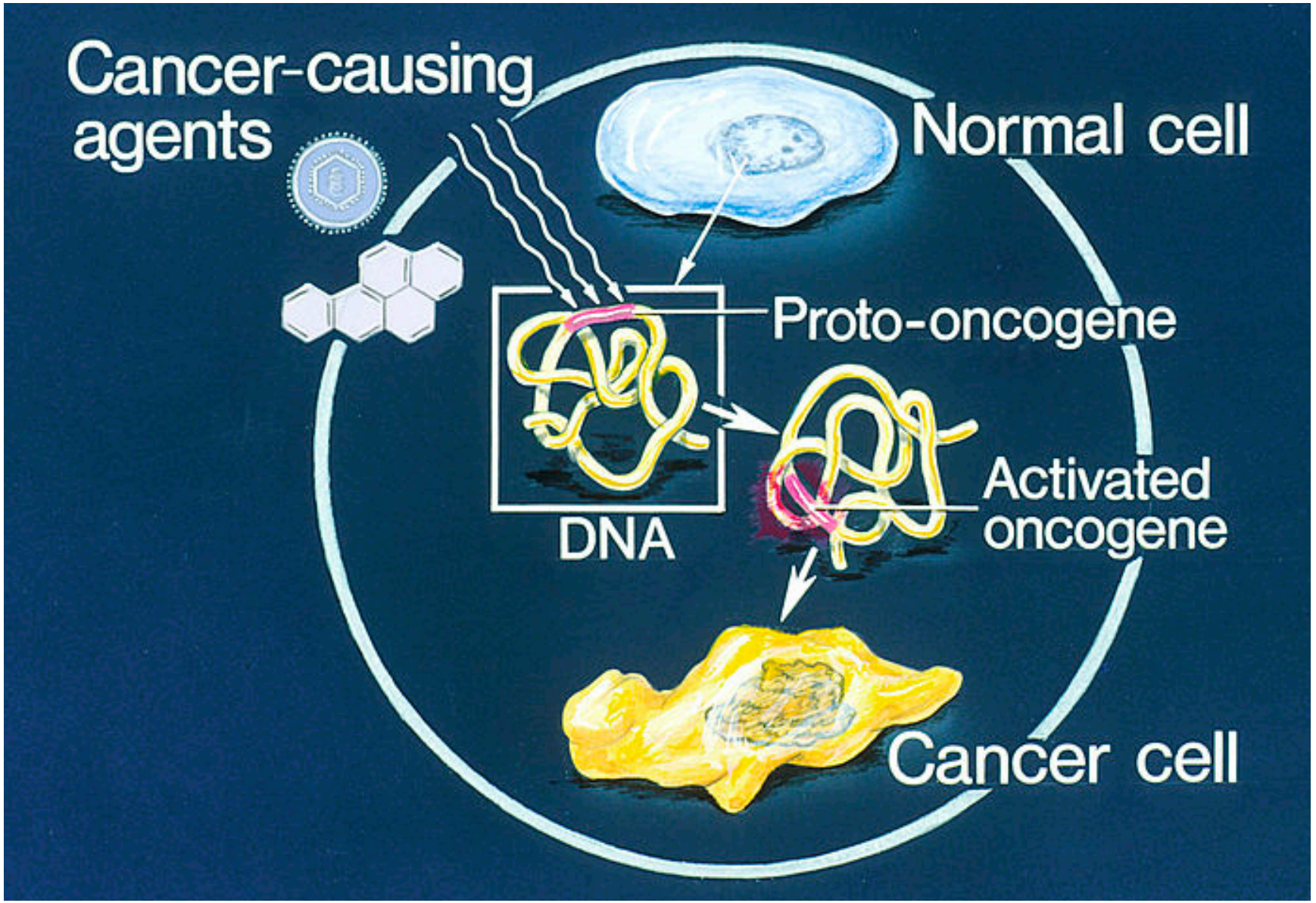
ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

ΠΜΣ «Εφαρμογές Μοριακής Βιολογίας – Γενετική.
Διαγνωστικοί δείκτες

Oncogenes,
Oncomirs,
RNA και καρκίνος

Νικόλαος Μπαλατσός


Cancer-causing agents
Normal cell
Proto-oncogene
DNA
Activated oncogene
Cancer cell

Oncogene history

1969 Term "***Oncogene***", J. Alexander and G. Torado, NIH

1970 **Discovery of *Src***, the first confirmed oncogene,
G.S. Martin, U. California, Berkeley

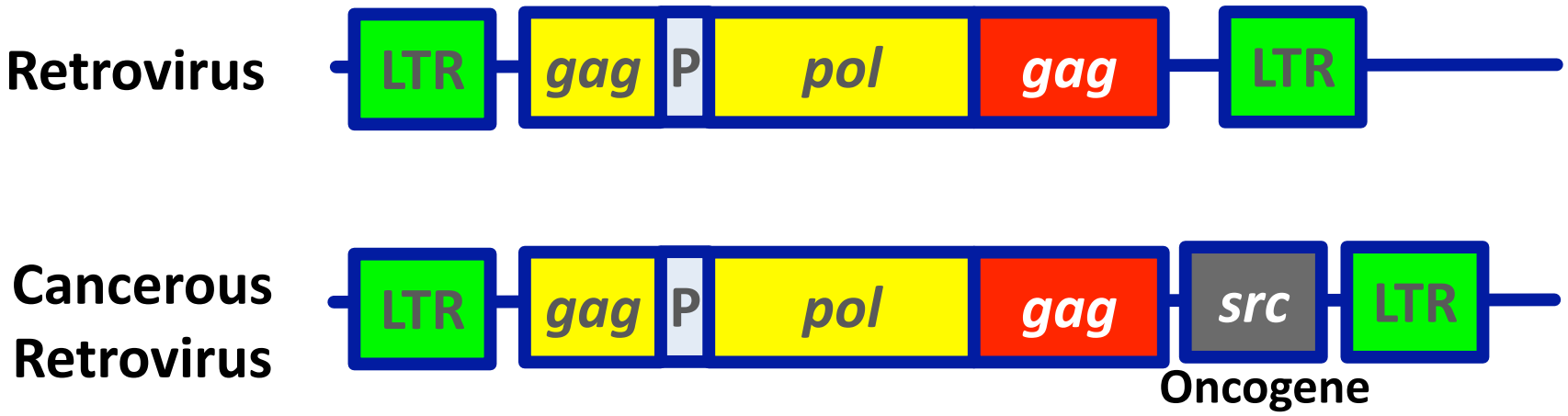
1976 **Oncogenes are activated *Proto-oncogenes***
D. Stehelin, J.M. Bishop, H.E. Varmus,
U. California, San Francisco.

Oncogene, Proto-oncogene

- Oncogenes: a gene that has the potential to cause cancer
- Proto-oncogenes: a normal gene that can become an oncogene due to mutations or increased expression.
- Oncoprotein: the resultant protein

Retroviruses and oncogenes

- Retroviruses: family of RNA viruses that cause cancer including humans
- Retrovirus: consists of 3 main genes *gag, pol & env* that are required for *virus replication* but not play role in *cell transformation.*
- Retrovirus: can *transform* cells from normal to cancer, if they include a specific gene known as <u>"Oncogene".</u>



Retrovirus oncogene

- Two main types of oncogenes:
  - *Viral oncogene*: gene from the retrovirus itself
  - *Non-Viral oncogene (Cellular oncogene):* genes derived from the genes of the host cell that are in an inactive form usually. Occasionally if the gene incorporates with the viral genome will form a highly oncogenic virus.
- Proto-oncogenes: are the form of cellular genes that inactive normally but can incorporate with the viral genome to produce a highly oncogenic virus.

How does a Proto-oncogene become an Oncogene?

Proto-Oncogene → Oncogene

1.Mutation

2. Abnormal Activity

3.Gene Translocation

4. Amplification

Proto-Oncogene ⟶ Oncogene

1. Mutation:
   - *Ras gene*
     - Continuous activation of Ras (constitutively in GTP-bound conformation)
     - ↓
     - Unregulated cell proliferation
     - ↓
     - Cell transformation.

WT	DNA	GTG	GGC	GCC	G**G**C	GGT	GTG
	Protein	Val	Gly	Ala	**Gly**	Gly	Val
MUTANT	DNA	GTG	GGC	GCC	G**T**C	GGT	GTG
	Protein	Val	Gly	Ala	**Val**	Gly	Val

Proto-Oncogene ⟶ Oncogene

2. Abnormal Activity:

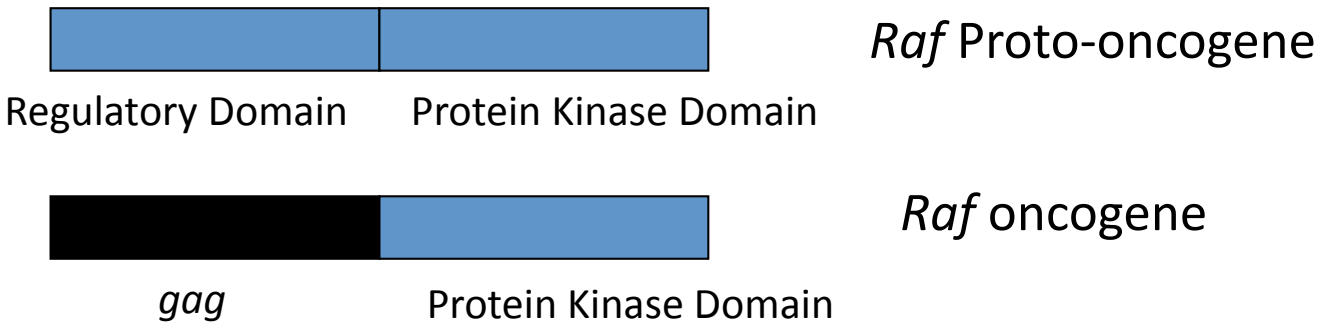
Example: Removal of the Regulatory domain in the *Raf gene* and replaced by *gag* gene

↓

Raf kinase domain consciously active

↓

Cell transformation



Proto-Oncogene ⟶ Oncogene

3. Gene translocation:

Example: *c-myc gene*

Translocation from

chromosome 8 to chromosome 14

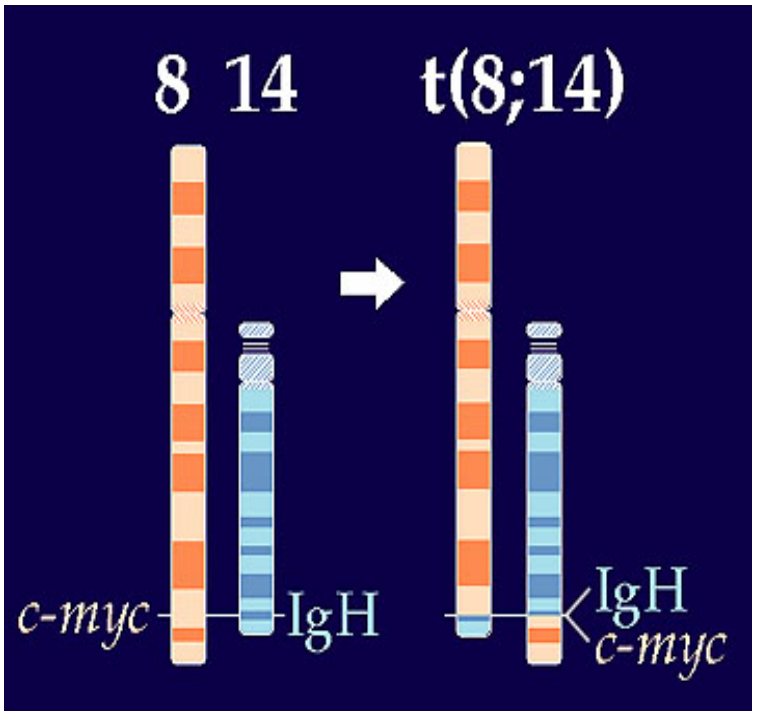
t(8;14)

↓

abnormal c-myc expression

↓

Cell transformation



Proto-Oncogene ⟶ Oncogene

4. Amplification:

Example: Amplification of *n-myc* ⟶ neuroblastoma.

Amplification of *erbB-2* ⟶ Breast & ovarian carcinomas

Classification

Category	Examples	Cancers	Gene functions
Growth Factors or mitogens	c-Sis	glioblastomass, fibrosarcomass, osteosarcomass, breast carcinomass, and melanomas	induces cell proliferation.
Receptor tyrosine kinases	EGFR PDGFR, and VEGFR, HER2/neu	Breast cancer, gastrointestinal stromal tumours, non-small-cell lung cancer and pancreatic cancer	transduce signals for cell growth and differentiation
Cytoplasmic tyrosine kinases	Src-family, Syk-ZAP-70 family, and BTK family of tyrosine kinases, Abl gene in CML–Philadelphia Chromosome	colorectal and breast cancers, melanomas, ovarian cancers, gastric cancers, head and neck cancers, pancreatice cancer, lung cancer, brain cancers, and blood cancers	mediate the responses to, and the activation receptors of cell proliferation, migration, differentiation, and survival
Cytoplasmic Serine/threonine kinases and their regulatory subunits	Raf kinasee, and cyclin-dependent kinases (overexpressionn).	malignant melanoma, papillary thyroid cancer, colorectal cancer, and ovarian cancer	Oorganism development, cell cycle regulation, cell proliferation, differentiation, cells survival, and apoptosis
Regulatory GTPases	Ras protein	adenocarcinomas of the pancreas and colon, thyroid tumors, and myeloid leukemia	involved in signalling a major pathway leading to cell proliferation
Transcription Factors	Myc gene	malignant T-cell lymphomas and acute myeloid leukemias, breast cancer, pancreatic cancer, retinoblastoma, and small cell lung cancer	They regulate transcription of genes that induce cell proliferation.
microRNAs, non-coding RNAs	miR-17, miR-19, miR155, etc	Most types	Deregulation of gene expression

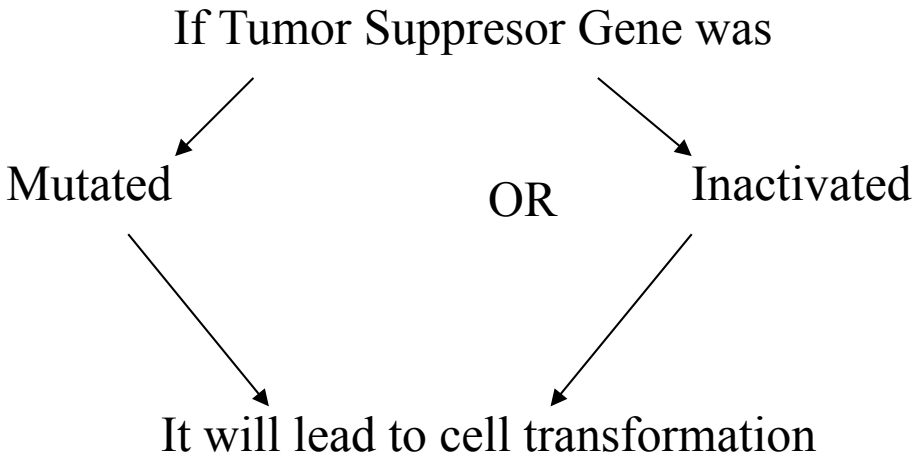
Based on http://en.wikipedia.org/wiki/Oncogene

Oncogenes

- Oncogene causes cancer by affecting:
  1. Cell Proliferation: (example; *Ras, Raf, EGF*)
  2. Cell differentiation (example, *PML/RAR* that inhibits the differentiation of promyelocyte to granulocyte which will maintain the cell in its active proliferate state)
  3. Cell Survival (example; *Pl-3/AKT* which will activate *BCL-2* ⟶ inhibit Apoptosis & maintain cell survival.

Tumour Suppressor Genes

- *Tumour Suppressor genes*: are genes that act to inhibit cell proliferation and tumour development.

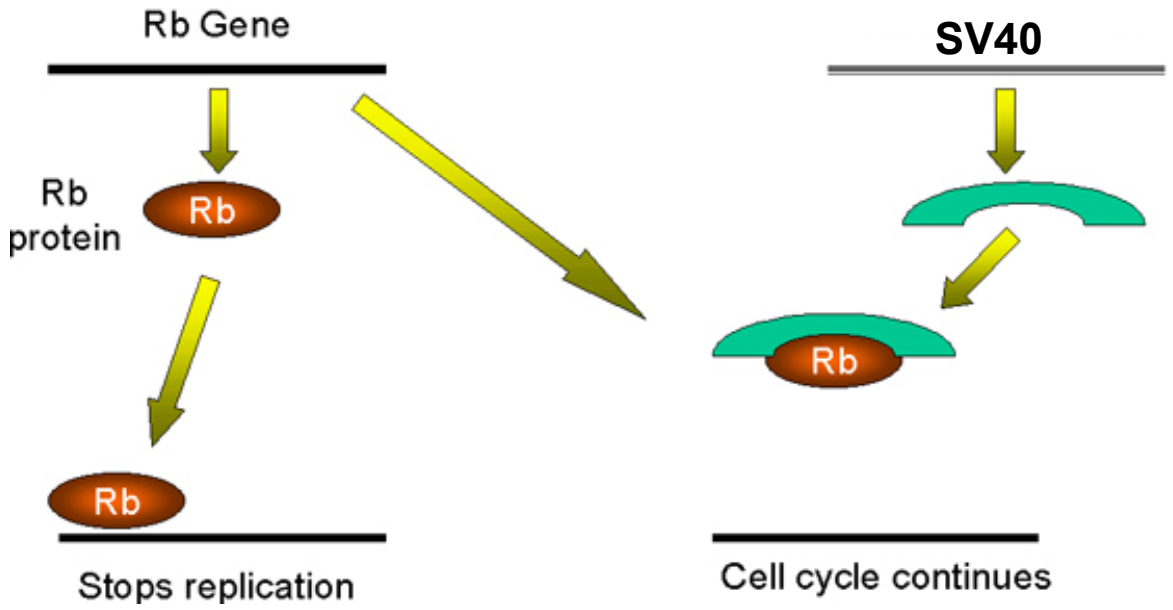


Tumour Suppressor Genes

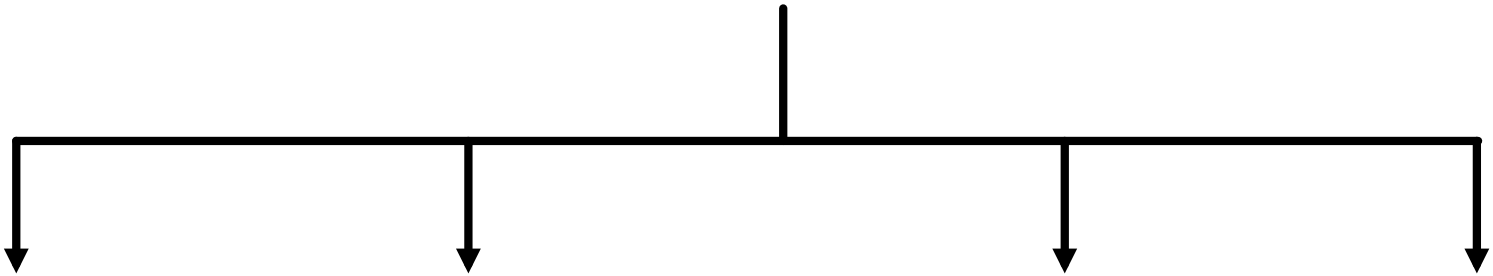
- Mutation of the tumour suppressor gene will cause cancer.
  - Example; deletion of Rb gene will cause retinoblastoma. The development of retinoblastoma can be either:
    - Hereditary: a defective copy of Rb gene is inherited from the affected parents.
    - Nonhereditary: in which 2 normal Rb genes are inherited and develop mutation during life.
  - Retinoblastoma is developed if 2 somatic mutations inactivate both copies of Rb in the same cell.

Tumor Suppressor Genes

- Inactivation of Tumour suppressor gene will cause cancer.
  - If the Rb gene interact with DNA tumour virus (SV40) it will induce cell transformation.



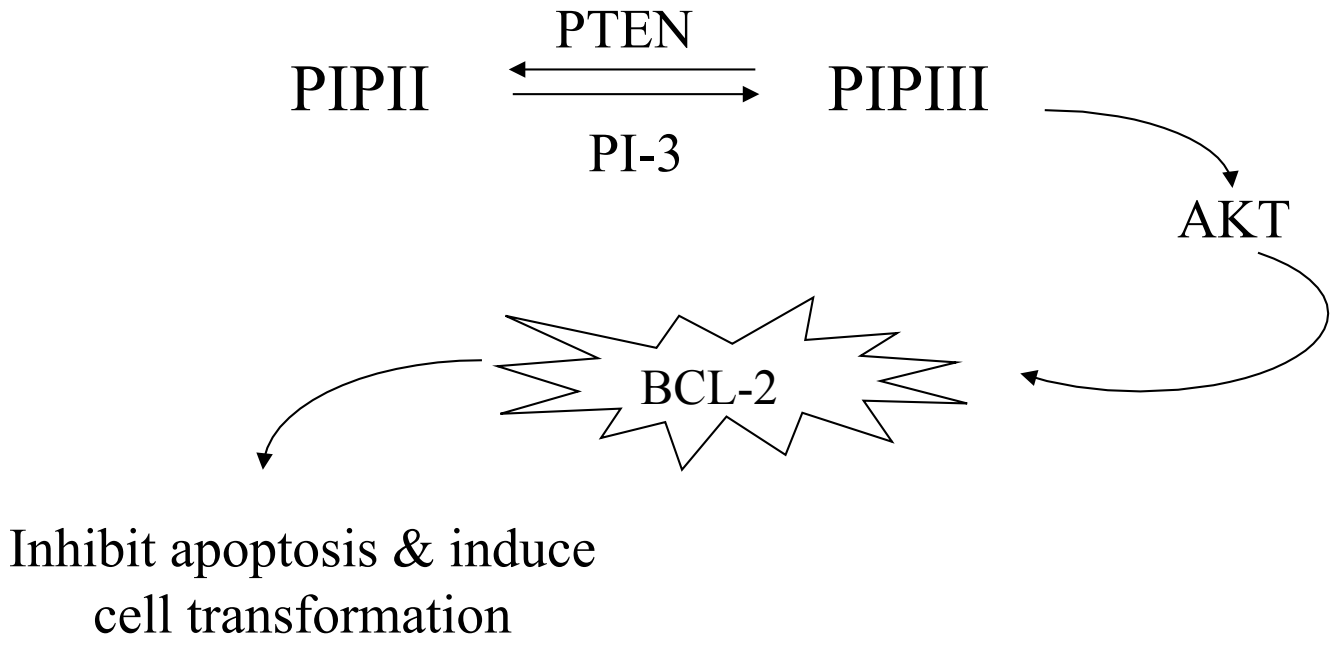
Tumor Suppressor Functions



1. **Antagonizes oncogene**
2. **Transcription Factor**
3. **Cell Cycle regulation**
4. **Apoptosis induction**

Function of Tumour Suppressor gene

1. *Antagonize the action of oncogene*. (ex.PTEN which converts PIPIII to PIPII because PIPIII will activate Pl-3/ AKT which will activate BCL-2 that will inhibit apoptosis and induce cell transformation)



Function of Tumour Suppressor gene

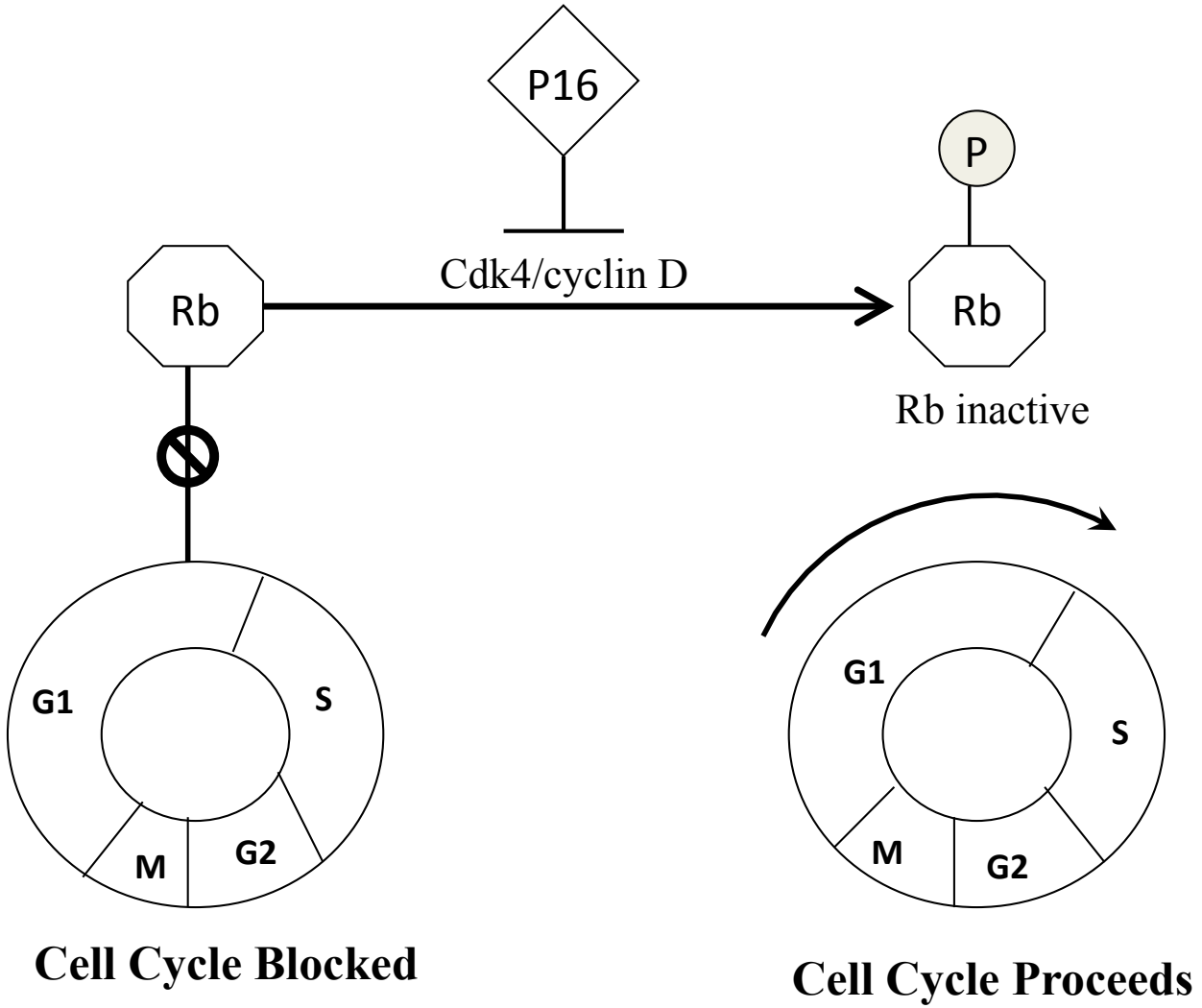
2. *Transcription factors*
   - Repressor transcription factors: example; WT1 is a repressor that appears to suppress transcription factor (Insulin like growth factor) which will contribute in the development of tumour.
   - Activator transcription factors: example; SMAD family that are activated by TGF-β, leading to inhibition of cell proliferation.

