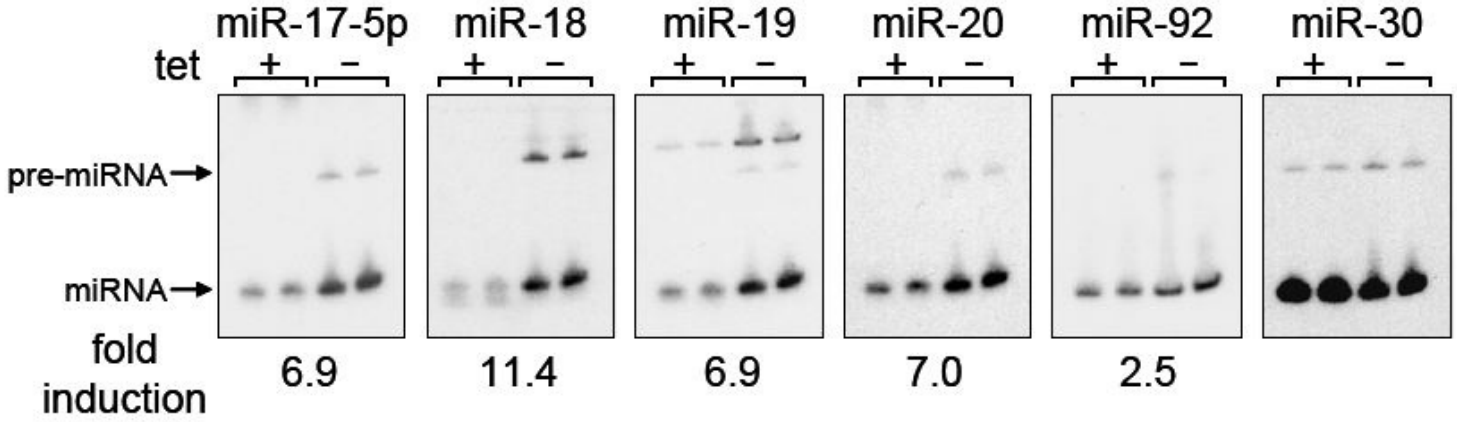
cMyc & miRNAs Regulate E2F1 Cell Cycle TF
Zhang et al Dev Biol. 2007 Feb 1;302(1):1-12



Zhang et al Dev Biol. 2007 Feb 1;302(1):1-12

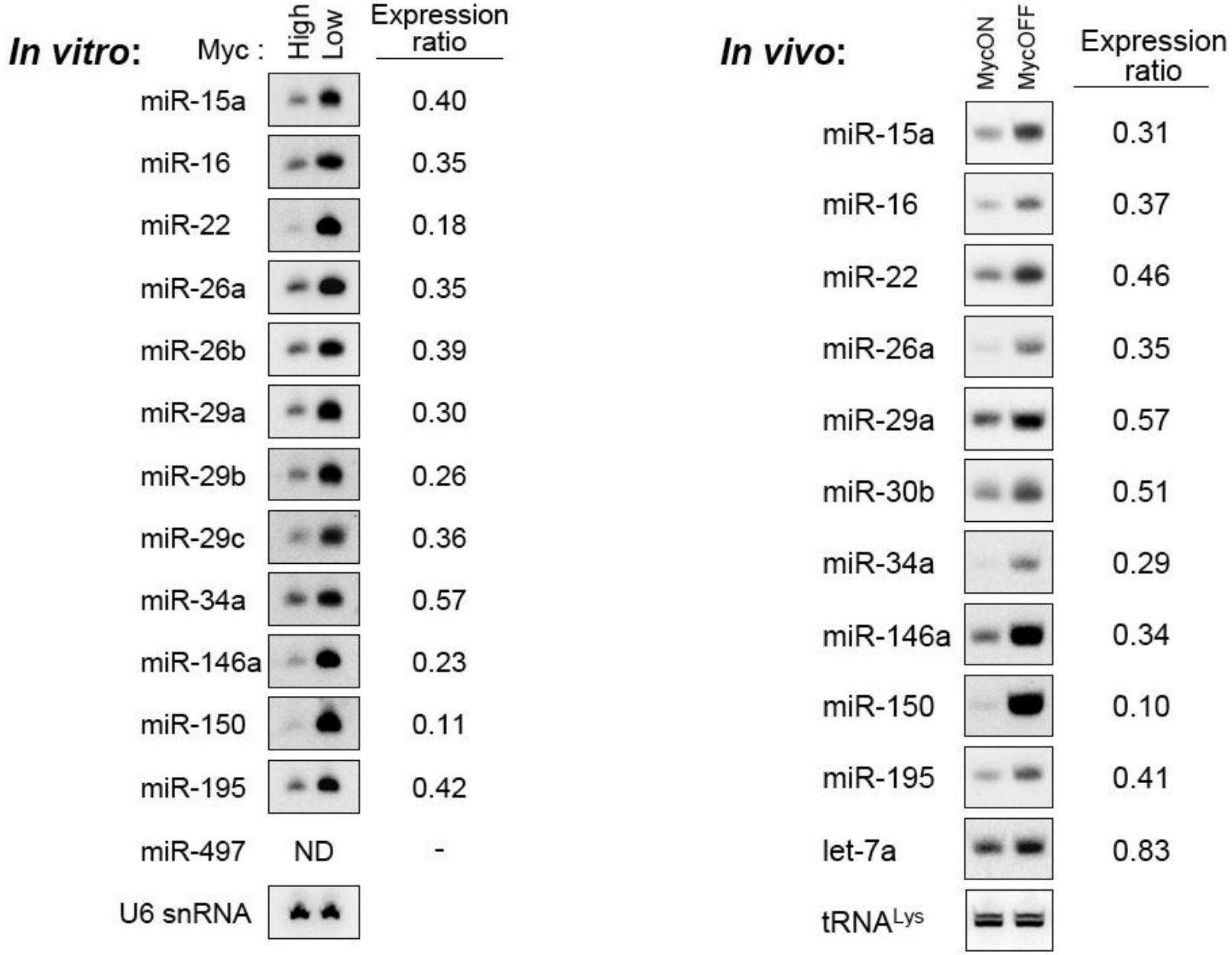
The miR-17-92 cluster is activated by Myc





