Biology I

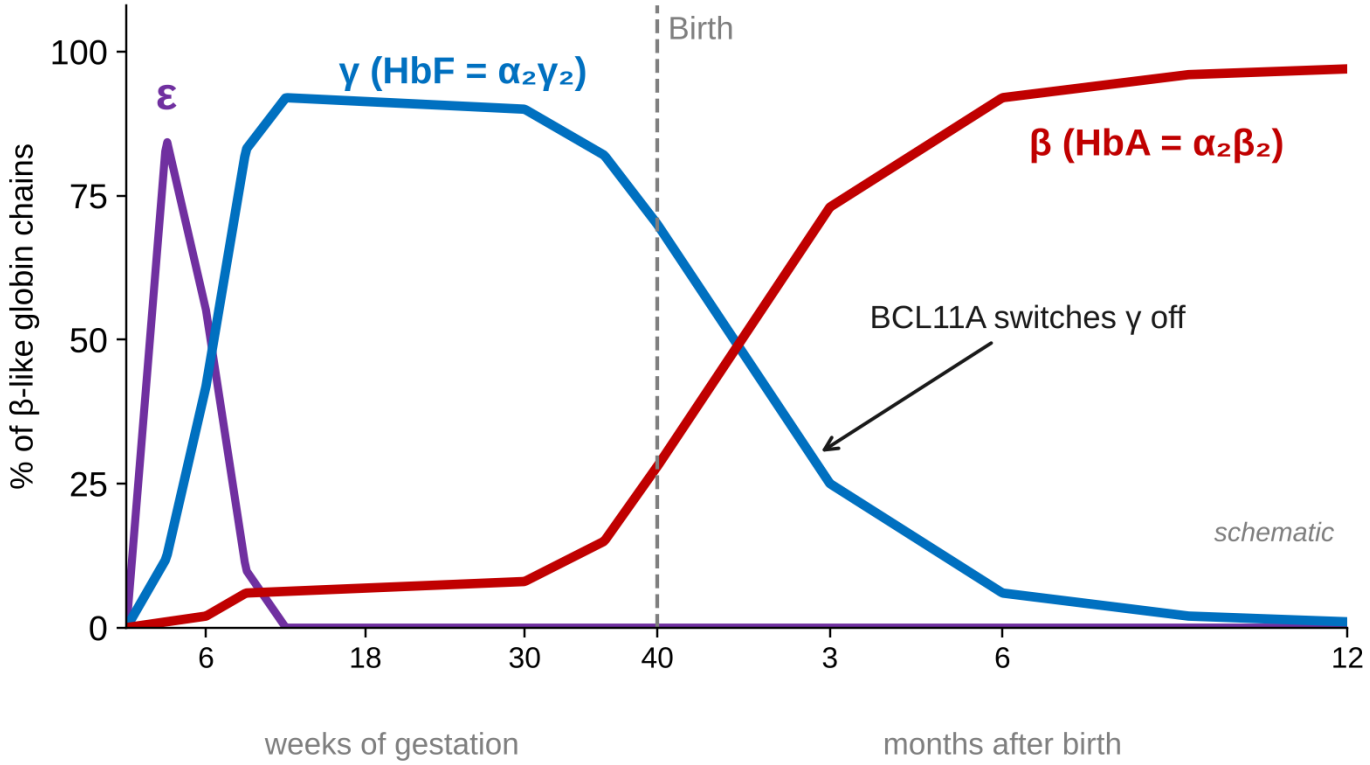
Basic Principles of Cell Biology

Regulation of gene expression

Transcription regulators, cell memory and regulatory RNAs

Yiannis Drosos
Assistant Professor
Laboratory of Biology
Department of Medicine
University of Patras
idrosos@upatras.gr

A gene that falls silent after birth: fetal hemoglobin



~7.5%

of the population in Greece are β-thalassemia carriers

91%

of patients with β-thalassemia transfusion-free after exa-cel (Casgevy)

Dec 2023

first FDA approval of a CRISPR therapy — it targets an enhancer

The γ-globin gene is not lost in the adult — it is simply "silenced". If we "wake it up", it replaces the missing β chain.

Sankaran et al., Science 2008 · Locatelli et al., NEJM 2024 · Loukopoulos, Indian J Med Res 2011 · FDA, Dec 2023

Three questions for the next three hours

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1

How does a cell “decide” which genes to express — and how does a protein find its target in DNA?

→ Part A (1 hour): principles, transcription regulators, operons

2

How is transcription controlled in humans — and how does a cell “remember” its identity?

→ Part B1: enhancers, combinatorial control, memory, iPS, diseases

3

How do small and long RNAs regulate expression — and how did they become drugs?

→ Part B2: miRNA, siRNA, lncRNA, RNA therapies

From previous lectures: chromatin, histone modifications, DNA methylation (Chromosomes) · transcription, splicing, translation (From DNA to proteins). **Today: who decides when, where and how much.** Alberts, Essential Cell Biology, Ch. 8.

Key concepts

Gene expression and its regulation

The path from DNA to a functional product; regulation decides which genes are expressed, when, where and how much.

Regulatory sequence (cis)

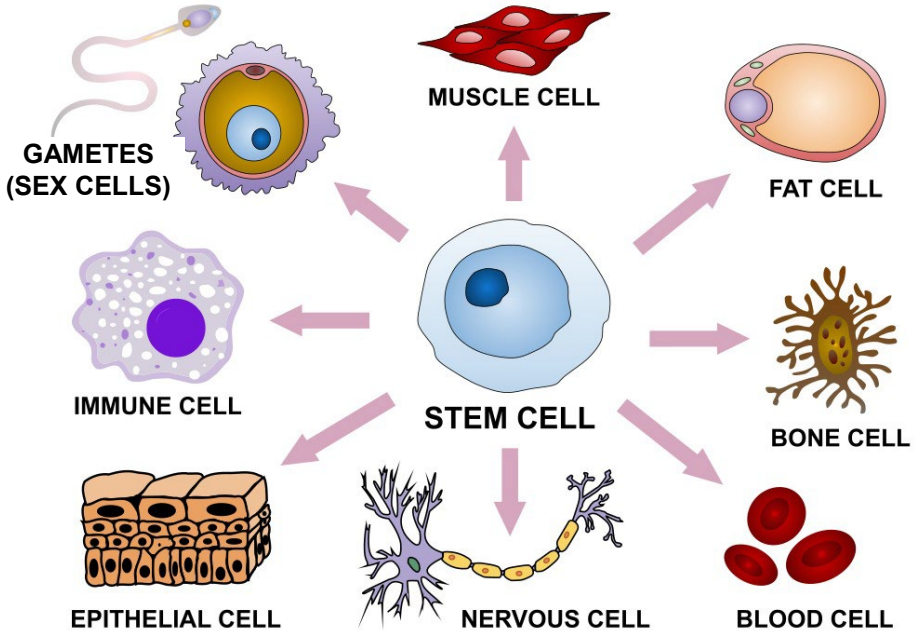
A short stretch of DNA on the same molecule as the gene it controls — promoter, enhancer, operator.

Transcription regulator (trans)

A protein that recognizes a regulatory sequence and activates or represses transcription.

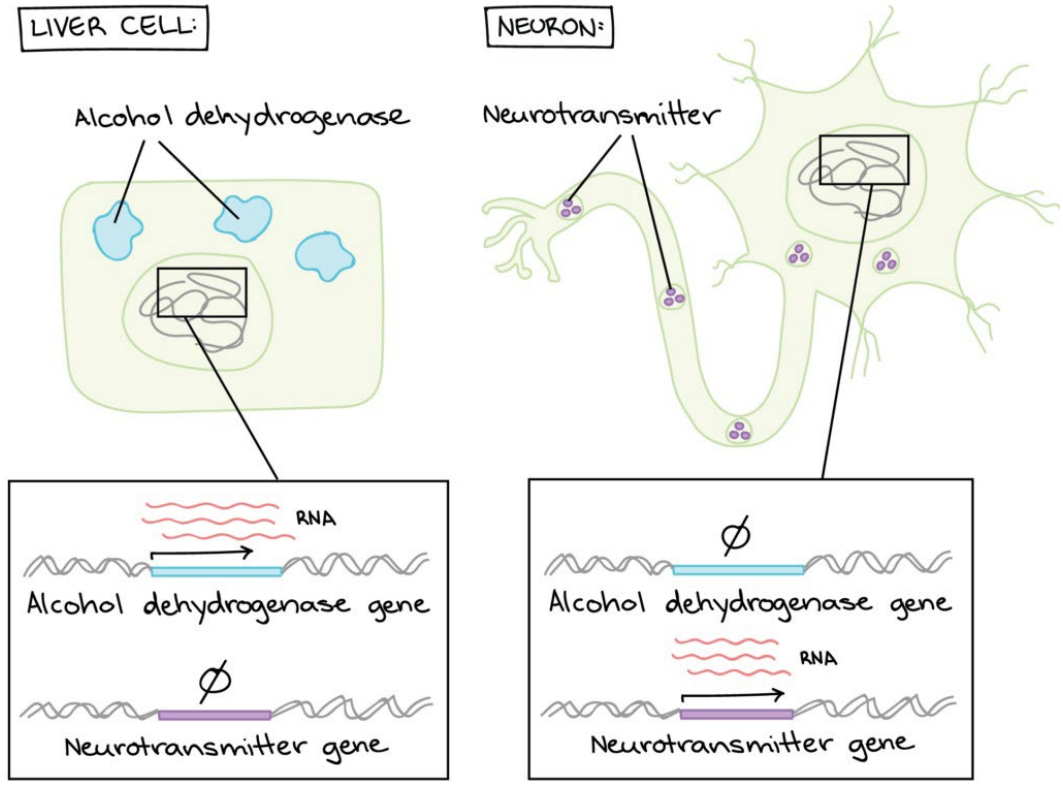
Regulatory non-coding RNA

An RNA that is not translated but controls other genes — miRNA, siRNA, lncRNA.



Alberts et al., Essential Cell Biology, 6th ed., Ch. 8

Same genome, a different “program” in every cell



Hepatocyte and neuron: same genes, different genes switched on

~37 trillion

cells in the human body

~400

major cell types

~44%

of protein-coding genes are expressed in all tissues

Housekeeping genes

RNA polymerases, ribosomal proteins, cytoskeleton, enzymes of basic metabolism and of DNA repair.

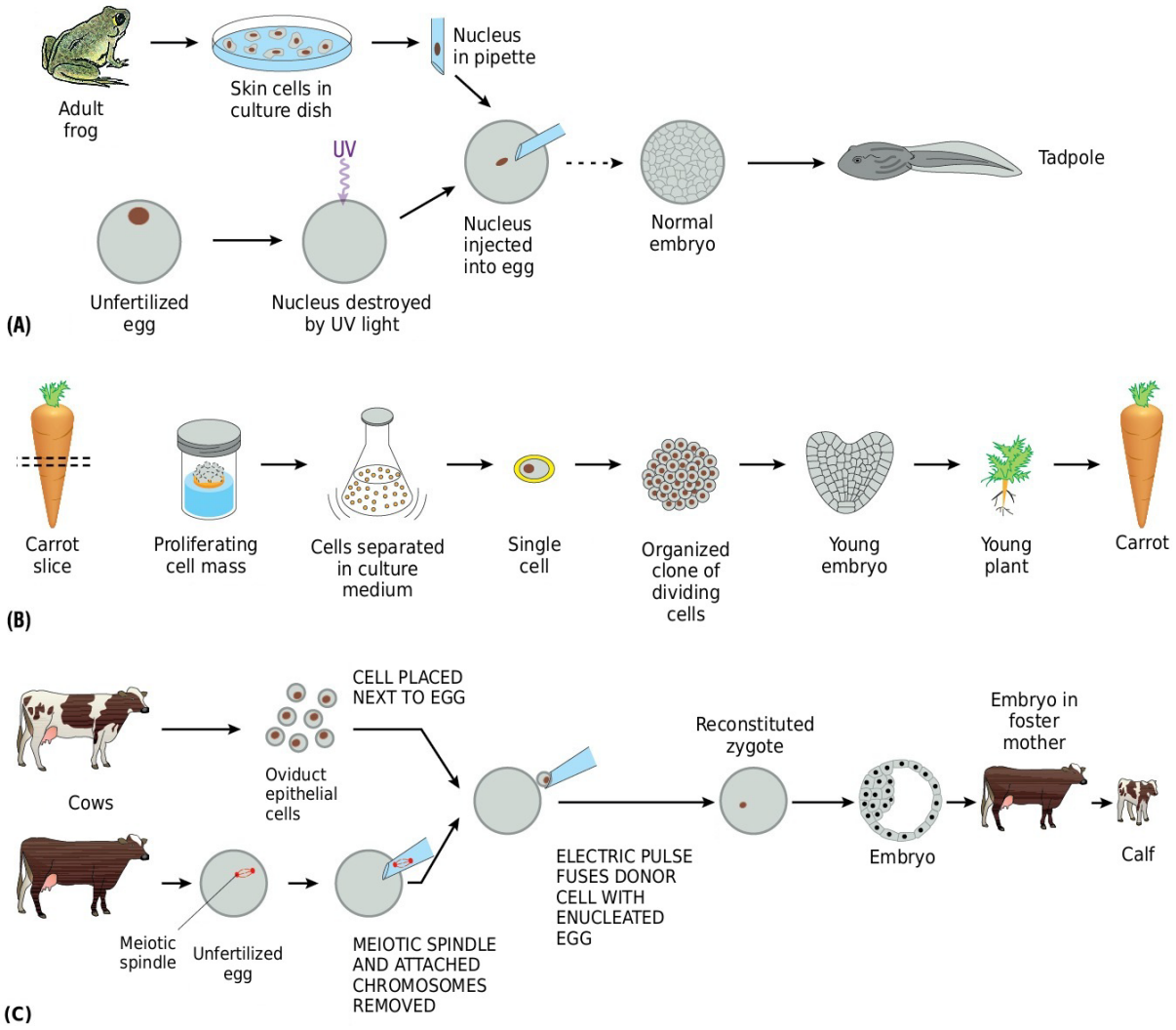
Cell-type-specific genes

Hemoglobin (erythroblasts) · insulin (β cells) · glucagon (α cells) · antibodies (plasma cells) · albumin (hepatocytes).

