REPÚBLICA PORTUGUESA
SAÚDE

Masters in Biochemistry and Biomedicine
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Oncobiology research–

Investigating the role of RAC1B in colorectal cancer

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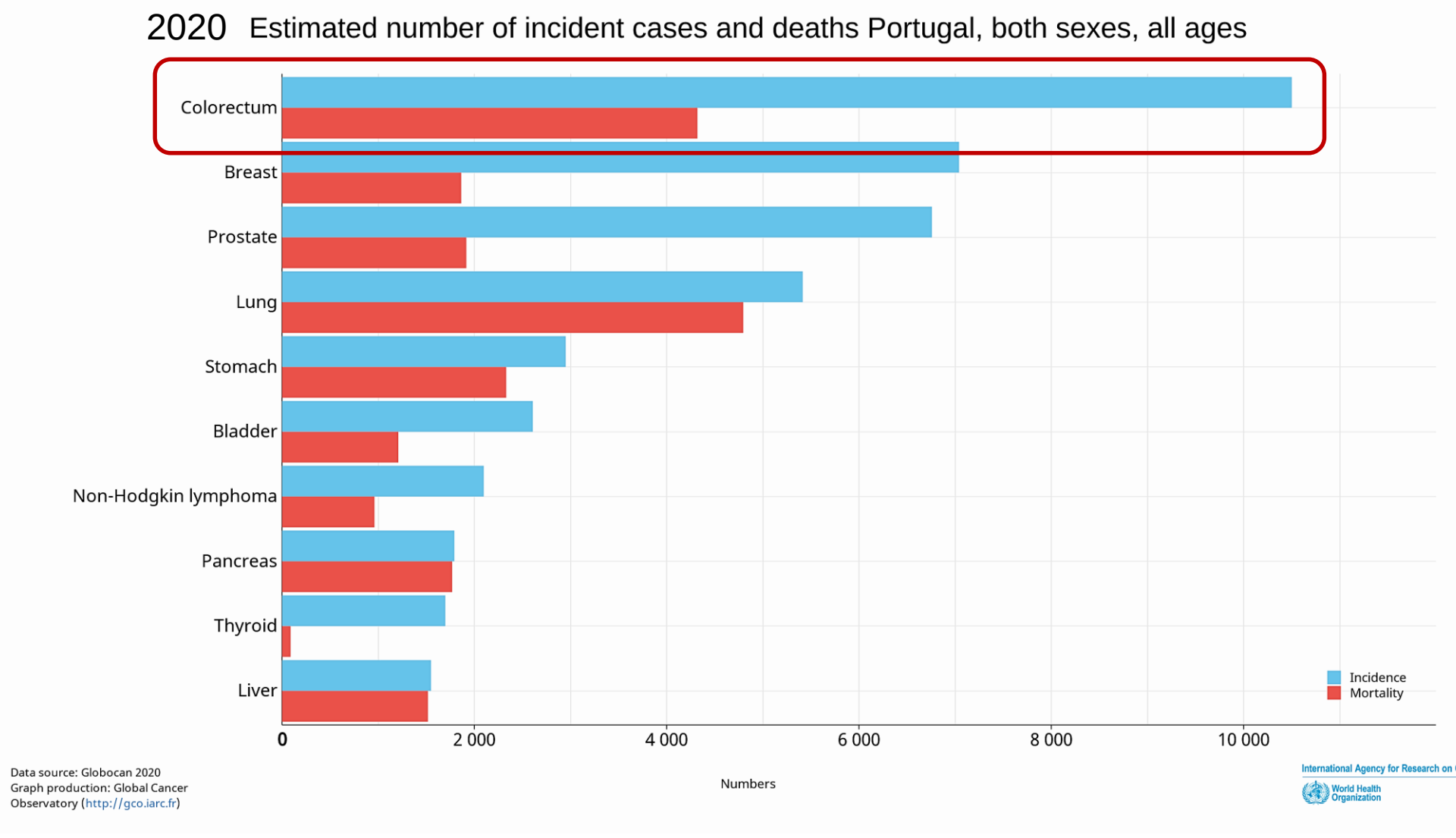
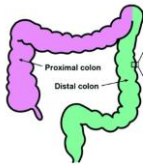


https://gco.iarc.fr/today/online-analysis-multi-bars?v=2020&mode=cancer&mode_population=countries&population=900&populations=620&key=total&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=10&group_cancer=1&include_nmsc=1&include_nmsc_other=1&type_multiple=%257B%2522inc%2522%253Atrue%252C%2522mort%2522%253Atrue%252C%2522prev%2522%253Afalse%257D&orientation=horizontal&type_sort=0&type_nb_items=%257B%2522top%2522%253Atrue%252C%2522bottom%2522%253Afalse%257D

colorectal cancer is not one disease,

but presents as

genetically distinct subtypes



Location in colon

distal

proximal

Polyp of origin

adenomatous

serrated

Karyotype

instable (CIN)

stable

DNA sequence stability

MSS/MSI-L

MSI-H

CpG island methylation

negative or CIMP-L

CIMP-H

KRAS/BRAF mutation

wt

KRAS

BRAF

0

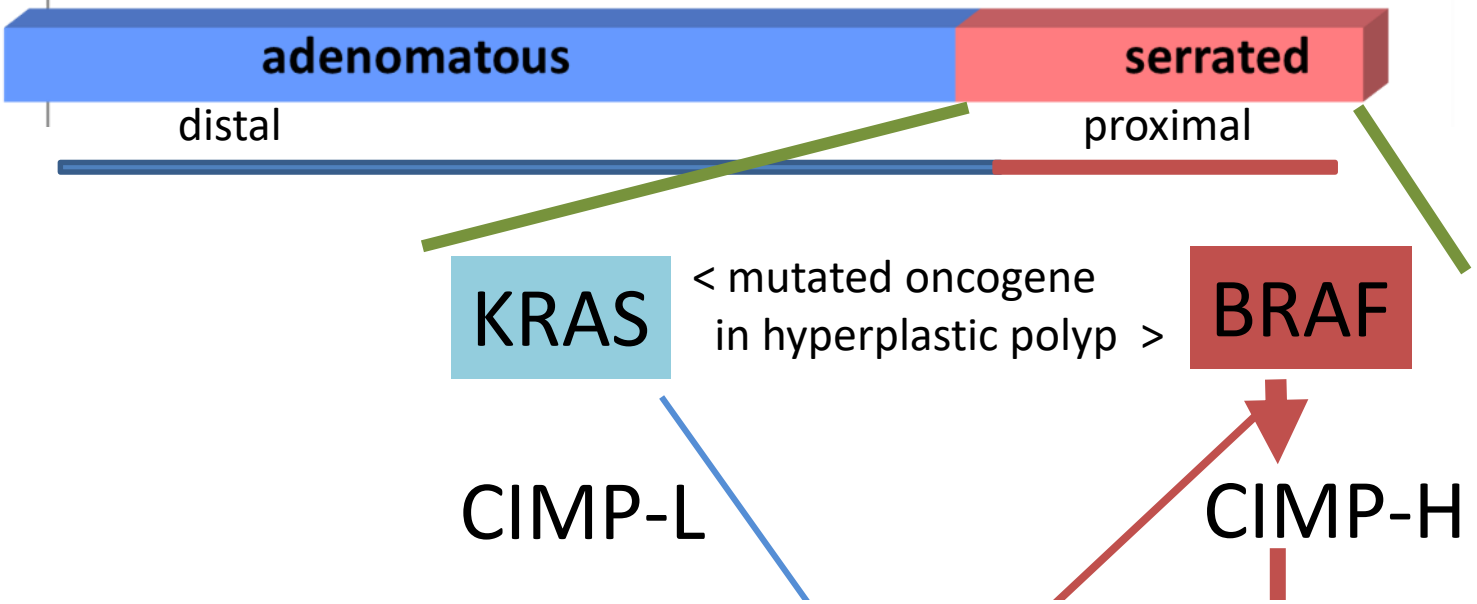
50

100

% of sporadic CRC cases

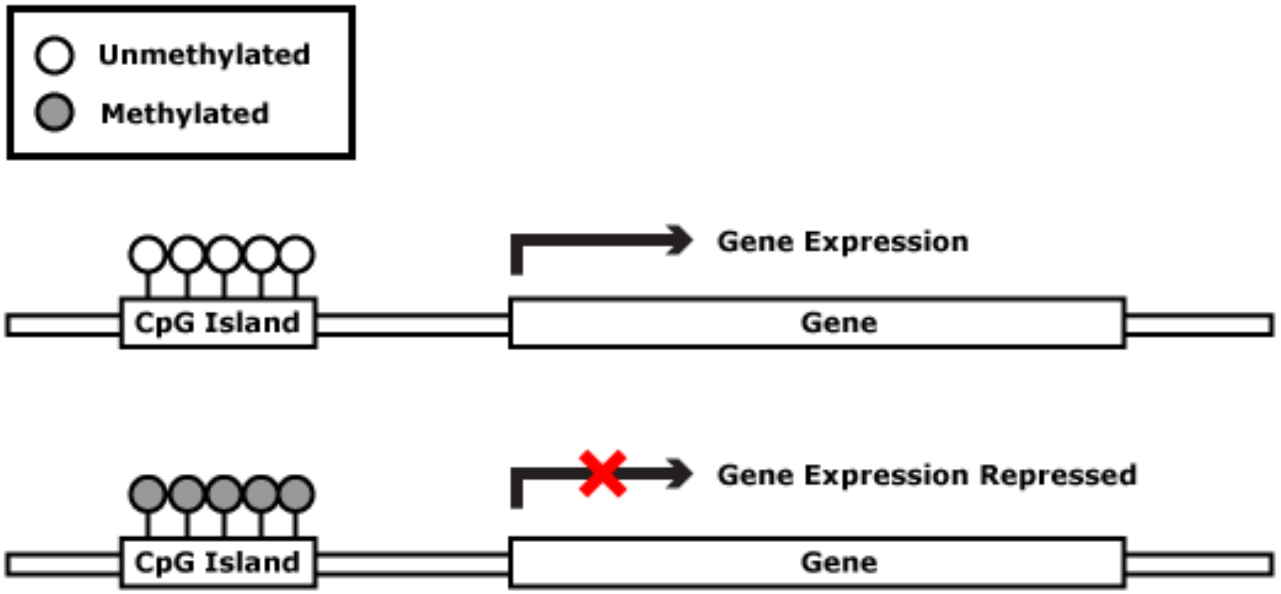
Matos (2016) BBA-Rev Cancer 1866:51–63

The Serrated pathway subtypes account for ~30% of all CRCs



Pai et al. in: *WHO Classification of Tumours of the Digestive System*. IARC Press, Lyon, France; 2019:163-169.

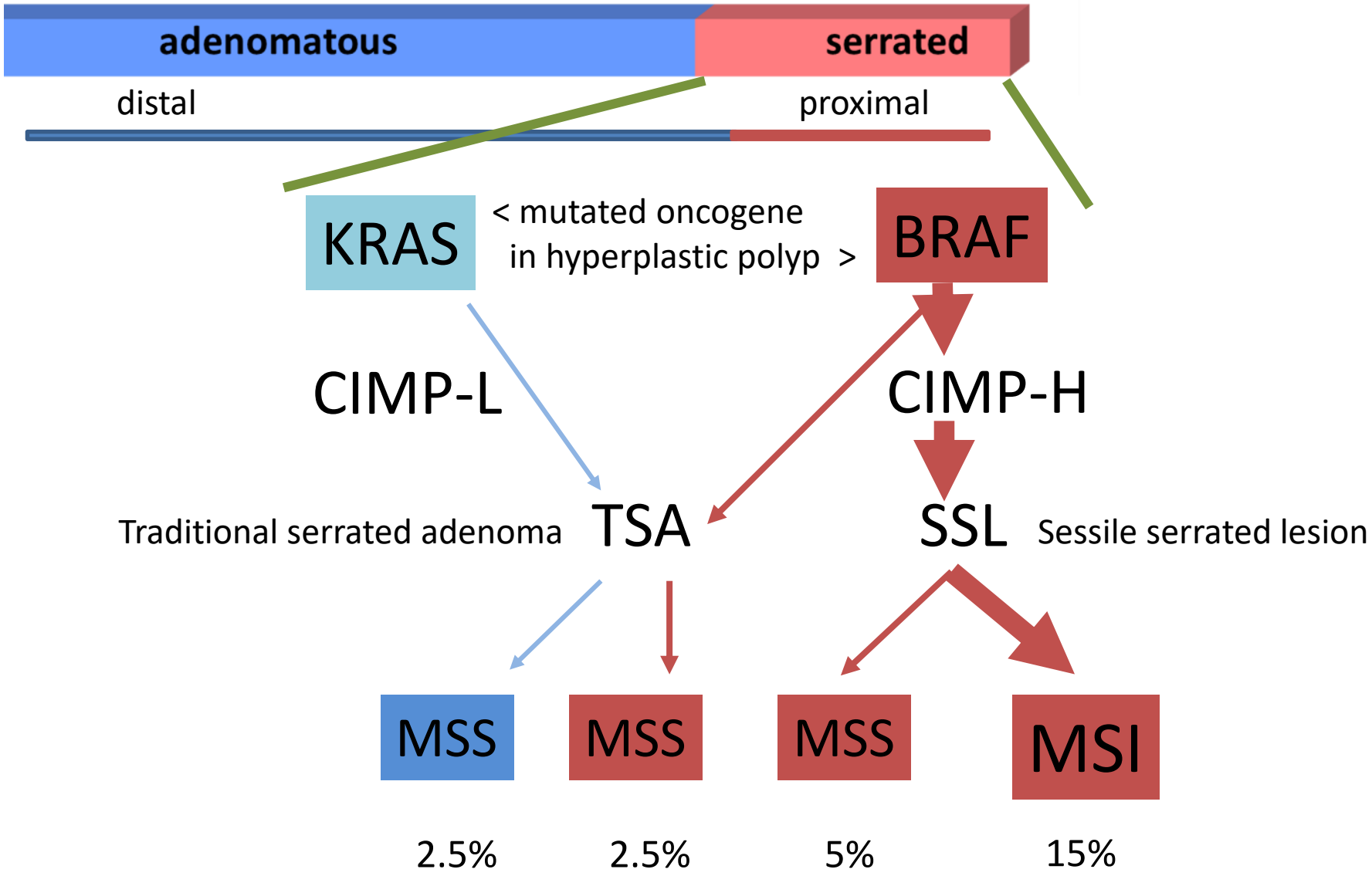
CIMP- **C**pG **I**sland **M**ethylator **P**henotype



CIMP= Genome-wide epigenetic imbalance;

…eventually will affect promoters of tumour suppressor genes, such as cell cycle regulator **p16**INK4a, or mismatch DNA repair gene **MLH1**, …

The Serrated pathway subtypes account for ~30% of all CRCs



Pai et al. in: *WHO Classification of Tumours of the Digestive System*. IARC Press, Lyon, France; 2019:163-169.