O'Donnell et al. Nature 2005 435, 839-843

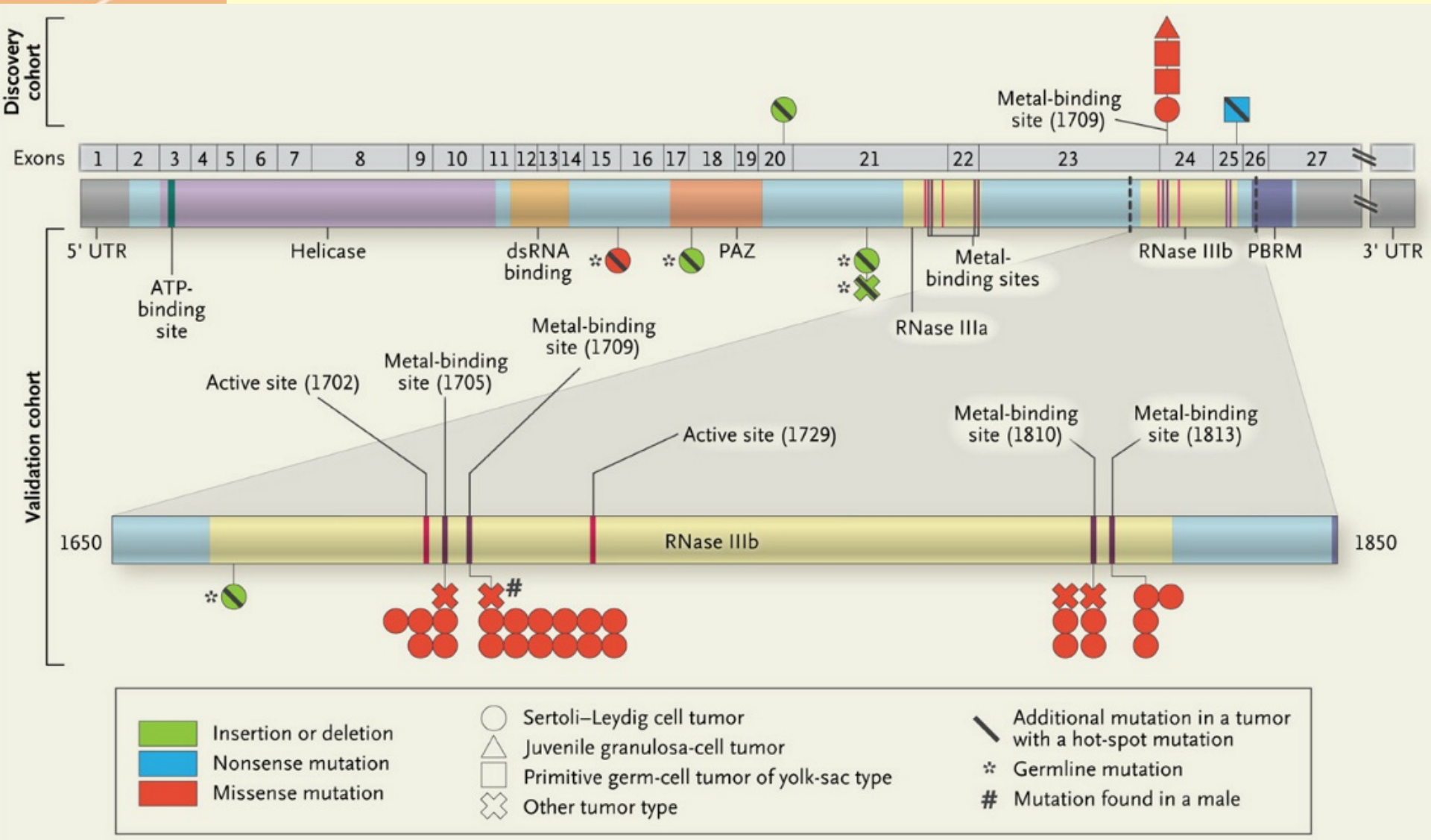
Myc represses the expression of many miRNAs



Chang *et al. Nature Genetics*, 2008

© George Calin

DICER1 mutations in cancers



N Engl J Med 2012; 366:234-242January 19, 2012

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miRNA Oncogenes or Tumor Suppressor Genes (Croce Nat Rev Genet. 2009 Oct;10(10):704-14.)

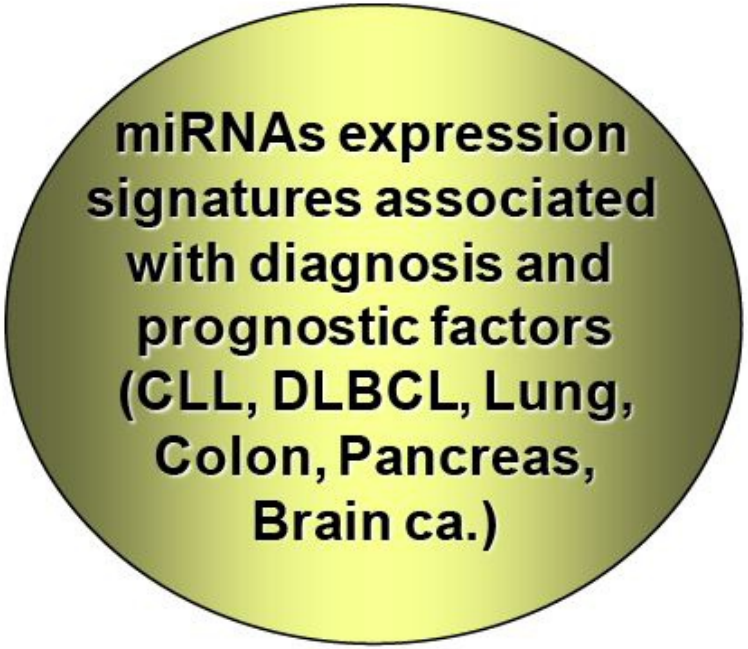
Table 1 | **MicroRNAs that function as oncogenes or tumour suppressor genes in human cancers**