The differences are qualitative (which genes) and quantitative (how much) — and they also involve RNAs that do not code for proteins.

Bianconi et al., Ann Hum Biol 2013 · Hatton et al., PNAS 2023 · Human Protein Atlas (v25) · Alberts et al., ECB, Ch. 8

Differentiated cells keep all their genes



1962 · Gurdon

Nucleus from a tadpole intestinal cell into an enucleated egg → normal tadpole and frog (cf. panel A).

1997 · Dolly

Nucleus from a mammary gland cell of an adult sheep → the first mammal cloned from an adult cell.

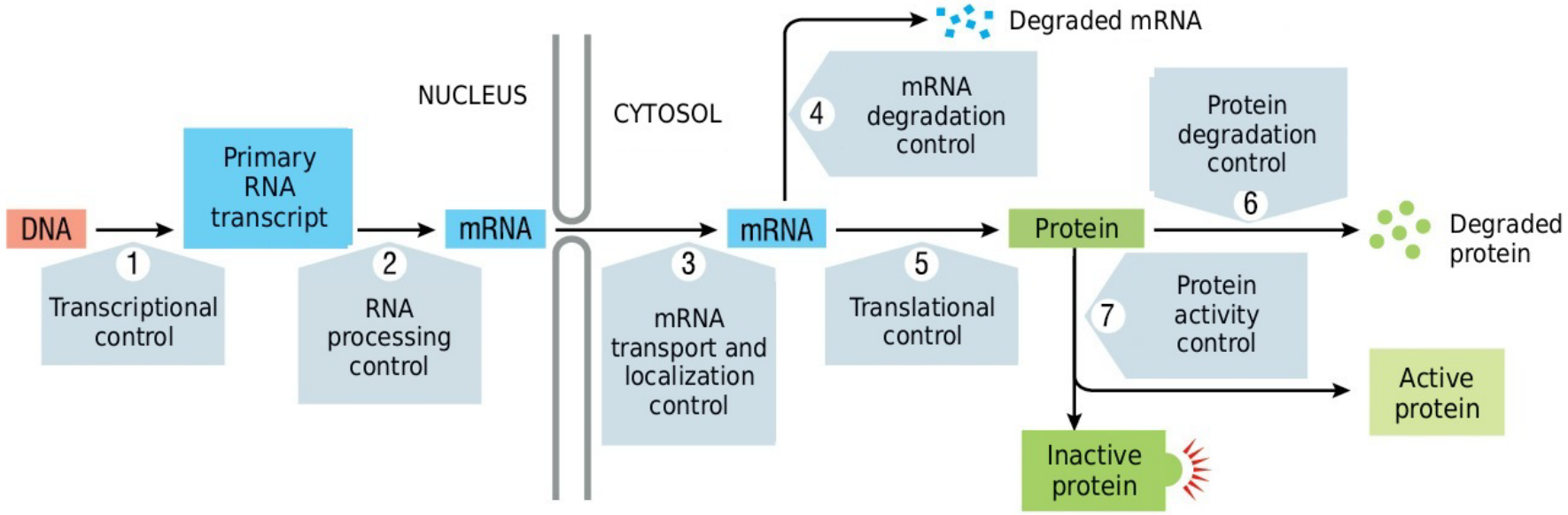
Nobel 2012 · Gurdon and Yamanaka

Mature cells can be reprogrammed (→ iPS, Part B1).

Differentiation changes gene expression, not DNA.
Exceptions: B and T lymphocytes rearrange their antibody and T-cell receptor genes; red blood cells lose their nucleus.

Gurdon, J Embryol Exp Morphol 1962 · Wilmut et al., Nature 1997 · Alberts et al., ECB, Fig. 8-2 (labels translated)

Where can the expression of a gene be regulated?



Today

(1) Transcriptional control: Parts A and B1

(4)–(5) mRNA degradation and translation by small RNAs: B2

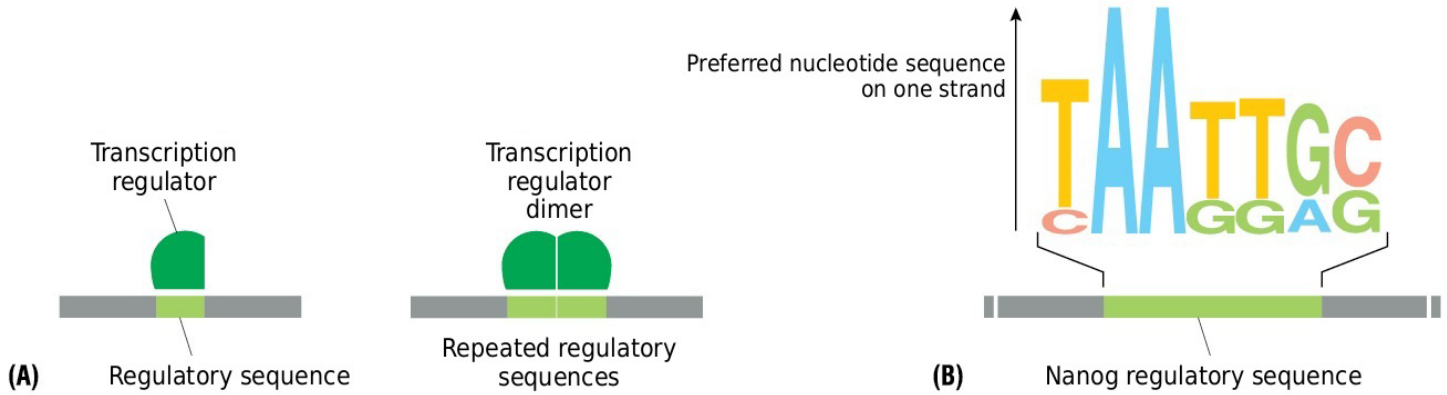
Already covered

Chromatin and epigenetic marks (Chromosomes) · (2) splicing, (3) export, NMD, (6) proteasome (From DNA to proteins)

For most genes the main control point is (1): the initiation of transcription.

Alberts et al., ECB, Fig. 8-3 (labels translated)

Regulatory sequences and transcription regulators



1,639

transcription factors in humans (~8% of protein-coding genes)

~20

protein–DNA contacts at one site: H-bonds, ionic, hydrophobic — without opening the helix

6–12 bp

typical length of a binding site

cis · regulatory sequences

Promoter, enhancers, operator; on the same DNA molecule as the gene, nearby or very far away.

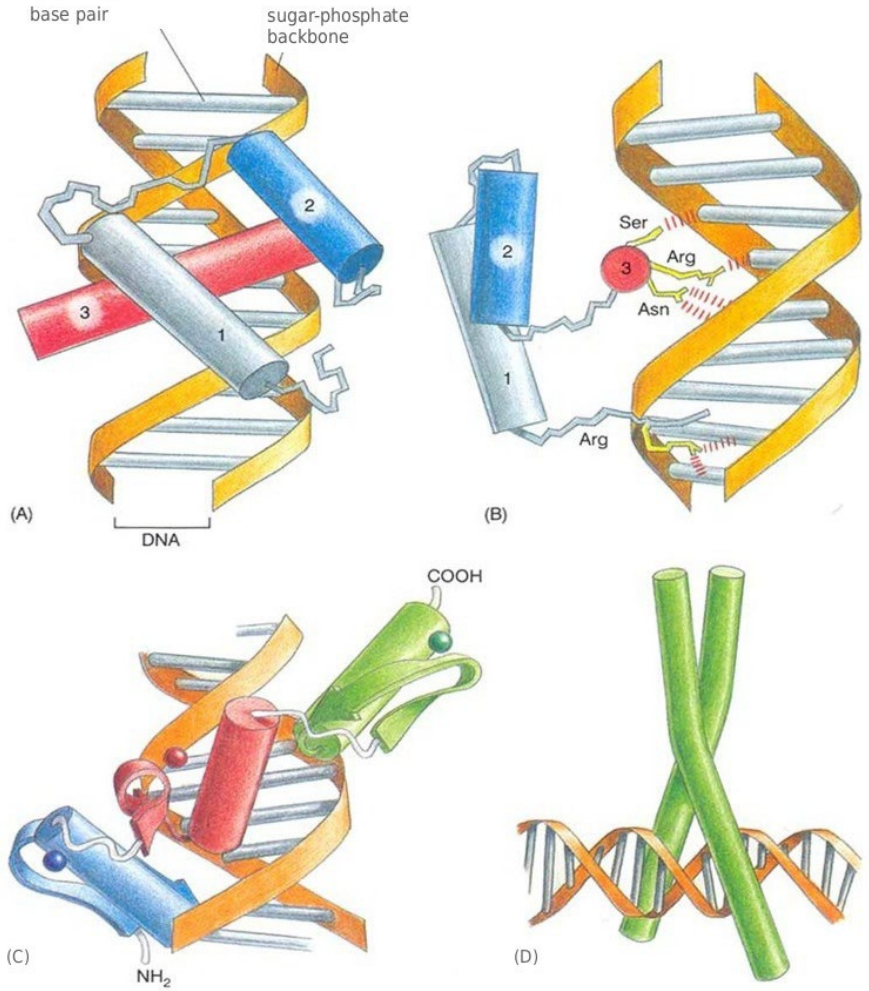
trans · transcription regulators

Proteins that “read” the bases in the major groove; activators or repressors, often as dimers.

A mutation in a regulatory sequence → changes how much protein is made, not its amino acid sequence.

Lambert et al., Cell 2018 · Alberts et al., ECB, Figs 8-4 and 8-5 (labels translated)

DNA-binding motifs — and what happens when they are mutated



Homeodomain · panels A, B

Three α-helices; helix 3 sits in the major groove. HOX and PAX genes that set up the body plan. Clinical: PAX6 → aniridia · HOXD13 → synpolydactyly.

Zinc finger · panel C

An α-helix and a β-sheet held together by a Zn^{2+} ion, often in series · WT1 → Wilms tumor. Nuclear receptors (glucocorticoid, estrogen) use a related type.

Leucine zipper · panel D

Two α-helices from two proteins zip together and bind as a dimer, “like a clothespin on a line”. FOS/JUN (AP-1), CREB · MYC/MAX → MYC oncogene (Burkitt lymphoma).

