Encoding Contingency

in Multicellular Organisms

Kenneth Kosik, UCSB May 18, 2011 KITP Colloquium

Contingency over Evolutionary Time Scales

Stephen Jay Gould: replaying the "tape of life" from some point in the distant past would yield a living world far different from the one we see today. Accidents and happenstance shape the course of evolution.

Simon Conway Morris: natural selection constrains organisms to a relatively few highly adaptive options despite the vagaries of history, so that "the evolutionary routes are many, but the destinations are limited." Evolution is broadly repeatable, and multiple replays would reveal striking similarities in important features, with contingency mostly confined to minor details.

A Contingency Frequency Timeline

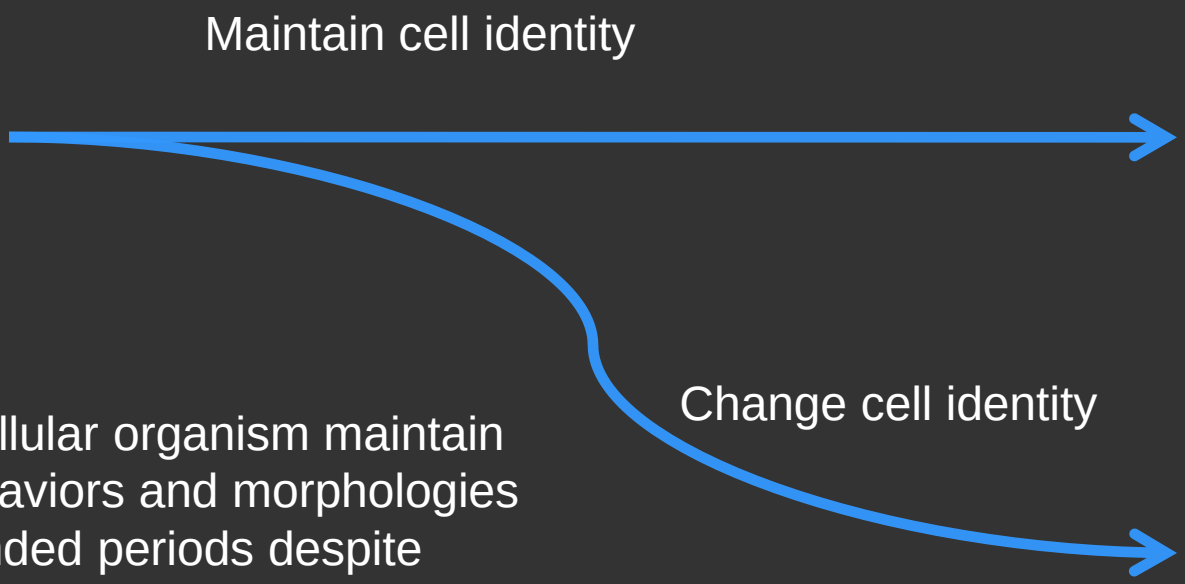
Cells face an environment that is many times more complex than the biological repertoire available within the genome.

preparedness for contingencies

Constancy and Change in the Face of Contingencies

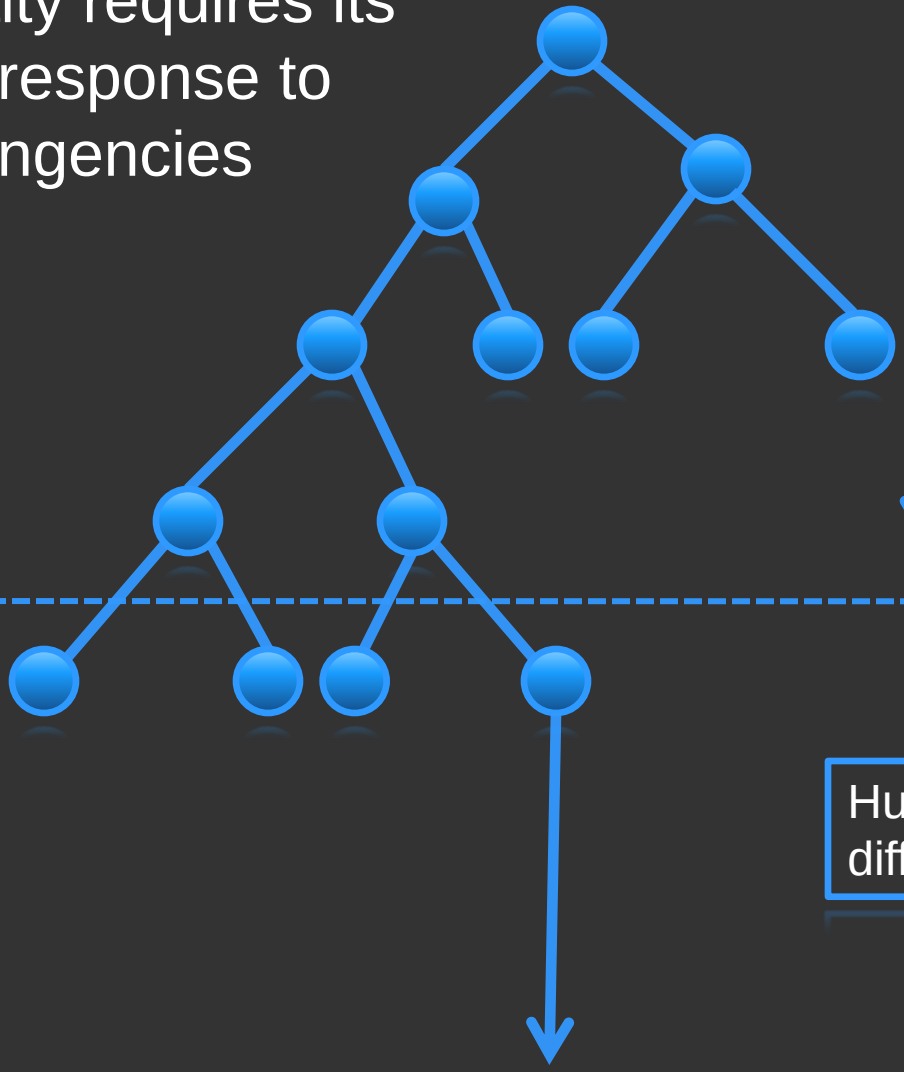
1. Evolutionary time scales--genetic drift v natural selection

2. Biological time scales--



Cell types in a multicellular organism maintain strikingly different behaviors and morphologies that persist over extended periods despite essentially identical genomes.

Each unique cell identity requires its own response to contingencies



pluripotent

Hundreds of precursor cell types

multipotent

terminal differentiation

Hundreds of terminally differentiated cell types

Extreme options-malignancy, apoptosis transdifferentiation

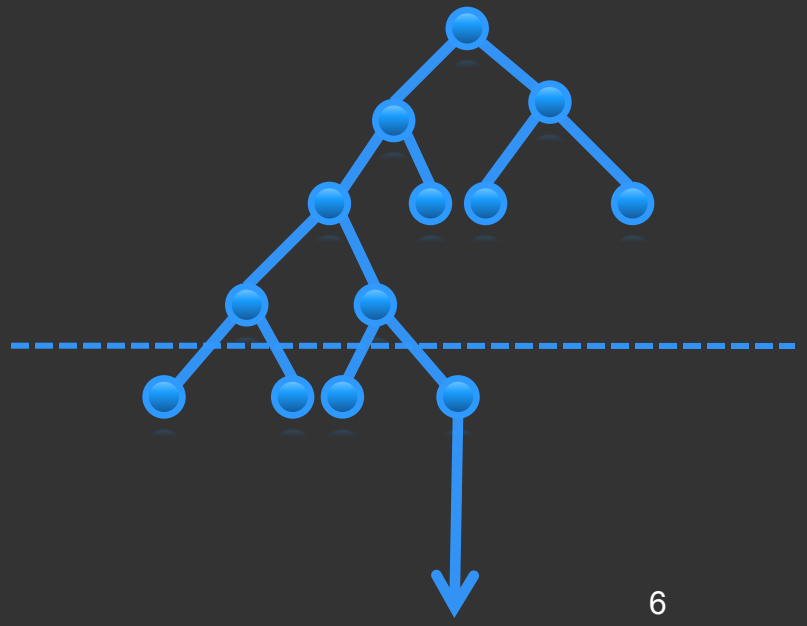
Terminal differentiation

homeostasis--preserve constant the conditions of the internal environment

"La fixité du milieu intérieur est la condition d'une vie libre et indépendante."
Claude Bernard

allostasis—stability through change

parameters are not constant, variation anticipates demand and thereby reduces error
Sterling & Eyer, 1988

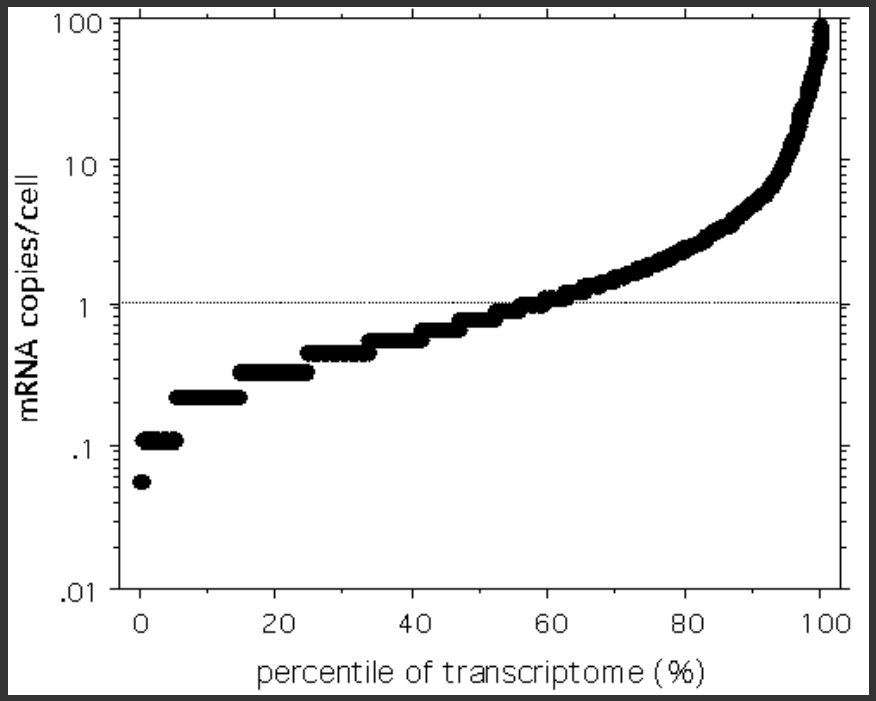


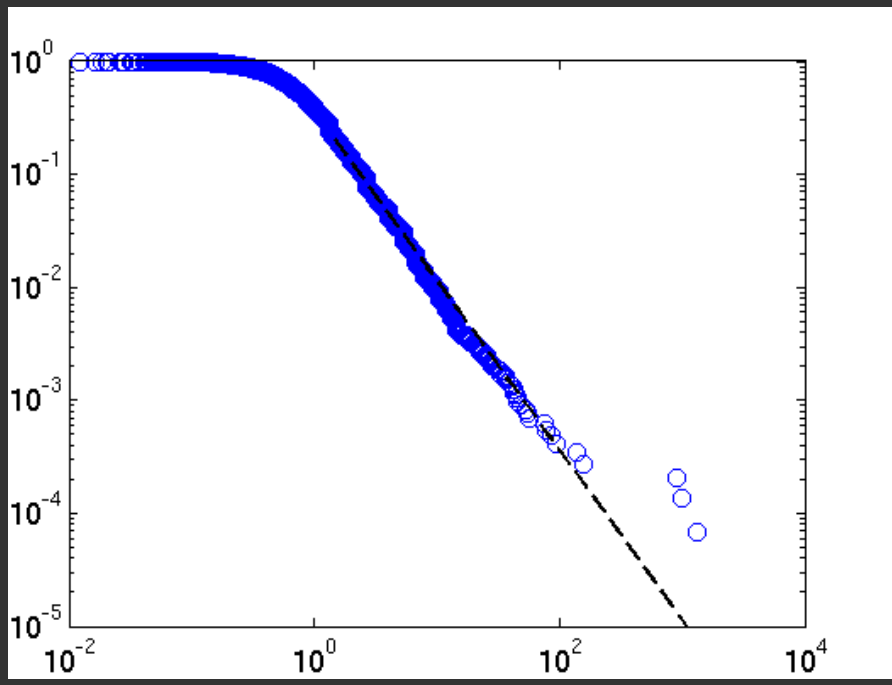
Inventory of parameters to implement the genome

	Number of individual elements	Total individual elements in a mammalian cell	Copy number distribution
1. genes	~25,000	~25,000	flat
epigenetic marks (histone code)			
2. transcriptome			
mRNAs	~10^4	3-5 x 10^5	Exponential
miRNAs	1000	~10^5	Exponential
piRNAs			
3. proteome (post translational modifications)		10^9	Exponential
4. metabolome			

5460 transcripts
15000 polyA-RNA's per cell
Average level: 2.8 copies/cell
Median level: 0.79 copies/cell
80% of the transcriptome is expressed at 0.1 - 2 mRNA copies/cell