MicroRNA	Dysregulation	Function	Validated targets	Oncogene (ONC) or tumour suppressor (TS)	Refs
miR-15a and *miR-16-1*	Loss in CLL, prostate cancer and multiple myeloma	Induces apoptosis and inhibits tumorigenesis	BCL2, WT1 RAB9B and MAGE83	TS	15,20,23, 30,52,69
let-7 (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *i*)	Loss in lung and breast cancer and in various solid and haematopoietic malignancies	Induces apoptosis and inhibits tumorigenesis	RAS, MYC and HMGA2	TS	22,26, 42,70
miR-29 (*a*, *b* and *c*)	Loss in aggressive CLL, AML (11q23), MDS lung and breast cancers and cholangocarcinoma	Induces apoptosis and inhibits tumorigenicity. Reactivates silenced tumour suppressor genes	TCL1, MCL1 and DNMTs	TS	30,64, 71,72
miR-34	Loss in pancreatic, colon, breast and liver cancers	Induces apoptosis	CDK4, CDK6, cyclin E2, EZF3 and MET	TS	56–58
miR-145	Loss in breast cancer	Inhibits proliferation and induces apoptosis of breast cancer cells	ERG	TS	31
miR-221 and *miR-222*	Loss in erythroblastic leukaemia	Inhibits proliferation in erythroblasts	KIT	TS	30
miR-221 and *miR-222*	Overexpression in aggressive CLL, thyroid carcinoma and hepatocellular carcinoma	Promotes cell proliferation and inhibits apoptosis in various solid malignancies	p27, p57, PTEN and TIMP3	ONC	43,51,73
miR-155	Upregulated in aggressive CLL, Burkitt's lymphoma and lung, breast and colon cancers	Induces cell proliferation and leukaemia or lymphoma in mice	MAF and SHIP1	ONC	32–34, 36,37
miR-17–92 cluster	Upregulated in lymphomas and in breast, lung, colon, stomach and pancreatic cancers	Induces proliferation	E2F1, BIM and PTEN	ONC	19,34,35, 40,41
miR-21	Upregulated in glioblastomas, AML (11q23), aggressive CLL and breast, colon, pancreatic, lung, prostate, liver and stomach cancers	Inhibits apoptosis and increases tumorigenicity	PTEN, PDCD4, TPM1 and TIMP3	ONC	31,37–39, 44–50
miR-372 and *miR-373*	Upregulated in testicular tumours	Promotes tumorigenicity in cooperation with RAS	LATS2	ONC	74

AML, acute myeloid leukaemia; BCL2, B cell leukaemia/lymphoma 2; BIM, Bcl2-interacting mediator of cell death; CLL, chronic lymphocytic leukaemia; DNMT, DNA methyltransferase; HMGA2, high mobility group AT-hook 2; LATS2, large tumour suppressor homologue 2; MCL1, myeloid cell leukaemia sequence 1; MDS, myelodysplastic syndrome; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; SHIP1, SH2 domain-containing inositol-5′-phosphatase 1; TCL1, T cell lymphoma breakpoint 1; TIMP3, tissue inhibitor of metalloproteinases 3; TPM1, tropomyosin 1; WT1, Wilms tumour 1.

Croce Nat Rev Genet. 2009 Oct;10(10):704-14

MIRNA PROFILING AS A NEW DIAGNOSTIC & PROGNOSTIC TOOL FOR CANCER PATIENTS



(Michael et al, Molec Cancer Res 2003; Lu et al, Nature, 2005; Eis et al, PNAS, 2005
Lui et al, Cancer Res 2007, Bloomston et al, JAMA 2007; Mi et al, PNAS, 2007; Garzon et al, Blood in press 2008)

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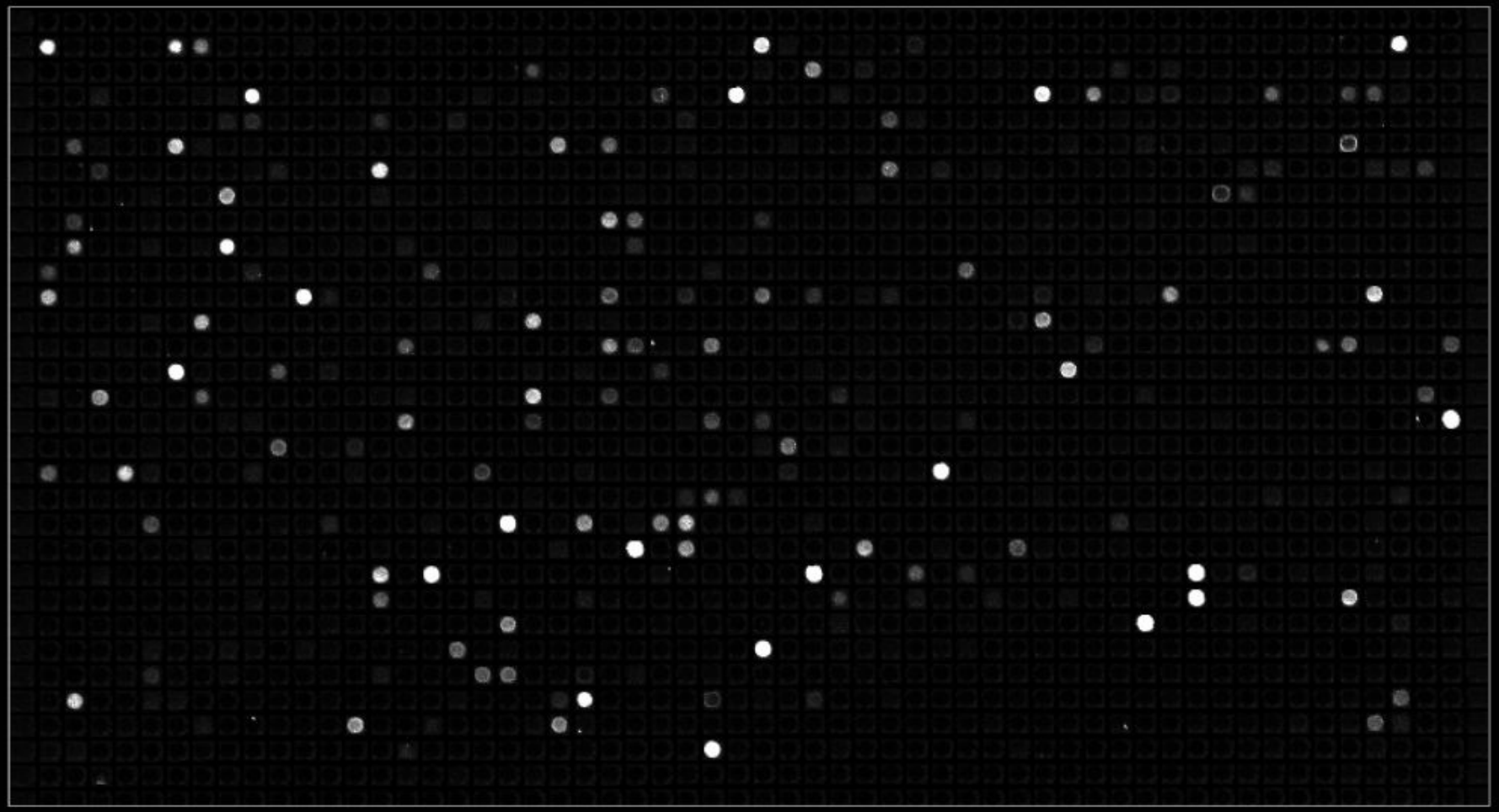
Profiling miRNA expression using custom microarrays