MSI- mutator phenotype

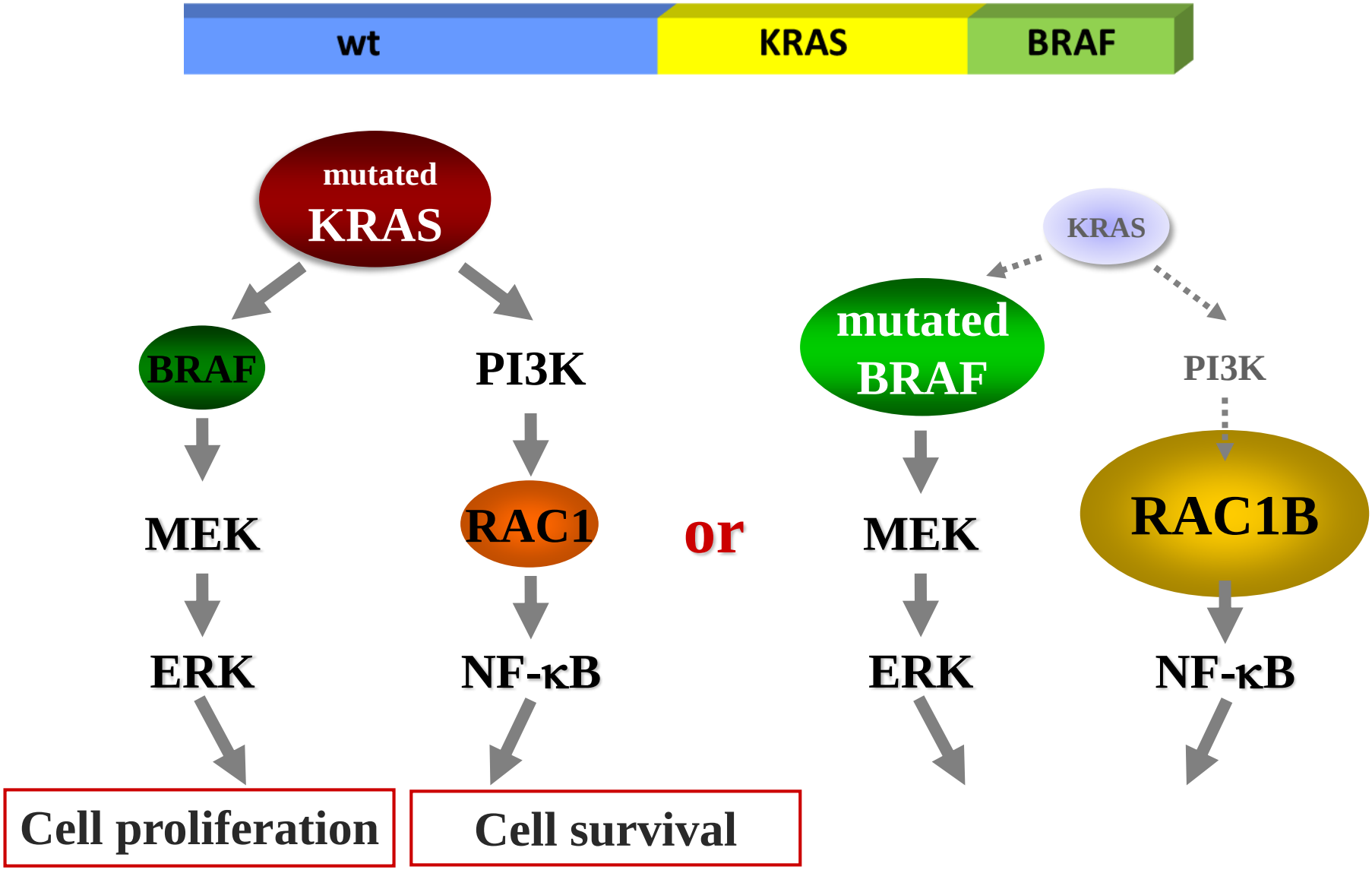
..mismatch replication errors in repetitive microsatellite sequences,

…but also happens genome-wide, including coding sequences of some tumor supressor genes

Nucleotide repeats:
GGG- Gly
CCC- Pro
AAA- Lys

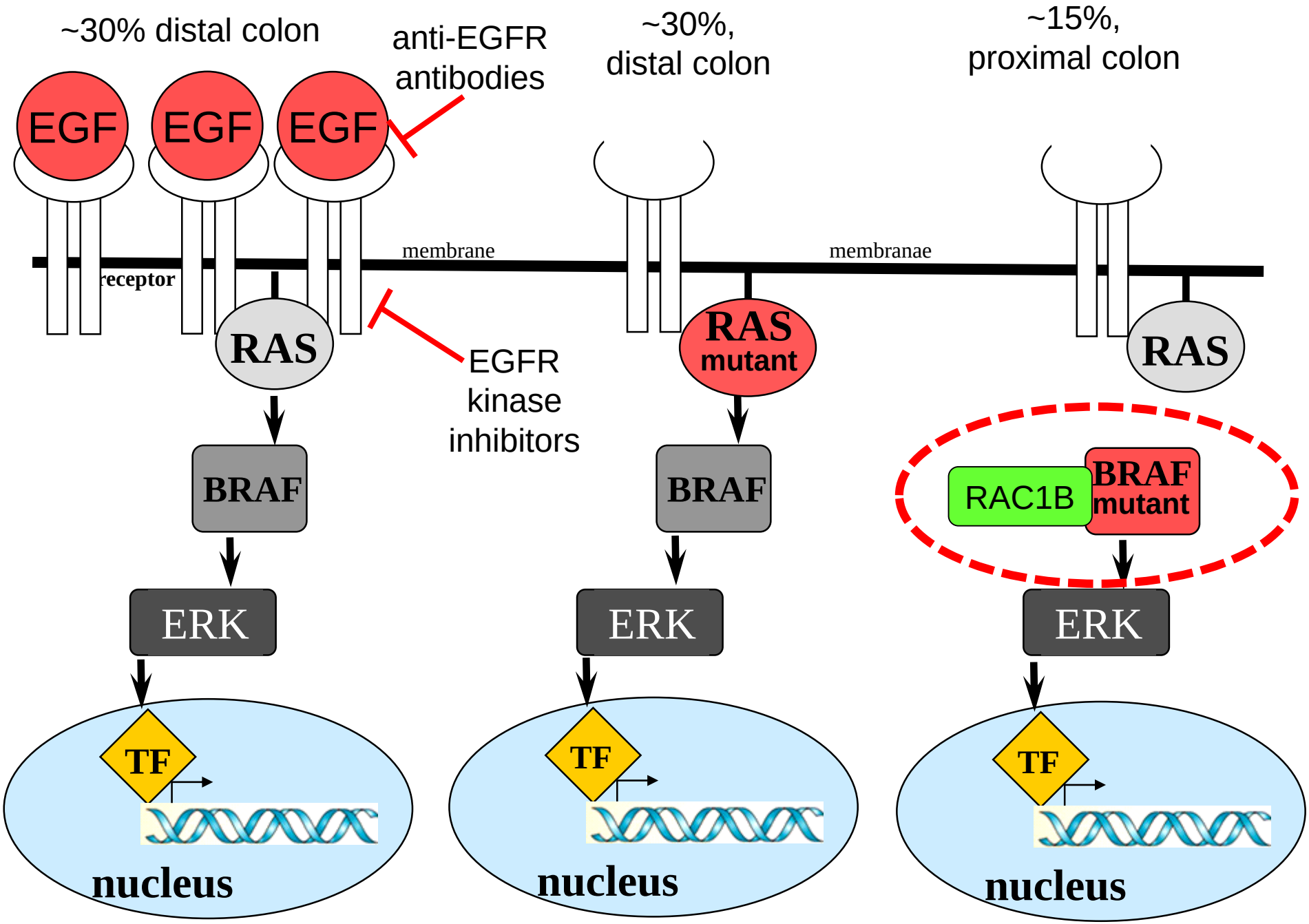
	BAX	--AATGGGGGGGGAGG-- → --AATGGGGGGGGGAGG-- (+1 base)	(G)8
	MSH6	--ATACCCCCCCCTTC-- → --ATACCCCCCCTTC-- (–1 base)	(C)8
p.E384fs	*TCF7L2*	--GAGAAAAAAAAAGTG-- → --GAGAAAAAAAAGTG-- (–1 base)	(A)9
p.E125fs	*TGFβRII*	--AGGAAAAAAAAAAGCC-- → --AGGAAAAAAAAAGCC-- (–1 base)	(A)10

MSI phenotype in endometrial, colon and gastric cancer

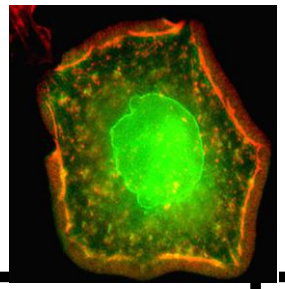


An alternative oncogenic pathway in CRC

Targeted therapy



RAC1 signaling



lamellipodia

RAC1
GTP

actin

PAK

JNK

NF -kB
STATs

$G_1 \rightarrow S$

nucleus

Adherens
junction

actin

Focal
adhesion

actin

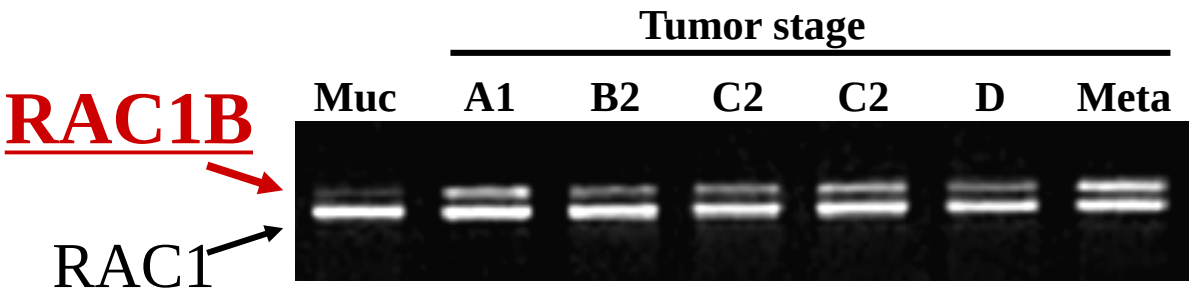
ECM

-adhesion
-motility
-proliferation
-gene expression

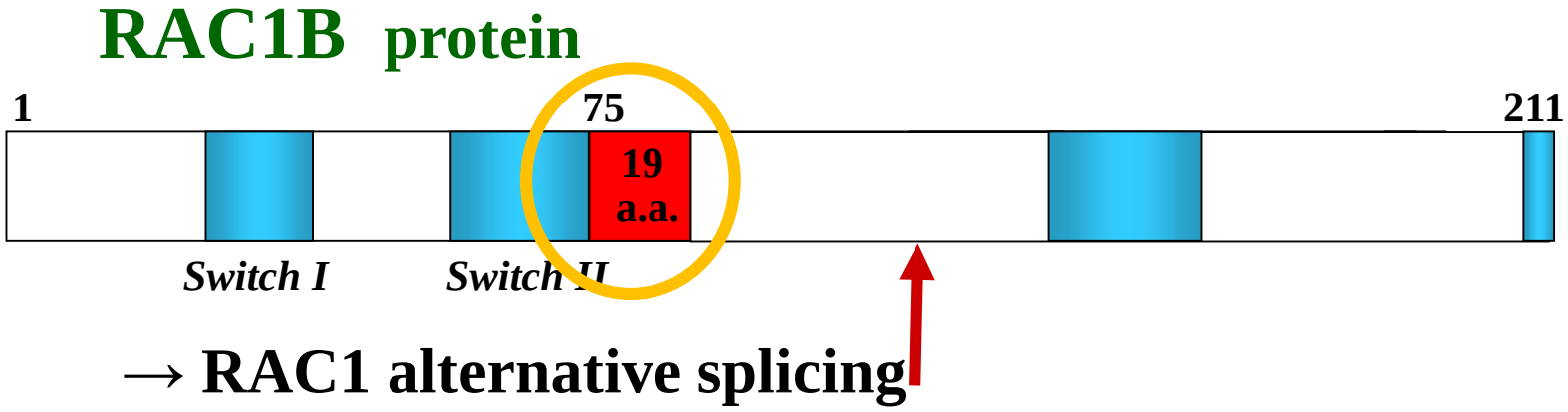
↓

Oncogenic
RAC1 mutations
in tumours ??

RAC1 expression in colorectal cancer (CRC): discovery of RAC1B

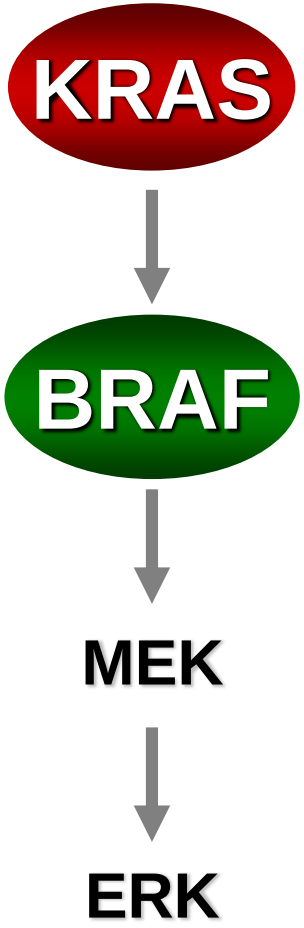


Jordan 1999, Oncogene 18:6835-39



Analysis of colorectal cancer cell lines

	Expression		Genotype	
Cell line	RAC1	RAC1B	BRAF	KRAS
V9P	+	–	wt	wt
SW48	+	–	wt	wt
LS174	+	-	wt	G12D
Lovo	+	-	wt	G13D
TC71	+	-	wt	G12D
HCT116	+	-	wt	G13D
HCT15	+	-	wt	G13D
IS513	+	-	wt	G12D
IS2	+	-	wt	G12D
Colo320	+	-	wt	G12D
SW480	+	-	wt	G12V
CO115	+	+	V600E	wt
HT29	+	+	V600E	wt
RKO	+	+	V600E	wt
Colo205	+	+	V600E	wt
Caco2	+	+	wt	wt



RAC1B overexpression in ~80% of CRC tumours with mutation in *BRAF*

One gene → **2 splicing variants** → **2 related proteins**

RAC1

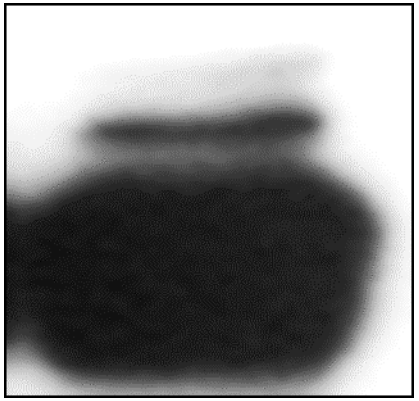
mRNA RAC1
mRNA RAC1B

Total RAC1 protein

GTP-loaded RAC1??