Function of Tumour Suppressor gene

3. Regulate cell cycle :

- *Rb gene:* that inhibits the cell cycle in the G1 phase decrease cell proliferation. ⟶
- *INK-4 gene:* that produces P16 that inhibits cdk4/cyclin D action (to phosphorylate Rb gene to inactivate it's action)
- *P53:* that produces P21 that has the same action of P16 in inhibiting the action of cdk4/cyclin D

Regulate cell cycle



Function of Tumour Suppressor gene

4. Induce apoptosis:

P53 release will

↓

increase Bax

↓

Release cytochrom c

↓

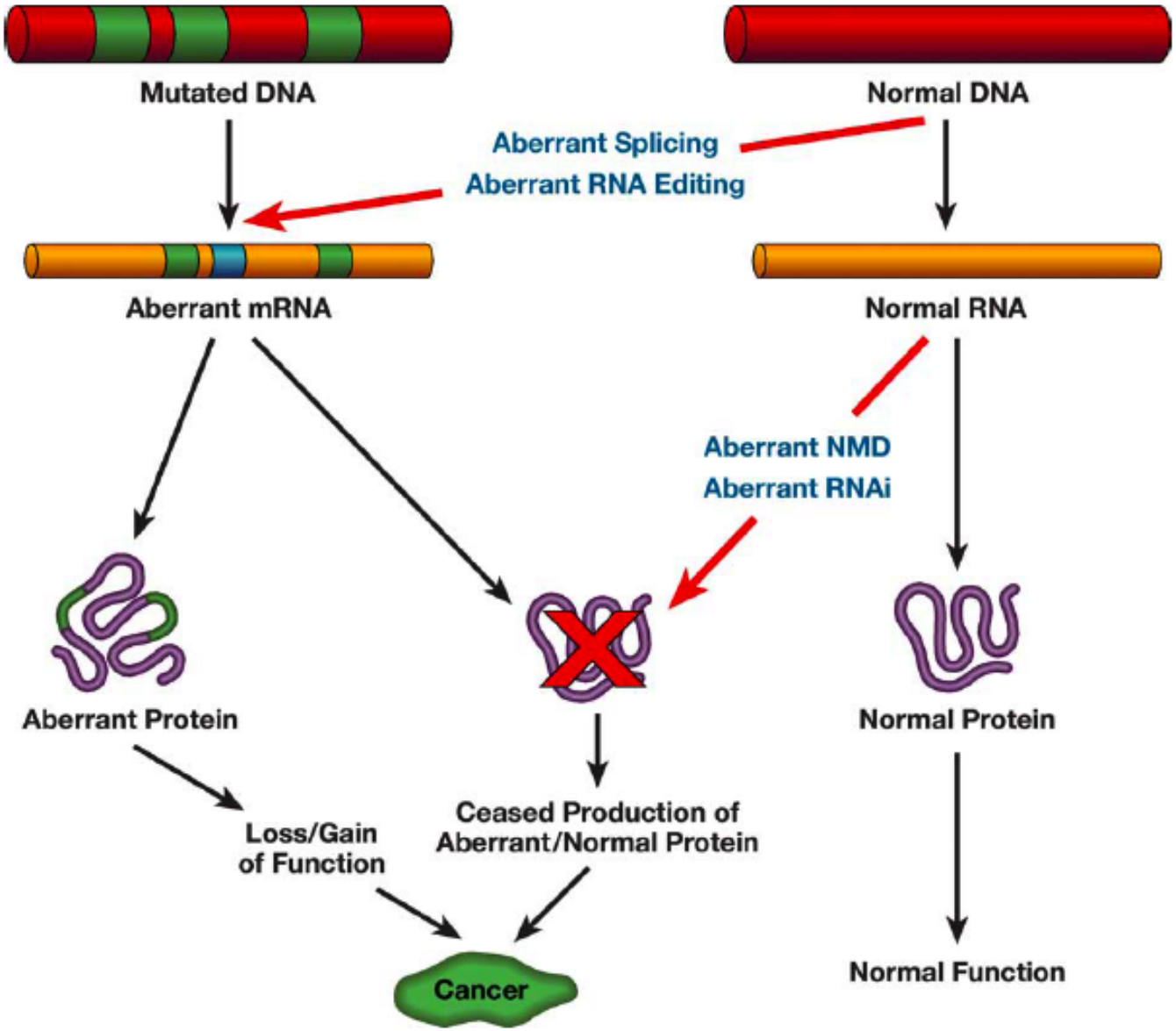
activate apoptosis

Cancer Detection

- Cancer detection :
  - *Clinical detection*
  - *Molecular detection*

RNA and cancer

RNA-based mechanisms - potential cancer development



Can RNA cause cancer?

- Epigenetic alterations in chromatin structure may influence
  gene expression independently of DNA mutations/alterations

- Epigenetic alterations may directly lead to cancer cell transformation

- mRNA surveillance link «mRNA quality» with processes
  including DNA metabolism.
  NMD involves hSMG-1 kinase (an ATM-related)
  ATM is involved in ivolved in DNA damage response detected early in tumorigenesis

- DNA damage conrol and mRNA surveillance may be connected

- ncRNAs: link between DNA and RNA.

Questions on the role of RNA in cancer

- Exact role of ncRNAs in the regulation of DNA- and RNA-related processes.
- Deregulation of RNA surveillance and change of transcriptome output
- How these processes are altered in the early stages of tumorigenesis
- Are the aberrant processing of pre-(m)RNAs the functional genome output, responsible *per se* for cancer development?

RNA and cancer

- alternative splicing
- Nonsense-mediated decay, NMD
- RNA editing
- RNA interference, RNAi

mRNA is not a passive intermediate product

- NUCLEUS
  - Quality control
    - capping, polyadenylation, splicing, editing, export
    - Nonsense-mediated decay

- CYTOPLASM
  - mRNA turnover
  - mRNA translation efficiency

- MicroRNAs (miRNAs)
  - translation interference
  - DNA methylation

- Non-coding RNAs (ncRNAs)
  - translation interference (antisense mechanism)
  - DNA methylation, mRNA processing - translation
  - Faster control of mRNA/protein expression than solely
    - protein-based regulation

Alternative splicing and cancer

PTC: Premature Termination Codon

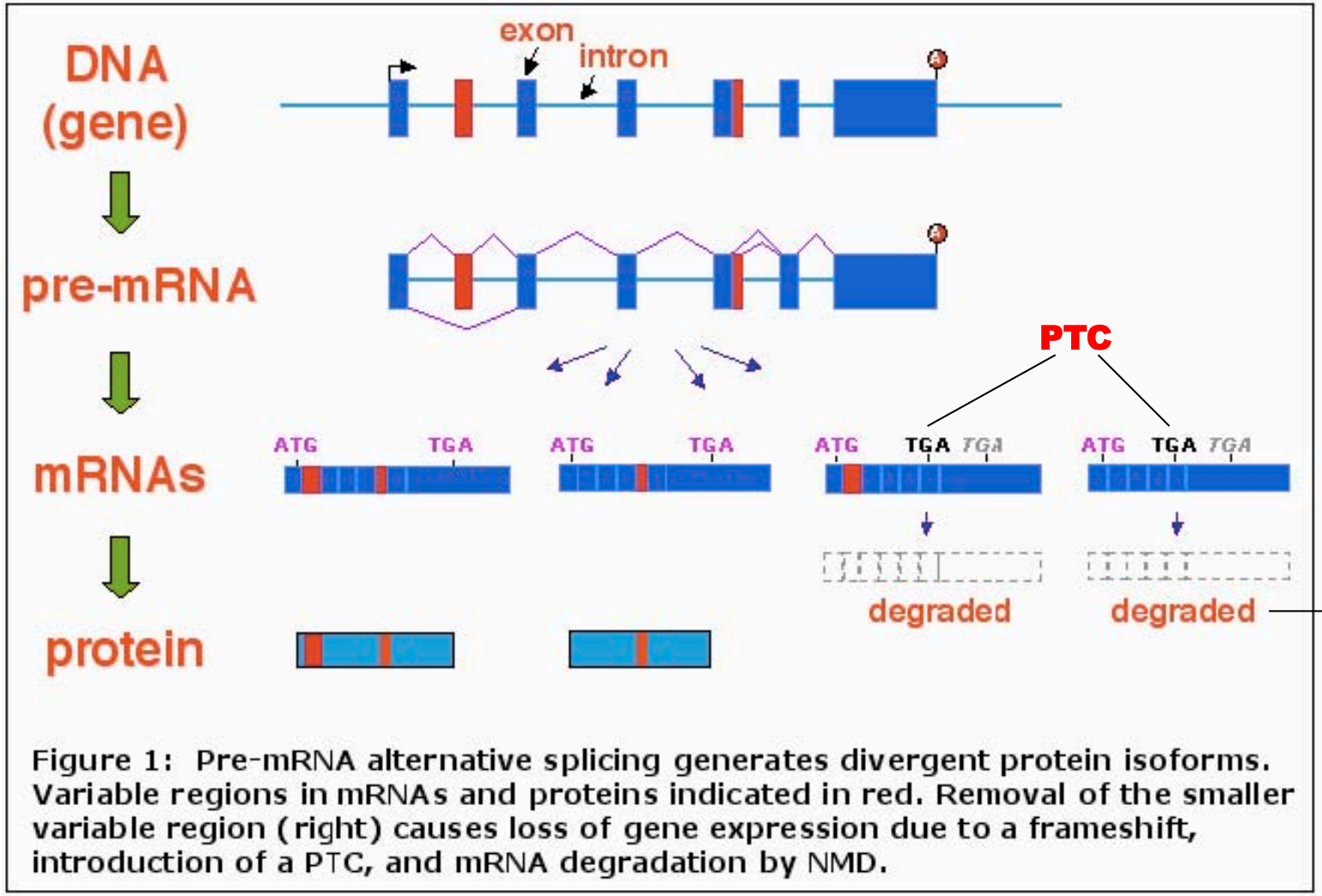
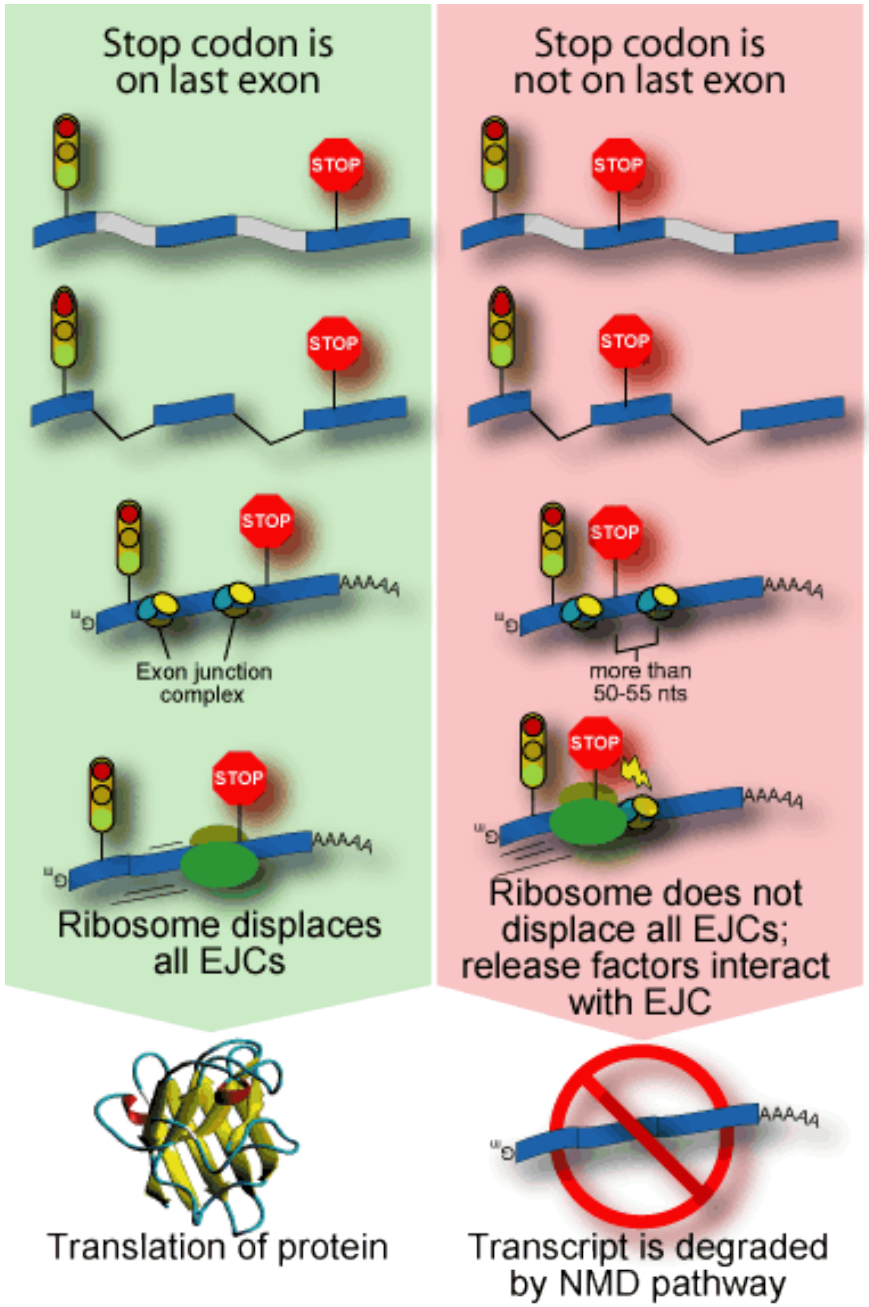


Figure 1: Pre-mRNA alternative splicing generates divergent protein isoforms. Variable regions in mRNAs and proteins indicated in red. Removal of the smaller variable region (right) causes loss of gene expression due to a frameshift, introduction of a PTC, and mRNA degradation by NMD.

http://www.bcm.edu/pathology/labs/cooper/files/images/Alternative_splicing_img_0.jpg

Nonsense-mediated mRNA decay, NMD



NMD modulates cancer-related mRNA expression in vivo

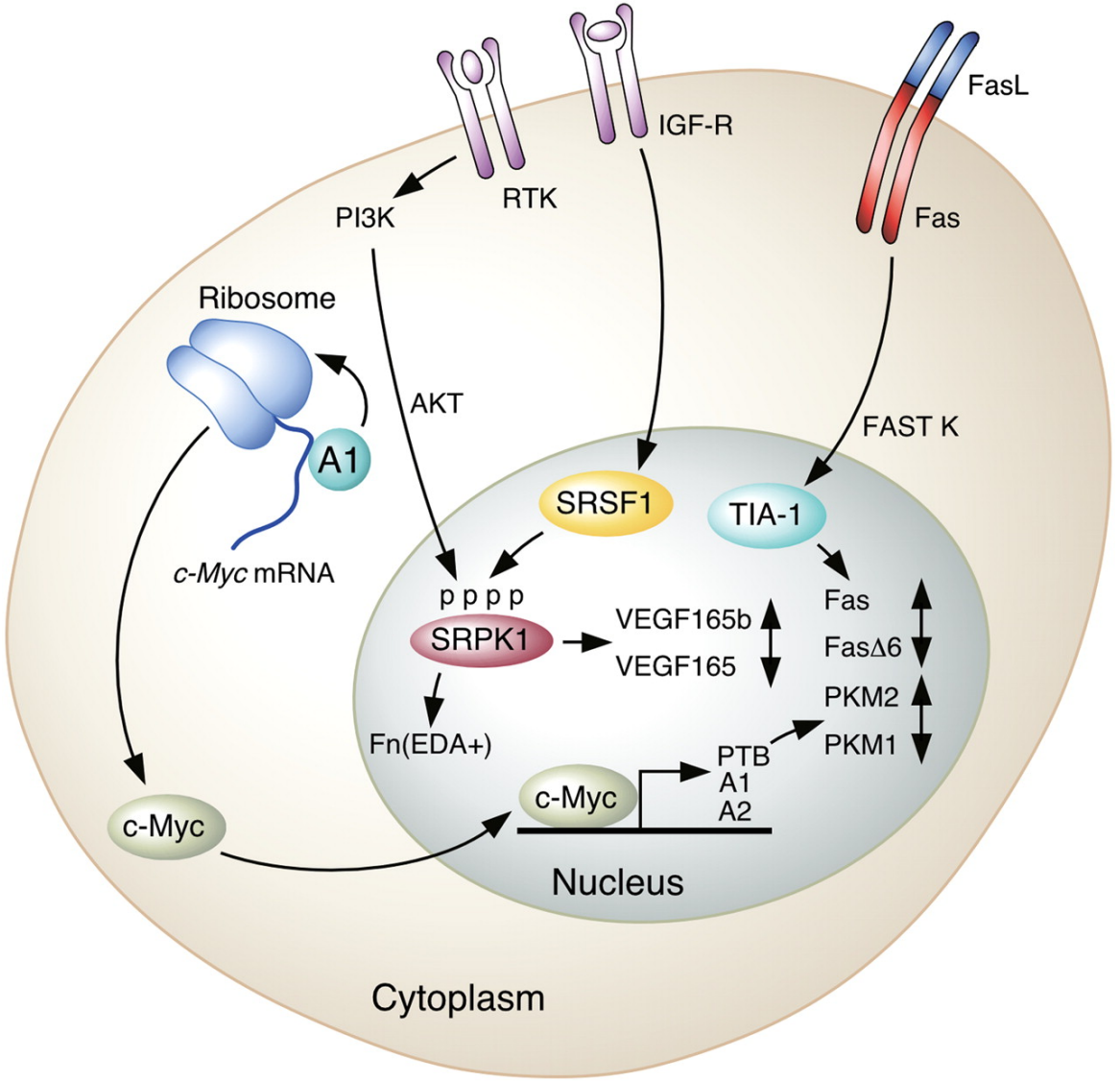
- Down-regulation of the majority of BRCA1 mRNAs
- UV-resistance associated gene (UVRAG)
- p300 genes
- Hexoaminidase B

Deregulation of NMD may lead to translation of
PTC-containing mRNAs producing functional aberrant proteins
that may influence many cellular processes
including tumorigenesis

Selected examples of cancer-specific alternative splicing categorized by affected tissue

Cancer tissue	Gene	Function	Transacting factor
Leukemia	Fyn	Tyrosine kinase	Rex
Leukemia	Caspase 8	Apoptosis	
Leukemia	PASG	Chromatin modelling	
Thyroid	MUC1	Adhesion, metastasis	
Thyroid, colon	Insulin receptor	Tyrosine kinase	
Colorectal	Rac1	Signalling GTPase	
Gastric	KAI1/CD82	Metastasis	
Gastric	WISP1	Invasion	
Pancreas	Secretin receptor	Growth inhibitor	
Pancreas	Gastrin receptor	Proliferation	U2AF
Liver	DNMT3b4	Chromatin modelling	
Liver	SVH	Novel	
Lung	NRSF	Transcription factor	
Lung	C-CAM	Adhesion	
Lung	VEGF	Angiogenesis	
Lung	Actinin-4	Adhesion, metastasis	
Endometrium	SHBG	Hormone signalling	
Endometrium	Integrin β1C	Adhesion	
Breast	AIB1	Hormone signalling	
Breast	Androgen receptor	Transcription Factor	
Breast	Estrogen receptor	Transcription Factor	
Breast	Syk	Metastasis	
Breast	uPAR	Adhesion, proteolysis	
Breast, brain	FGFR1	Growth signalling	PTB
Brain	Crk	Migration, invasion	
Brain	NF1	Signalling GTPase	
Many	TSG101	Proteolysis	
Many	MDM2	Proteolysis	
Many	CD44	Proliferation, angiogenesis	9G8, SAM68
Many	Tenascin-C	Adhesion inhibitor	
Many	Fibronectin	Angiogenesis	SRp40

Selected signal transduction pathways that affect Alternative Splicing of transcripts of genes

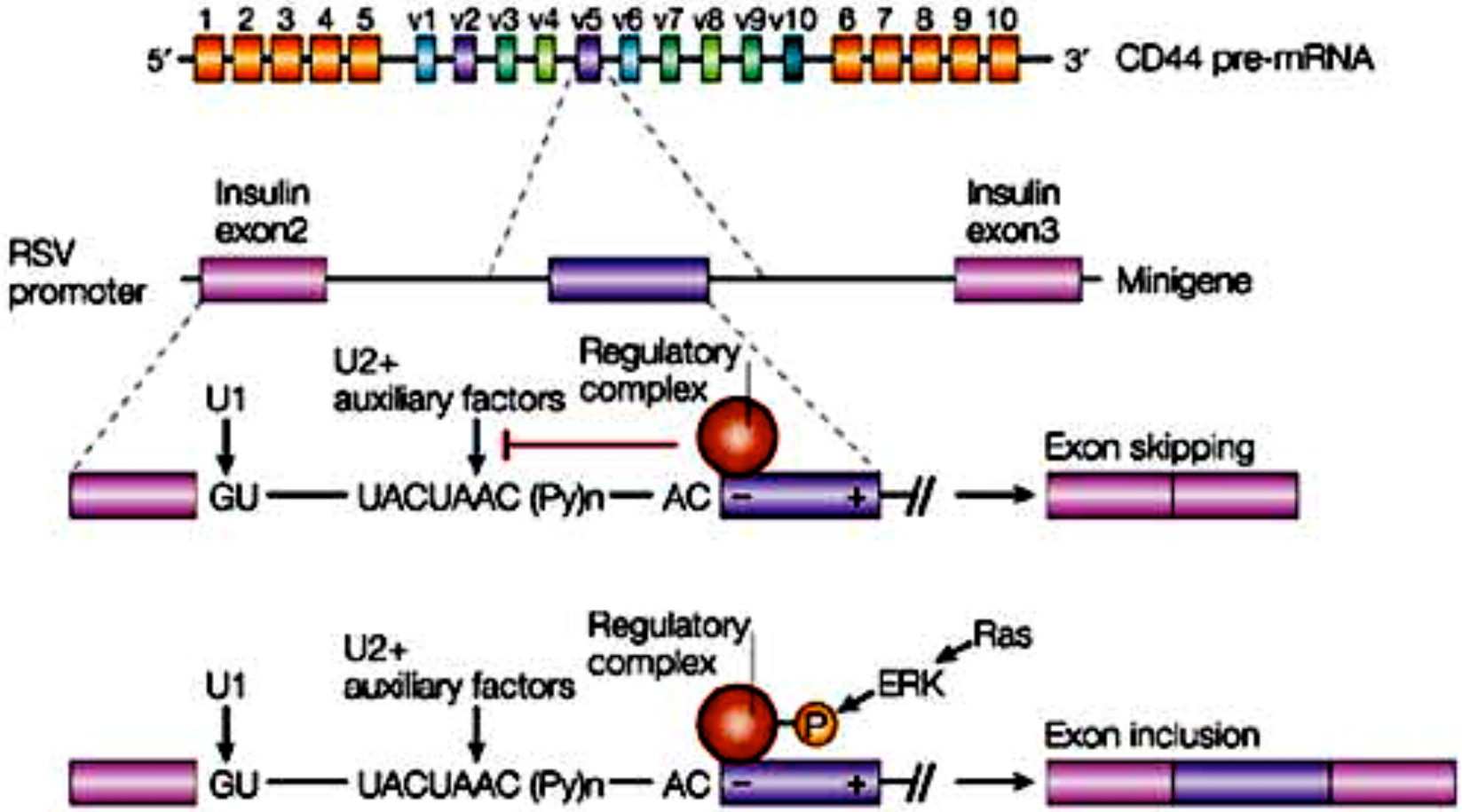


David C J , Manley J L Genes Dev. 2010;24:2343-2364

Alternative splicing and cancer

Can cancer-prone isoforms be produced without mutations in genomic DNA influenced only by deregulation of alternative splicing?

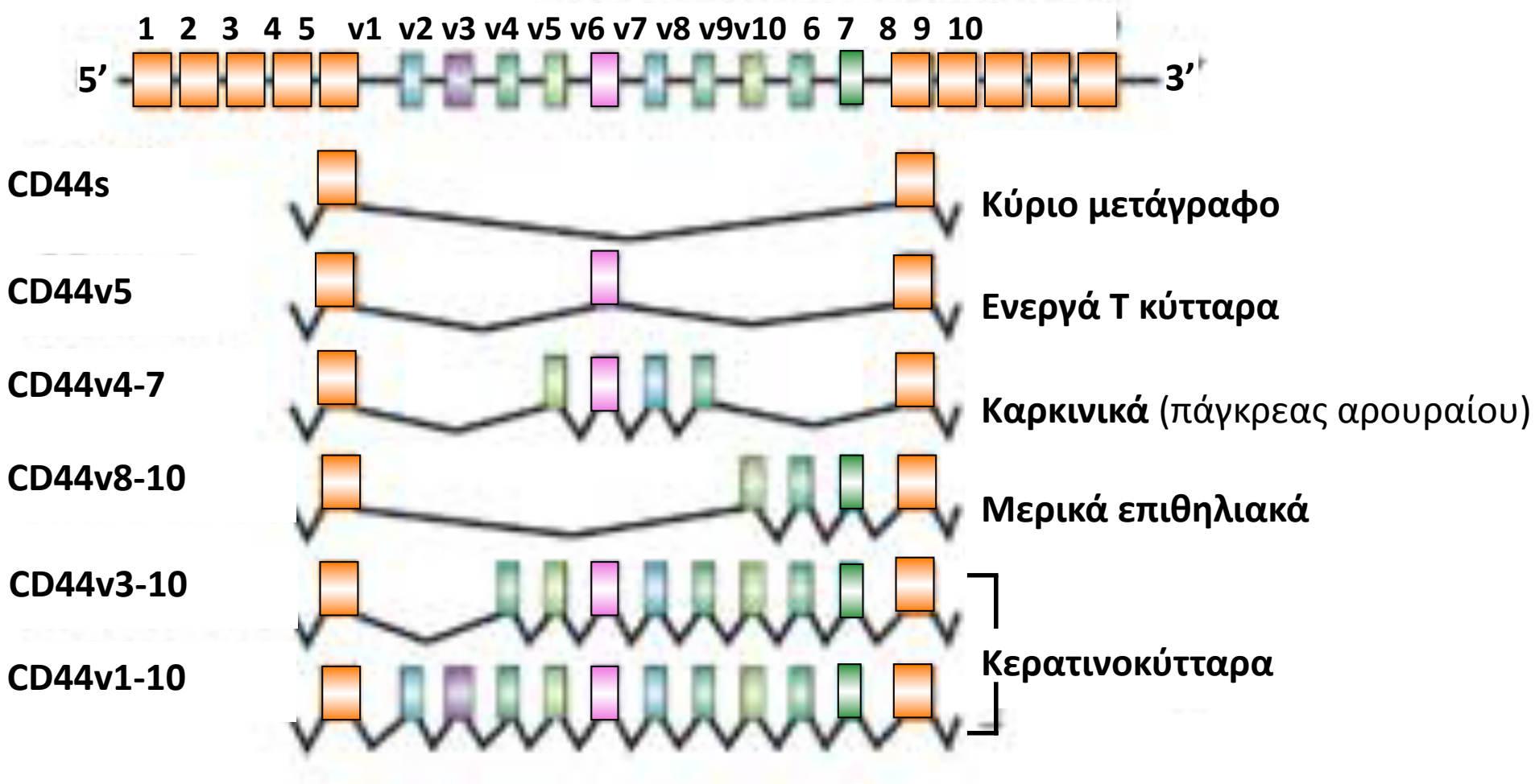
The CD44 family transcripts



Ponta H et al. Nat Rev MCB 4, 33-45 (2003)

Τα μετάγραφα της οικογένειας CD44

CD44 pre-mRNA



Ponta H et al. Nat Rev MCB 4, 33-45 (2003)

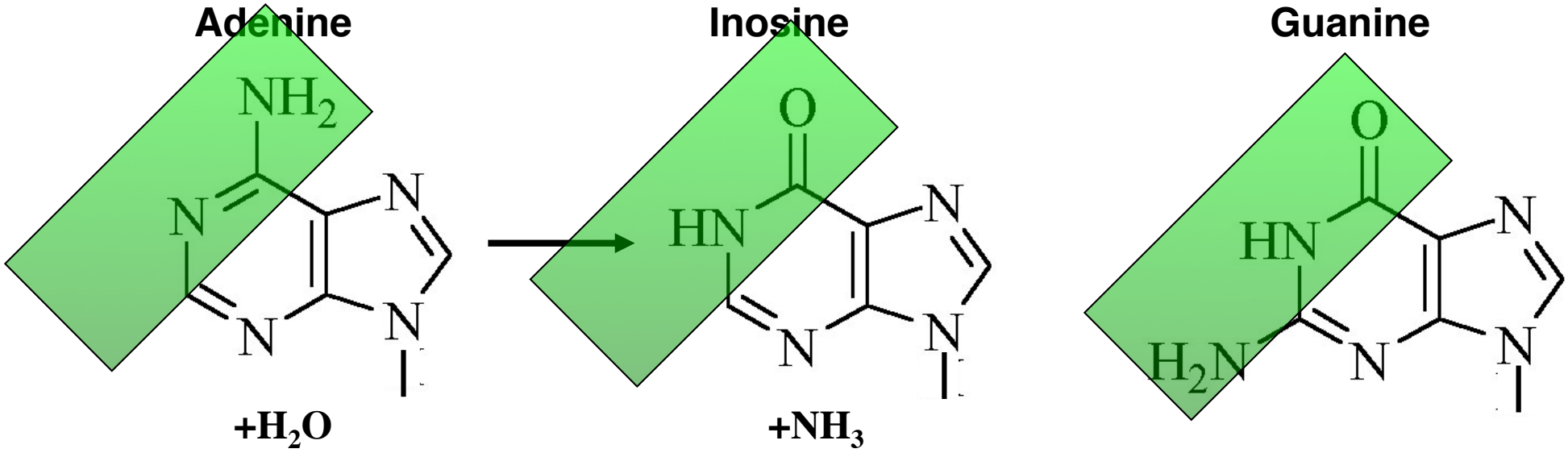
RNA editing (Επιμέλεια RNA).