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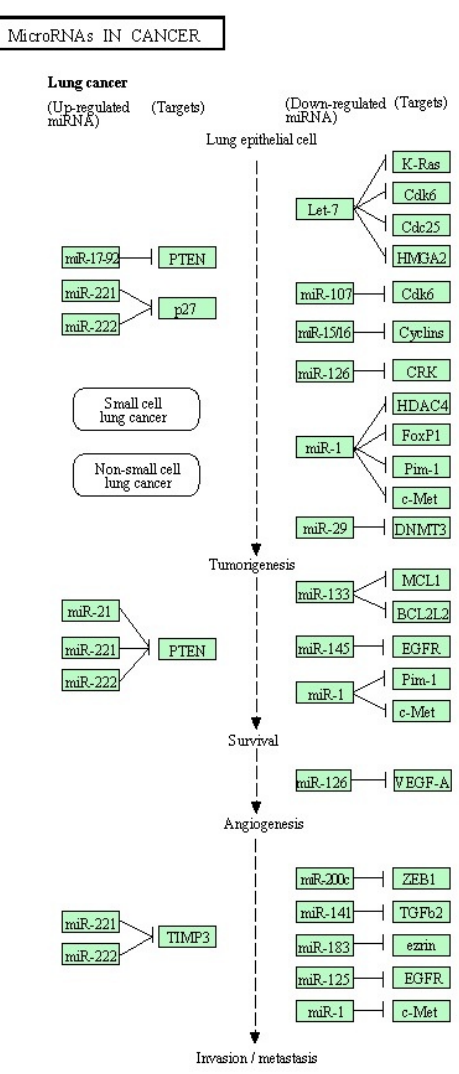
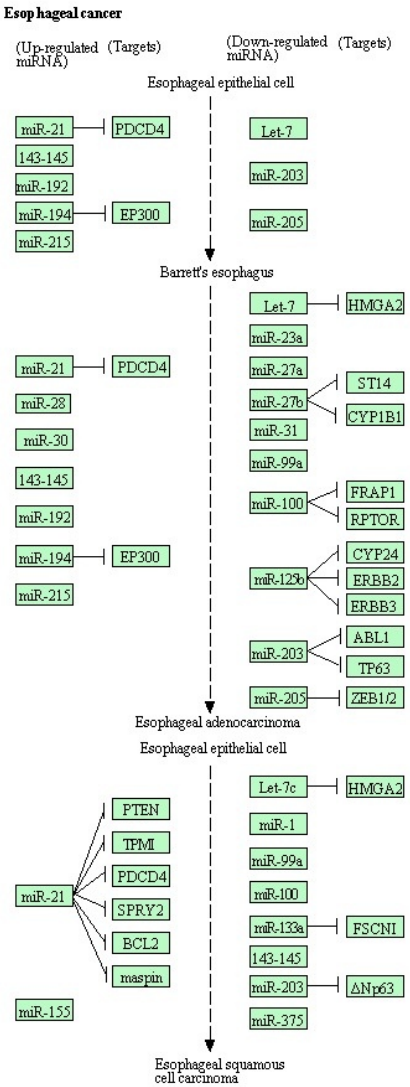
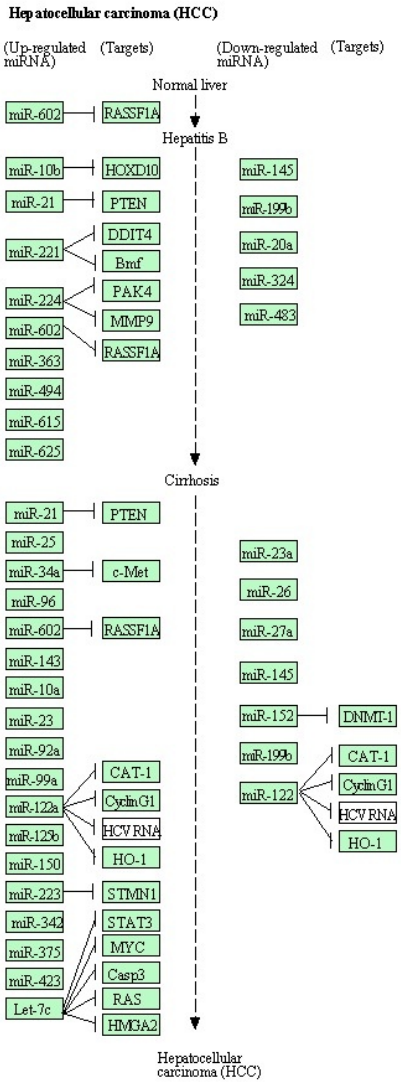
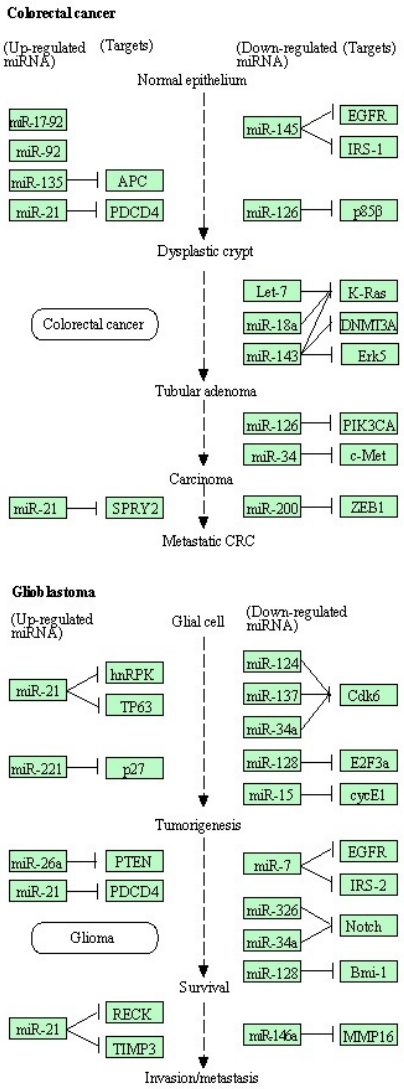
miRNAs in Cancer