Demosponge
~21,000 transcripts

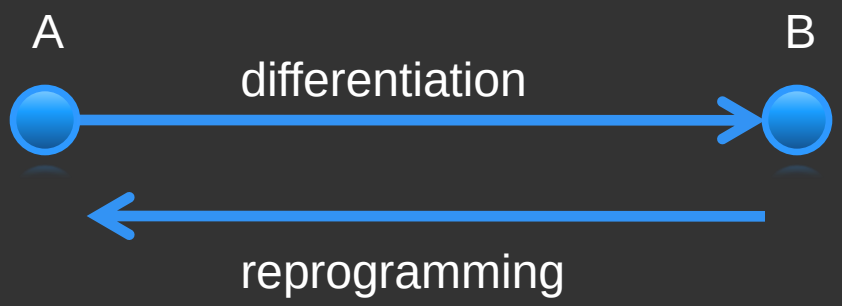




Rick Young Cell 95:717-28, 1998

Dani Bassett
Cecilia Conaco

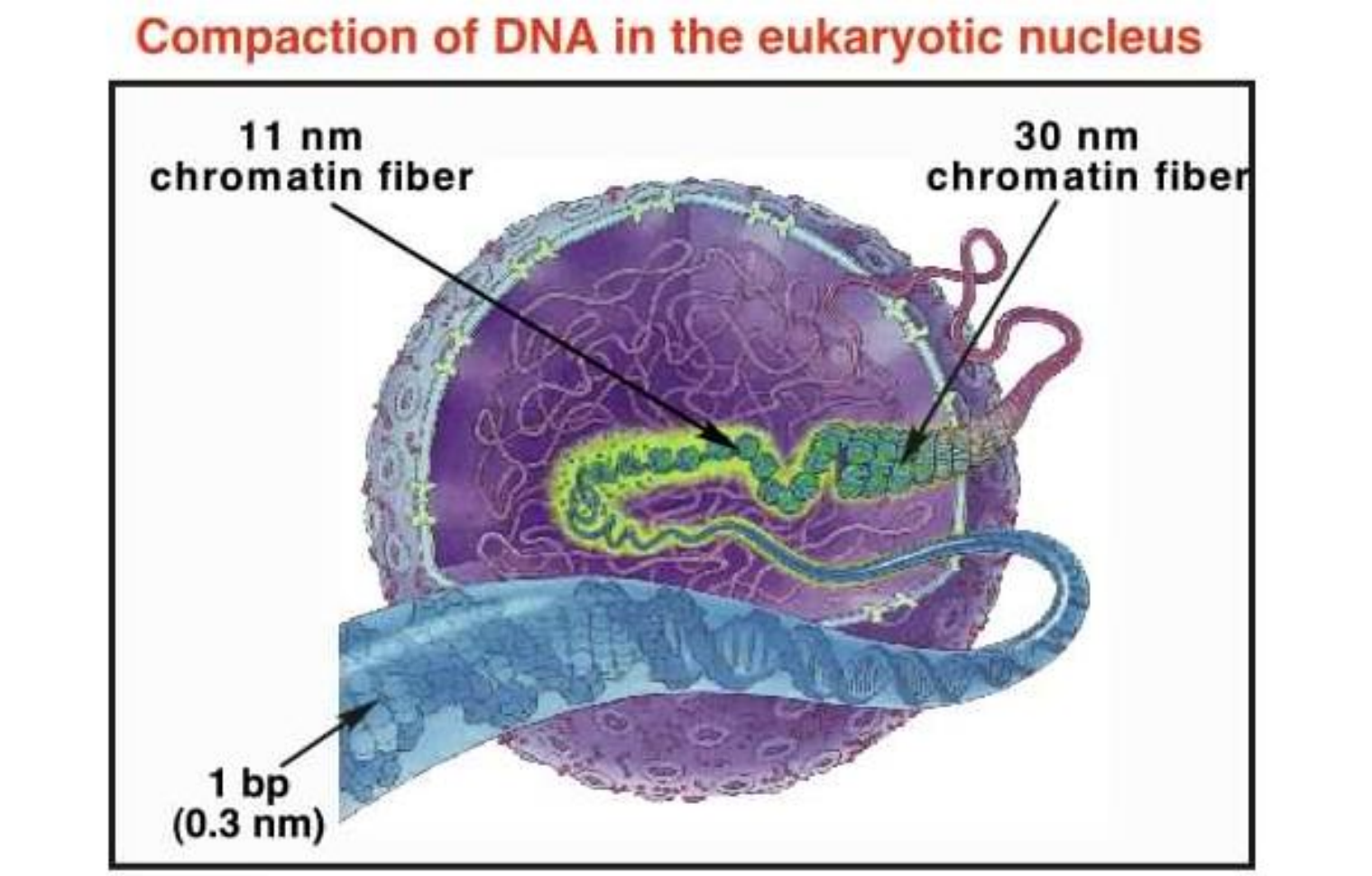
Identity change



Directional drivers = transcription factors

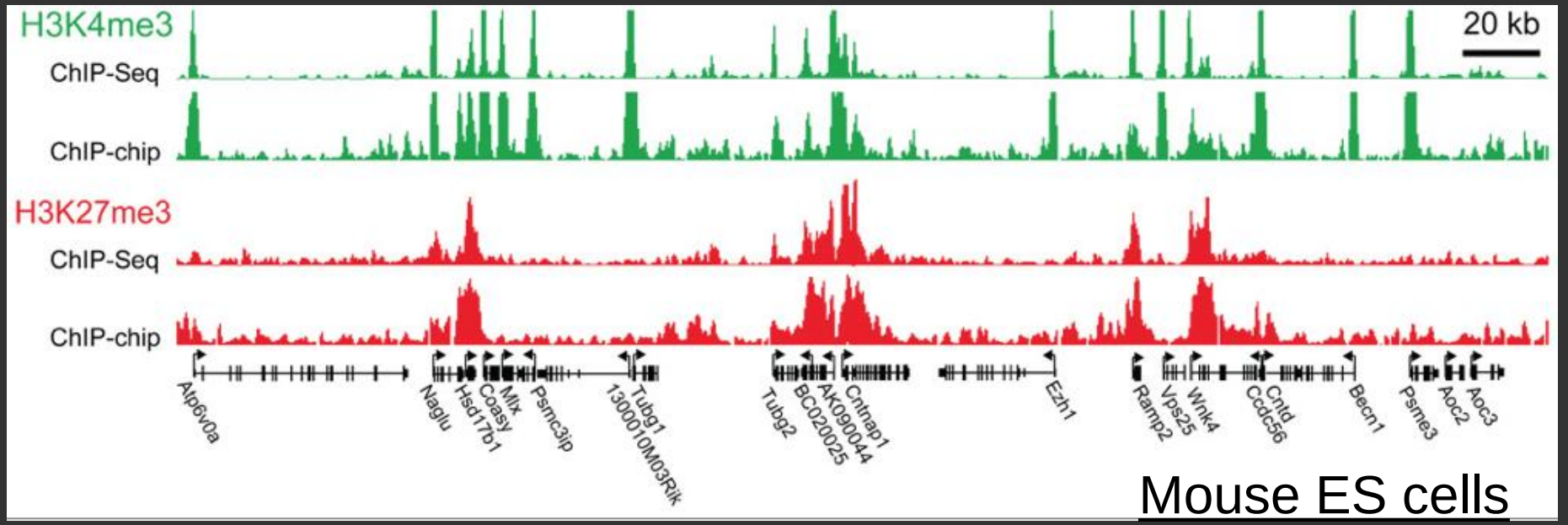
Core pluripotency factors —
Oct 4, Klf 4, Sox 2, c-Myc

Cellular identity may be closely related to ‘chromatin state’



Histone modifications:
pan-H3, H3K4me3, H3K9me3, H3K27me3,
H3K36me3, H4K20me3, RNA polymerase II

ChIP-Seq maps of specific histone modifications show marked enrichment at specific locations in the genome



H3K4me3 is catalyzed by trithorax-group (trxG) proteins -- activation

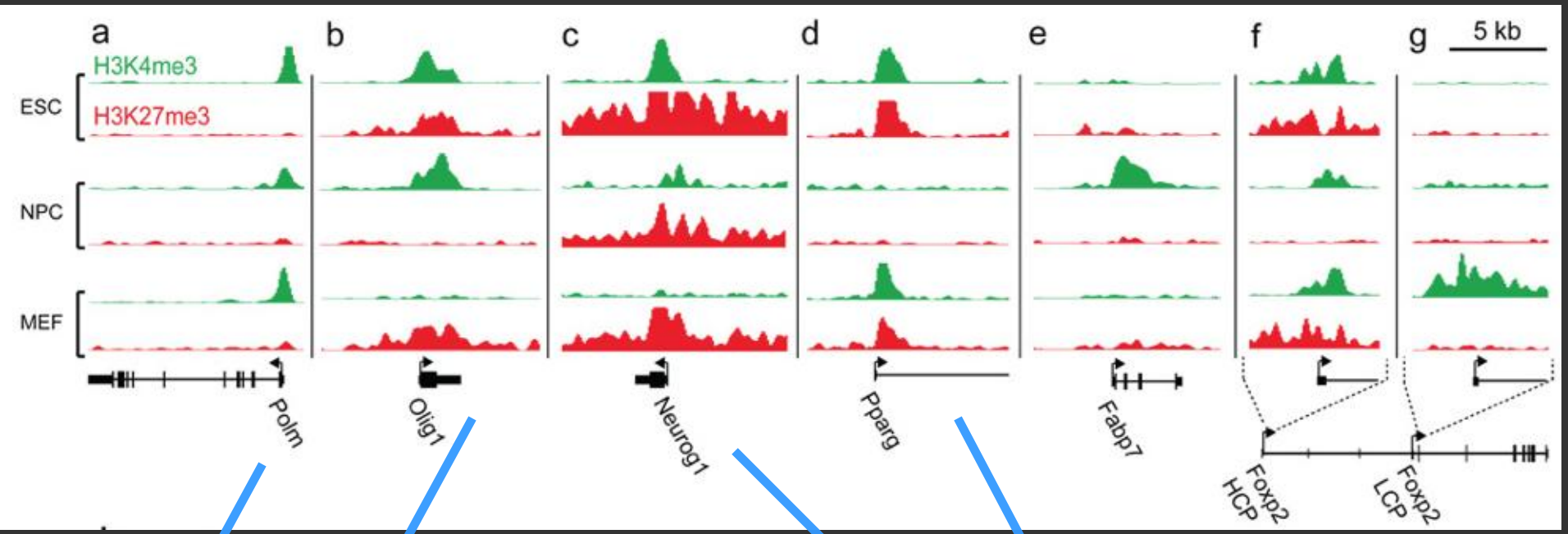
H3K27me3 is catalyzed by Polycomb-group (PcG) proteins -- silencing

Monovalent promoters (H3K4me3) regulate genes with 'housekeeping' functions including replication and basic metabolism.

'bivalent' chromatin mark: Promoters in ES cells with both H3K4me3 and H3K27me3 -- key developmental genes poised for lineage-specific activation or repression

Nature 448: 553, 2007

bivalent marks in ES cells resolve to a monovalent status in committed cells



housekeeping

Neural transcription factor

Neurogenesis Transcription factor

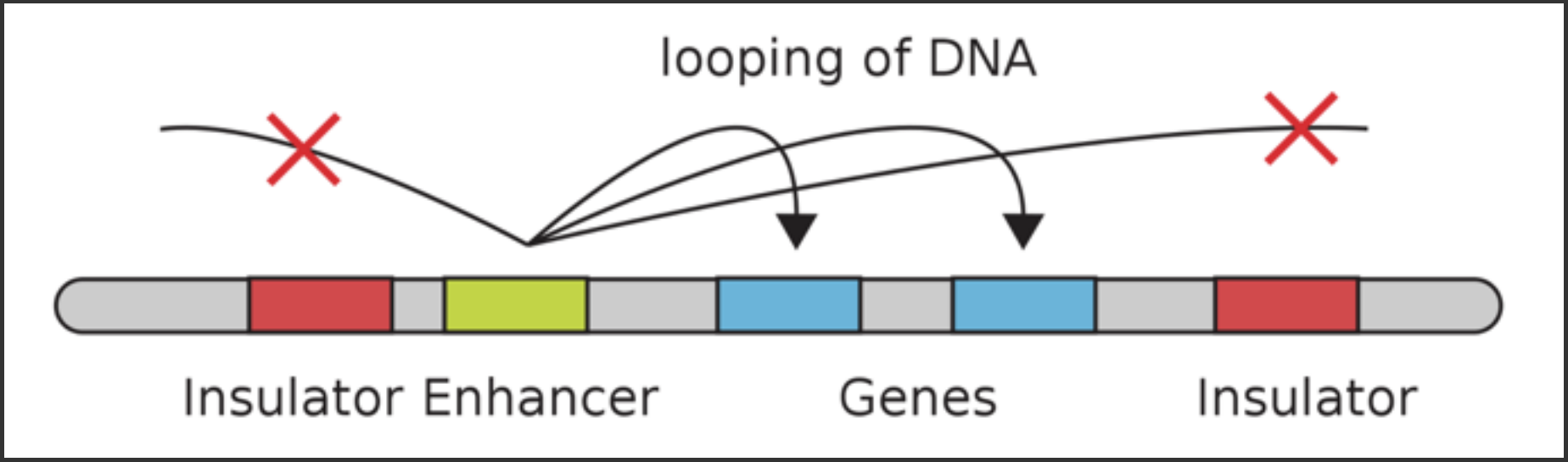
Adipogenesis Transcription factor

ES cells
neural progenitor cells (NPCs)
embryonic fibroblasts (MEFs)

Enhancer elements—cell identity

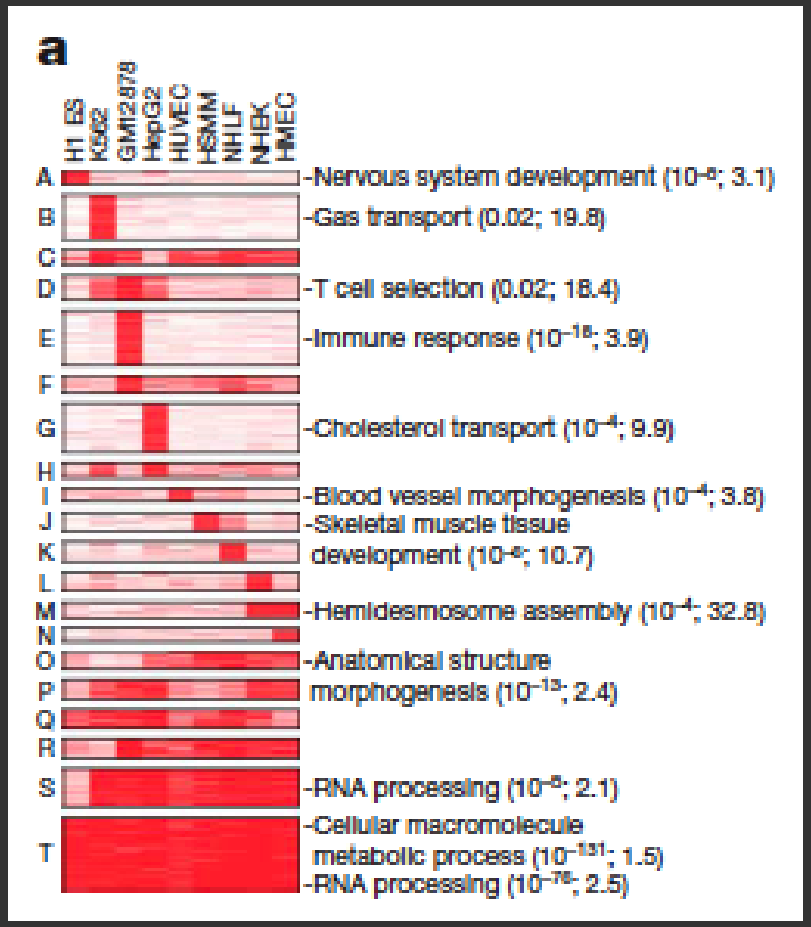
Specific histone modifications correlate with regulator binding, transcriptional initiation and elongation, enhancer activity and repression. Combinations of modifications can provide even more precise insight into chromatin state.

Recurrent combinations of marks define repressed, poised and active promoters, strong and weak enhancers, putative insulators, transcribed regions, and large-scale repressed and inactive domains.

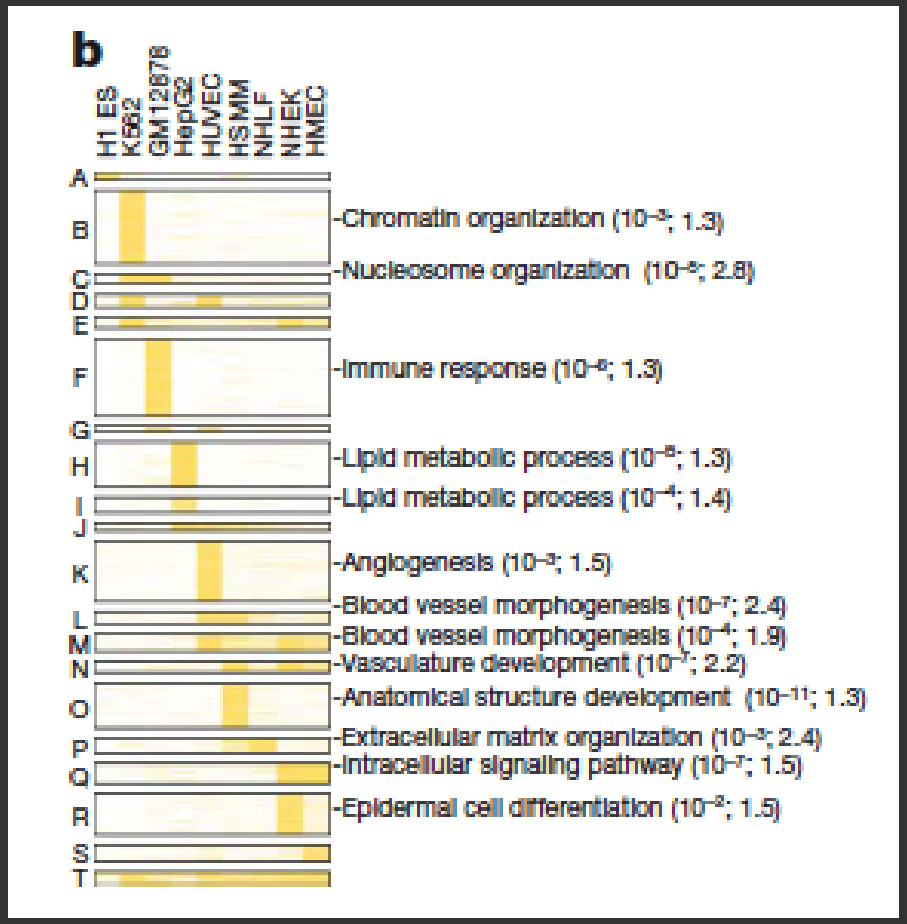


Ernst J et al. Nature 473:43, 2011

Promoters



Enhancers



Enhancer clusters are significantly more cell type specific than promoters, with few regions showing activity in more than two cell types and a majority being specific to a single cell type.

microRNAs

1. post-transcriptional regulators that imperfectly bind to complementary sequences in the 3' UTRs of target mRNAs, and usually result in gene silencing