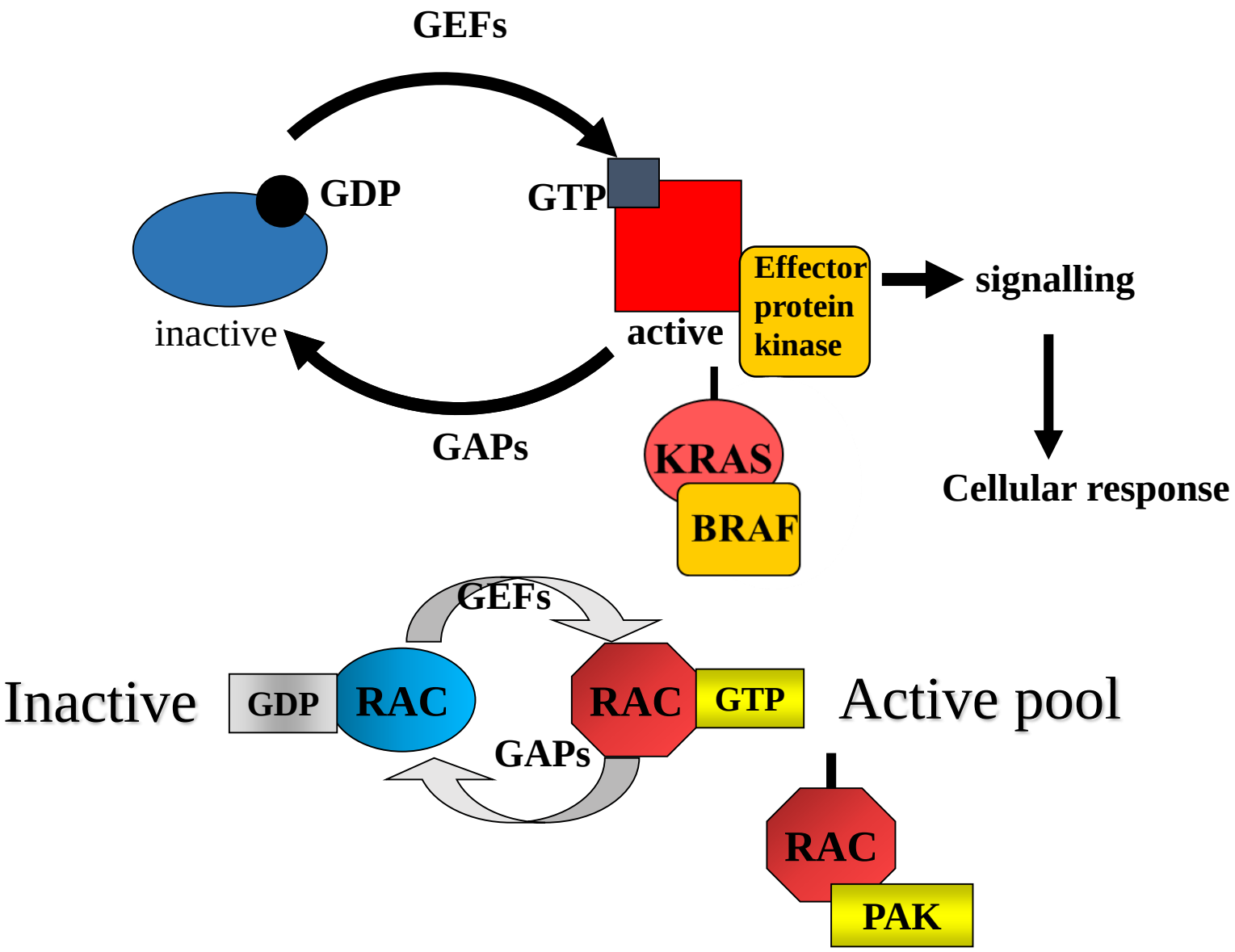
RAC1B →

RAC1 →

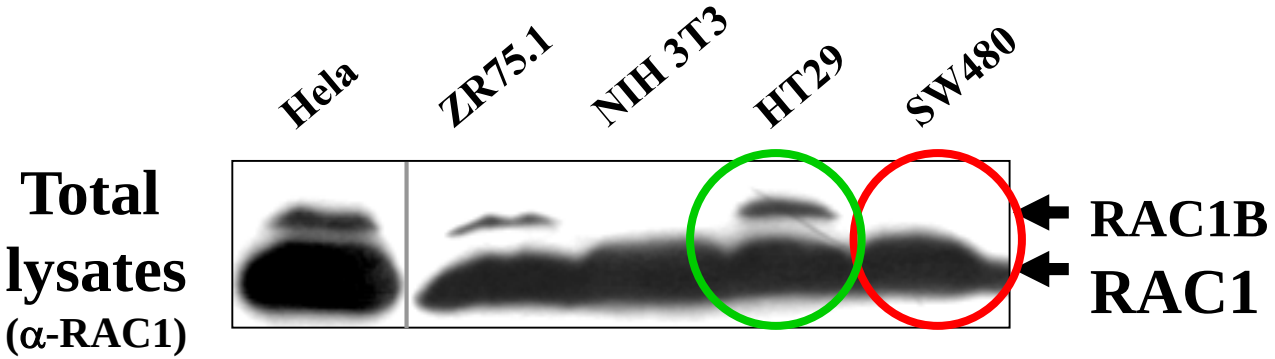


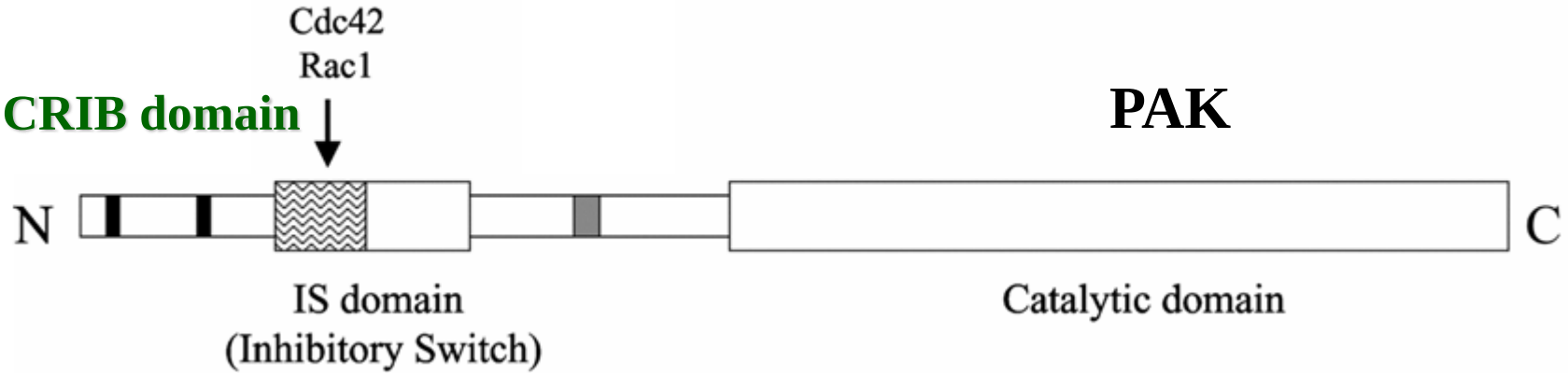
HT29 cell lysates

The GTPase cycle of the RAS superfamily



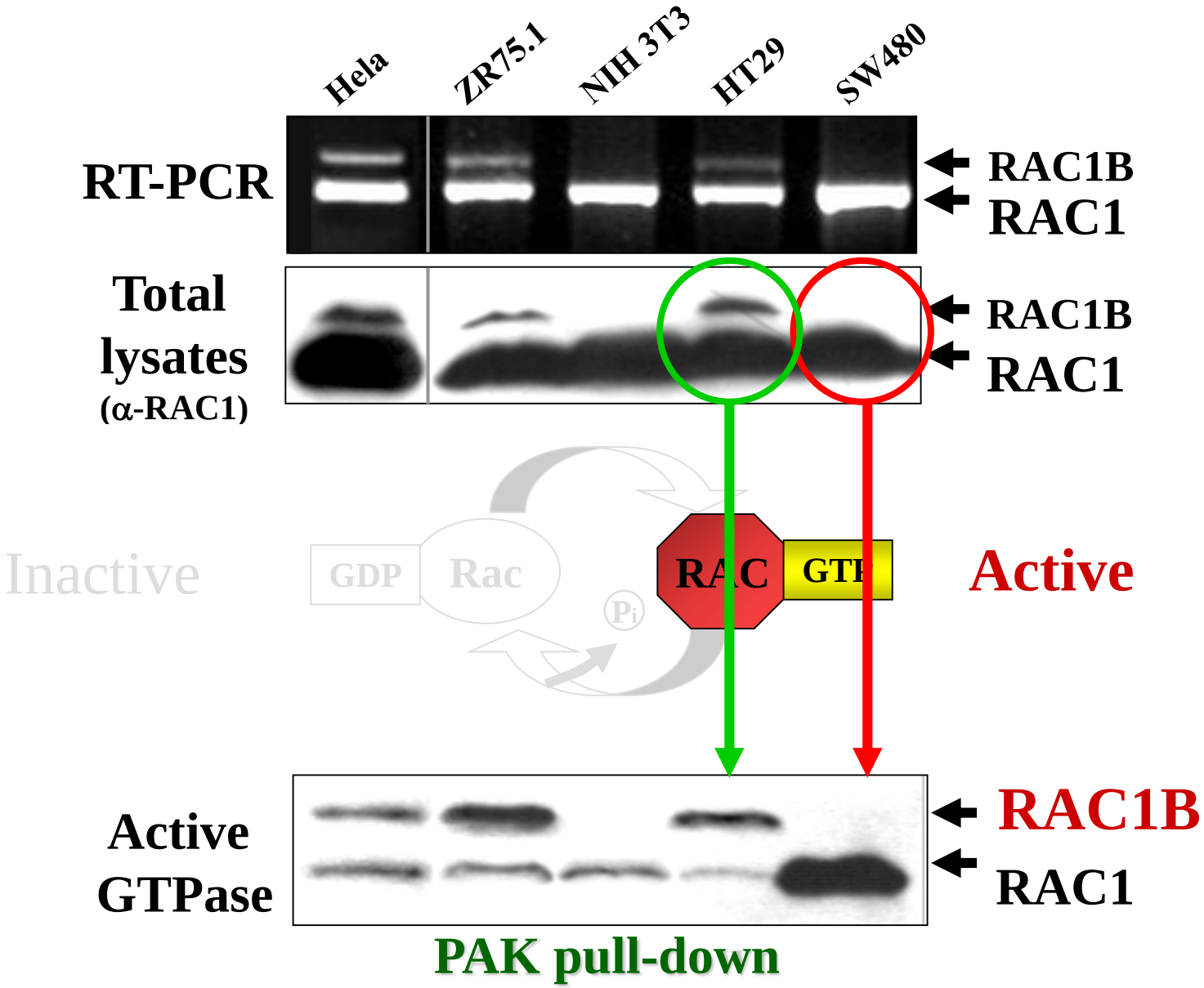
Testing RAC1B activation in cell lines





Recombinant GST-CRIB domain peptide → **PAK pull-down assay**

Testing RAC1B activation in cell lines



Matos 2003, J Biol Chem 278:50442–48.

One gene → **2 splicing variants** → **2 related proteins but with different activities**

RAC1

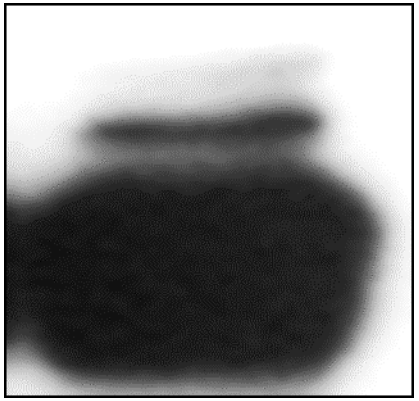
mRNA RAC1
mRNA RAC1B

Total RAC1 protein

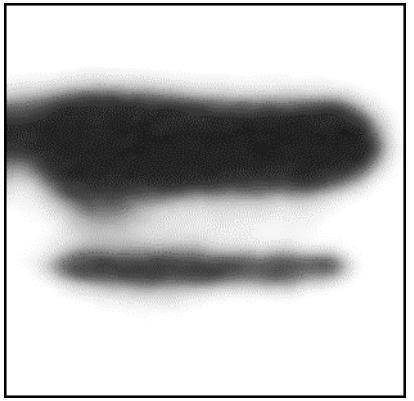
Pool of GTP-loaded RAC1 (= signalling-competent state)

RAC1B →

RAC1 →



HT29 cell lysates



PAK-pull-down assay

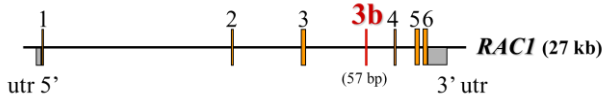
RAC1B predominantly in signalling-competent state

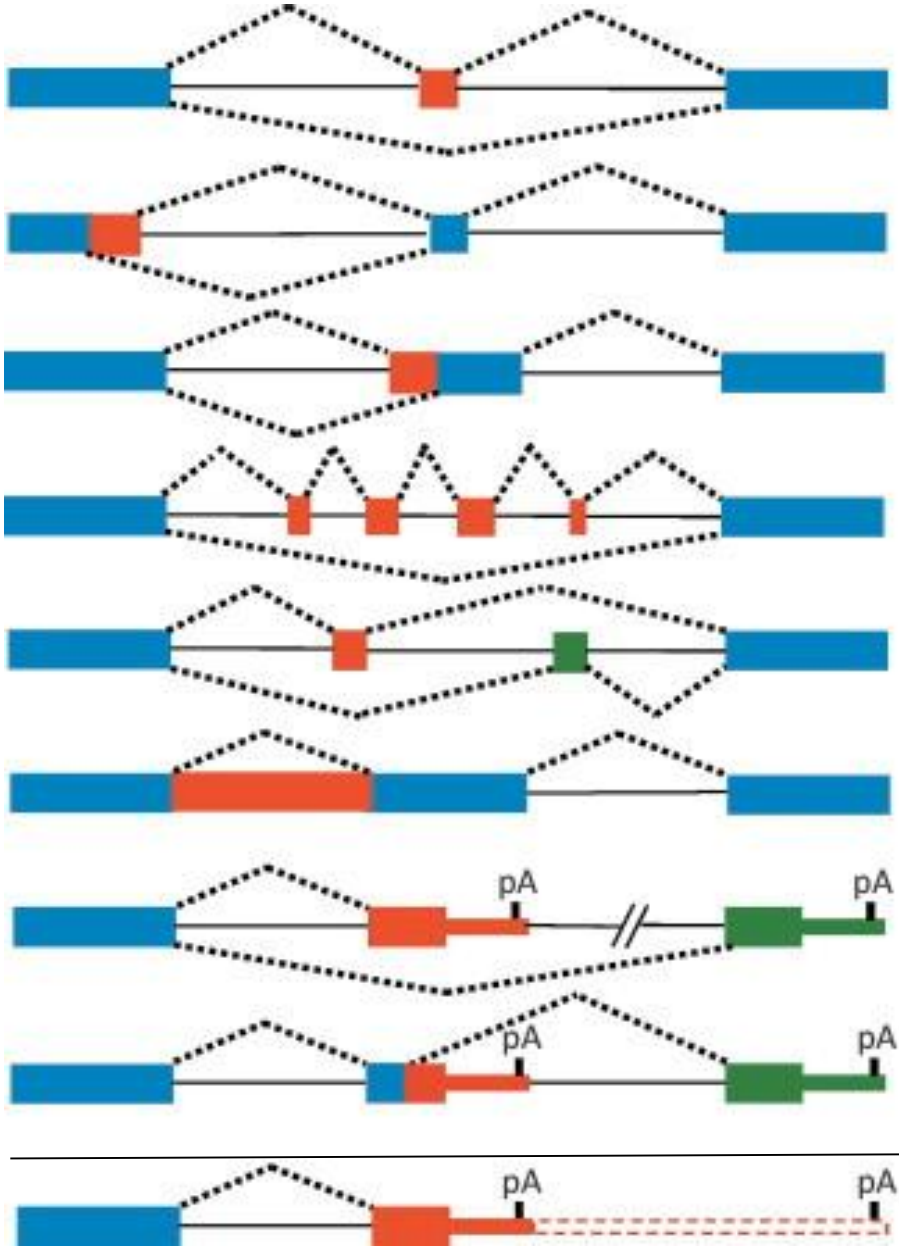
Alternative splicing

as a

tumour-promoting event

Alternative splicing ….