http://www.kegg.jp/pathway/hsa05206









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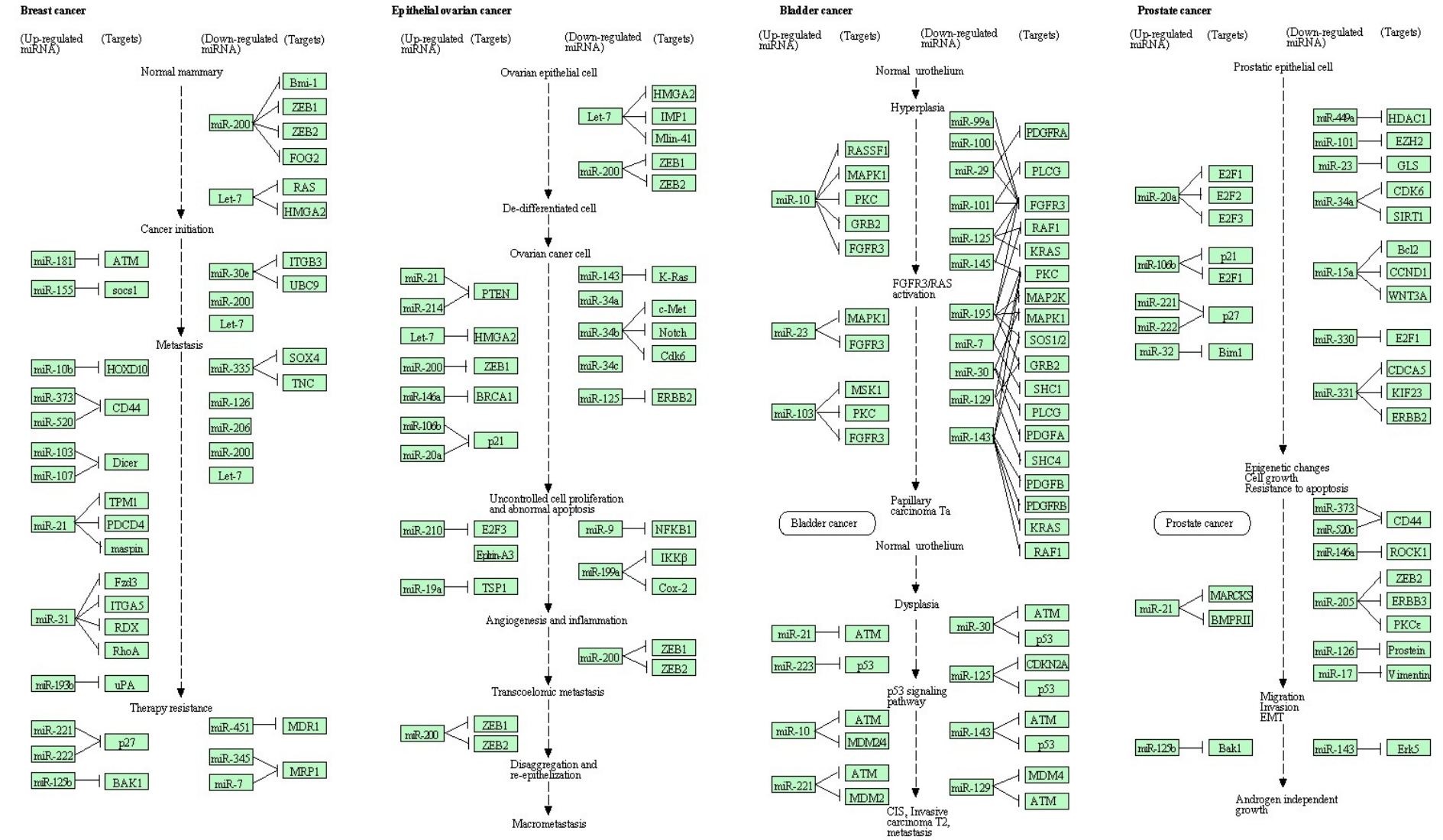
http://www.kegg.jp/pathway/hsa05206



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miRNAs and Cancer – A Summary

- miRNAs control cell cycle, cell differentiation and apoptosis by regulating oncogenes and tumor supressor genes
- •miRNAs are misexpressed in cancer and are therefore excellent diagnostic/prognostic markers in cancer
- •Some miRNAs e.g. *mir-155, can cause cancer and oncogenic miRNAs may be therapeutic targets in cancer*
- •Other miRNAs like *let-7, may prevent cancer and may be therapeutic molecules themselves.*
- •MicroRNAs could augment current cancer therapies.

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Important Micro RNA Web Sites

- miRBase: http://www.mirbase.org/
- MicroCosm: http://www.ebi.ac.uk/enright-srv/microcosm/
- miRNAminer: http://groups.csail.mit.edu/pag/mirnaminer
- miRNAviewer: http://people.csail.mit.edu/akiezun/miRviewer
- Patrocles: http://www.patrocles.org/
- TargetRank: http://hollywood.mit.edu/targetrank
- TargetScanS: http://www.targetscan.org/

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miRNA Dysregulation in Cancer