Editing = Changes to the RNA nucleotides that change the coding potential

Three main types:

- Many Uridines (U) are inserted and deleted in trypanosomes kinetoplast genes.
- Some Adenines (A) are converted to Inosines (I) in metazoans.
- Some Cytidines (C) are converted to Uridines (U) in metazoans.

A to I deamination.

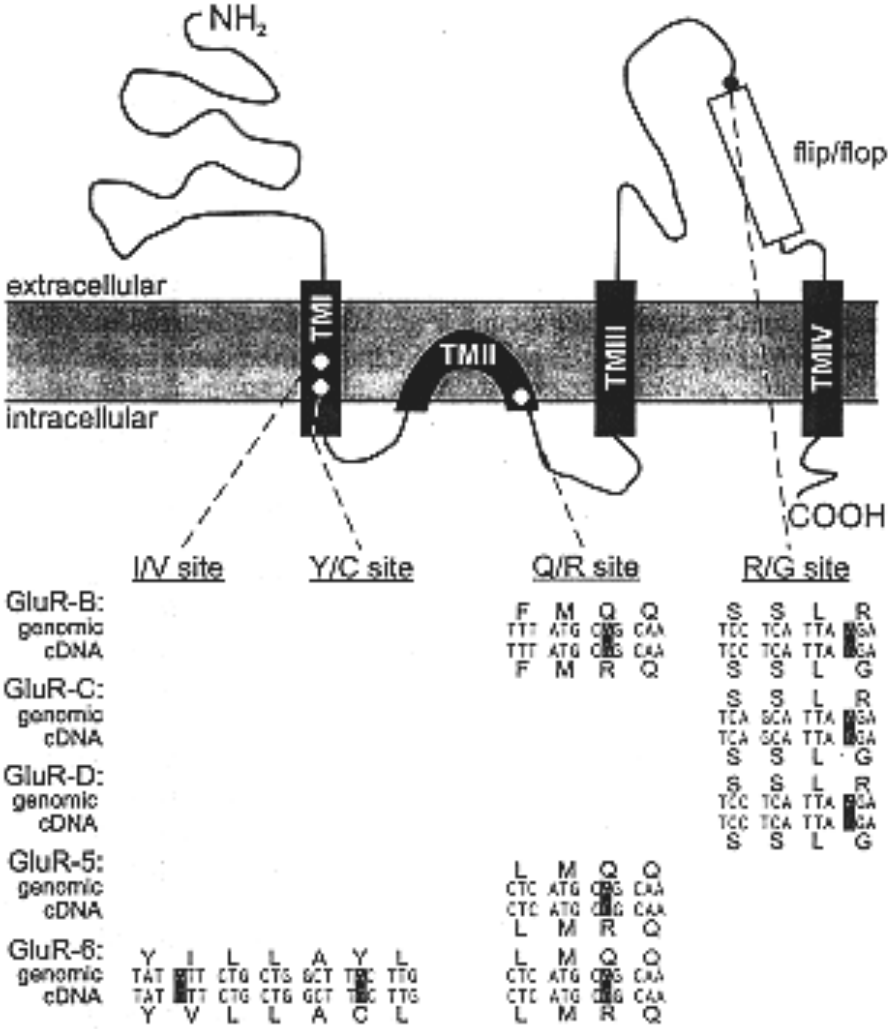


I base pairs like G: different amino acid inserted during translation.

example: Amino acid change in human glutamate receptors.

example: worm mutants missing Adenine deaminase show aberrant behavior.

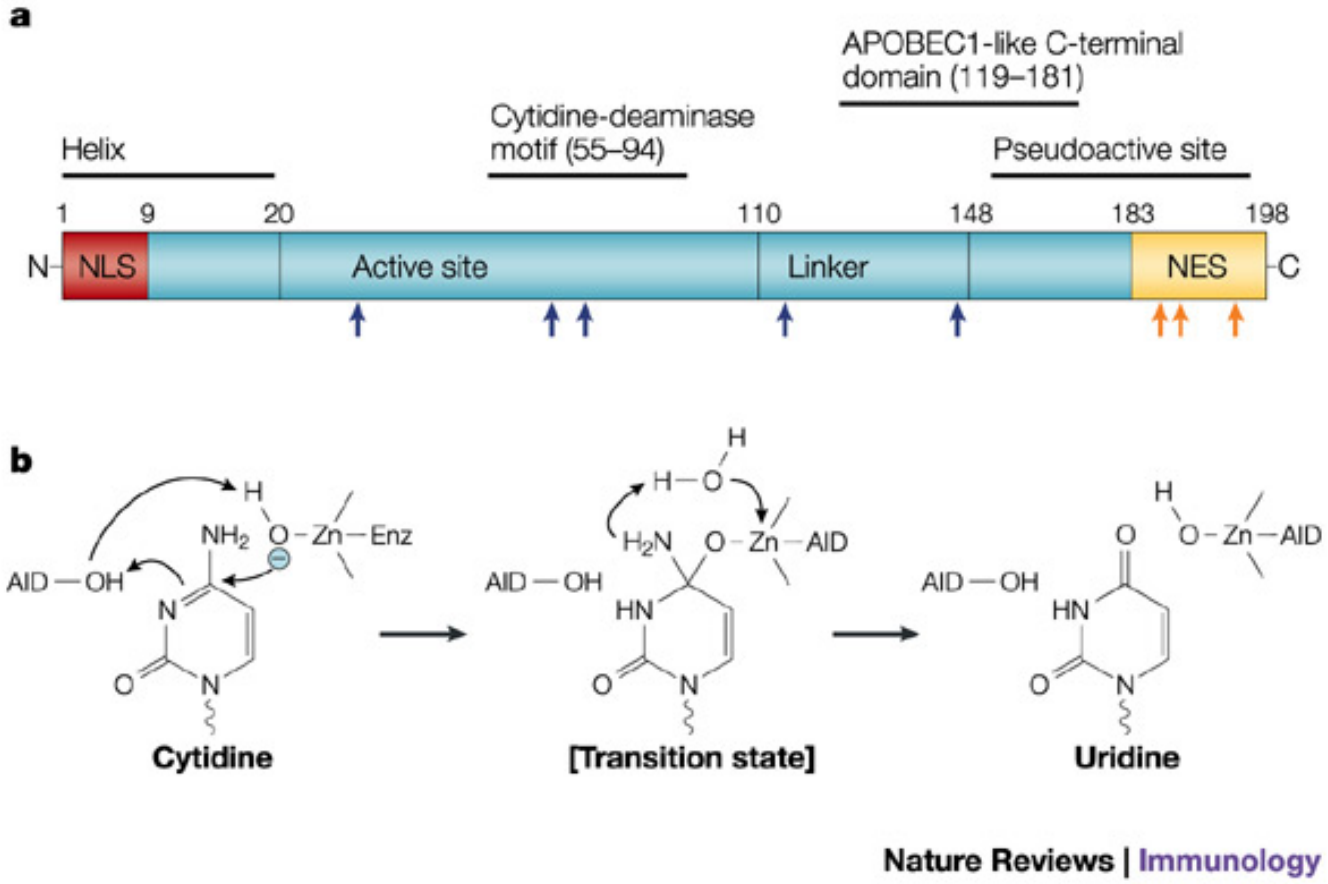
A to I deamination.



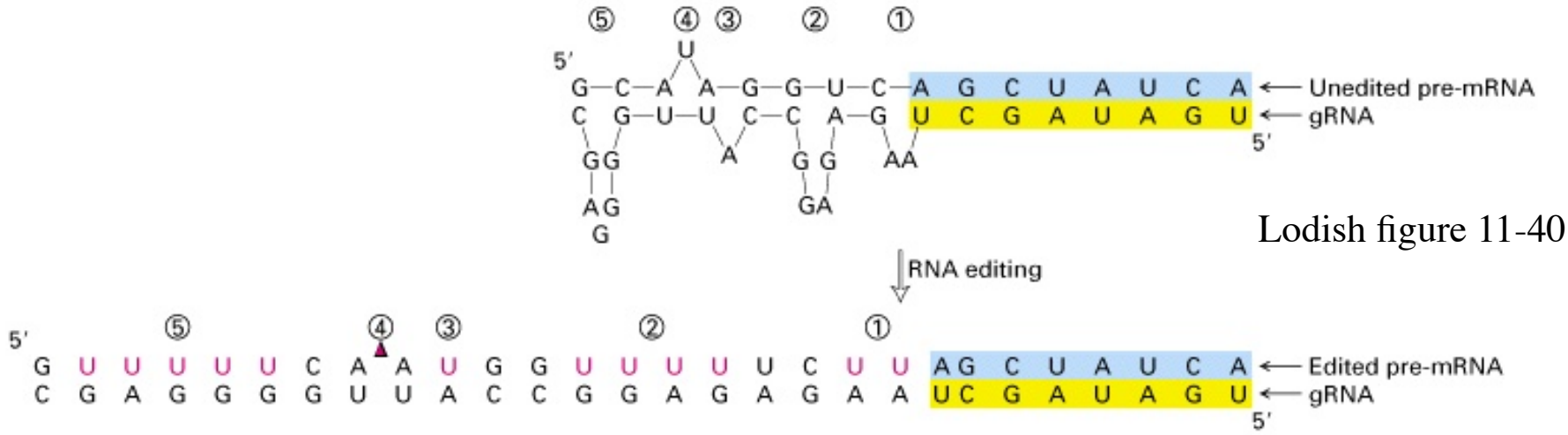
example: Amino acid changes in human glutamate receptors.

RNA editing and cancer

- CDARs (Cytidine Deaminase Acting on RNAs)
- APOBEC1, homolog of AID best characterized CDAR
- ADARs (Adenine Deaminases Acting on RNAs)



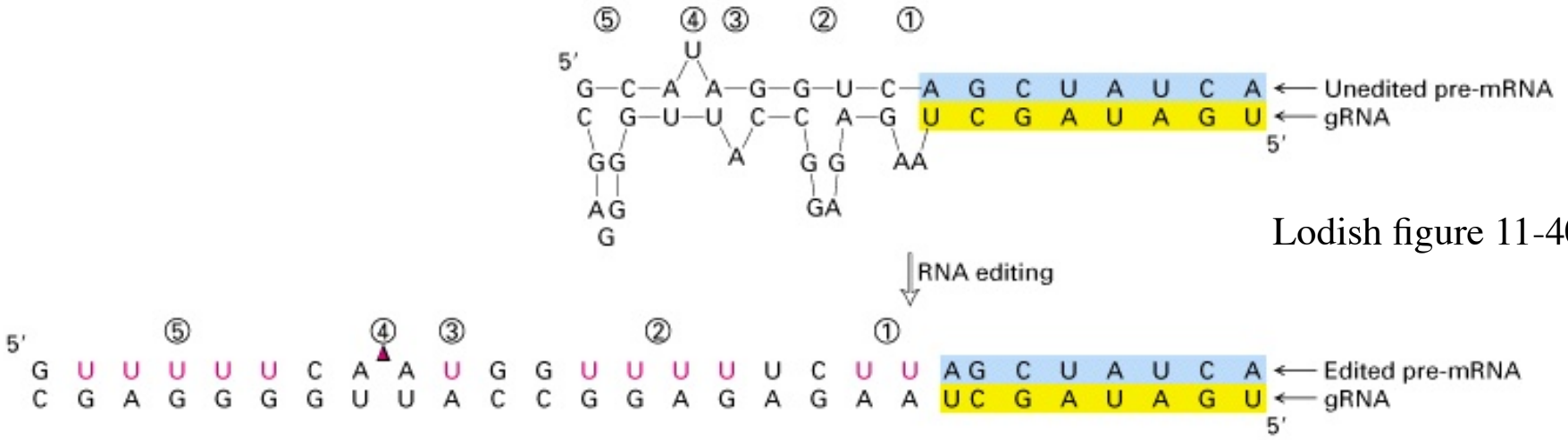
U insertion and deletion is encoded in guide RNAs



Lodish figure 11-40

- Editing requires a dsRNA around the editing site
- This can be formed by neighboring exonic and/or intronic sequences
- Therefore, editing takes place in the nucleus, and precedes splicing

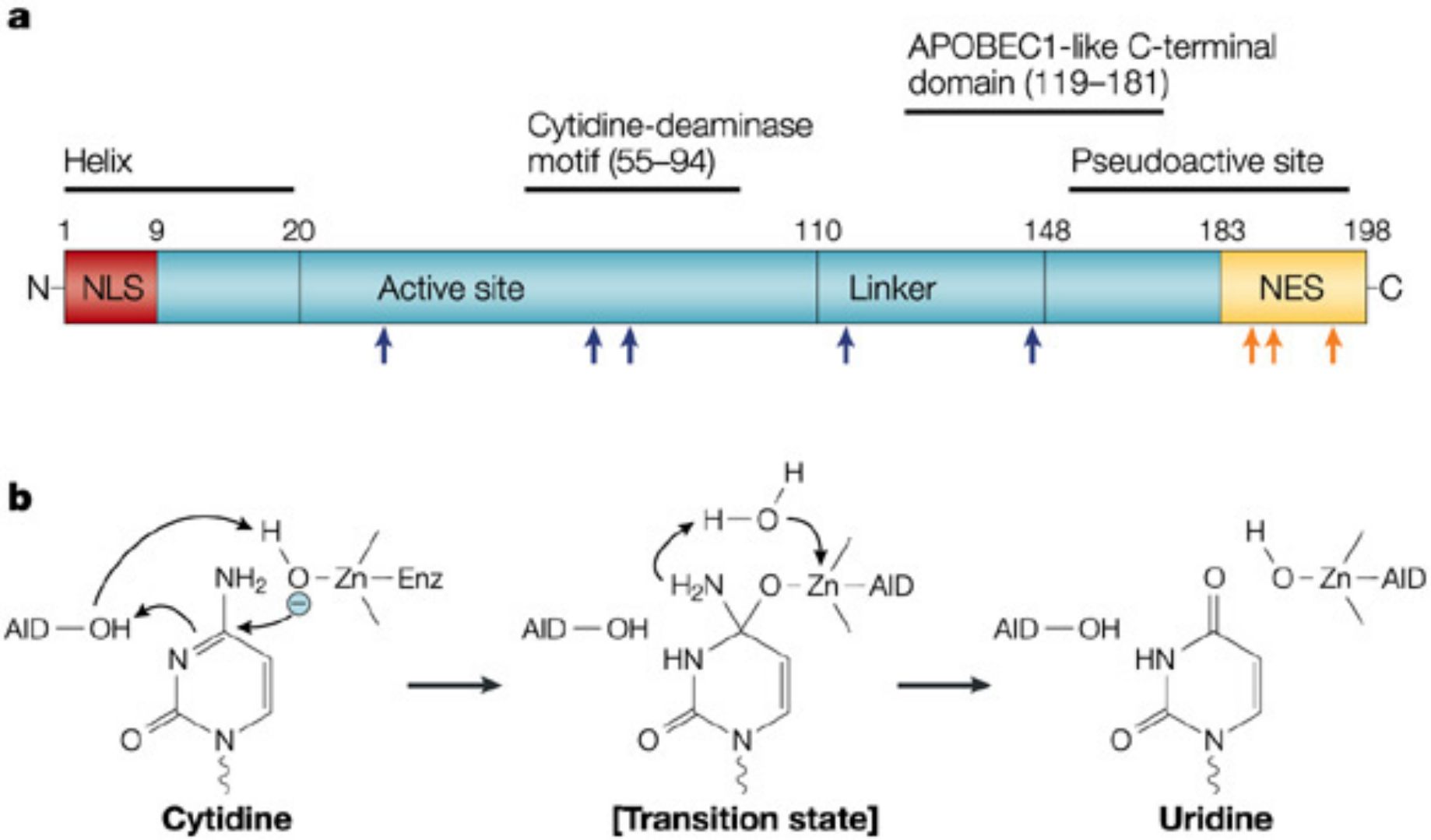
RNA editing and cancer



Lodish figure 11-40

- Editing requires a dsRNA around the editing site
- This can be formed by neighboring exonic and/or intronic sequences
- Therefore, editing takes place in the nucleus, and precedes splicing
- Deregulation of ADARs has been shown to occur in malignant neuroblastomas

AID and DNA deamination



Jayanta Chaudhuri & Frederick W. Alt (2004)

Nature Reviews | Immunology

AID (activation-induced cytidine deaminase)

Δράση σε υποδοχείς AMPA και κανάλια Ca^{2+}

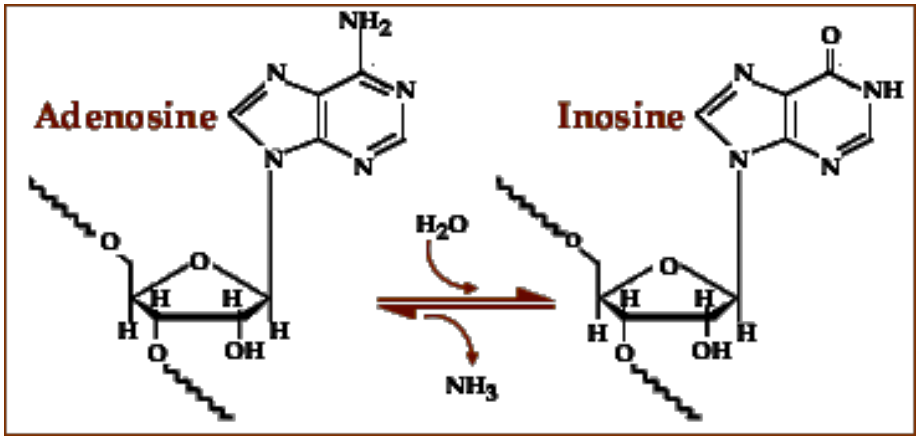
4 υπομονάδες: GluR1-4

Ελλειψη της GluR2

↓

Υψηλή διαπερατότητα σε Ca^{2+}, οφείλεται στην περιοχή Q/R.
Η αλλαγή Q→R οφείλεται σε επιμέλεια RNA (editing).

Q		R
CAA	⟶	CGA
CAG		CGG



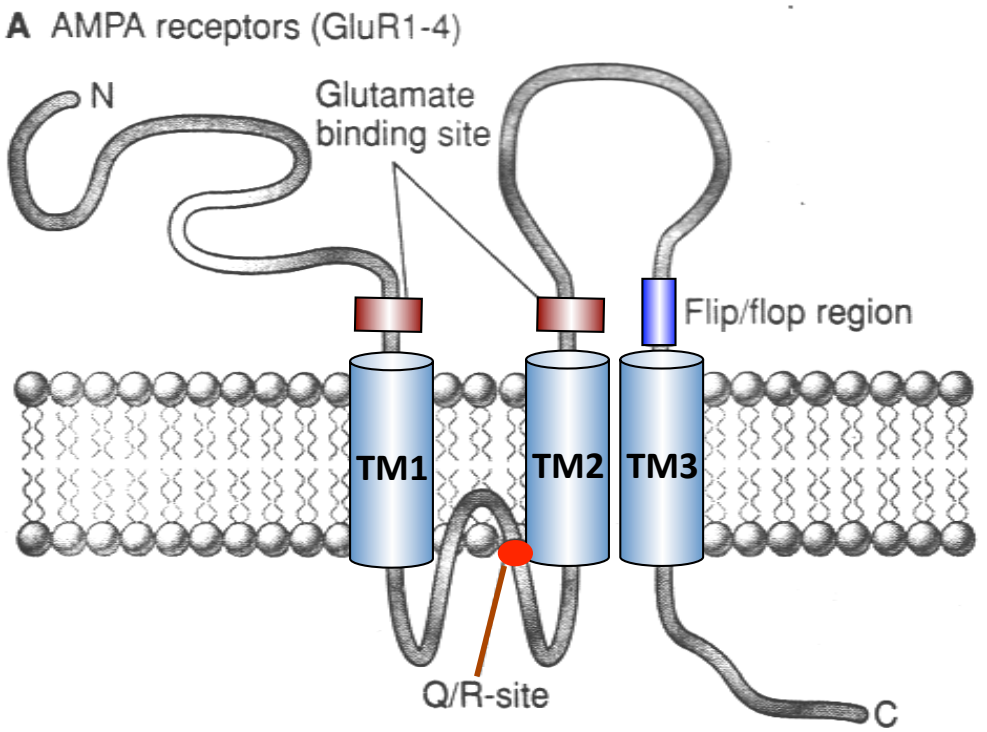


Figure 7–4. Membrane topology of AMPA glutamate, $GABA_A$, and glycine receptors. **A.** AMPA glutamate receptor subunits (GluR1-4) possess only three transmembrane spanning domains. The channel lining domain between TM1 and TM2 is a reentrant loop with both ends facing the cytoplasm. The Q/R site, which controls the Ca^{2+} permeability of AMPA receptor subunits, is located in this loop. The flip-flop site, located extracellularly between TM2 and TM3, yields two splice variants of each subunit. The glutamate binding site of AMPA receptors is formed by several amino acids in the N-terminal and extracellular loop. **B.** In contrast to AMPA receptors, $GABA_A$ and glycine receptors possess four putative membrane-spanning domains.

Δράση σε υποδοχείς GluR2 (AMPA υποδοχέας) και κανάλια Ca^{2+}

4 υπομονάδες: GluR1-4

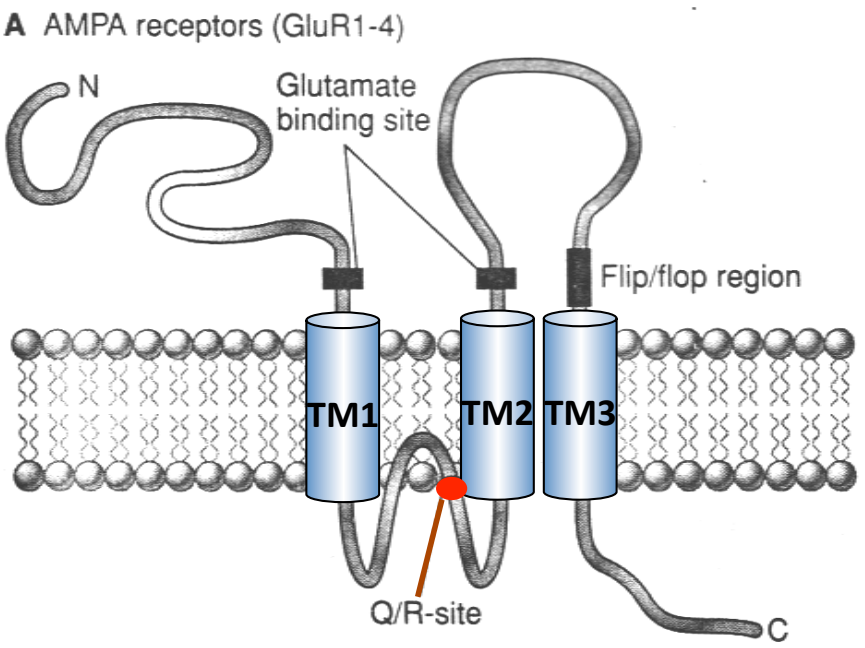
Ελλειψη της GluR2

↓

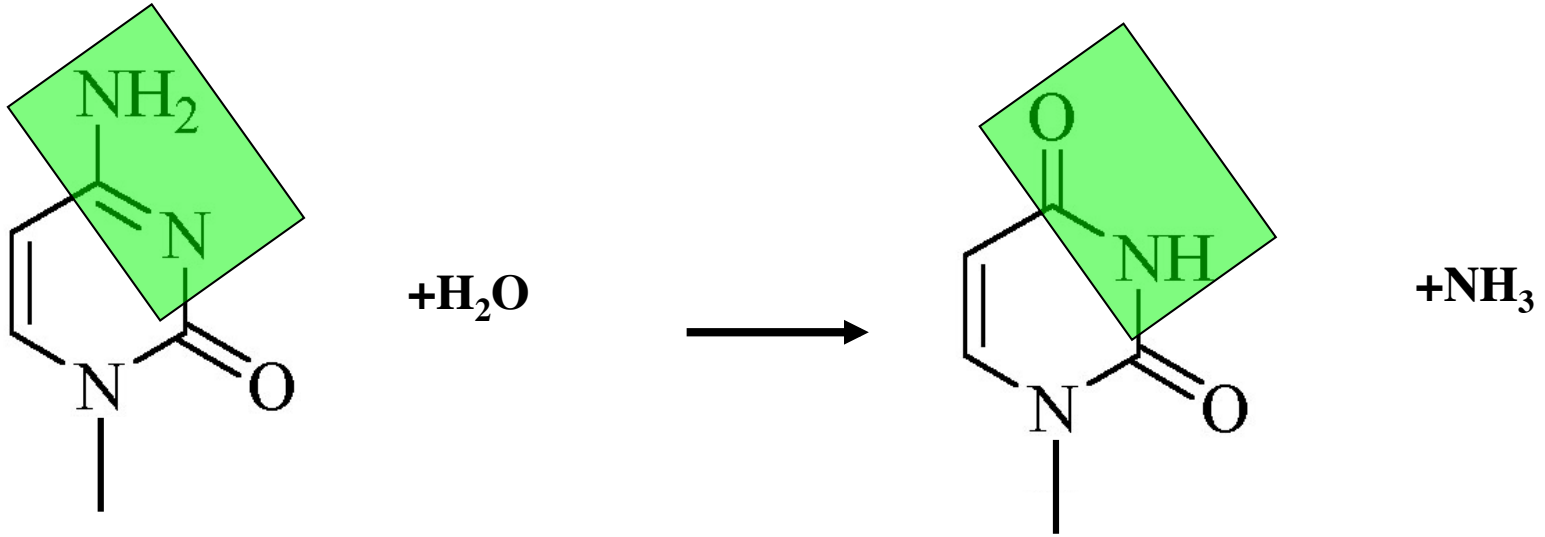
Υψηλή διαπερατότητα σε Ca^{2+},
οφείλεται στην περιοχή Q/R.
Η αλλαγή Q→R οφείλεται σε
επιμέλεια RNA (editing).

