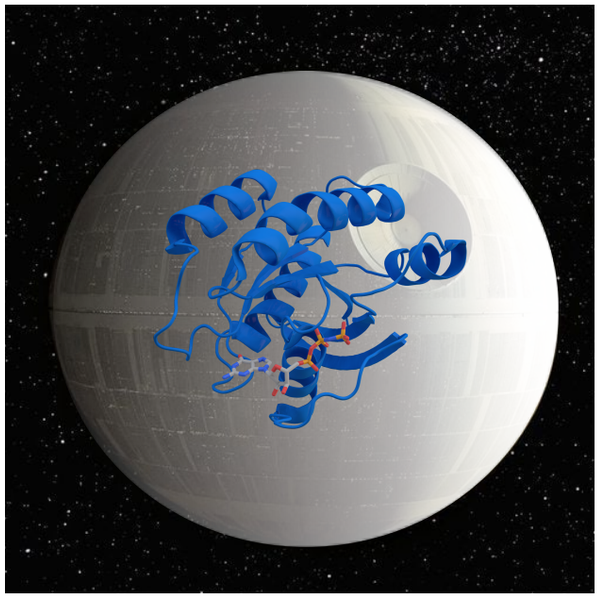
Oncogenes and the "Death Star" of Cancer



Sean Huth

MacMillan Group

Lit. Talk

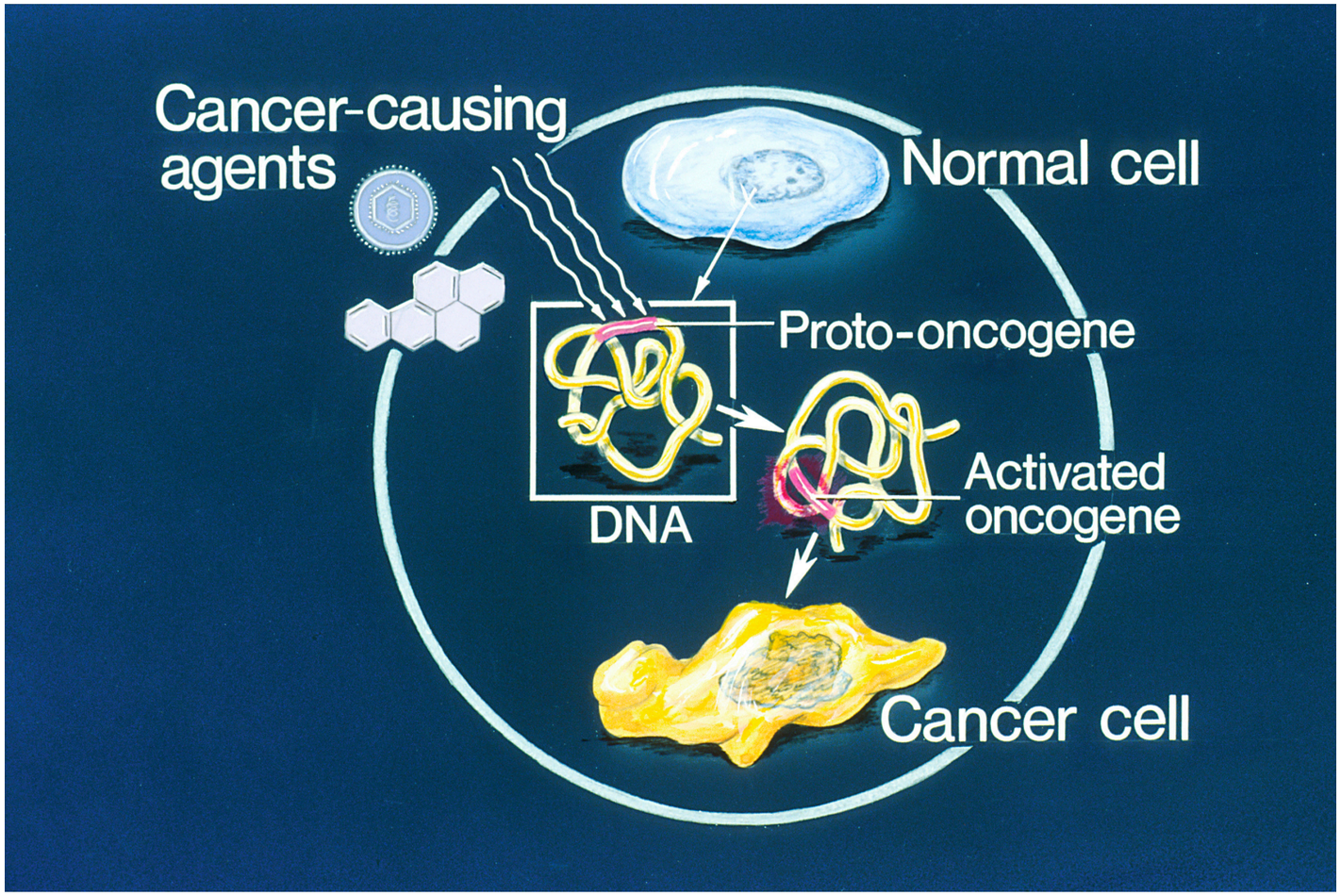
December 7th, 2021

Introduction

What is an Oncogene?

Mutated or over-expressed genes which contribute to causing cancer

Unmutated/normally expressed form referred to as a “proto-oncogene”



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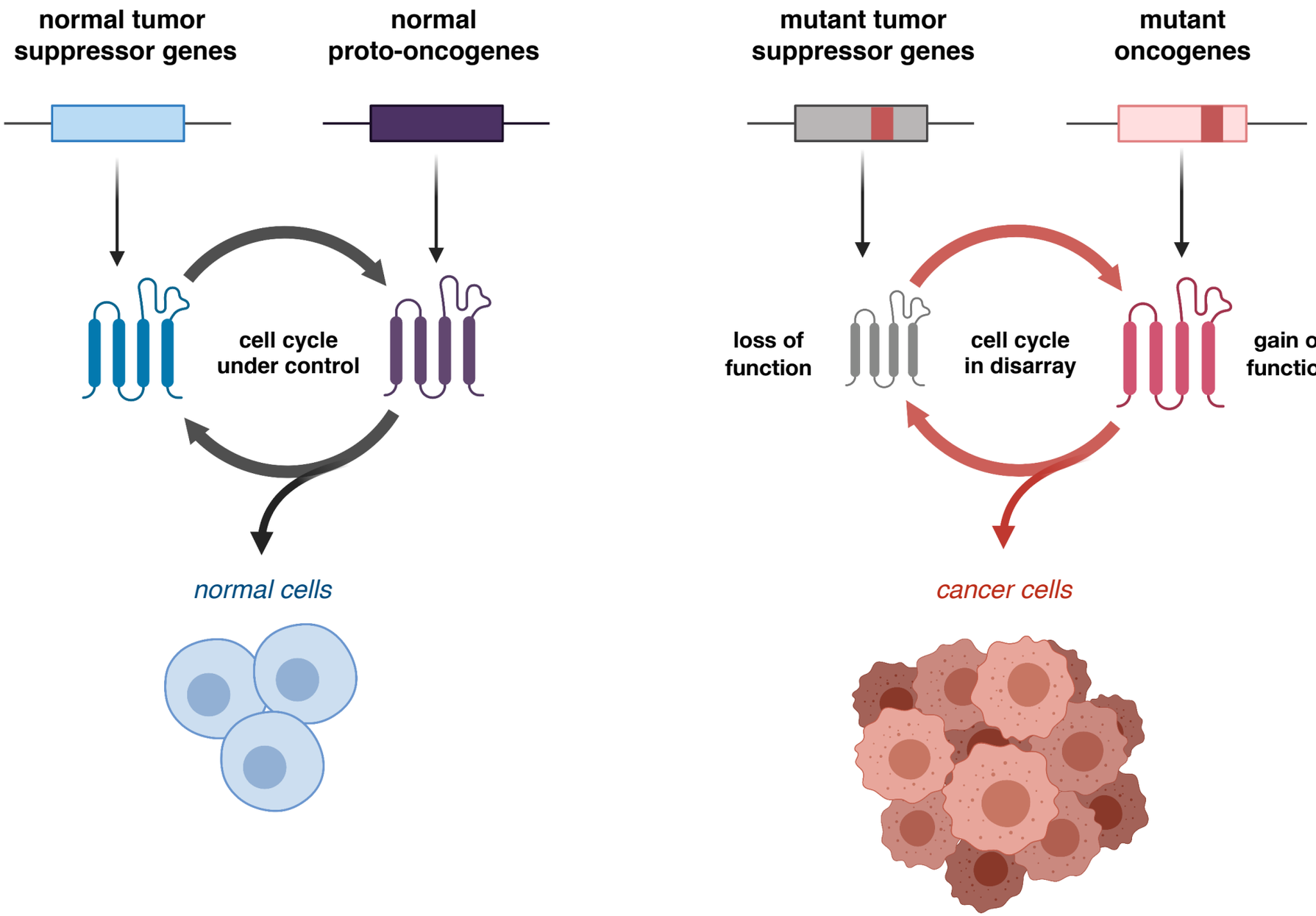

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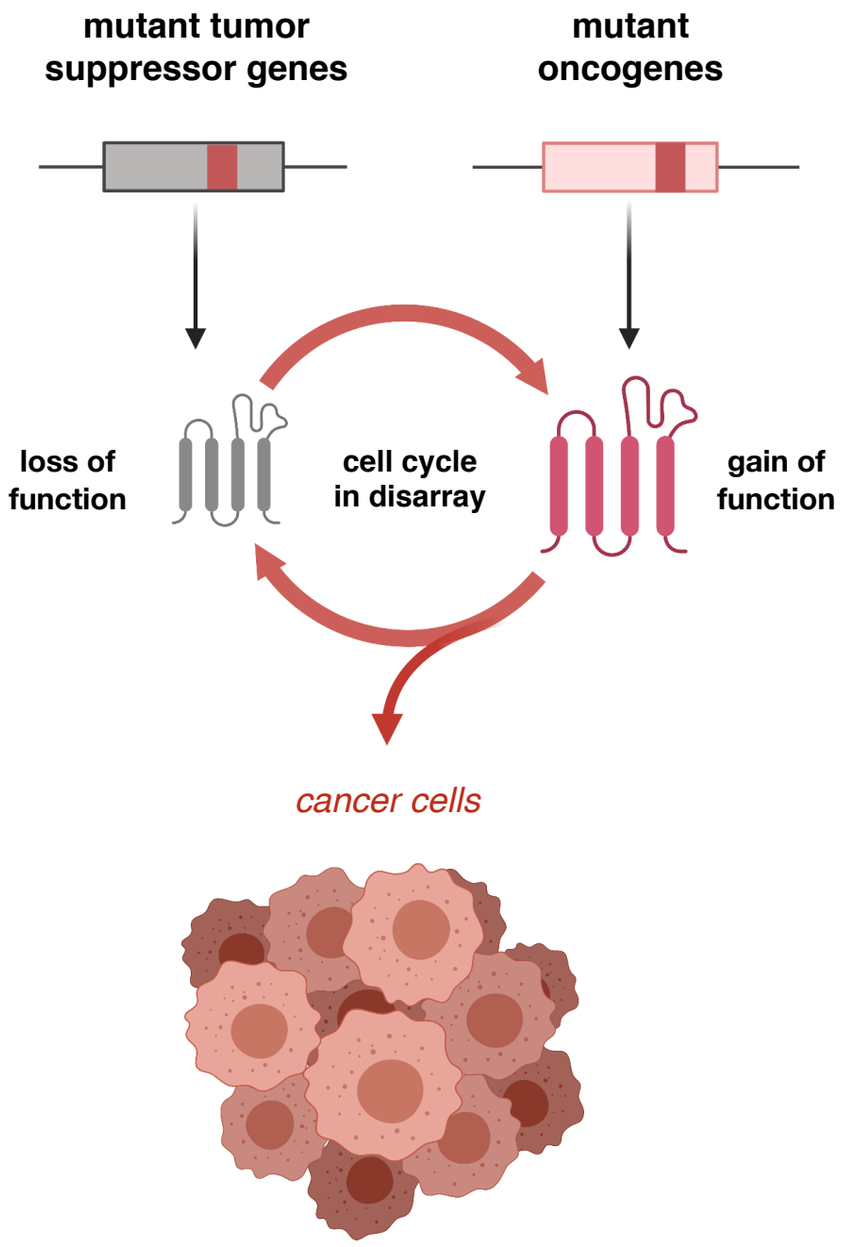

Cell division process as a car

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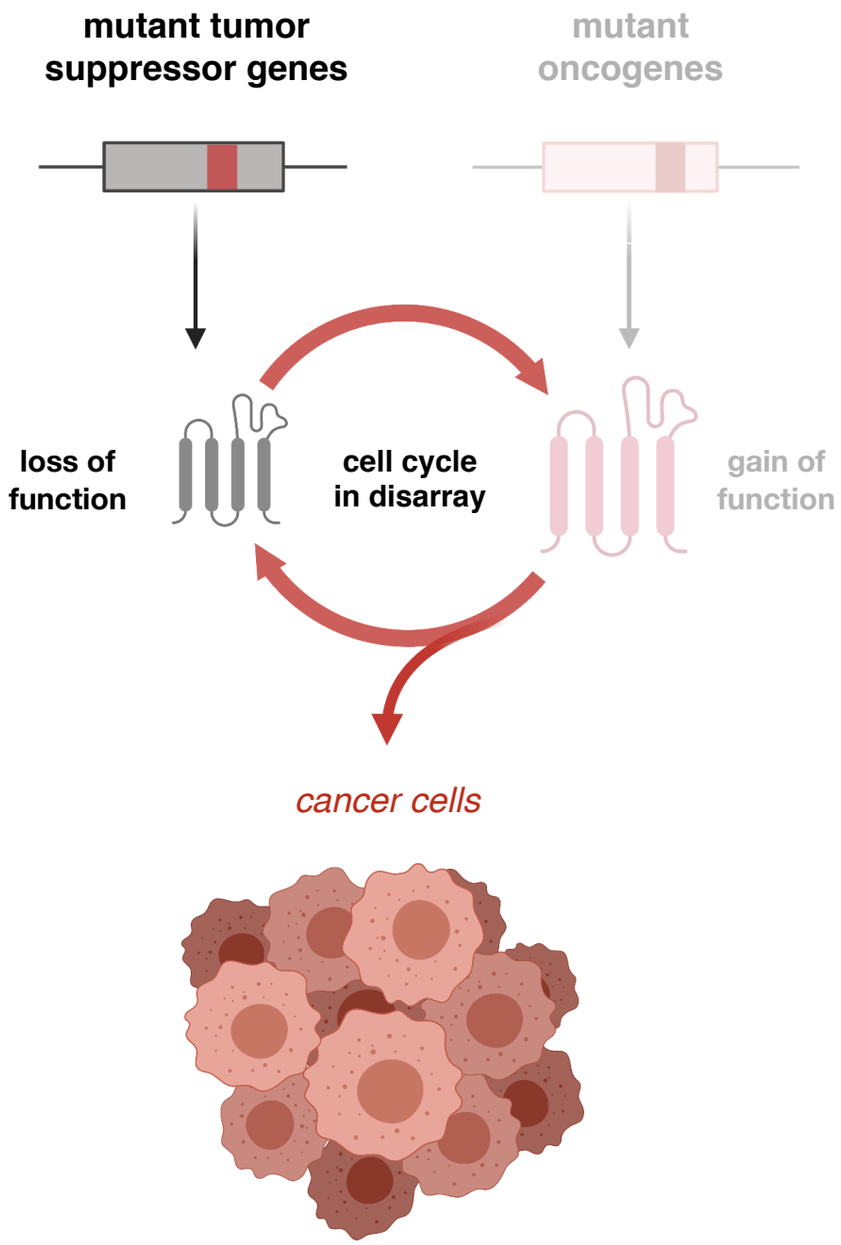