Simple cassette exon **~40%**

Alternative 5' splice sites **~8%**

Alternative 3' splice sites **~18%**

Tandem cassette exons

Mutually exclusive exons

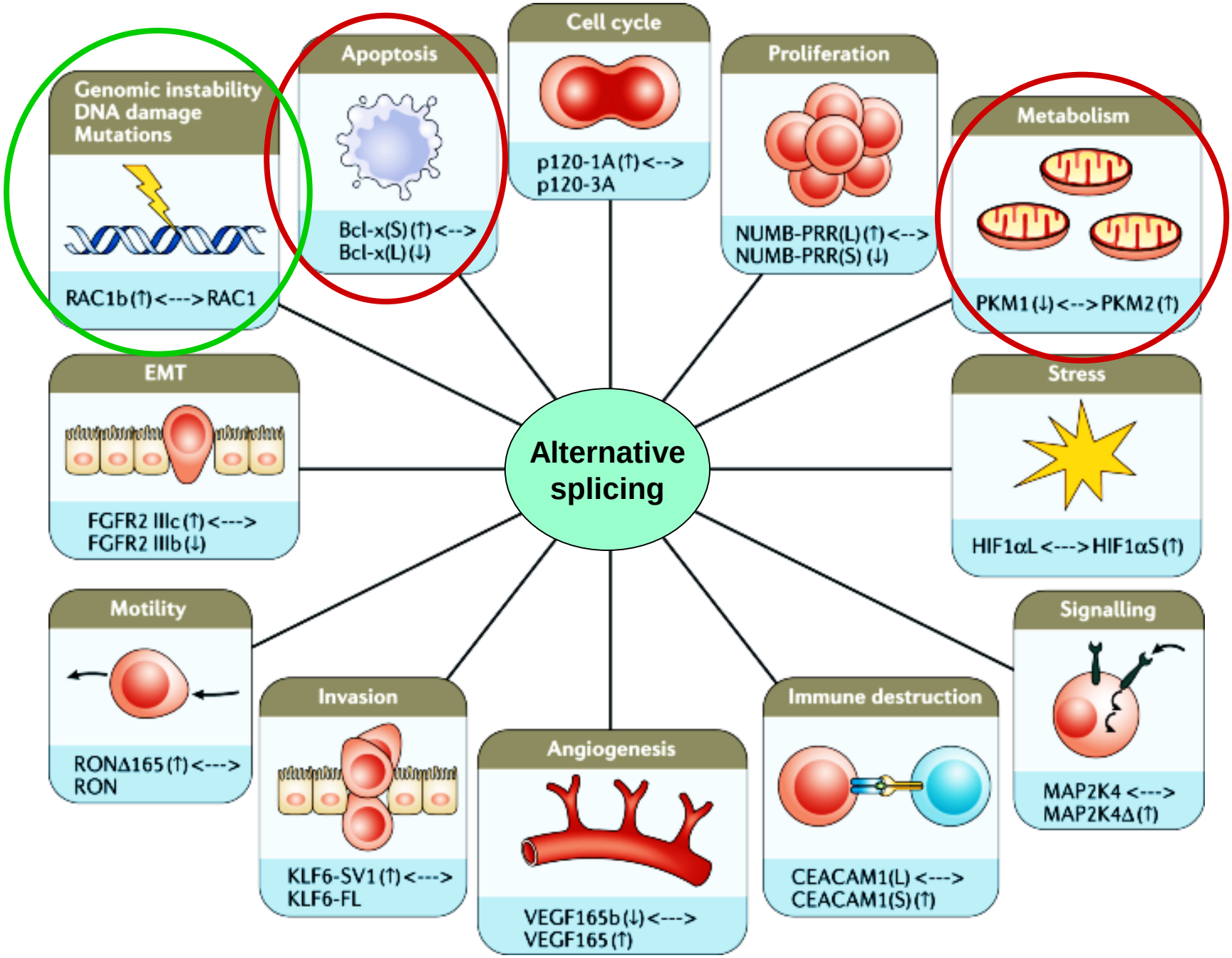
Intron retention **~3%**

Alternative 3' terminal exon/ polyA site (APA3)

Alternative 3' terminal exon/ polyA site (APA5)

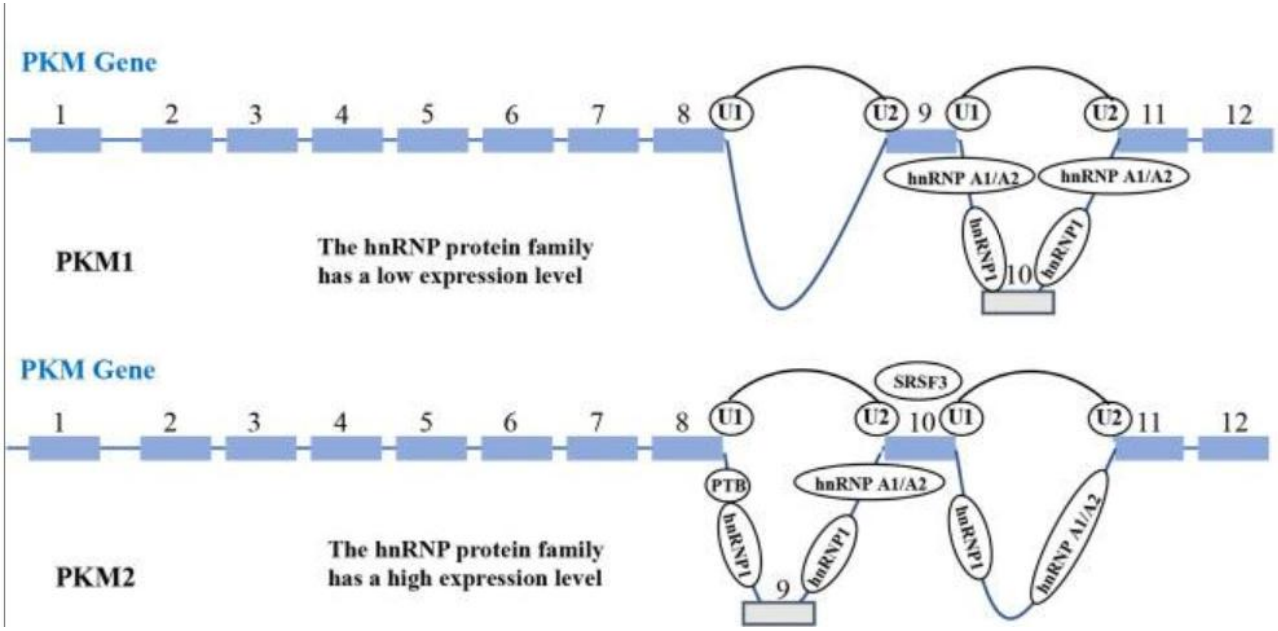
Alternative polyA (APA)

~50%



Valcárcel (2020) Nat Rev Clin Oncol, https://doi.org/10.1038/s41571-020-0350-x

Pyruvate Kinase Isoforms



PTB: Polypyrimidine Tract Binding Protein

hnRNP: Heterogeneous Nuclear Ribonucleoprotein

SRSF3: Serine/Arginine—Rich Splicing Factor 3

Exon 9 or 10

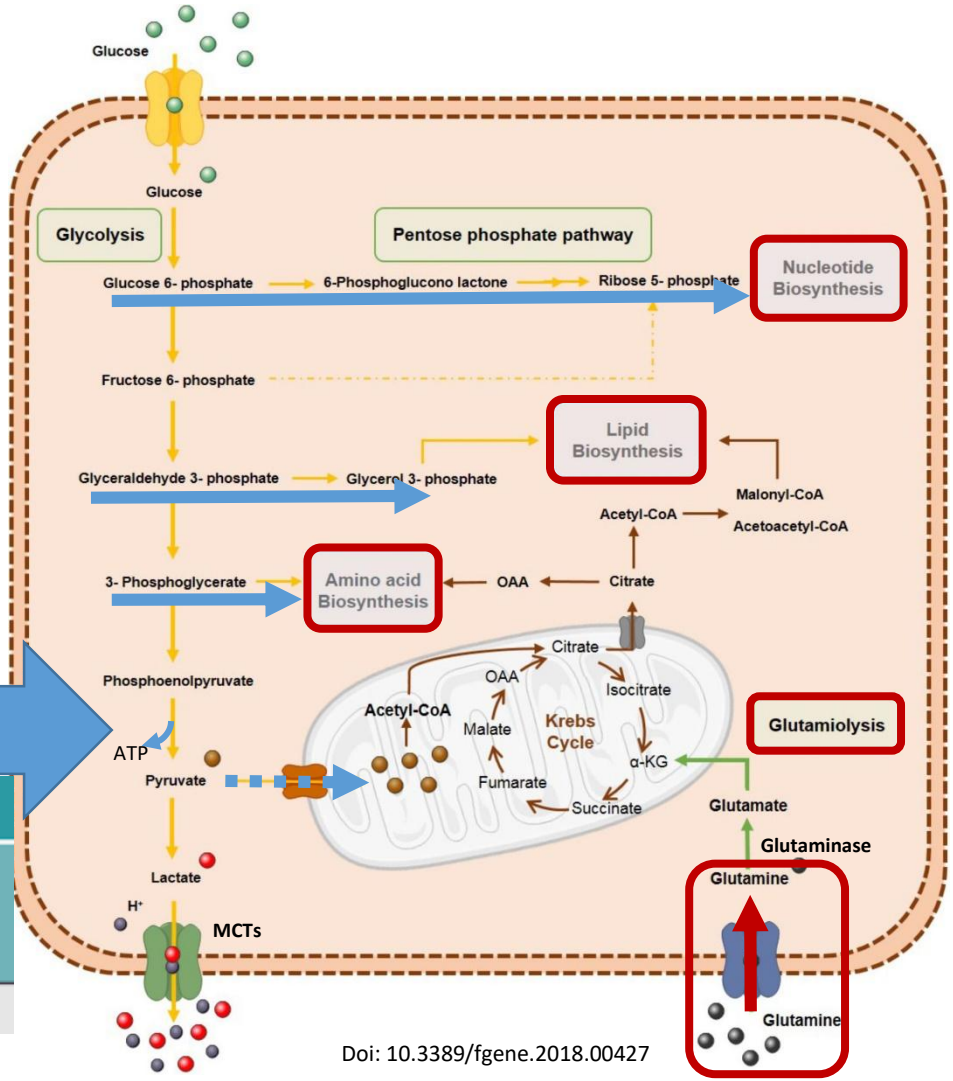
Mutually exclusive exons

Cancer cell metabolism

Due to PK-M2 expression, the glycolytic intermediates above pyruvate accumulate

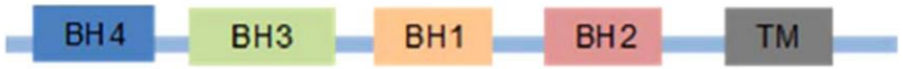
a) biosynthetic building blocks to sustain proliferation

b) Glutaminolysis feeds TCA



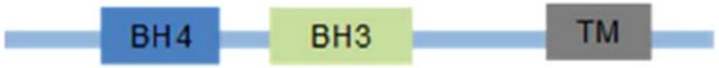
Doi: 10.3389/fgene.2018.00427

BCL-X_L

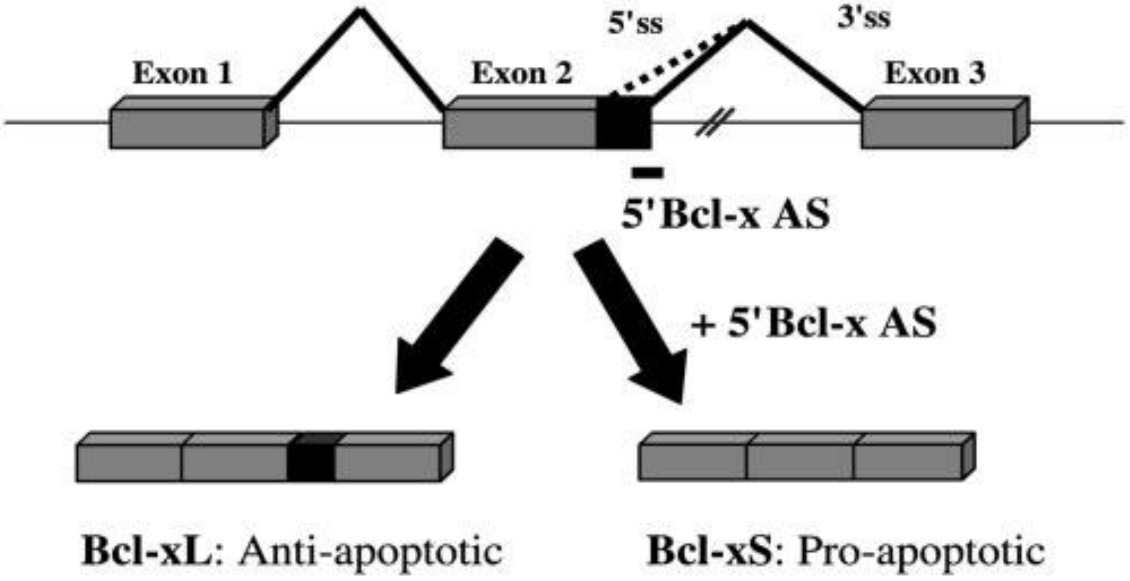


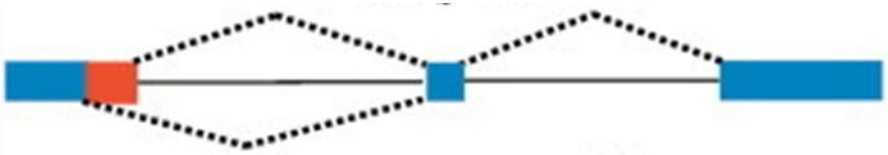
BCL-XL is anti-apoptotic by forming heterodimers with both BAX and BAK proteins in prevent pore formation at the mitochondrial memrbane;