Alberts et al., ECB, Fig. 8-4 (labels translated) · OMIM · Lambert et al., Cell 2018

IN-CLASS EXERCISE #1 · 4 MINUTES

Find the binding site

A transcription regulator with a leucine zipper binds to the following stretch of a promoter:

```
5′- G C A T T G A C G T C A G C C T -3′
3′- C G T A A C T G C A G T C G G A -5′
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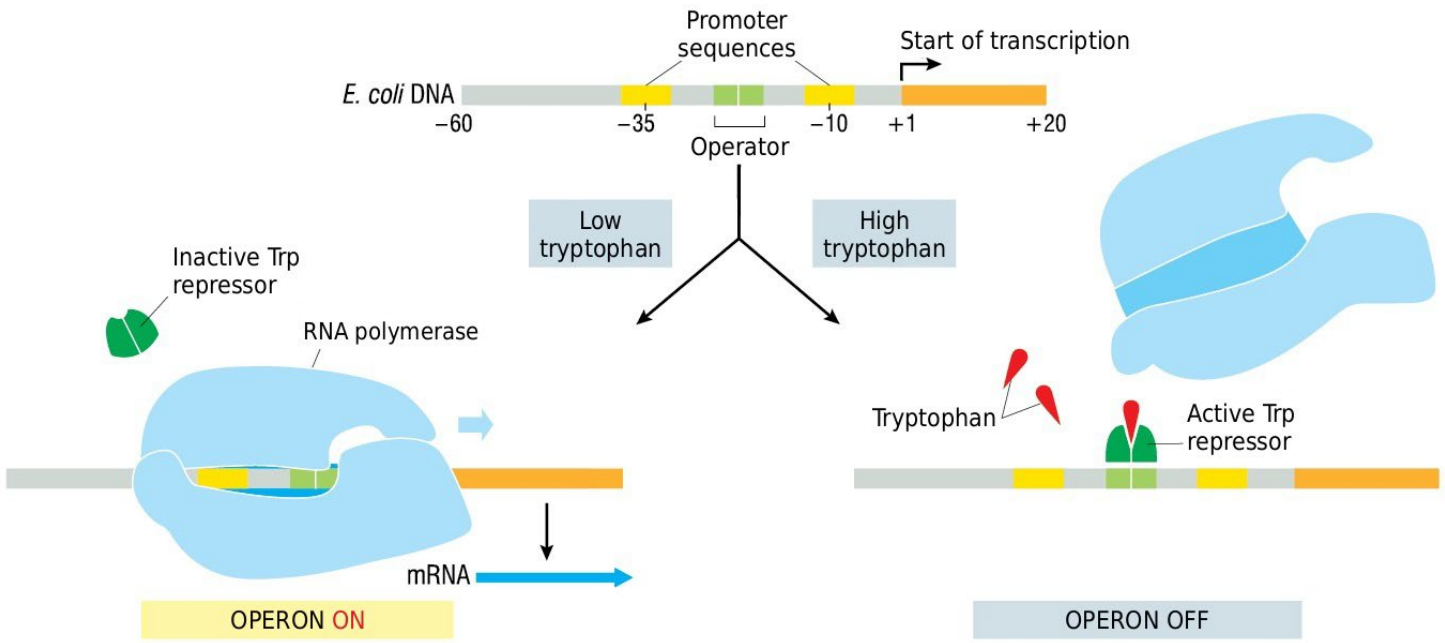
a Find the 8-nucleotide sequence that reads the same (5′→3′) on both strands.

b Why does this symmetry “suit” a regulator that binds as a dimer?

c How many times would it be expected to occur by chance in the genome (3.1×10^9 bp)? What does this mean for selectivity?

Hint: the probability of finding a specific 8-mer at a given position is $(1/4)^8$.

Bacteria: the tryptophan operon is controlled by a repressor



Operon

Bacterial genes transcribed together into one mRNA from one promoter — e.g., the 5 genes for the enzymes that make tryptophan.

Negative control

Tryptophan activates the repressor → it binds the operator → Pol does not transcribe.

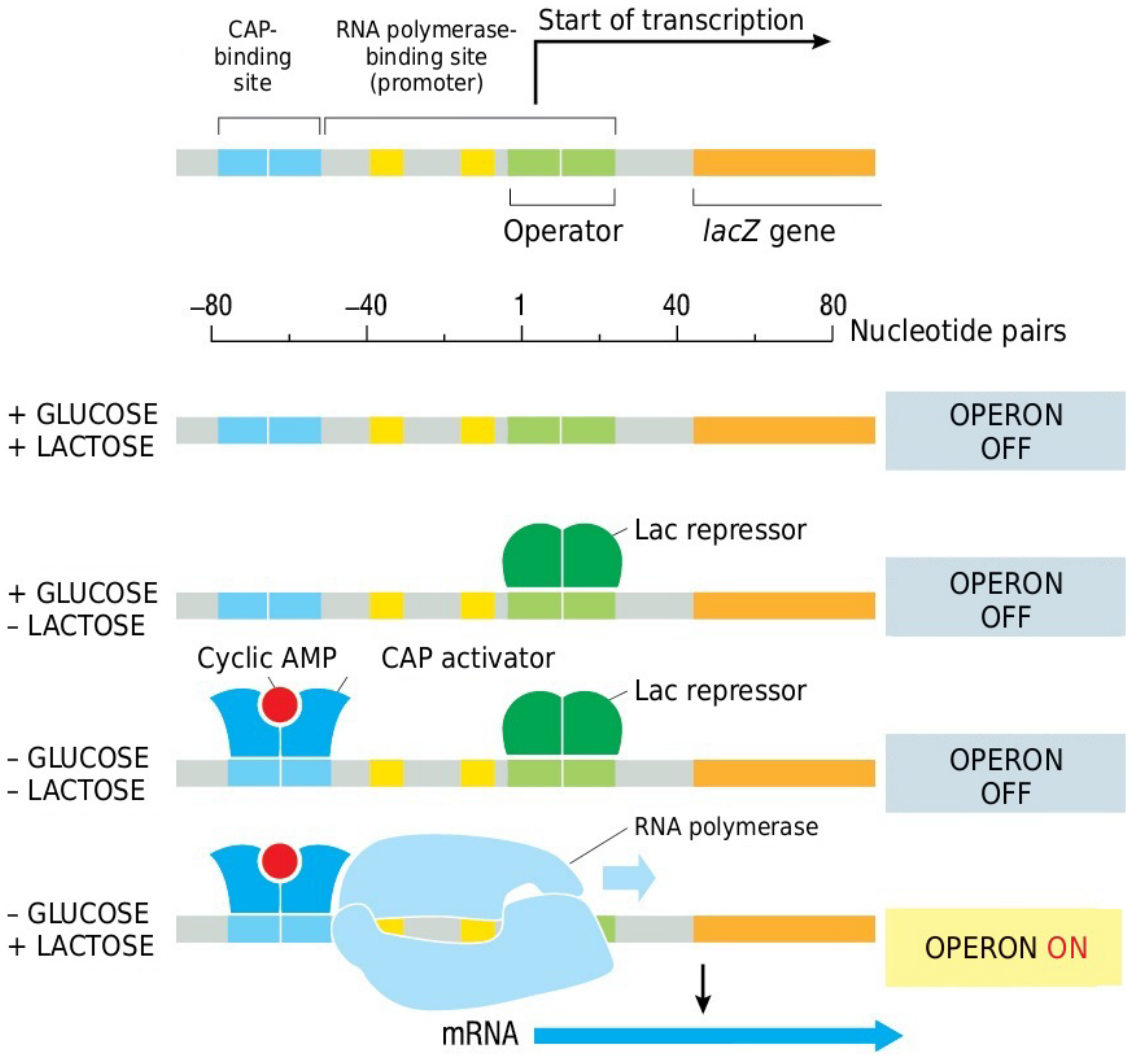
Positive control

Activators help Pol to bind.

The logic: "when the product is there, don't make the enzymes". The metabolite itself flips the switch (negative feedback).

Alberts et al., ECB, Figs 8-6 and 8-7 (labels translated)

The lactose operon: one repressor and one activator



Lac repressor

Binds the operator when lactose is absent; allolactose removes it (inducer).

CAP activator

When glucose falls, cAMP rises; the cAMP–CAP complex "brings in" the polymerase.

An "AND" logic gate

High transcription only with lactose AND without glucose.

Jacob & Monod · Nobel 1965. The lac promoter and the inducer IPTG are used to produce recombinant proteins.

Trap: glucose does NOT bind the repressor; it acts through cAMP–CAP.

Alberts et al., ECB, Fig. 8-9 (labels translated) · Jacob & Monod, J Mol Biol 1961

IN-CLASS EXERCISE #2 · 5 MINUTES · DISCUSS WITH YOUR NEIGHBOR

Predict transcription of lacZ

For each E. coli strain: high, low or almost zero?

a Wild type · with glucose and with lactose.

b lacI⁻ (no functional repressor) · no glucose, no lactose.

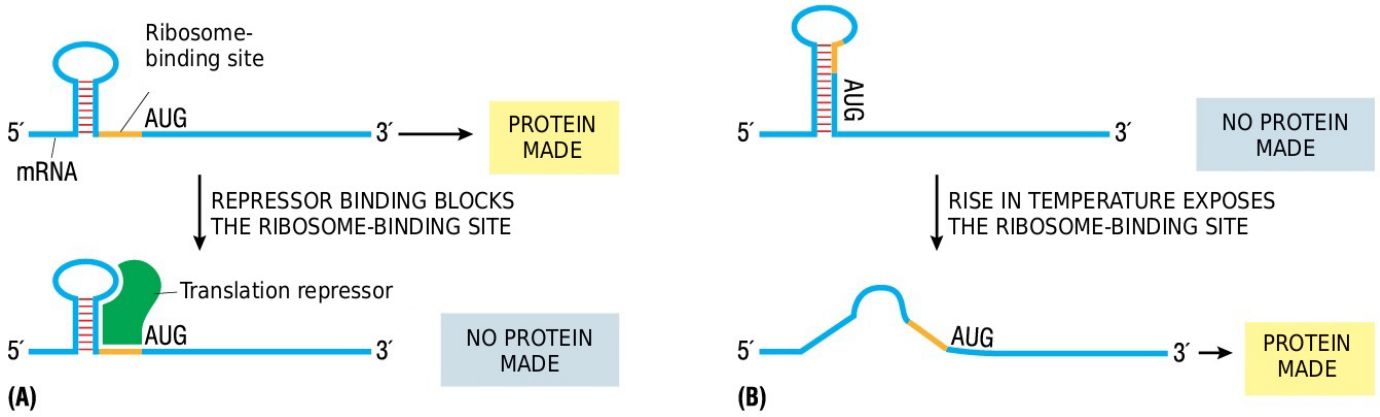
c Operator mutation (the repressor cannot bind) · with glucose, no lactose.

d CAP mutation · no glucose, with lactose.

e In b and c we add a normal lacI gene on a plasmid. Which strain is “rescued”? Why?

Hint: cis = on the same DNA molecule · trans = a diffusible product (protein).

Regulation in bacteria and infections: when the switch is RNA



Inducible AmpC β-lactamase

Enterobacter, Citrobacter: ampC is normally repressed. During treatment, mutants with permanent derepression are selected → resistance to 3rd-generation cephalosporins.

RNA thermometer · Listeria

At 37 °C the hairpin at the 5′ end of the prfA mRNA “melts” → PrfA is translated → virulence genes (panel B).

Riboswitches

A metabolite binds directly to the mRNA and changes its structure. Target of new antibiotics (ribocil, preclinical).

19%

of patients with Enterobacter bacteremia treated with a 3rd-generation cephalosporin developed resistance

Gene regulation in a microbe determines when it becomes dangerous — and how it escapes antibiotics.

Johansson et al., Cell 2002 · Howe et al., Nature 2015 · Chow et al., Ann Intern Med 1991 · Alberts et al., ECB, Fig. 8-26 (labels translated)

Summary of Part A

1. All cells have almost the same genome; their identity comes from differential expression (Gurdon, Dolly).
2. Expression can be controlled at many points; for most genes the main one is the initiation of transcription.
3. Transcription regulators (trans) recognize short regulatory sequences (cis) with conserved motifs, often as dimers.
4. Bacterial operons are controlled by repressors and activators; lac is an “AND” logic gate.
5. RNA can also be a switch (RNA thermometers, riboswitches) — which matters for infections and antibiotics.

Part B: Gene regulation in humans

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B1

Hour 1 · Transcriptional control and cell memory

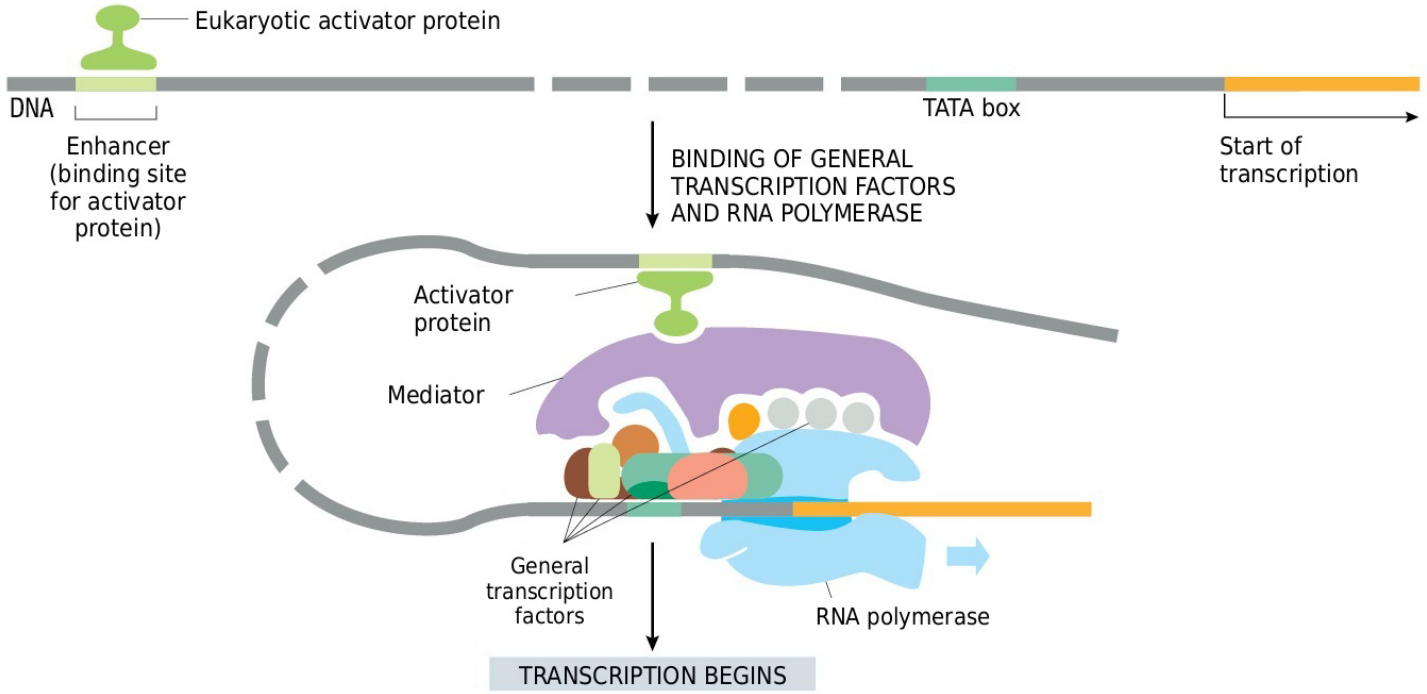
- Enhancers, Mediator, activators and repressors
- Combinatorial control · one regulator, many genes
- Enhancer diseases · fetal hemoglobin and Casgevy