Cell division process as a car

brake doesn't work - unable to slow down/stop

tumor suppressor gene malfunction causes uncontrolled division

Introduction

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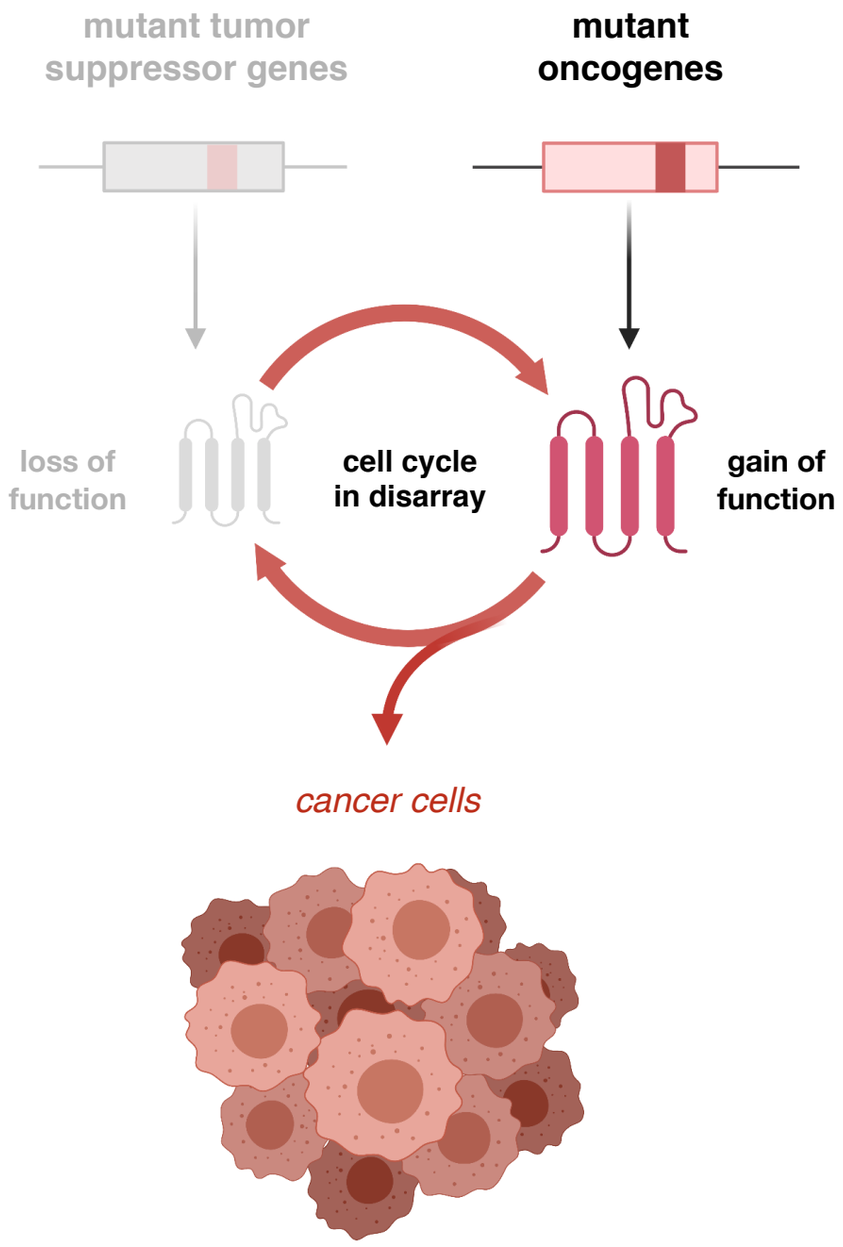
Mutated or over-expressed genes which contribute to causing cancer



Cell division process as a car

gas pedal stuck - uncontrolled acceleration

oncogenes cause uncontrolled division

Introduction

Classes of Oncogenes

Many classes of oncogenes with various mechanisms

Category	Examples	Gene function
growth factors, mitogens	c-sis	induces cell proliferation
receptor tyrosine kinases	EGFR, VEGFR, HER2	signalling for cell proliferation
cytoplasmic tyrosine kinases	src family, Abl, BTK	mediate responses to cell signals
serine, threonine kinases	Raf, CDKs	cell cycle signalling
regulatory GTPases	Ras family	cell proliferation pathway signalling
transcription factors	myc family	regulate transcription of proliferation genes

Introduction

How does a proto-oncogene become an oncogene?

Point mutations, deletions, or insertions

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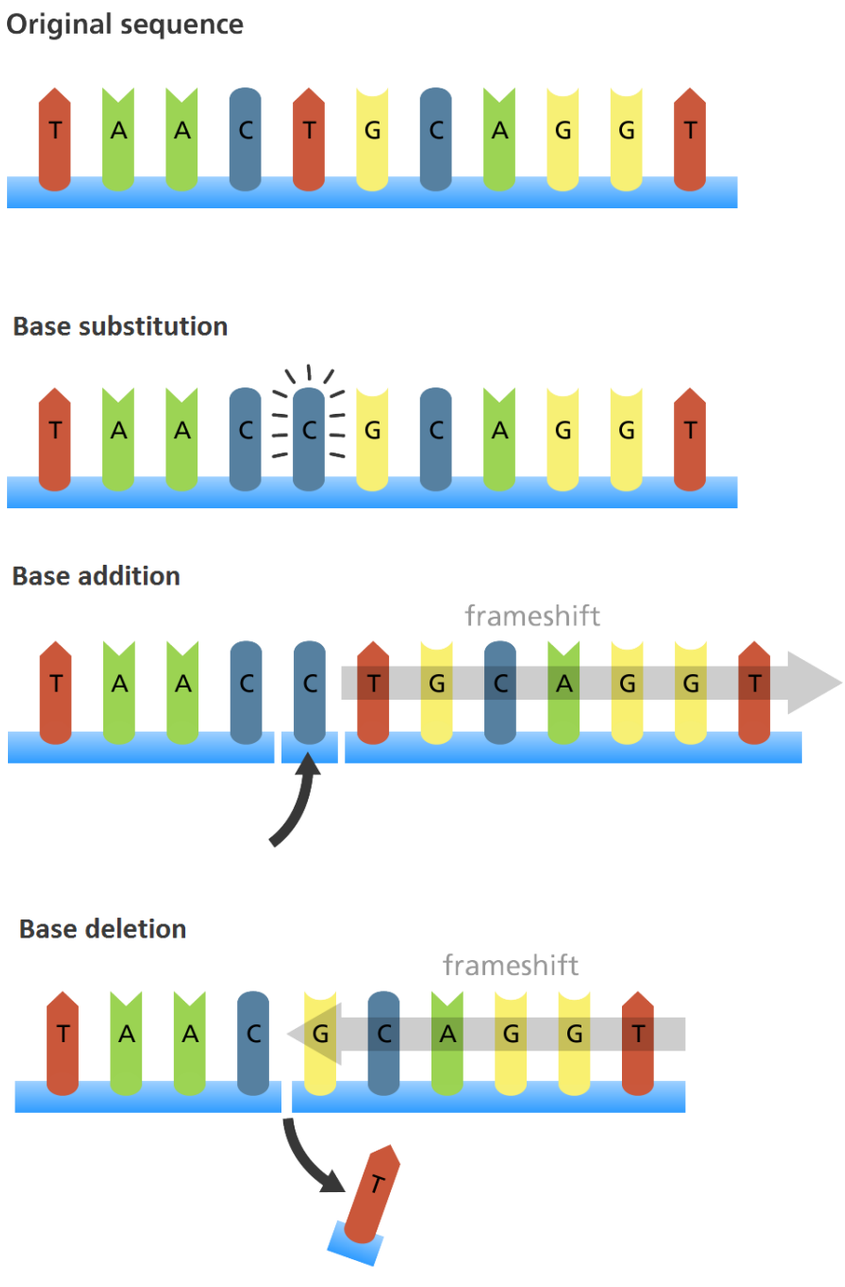
Point mutations, deletions, or insertions



Introduction

How does a proto-oncogene become an oncogene?

→ Point mutations, deletions, or insertions

External Causes:

Radiation

UV

ionizing radiation

Chemicals

alkylating reagents

polycyclic aromatic hydrocarbons (PAH)

Biological Agents

HPV

Helicobacter pylori

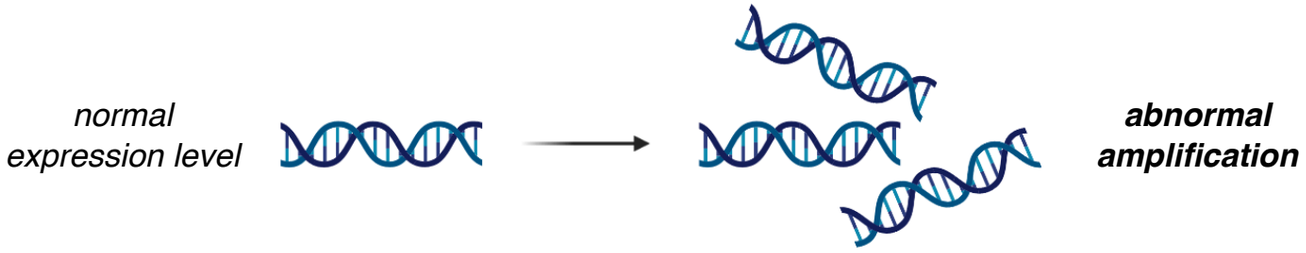
Sturm, N. “DNA Damage and Repair” *CSU*. **2019**.

Introduction

How does a proto-oncogene become an oncogene?

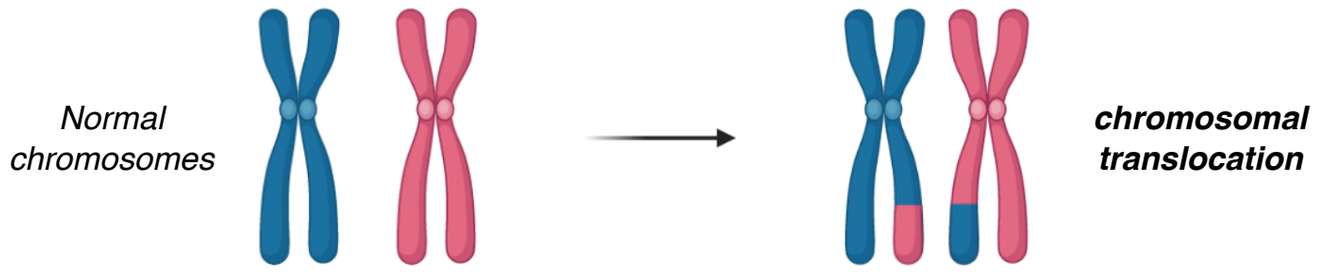
→ Point mutations, deletions, or insertions

→ Gene amplification leading to extra copies of a protein



→ Chromosomal translocation leading to higher expression

→ Chromosomal translocation leading to a fusion protein



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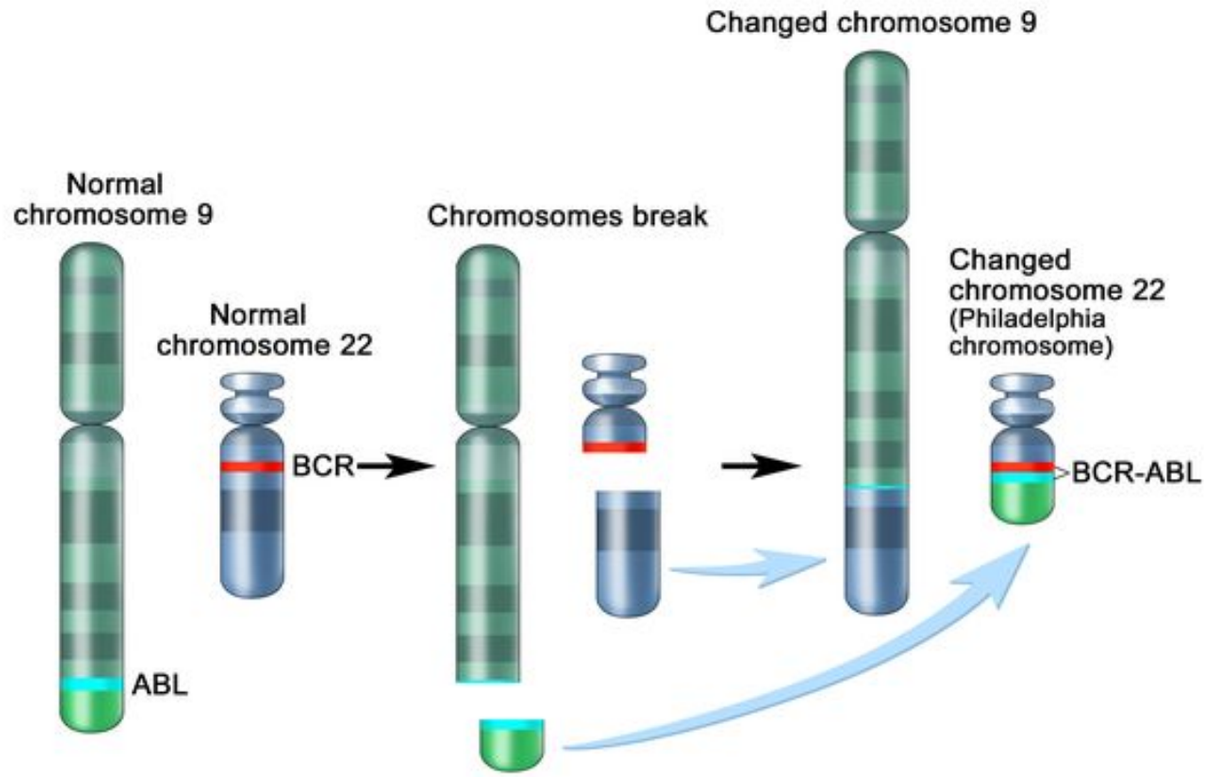
How does a proto-oncogene become an oncogene?