BCL-X_S



BCL-Xs does not form these heterodimers





Alternative 5’ splice sites

Intrinsic pathway to apoptosis

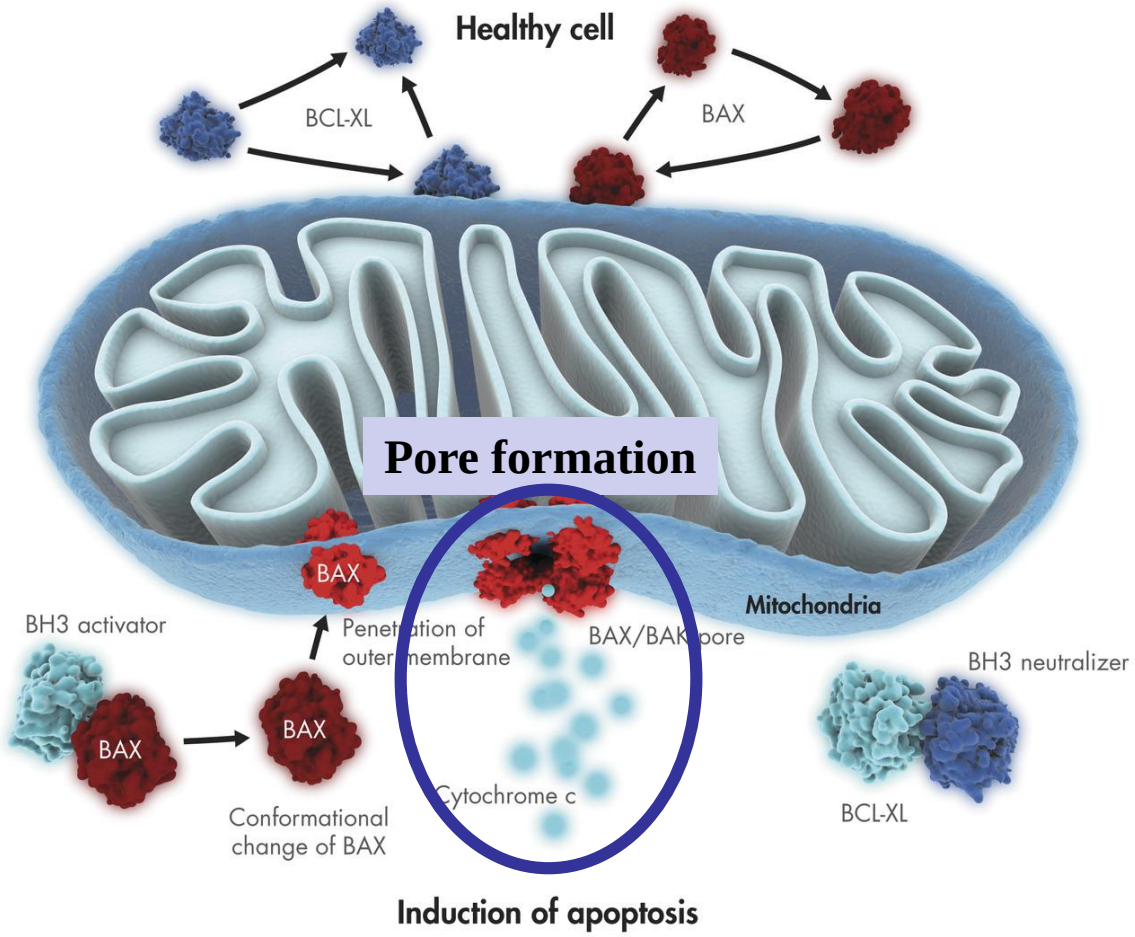


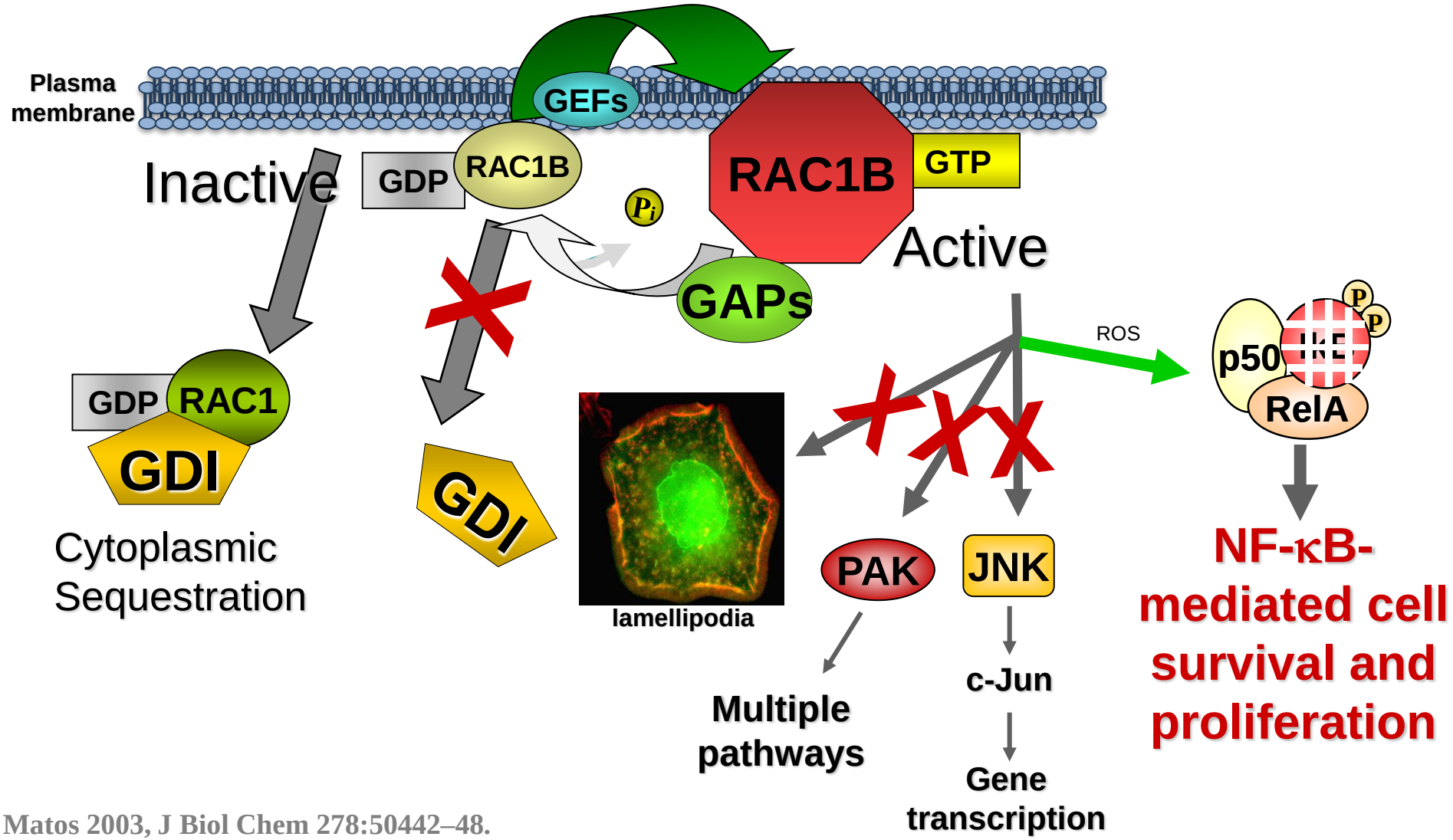
Image: https://www.qiagen.com/~/media/nextq/image%20library/illu/03/41/illu_0341_rt2profiler/1_5.ashx?la=en&hash=BF639C1F0C3B0A3D70DD3FE2EA7A12740F98957F

Main findings so far…

- **Transcription of the *RAC1* gene can generate two mRNAs by alternative splicing: RAC1 and RAC1B**

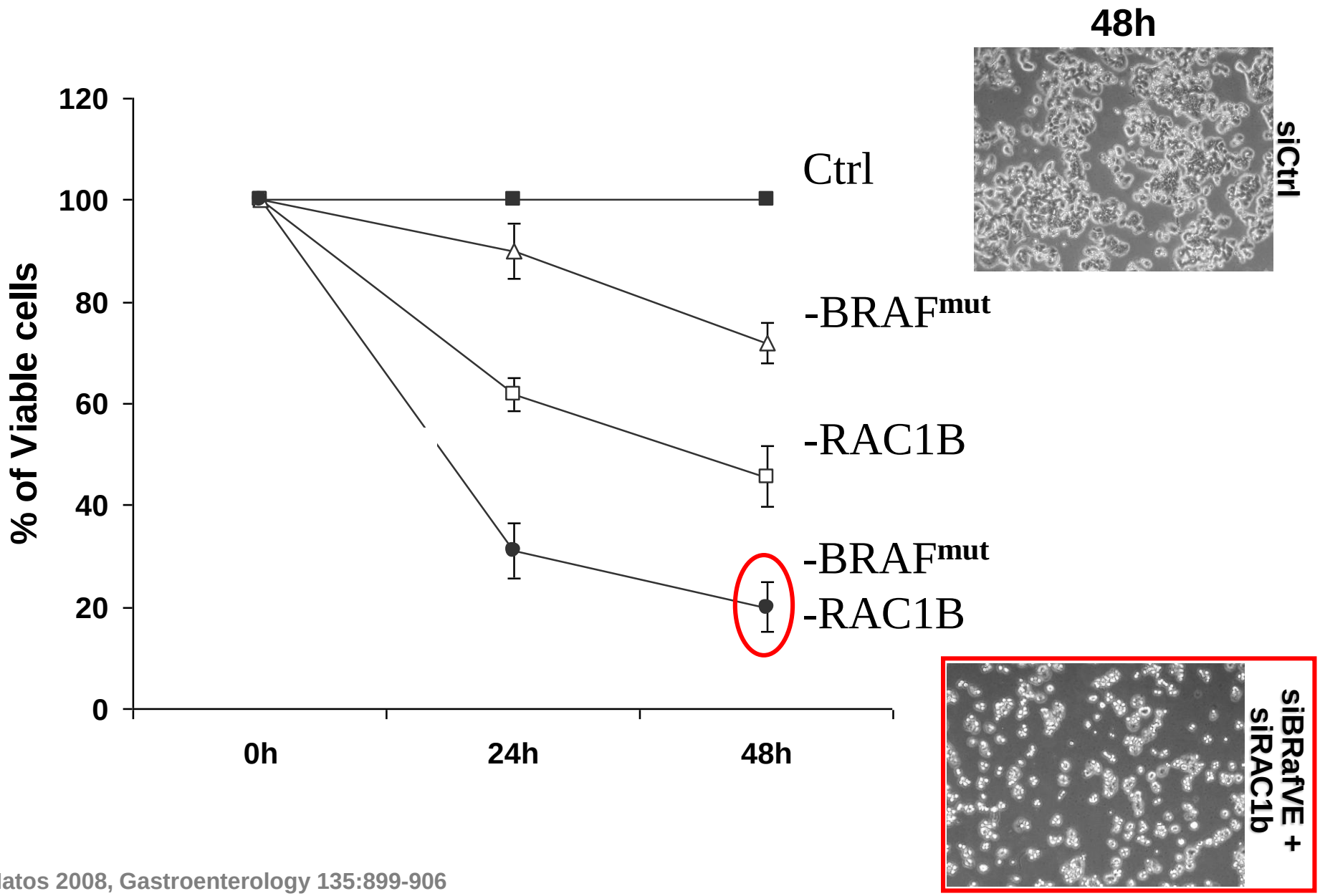
- **Overexpression of RAC1B ocurrs in ~80% of CRC tumours with mutation in *BRAF* (~10-15% of cases)**

Summary of the unique properties of alternative spliced RAC1B



Matos 2003, J Biol Chem 278:50442–48.

Cell viability after suppressing RAC1B and BRAF in HT29 cells



Matos 2008, Gastroenterology 135:899-906

Main findings so far…

- Transcription of the *RAC1* gene can generate two mRNAs by alternative splicing: RAC1 and RAC1B

- Overexpression of RAC1B ocurrs in ~80% of CRC tumours with mutation in *BRAF* (~10-15% of cases)

- **RAC1B protein differs in regulation and signalling**

- **Overexpression of RAC1B cooperates with mutant BRAF to sustain the survival of colorectal tumour cells**

RAC1B overexpression in other tumors

Colon- **associated with BRAF-V600E, stimulates NF-kB**
Jordan et al. 1999; Matos et al. 2008

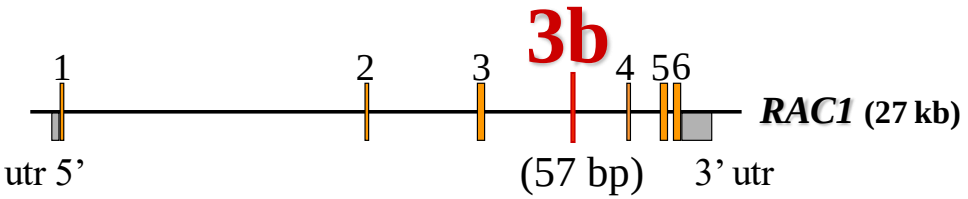
Breast- **promoted ROS and EMT when MMP3-stimulated**
Schnelzer et al. 2000; Radisky et al, 2005

Lung- **associated with KRAS mutation**
Stallings-Mann et al. 2012; Zhou et al. 2013

Thyroid- **associated with BRAF-V600E in papillary TC**
Silva et al. 2013

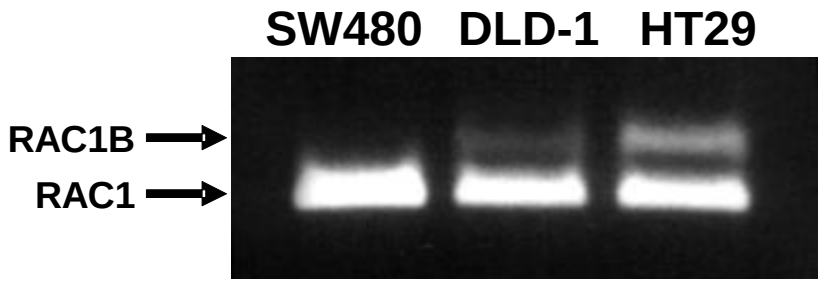
Pancreas- **inhibitor of TGF-β dependent EMT**
Ungefroren et al. 2018

What leads to overexpression of RAC1B in colorectal tumor cells ?