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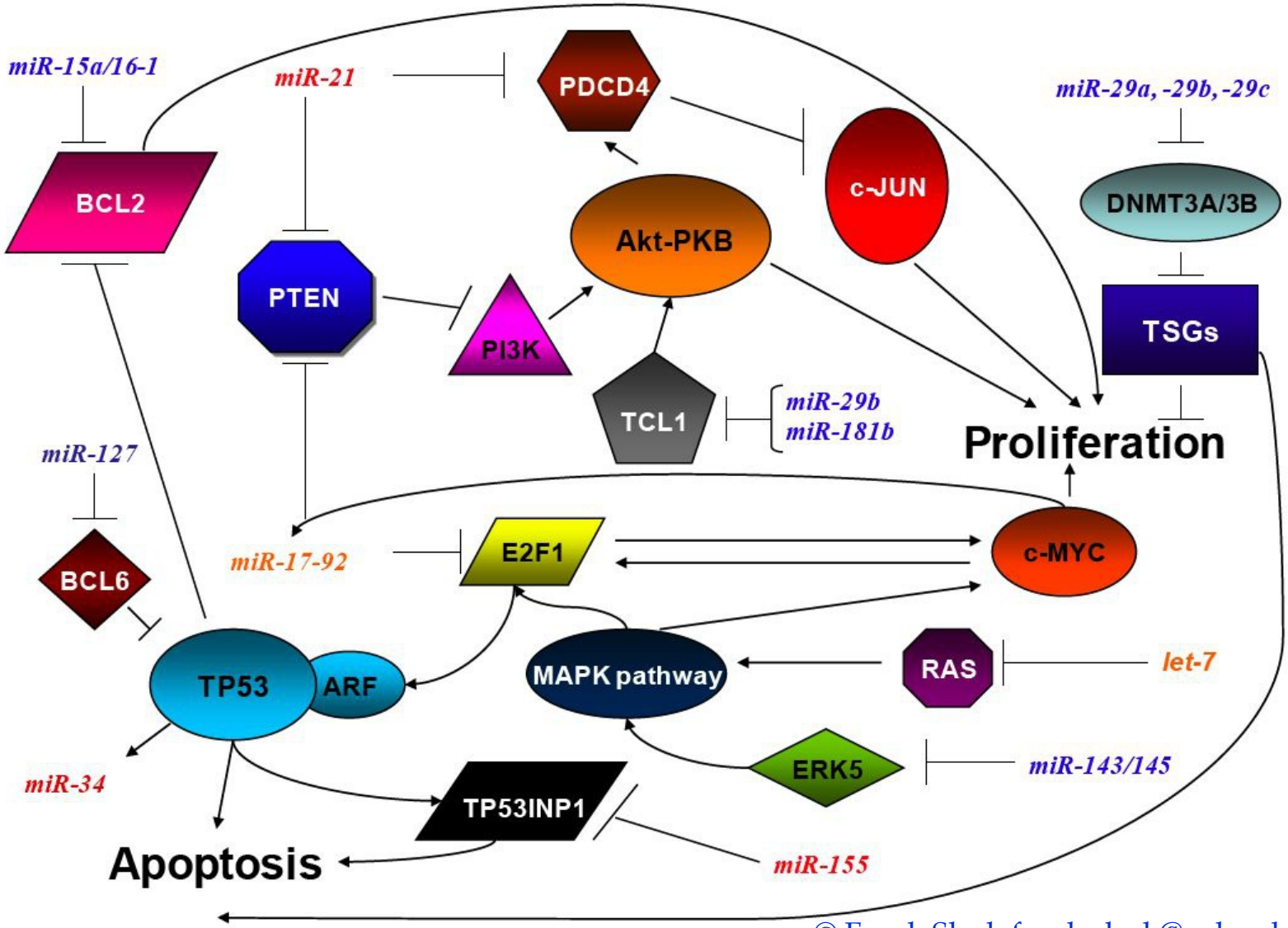
Table 2 | **Consequences of microRNA dysregulation in human cancers**

MicroRNA dysregulation	Targets	Consequences
MicroRNA overexpression	Tumour suppressors	Downregulation of tumour suppressors — for example, PTEN, p22, p57, TIMP3 and PDCD4
MicroRNA loss	Oncogenes	Upregulation of oncogenes — for example, *BCL2*, *MCL1*, *RAS*, *HMGA2*, *MYC* and *MET*
MicroRNA loss	DNA methyltransferases	Downregulation of tumour suppressors — for example, p16, FHIT and WWOX
MicroRNA loss	Chromatin silencers	Downregulation of tumour suppressors

BCL2, B cell leukaemia/lymphoma 2; FHIT, fragile histidine triad protein; *HMGA2*, high mobility group AT-hook 2; *MCL1*, myeloid cell leukaemia sequence 1; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; TIMP3, tissue inhibitor of metalloproteinases 3; WWOX, WW domain-containing oxidoreductase.

Micro RNAs Regulate Cell Growth and Death



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MicroRNAs Commonly Associated with Human Cancer

miRNA	Gene Loci	Cancer association	Function*	References
miR15, miR-16	chromosome 13q14	Frequently deleted/downregulated in B-cell chronic lymphocytic leukemia. Negatively regulates the anti-apoptotic gene, BCL2.	TS	Calin, 2002 Cimmino, 2005
miR-143, miR-145	chromosome 5q3233	Decreased abundance in colorectal cancer. Down-regulated in breast, prostate, cervical, and lymphoid cancer celllines. miR-145 decreased in breast cancer.	TS	Michael, 2003 Iorio, 2005
miR-21	chromosome 17q23.2	Antiapoptotic factor. Upregulated in glioblastomas and breast cancer.	OG	Chan, 2005 Ciafre, 2005 Iorio, 2005
let-7	multiple loci	Negatively regulates the Ras oncogene. Directs cell proliferation, differentiation. Decreased abundance in lung cancer.	TS	Johnson, 2005 Takamizawa, 2004
miR-142	chromosome 17q22	t(8,17) translocation that places the MYC oncogene downstream of the *mir-142* hairpin resulting in an aggressive B cell leukemia due to MYC over-expression.	N/A	Lagos-Quintana, 2002
BIC/miR-155	chromosome 21q21	Upregulated in pediatric Burkitt's lymphomas, Hodgkins, primary mediastinal and diffuse large B cell lymphomas. Upregulated in human breast cancer.	OG	Eis, 2005 Kluiver, 2005 van den Berg, 2003 Metzler, 2003 Iorio, 2005
miR-17-19b cluster	chromosome 13q3132	Upregulated by the c-Myc oncogene Negatively modulates E2F1 oncogene. Loss-of-heterozygosity of cluste in hepatocellular carcinoma. Over-expressed in B-cell lymphomas.	TS/ OG	He, 2005 O'Donnell, 2005