Q		**R**
CAA	⟶	CGA
CAG		CGG

Η αλλαγή αυτή μειώνει
τη διαπερατότητα των μεμβρανών
σε ιόντα Ca.

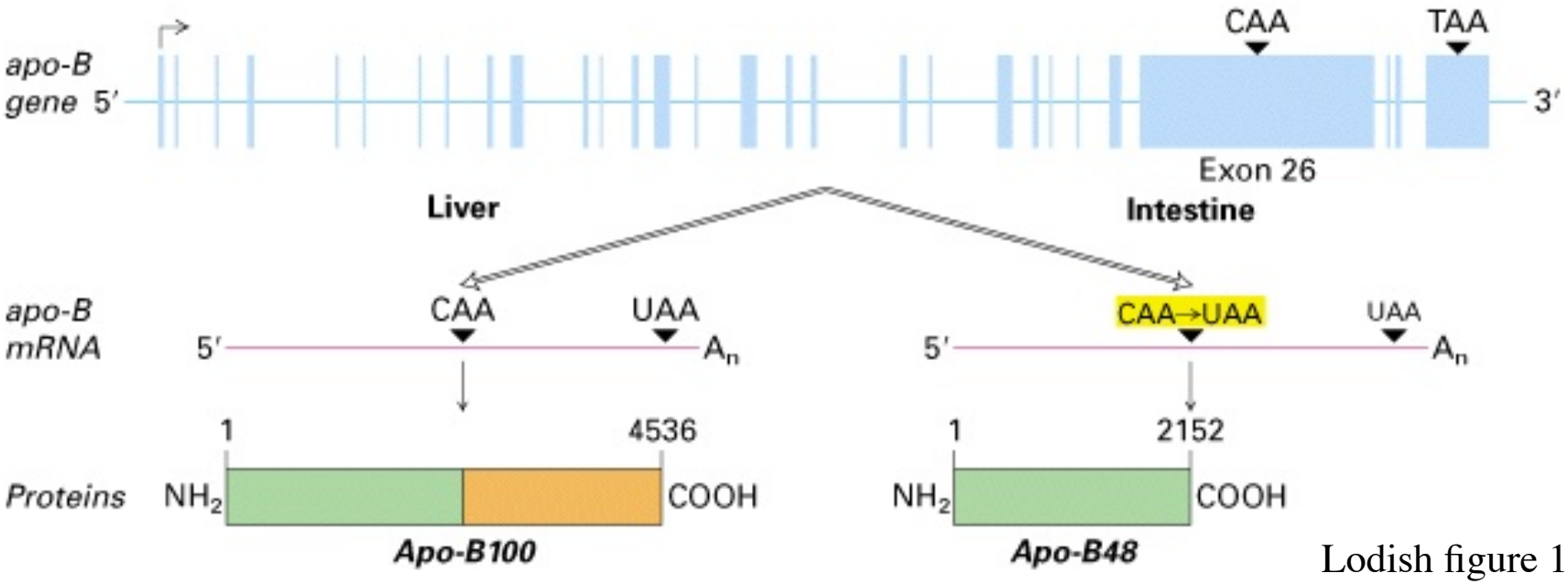


C to U deamination.



Changes one codon in human apoB mRNA to a stop codon: different functions for liver 100K protein and gut 48K protein.

C to U deamination.

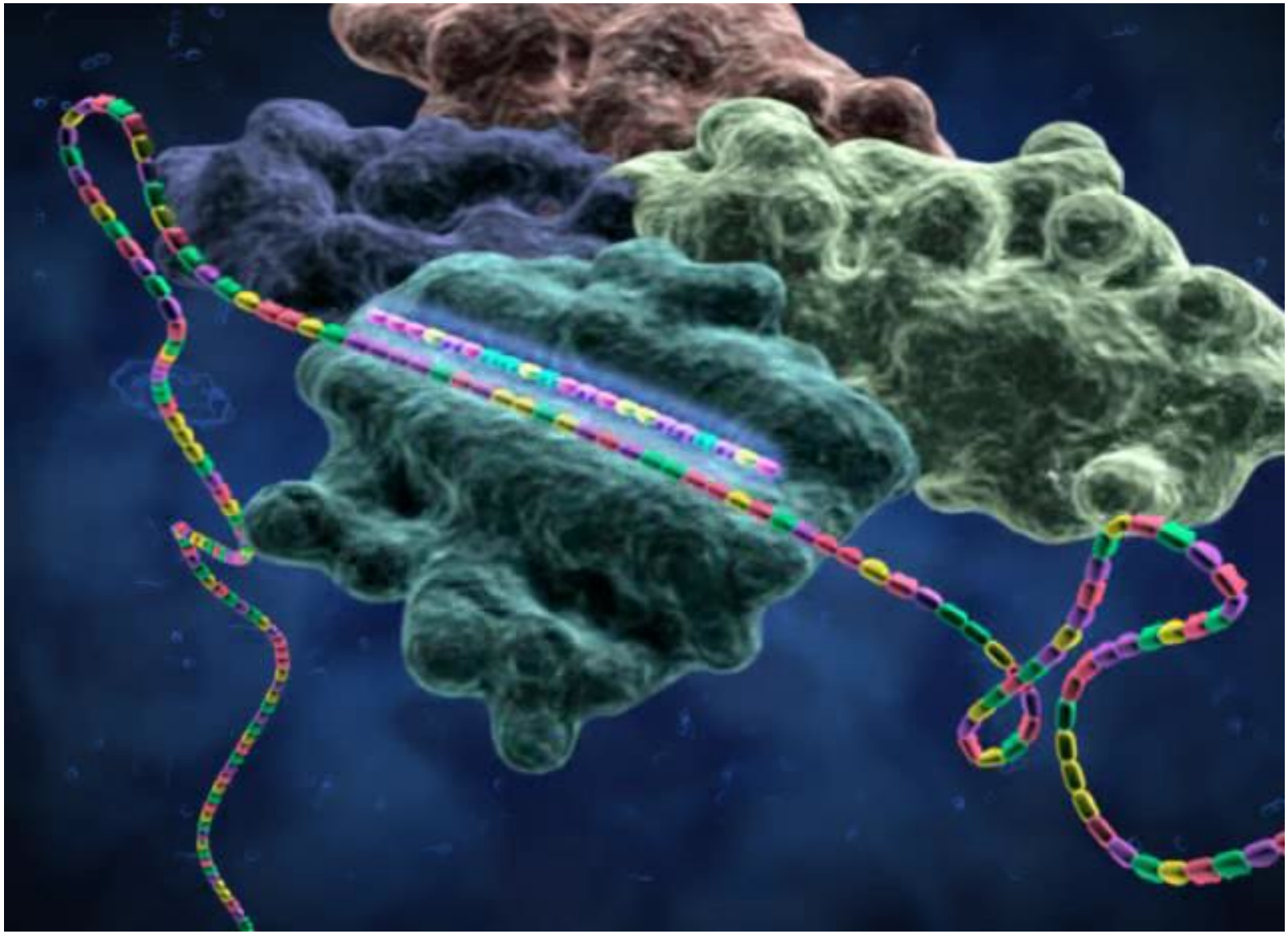


Lodish figure 11-39

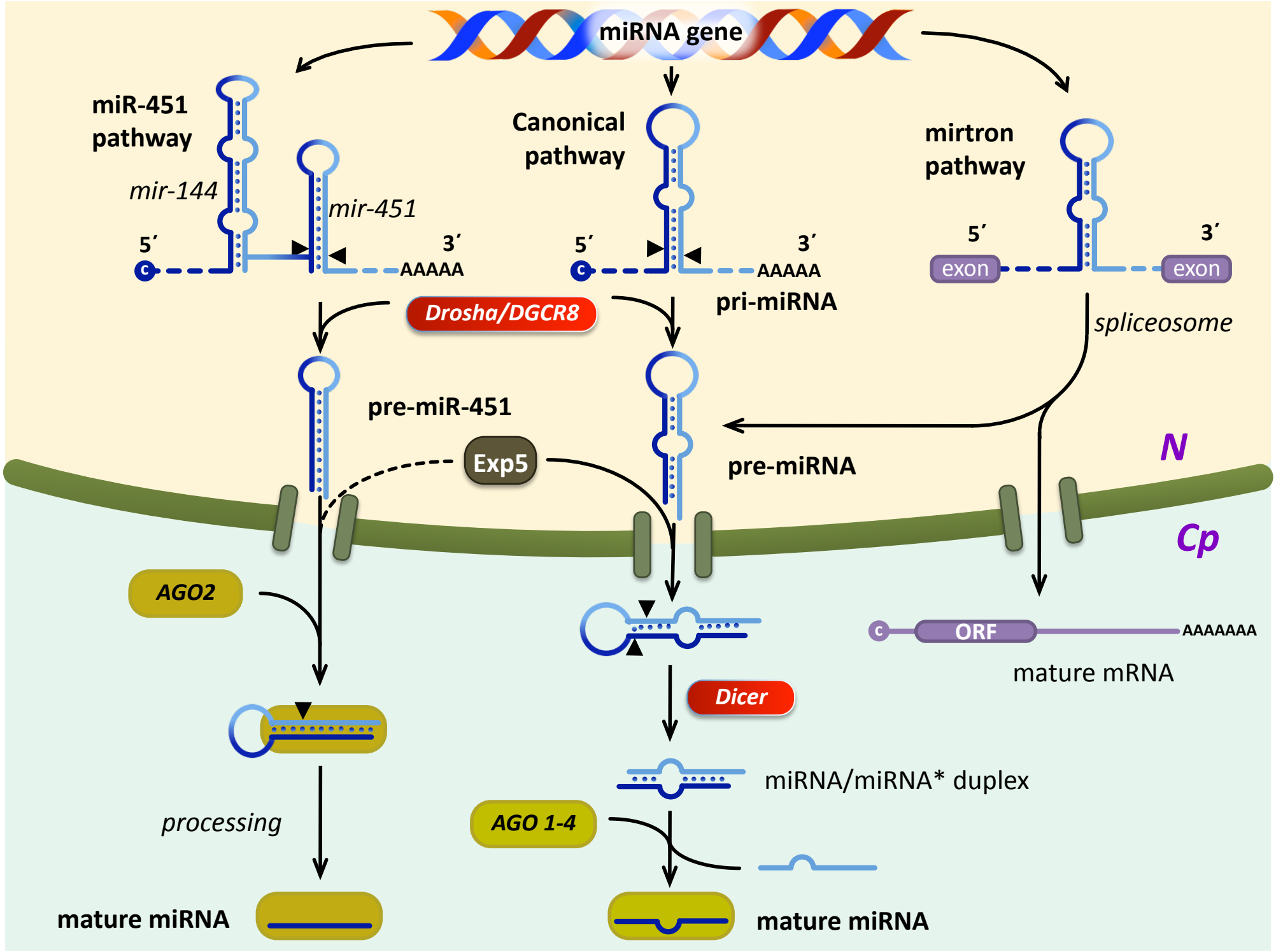
Changes one codon in human apoB mRNA to a stop codon:
different functions for liver 100K protein and gut 48K protein.

RNAi

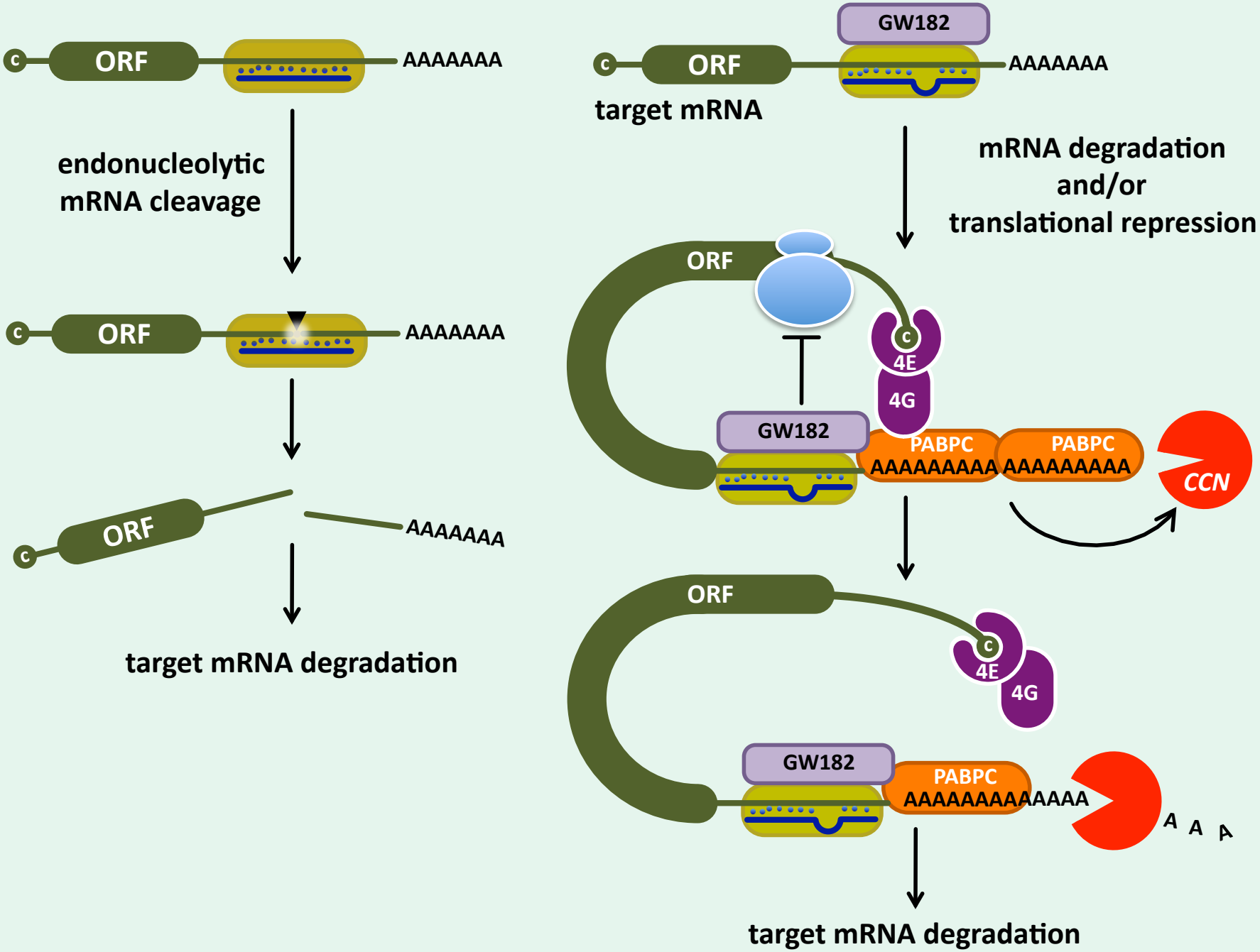
ncRNAs, miRNAs, siRNAs,…



http://cdn.medgadget.com/img/sirna.jpg


miRNA gene
miR-451 pathway
mir-144
mir-451
5′
3′
AAAAA
Canonical pathway
pri-miRNA
mirtron pathway
exon
exon
spliceosome
Drosha/DGCR8
pre-miR-451
Exp5
pre-miRNA
N
Cp
AGO2
ORF
AAAAAAA
mature mRNA
Dicer
miRNA/miRNA* duplex
processing
AGO 1-4
mature miRNA
mature miRNA

Mechanisms of miRNA-mediated gene silencing

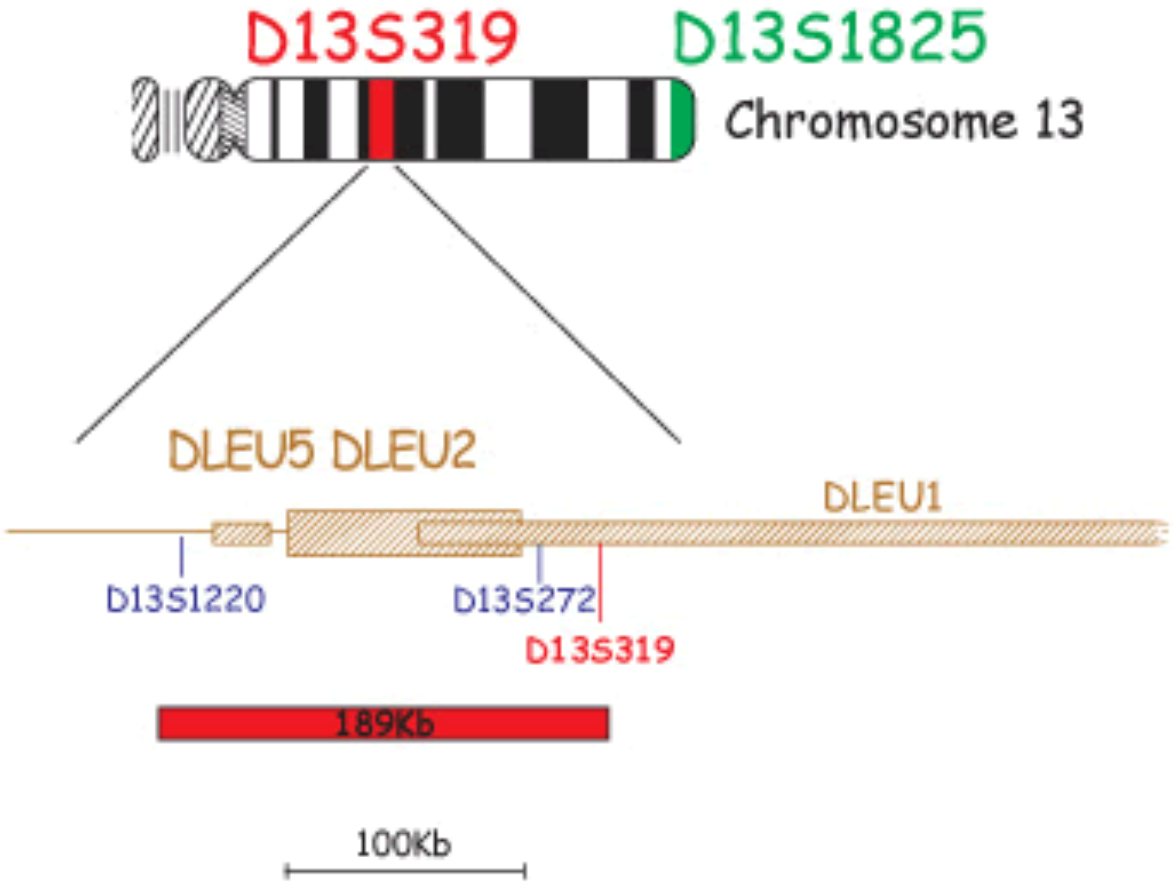


Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia

George Adrian Calin*, Calin Dan Dumitru*, Masayoshi Shimizu*, Roberta Bichi*, Simona Zupo[†], Evan Noch*, Hansjuerg Aldler*, Sashi Rattan*, Michael Keating[‡], Kanti Rai[§], Laura Rassenti[¶], Thomas Kipps[¶], Massimo Negrini*, Florencia Bullrich*, and Carlo M. Croce*[‖]

PNAS | November 26, 2002 | vol. 99 | no. 24 | 15524–15529

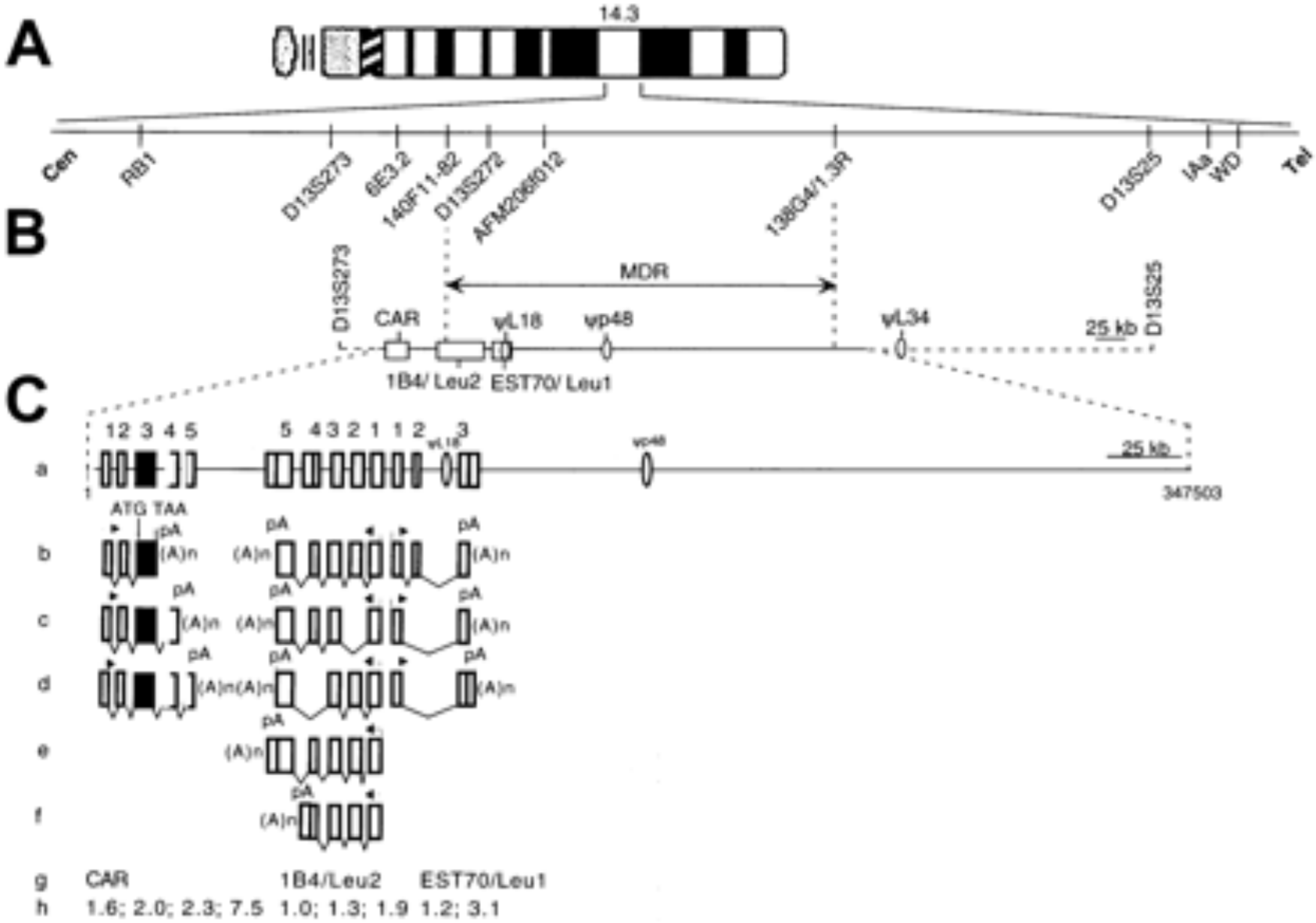
B-cell chronic lymphocytic leukemia, B-CLL



Chromosomal abnormalities on chromosome 13q occur in 16 - 40% of multiple myeloma cases and are associated with poor prognosis. Deletions affecting the 13q14 band are also the most frequent genetic abnormalities of B-CLL chronic lymphocytic leukaemia (B-CLL). Two non-coding RNA genes, DLEU1 and DLEU2 and the genetic marker D13S319 span the pathogenic critical region 13q14.36. DLEU1 is considered to be the most likely CLL-associated candidate tumour suppressor gene within the 13q14 region. Subsequently the locus D13S319, located between the RB1 gene and D13S25 and within the DLEU1 locus, was found to be deleted in 45% of CLL cases. The Cytocell D13S319 deletion probe covers the marker and the centromeric end of the DLEU1 locus.