1. ~22 nucleotides long

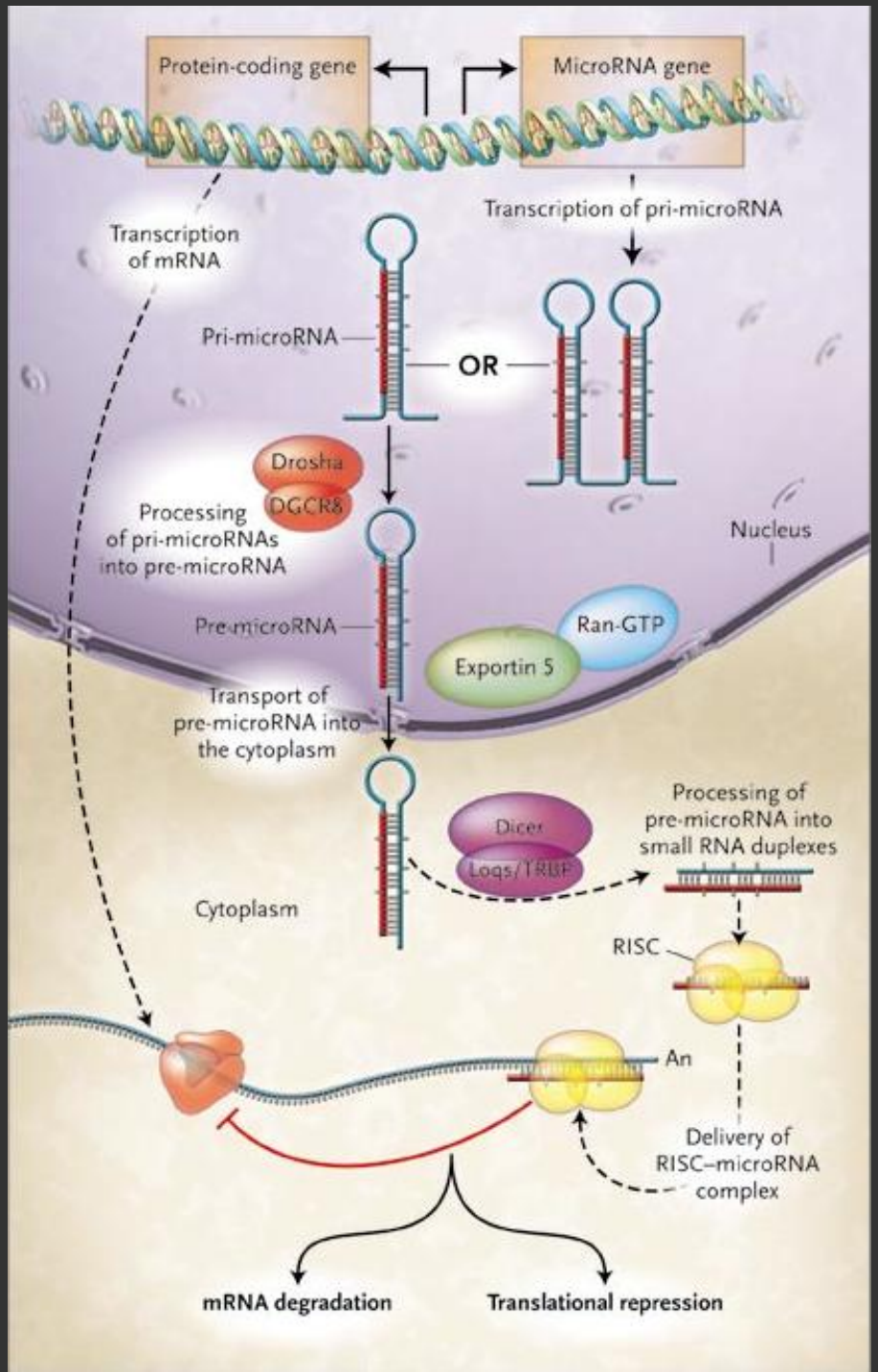
2. Human genome encodes over ~1000 miRNAs which target about 60% of mammalian genes. Each miRNA may repress hundreds of mRNAs

3. Cytoplasmic regulators of gene expression with comparable complexity to transcription factors (nuclear regulators)

4. Evolutionarily ancient and extremely low rate of evolution

The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Lee RC, Feinbaum RL, Ambros V. Cell 75:843-54, 1993

microRNA Biogenesis



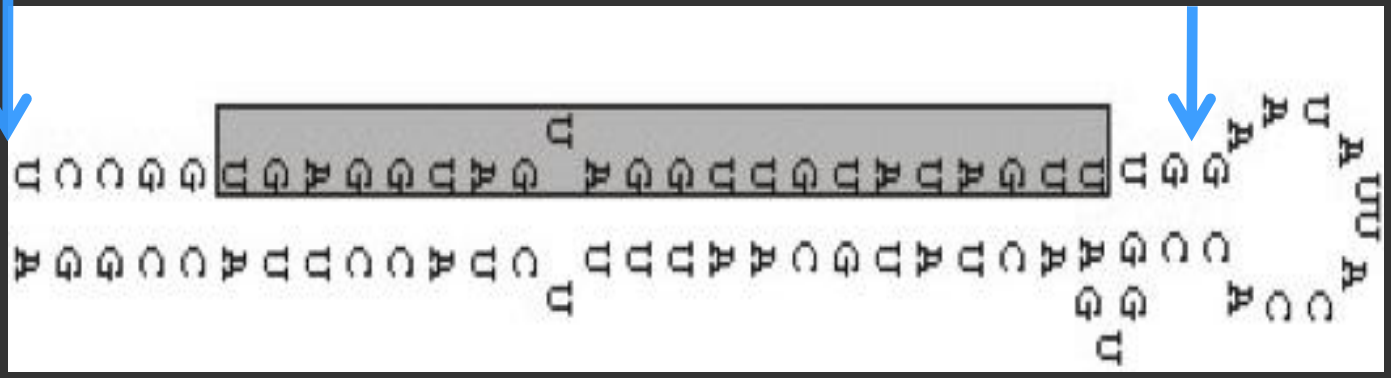
Chang-Zheng Chen C-Z
New Eng. J. Med.
353:1768-1771, 2005

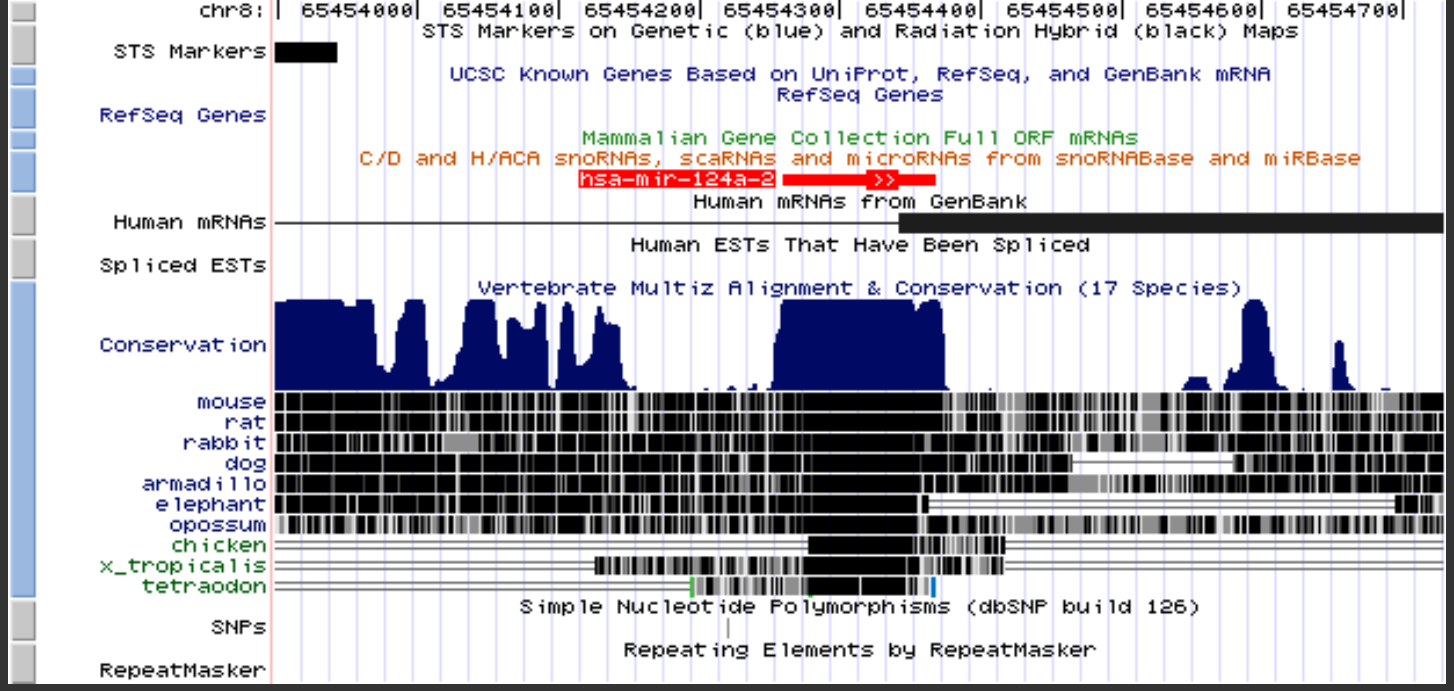
Rnase III endonuclease

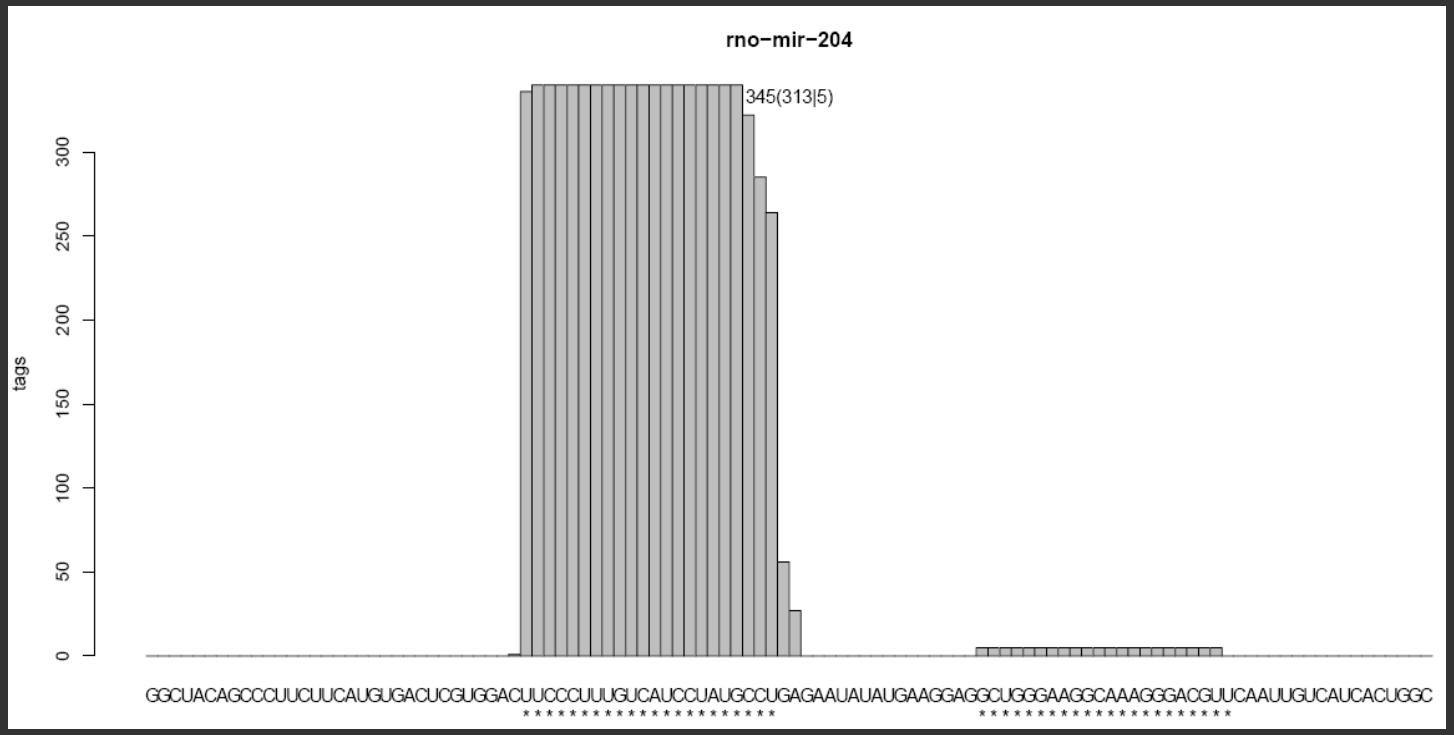
leaves a 2 nt 3’ overhang

Nuclear cut by Drosha defines this end

Cytoplasmic cut by Dicer defines this e




chr8: | 65454000| 65454100| 65454200| 65454300| 65454400| 65454500| 65454600| 65454700|
STS Markers
STS Markers on Genetic (blue) and Radiation Hybrid (black) Maps
UCSC Known Genes Based on UniProt, RefSeq, and GenBank mRNA
RefSeq Genes
RefSeq Genes
Mammalian Gene Collection Full ORF mRNAs
C/D and H/ACA snoRNAs, scaRNAs and microRNAs from snoRNABase and miRBase
hsa-mir-124a-2
Human mRNAs
Human mRNAs from GenBank
Spliced ESTs
Human ESTs That Have Been Spliced
Conservation
Vertebrate Multiz Alignment & Conservation (17 Species)
mouse
rat
rabbit
dog
armadillo
elephant
opossum
chicken
x_tropicalis
tetraodon
SNPs
Simple Nucleotide Polymorphisms (dbSNP build 126)
RepeatMasker
Repeating Elements by RepeatMasker


rno-mir-204
345(313|5)
tags
0
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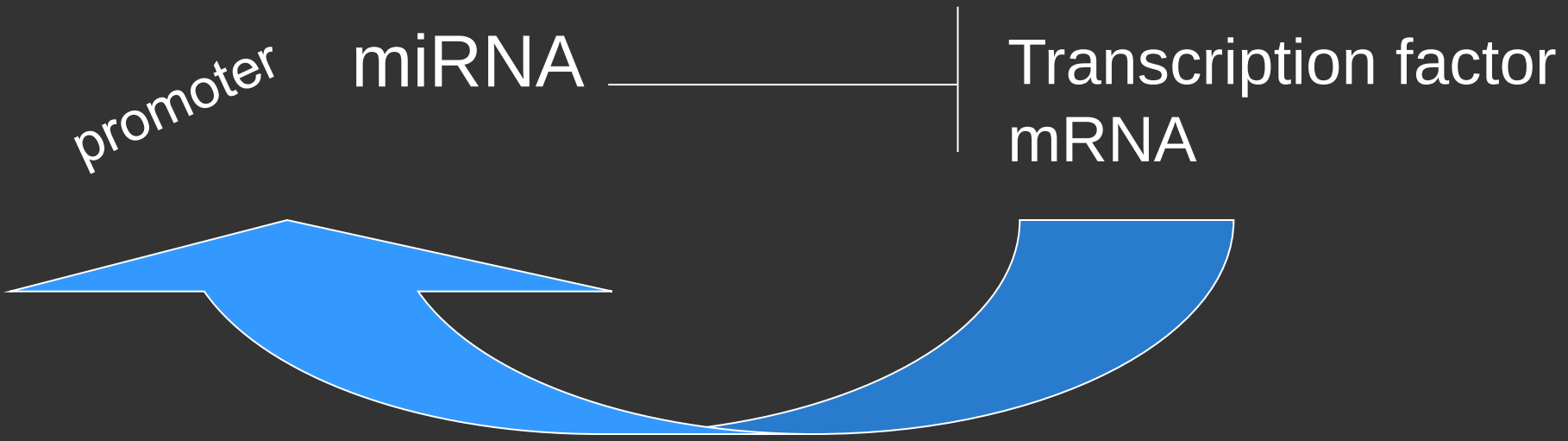
GGCUACAGCCCUUCUUCAUGUGACUCGUGGACUUCCCUUUGUCAUCCUAUGCCUGAGAAUAUAUGAAGGAGGCUGGGAAGGCAAAGGGACGUUCAAUUGUCAUCACUGGC