→ Chromosomal translocation leading to a fusion protein



BCR-ABL fusion protein is an oncogene

Winslow, T. NIH. *National Cancer Institute*. Web.

Outline

- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib
- Future directions/Outlook

Outline

- Common oncogenes and their functions
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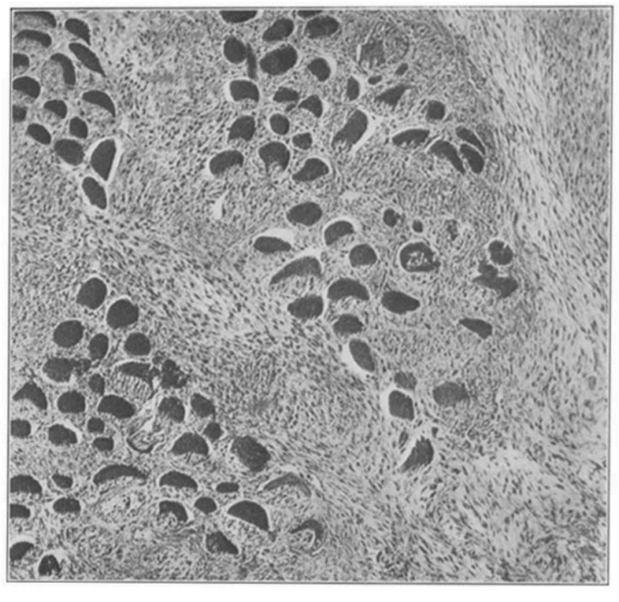
An overview of common oncogenes

src

1911 - Discovery of a “filterable agent” that causes sarcoma in chickens

- Chicken tumor tissue transplant - 1.5 cm tumors within a week
- Isolate tumor tissue and filter it to be cell free

10 days to 3 weeks - tumors appear



Suggested transmission of cancer via an infectious agent

↓

first evidence of the v-src viral oncogene

Peyton Rous

Rous, P. *J. Exp. Med.* **1911**, 13 (4): 397–411.

An overview of common oncogenes

src

1911 - Discovery of a “filterable agent” that causes sarcoma in chickens

- Controversial finding at the time - cancer thought to be endogenous

> *“But, my dear fellow, don’t you see, this can’t be cancer because you know its cause”*

Peyton Rous

Rous, P. *J. Exp. Med.* **1911**, 13 (4): 397–411.

An overview of common oncogenes

src

1911 - Discovery of a “filterable agent” that causes sarcoma in chickens

- Controversial finding at the time - cancer thought to be endogenous
- Wasn’t widely accepted until 10 years later
- 40 years later - Nobel Prize in Medicine

1966

"for his discovery of tumour-inducing viruses."

Peyton Rous

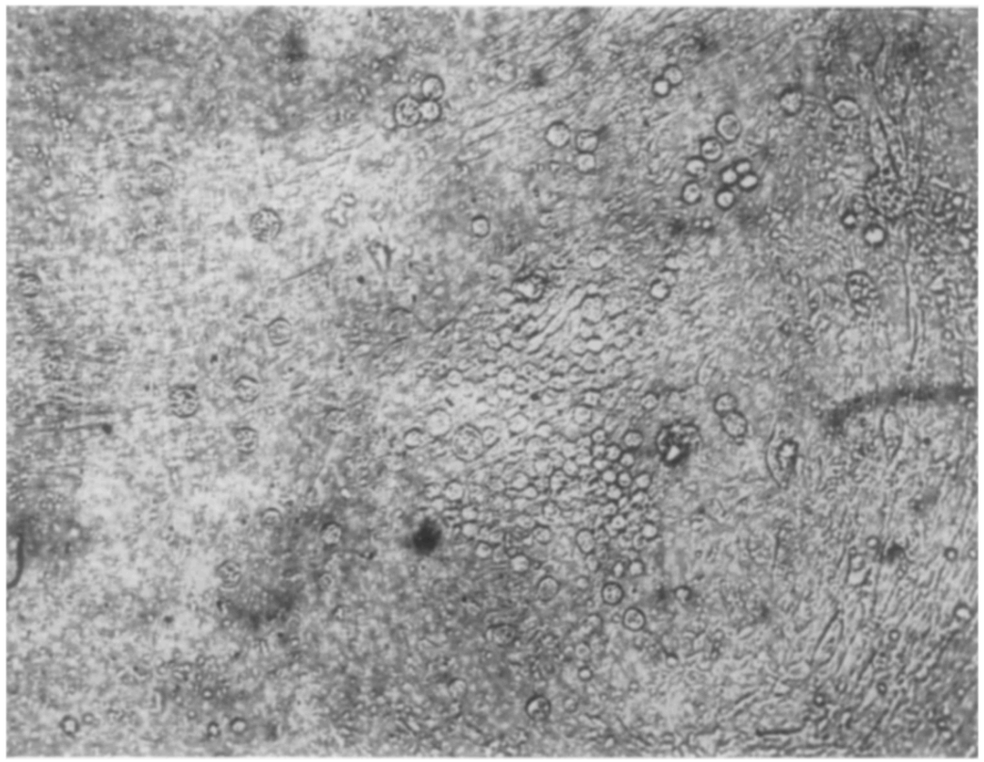
Rous, P. *J. Exp. Med.* **1911**, 13 (4): 397–411.

An overview of common oncogenes

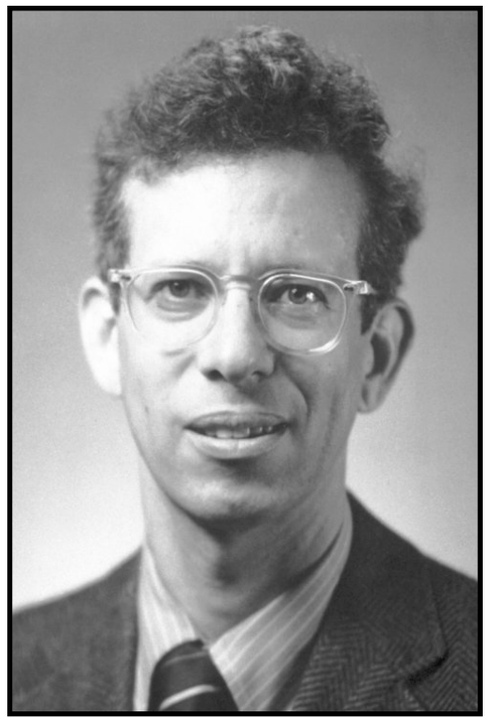
src

Late 1950s - Discovery that genetic property of viruses causes morphology alterations

- Developed the first RSV assays using chicken embryo cells
- Recognized cancer morphology changes in infected cells



Secondary chicken embryo fibroblasts, 100X



Howard Temin

Temin, H. *Virology*. **1960**, 10, 2, 182-197.

Temin, H; Rubin, H. *Virology*. **1958**, 6, 3, 669-688.

An overview of common oncogenes

src

Late 1950s - Discovery that genetic property of viruses causes morphology alterations

- Developed the first RSV assays using chicken embryo cells
- Recognized cancer morphology changes in infected cells
- Different viral mutations caused different morphologies
- Important conclusion drawn from study in discussion:

"There are two means by which RSV could operate:
either it could contribute genetic information directly to the cell
to enable it to become tumorous,
or it could activate a tumorous state of the cell."

Howard Temin

Temin, H. *Virology*. **1960**, 10, 2, 182-197.

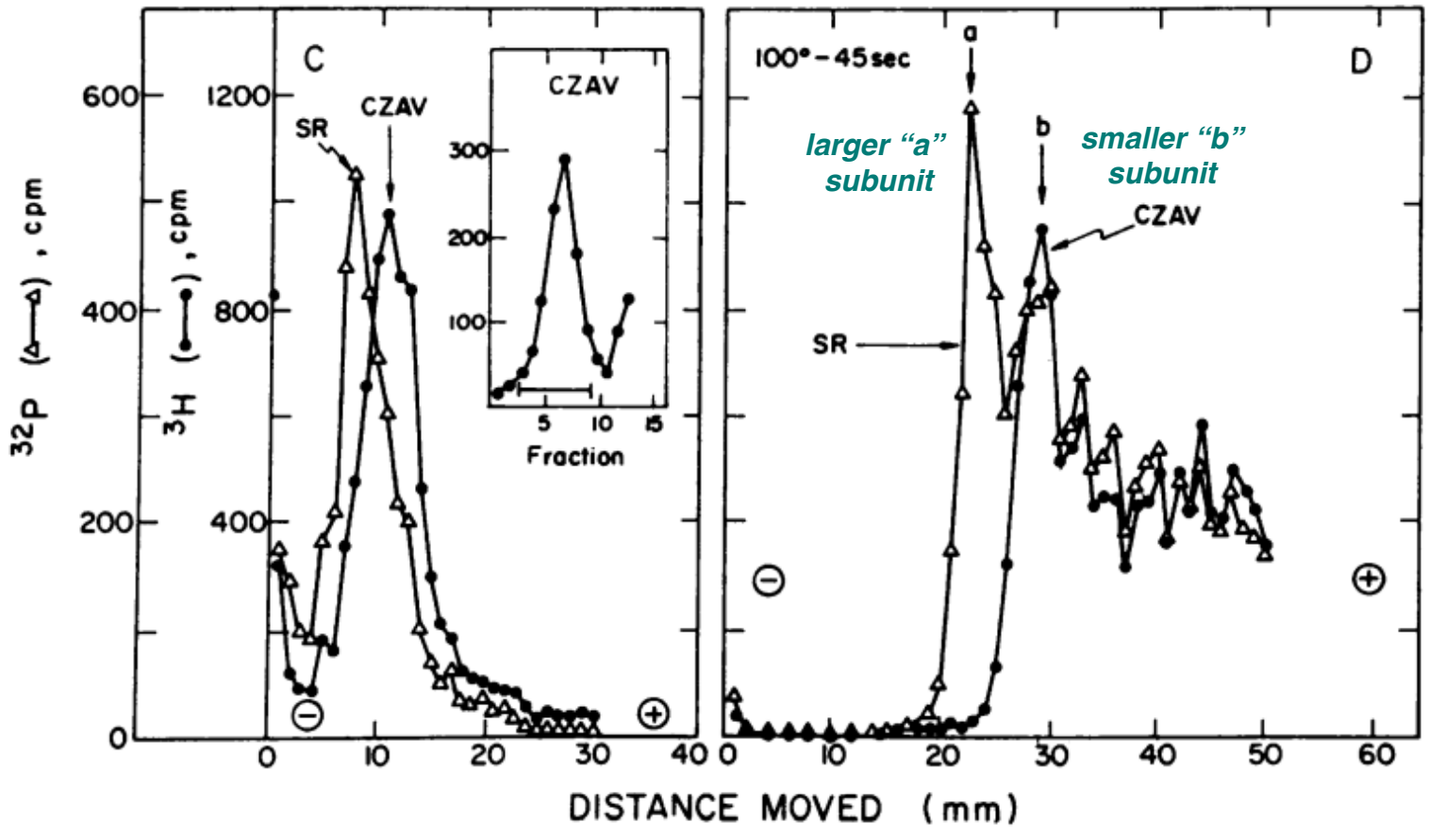
Temin, H; Rubin, H. *Virology*. **1958**, 6, 3, 669-688.

An overview of common oncogenes

src

1960-1970s - Discovery of the physical v-src gene

- Transforming and non-transforming viral strains known
- Gel electrophoresis revealed size differences between their RNAs



Peter K. Vogt
Peter Duesberg

Duesberg P. H.; Vogt, P. K. *Proc. Natl. Acad. Sci.* **1970**, 67, 4, 1673-1680.

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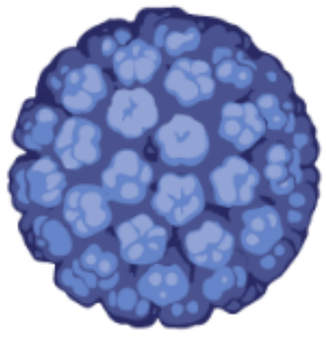
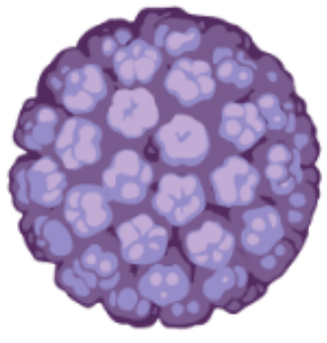
Transforming



a and b

Transformation deficient



b only

Peter K. Vogt
Peter Duesberg

"a = b + x"

it was known that b was a part of a

Duesberg P. H.; Vogt, P. K. *Proc. Natl. Acad. Sci*. **1970**, 67, 4, 1673-1680.

An overview of common oncogenes

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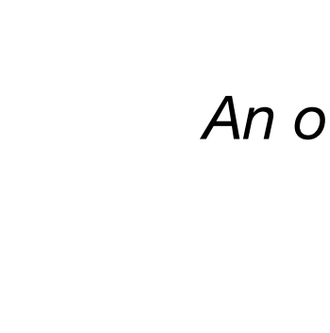
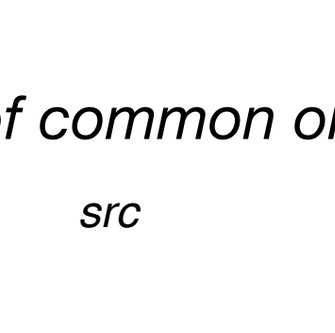


a and b

Transformation deficient



b only

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Peter Duesberg

“a = b + x”

what is x?