*Abbreviations: TS, tumor-suppressor gene; OGoncogene; N/A, not applicable

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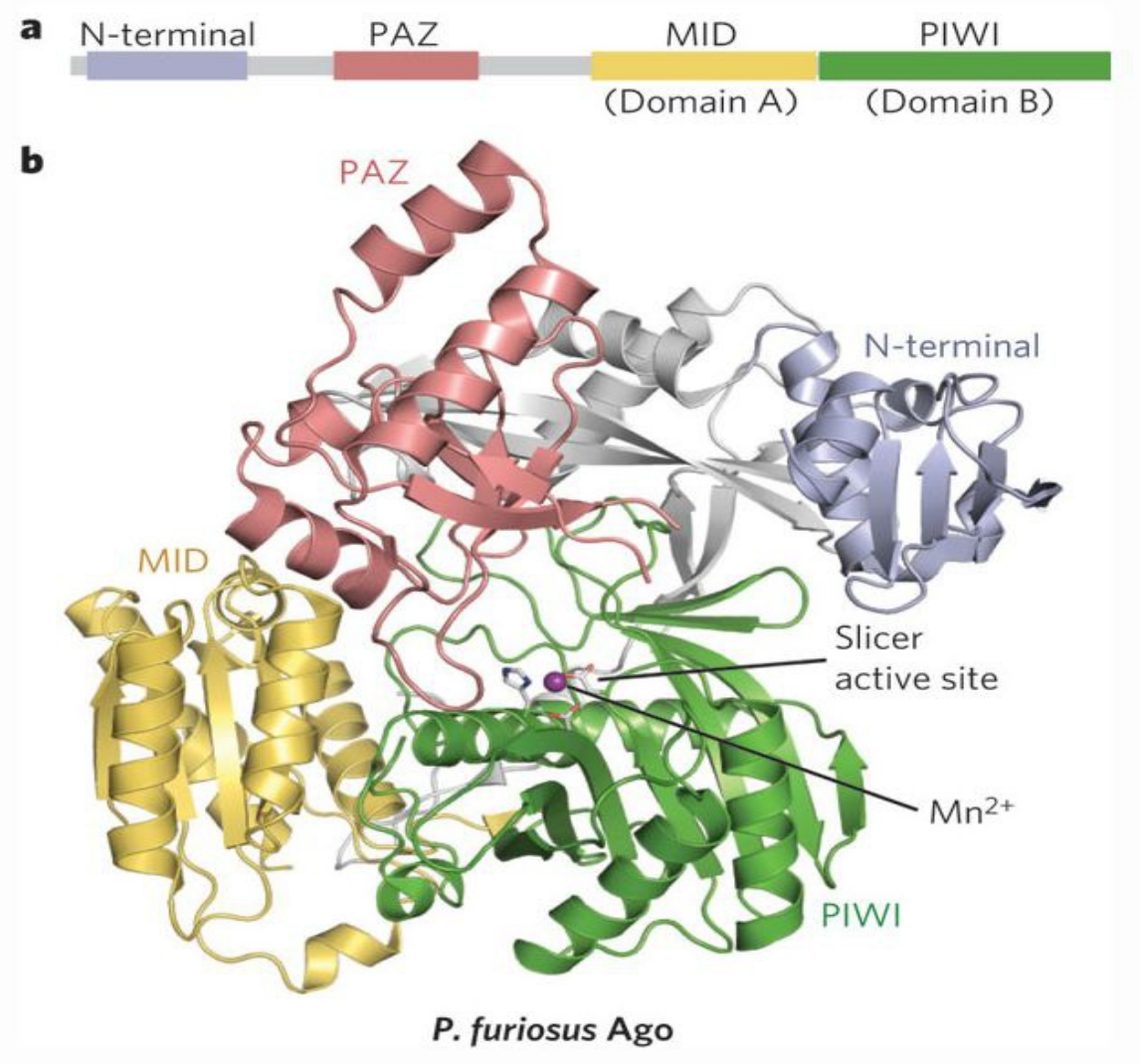
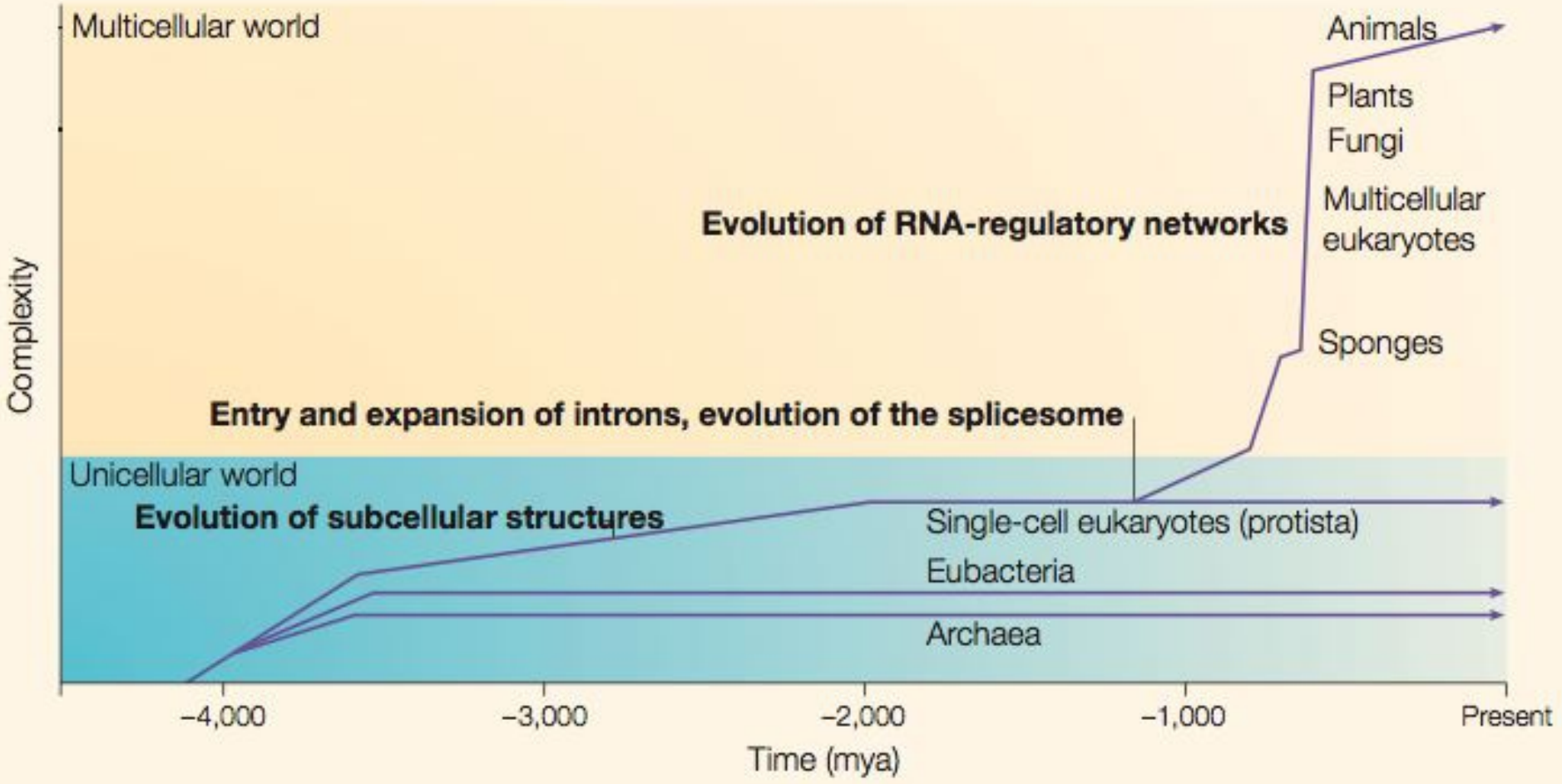
Argonaute Structure and Function