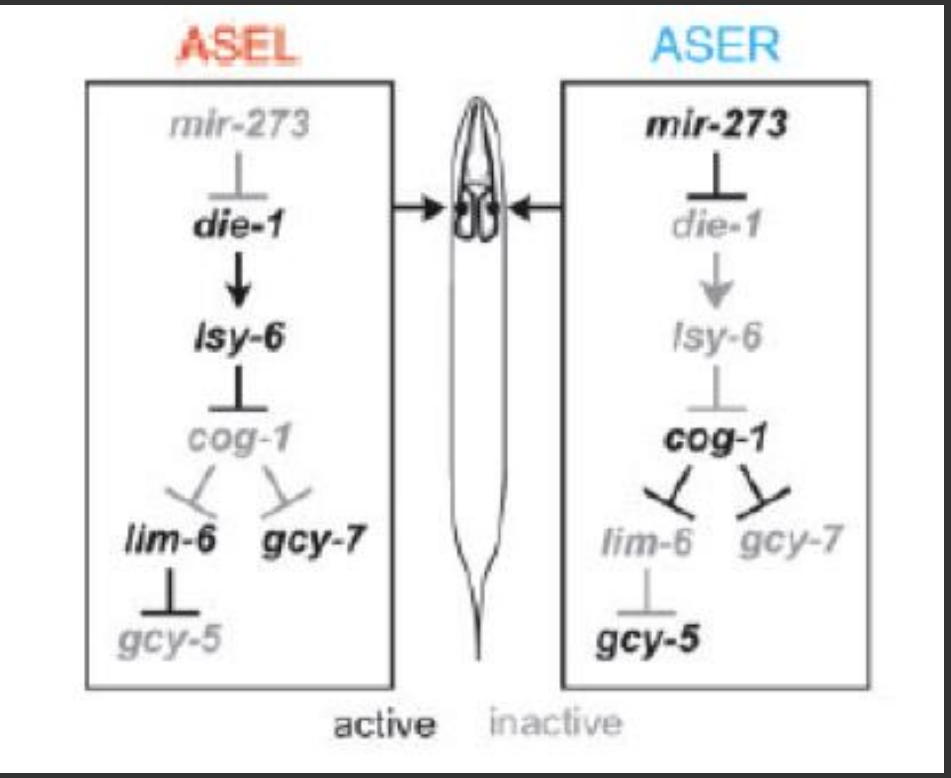
Systems Functions of miRNAs

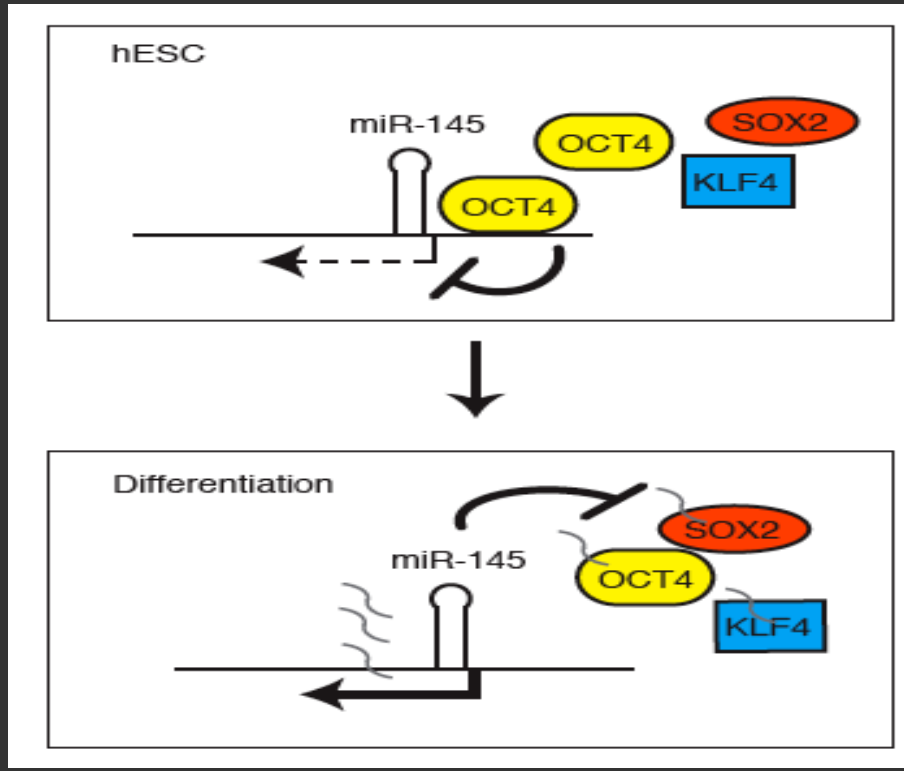
1. **Feedback Loops**

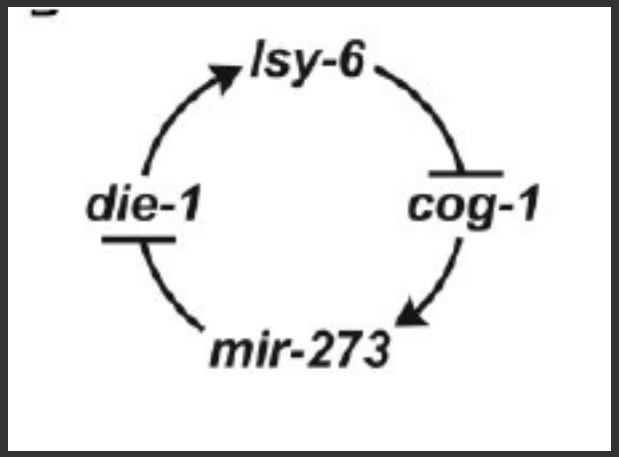
2. Distributed Network Effects

Changes in Cell Identity via Transcriptional Feedback loops





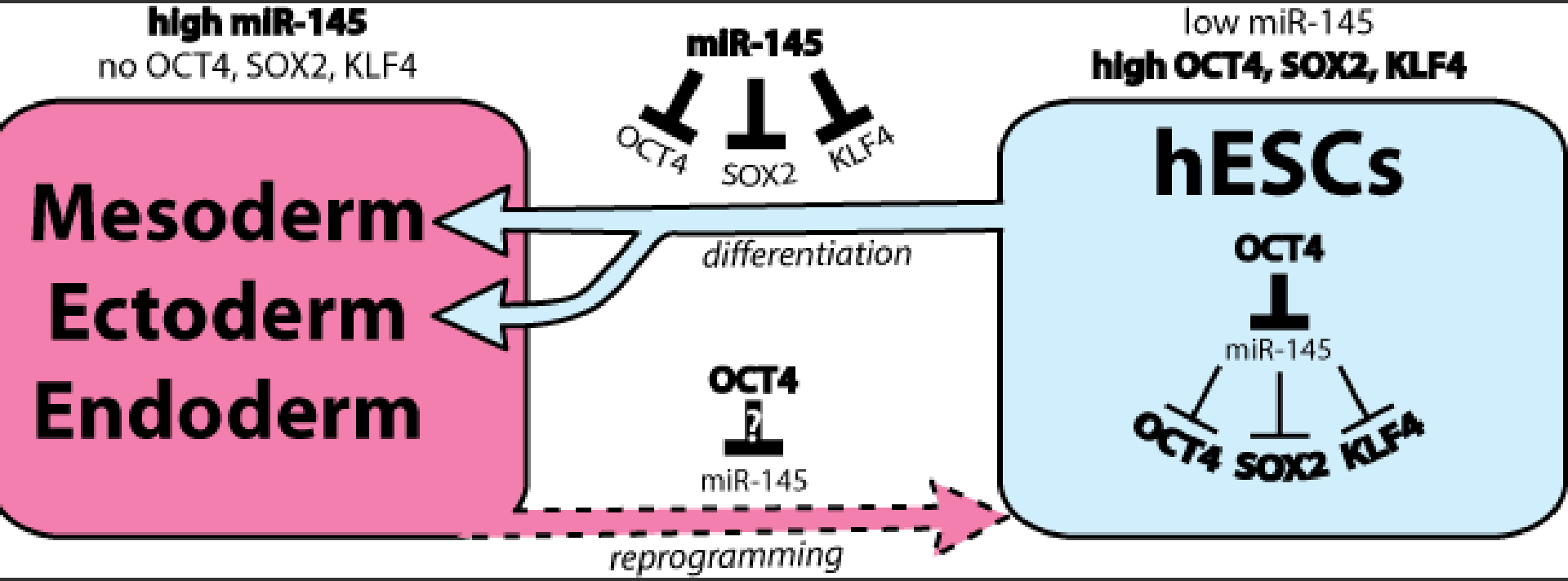




Xu N et al., Cell 2009

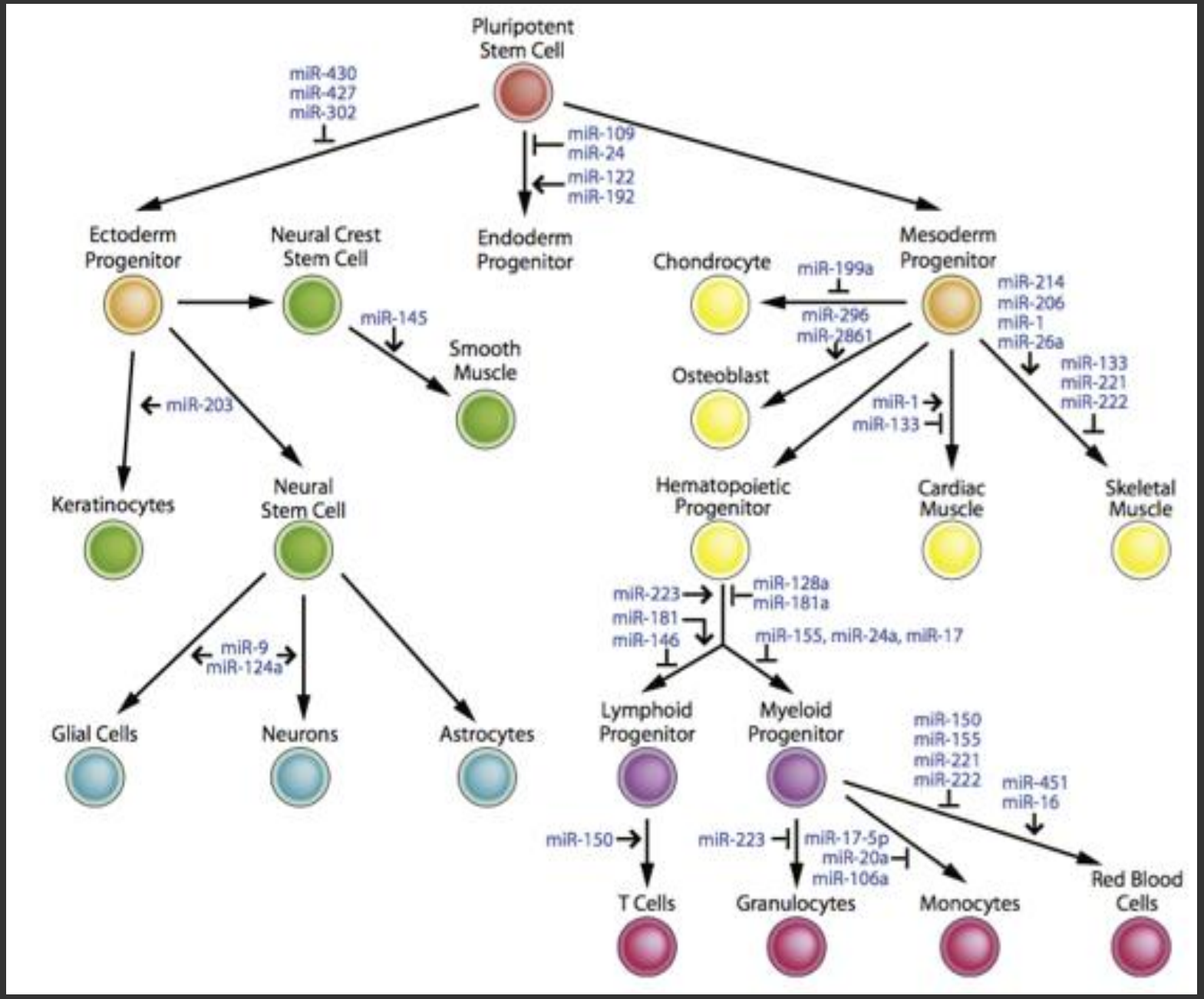
Johnston RJ Jr et al PNAS 2005

miR-145 inhibits ESC self-renewal while concurrently promotes cellular differentiation



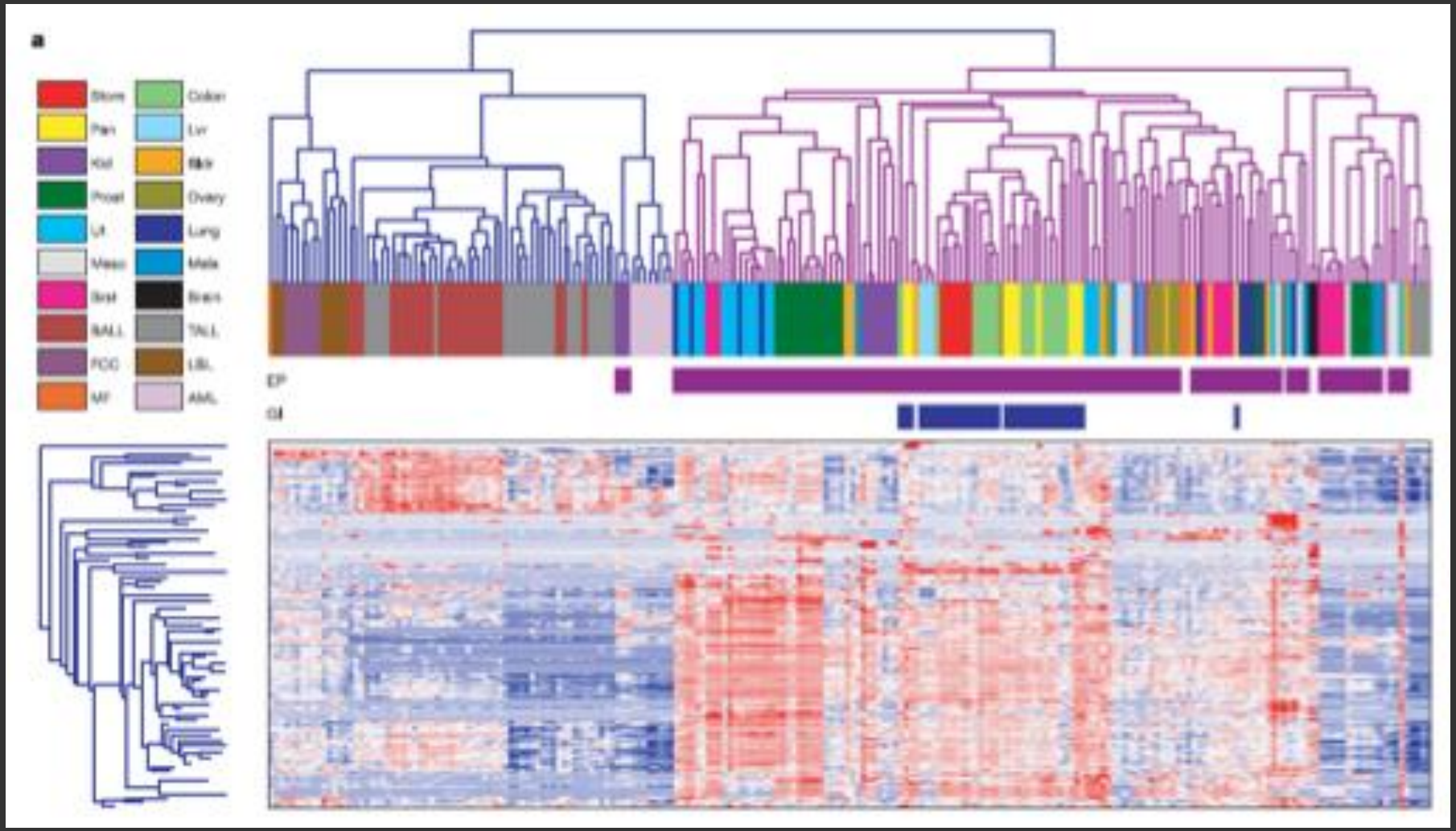
Double negative feedback loop → Bistability ?