Duesberg P. H.; Vogt, P. K. *Proc. Natl. Acad. Sci.* **1970**, 67, 4, 1673-1680.

An overview of common oncogenes

src

1960-1970s - Discovery of the physical v-src gene

- Transforming and non-transforming viral strains known
- Gel electrophoresis revealed size differences between their RNAs
- Further validated via oligonucleotide fingerprinting
- Indicative of a single gene - this is the v-src gene

Peter K. Vogt
Peter Duesberg

v-src! ⟵ **what is x?**

Duesberg P. H.; Vogt, P. K. *Proc. Natl. Acad. Sci.* **1970**, 67, 4, 1673-1680.

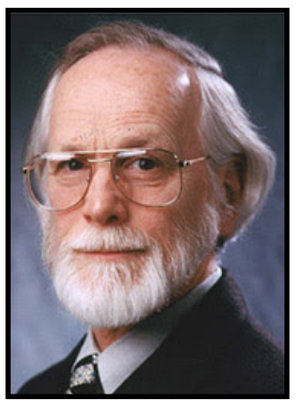
An overview of common oncogenes

src

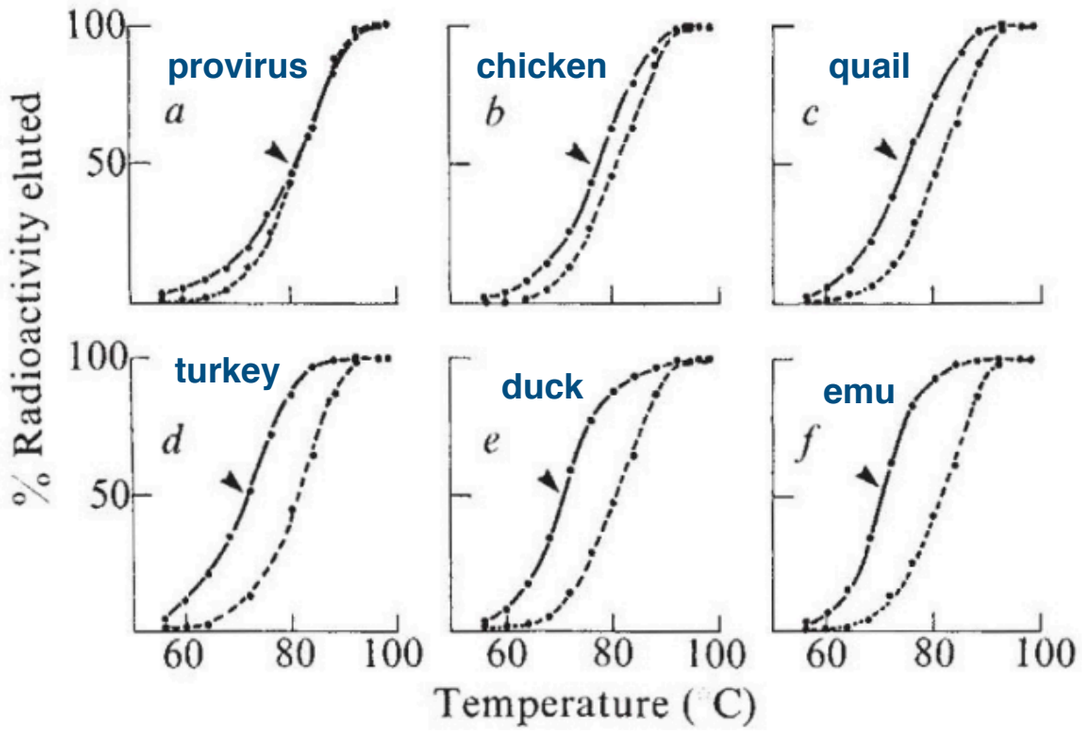
1976 - The discovery of v-src as an oncogene

- Generation of a complementary DNA probe to viral v-src DNA
- Testing against a variety of native avian DNAs
- DNA hybridized with a variety of avian DNAs

Harold Varmus



Mike Bishop



radioactive elution of tritium labeled DNA measured

stability of DNA duplexes

higher stability indicated greater duplex formation

Stehelin, D.; Varmus, H.; Bishop, M. *Nature*. **1976**, 260, 170-173.

An overview of common oncogenes

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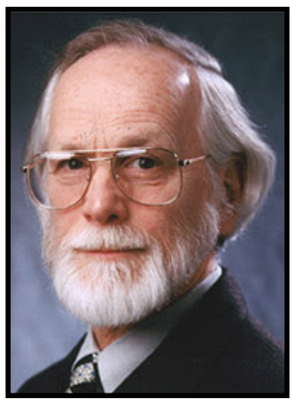


Mike Bishop

Table 1 Homology between $cDNA_{sarc}$ and normal DNAs

Assay	Hybridisation conditions		Extent of reaction between $cDNA_{sarc}$ and DNA from						
	$[Na^+]$	Temperature	Chicken	Quail	Turkey	Duck	Emu	Mouse	Calf
S1	0.9 M	68°	52%	46%	48%	45%	24%	<2%	<2%
HAP	0.9 M	68°					36%		<5%
HAP	1.5 M	59°					54%		

Stehelin, D.; Varmus, H.; Bishop, M. *Nature*. **1976**, 260, 170-173.

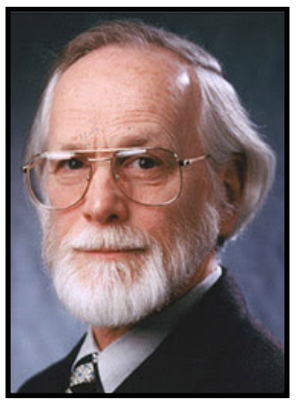
An overview of common oncogenes

src

1976 - The discovery of v-src as an oncogene

- Generation of a complementary DNA probe to viral v-src DNA
- Testing against a variety of native avian DNAs
- DNA hybridized with a variety of avian DNAs
- Suggested cellular origin of the v-src gene

Harold Varmus



Mike Bishop

1989

"for their discovery of the cellular origin of retroviral oncogenes."

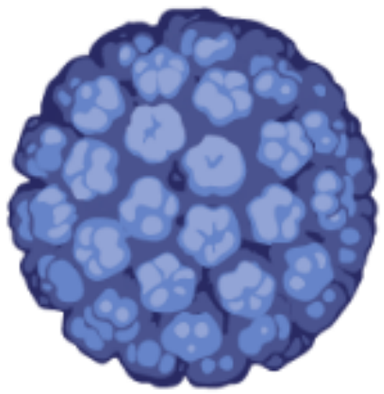
Stehelin, D.; Varmus, H.; Bishop, M. *Nature*. **1976**, 260, 170-173.

An overview of common oncogenes

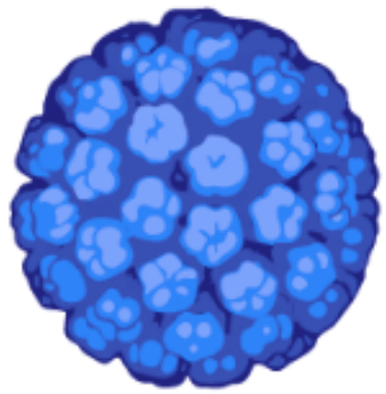
src

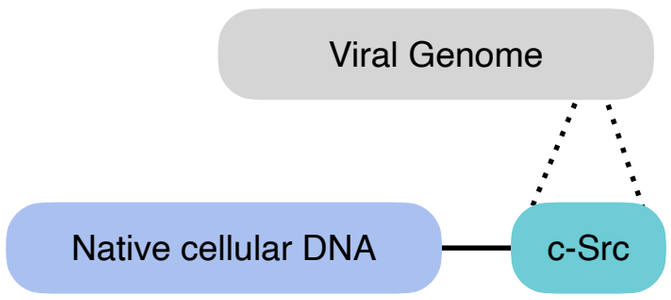
Origin of src revealed

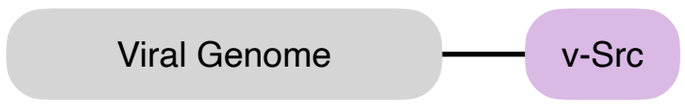
rous sarcoma virus
acquires mutated c-src
from native genome



this new v-src
causes cancer in chickens
due to mutations







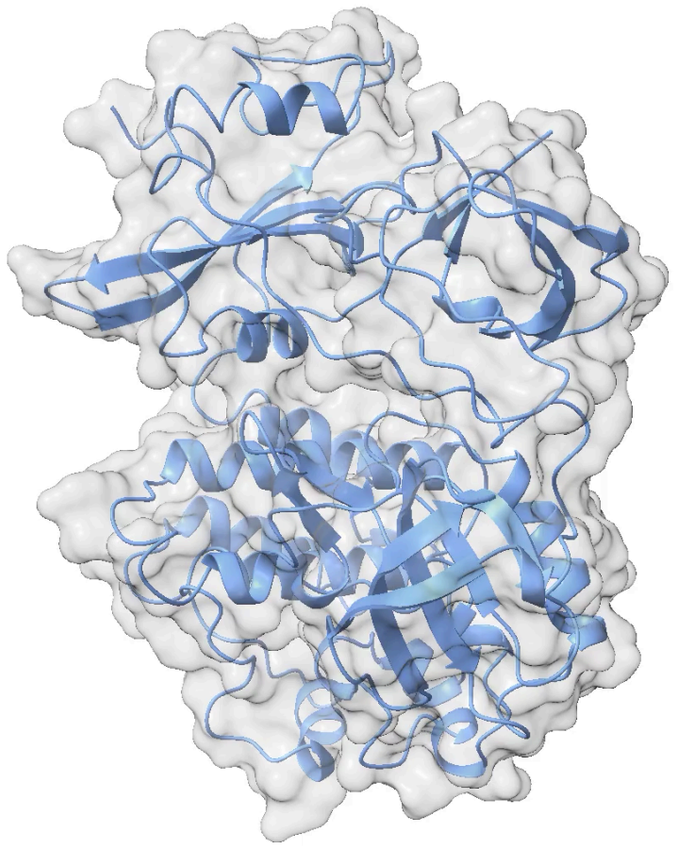
mutation causes oncogenic activity

An overview of common oncogenes

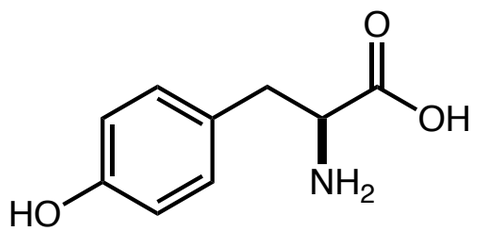
src

Late 1970s - Src protein identity and function

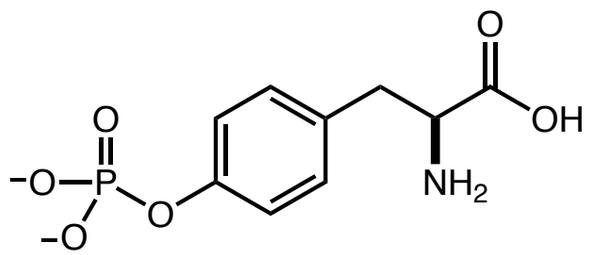
Src



- First discovered tyrosine kinase



src, ATP



Eckhart, W. Hutchinson, M.A. Hunter, T. *Cell*. **1979**, 18, 925-933.

An overview of common oncogenes

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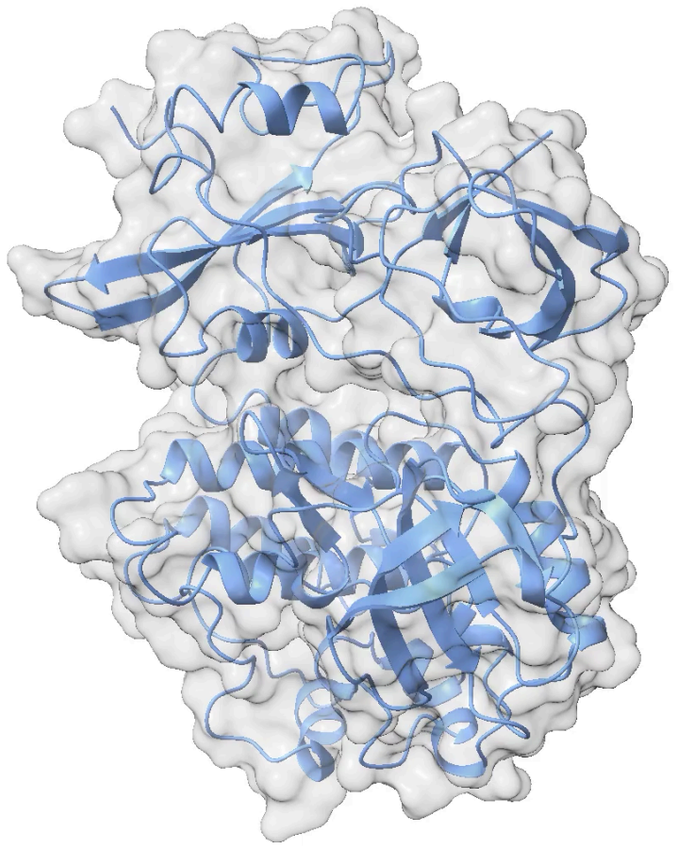
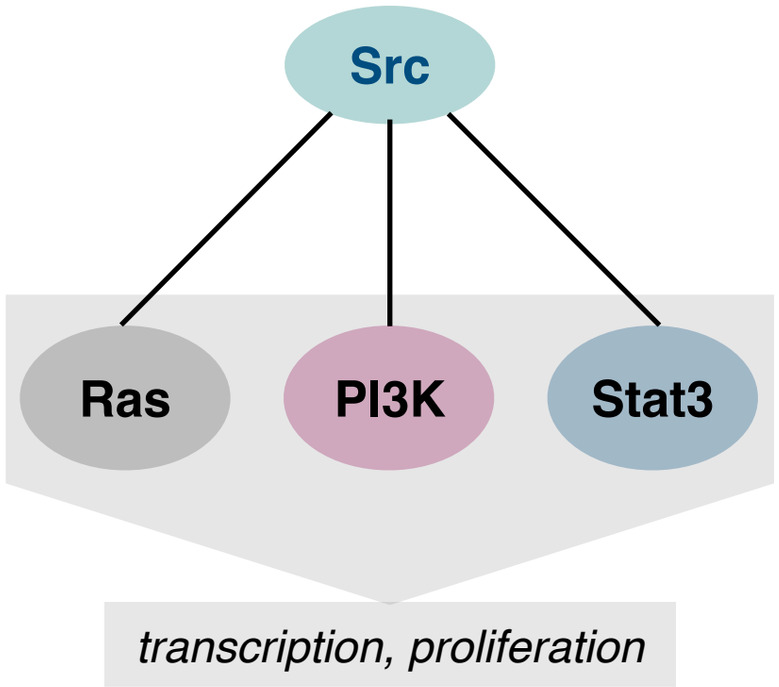