- Cell memory · master regulators · iPS
- Transcription factors, cancer and drugs

B2

Hour 2 · Regulatory RNAs

- The world of non-coding RNAs
- miRNAs: discovery, biogenesis, target recognition
- miRNAs in disease and as biomarkers
- siRNA, RNA interference and RNA drugs
- lncRNA, circRNA · circuits with transcription factors

Eukaryotes: activating transcription from a distance



Enhancer

Binding site for activators; up to ~1 Mb away, upstream or downstream of the gene, even in an intron of another gene.

DNA loop

Brings the activator close to the promoter; usually within the same TAD (see Chromosomes).

Mediator

A complex of ~30 subunits; a “bridge” between the activators and the general transcription factors (GTFs) and Pol II.

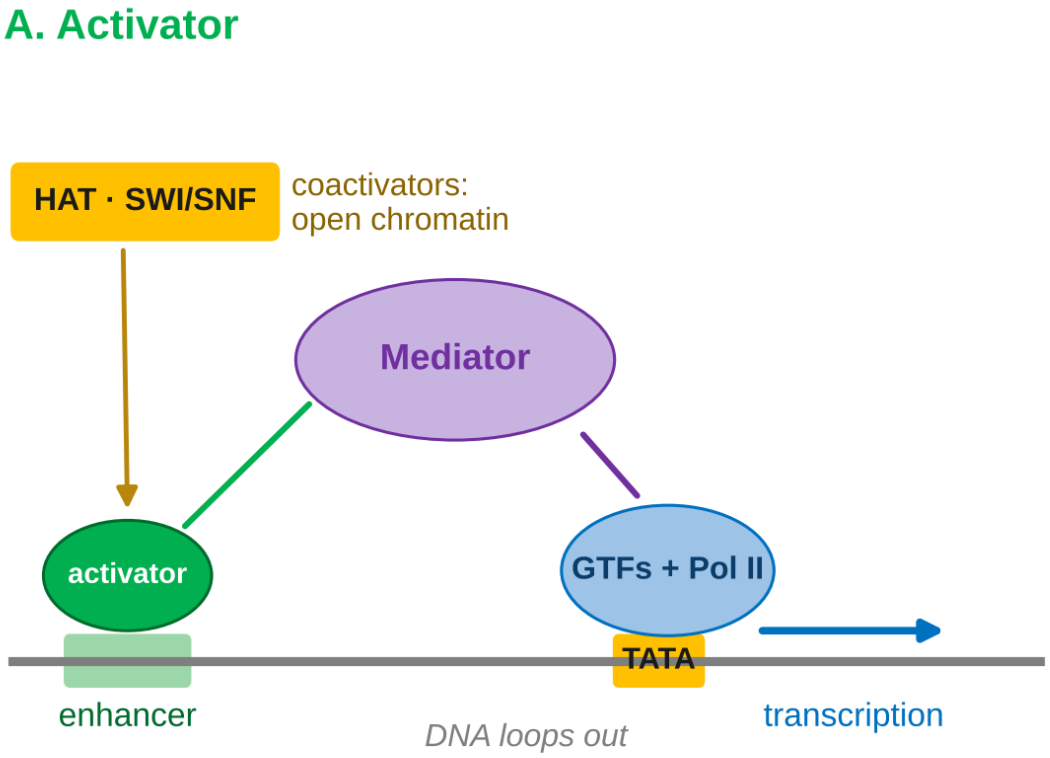
~926,000

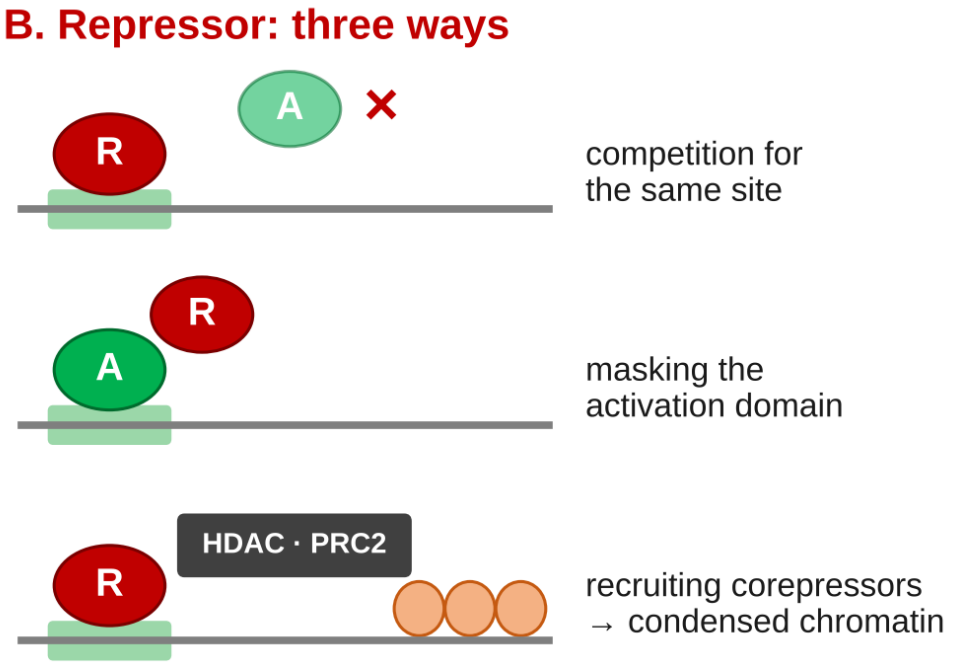
candidate regulatory elements (ENCODE)

Enhancers are often tissue-specific: a gene has a different enhancer for each tissue or developmental stage.

Alberts et al., ECB, Fig. 8-10 (labels translated) · ENCODE Project Consortium, Nature 2020

How activators and repressors work





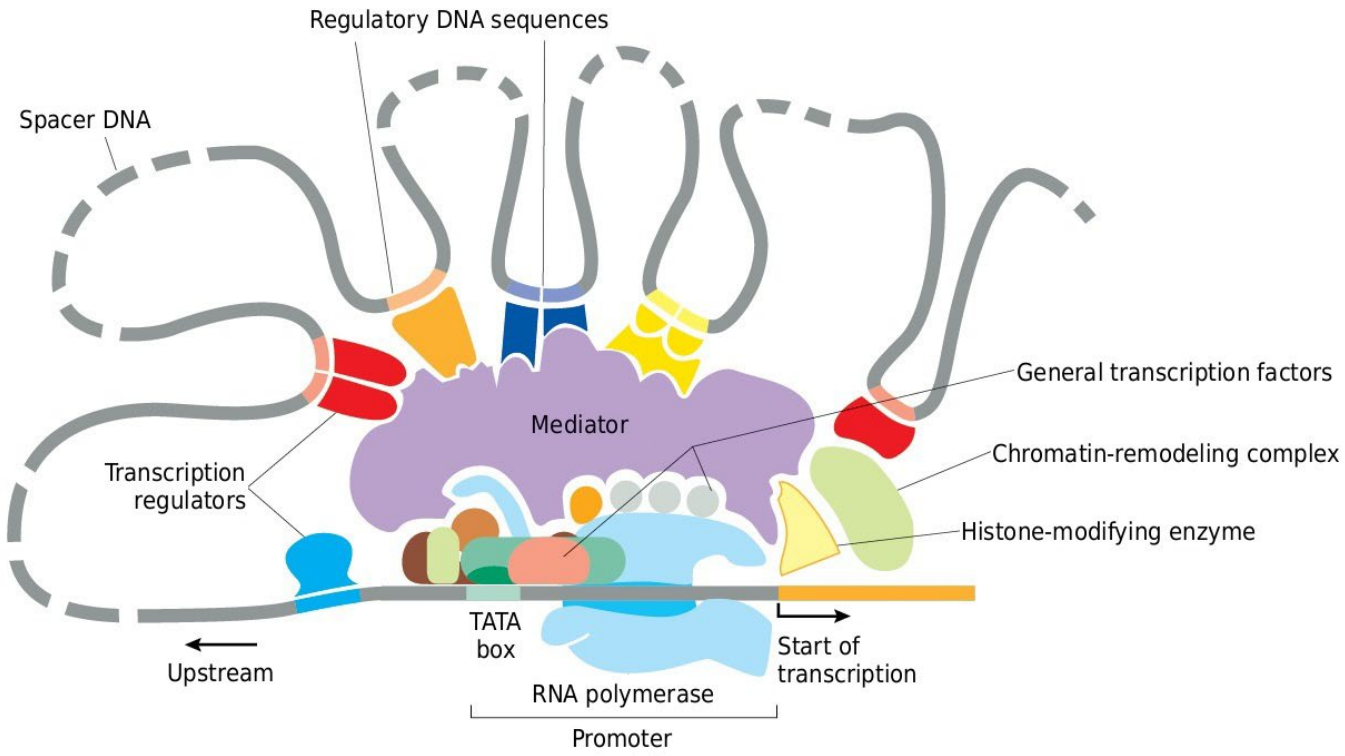
Coactivators and corepressors

They do not bind DNA; we met their enzymes (HAT, HDAC, PRC2, SWI/SNF) in the chromatin lecture.

Clinical: estrogen receptor (ER)

Tamoxifen changes the shape of ER: it recruits corepressors instead of coactivators (ER+ breast cancer).

Combinatorial control: every gene has its own “code”



The “code”

A gene switches on only when the right regulators come together at its enhancers — none of them is enough on its own.

Example: the hepatocyte

HNF1A, HNF4A, FOXA, C/EBP: a network that keeps liver genes active (albumin, clotting factors).

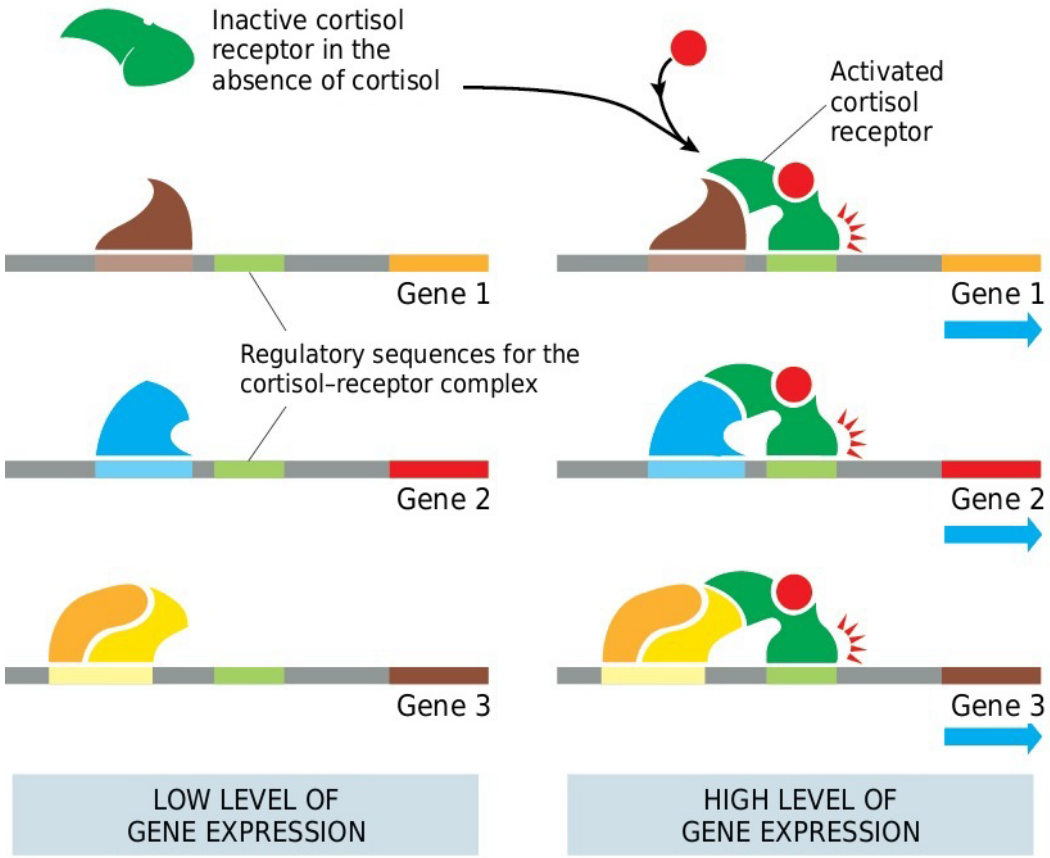
1,639 regulators × 100,000s of enhancers

→ enough combinations for every cell type

Back to exercise #1: ~47,000 random sites for one regulator — selectivity comes from the combination.

Alberts et al., ECB, Fig. 8-13 (labels translated) · Lambert et al., Cell 2018

One regulator, many genes: the glucocorticoid receptor



Mechanism

Cortisol → receptor (Zn fingers) → nucleus → response elements (GREs) in many genes → in the liver, conversion of amino acids to glucose (e.g., tyrosine aminotransferase) during fasting or stress.

Clinical 1 · Steroid-induced hyperglycemia

Dexamethasone and prednisolone “switch on” the same liver genes → raised glucose, common in hospitalized patients.

Clinical 2 · Anti-inflammatory action

In immune cells the same receptor represses the factors NF-κB and AP-1.

Same hormone, different result in a different tissue: in fat cells tyrosine aminotransferase goes down — it all depends on the “partners”.

Alberts et al., ECB, Fig. 8-16 (labels translated) · Alberts et al., MBoC, 7th ed., Ch. 7

When the mutation is in an enhancer: diseases of regulation

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Lactase persistence

Adults: −13910 C>T in an LCT enhancer, inside an intron of the neighboring MCM6: lactase stays active after weaning.

Preaxial polydactyly

Point mutations in the ZRS enhancer, ~1 Mb from SHH: SHH is expressed in the wrong place in the developing limb.

~93%

of disease-associated variants (GWAS) lie in non-coding DNA — mostly in regulatory elements

Cancer: the TERT promoter

Point mutations create new ETS sites → telomerase is reactivated (melanoma, glioblastoma).

Hemoglobinopathies

Common variants in the erythroid enhancer of BCL11A set the level of HbF — and the severity of β-thalassemia.

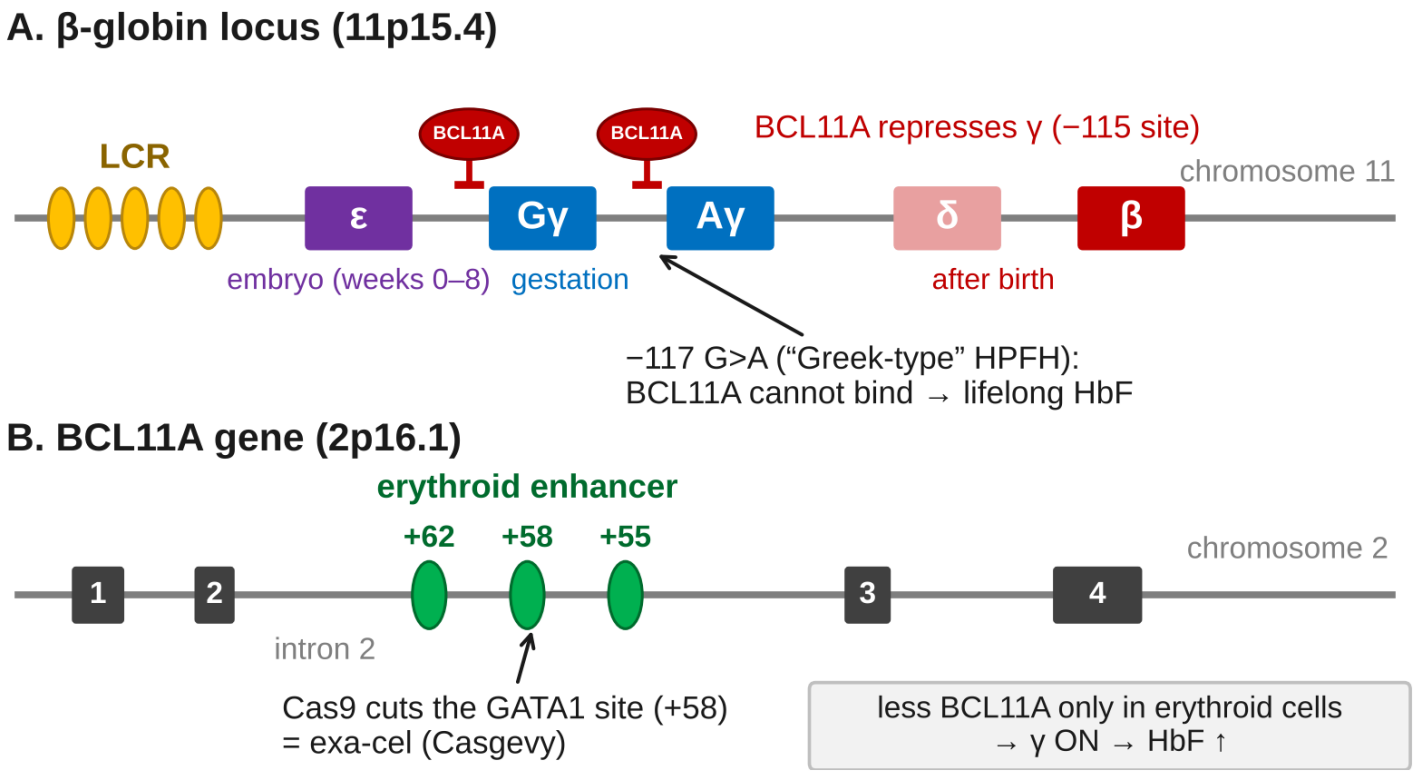
71%

of melanomas carry a TERT promoter mutation

Exome sequencing (WES) misses such mutations — they need whole-genome sequencing (WGS) and functional interpretation.

Enattah et al., Nat Genet 2002 · Lettice et al., Hum Mol Genet 2003 · Huang et al. and Horn et al., Science 2013 · Bauer et al., Science 2013 · Maurano et al., Science 2012

Fetal hemoglobin: from repressor to CRISPR therapy



The “switch”

After birth the repressor BCL11A binds the γ promoters and silences them.

“Greek-type” HPFH

−117 G>A: BCL11A cannot bind → lifelong HbF, no symptoms.

Casgevy (exa-cel)

Autologous blood stem cells; CRISPR at the erythroid enhancer of BCL11A.

91%

β-thalassemia: transfusion-free after exa-cel

97%