Gabriele Lillacci
Mustafa Khammash



Ivey & Srivastava, 2010

miRNAs can classify different cancer types

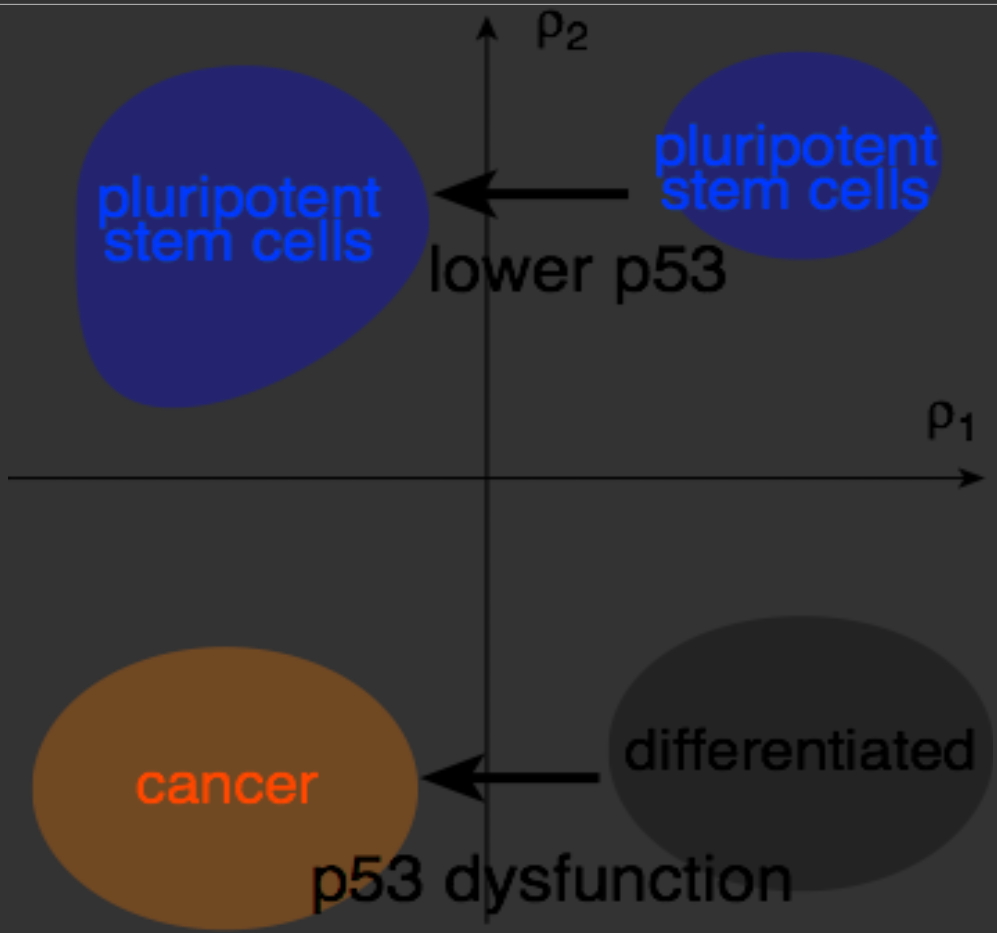


Lu J. et al, Nature, 2005

Targeting of transcripts that operate near thresholds

Low p53 → High p53

miRMap



Reducing high dimensional miRNA data to categories that classify cell identities

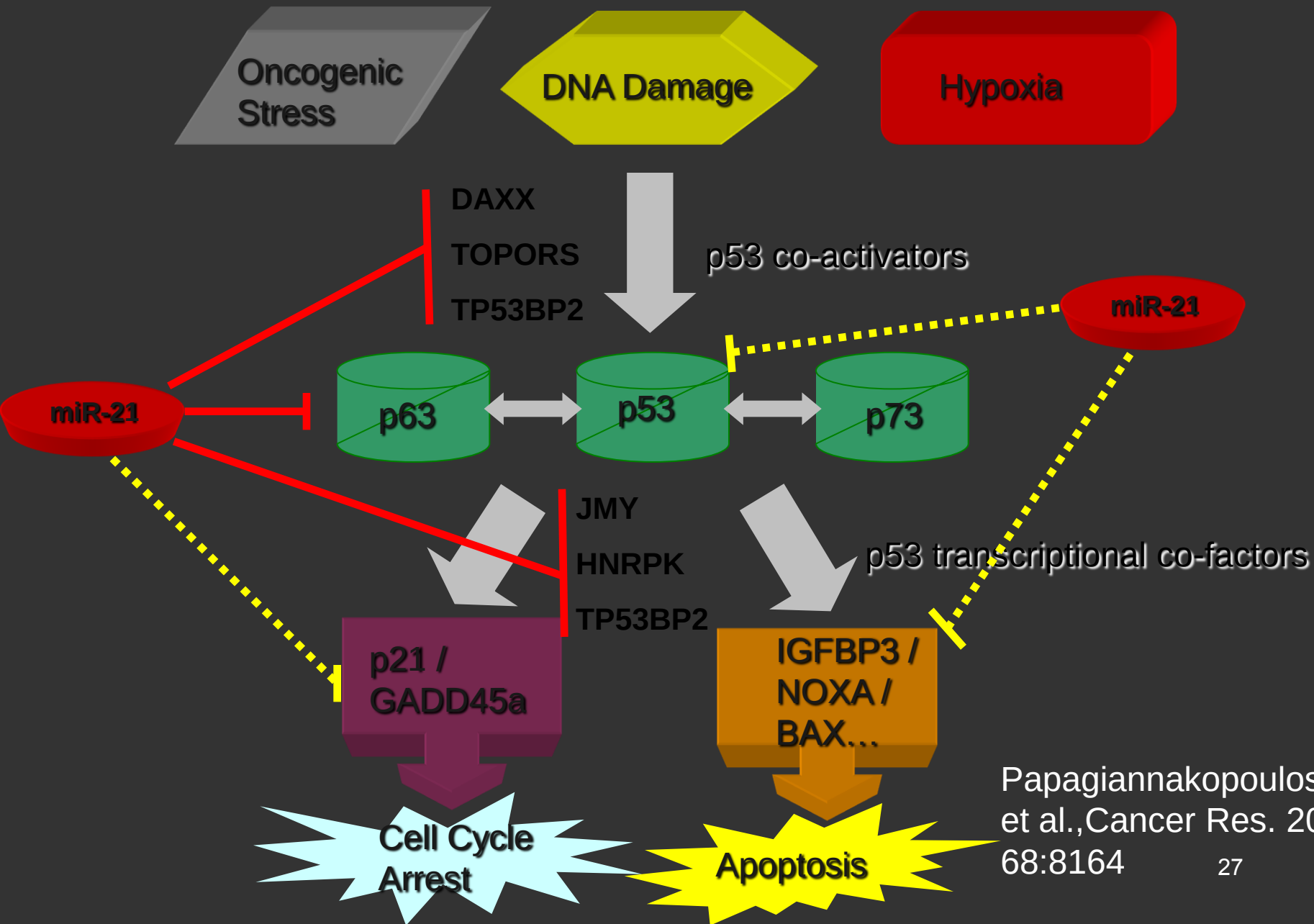
Neveu et al.,
Cell Stem Cell 7:671, 2010

Systems Functions of miRNAs

1. Feedback loops

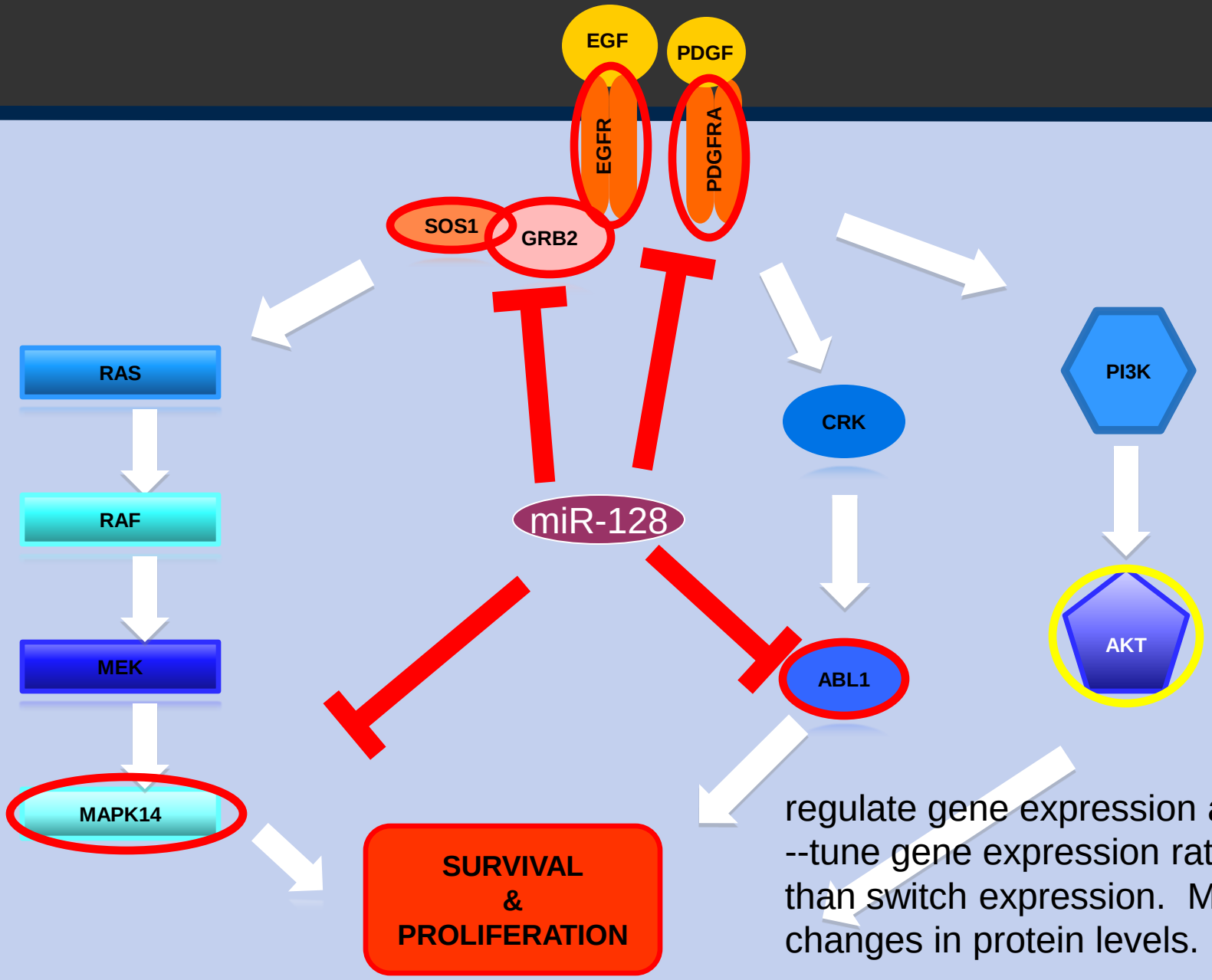
2. **Distributed Network Effects**

miR-21 network targeting



Papagiannakopoulos T, et al.,Cancer Res. 2008, 68:8164

miR-128 targets Mitogenic RTK Signaling



regulate gene expression additively --tune gene expression rather than switch expression. Make small changes in protein levels.

miR-7 RNA sequence is perfectly conserved from annelid to human

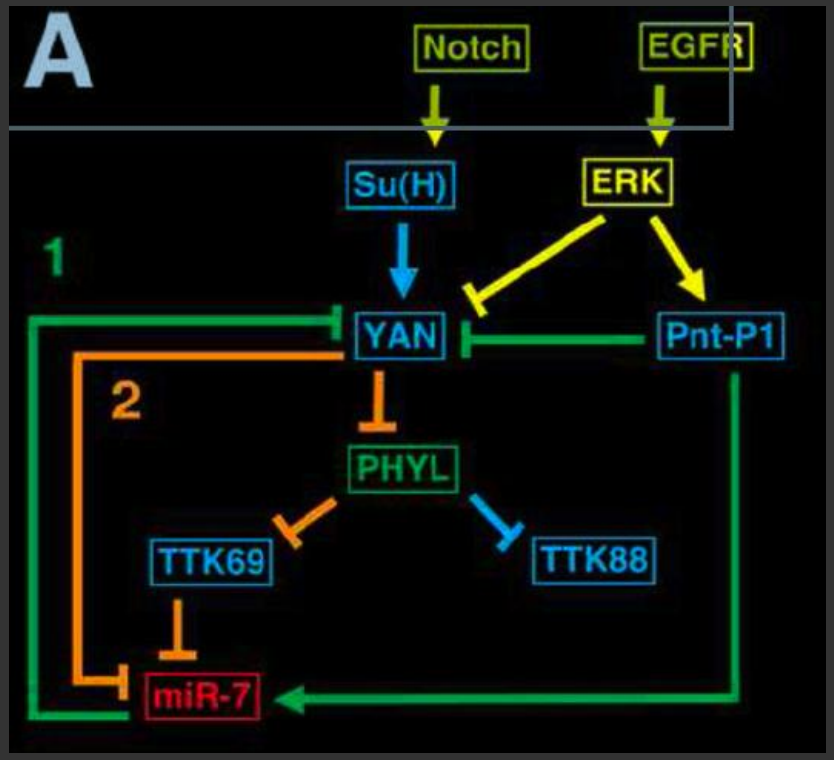
In *Drosophila miR-7* is in gene networks that determine sensory organ fate & is expressed in developing sensory organs

loss of *miR-7* --little or no detectable impact on sensory organ development

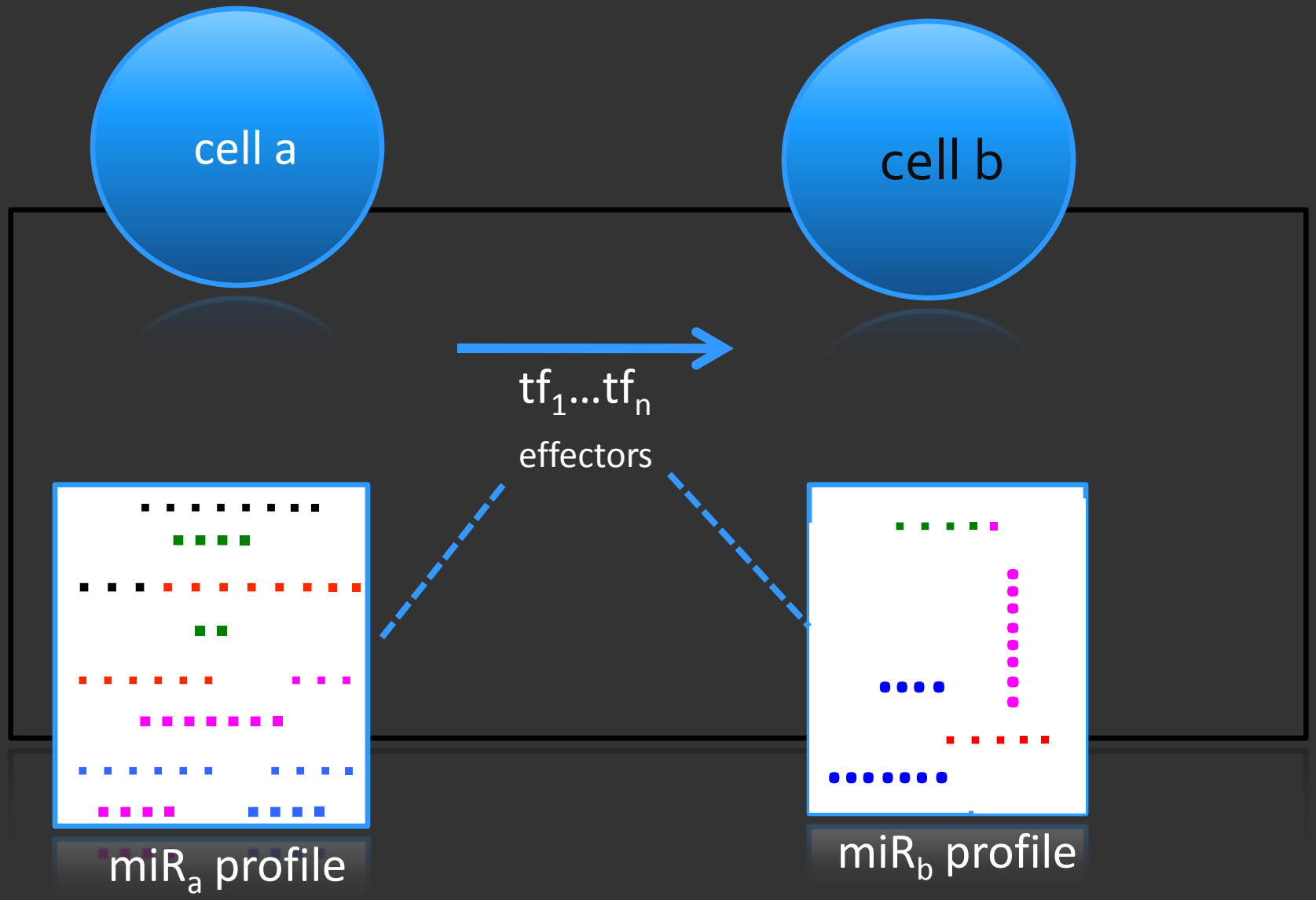
developing *Drosophila larvae*
fluctuate the environmental temperature between 31˚ C and 18˚ C every ~ 1.5 hrs