- First discovered tyrosine kinase
- Signal transducer - proliferation, differentiation



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An overview of common oncogenes

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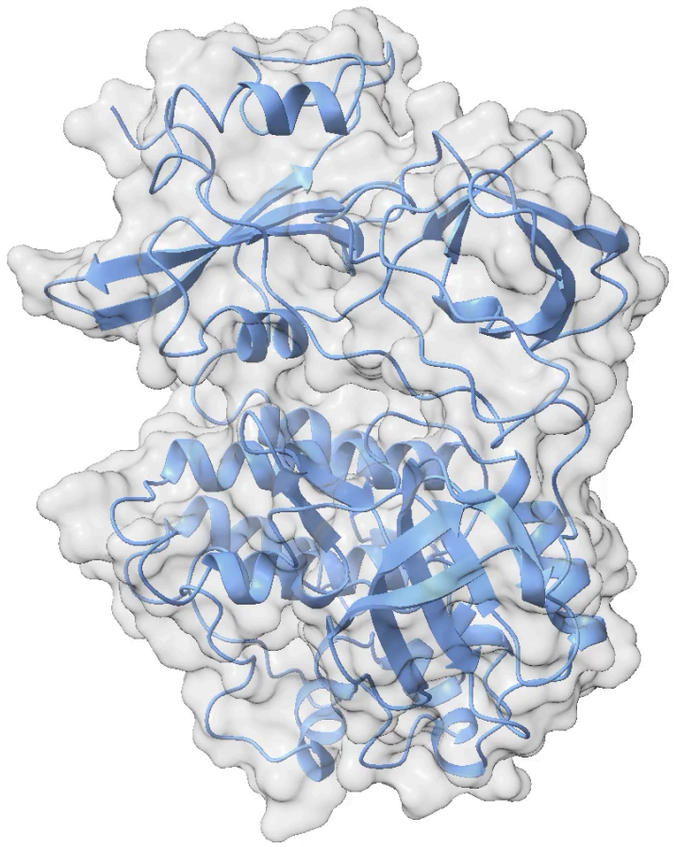
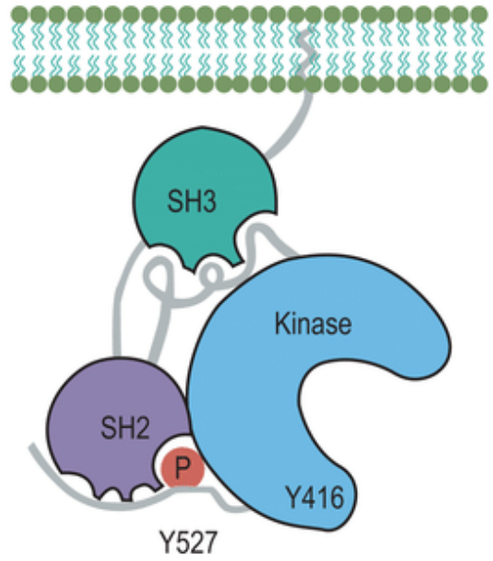


- First discovered tyrosine kinase
- Signal transducer - proliferation, differentiation
- c-Src has inhibitory phosphorylation site - Y527



dephosphorylation activates c-Src

Hunter, T. *Cell*. **1987**, 49, 1-4.

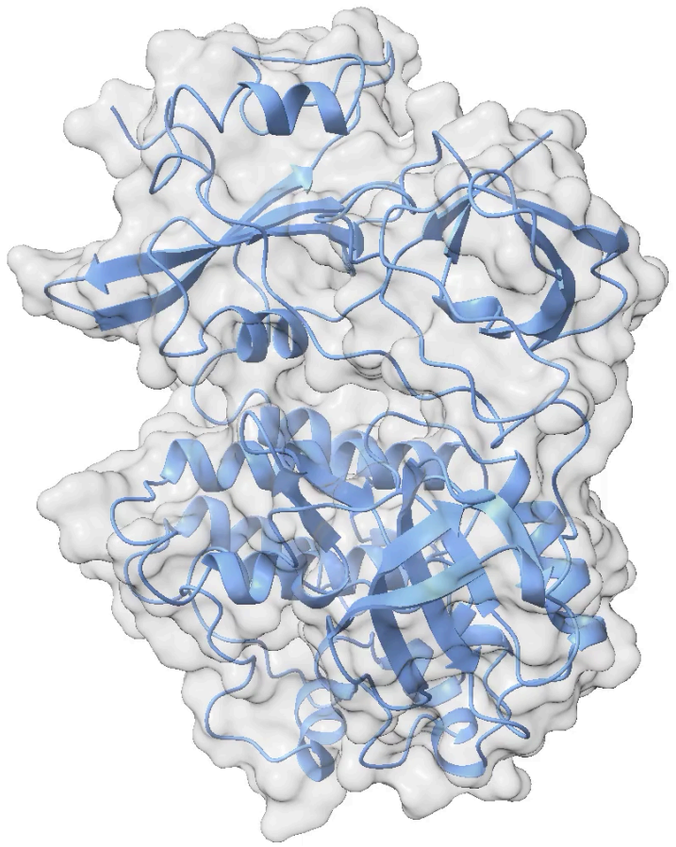
Dunn, E. et al. *The Oncologist*. **2009**, 14, 7, 667-78.

An overview of common oncogenes

src

Late 1970s - Src protein identity and function

Src



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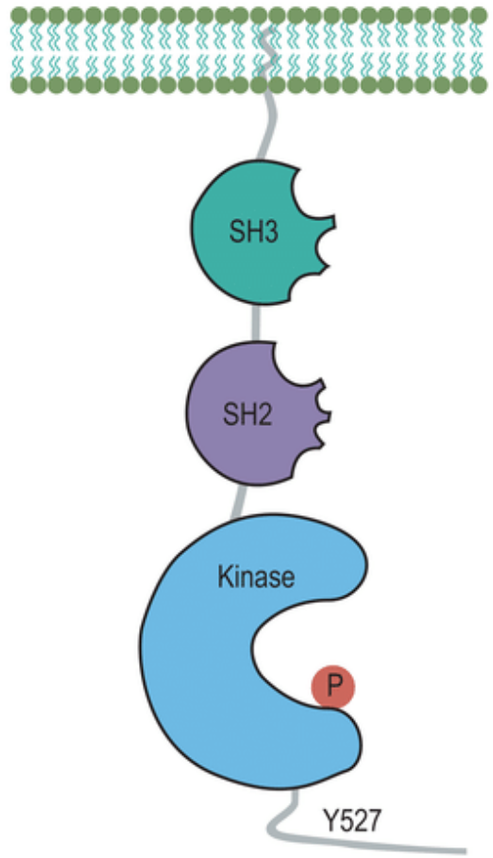

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An overview of common oncogenes

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Late 1970s - Src protein identity and function

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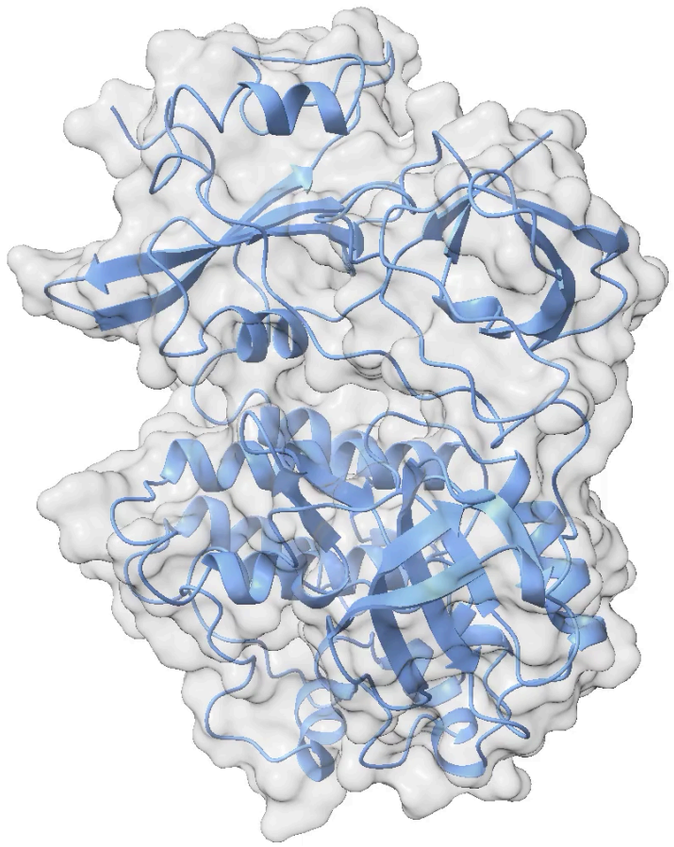


- First discovered tyrosine kinase
- Signal transducer - proliferation, differentiation
- c-Src has inhibitory phosphorylation site - Y527
- v-Src lacks this inhibitory site

→ ***"constitutionally active"* Src**

uncontrolled signalling,
uncontrolled growth

currently evidence for Src role in human tumor proliferation, research is ongoing

Hunter, T. *Cell*. **1987**, 49, 1-4.

Outline

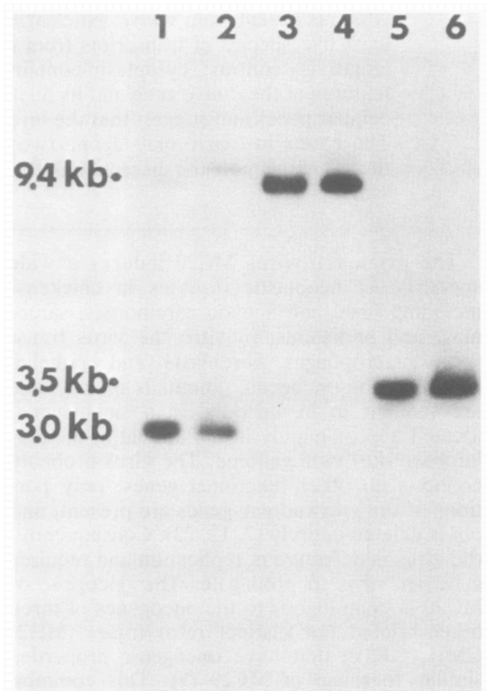
- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib
- Future directions/Outlook

An overview of common oncogenes

myc

Discovery of myc as an oncogene

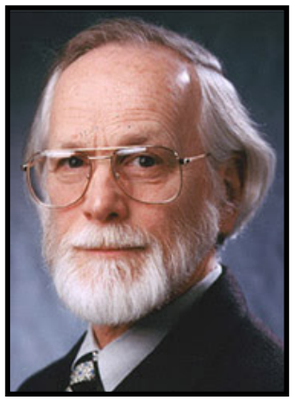
- First described in chicken viruses - similar to src
- DNA hybridization experiments - heteroduplex formation with native DNA



cloning of chromosomal myc DNA

↓

comparison of native DNA to cloned plasmid



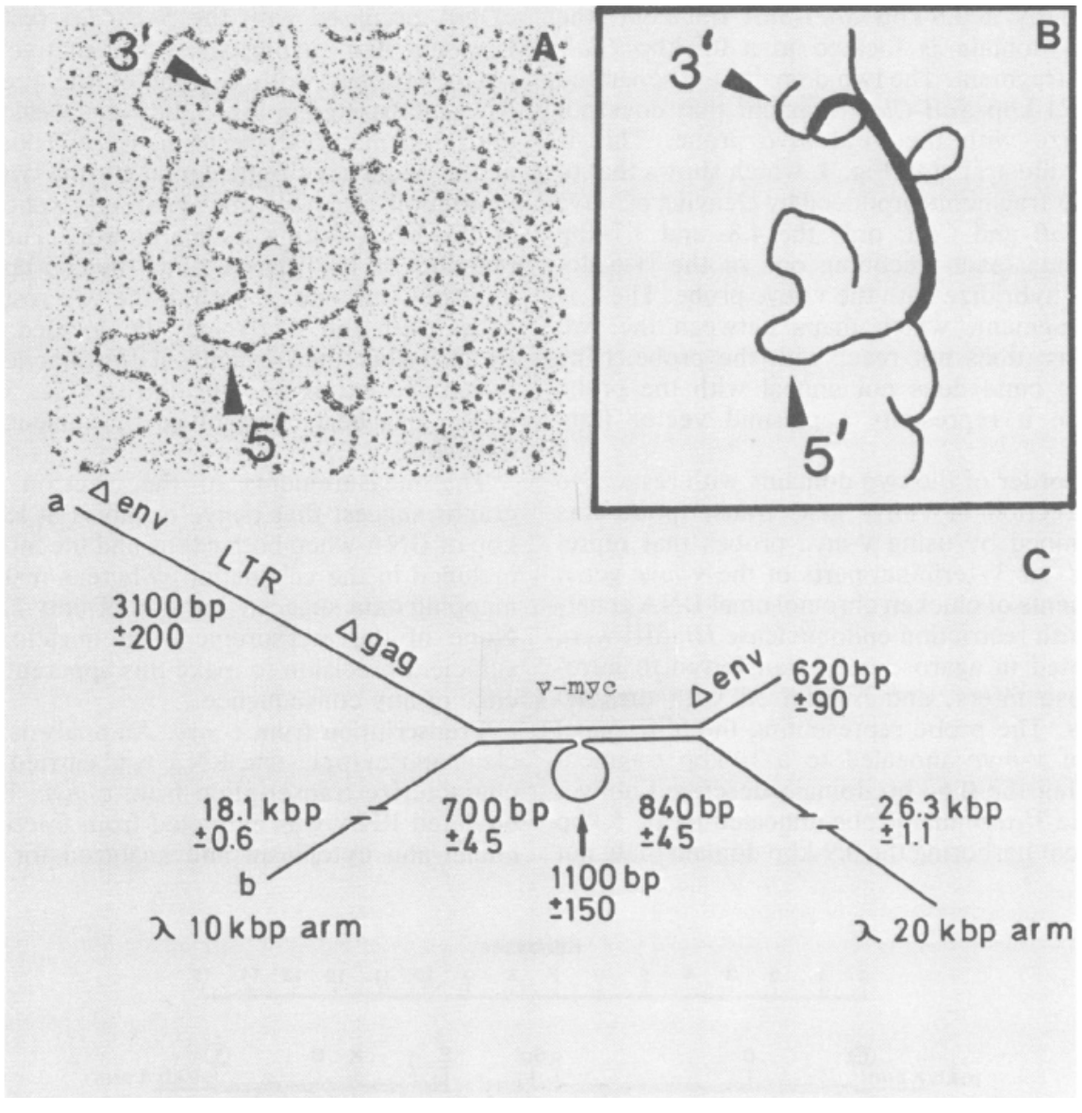
Mike Bishop

Vennstrom, B.; Sheiness, D.; Zabielski, J.; Bishop, M. *Journal of Virology*. **1982**, 42, 3, 773-779.