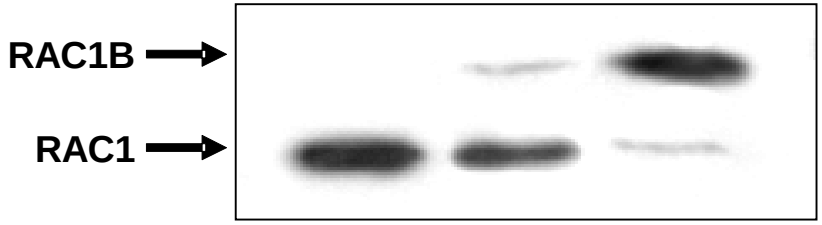
alternative splicing

Do genomic mutations cause increased exon 3b inclusion?



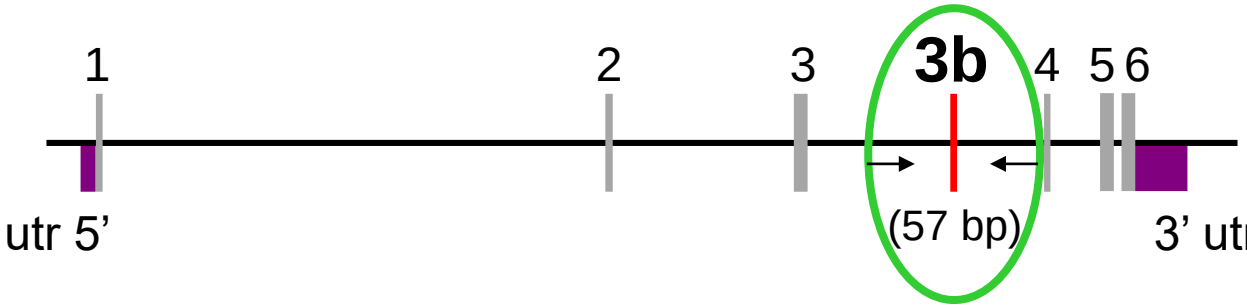
mRNA
(RT-PCR)

Endogenous expression



Protein
(anti-RAC1)

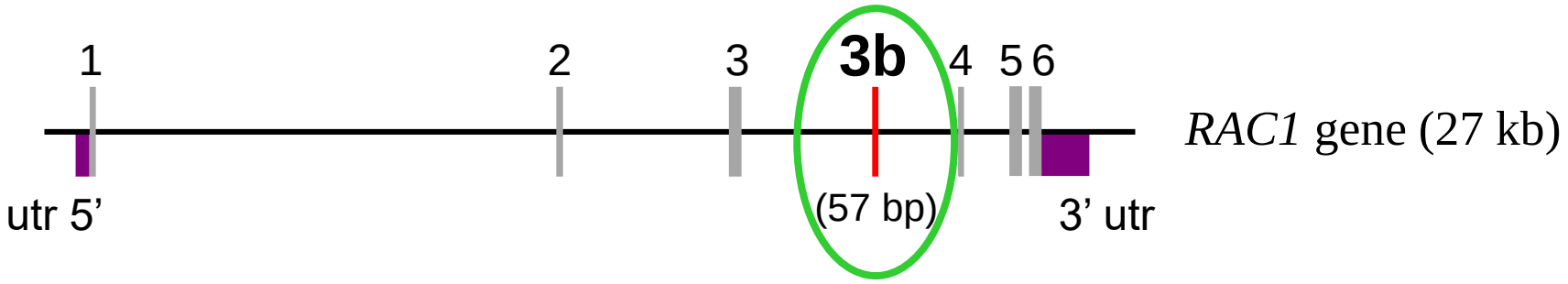
GTP-loaded RAC



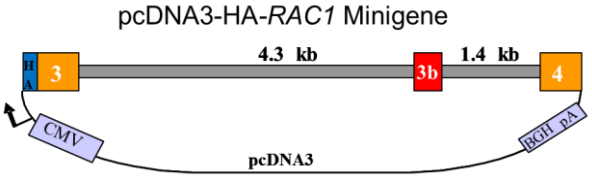
RAC1 gene (27 kb)

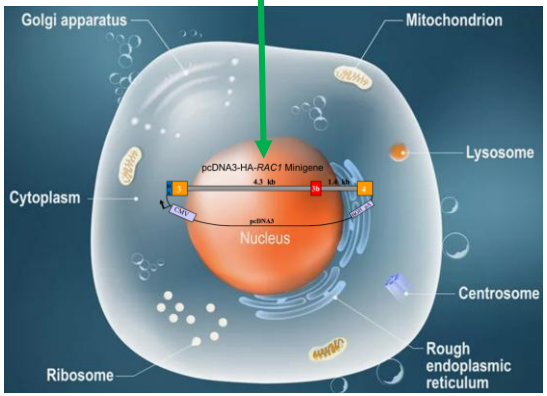
→ **Cell lines have identical genomic *RAC1* sequence**

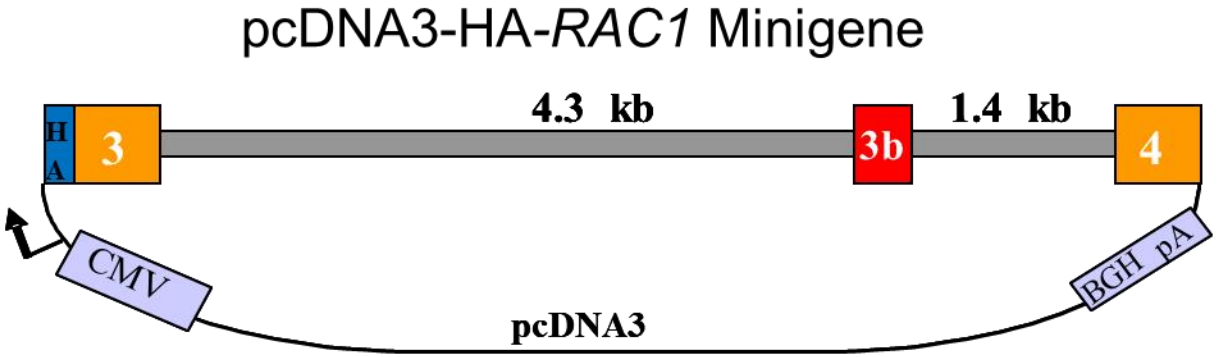
Instead of gene mutations, alternative splicing of RAC1B could be regulated by altered signalling mechanisms in colorectal cancer cells

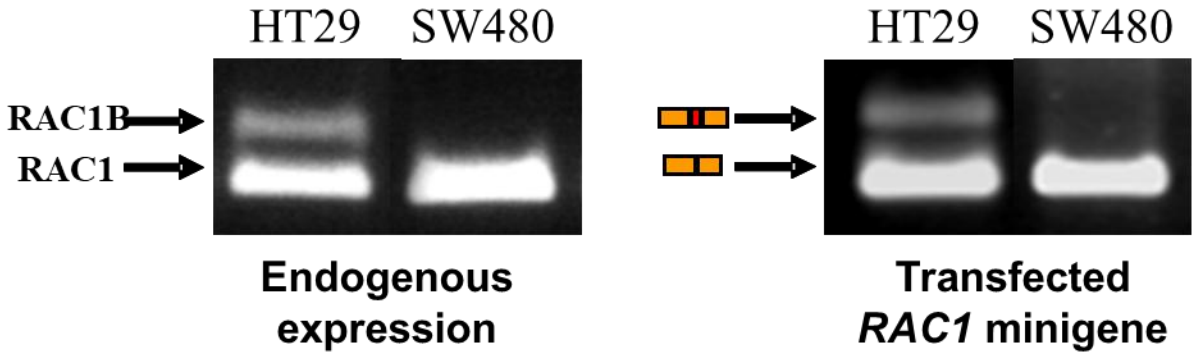


Designing a minigene to study what determines exon 3b inclusion or skipping



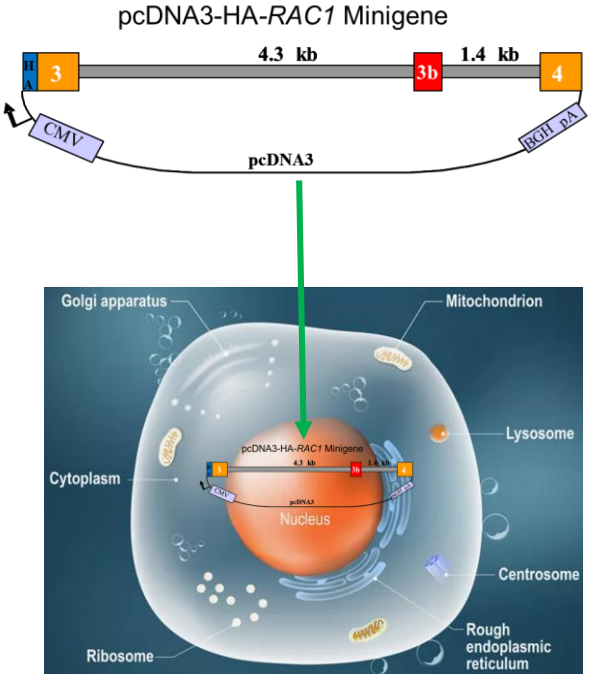


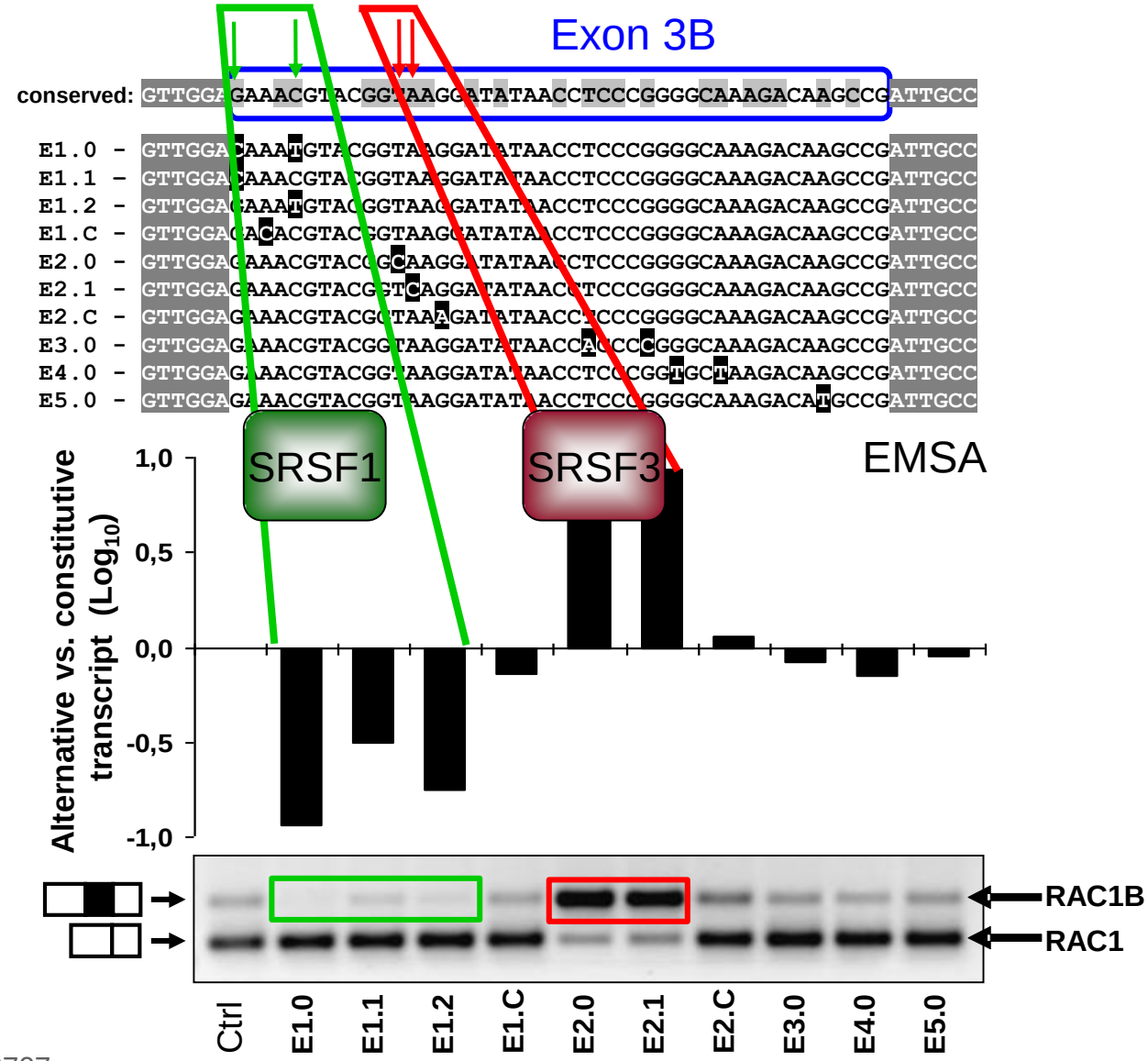




Gonçalves 2009, Hum Mol Genet 18:3696-3707

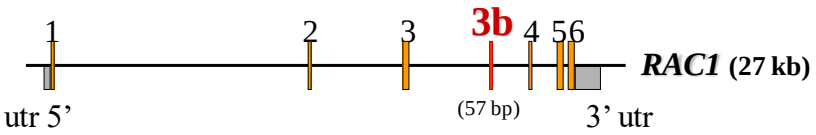
Two regulatory sequence elements determine exon 3b inclusion or skipping