wild-type larvae --no defects in expression of *ato and yan.*

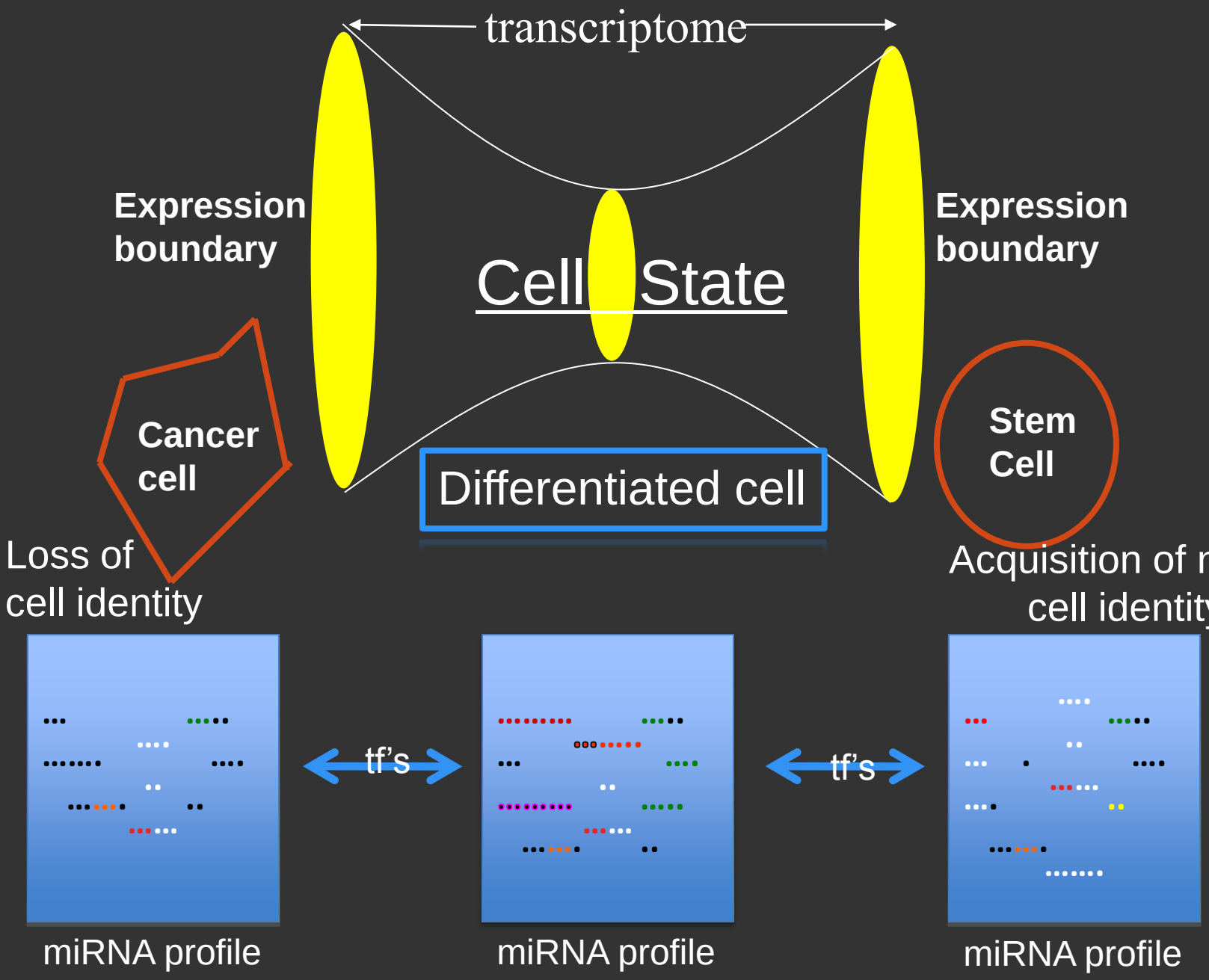
miR-7 mutant eyes fails to activate ato and repress yan.



Carthew lab
Cell 137:273–282, 2009.

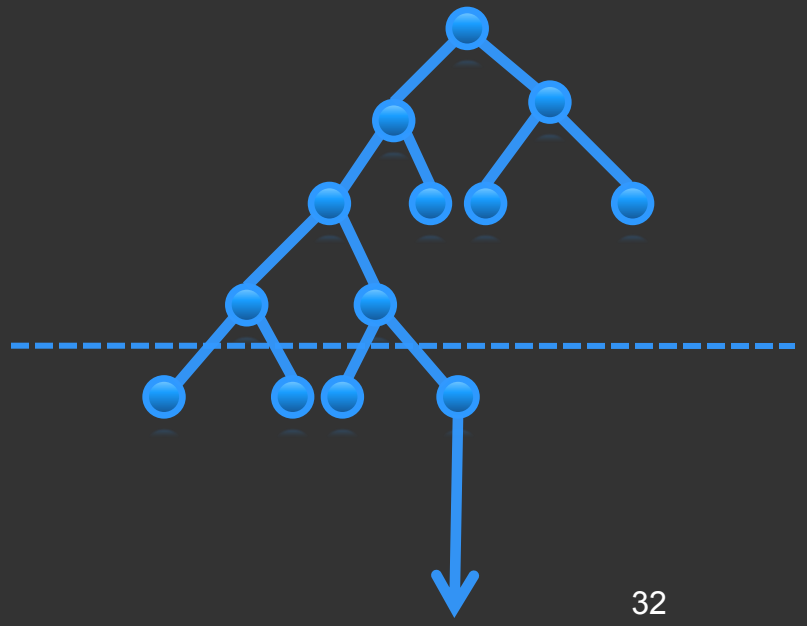


Kosik KS Cell 2010 Oct 1;143(1):21-6.



Kosik KS Cell 143:21, 2010

miRNAs may capacitate the emergence of large numbers of precursor cell types capable of honing developmental processes toward highly specialized identities and precise cell numbers.



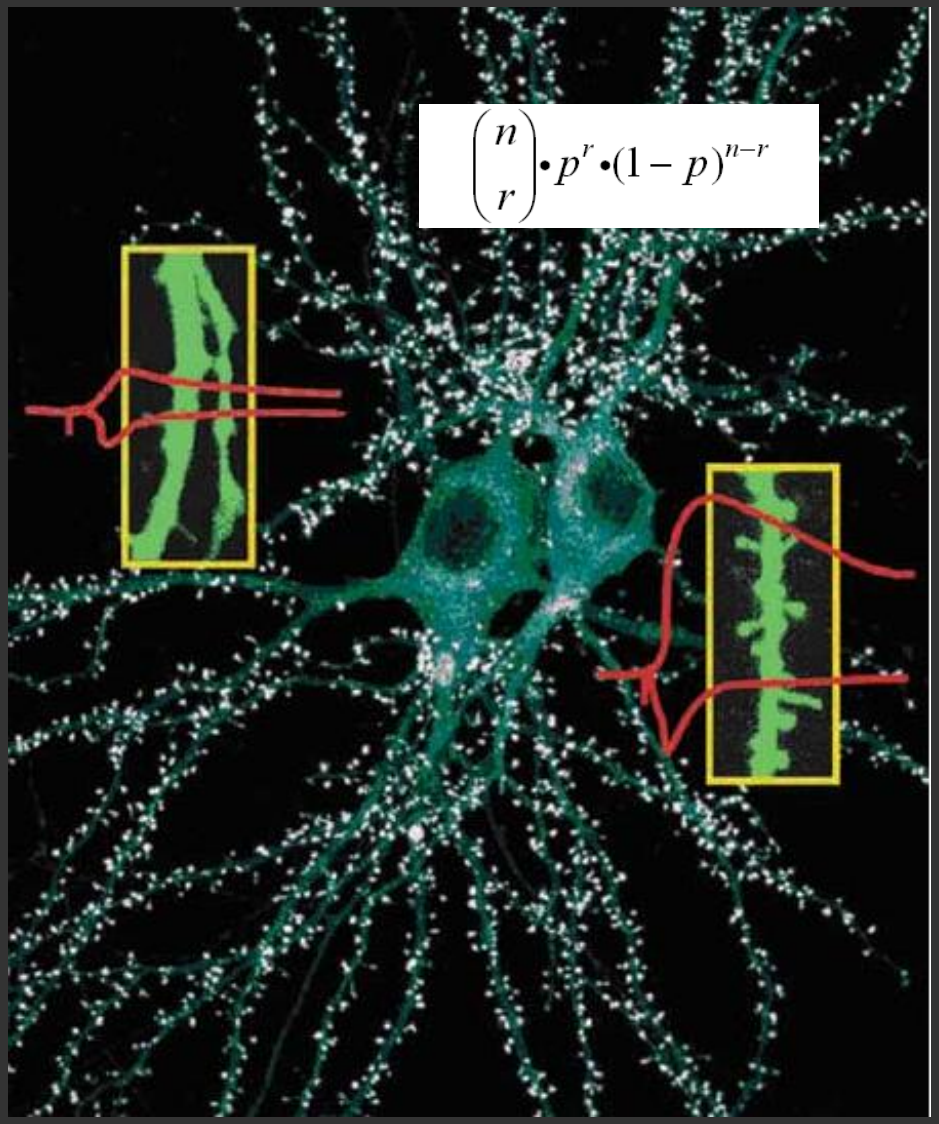
Neurons recruit the miRNA system for a specialized role at synapses

“why miRNA gene regulation” instead of using more transcription factors?

1. Rate of biogenesis is more rapid than proteins
2. Affect expression with less delay than factors that regulate nuclear events

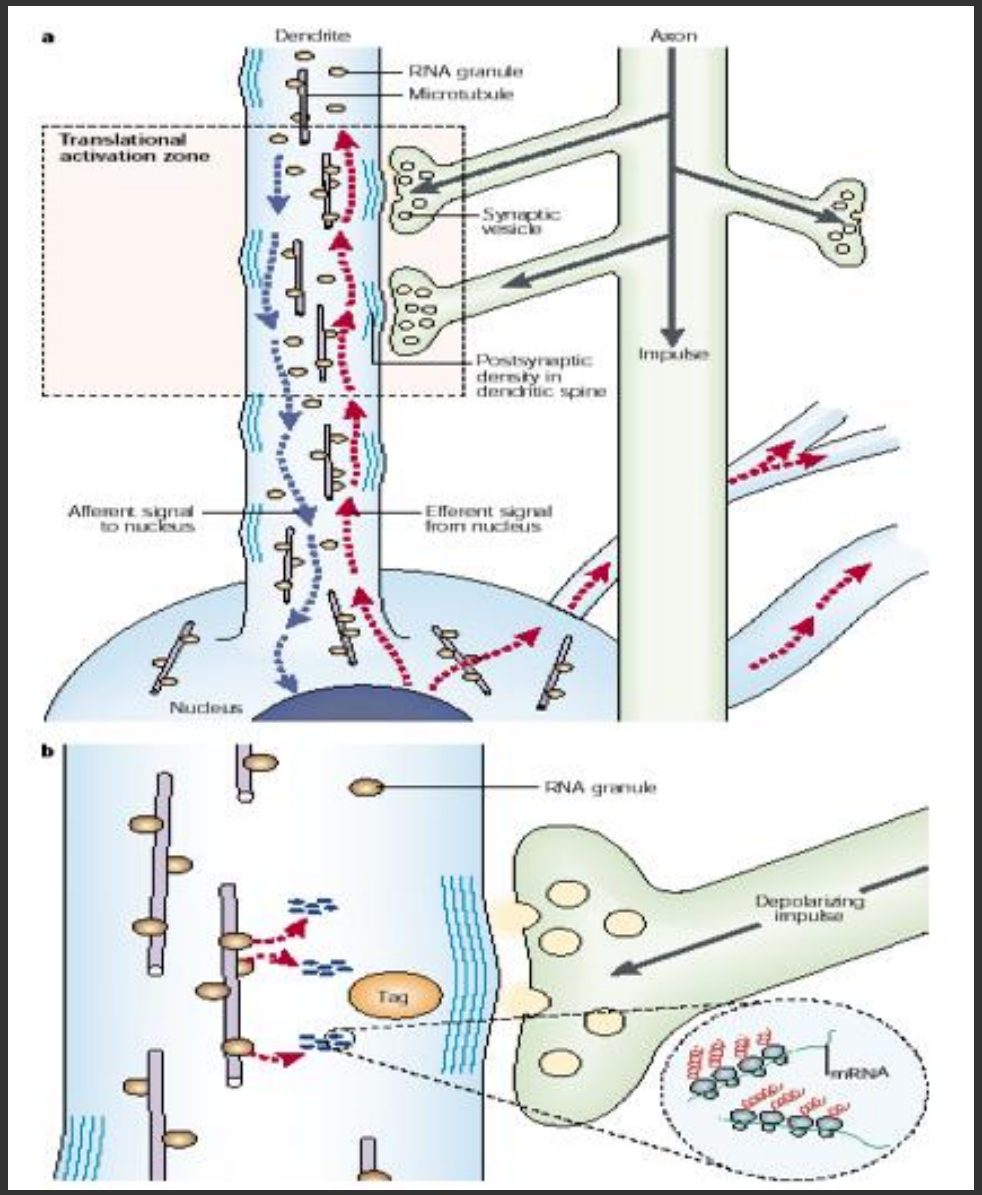
Therefore, miRNAs can produce more rapid responses