Martin Jinek & Jennifer A. Doudna *Nature 457, 405-412(22 January 2009)*

A Simplified History of Life on Earth



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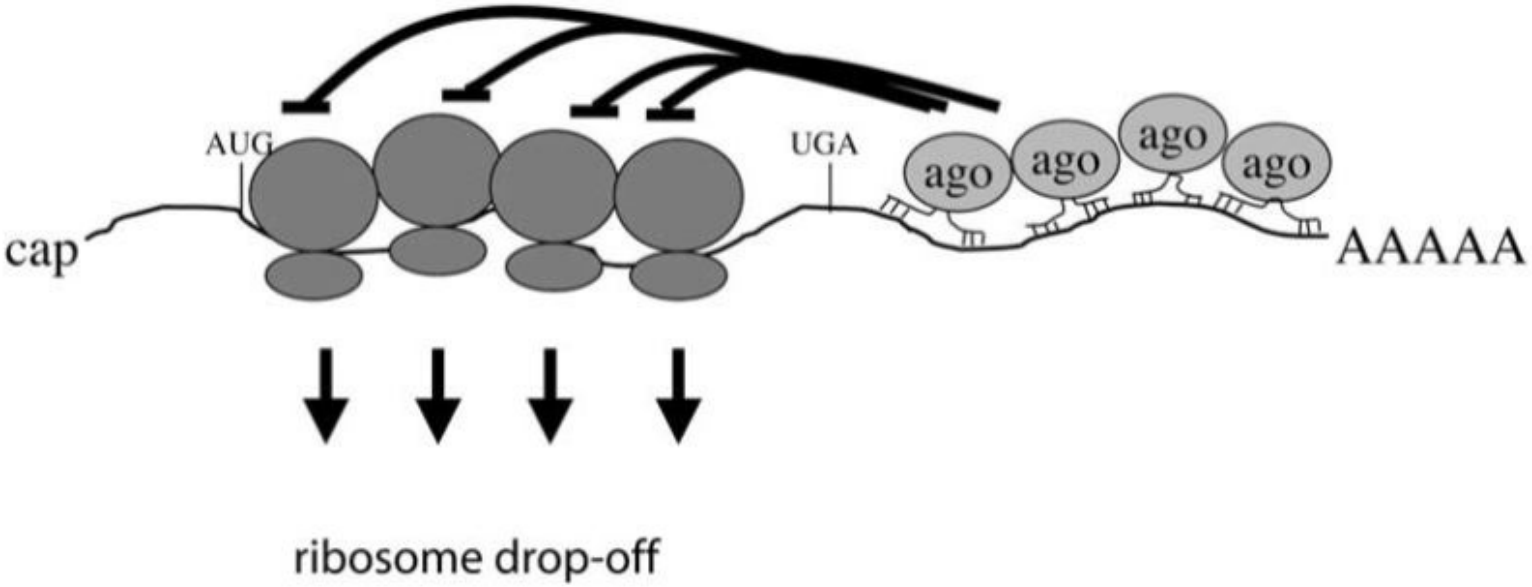
miRNAs Inhibit Translation by Inducing Ribosome Drop-Off



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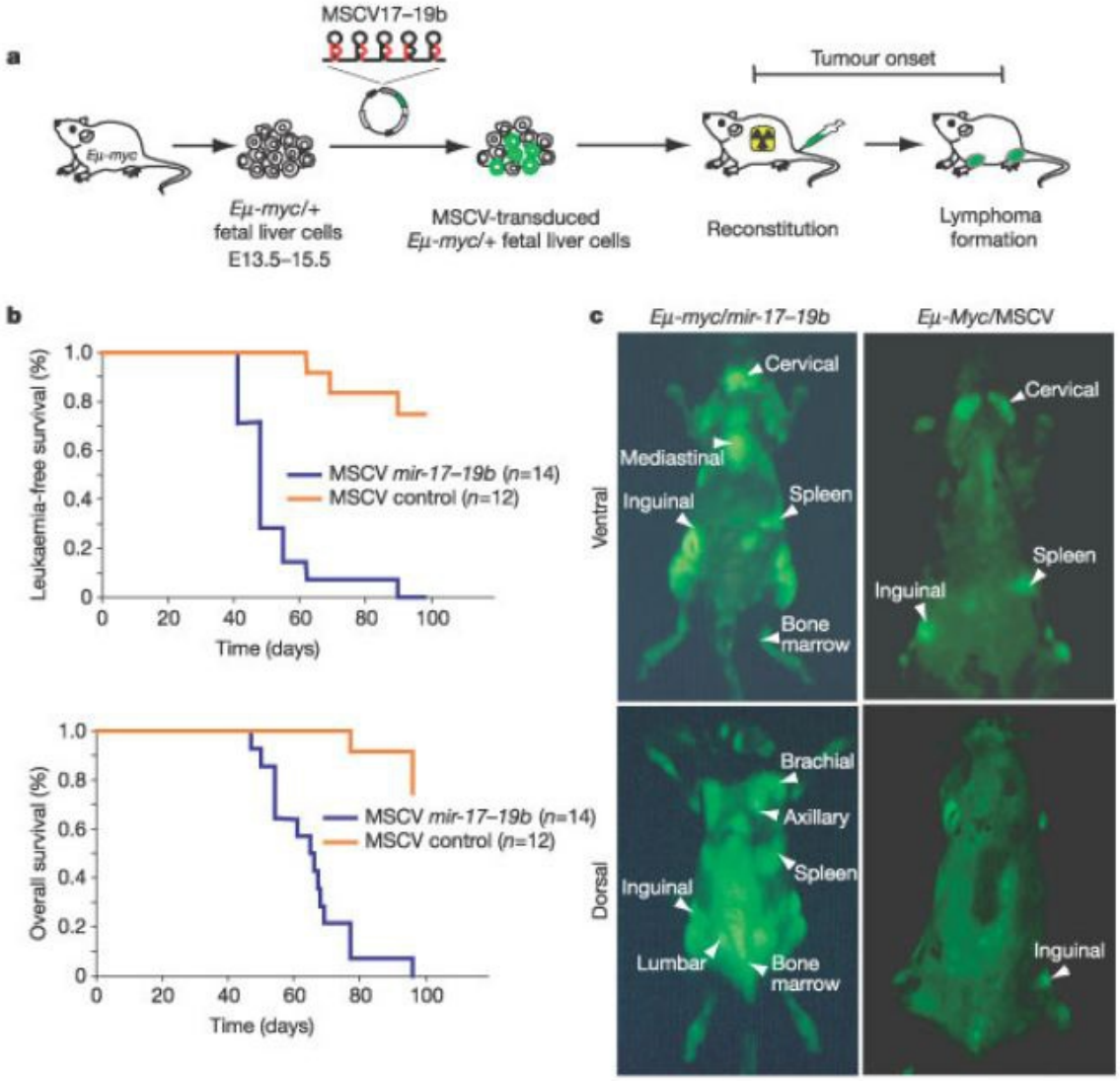

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