sickle cell disease: free of vaso-occlusive crises ≥12 months

12/2023 · 1/2024

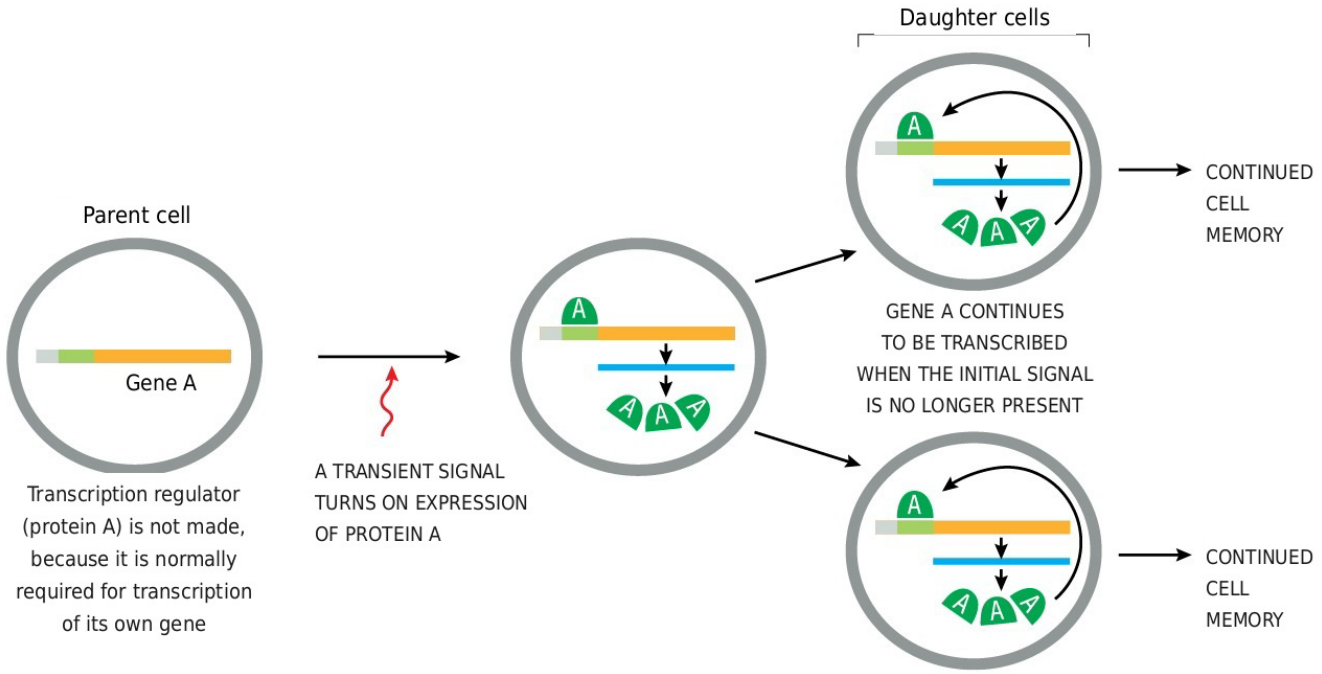
FDA approval (sickle cell · β-thalassemia); EU: 2/2024

1998

hydroxyurea: the first drug that raises HbF

Sankaran et al., Science 2008 · Collins et al. and Gelinas et al., Nature 1985 · Martyn et al., Nat Genet 2018 · Locatelli et al. and Frangoul et al., NEJM 2024

Cell memory: how cells keep their identity



Bacteria

The response lasts only as long as the signal (e.g., lac).

Multicellular organisms

A cell's "decision" is kept for tens of divisions.

Epigenetic inheritance: a change in expression, not in sequence, is passed on.

1 · Positive feedback

The regulator activates its own gene (figure) — e.g., MyoD in muscle, GATA1 in erythroid cells.

2 · Histone modifications

Read–write: the marks are copied onto the new nucleosomes (see Chromosomes).

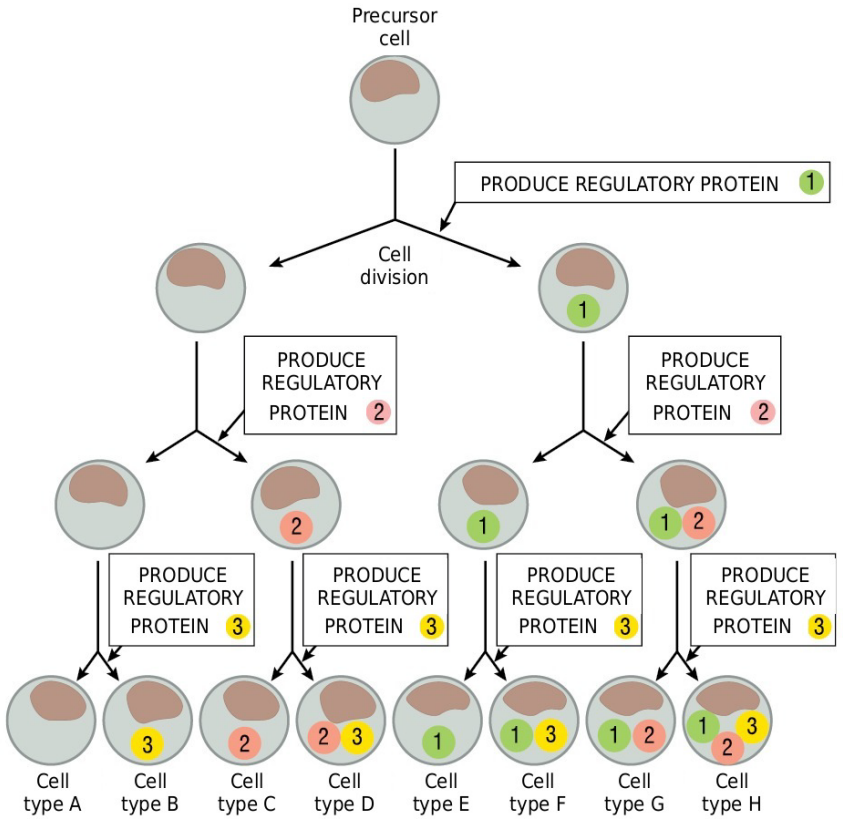
3 · DNA methylation

DNMT1 recognizes hemimethylated CpGs after replication and methylates the new strand.

Alberts et al., ECB, Fig. 8-22 (labels translated) · Allis & Jenuwein, Nat Rev Genet 2016

Master regulators define cell types





MyoD (1987)

A single regulator converts fibroblasts into myoblasts.

Eyeless / PAX6 (1995)

Expression in the fly leg → ectopic eye (left figure).
In humans: PAX6 haploinsufficiency → aniridia.

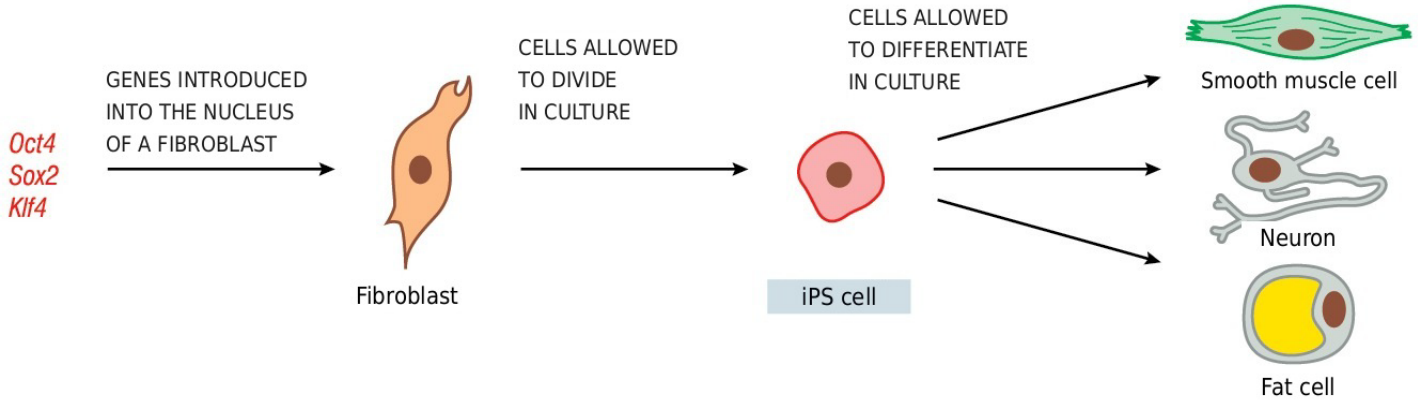
Combination

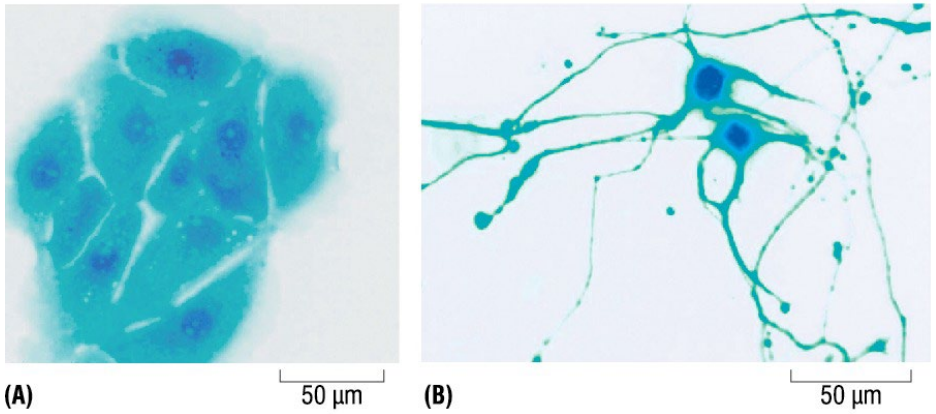
3 regulators → 8 cell types (center figure).

Master regulator: a transcription factor that switches on a whole program — often by activating other regulators.

Davis et al., Cell 1987 · Halder et al., Science 1995 · Alberts et al., ECB, Figs 8-17 and 8-19 (labels translated)

Reprogramming: from fibroblast to iPS cell





Direct conversion: hepatocytes (A) → neurons (B) with three neural regulators

Yamanaka · 2006–2007

Oct4, Sox2, Klf4 (+ c-Myc) → induced pluripotent stem (iPS) cells. Nobel 2012.

In the clinic

Macular degeneration: autologous iPS-derived RPE (2017) · Parkinson's: dopaminergic progenitors from iPS or ES cells (2025) · T1D: islets from chemically induced iPS cells (2024).

"Disease in a dish"

Patient iPS cells → neurons, cardiomyocytes for research.

Challenges: safety (c-Myc is an oncogene; risk of tumors), cost, production time.

Takahashi & Yamanaka, Cell 2006 · Takahashi et al., Cell 2007 · Mandai et al., NEJM 2017 · Sawamoto et al. and Tabar et al., Nature 2025 · Wang et al., Cell 2024

Transcription factors, cancer and drugs

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TP53

The “guardian of the genome” is a transcription factor; mutated in almost half of all cancers.

MYC

Amplified or translocated (Burkitt: t(8;14) next to immunoglobulin enhancers) → drives proliferation.

97%

event-free survival (2 years) in low/intermediate-risk APL with ATRA + arsenic, without chemotherapy

Fusion factors

PML::RARA (acute promyelocytic leukemia) · EWSR1::FLI1 (Ewing sarcoma).

Nuclear receptors

They have a hormone-binding site → “druggable”: tamoxifen (ER), enzalutamide (AR), dexamethasone (GR).

Differentiation therapy: ATRA and arsenic send PML::RARA for degradation; the promyelocytes are “unblocked” and differentiate.

Transcription factors were considered “undruggable” — today we target them through hormones, degradation (molecular glues, PROTACs) or differentiation.

Lo-Coco et al., NEJM 2013 · Lee & Young, Cell 2013 · Lambert et al., Cell 2018

Summary of B1

1. Activators bind enhancers, even ~1 Mb away; through loops and Mediator they recruit Pol II and open chromatin.

2. Repressors compete, “mask” or recruit corepressors; the combination of regulators gives each gene its “code”.

3. Mutations in enhancers and promoters change expression without changing the protein (lactase, ZRS, TERT, BCL11A); an enhancer is the target of Casgevy.

4. Positive feedback, histone modifications and DNA methylation give cell memory; master regulators define identities (MyoD, PAX6, iPS).

5. Transcription factors are involved in cancer and monogenic diabetes — they are drug targets (APL, ER) or guide the choice of treatment (MODY).

B2 · Regulatory RNAs: when RNA controls other genes

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1

How can an RNA of 22 nucleotides control hundreds of genes?

2

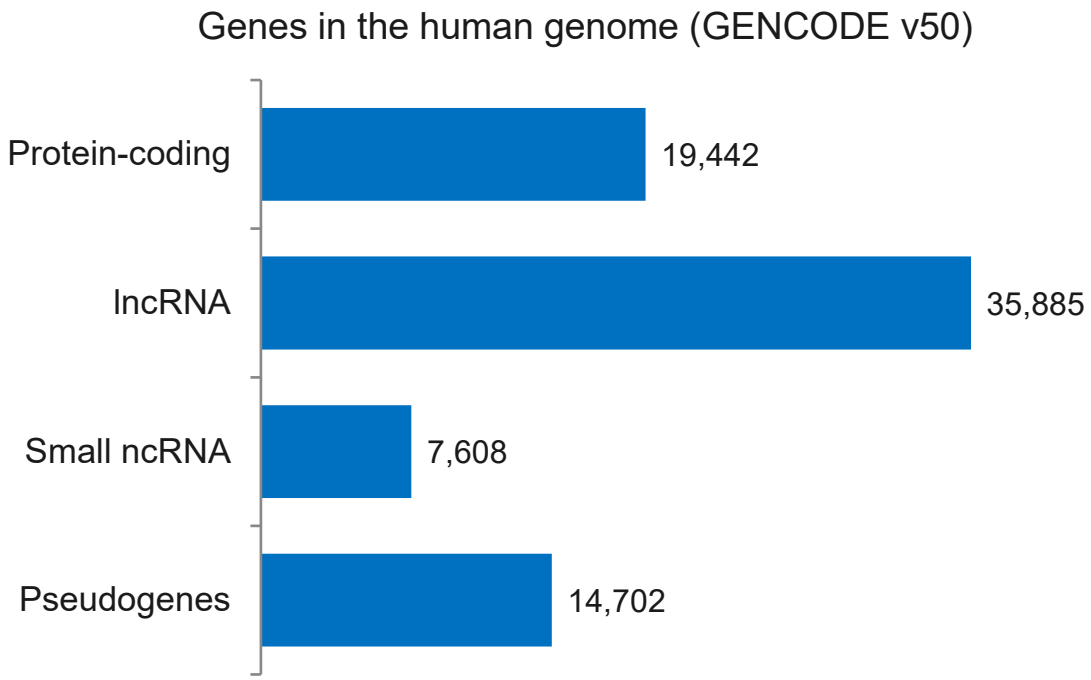