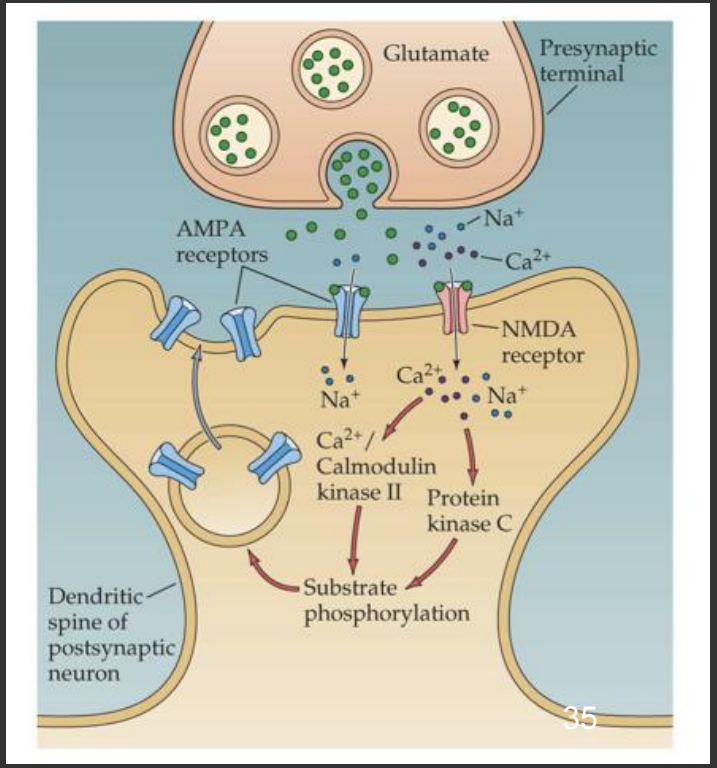
Neural Firing is a contingency event



depends on chance contingencies of environmental exposure, free will…

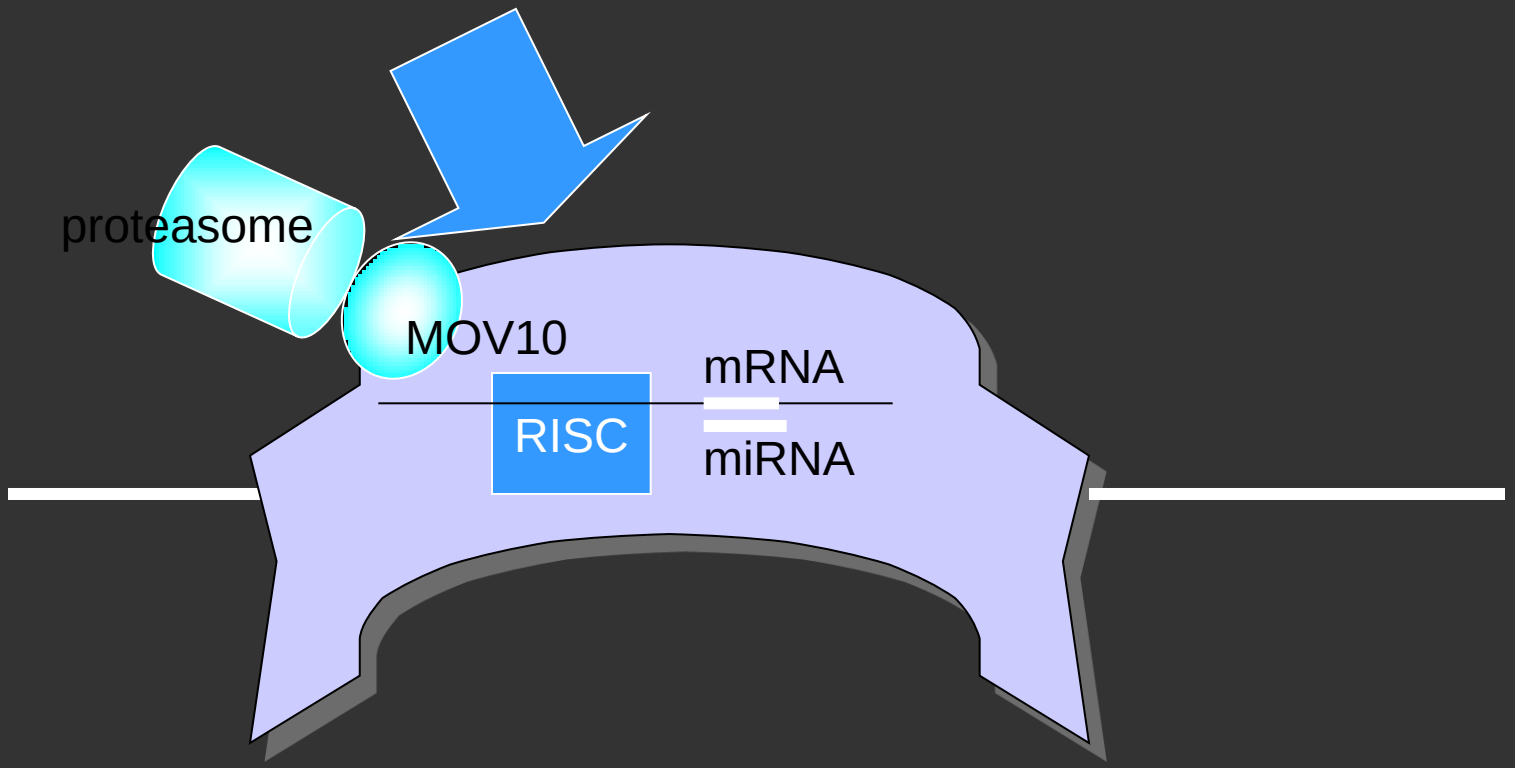
Synapse Utilization



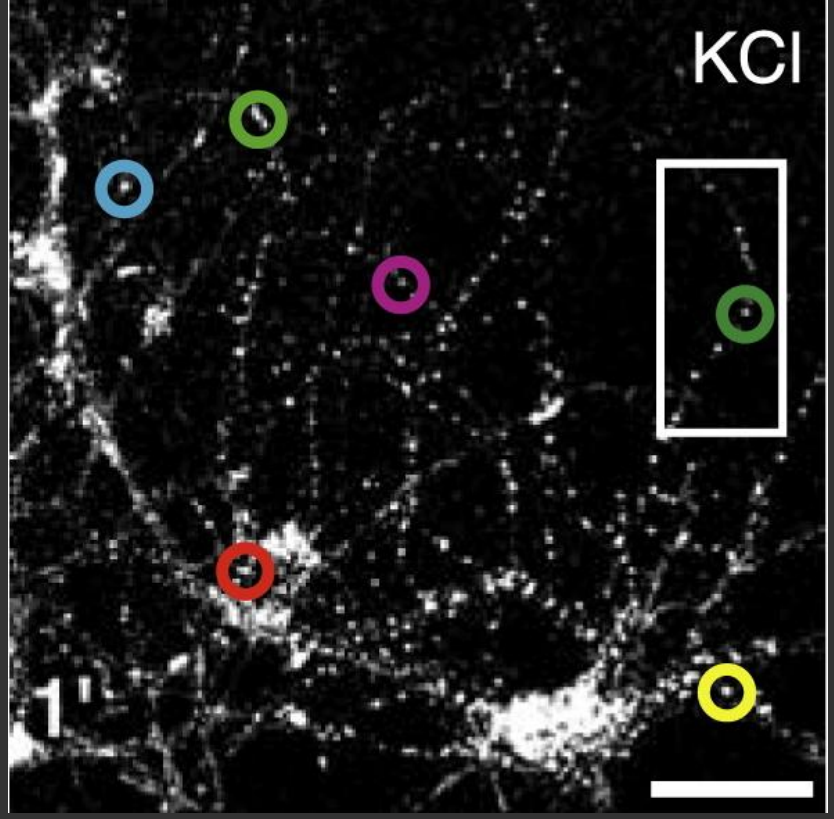


Martin KC, Kosik KS: Synaptic tagging -- who's it?
Nat Rev Neurosci. 3:813-20, 2002

Degradation at the synapse relieves miRNA suppression and permits protein translation



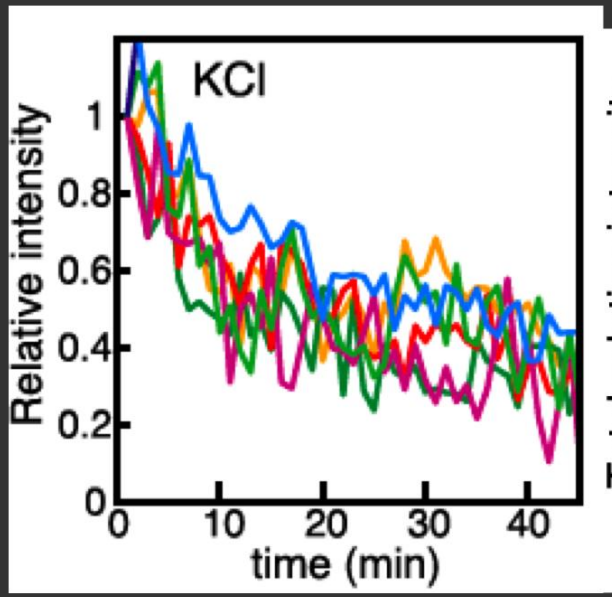
stable miRNA/mRNA duplexes allows an entire control layer to lie poised for the rapid release of a networked set of mRNAs to undergo translation and achieve a smooth and coordinated identity transition or change in synaptic thresholds.

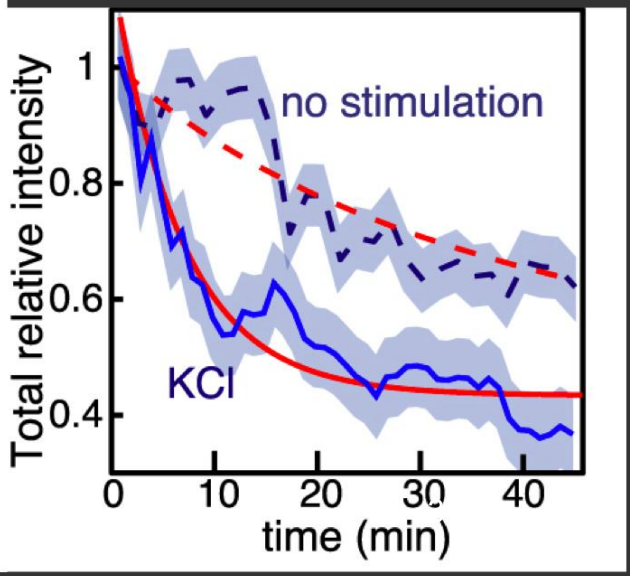


Single Puncta Analysis of MOV10 Degradation

[MOV10] $\xrightarrow{k_1=1/\tau}$ [MOV10]

τ=8.6 sec

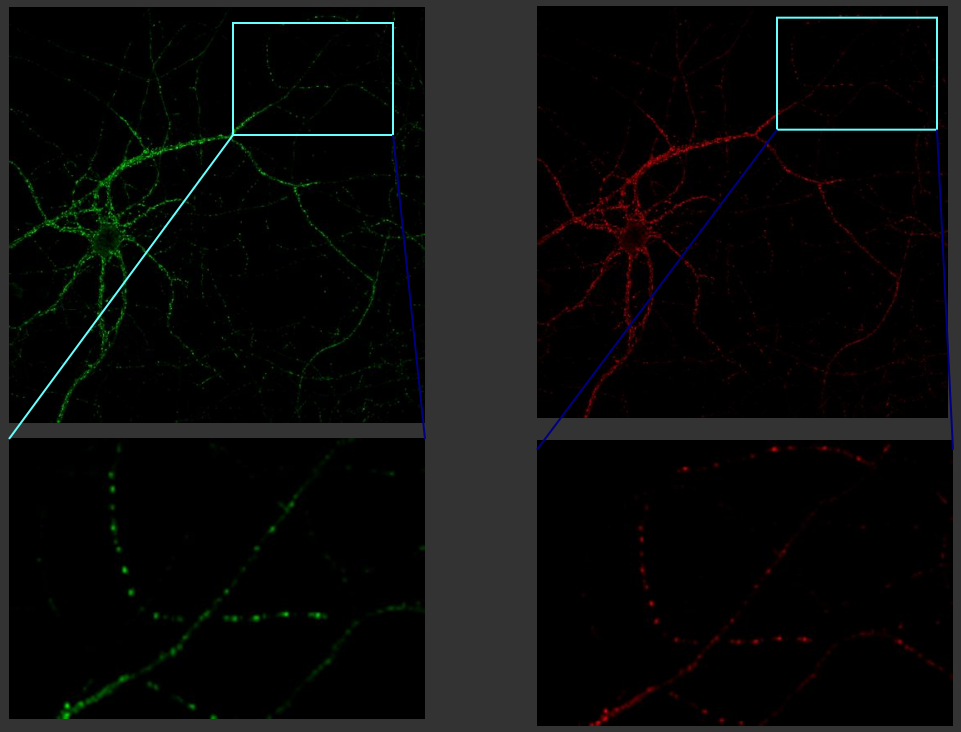




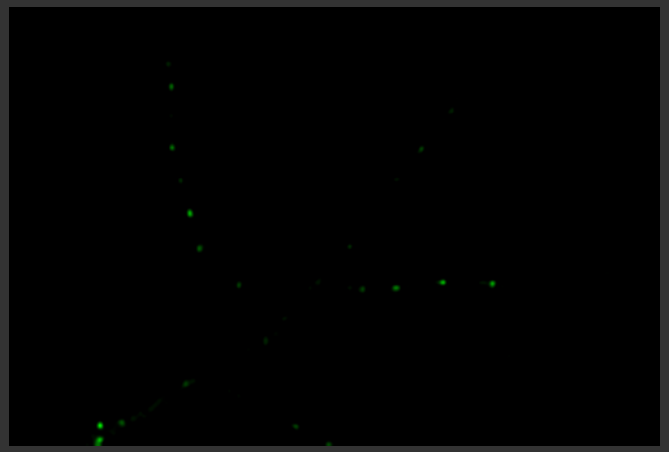
Banerjee S, et al.,
Neuron, 2009

Evaluation of Translationally Trapped mRNAs: New Protein Synthesis Reporter

Kaede is a photoconvertible fluorescence protein that changes from green to red upon exposure to uv light.