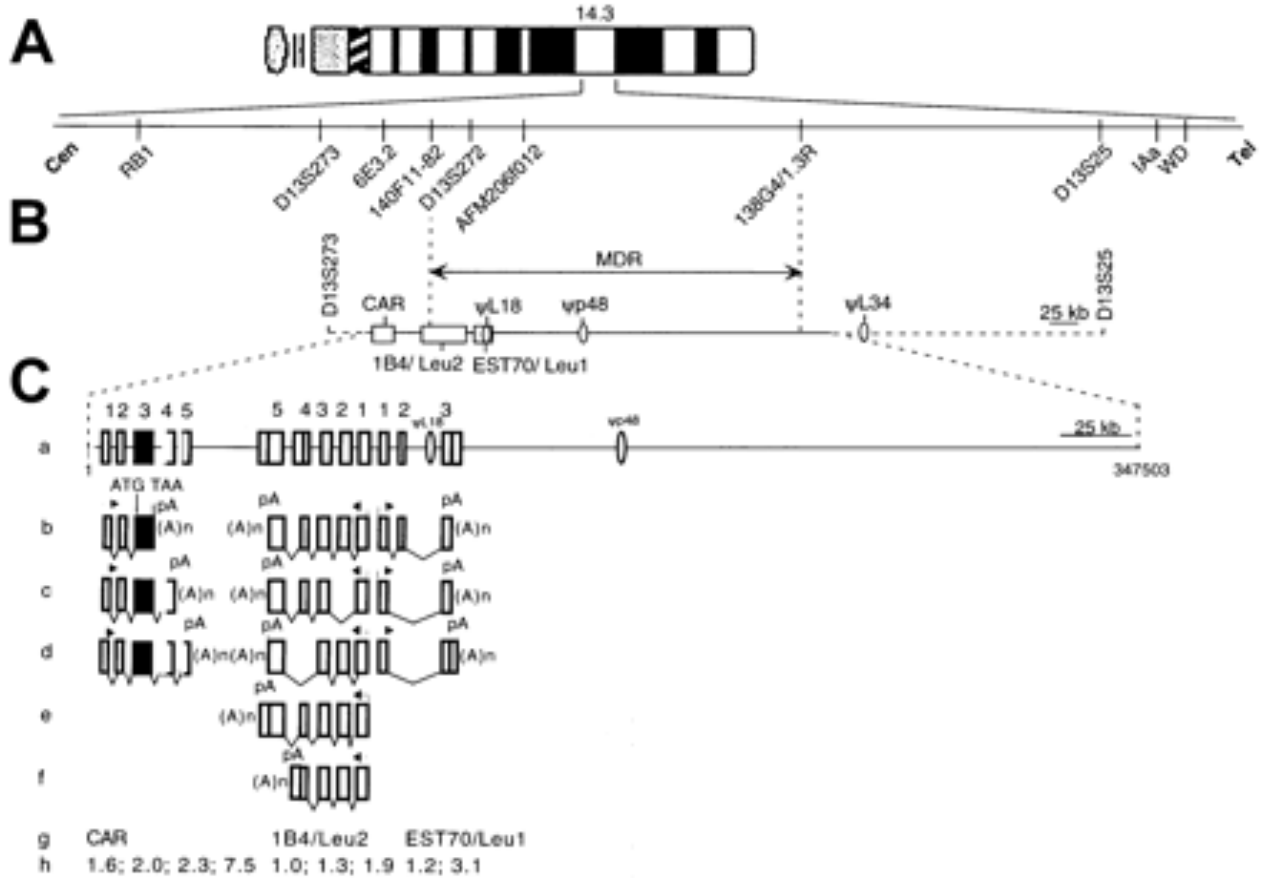
http://www.cytocell.com/products/aquarius/haematology-probes/by-disease-state/chronic-lymphocytic-leukaemia-probes/D13S319-Deletion-probe.asp

13q14 chromosomal region deleted in B-CLL



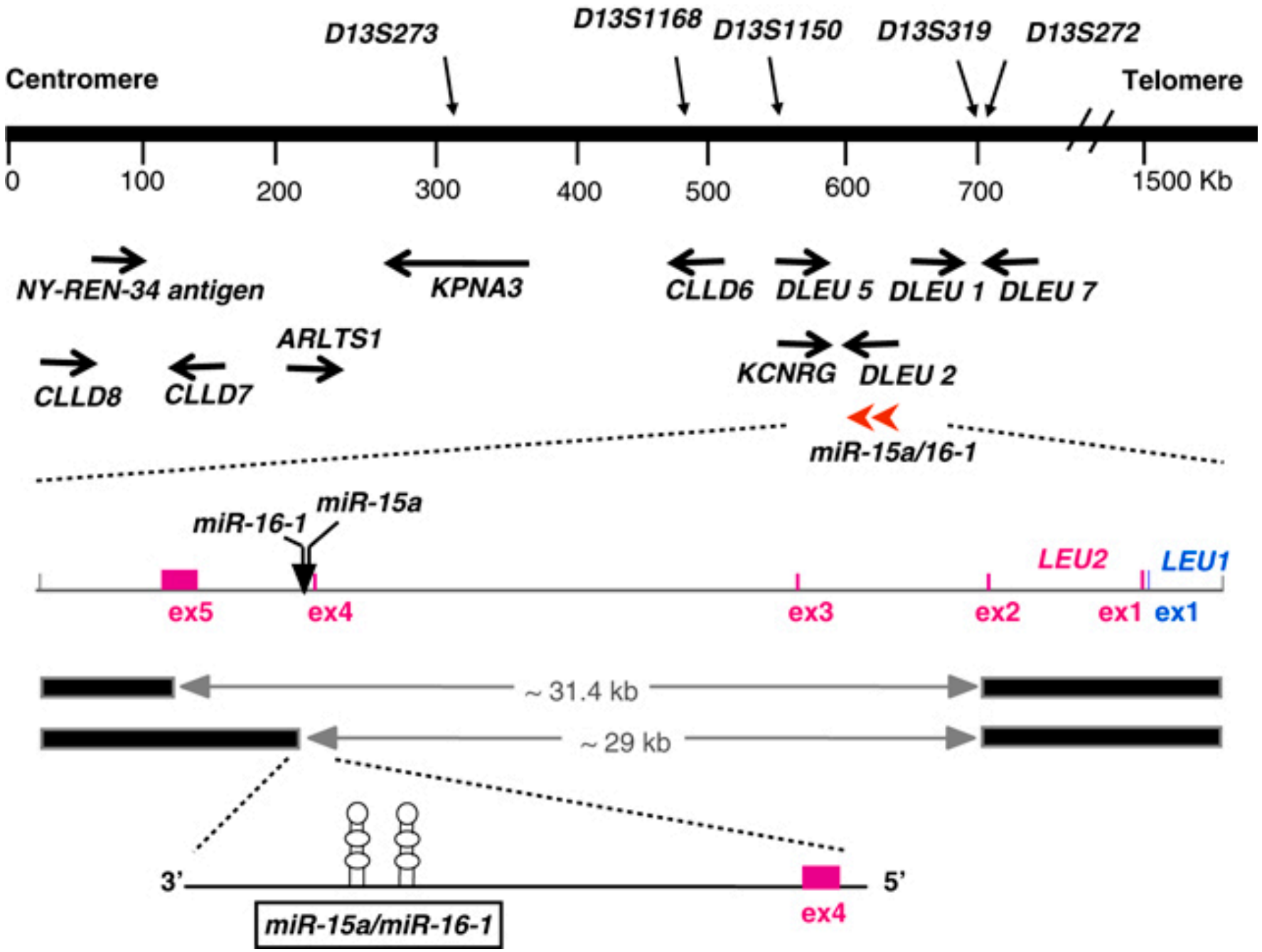
Migliazza et al, *Neoplasia,* 2001 (http://bloodjournal.hematologylibrary.org/content/97/7/2098/F1.expansion)

13q14 chromosomal region deleted in B-CLL



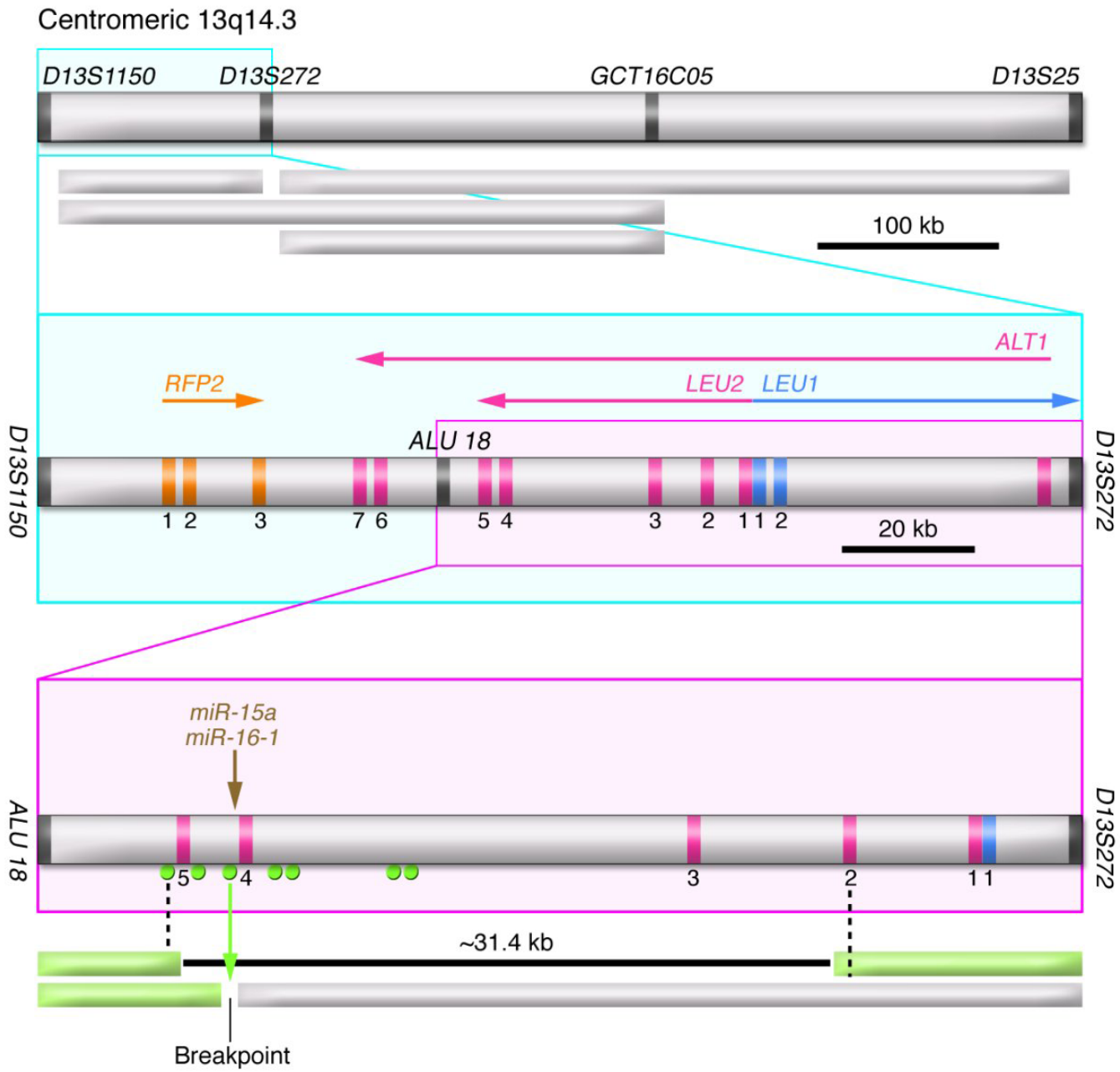
Schematic representation of the 13q14 chromosomal region deleted in B-CLL. (A) The chromosome 13 ideogram is shown with approximate location of reference markers used in previous studies for deletion analysis in B-CLL samples. Markers 140F11-82 and 138G4/1.3R border the MDR region of approximately 300 kb as defined previously,10 located between the retinoblastoma 1 gene locus (RB1) and the Wilson disease (WD) locus. (B) Distribution of genes in an approximate 600-kb region (between markers D13S273 and D13S25), spanning the MDR (indicated by an arrow): 3 transcribed sequences (CAR, 1B4/Leu2, and EST70/Leu1) and 3 pseudogenes (vL18, vp48, and vL34) were identified. The continuous line corre- sponds to the fully sequenced region of 347 503 bp; the dashed line indicates a region partially sequenced. (C) Row a. The region of approximate 347 kb completely sequenced and including the MDR is shown with various gene annotations: the gene exons (rectangles: empty, noncoding; filled, coding) and their relative positions along the MDR are indicated. The 2 pseudogenes located within the MDR are also shown. Rows b-c. Diagram of various mRNA splicing variants identified. The transcriptional start site as well as the transcriptional orientation for each candidate gene is indicated by an arrow. pA indicates the presence of a typical polyA [(A)n] addiction signal within the gene sequences. Row g. Gene names. Row h. Transcripts sizes of the detected mRNA for each gene.

Migliazza et al, *Neoplasia,* 2001 (http://bloodjournal.hematologylibrary.org/content/97/7/2098/F1.expansion)



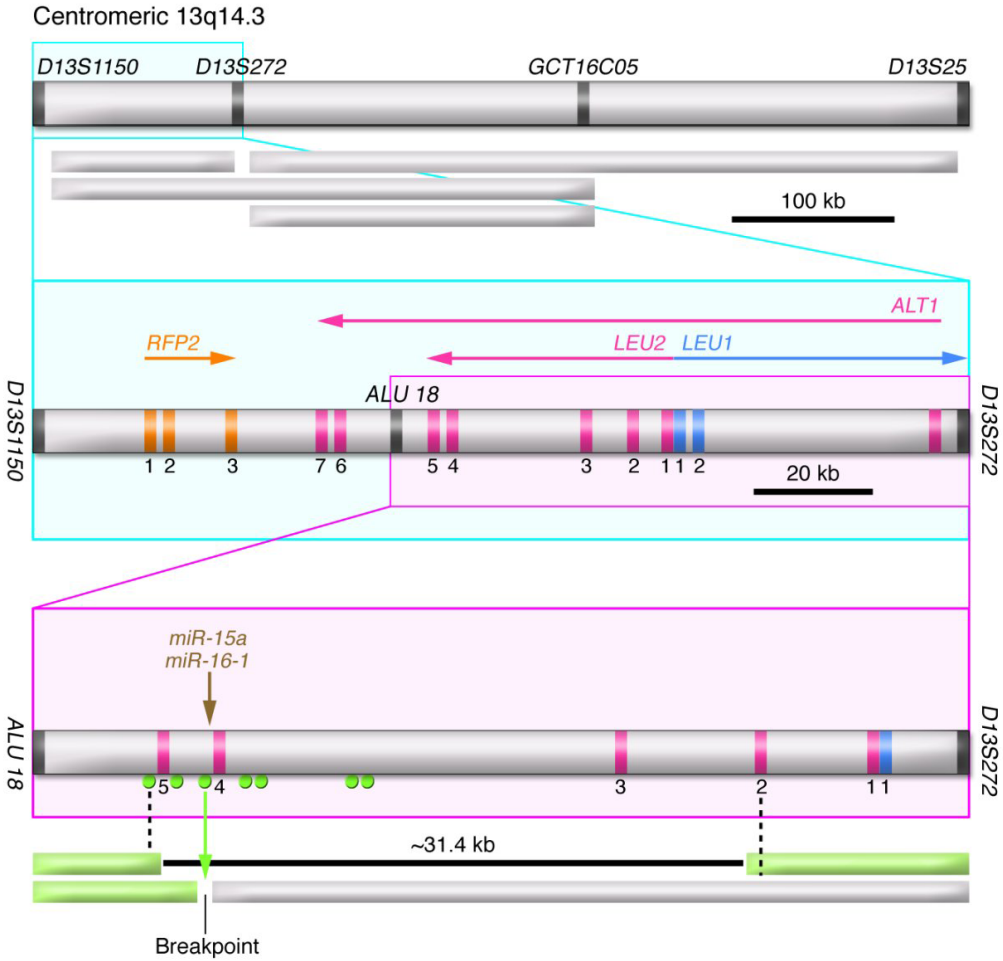
Aqeilan et al., *Cell Death Differentiation,* 2009. (http://www.nature.com/cdd/journal/v17/n2/fig_tab/cdd200969f1.html)

B-cell chronic lymphocytic leukemia, B-CLL



Calin and Croce, *JCI,* 2007 (http://www.jci.org/articles/view/32577/figure/2)

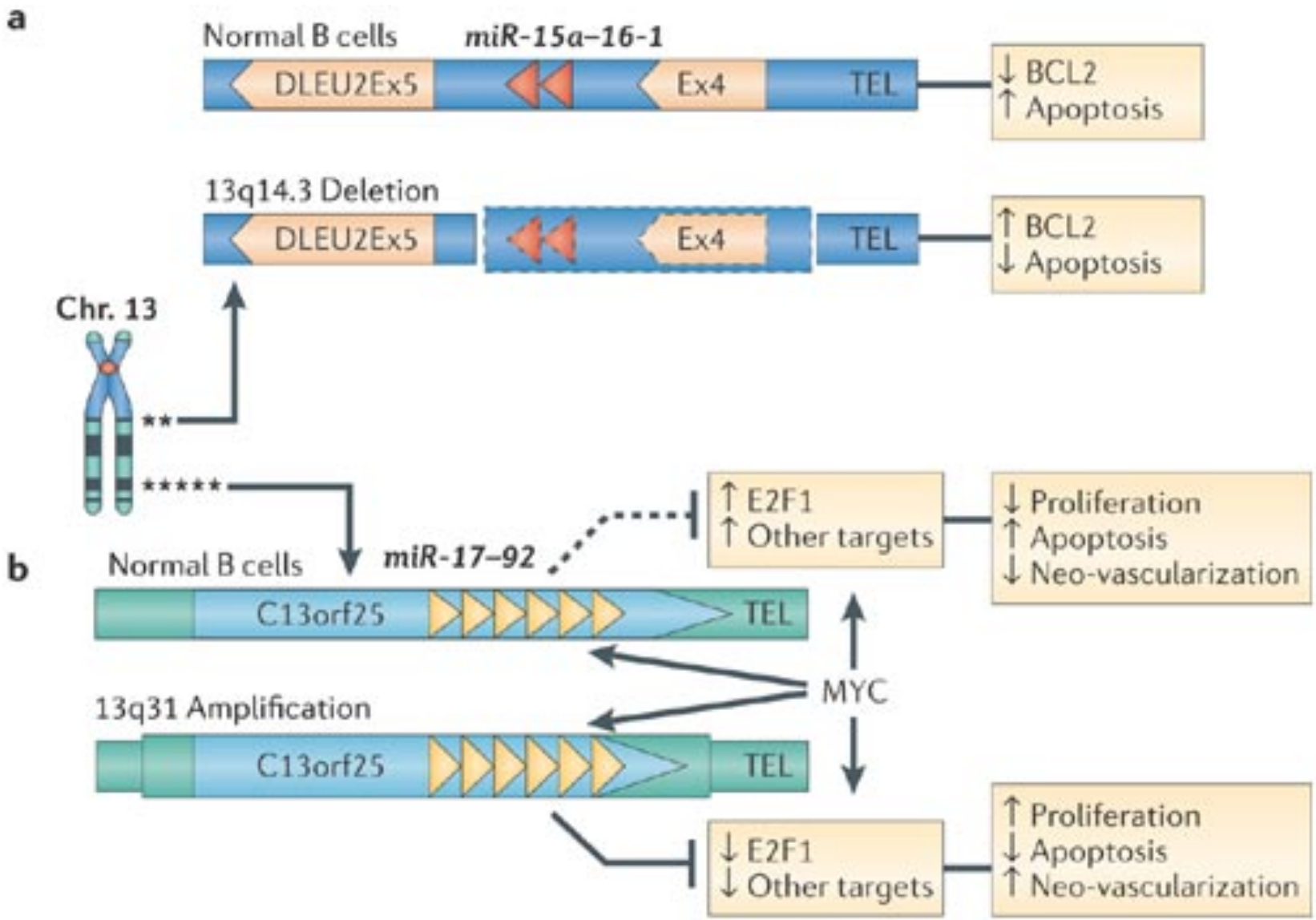
B-cell chronic lymphocytic leukemia, B-CLL



The t(2;13)(q32;q14) translocation involving miR-15a and miR-16-1 in indolent CLL. The genomic map of the 13q14.3 locus between ALU 18 and D13S272 markers is shown. Exons for LEU2/ALT1 and LEU1 are indicated by numbers. The brown arrow marks the position of miR-15a and miR-16-1 genes. Green circles mark the positions of PCR primers used to screen somatic cell hybrid clones derived from a fusion of two independent leukemia cases. The green arrow represents the position of the breakpoint in CLL carrying a t(2;13)(q32;q14) translocation. Green boxes represent portions of chromosome 13 present in the hybrids. The 31.4-kb deletion was present in a clone derived from a patient with CLL, bilateral retinoblas- toma, and ulcerative colitis. Figure modified from *Proceedings of the National Academy of Sciences of the United States of America* (32).

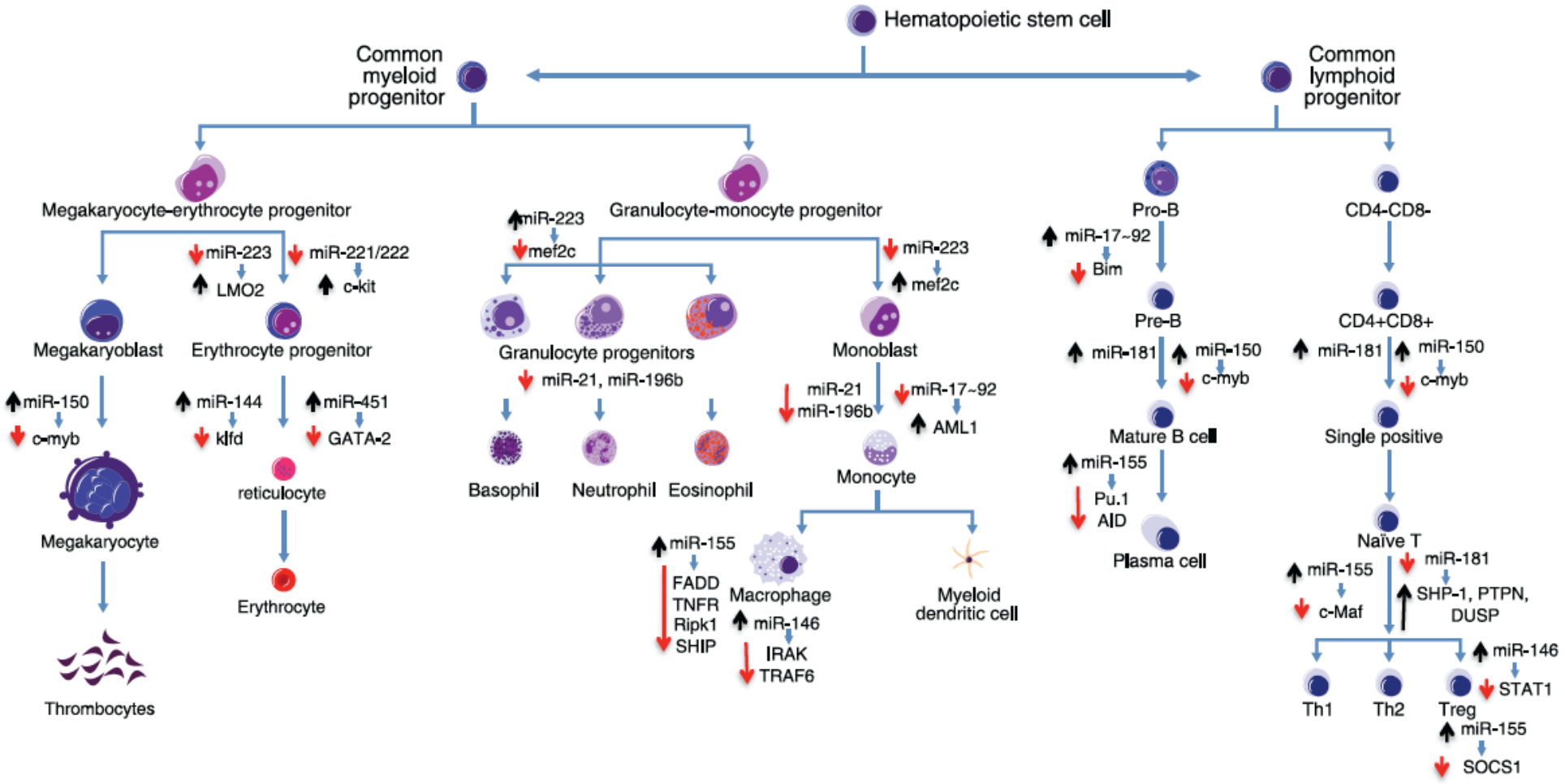
Calin and Croce, *JCI,* 2007 (http://www.jci.org/articles/view/32577/figure/2)

Chromosomal alterations at microRNA loci



Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

miRNAs in hematopoietic system development



Sayed and Abdellatif, *Physiol Rev* 2011

Oncogenic miRNAs, OncomiRs

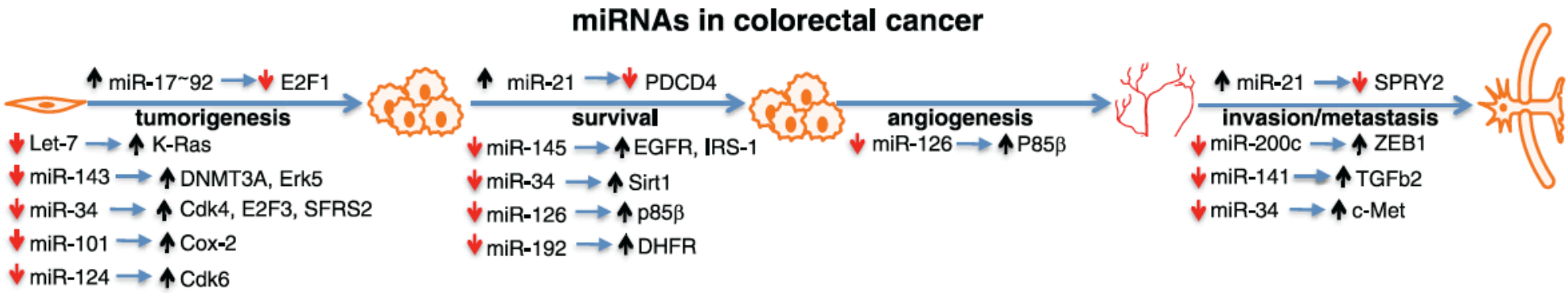
- **miRNAs that play crucial role in the initiation and progression of cancer**
- **act as tumor suppressors oncogenes**

OncomiRs

microRNAs	Tumorigenesis	Diagnosis	Prognosis
miR-9	Neuroblastoma		
miR-10b	Breast cancer		
miR-15, miR-15a	Leukemia, pituitary adenoma		
miR-16, miR-16-1	Leukemia, pituitary adenoma		
miR-17-5p, miR-17-92	Lung cancer, lymphoma		
miR-20a	Lymphoma, lung cancer		
miR-21	Breast cancer, cholangiocarcinoma, head & neck cancer, leukemia, cervical cancer		Pancreatic cancer
miR-29, miR-29b	Leukemia, cholangiocarcinoma		
miR-31	Colorectal cancer		
miR-34a	Pancreatic cancer		Neuroblastoma
miR-96	Colorectal cancer		
miR-98	Head & neck cancer		
miR-103	Pancreatic cancer		
miR-107	Leukemia, pancreatic cancer		
miR-125a, miR-125b	Neuroblastoma, breast cancer		
miR-128	Glioblastoma		
miR-133b	Colorectal cancer		
miR-135b	Colorectal cancer		
miR-143	Colon cancer, cervical cancer		
miR-145	Breast cancer, colorectal cancer		
miR-146	Thyroid carcinoma		
miR-155	Breast cancer, leukemia, pancreatic cancer		Lung cancer

Cho W, Mol Cancer 2007

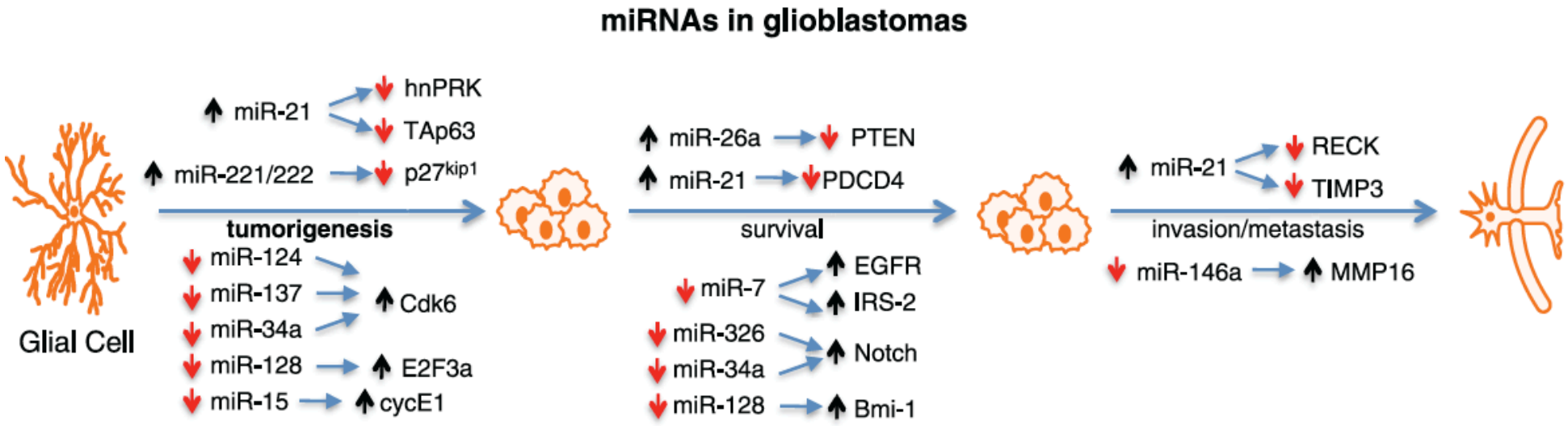
miRNAs in colorectal cancer



The diagram displays the different miRNAs and their targets that are involved in transformation, survival, angiogenesis, and invasion/metastasis of colorectal cancer. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: E2F transcription factor 1 (E2F1), K-Ras, DNA methyltransferase 3A (DNMT3A), mitogen-activated protein kinase 7 (Erk5), cyclin-dependent kinase 4 (Cdk4), E2F transcription factor 3 (E2F3), splicing factor, arginine/serine-rich 2 (SFRS2), Cox-2, cyclin-dependent kinase 6 (Cdk6), epidermal growth factor receptor (EGFR), insulin receptor substrate 1 (IRS-1), Sirtuin 1 (Sirt1), phosphoinositide-3-kinase, regulatory subunit 2 (beta) (p85"), dihydrofolate reductase (DHFR), zinc finger E-box binding homeobox 1 (ZEB1), transforming growth factor beta 2 (TGF"2), and hepatocyte growth factor receptor (c-Met).