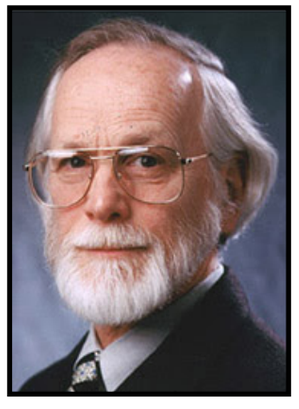
An overview of common oncogenes

myc

Discovery of myc as an oncogene



Heteroduplex formation with native DNA showed cellular origin for myc



Mike Bishop

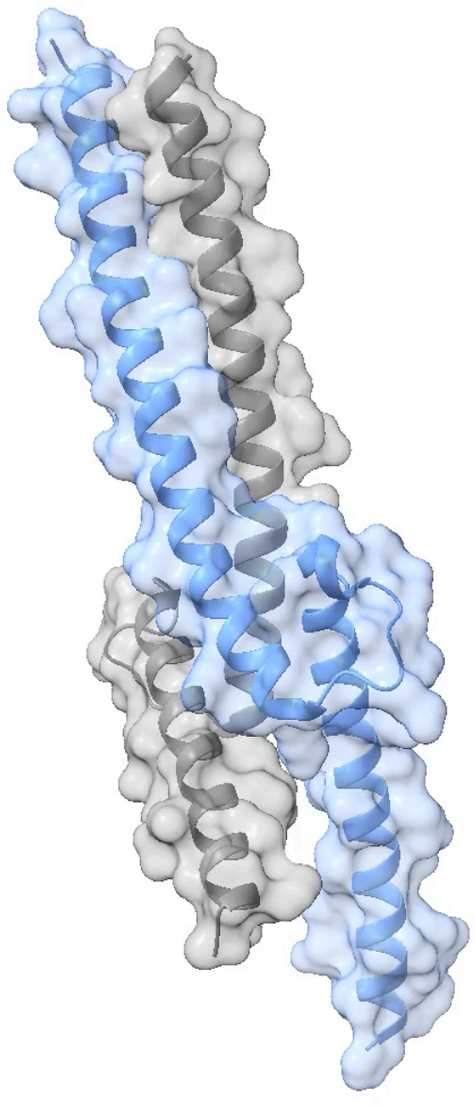
Vennstrom, B.; Sheiness, D.; Zabielski, J.; Bishop, M. *Journal of Virology*. **1982**, 42, 3, 773-779.

An overview of common oncogenes

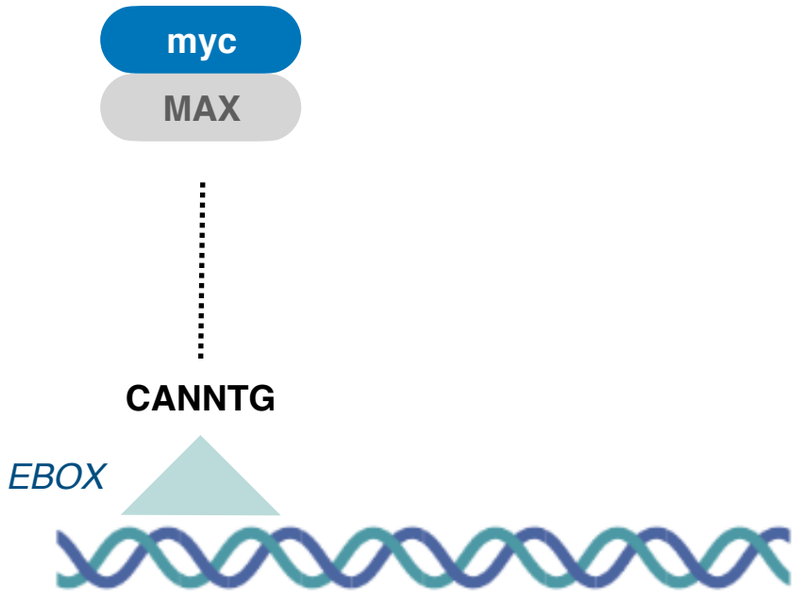
myc

myc protein identity and function

myc - MAX



- transcription factor - regulates over 1500 genes
- Forms a dimer with MAX to form a helix like structure
- Recognizes and binds to EBOX sequences in DNA



Mason, J. et al. *Molecular Cancer*. **2021**, 20, 3.

An overview of common oncogenes

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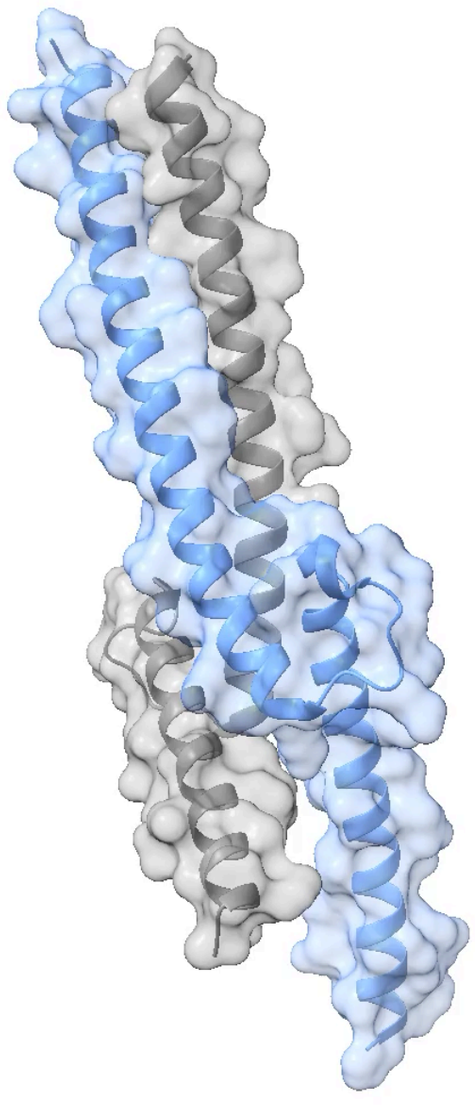

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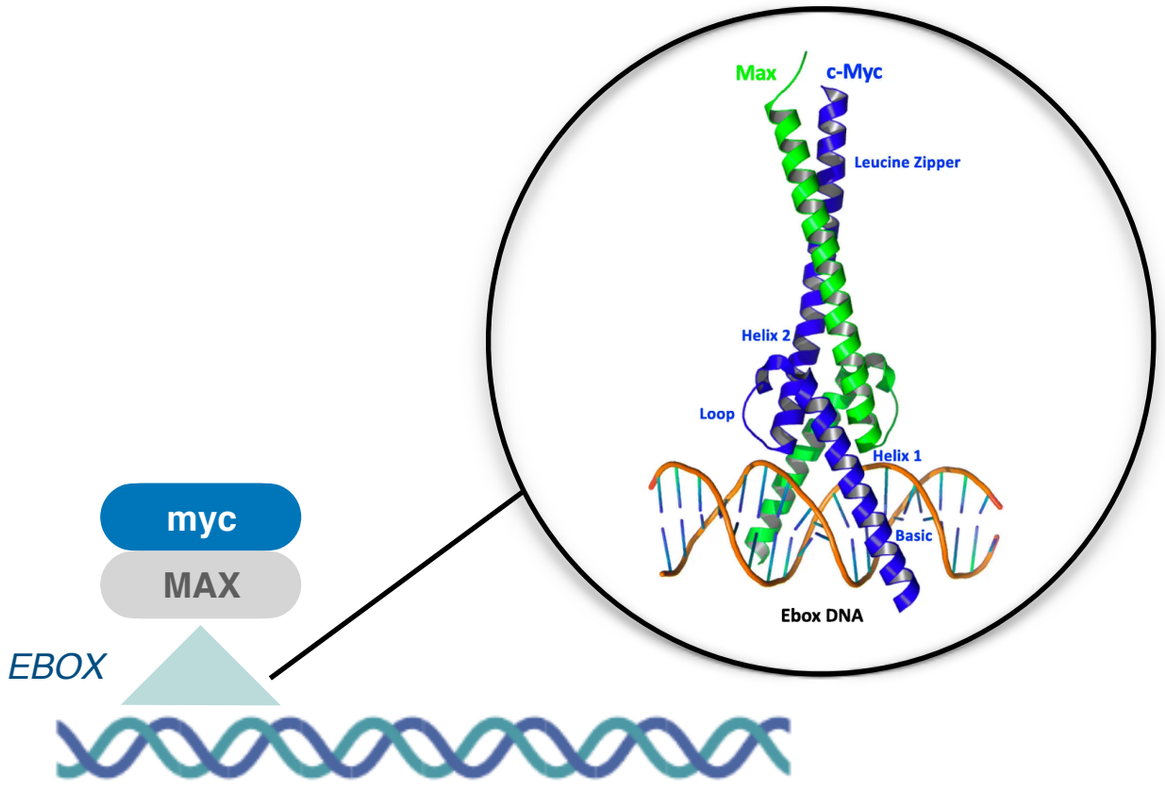

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An overview of common oncogenes

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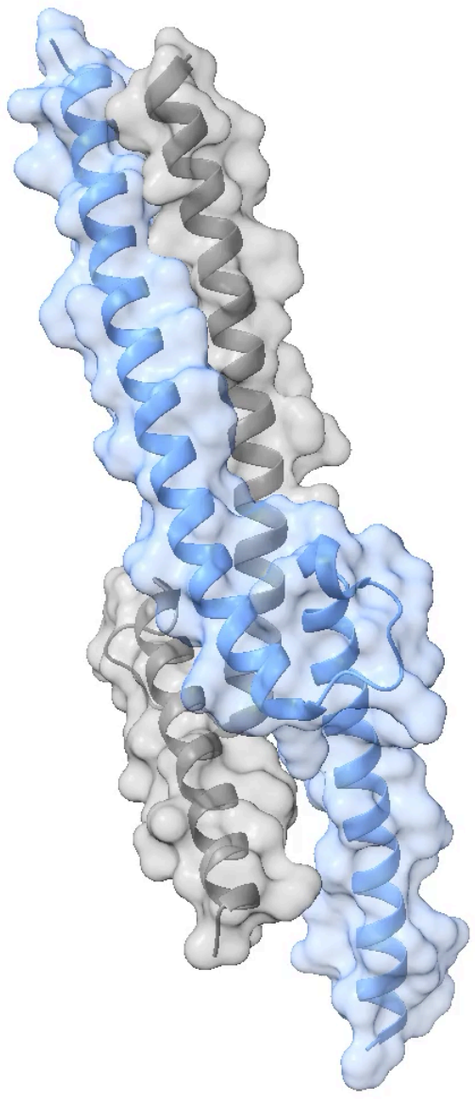

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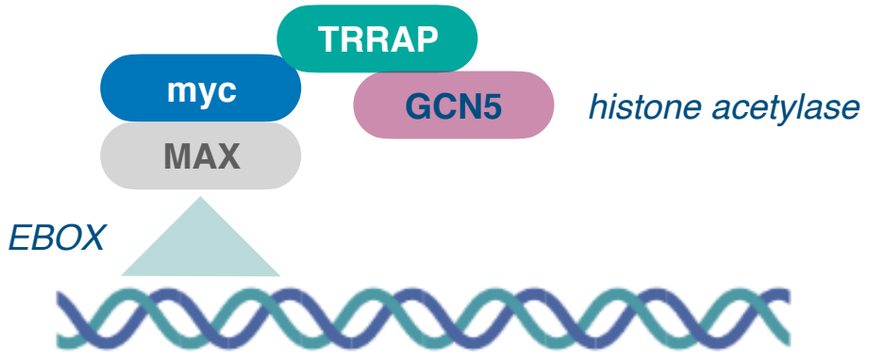
chromatin modifying complex formed



Dang, C. *Cell*. **2012**, 149, 1, 22–35.