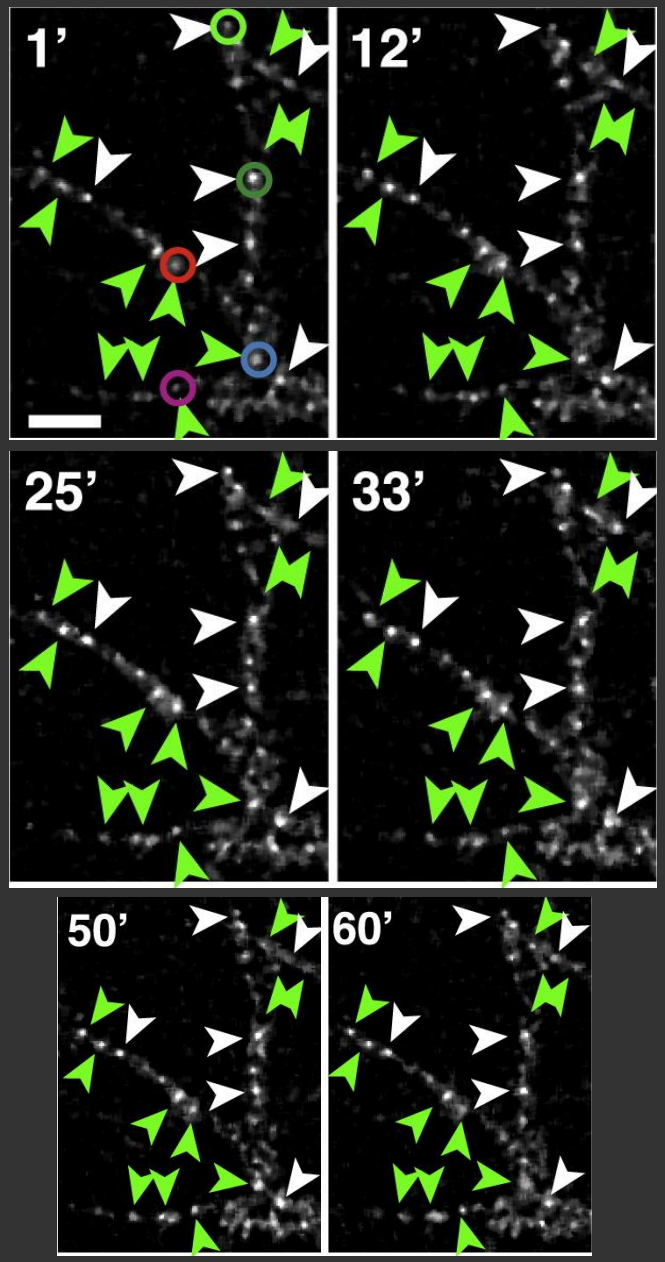
Photoconversion

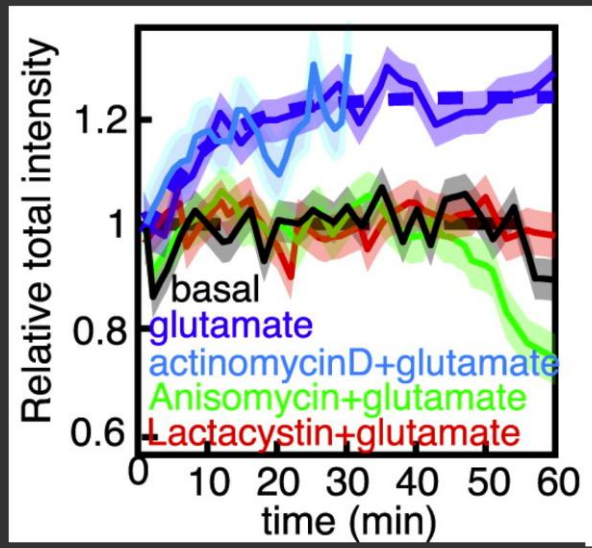


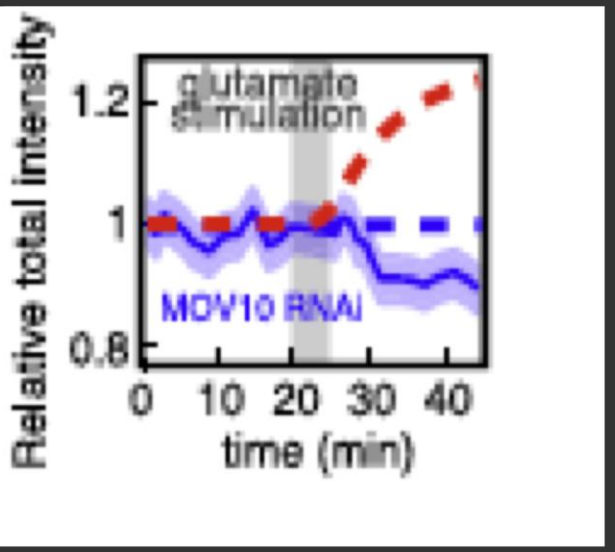
Stimulation

Kaede | Lypla1 3' UTR

Activity-regulated Proteasomal Control of Localized Synthesis

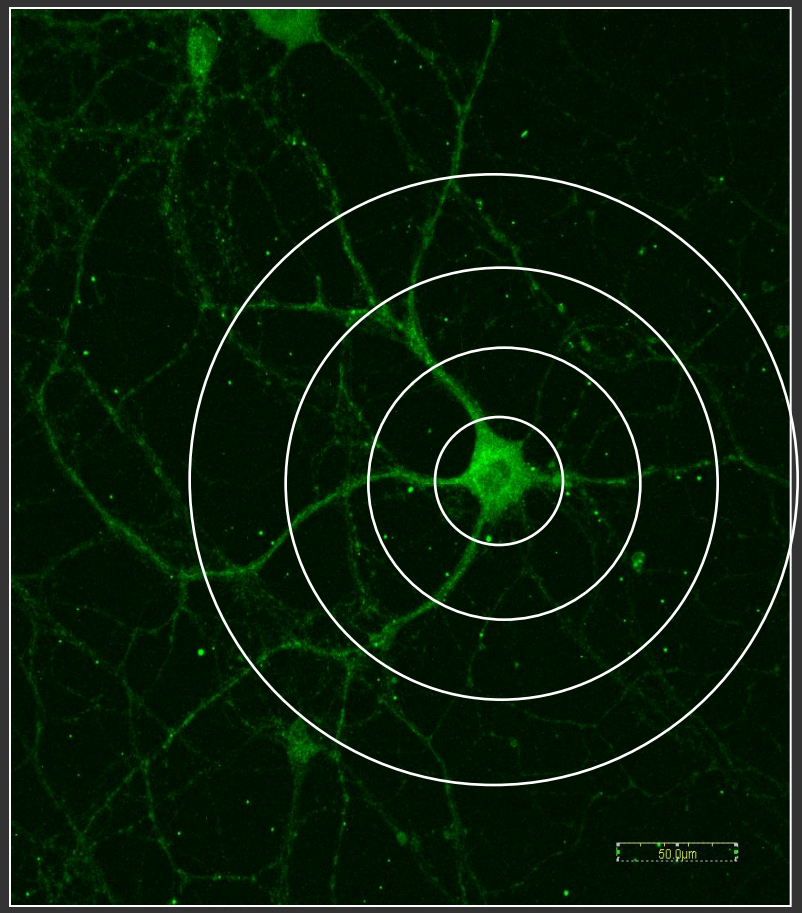




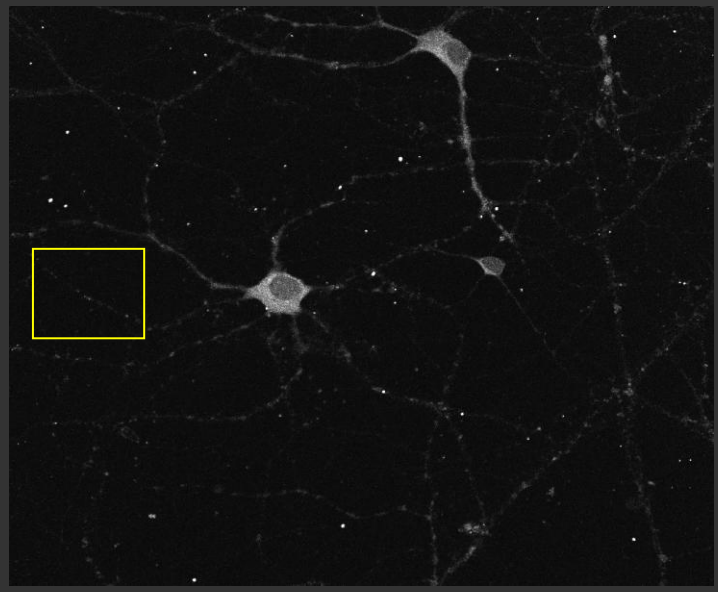


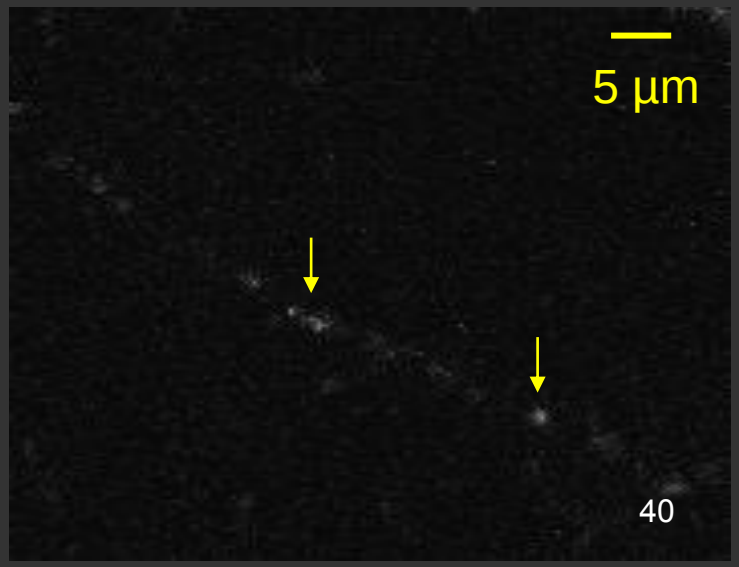
Banerjee S, Neveu P, Kosik KS. Neuron 64:871-84, 2009

Specialized Roles for miRNAs in Neurons

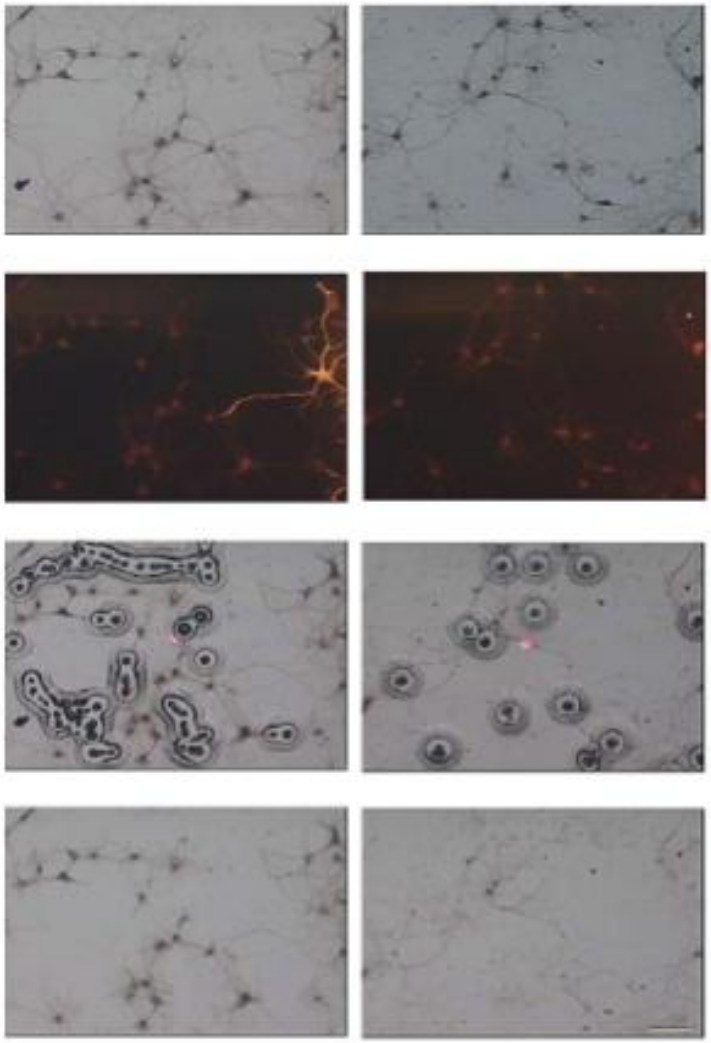


rno-miR-26a





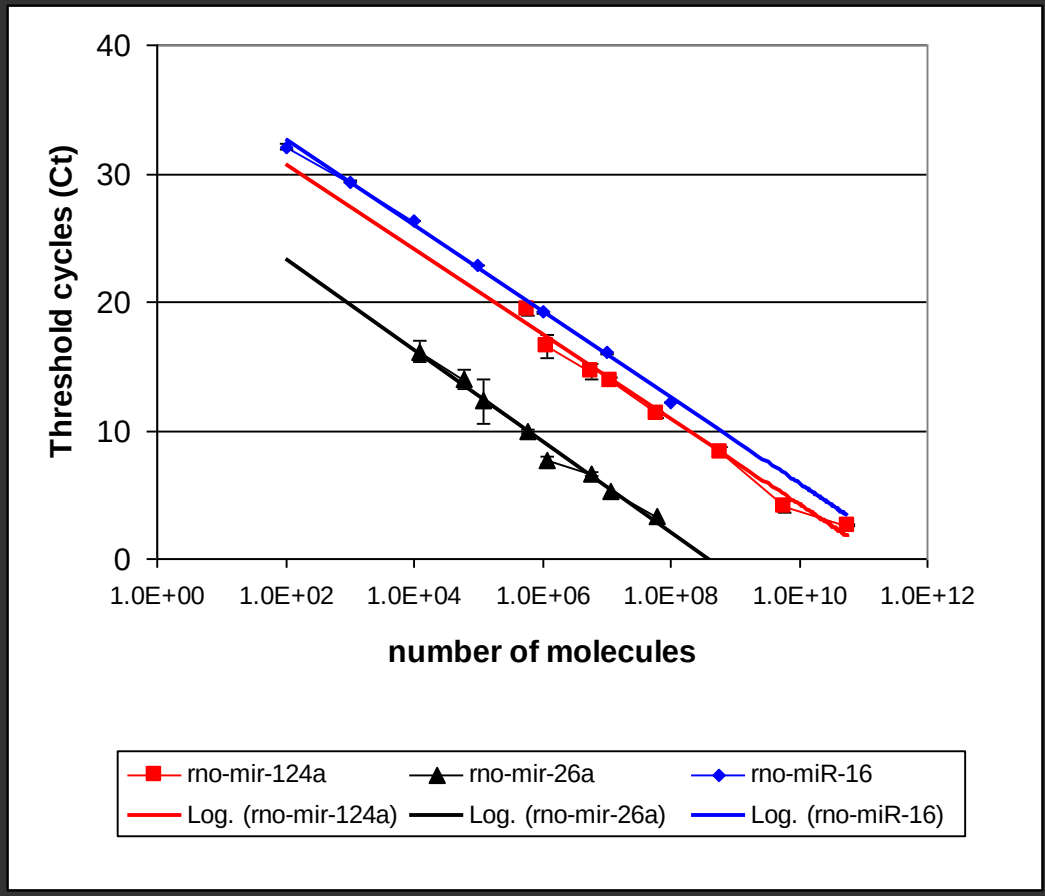
Biology at low copy number



rno-miR-124a: 20864 ± 3654 / cell

rno-miR-26a: 4118.9 ± 142.2 / cell

rno-miR-16: 3224.5 ± 656.8 / cell



Relative to the number of synapses
neuronal firing is relatively low frequency event.

High cost of maintaining synaptic machinery
available for low frequency contingencies

The brain is an expensive organ:

The adult human brain = 2% of the body weight

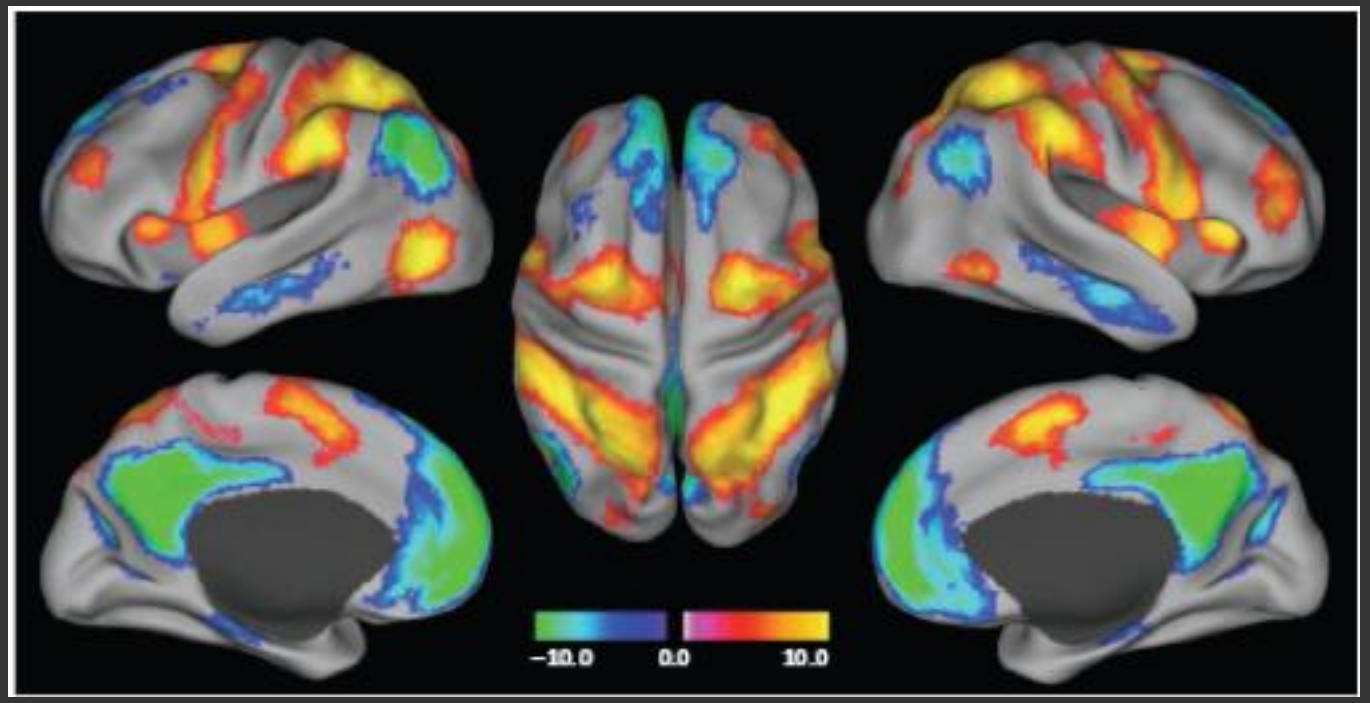
but uses 20% of the body's total energy consumption

Stochastic availability of neural machinery
to respond to the contingencies of synaptic firing

Advantages: reduce resource requirements at each synapse
reduce noise by coincidence detection

The additional energy the brain expends for task directed behavior is extremely small compared to the ongoing amount of energy that the brain continuously expends.

60 - 80% of the brain energy budget is basal/intrinsic activity. The additional energy burden associated with momentary demands of the environment is 0.5 to 1.0% of the total energy budget.



Default network

Marcus E. Raichle The Brain's Dark Energy. Science 314:1249, 2006

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