How did a defense mechanism against viruses become a family of drugs?

3

What do the tens of thousands of RNAs that do not code for proteins do?

RNA is not only a messenger: it is also a regulator — and a drug.

The world of non-coding RNAs



RNA	Size	Main action
miRNA	~22 nt	represses mRNAs (seed)
siRNA	~21 nt	cleaves its target · defense, drugs
piRNA	24–31 nt	silences transposons (germline)
lncRNA	>200 nt	guide, scaffold, decoy
circRNA	variable	“sponges” for miRNAs

~75%

of the genome is transcribed in some cell

~1,900

miRNA precursors in miRBase (~500 high-confidence)

There are more lncRNA genes than protein-coding genes — but for most of them the function is not yet known.

GENCODE v50 (2026) · Djebali et al., Nature 2012 · miRBase v22 · MirGeneDB 3.0 · Statello et al., Nat Rev Mol Cell Biol 2021

From *C.elegans* to the Nobel Prize: the story of small RNAs

1993 — lin-4: a small RNA represses lin-14 (Ambros · Ruvkun)

1998 — RNA interference: double-stranded RNA silences genes (Fire & Mello)

2000 — let-7: conserved from worm to human

2002 — First link to cancer: miR-15/16 in CLL

2005 — Hepatitis C virus needs liver miR-122

2006 — Nobel Prize: RNA interference

2018 — First siRNA drug (patisiran)

2024 — Nobel Prize: Ambros & Ruvkun (miRNA)

Why was the discovery so slow?

In C. elegans, lin-4 did not code for a protein; it gave a 22-nucleotide RNA complementary to sites in the 3′UTR of lin-14. For 7 years it was seen as a “quirk” of the worm — until let-7 was also found in humans. Today we know that miRNAs regulate most of our genes.

The Nobel Prize in Physiology or Medicine 2024



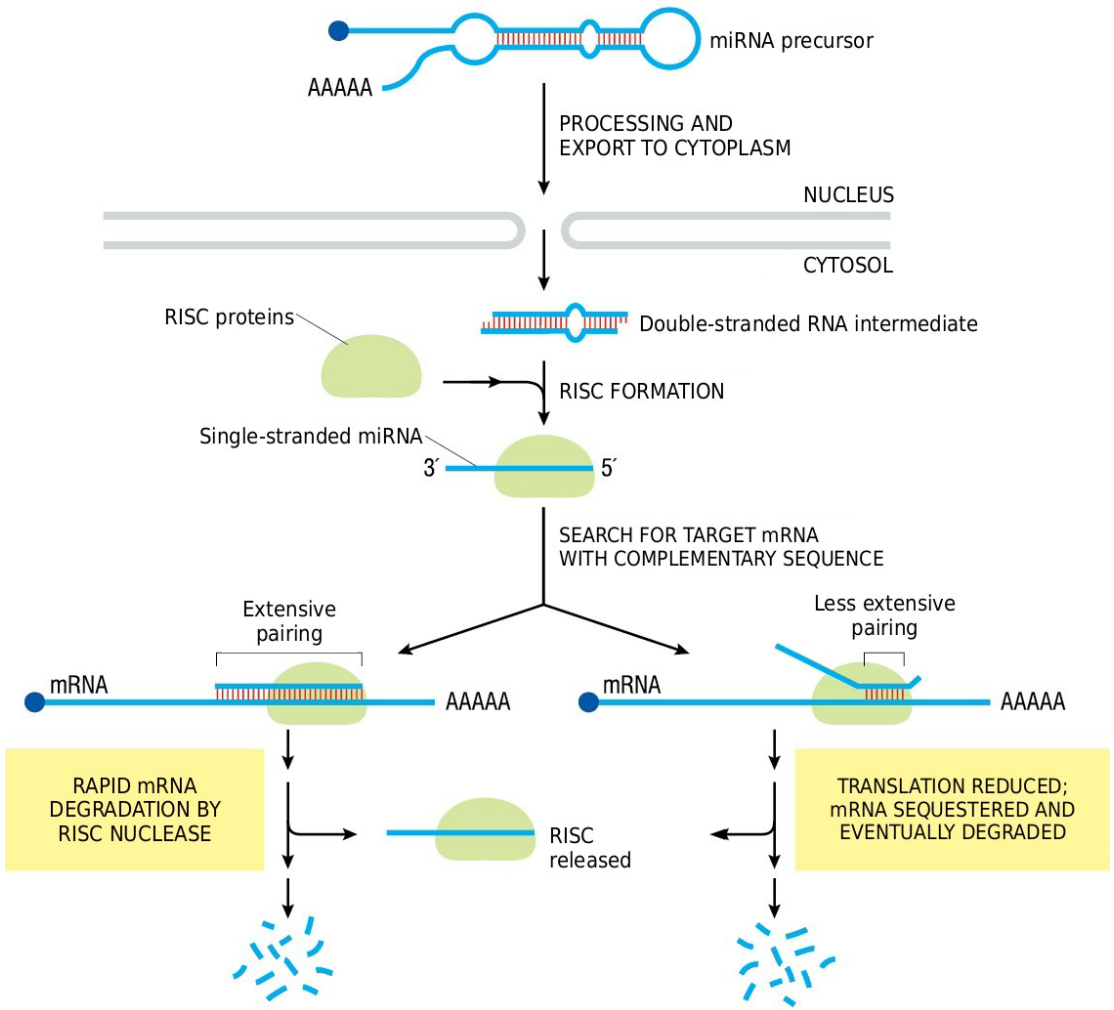
Ill. Niklas Elmehed © Nobel Prize Outreach
Victor Ambros



Ill. Niklas Elmehed © Nobel Prize Outreach
Gary Ruvkun

Lee et al. and Wightman et al., Cell 1993 · Fire et al., Nature 1998 · Reinhart et al. and Pasquinelli et al., Nature 2000 · Calin et al., PNAS 2002 · Jopling et al., Science 2005 · nobelprize.org

How a miRNA is made



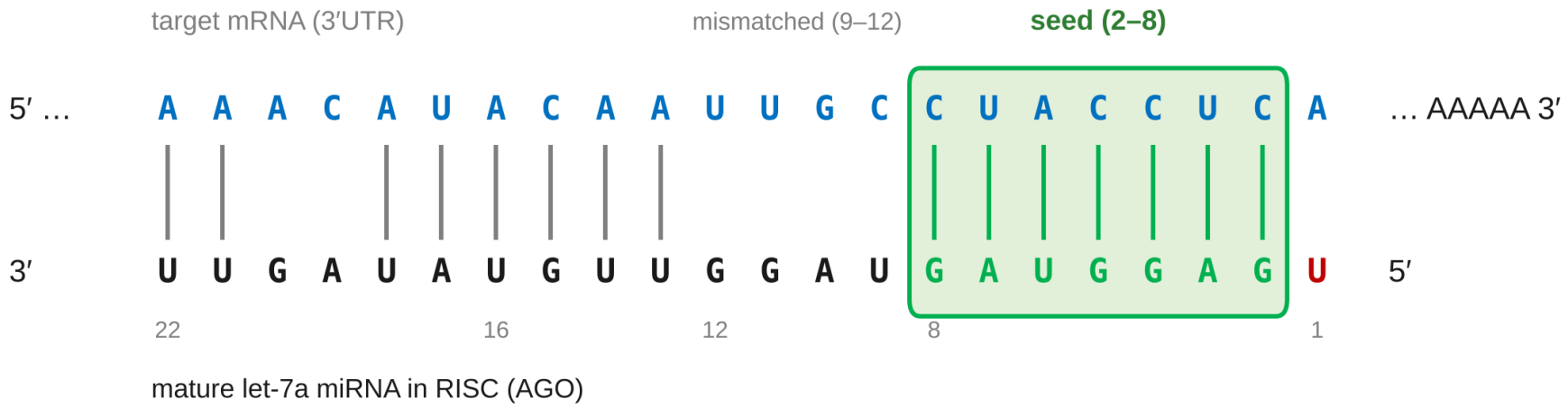
1. Pol II transcribes the pri-miRNA, with one or more hairpins (often within introns).
2. In the nucleus, the Drosha–DGCR8 complex cuts the hairpin → pre-miRNA ~70 nt.
3. Exportin-5 exports it to the cytoplasm; Dicer cuts it → a ~22 nt duplex.
4. AGO keeps one strand (the “guide”) and forms RISC; the other is degraded.

Clinical

DGCR8 lies in the 22q11.2 region (DiGeorge syndrome) · DICER1 syndrome: germline mutations → pleuropulmonary blastoma, thyroid and ovarian tumors.

Alberts et al., ECB, Fig. 8-27 (labels translated) · Bartel, Cell 2018 · Hill et al., Science 2009

How a miRNA finds its targets: the “seed”



The “seed”

Nucleotides 2–8 pair perfectly, usually in the 3′UTR; the rest of the molecule tolerates mismatches.

Partial pairing

Translational repression + deadenylation → decay; in mammals ≥84% of the effect is loss of mRNA.

Full pairing

AGO2 cleaves the mRNA in the middle of the site — as siRNAs do.

>60%

of protein-coding genes are conserved miRNA targets

A miRNA rarely “switches off” a gene; it usually “turns down the volume” of hundreds of genes together.

Bartel, Cell 2018 · Friedman et al., Genome Res 2009 · Guo et al., Nature 2010

miRNAs and disease: a broken “volume knob”

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

Deafness · miR-96

Mutations in the miR-96 seed → autosomal dominant, progressive hearing loss.

Feingold syndrome 2 · MIR17HG

Deletion of the miR-17~92 cluster → microcephaly, short stature, digital anomalies.

1 → 100s

targets per miRNA: one mutation, a multisystem phenotype

DICER1 syndrome

Germline mutation in Dicer → pleuropulmonary blastoma, thyroid and ovarian tumors.

Cancer

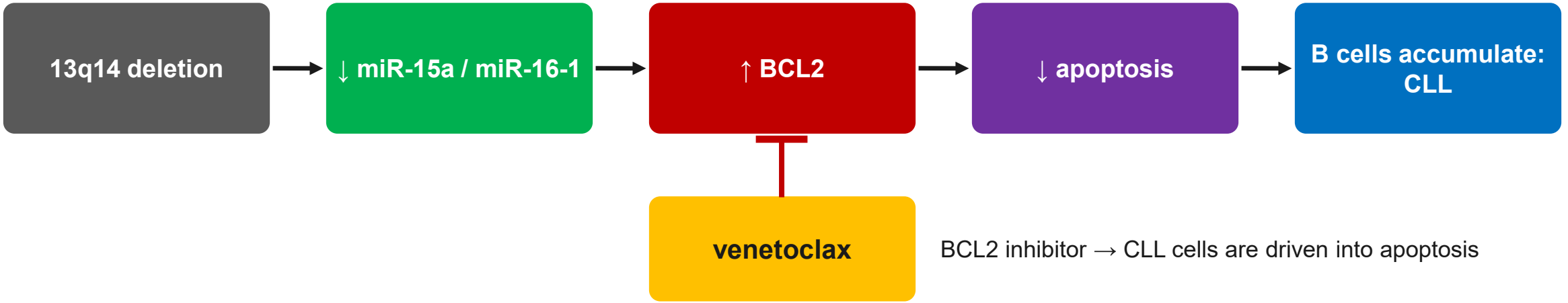
The miR-17~92 cluster is amplified in lymphomas (“oncomiR”); let-7 and miR-15/16 act as tumor suppressors.

Two ways to go wrong: loss of the miRNA (its targets “escape”) or a change in the seed (the miRNA finds new, wrong targets).

In CLL the most common chromosomal lesion (13q14 deletion) removes two miRNAs → next slide.

Mencía et al., Nat Genet 2009 · de Pontual et al., Nat Genet 2011 · Hill et al., Science 2009 · Bartel, Cell 2018

Chronic lymphocytic leukemia: from 13q14 to venetoclax



~68%

of CLL cases: loss or reduction of miR-15/16