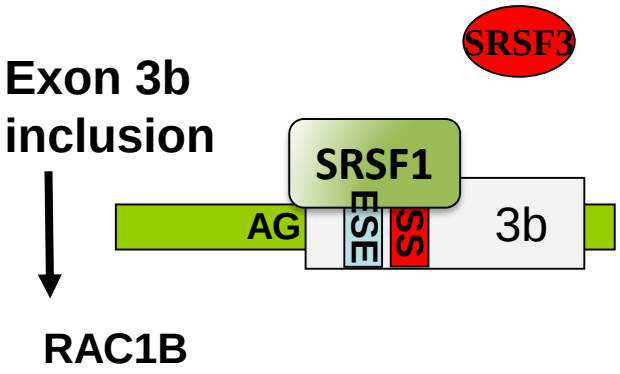


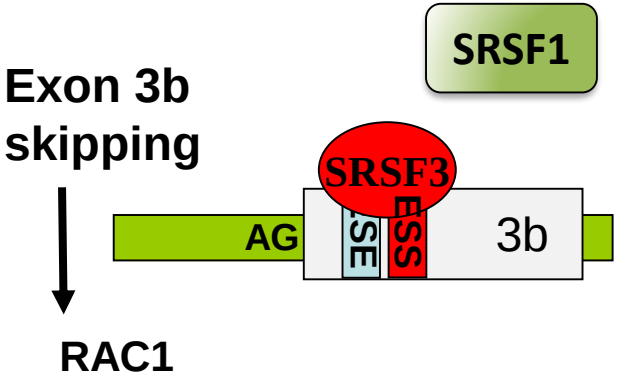


Gonçalves 2009, Hum Mol Genet 18:3696-3707

Model for regulation of *RAC1* alternative splicing in CRC



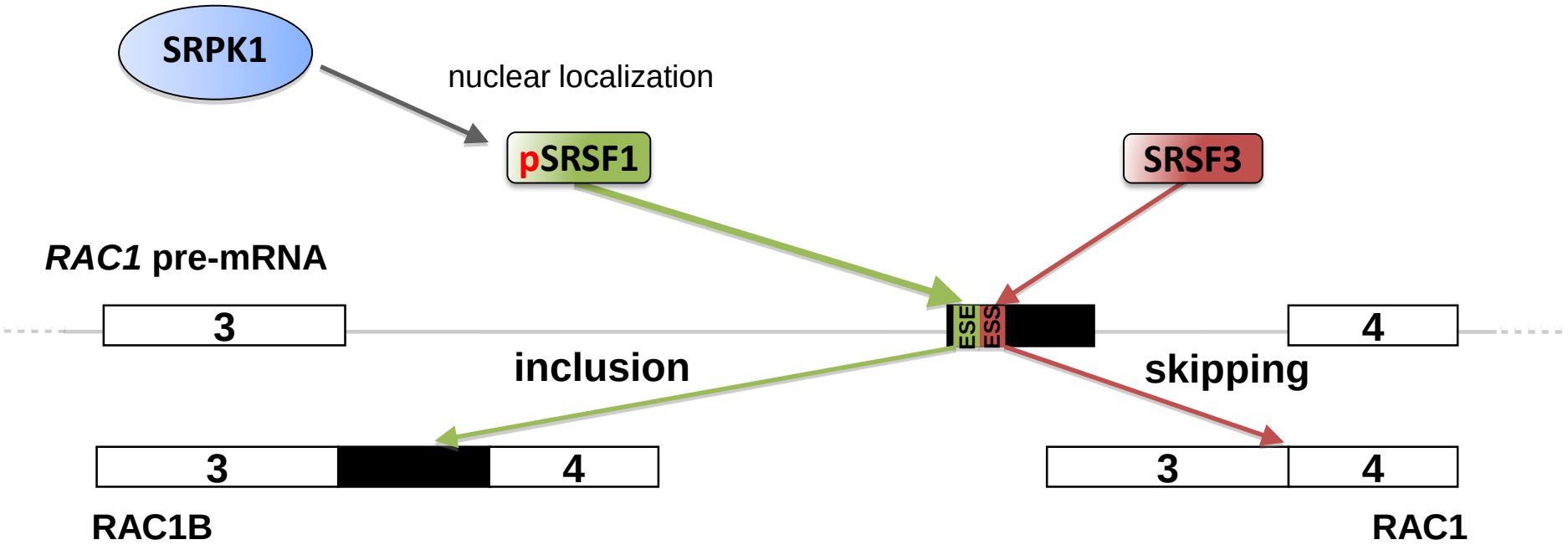


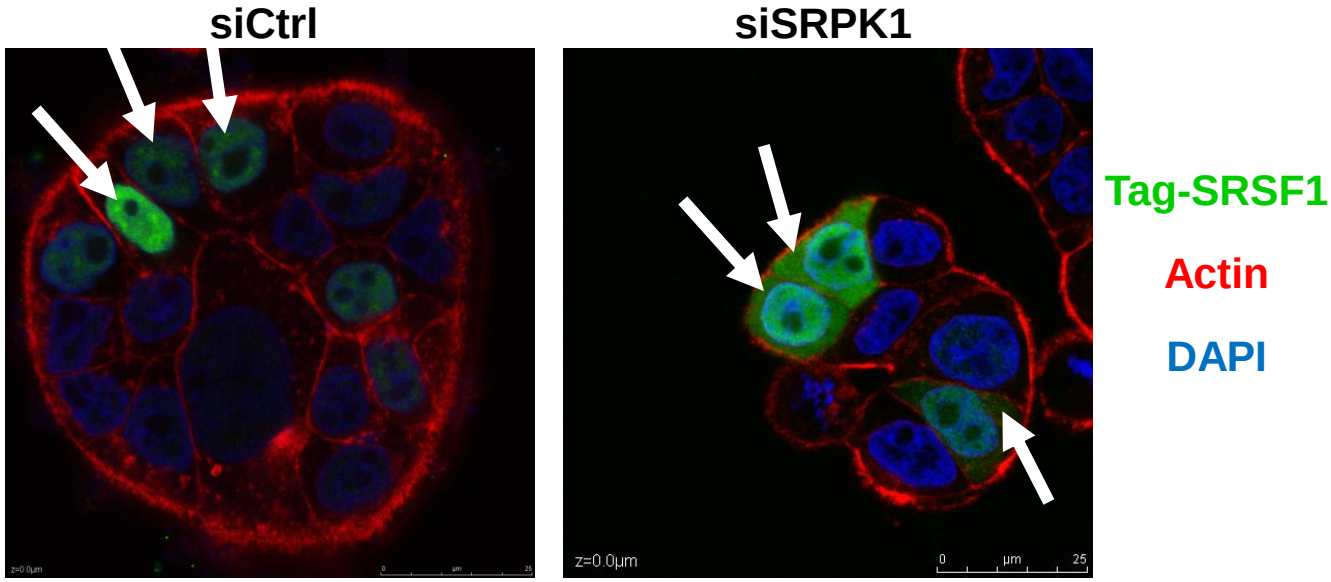


How is the binding of SRSF1 and SRSF3 regulated?

What is different in colorectal tumor cells?

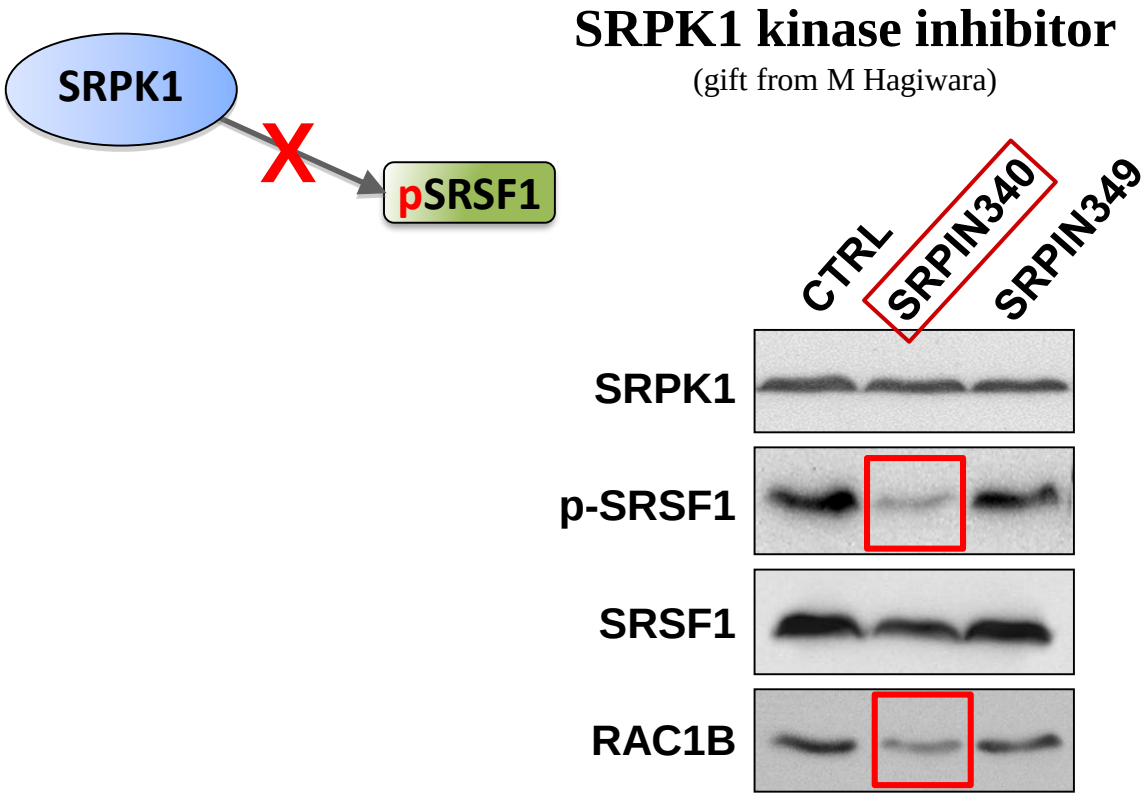
Role of protein kinase SRPK1 in RAC1B overexpression





Gonçalves 2014,
RNA 20:474-82

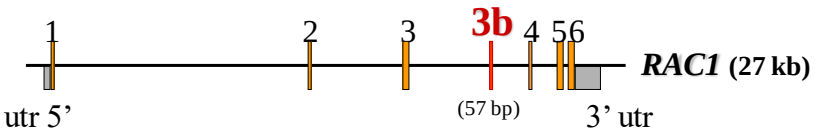
Role of protein kinase SRPK1 in RAC1B overexpression

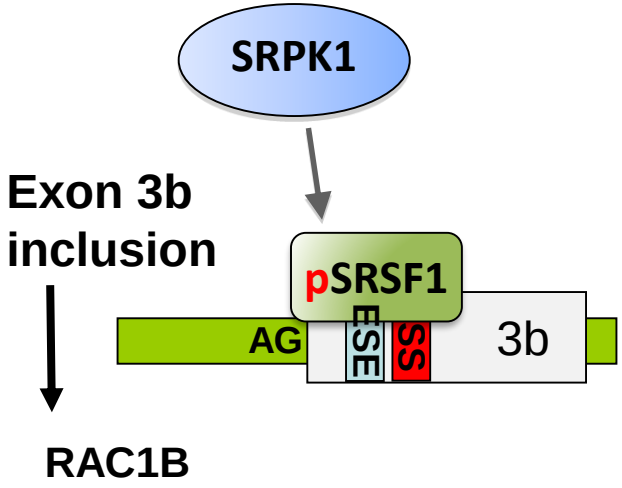


Inhibition of SRPK1 activity with small-molecule drugs decreased phosphorylation of SRSF1 and reversed the overexpression of RAC1B