Sayed and Abdellatif, *Physiol Rev* 2011

miRNAs in brain tumors

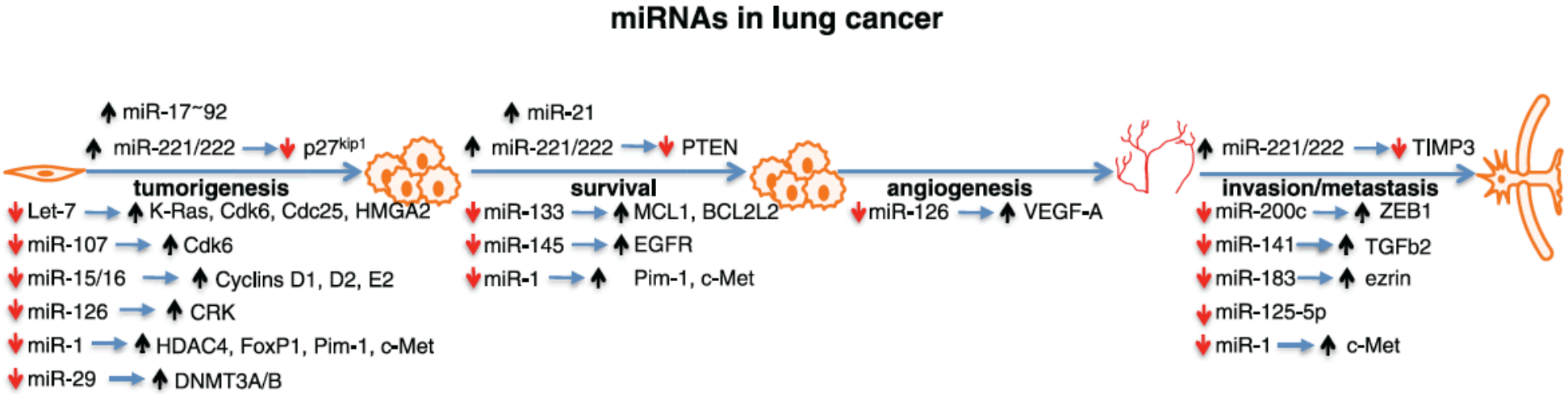


The diagram displays the different miRNAs and their targets that are involved in transformation, survival, and invasion/metastasis of glioblastomas.

Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: heterogeneous nuclear ribonucleoprotein K (hnRPK), tumor protein p63 (TAp63), cyclin-dependent kinase 6 (Cdk6), E2F transcription factor 3 (E2F3a), phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), epidermal growth factor receptor (EGFR), insulin receptor substrate 2 (IRS-2), BMI1 polycomb ring finger oncogene (Bmi-1), reversion-inducing-cysteine-rich protein with kazal motifs (RECK), tissue inhibitor of metalloproteinase 3 (TIMP3), and matrix metallopeptidase 16 (MMP16).

Sayed and Abdellatif, *Physiol Rev* 2011

miRNAs in lung cancer



The diagram displays the different miRNAs and their targets that are involved in transformation, survival, angiogenesis, and invasion/metastasis of lung cancer. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: cell division cycle 25 (Cdc25), cyclin-dependent kinase 6 (Cdk6), high mobility group AT-hook 2 (HMGA2), K-Ras, v-crk sarcoma virus CT10 oncogene homolog (CRK), histone deacetylase 4 (HDAC4), forkhead box P1 (FoxP1), proviral integration site 2 (Pim-1), hepatocyte growth factor receptor (c-Met), DNA methyltransferase 3A/B (DNMT3A/B), myeloid cell leukemia sequence 1 (MCL1), Bcl2-like 2 (BCL2L2), epidermal growth factor receptor (EGFR), vascular endothelial growth factor A (VEGF-A), zinc finger E-box binding homeobox 1 (ZEB1), and transforming growth factor beta 2 (TGFb2).

Sayed and Abdellatif, *Physiol Rev* 2011

miRNAs dysregulated in lung cancer

Table 1
Summary of the miRNAs discussed in the manuscript, with the identified translational implications of their dysregulation in cancer.

miRNA	Chromosome location	Type of deregulation	Lung cancer histotype	Lung cancer target	Clinical correlation	References
miR-155	21q21.3	↑	NSCLC		Distinguish between NSCLC and healthy control and screening test for adenocarc.	[5,9]
Let-7a	9q22.32 (Let-7a-1) 11q24.1 (Let-7a-2) 22q13.31 (Let-7a-3)	↓	NSCLC	KRAS	Distinguish between NSCLC and healthy control, non-invasive early detection of lung cancer and histological lung cancer sub-types	[5,6,11,14,23]
miR-29	7q32.3 (miR-29a, 29b) 1q32.2 (miR-29c)	↓	NSCLC	Dnmt3A Dnmt3B	Distinguish between NSCLC and healthy control	[7]
miR-23a	19p13.13	↑	NSCLC		Distinguish between NSCLC and healthy control	[8]
miR-205	1q32.2	↑	Squamous NSCLC		Distinguish between NSCLC and healthy control and histological lung cancer sub-types	[8,9,11,18]
miR-145-3p	5q32	↓	NSCLC		Distinguish between histological lung cancer sub-types	[11]
miR-210	11p15.5	↑	SCC		Distinguish between SCC and healthy control	[12]
miR-182	7q32.2	↑	SCC		Distinguish between SCC and healthy control	[12]
miR-486-5p	8p11.21	↓	NSCLC and SCC		Distinguish between NSCLC and healthy control, SCC and healthy control. Moreover, distinguish between different survival of NSCLC	[12,16,29]
miR-30a	6q13	↓	SCC		Distinguish between SCC and healthy control	[12]
miR-140-3p	16q22.1	↓	SCC		Distinguish between SCC and healthy control	[12]
miR-31	9p21.3	↑	SCC	DICER1	Distinguish between SCC and healthy control	[12]
Let-7e	19q13.41	↓	SCC		Distinguish between SCC and healthy control	[13]
miR-34a	1p36.22	↓	SCC		Distinguish between SCC and healthy control	[13]
miR-34c-5p	11q23.1	↓	SCC		Distinguish between SCC and healthy control	[13]

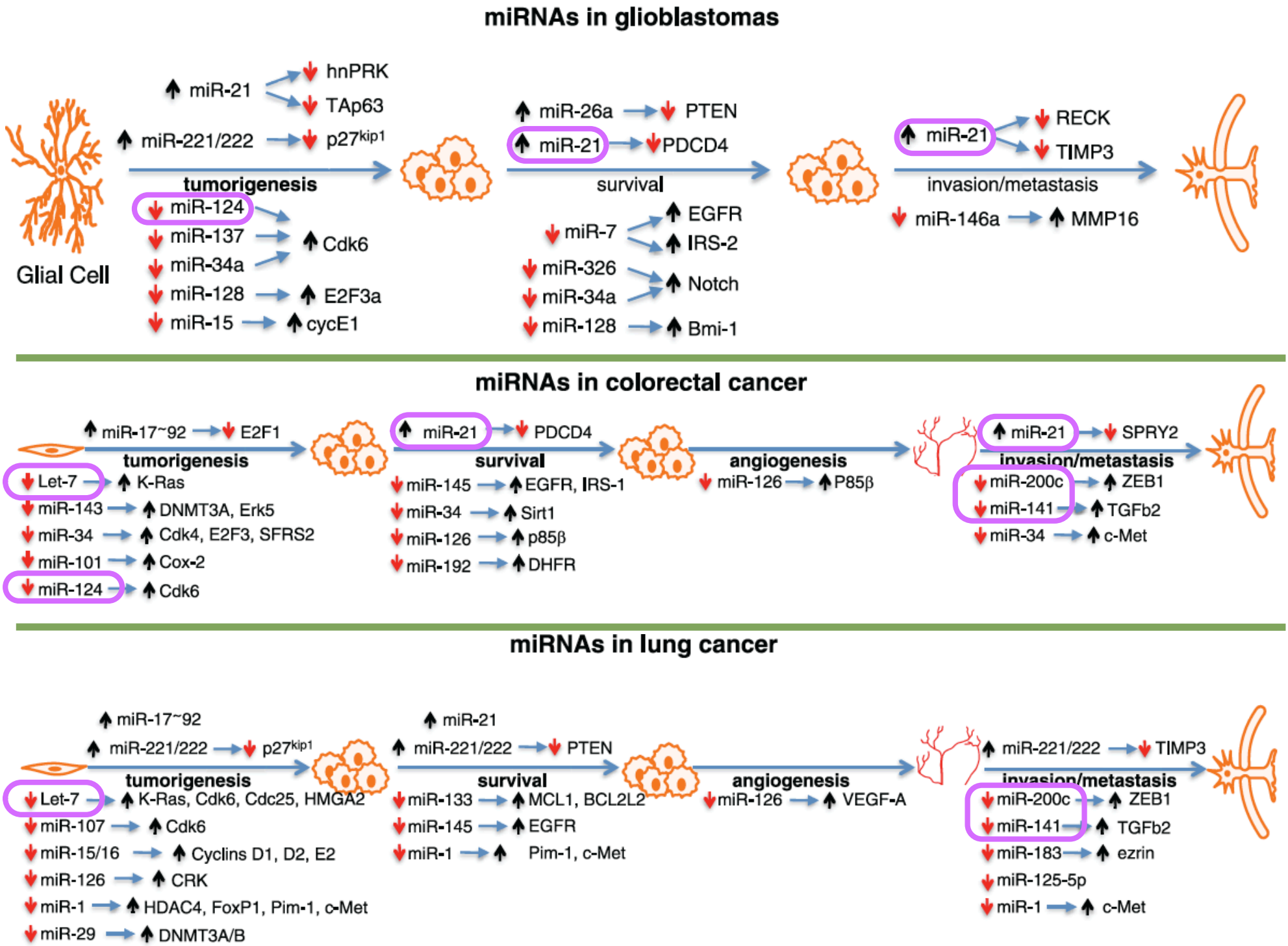
Vannini et al., *Clin Biochem* 2013

miRNAs dysregulated in lung cancer

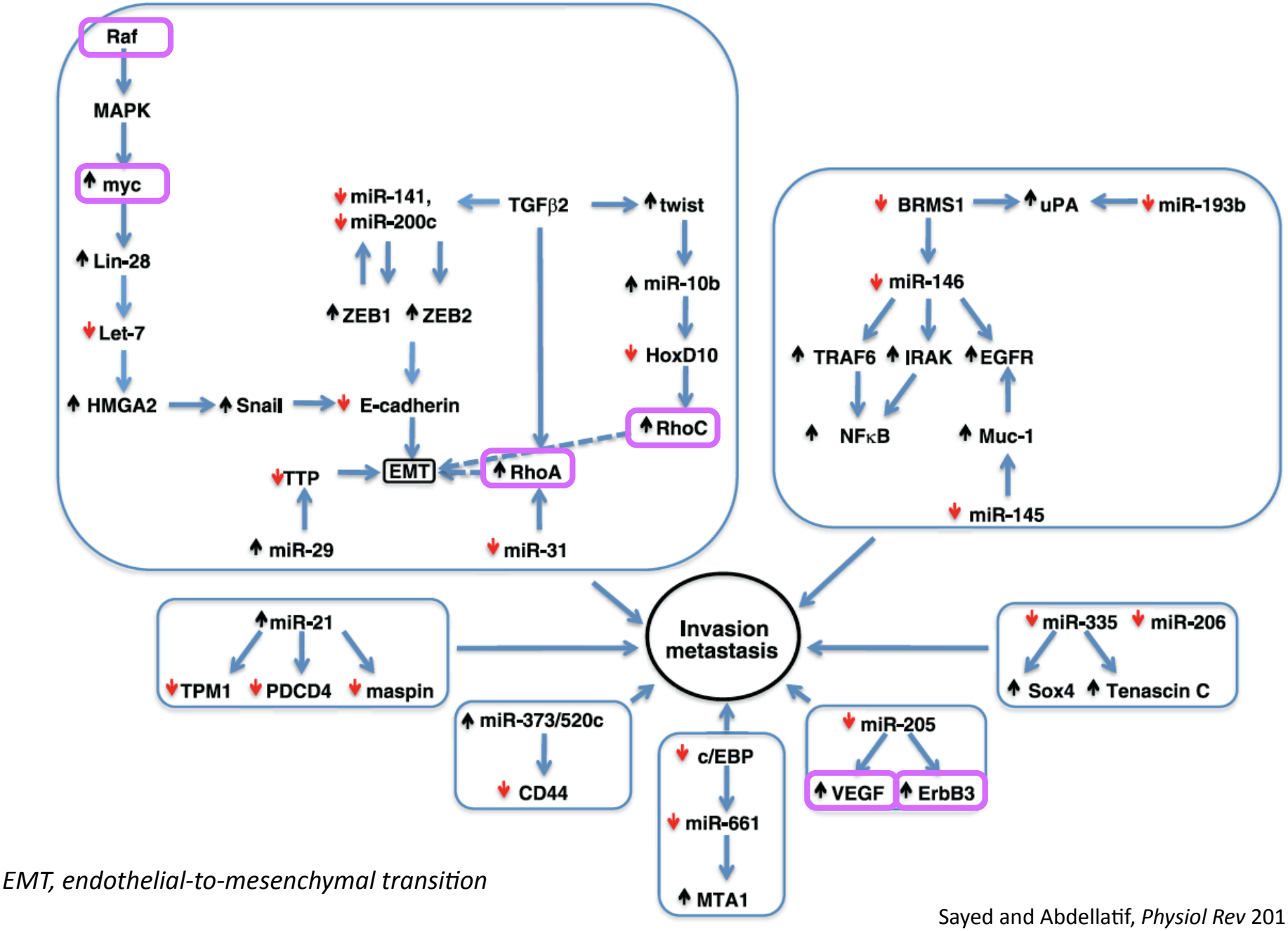
Table 1
Summary of the miRNAs discussed in the manuscript, with the identified translational implications of their dysregulation in cancer.

miRNA	Chromosome location	Type of deregulation	Lung cancer histotype	Lung cancer target	Clinical correlation	References
miR-25	7q22.1	↓	SCC		Distinguish between SCC and healthy control	[13]
miR-191	3p21.31	↓	SCC		Distinguish between SCC and healthy control	[13]
miR-191	3p21.31	↑	NSCLC		Screening test for adenocarc.	[9]
miR-30d	8q24.22	↓	NSCLC		Distinguish between different survival of NSCLC	[16]
miR-499	20q11.22	↓	NSCLC		Distinguish between different survival of NSCLC	[16]
miR-374a	Xq13.2	↓	NSCLC		Prognosis of poor survival	[20]
miR-1254	10q21.3 (miR-1254-1) 10 (miR-1254-2)	↑	NSCLC		Serum-based biomarkers for diagnostic evaluation	[28]
miR-574-5p	4	↑	NSCLC		Serum-based biomarkers for diagnostic evaluation	[28]
miR-21	17q23.1	↑	NSCLC		Distinguish between NSCLC and healthy control	[29,31,36]
miR-210	11p15.5	↑	NSCLC		Distinguish between NSCLC and healthy control	[29,31]
miR-126	9q34.3	↓	NSCLC		Distinguish between NSCLC and healthy control	[29]
miR-17-3p	13q31.3	↑	NSCLC		Screening test for adenocarc.	[9]
miR-106a	Xq26.2	↑	NSCLC		Screening test for adenocarc.	[9]
miR-146	5q34 (miR-146a) 10q24.32 (miR-146b)	↑	NSCLC		Screening test for adenocarc.	[9]
miR-192	11q13.1	↑	NSCLC		Screening test for adenocarc.	[9]
miR-203	14q32.33	↑	NSCLC		Screening test for adenocarc.	[9]
miR-212	17p13.3	↑	NSCLC		Screening test for adenocarc.	[9]
miR-214	1q24.3	↑	NSCLC		Screening test for adenocarc.	[9]
miR-128b	3p22.3	↓	NSCLC	EGFR	Clinical response and survival following TKI treatment	[34]
miR-221	Xp11.3		NSCLC	PTEN	Response to TRAIL treatment	[38]
miR-222				TIMP3		

Vannini et al., *Clin Biochem* 2013


miRNAs in glioblastomas
Glial Cell
miR-21
hnPRK
TAp63
miR-221/222
p27kip1
tumorigenesis
miR-124
miR-137
miR-34a
Cdk6
miR-128
E2F3a
miR-15
cycE1
miR-26a
PTEN
miR-21
PDCD4
survival
miR-7
EGFR
IRS-2
miR-326
miR-34a
Notch
miR-128
Bmi-1
miR-21
RECK
TIMP3
invasion/metastasis
miR-146a
MMP16
miRNAs in colorectal cancer
miR-17~92
E2F1
tumorigenesis
Let-7
K-Ras
miR-143
DNMT3A, Erk5
miR-34
Cdk4, E2F3, SFRS2
miR-101
Cox-2
miR-124
Cdk6
miR-21
PDCD4
survival
miR-145
EGFR, IRS-1
miR-34
Sirt1
miR-126
p85β
miR-192
DHFR
angiogenesis
miR-126
P85β
miR-21
SPRY2
invasion/metastasis
miR-200c
ZEB1
miR-141
TGFb2
miR-34
c-Met
miRNAs in lung cancer
miR-17~92
miR-221/222
p27kip1
tumorigenesis
Let-7
K-Ras, Cdk6, Cdc25, HMGA2
miR-107
Cdk6
miR-15/16
Cyclins D1, D2, E2
miR-126
CRK
miR-1
HDAC4, FoxP1, Pim-1, c-Met
miR-29
DNMT3A/B
miR-21
miR-221/222
PTEN
survival
miR-133
MCL1, BCL2L2
miR-145
EGFR
miR-1
Pim-1, c-Met
angiogenesis
miR-126
VEGF-A
miR-221/222
TIMP3
invasion/metastasis
miR-200c
ZEB1
miR-141
TGFb2
miR-183
ezrin
miR-125-5p
miR-1
c-Met

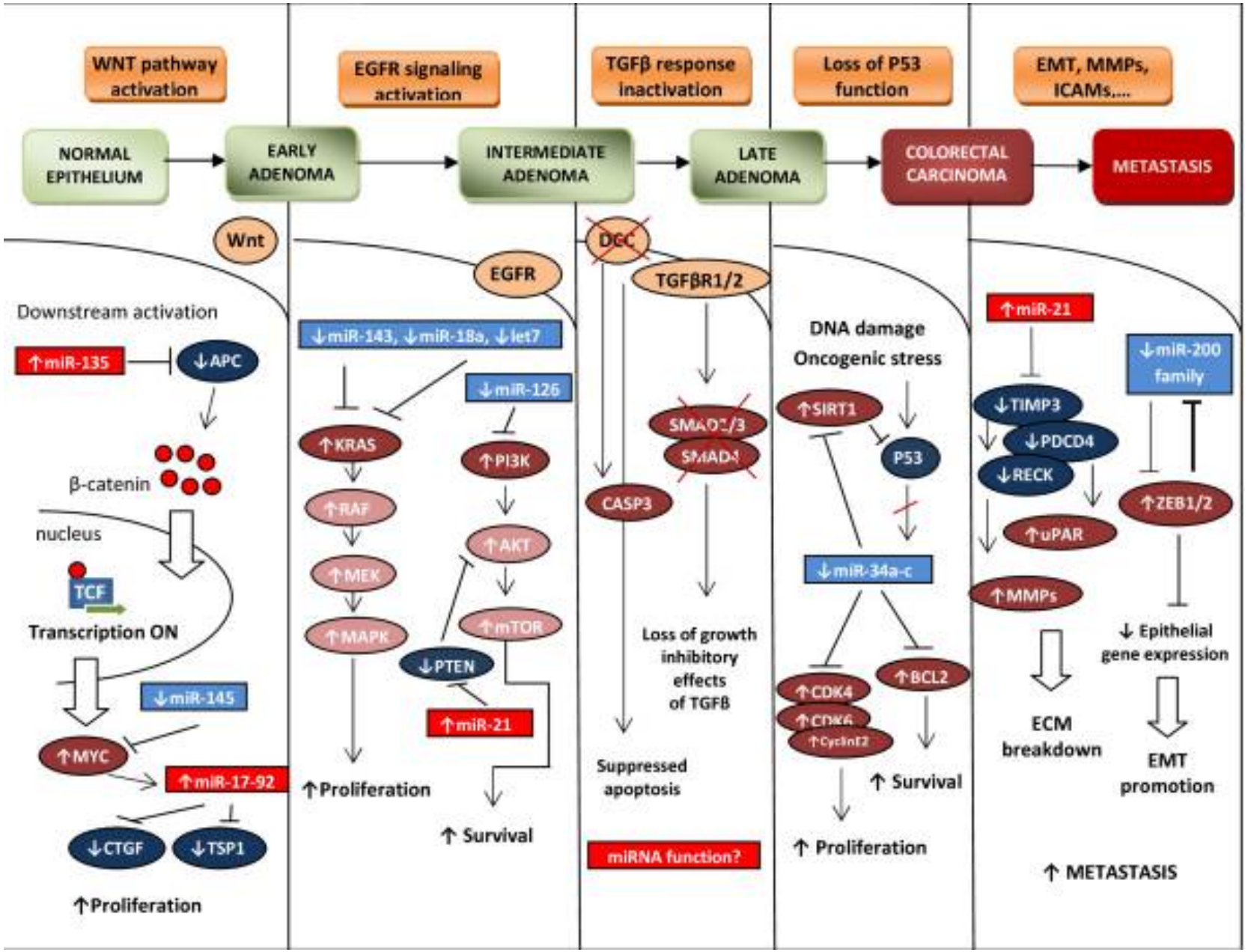
miRNAs in breast cancer cell invasion and metastasis



EMT, endothelial-to-mesenchymal transition

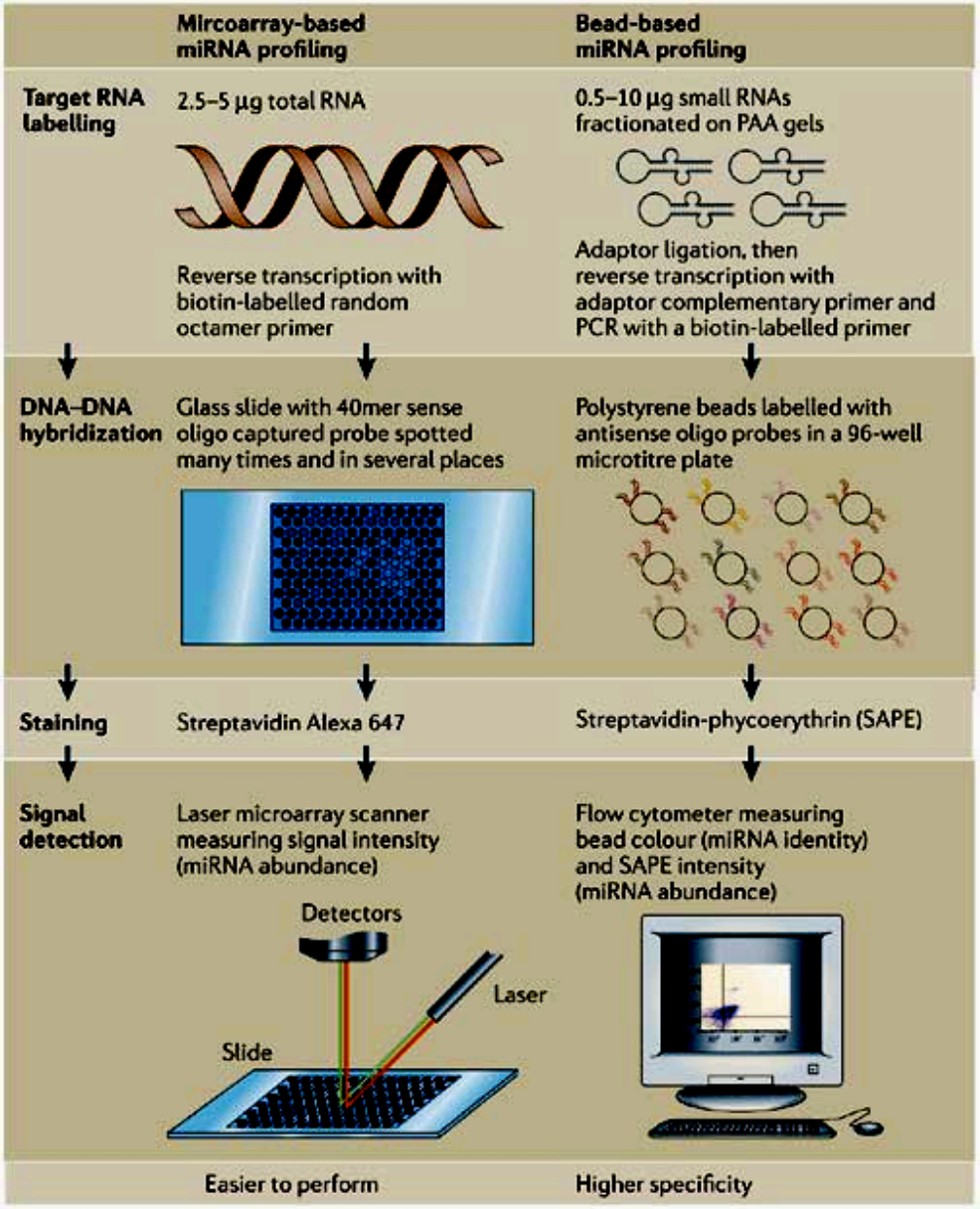
Sayed and Abdellatif, *Physiol Rev* 2011