BCL2

An anti-apoptotic protein; miR-15/16 keep it low. Without them, the cells become “dependent” on BCL2.

Timeline

2002: miR-15/16 at 13q14 · 2005: BCL2 is the target · 2016: venetoclax approved (FDA).

The loss of two small RNAs explains why CLL cells are so vulnerable to BCL2 inhibition.

Calin et al., PNAS 2002 · Cimmino et al., PNAS 2005 · Roberts et al., NEJM 2016 · FDA, Apr 2016

miRNAs in the blood: biomarkers and “signals” between organs

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

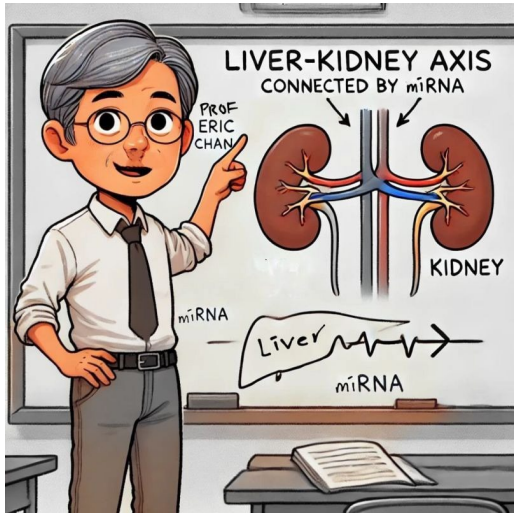


Research paper

Transfer of hepatocellular microRNA regulates cytochrome P450 2E1 in renal tubular cells

Olivia Matthews[1,†], Emma E. Morrison[1,†], John D. Tranter[1], Philip Starkey Lewis[2], Iqbal S. Toor[1], Abhishek Srivastava[3], Rebecca Sargeant[3], Helen Rollison[3], Kylie P. Matchett[4], Timothy J. Kendall[4], Gillian A. Gray[1], Chris Goldring[5], Kevin Park[5], Laura Denby[1], Neeraj Dhaun[1], Matthew A. Bailey[1], Neil C. Henderson[4,6], Dominic Williams[3], James W. Dear[1,*]

[1] Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, United Kingdom
[2] Medical Research Council Centre for Regenerative Medicine, University of Edinburgh, United Kingdom
[3] AstraZeneca, Clinical Pharmacology & Safety Sciences Department, Biopharmaceuticals Science Unit, Darwin Building 310, Cambridge Science Park, Milton Rd, Cambridge, CB4 0FZ. United Kingdom
[4] Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh, United Kingdom
[5] Department of Molecular and Clinical Pharmacology, MRC Centre for Drug Safety Science, University of Liverpool, United Kingdom
[6] MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XU, United Kingdom



Cartoon: the liver–kidney axis “connected” by miRNA (top: the paper by Matthews et al., 2020)

Why they make good biomarkers

Stable in plasma (inside extracellular vesicles or in complexes with AGO2) · many are tissue-specific (miR-122 ≈ liver).

Acetaminophen (paracetamol) poisoning

Serum miR-122 rises ~100-fold and correlates with ALT; it is being studied as a more specific marker of liver injury.

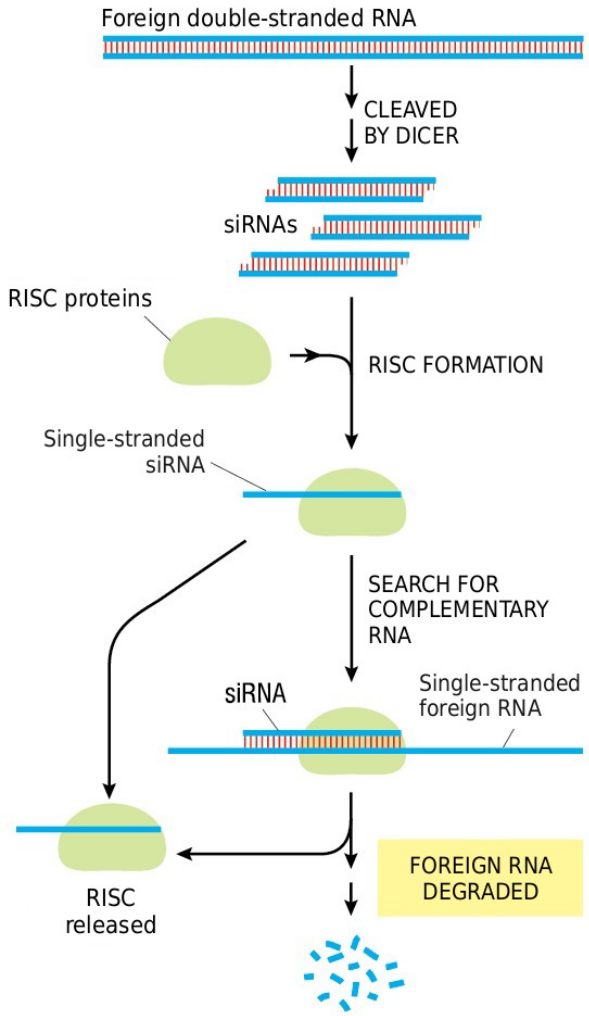
Liver–kidney axis (mice)

Hepatocyte miR-122 reaches the tubular cells of the kidney and represses CYP2E1; in liver injury the transfer increases.

Most blood miRNA tests are still investigational — standardization is the challenge.

Starkey Lewis et al., Hepatology 2011 · Matthews et al., EBioMedicine 2020

RNA interference: a defense system



1998 · Fire & Mello

Double-stranded RNA in C. elegans selectively silences the gene with the same sequence — far more strongly than single-stranded RNA.

The mechanism

Dicer → siRNA ~21 nt → RISC (AGO2) keeps one strand → full complementarity → cleavage.

Physiological role

Defense against viruses and transposons (plants, insects, worms); in mammals the interferon response dominates.

2001 · A tool and a drug

Synthetic 21-nt siRNAs silence genes in human cells.

The Nobel Prize in Physiology or Medicine 2006



Photo: L. Cicero
Andrew Z. Fire



Photo: J. Mottern
Craig C. Mello

RNA interference: silencing of a gene by double-stranded RNA with the same sequence. Specificity comes from complementarity.

Fire et al., Nature 1998 · Elbashir et al., Nature 2001 · Alberts et al., ECB, Fig. 8-28 (labels translated)

miRNA and siRNA: a comparison

	miRNA	siRNA
Origin	endogenous genes (hairpin)	long double-stranded RNA (viruses, transposons) or synthetic
Processing	Drosha + Dicer	Dicer
Protein	AGO (RISC)	AGO2 (RISC)
Pairing	partial (seed 2–8)	full
Result	translational repression, decay	cleavage of the target
Targets	hundreds	usually one

Bartel, Cell 2018 · Alberts et al., ECB, Ch. 8 · Alberts et al., MBoC, 7th ed., Ch. 7

Three ways to target RNA

Antisense oligonucleotides (ASOs)

Single-stranded, 16–20 nt

RNase H cleaves the DNA–RNA hybrid, or they change splicing by blocking sites.

Examples: Nusinersen (SMA) · tofersen (SOD1-ALS, 2023) · olezarsen (APOC3, 2024)

siRNA

Double-stranded, ~21 nt

Loaded onto RISC (AGO2), they catalytically cleave many mRNA molecules.

Examples: Inclisiran, vutrisiran, fitusiran, etc. — 8 approved

miRNA mimics and inhibitors

Mimics or anti-miRs

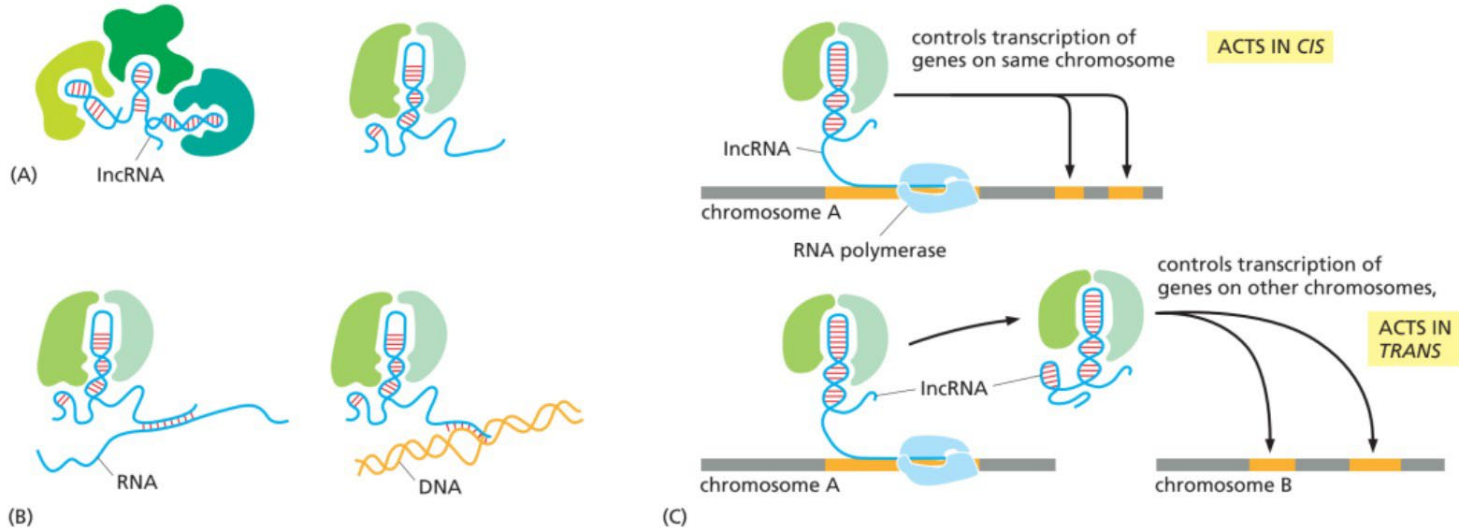
Restore a missing miRNA, or block one in excess.