An overview of common oncogenes

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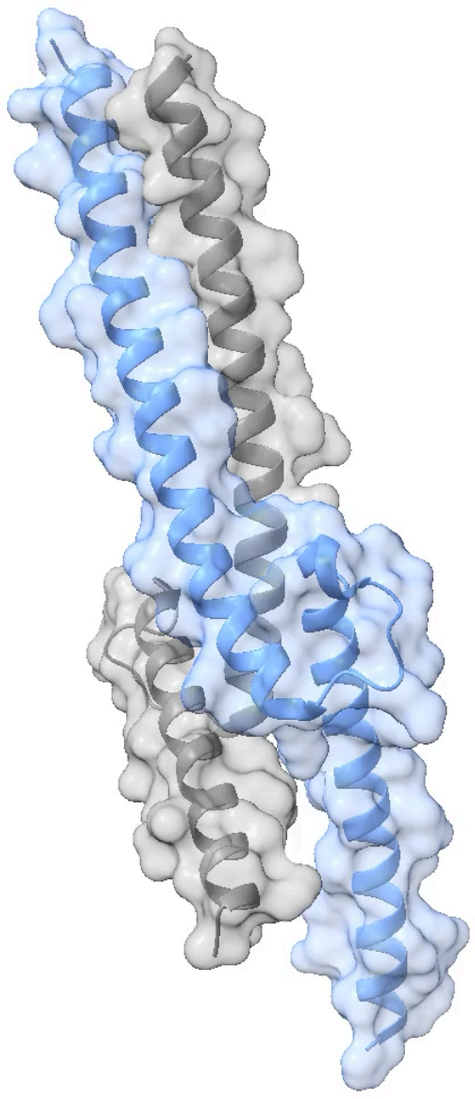
myc - MAX



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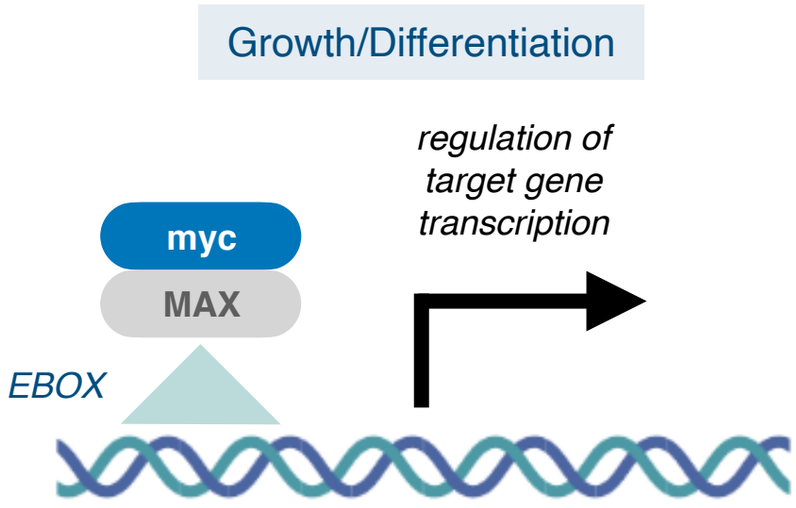


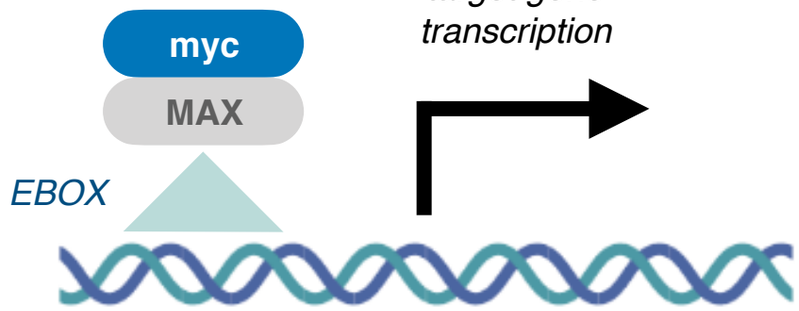
Dang, C. *Cell*. **2012**, 149, 1, 22–35.

An overview of common oncogenes

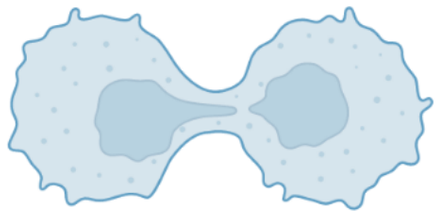
myc

myc protein identity and function

Growth/Differentiation



regulation of target gene transcription



target genes regulate proliferation, growth, etc.

crucial in embryonic development

myc is generally expressed as a result of mitogen stimulation

initially nature of amplification unclear - is myc an on/off switch?

Kim S. et al. *Mol Cell*. **2003**, 11, 1177–88.

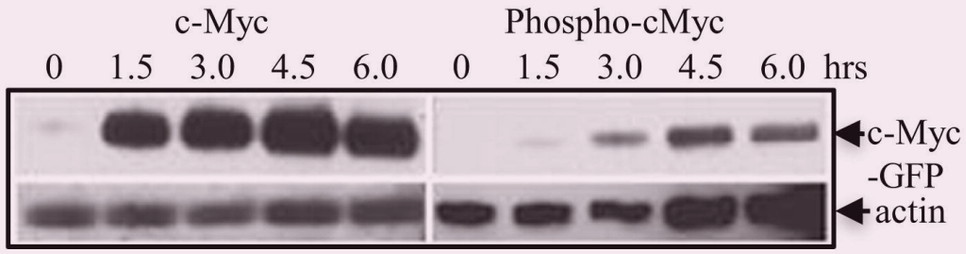
An overview of common oncogenes

myc

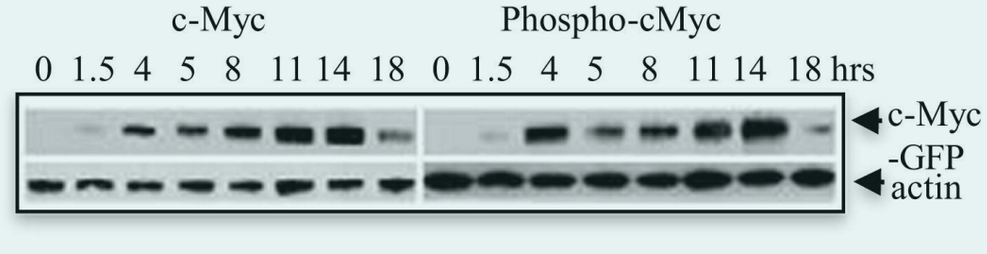
investigating the nature of myc

- myc-GFP fusion generated to investigate expression
- stimulation of lymphocytes with mitogen
- readout of c-Myc expression via western blot/flow cytometry

mouse B cells



mouse T cells



validate c-Myc construct expression - identify peak expression time points

Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

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- myc-GFP fusion generated to investigate expression
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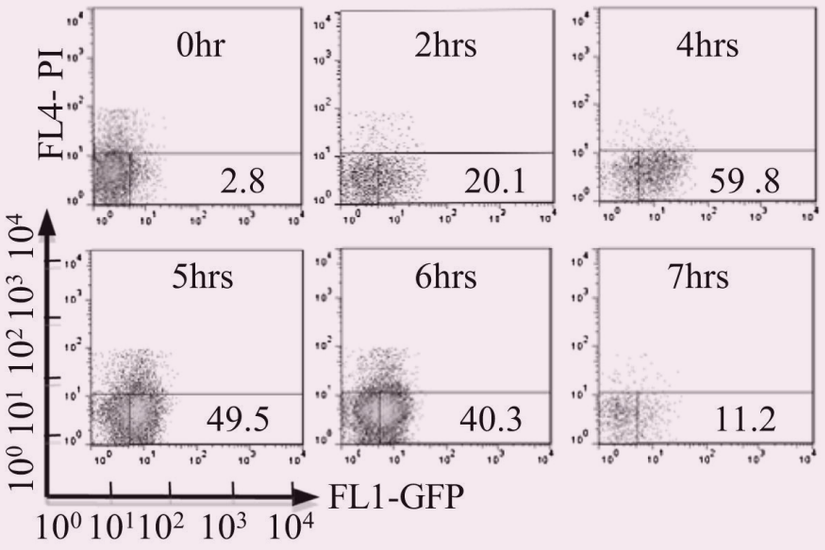
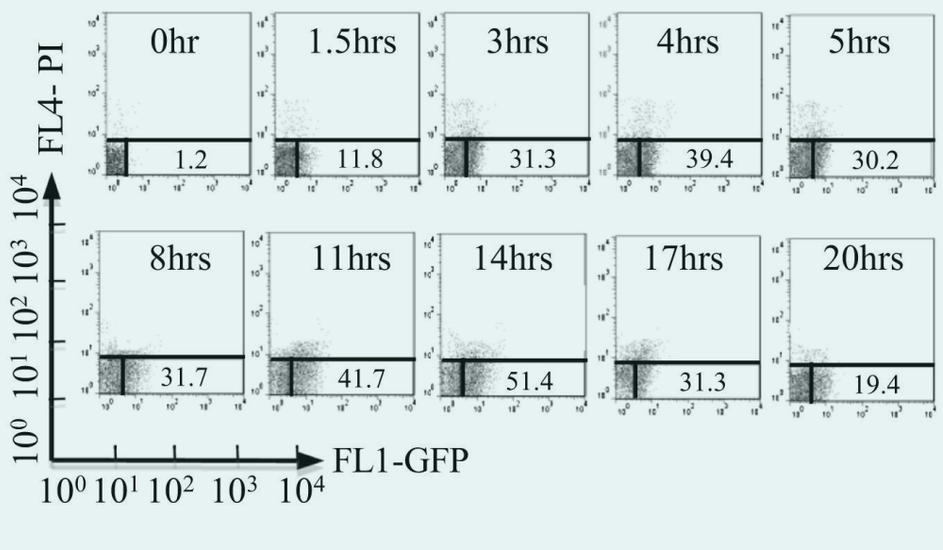


mouse T cells



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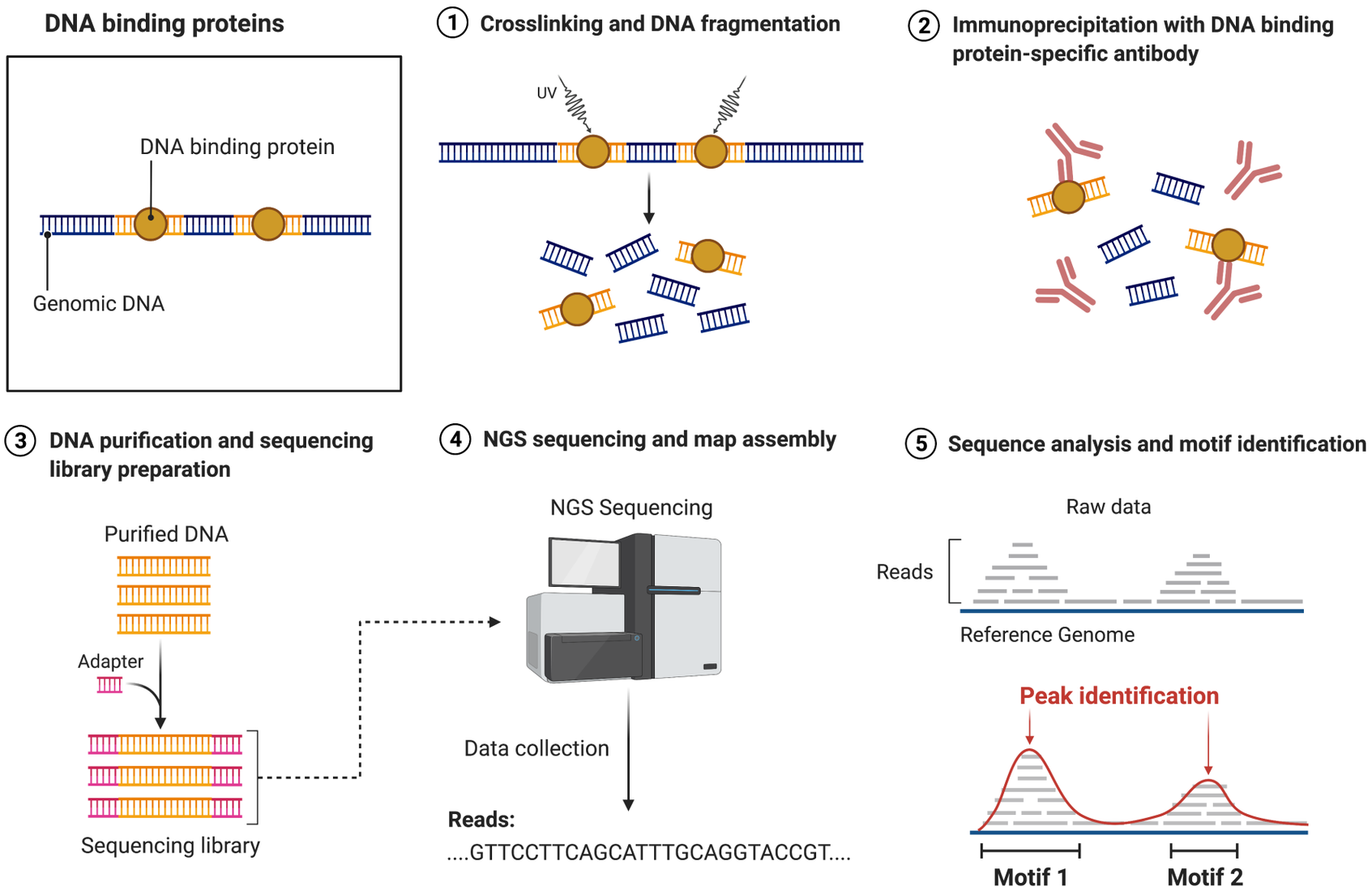
An overview of common oncogenes

myc

ChIP-seq investigation



Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

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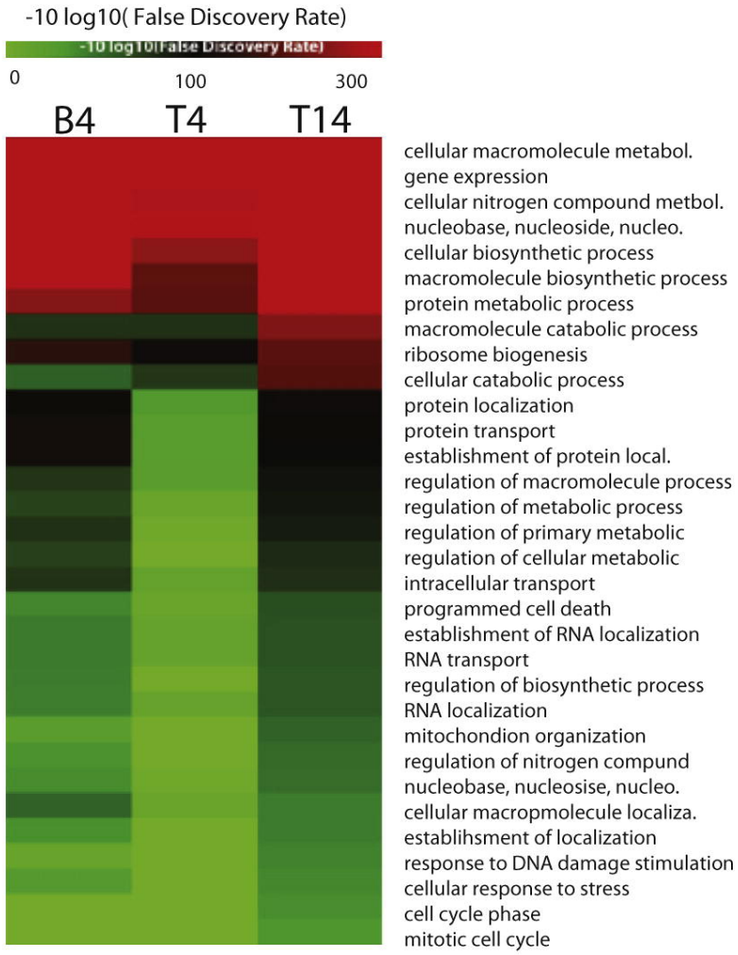