Vogelstein's model for role of miRs in colorectal cancer pathogenesis



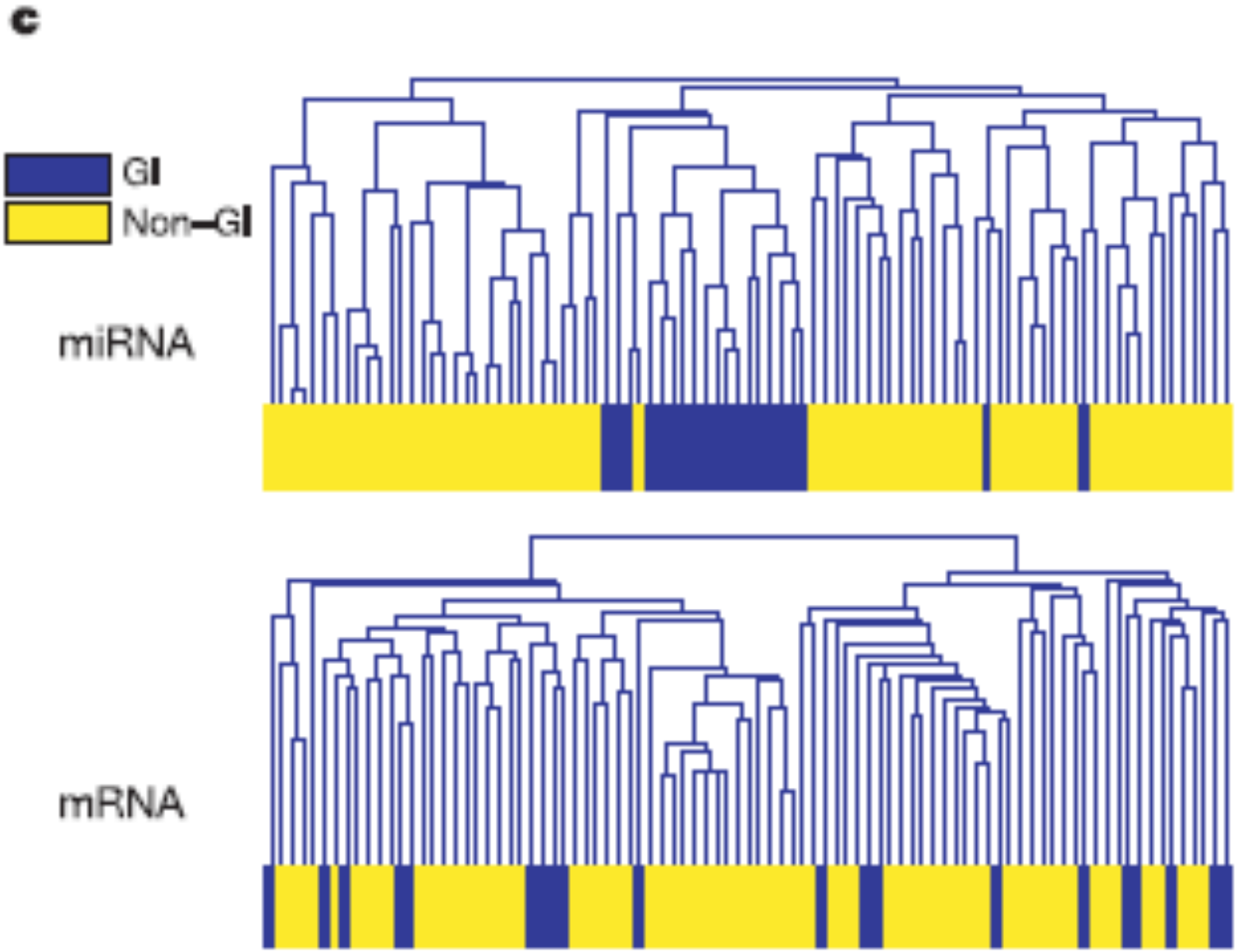
Slaby et al. Mol Cancer 2009; 8: 102-102.

RNA diagnostics - OncomiRs



Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

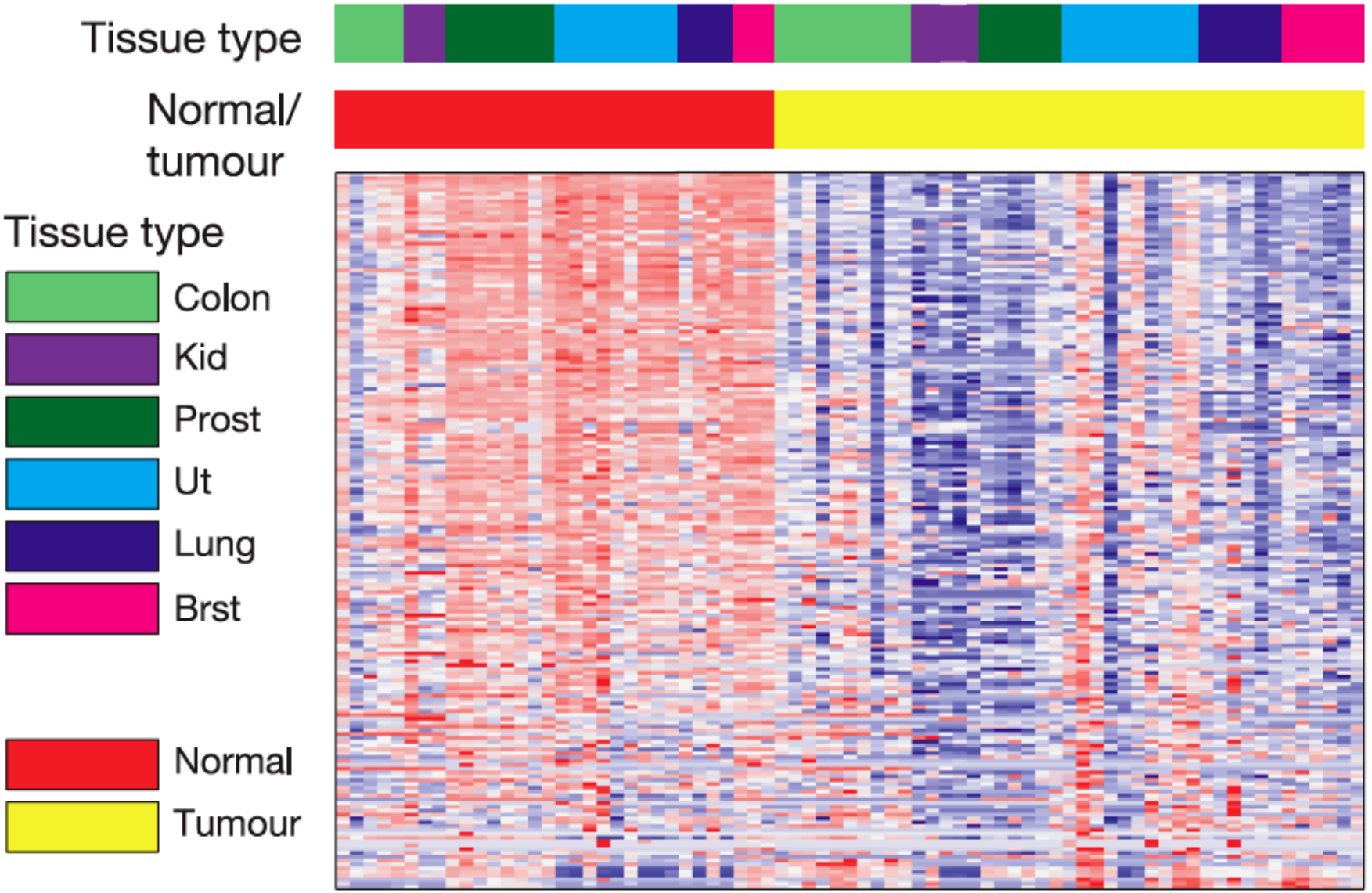
miRNA vs mRNA profiling



GI: Gastrointestinal Tract

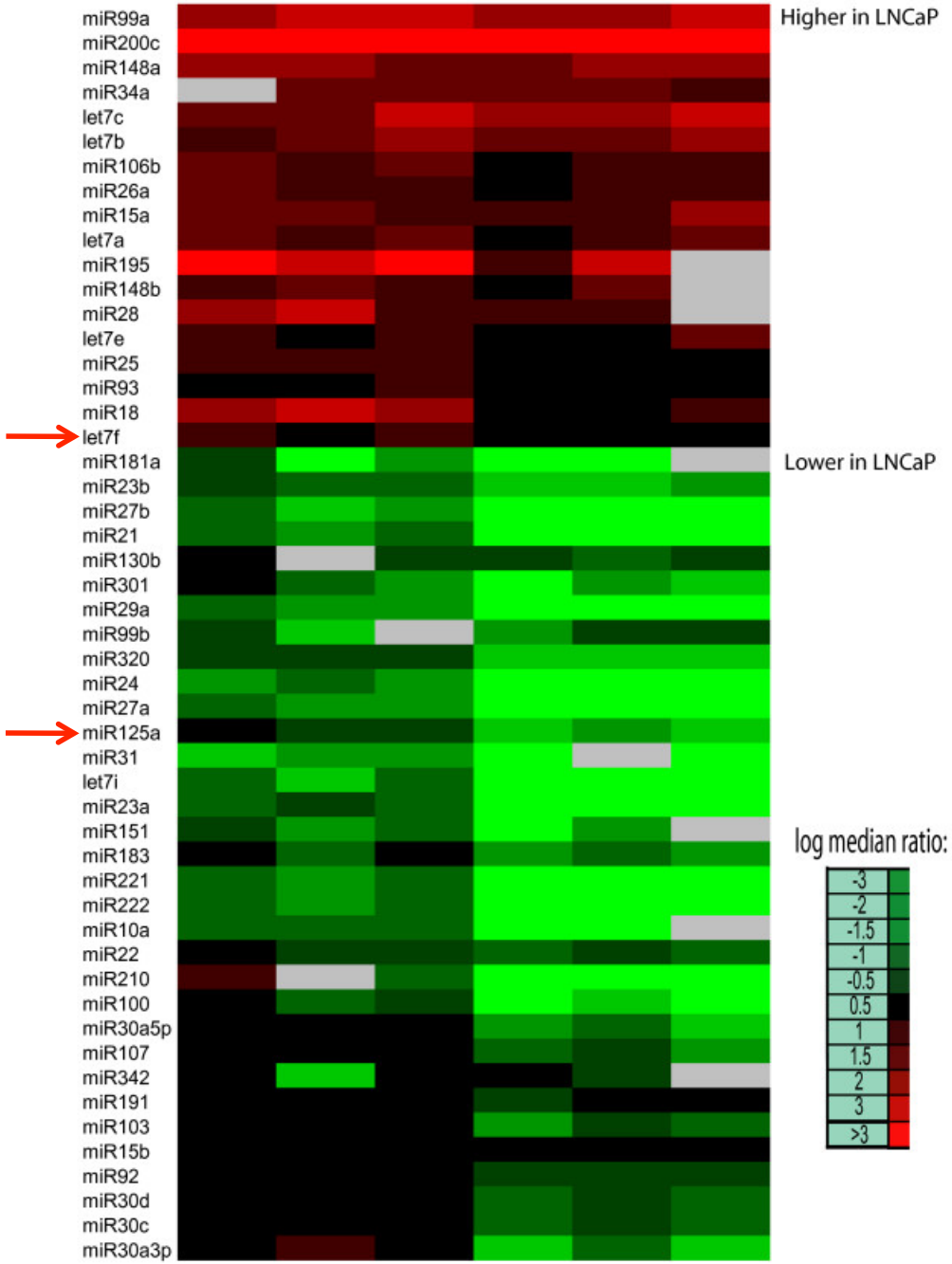
Lu J et al., Nature 435:834-838, 2005

miRNA expression profile classify human cancers



Lu J et al., Nature 435:834-838, 2005

miRNAs and breast cancer – profiling data



→: HER2-acossiated
(*let7f, let7g, miR107, miR10b, miR125a, miR125b, miR126, miR154 and miR195)*

Mattie MD, Mol Cancer 2006

microRNA-expression profiling in human cancers

Cancer type*	MiRNA profiling data	Significance	Refs
Chronic lymphocytic leukaemia	A unique signature of 13 genes associated with prognostic factors (ZAP70 and IgVH mutation status) and progression (time from diagnosis to therapy)	MiRNAs as diagnostic markers (the identification of two categories of patients)	49,35
Lung adenocarcinoma	Molecular signatures that differ with tumour histology; miRNA profiles correlated with survival (*miR-155* and *let-7*)	MiRNAs as prognostic and diagnostic markers	53
Breast carcinoma	MiRNA expression correlates with specific pathological features	MiRNAs as prognostic markers	50
Endocrine pancreatic tumours	A signature that distinguishes endocrine from acinar tumours; the overexpression of *miR-21* is strongly associated with both a high Ki67 proliferation index and the presence of liver metastases	MiRNAs as diagnostic and prognostic markers	54
Hepatocellular carcinoma	MiRNA expression correlated with differentiation	MiRNAs as prognostic markers	52
Papillary thyroid carcinoma	MiRNA upregulation (for example, *miR-221* and *miR-222*) in tumoral cells and normal cells adjacent to tumours, but not in normal thyroids without cancers	MiRNAs probably involved in cancer initiation	37 114
Glioblastoma	A specific signature compared with normal tissues	MiRNAs as diagnostic markers	51
Human cancers	MiRNA-expression profiles accurately classify cancers; an miRNA classifier classes poorly differentiated samples better than a messenger RNA classifier	MiRNAs as diagnostic markers	41
Human solid cancers	Common signature for distinct types of solid carcinomas	Specific miRNAs are involved in common molecular pathways	47

*Only data from microarray studies reporting results on human primary tumours were included in this table. IgV_H, immunoglobulin heavy-chain variable-region, MiRNA, microRNA. ZAP70, 70 kDa zeta-associated protein.

Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

miRNA expression profiles as diagnostic and prognostic markers of lung cancer

Expression of let-7 miRNA

Frequently reduced in human lung cancers
Reduced shorter postoperative survival.

***let-7 miRNA* overexpression in A549 lung adenocarcinoma cells**

inhibited lung cancer cell growth *in vitro.*

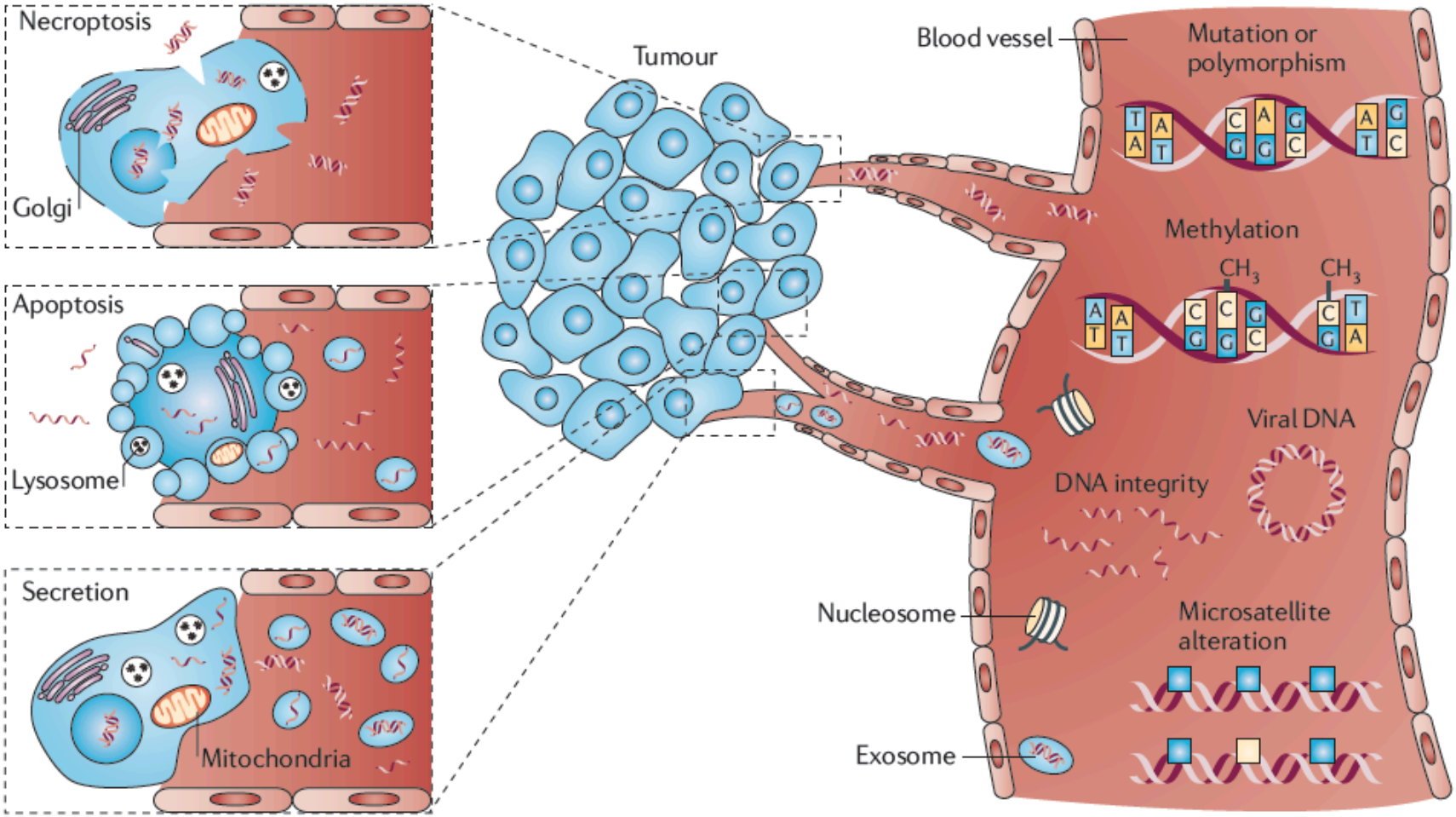
Takamizawa *et al. Cancer Res 2004*

miRNA expression profiles discriminate lung cancers from noncancerous lung tissues

Molecular signatures that differed in tumor histology
High *hsamiR-155 and low hsa-let-7a-2 precursor miRNA expression*
correlated with poor survival of lung adenocarcinomas

Yanaihara *et al. Cancer Cell 2006*

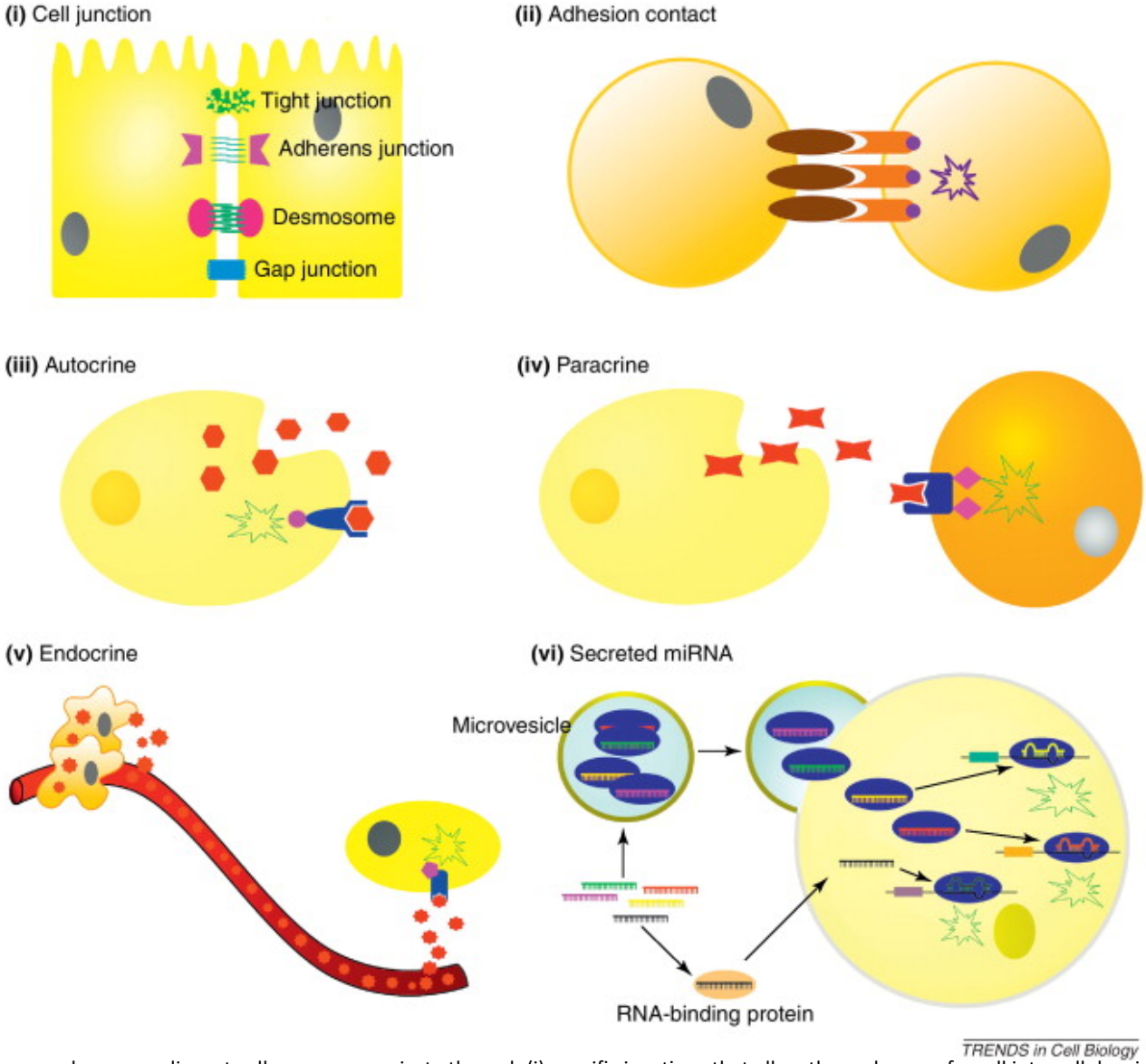
Cell-free nucleic acids in the blood



Mutations, methylation, DNA integrity, microsatellite alterations and viral DNA can be detected in cell-free DNA (cfDNA) in blood. Tumour-related cfDNA, which circulates in the blood of cancer patients, is released by tumour cells in different forms and at different levels. DNA can be shed as both single-stranded and double-stranded DNA. The release of DNA from tumour cells can be through various cell physiological events such as apoptosis, necrosis and secretion. The physiology and rate of release is still not well understood; tumour burden and tumour cell proliferation rate may have a substantial role in these events. Individual tumour types can release more than one form of cfDNA.

Schwarzenbach et al. *Nature Reviews Cancer* 2011; 11: 426.

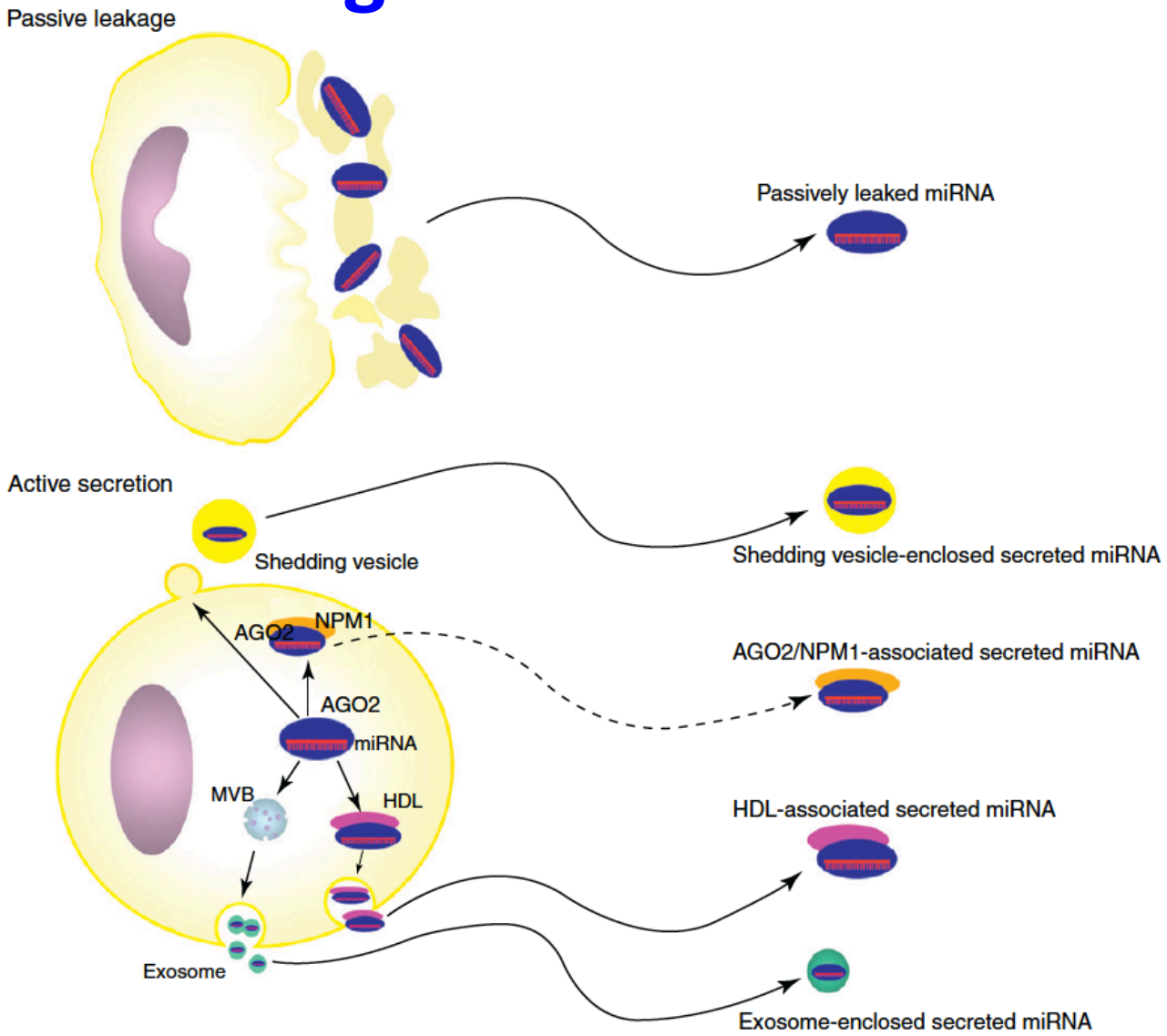
Secreted miRNA-mediated gene regulatory network as a novel form of intercellular communication



Cells can communicate by several means: adjacent cells can communicate through (i) specific junctions that allow the exchange of small intracellular signaling molecules or (ii) direct adhesion contacts between a membrane-bound signaling molecule on one cell and a receptor on the surface of another cell. Cells also can communicate via soluble messengers, such as hormones, cytokines and chemokines, which may act (iii) on the original cells (autocrine action) or (iv) on adjacent cells (paracrine action) or (v) travel long distances through intercellular nanotubes to affect target cells (endocrine action). In addition to these methods, (vi) secreted miRNA-mediated gene regulatory networks represent another type of intercellular communication in which a group of specific miRNAs can be transferred to target cells via microvesicles or RNA-binding proteins. These exogenous miRNAs can then activate myriad signaling events in the recipient cells by modulating expression of their target genes.