Examples: Miravirsen (anti-miR-122, HCV): discontinued after phase II · none approved

All three "find" their target by base complementarity — so they can be designed even for a single patient (milasen).

FDA (tofersen 4/2023 · olezarsen 12/2024) · Janssen et al., NEJM 2013 · Kim et al., NEJM 2019

Long non-coding RNAs (lncRNAs)



~35,900

lncRNA genes (GENCODE v50) — more than protein-coding genes

Properties

>200 nucleotides, usually capped and polyadenylated · often stay in the nucleus · low expression, highly tissue-specific · less conserved than protein-coding genes.

How they act

Guide (brings complexes to DNA sites) · scaffold (panel A) · decoy · in cis or in trans (panel C).

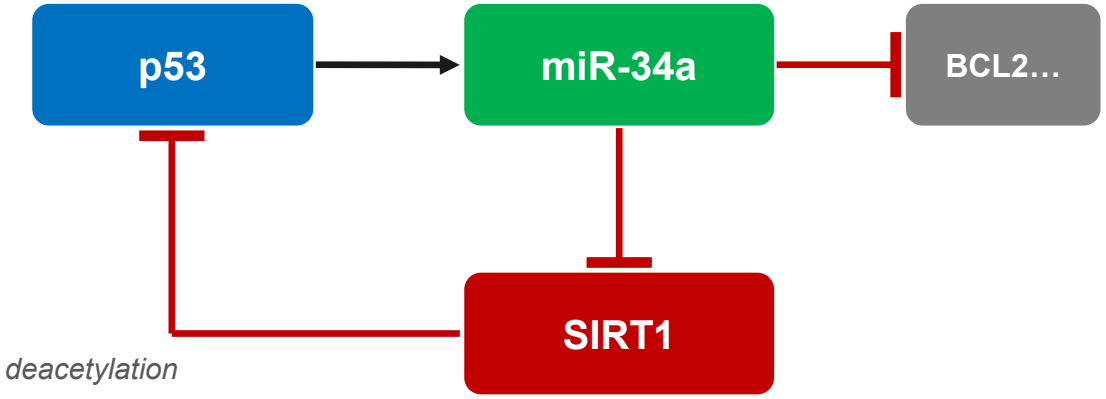
Well-known examples

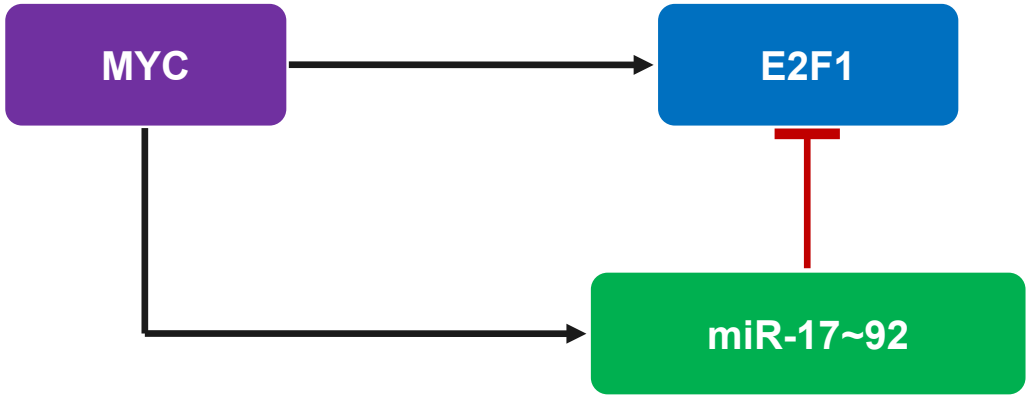
XIST (X inactivation — see Chromosomes) · H19 (imprinting) · MALAT1, NEAT1 (nuclear bodies).

Caution: many lncRNAs may be transcriptional "noise" — function must be demonstrated for each one.

Statello et al., Nat Rev Mol Cell Biol 2021 · GENCODE v50 · Alberts et al., MBoC, Ch. 7

Circuits: transcription factors and miRNAs





The p53 – miR-34a loop

p53 switches on miR-34a; this represses SIRT1, which inactivates p53 → positive feedback and apoptosis. miR-34a also represses BCL2.

MYC: accelerator and brake together

MYC switches on E2F1 and, at the same time, miRNAs that brake it → a controlled proliferation signal.

Beware of clinical translation: the miR-34a mimic MRX34 was stopped in phase 1 after 4 deaths from immune-related reactions.

He et al., Nature 2007 · Yamakuchi et al., PNAS 2008 · O'Donnell et al., Nature 2005 · Hong et al., Br J Cancer 2020

Summary of B2

1. Most of the genome is transcribed; there are more lncRNA genes than protein-coding genes.
2. miRNAs (Pol II → Drosha → Dicer → AGO/RISC) recognize targets through the “seed” (2–8) and modestly lower hundreds of mRNAs.
3. Mutations in miRNAs or their target sites cause disease or raise risk (miR-96, 13q14 in CLL, KRAS-LCS6); blood miRNAs are candidate biomarkers.
4. siRNAs cleave a single target with full complementarity; 8 siRNA drugs (GalNAc, LNP) target liver genes.
5. lncRNAs act as guides, scaffolds or decoys (XIST, UBE3A-ATS); circRNAs “soak up” miRNAs.

I. Comment briefly (1–3 sentences): true or false?

A When both glucose and lactose are present, the lac operon is inactive because glucose binds to the repressor.

B An activator bound to an enhancer 50,000 bp away can increase transcription through Mediator.

C A miRNA must be fully complementary to its target mRNA in order to reduce its expression.

D A hepatocyte and a neuron differ because each has lost the genes of the other.

E Dicer is needed for the maturation of both miRNAs and siRNAs.

II. Clinical case: a family with deafness

In a family, progressive hearing loss is inherited as an autosomal dominant trait. A single-nucleotide mutation was found within nucleotides 2–8 of mature miR-96, which is expressed in the hair cells of the inner ear.

a What is this region of the miRNA called, and why is it critical?

b Why can a single-base change in a 22-nucleotide RNA affect hundreds of genes? Give two consequences.

c Why is there a phenotype in heterozygotes?

d Propose a therapeutic strategy using oligonucleotides. What would you target?

For study and revision

Animations, simulations & tools

LabXchange: Prokaryotic Gene Regulation (operons)

HHMI BioInteractive: RNA interference

Nobel Prize 2024: microRNA (nobelprize.org)

TargetScan: miRNA target prediction

JASPAR: transcription factor binding sites

miRBase · GENCODE · ENCODE SCREEN

Reading

Alberts et al. Essential Cell Biology, 6th ed. (2023), Ch. 8 · MBoC, 7th ed., Ch. 7

Lambert et al., Cell 2018 · Lee & Young, Cell 2013 · Maurano et al., Science 2012

Sankaran et al., Science 2008 · Bauer et al., Science 2013 · Locatelli et al., NEJM 2024

Takahashi & Yamanaka, Cell 2006 · Lo-Coco et al., NEJM 2013 · Pearson et al., Lancet 2003

Bartel, Cell 2018 · Friedman et al., Genome Res 2009 · Calin et al., PNAS 2002

Fire et al., Nature 1998 · Ray et al., NEJM 2020 · Statello et al., Nat Rev Mol Cell Biol 2021

Full list with DOIs: in the lesson plan

Link to the lab: in the Bioinformatics lab you will search for binding sites and miRNA targets with the tools above. **Questions:** idrosos@upatras.gr