Gonçalves 2014, RNA 20:474-82

Model for regulation of *RAC1* alternative splicing in CRC

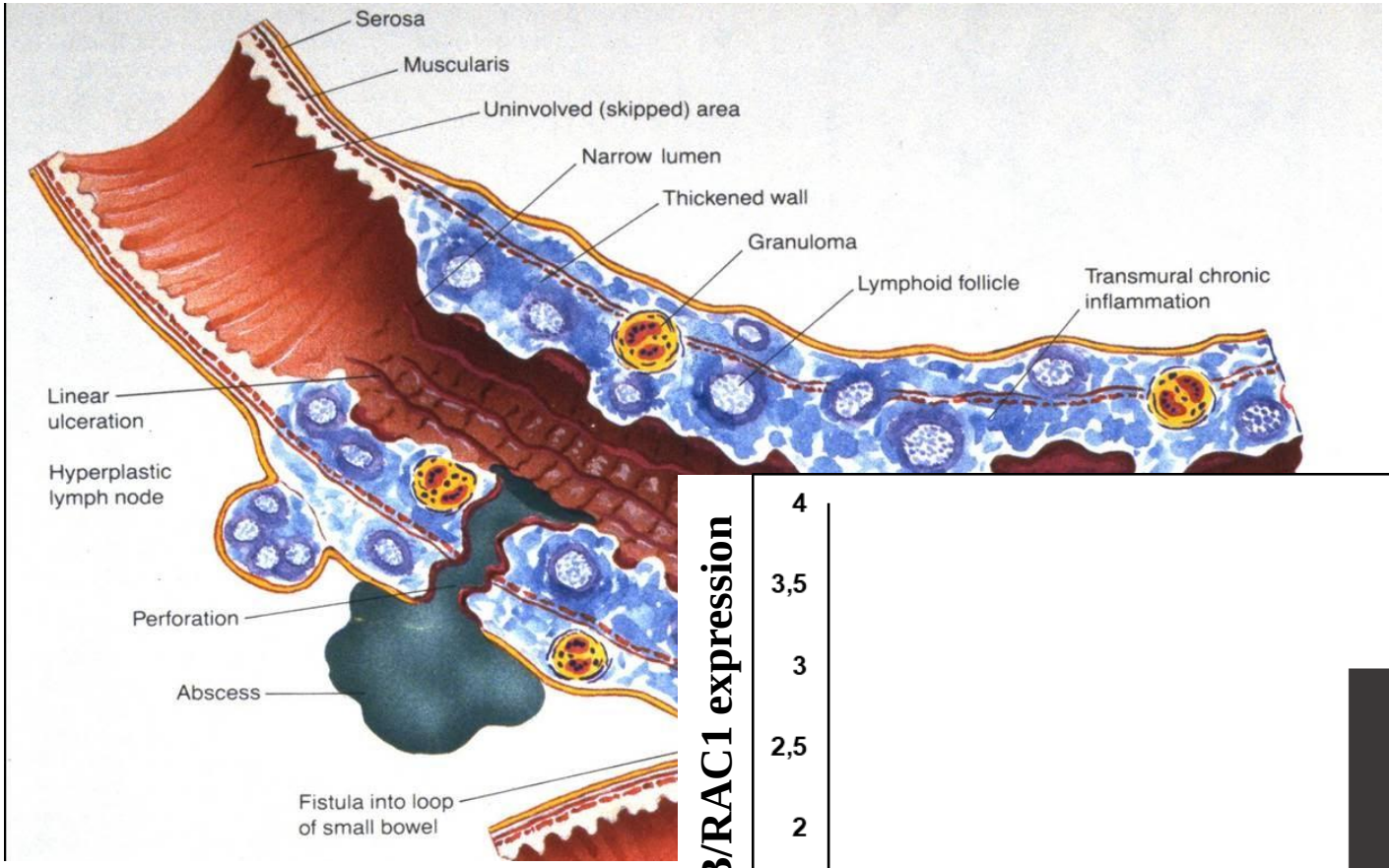




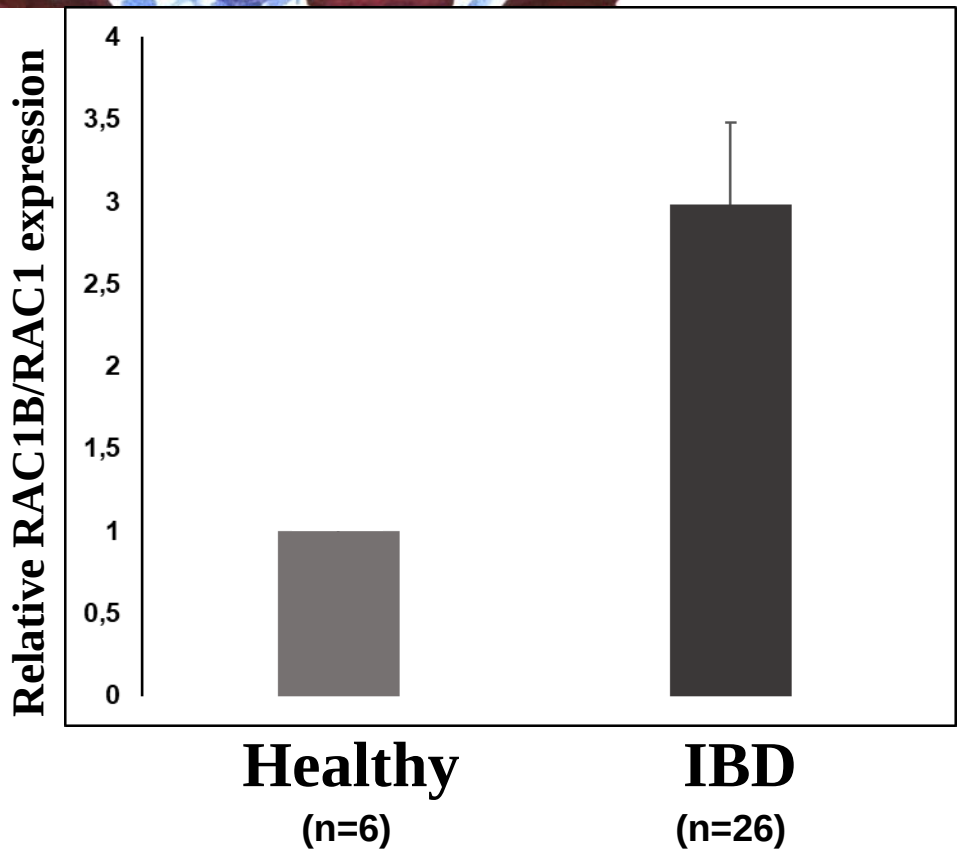
What determines the phosphorylation of SRSF1?

What is different in colorectal tumour cells?

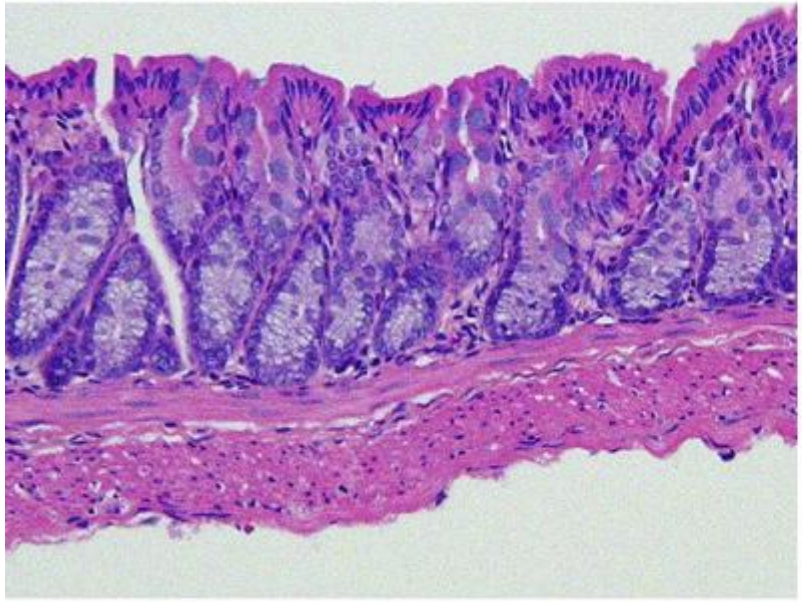
Inflammation



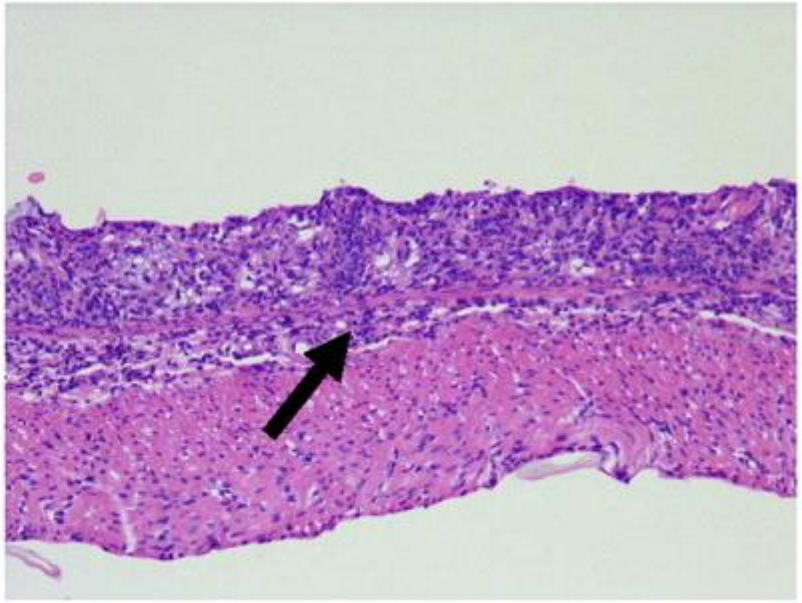
RNA extracted from 32 human IBD samples



Untreated Control



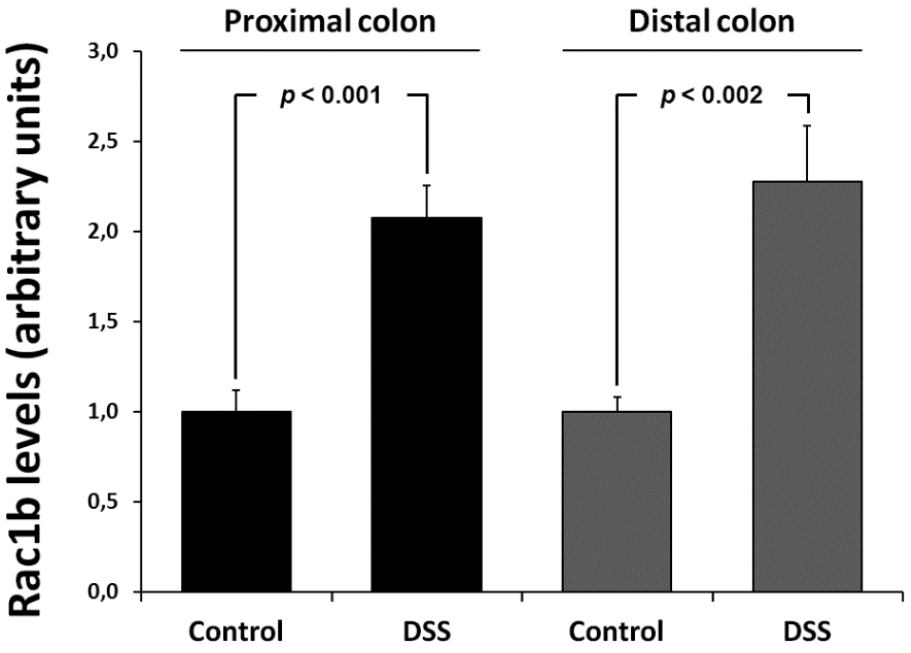
WT DSS



Colitis mouse model

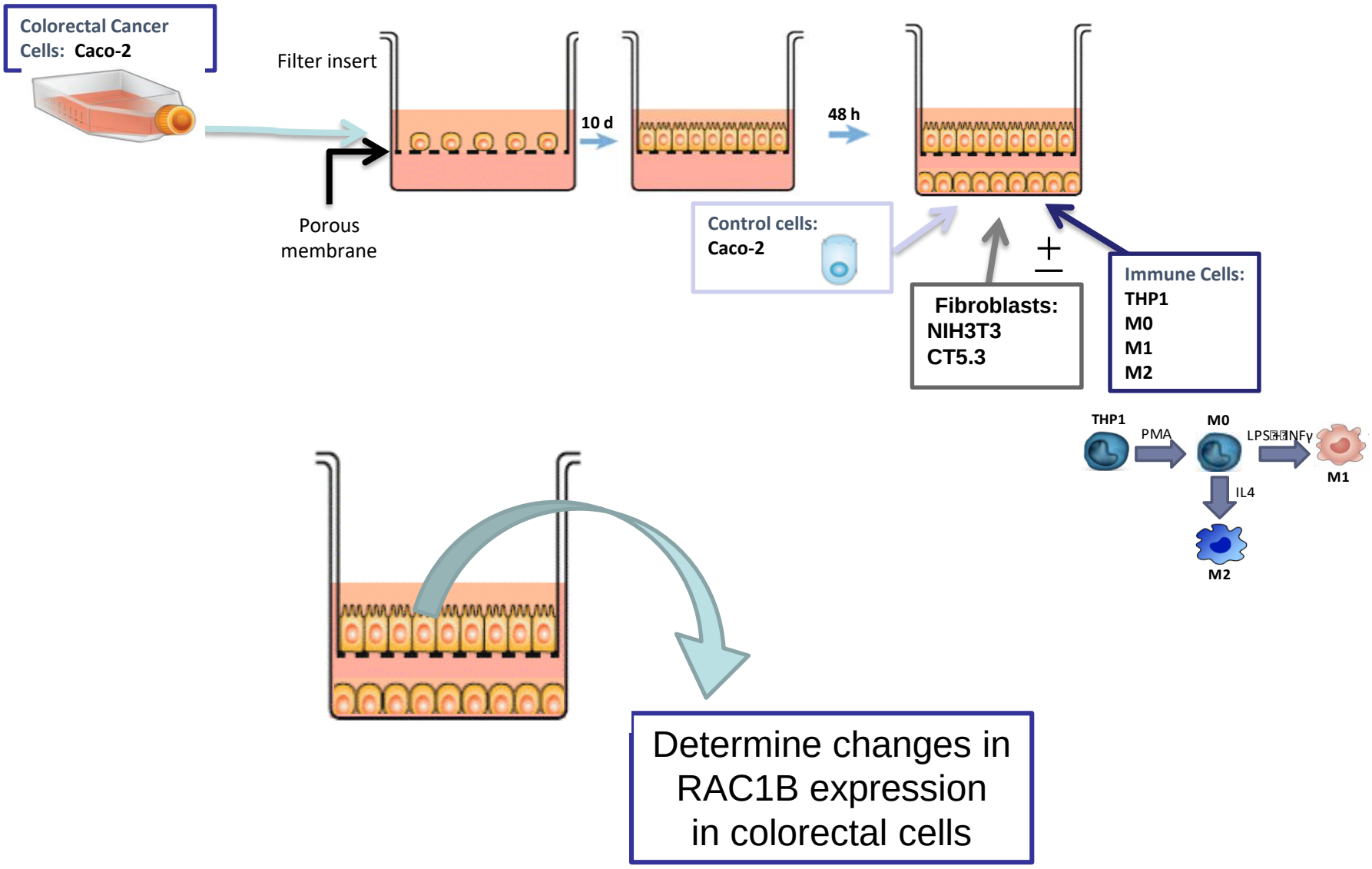
(mechanical mucosa irritation by DSS (dextran sulfate) in drinking water for 5 days)

(Collaboration Eric Chastre, INSERM Paris)



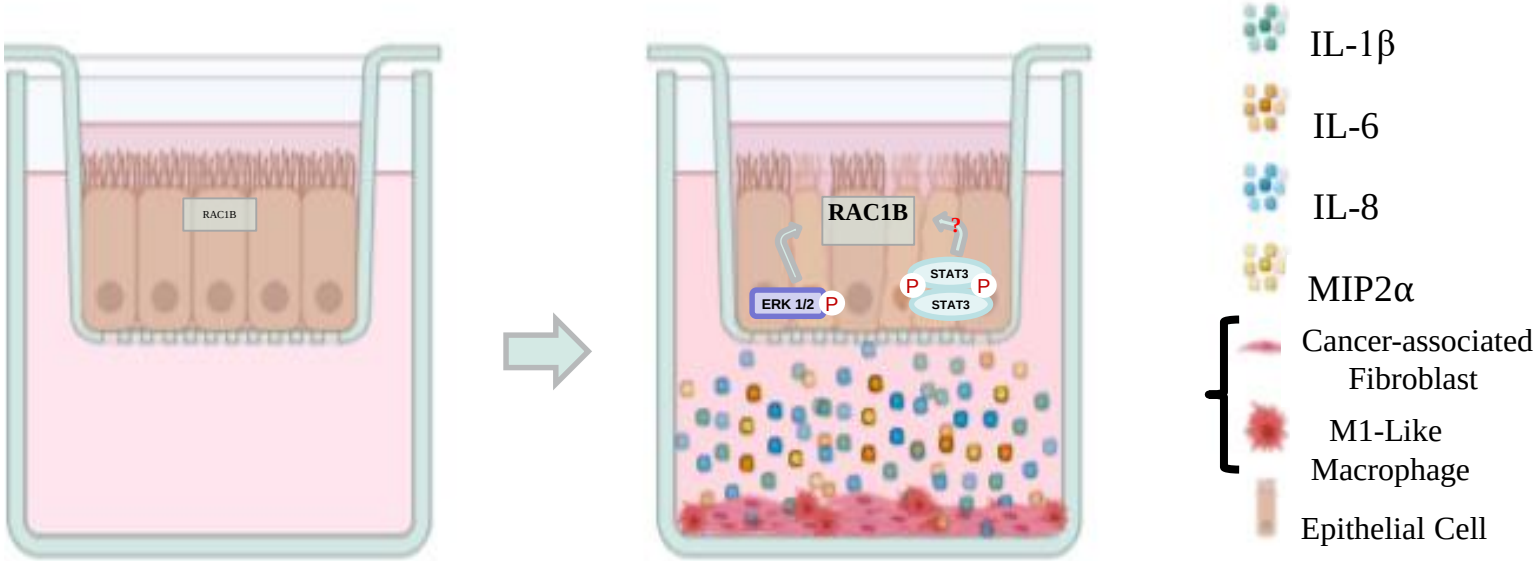
Neoplasia (2013) 15, 102–111

Identify whether inflammatory signals can induce RAC1B overexpression



Conclusions

- **Po-inflammatory IL-6 secreted into the medium triggers the increase in RAC1B in colon cells**
- **IL-6 activates signaling pathways in Caco-2 cells including STAT3 and MEK/ERK**



Pro-inflammatory signals from the microenvironment can induce RAC1B overexpression and promote tumorigenesis