Chen et al., *Trends in Cell Biology*, 2012; 22: 125

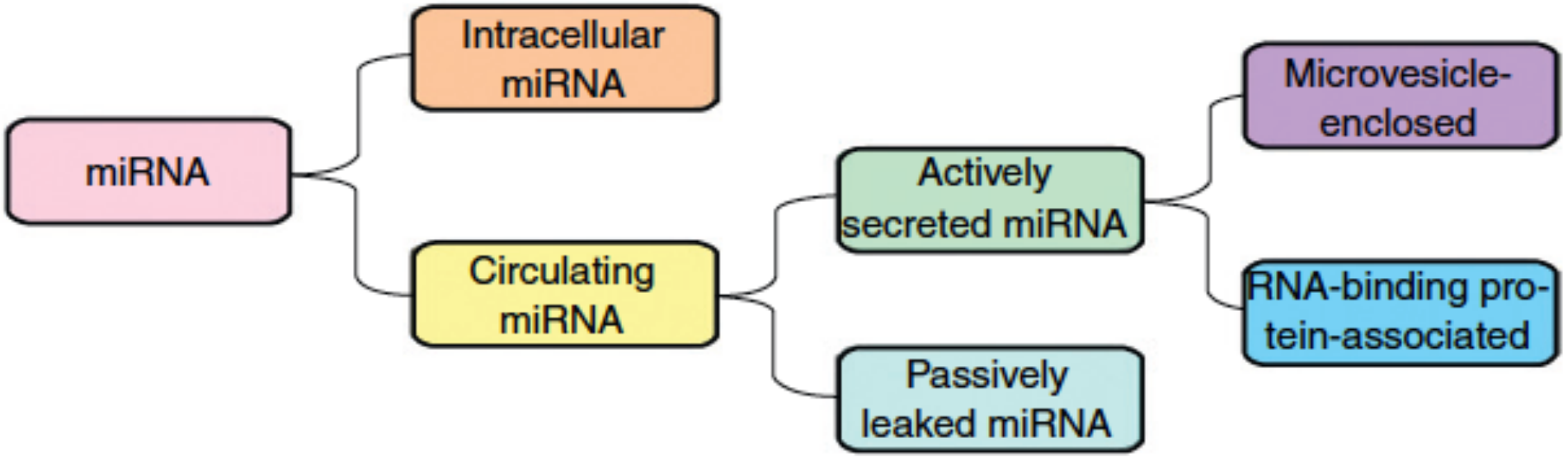
Circulating vs. secreted miRNAs



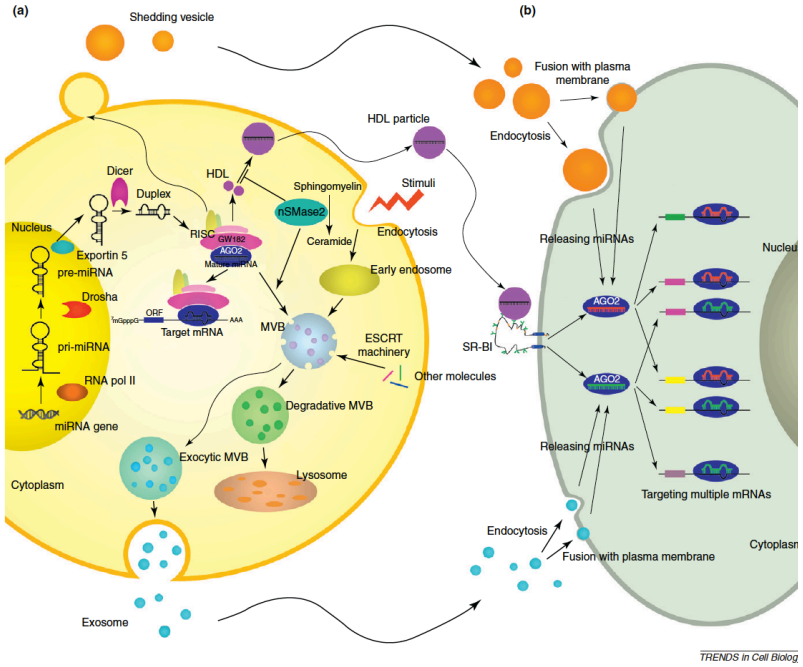
miRNAs can enter the circulation through three pathways: (i) passive leakage from broken cells; (ii) active secretion via microvesicles, including exosomes and shedding vesicles; and (iii) active secretion in conjunction with the RNA-binding protein high-density lipoprotein (HDL). Other RNA-binding proteins, including Argonaute2 (AGO2) and nucleophosmin 1 (NPM1), are found to bind circulating miRNAs; however, whether AGO2- or NPM1-bound miRNAs are actively released from cells and can be taken up by recipient cells is currently unclear. miRNA secretion via microvesicles and HDL is active and energy dependent, and this is the key characteristic that distinguishes secreted miRNAs from passively leaked miRNAs.

Chen et al., *Trends in Cell Biology*, 2012; 22: 125

Circulating vs. secreted miRNAs



Chen et al., *Trends in Cell Biology*, 2012; 22: 125



Schematic description of the sorting and release of secreted miRNAs. After being transcribed in the nucleus, exported to the cytoplasm and processed into a mature form, miRNAs can bind to complementary sequences on target mRNAs to repress translation or trigger mRNA cleavage. They can also be packaged and transported to the extracellular environment via three different pathways. (i) The generation of exosomal miRNAs requires ceramide production on the cytosolic side by neutral sphingomyelinase 2 (nSMase2), and other molecules that are targeted to lysosomes depend on the endosomal sorting complex required for transport (ESCRT) machinery. Thus, a ceramide-dependent, ESCRT-independent pathway may control the incorporation of miRNAs into exosomes. Furthermore, exosomes may deliver cellular components of the RNA-induced silencing complex (RISC), such as GW182 and Argonaute2 (AGO2), to enhance the biological function of the secreted miRNAs. After fusion of multivesicular bodies (MVBs) with the plasma membrane, exosomal miRNAs are released into the circulation accompanying the release of exosomes. (ii) Shedding vesicles are formed by the process of blebbing or shedding from the plasma membrane. However, it is currently unknown how miRNAs are shed at the cell surface. (iii) miRNA inside the donor cell can be stably exported in conjunction with RNA- binding proteins, such as high-density lipoprotein (HDL). nSMase2 represses cellular export of miRNAs to HDL. (b) Schematic description of the uptake of secreted miRNAs in recipient cells. Exosomes and shedding vesicles can donate their miRNAs to recipient cells by the process of endocytosis, phagocytosis or direct fusion with the plasma membrane. HDL-associated miRNAs are taken up by recipient cells through binding to scavenger receptor class B type I (SR-BI) receptors present at the recipient cellular membrane. Because one miRNA can target numerous mRNAs and numerous miRNAs can target one mRNA simultaneously, secreted miRNAs may function in networks that form a complex system regulating myriad signaling events in the target cells.

Chen et al., *Trends in Cell Biology*, 2012; 22: 125

Radiolabeling small RNA with technetium-99m for visualizing cellular delivery and mouse biodistribution

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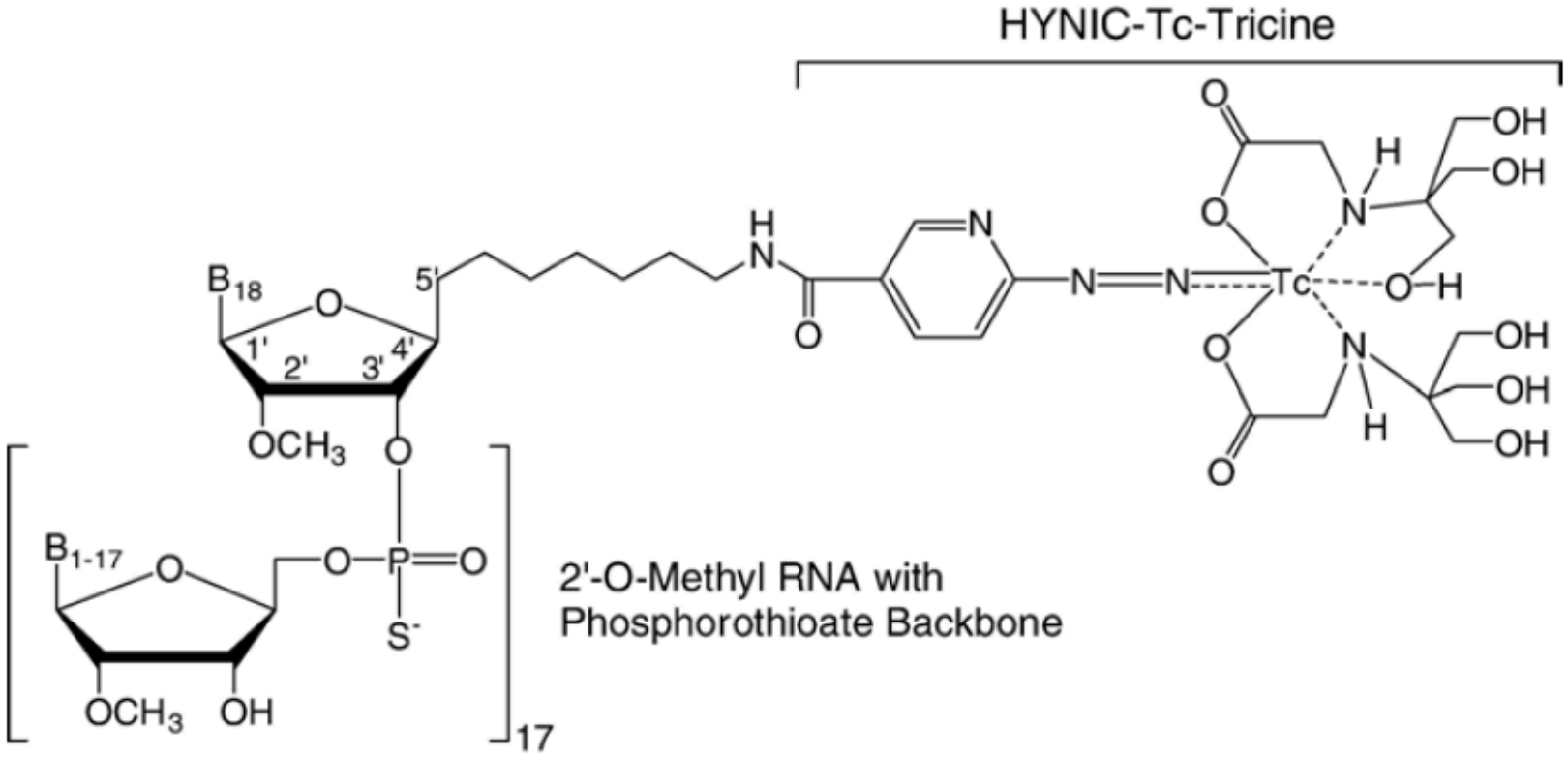
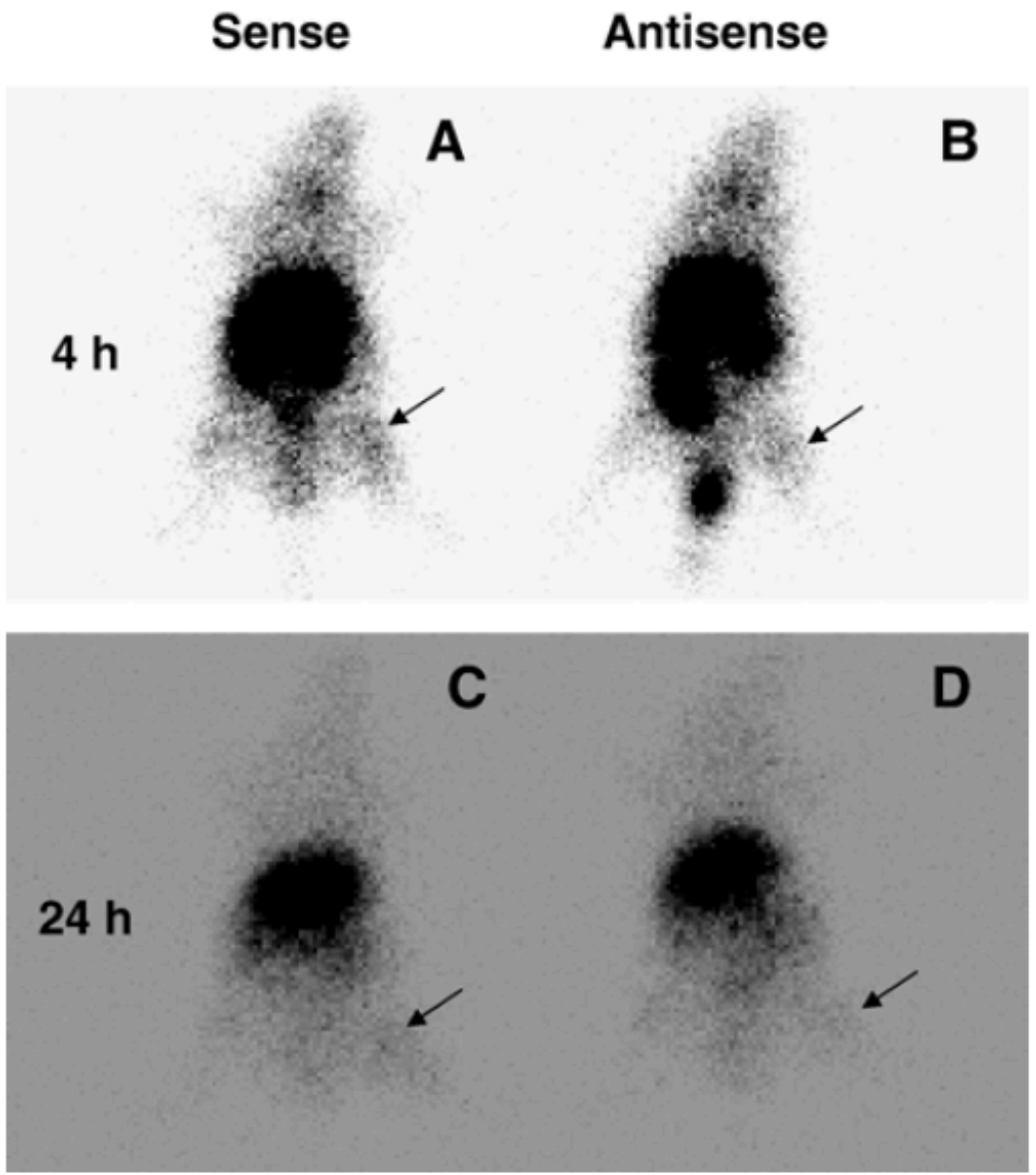
Nuclear Medicine and Biology 34 (2007) 399–404



Biodistribution of ^{99m}Tc-labeled miRNAs



In conclusion, using ^{99m}Tc radiolabeling, the delivery of small RNAs could be measured quantitatively in cultured cells and could be noninvasively visualized in living animals using a gamma camera.

Liu et al., *Nucl Med Biol* 2007; 34: 399

Proto-Oncogene ⟶ Oncogene

3. Gene translocation:

Example: *BCR* and *ABL* genes

Translocation

chromosome 9 and chromosome 22

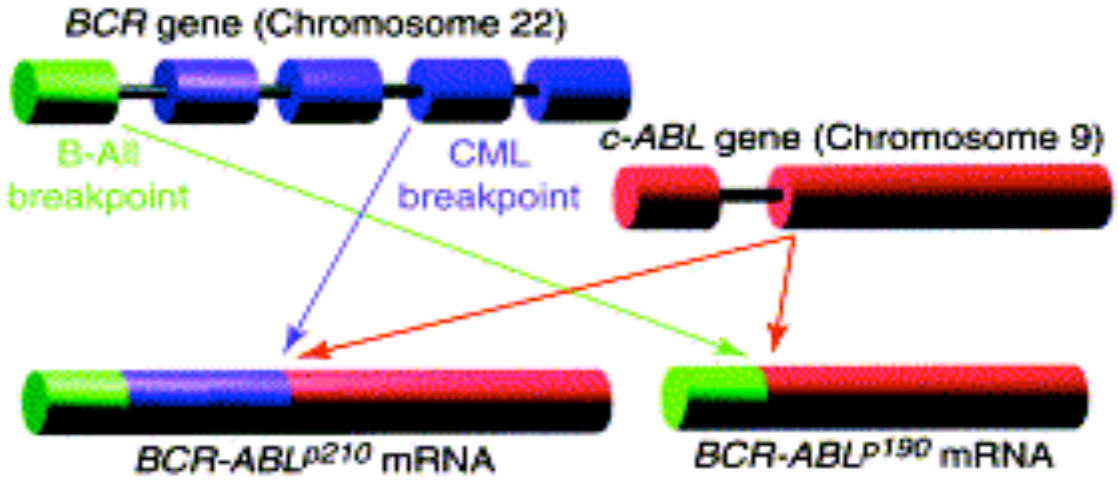
t(9;22)(q28;q11)

↓

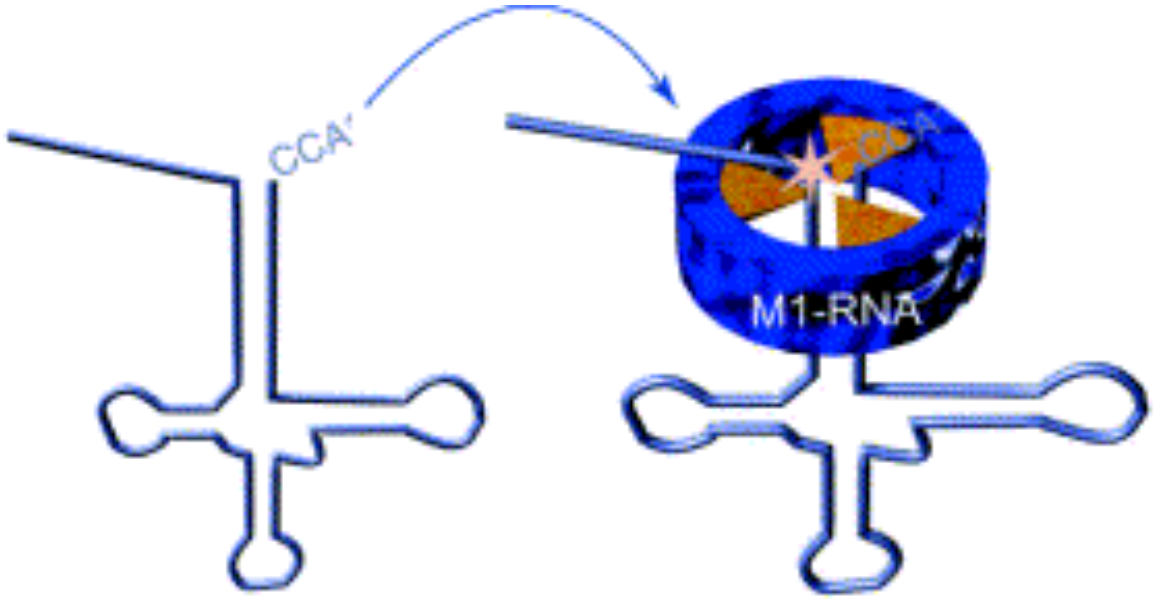
abnormal expression

↓

Chronic myelogenous leukemia



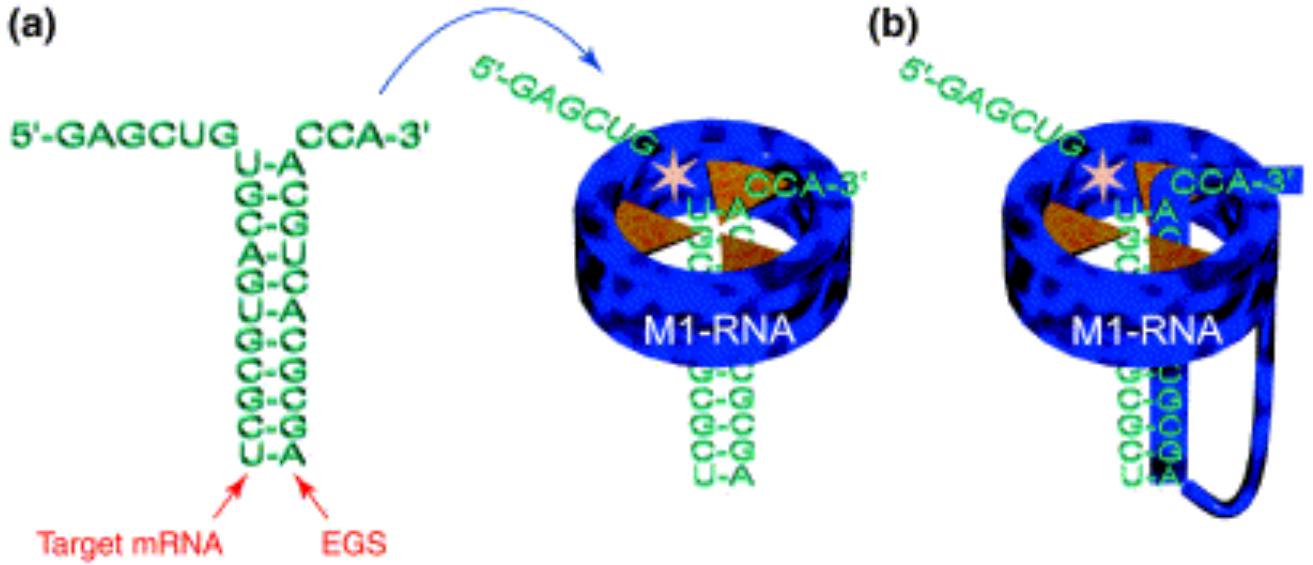
RNaseP and M1 RNA



Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

Fig. 1. Biological function of M1 RNA from *Escherichia coli* RNase P. Pre-tRNA (left) is the natural target of RNase P, which recognizes the double-stranded RNA structure of the pre-tRNA T-stem and the 3′ CCA unpaired stretch, and cleaves the 5′ leader to release the mature tRNA.

RNaseP, M1 RNA and (E)GS/IGS technology



Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

Fig. 2. M1 RNA as a gene therapy tool.
(a) Use of a external guide sequence (EGS) to anneal with the mRNA and to mimic the structure of M1 RNA natural target, by producing a 3′ CCA unpaired tail, a doublestranded RNA region and a 5′ leader-like region in the strand that has to be cut after interaction with the ribozyme. The cleavage site in the target mRNA is between U7 and G8.
(b) Structure of a M1 RNA with an internal guide sequence (IGS) covalently linked to its 3′ end, joined to its target substrate and providing all the necessary structural requirements to cleave it at the desired point.

chimeric oncogenic mRNA suppression by IGS technology

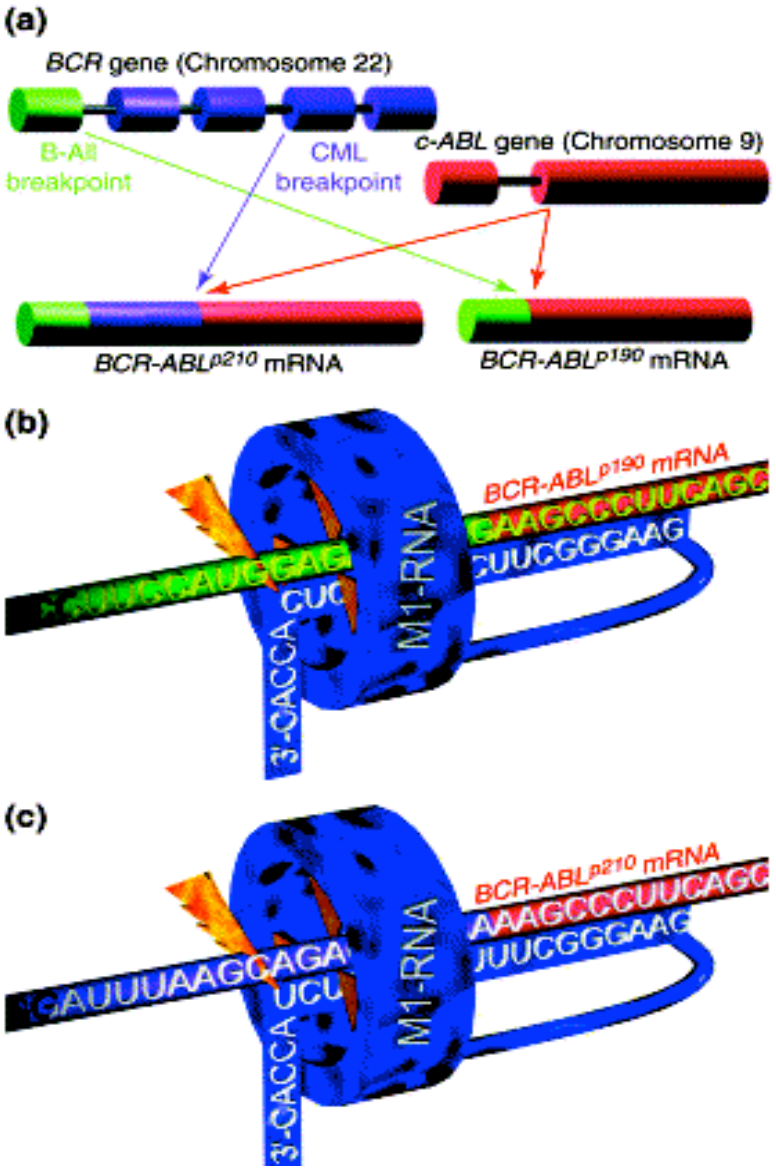


Fig. 3. Destruction of chimeric oncogenes by M1 RNA with an internal guide sequence (M1-RNA-IGS).
(a) The products of the t(9;22)(q28;q11) translocation. Different oncogenes can arise depending on the precise breakpoints in the BCRgene. Two are shown: BCR-ABLp190, which is associated with B-acute lymphoblastic leukaemias (B-ALL) and BCR-ABLp210, which produces chronic myelogenous leukaemia (CML).
They differ in the part coming from BCRgene but share the same region of ABLgene.
(b) The structure of the anti- BCR-ABLp190 M1-RNA-IGS. This is bound to the BCR-ABLp190 oncogene mRNA at its fusion region by base-pairing with the IGS. The point in which the target is cut by the ribozyme is shown. (c) The structure of anti- BCR-ABLp210 M1-RNA-IGS. This is bound to the BCR-ABLp210 oncogene mRNA at its fusion region by base-pairing with the IGS. The point in which the target is cut by the ribozyme is shown.

Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

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