- gene ontology analysis reveals target genes

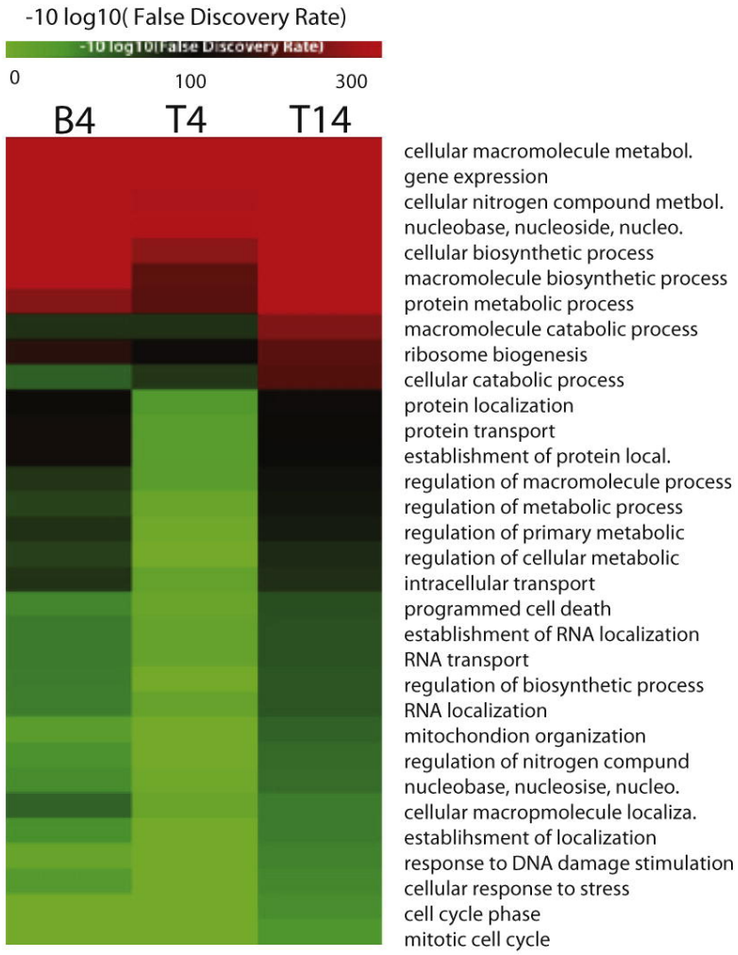
cellular macropmolecule localiza.
establihsment of localization
response to DNA damage stimulation
cellular response to stress
cell cycle phase
mitotic cell cycle

Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

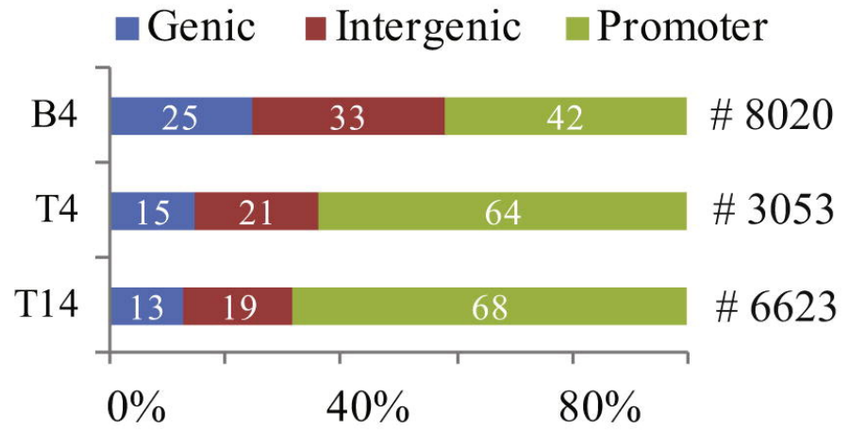
An overview of common oncogenes

myc

ChIP-seq investigation



- gene ontology analysis reveals target genes
- myc primarily binds to promoter regions



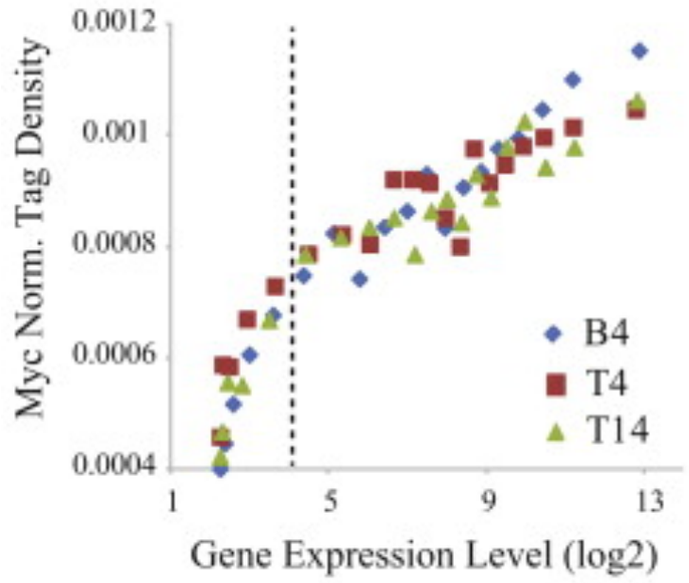
Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

ChIP-seq investigation

correlation of gene expression with myc density



linear log scale correlation between myc density at promoters and gene expression

myc amplifies expression in a non-linear fashion

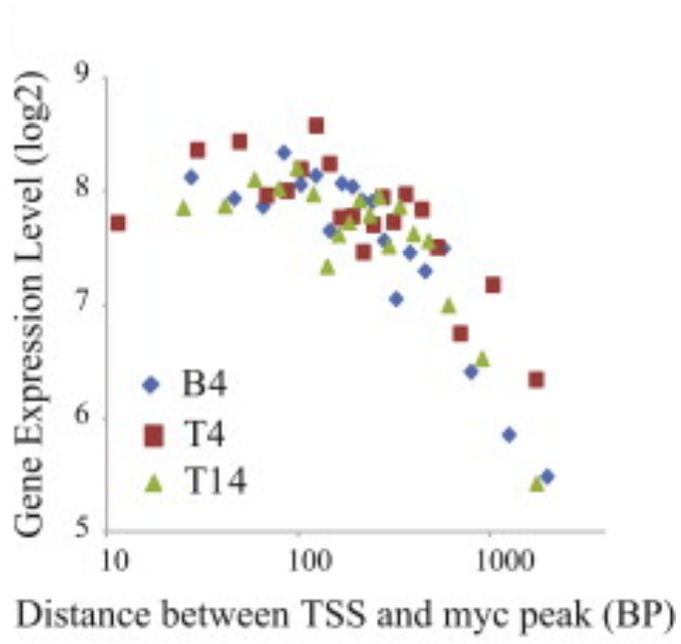
Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

ChIP-seq investigation

correlation of gene expression with myc density



gene expression level drops off quickly after around 250 BP from transcription start site (TSS)

myc has a range of around 250 BP for gene amplification

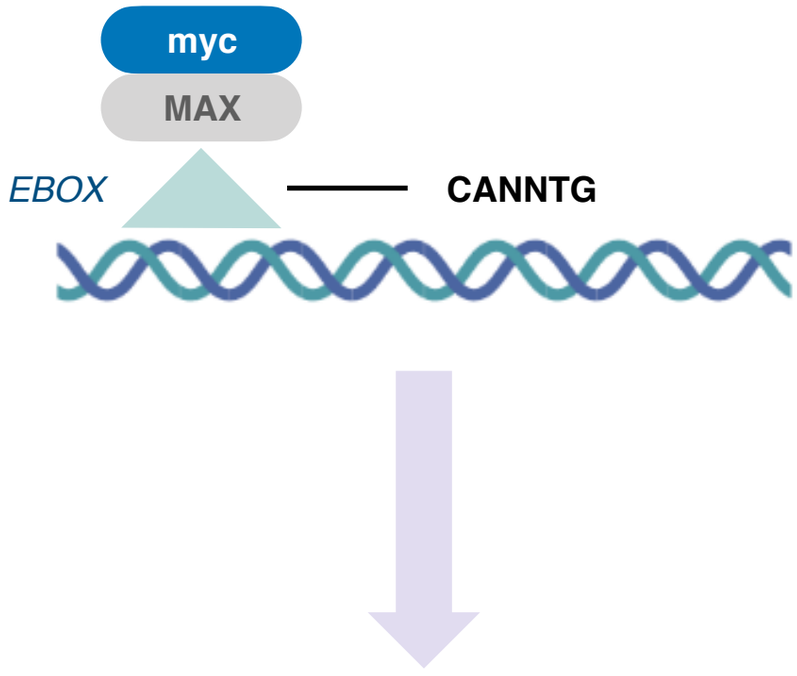
Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

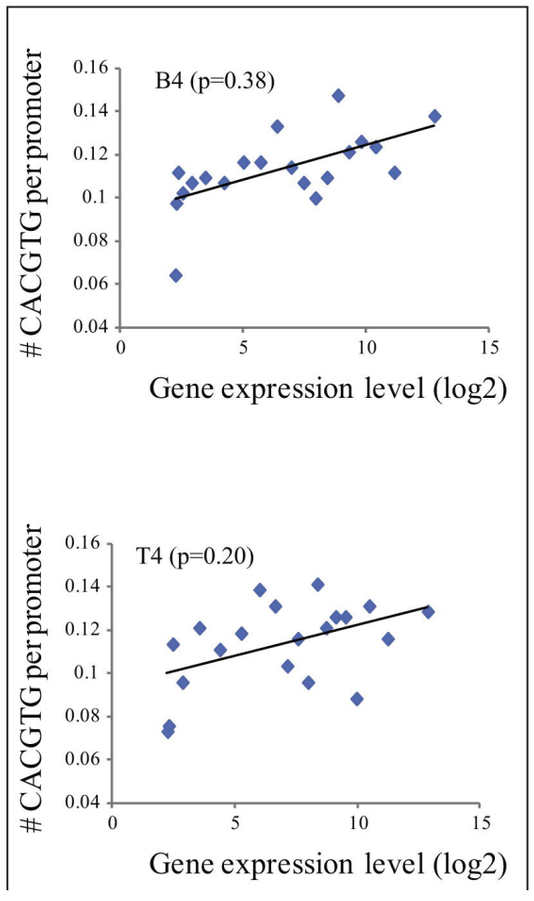
myc

ChIP-seq investigation

EBOX correlation is present but weak



“In principle, a 4/6 match to the canonical E-box would occur every 30 nucleotides in random 50% AT/GC DNA.”



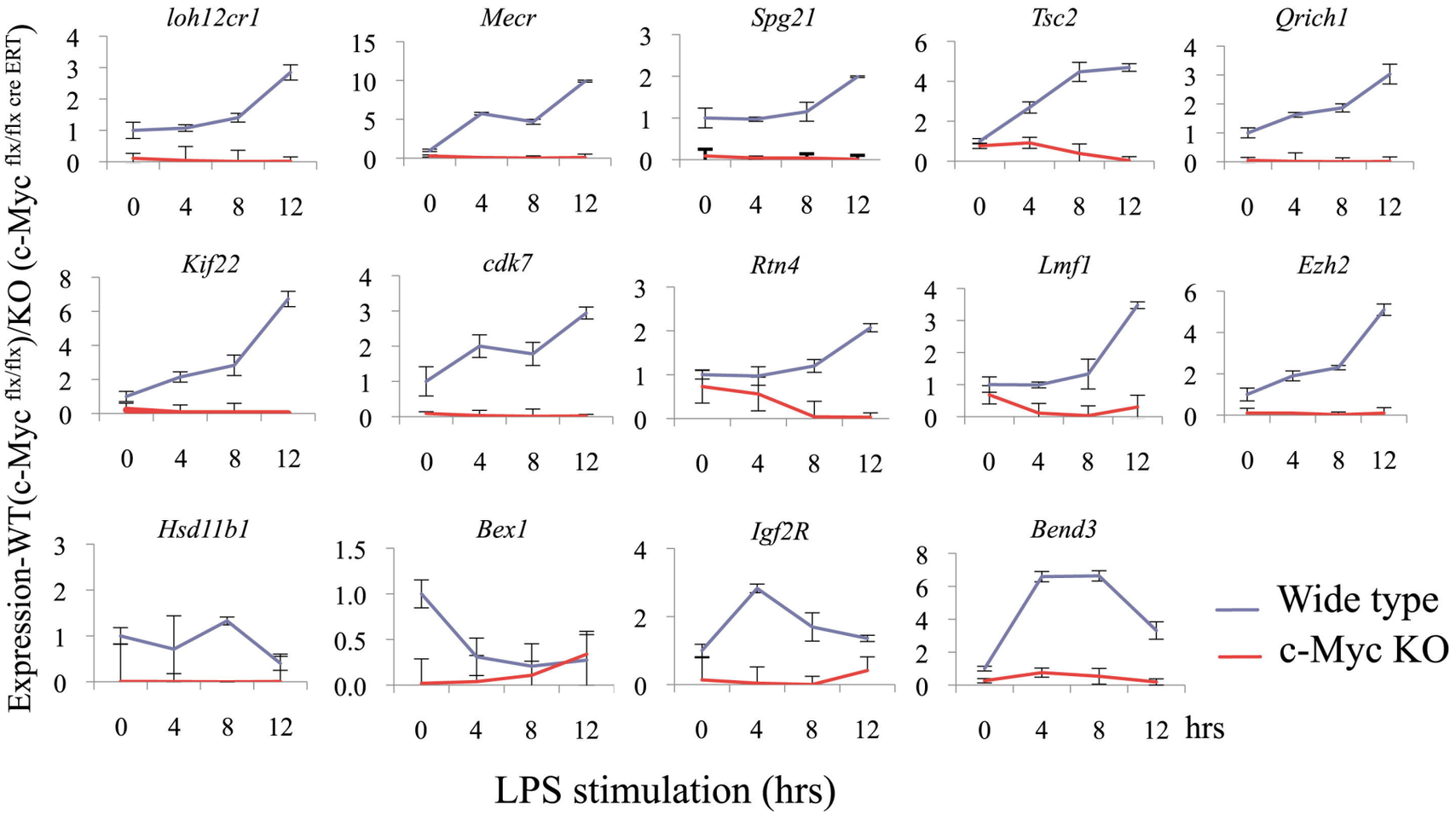
Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

ChIP-seq investigation

c-Myc correlates with universal amplification



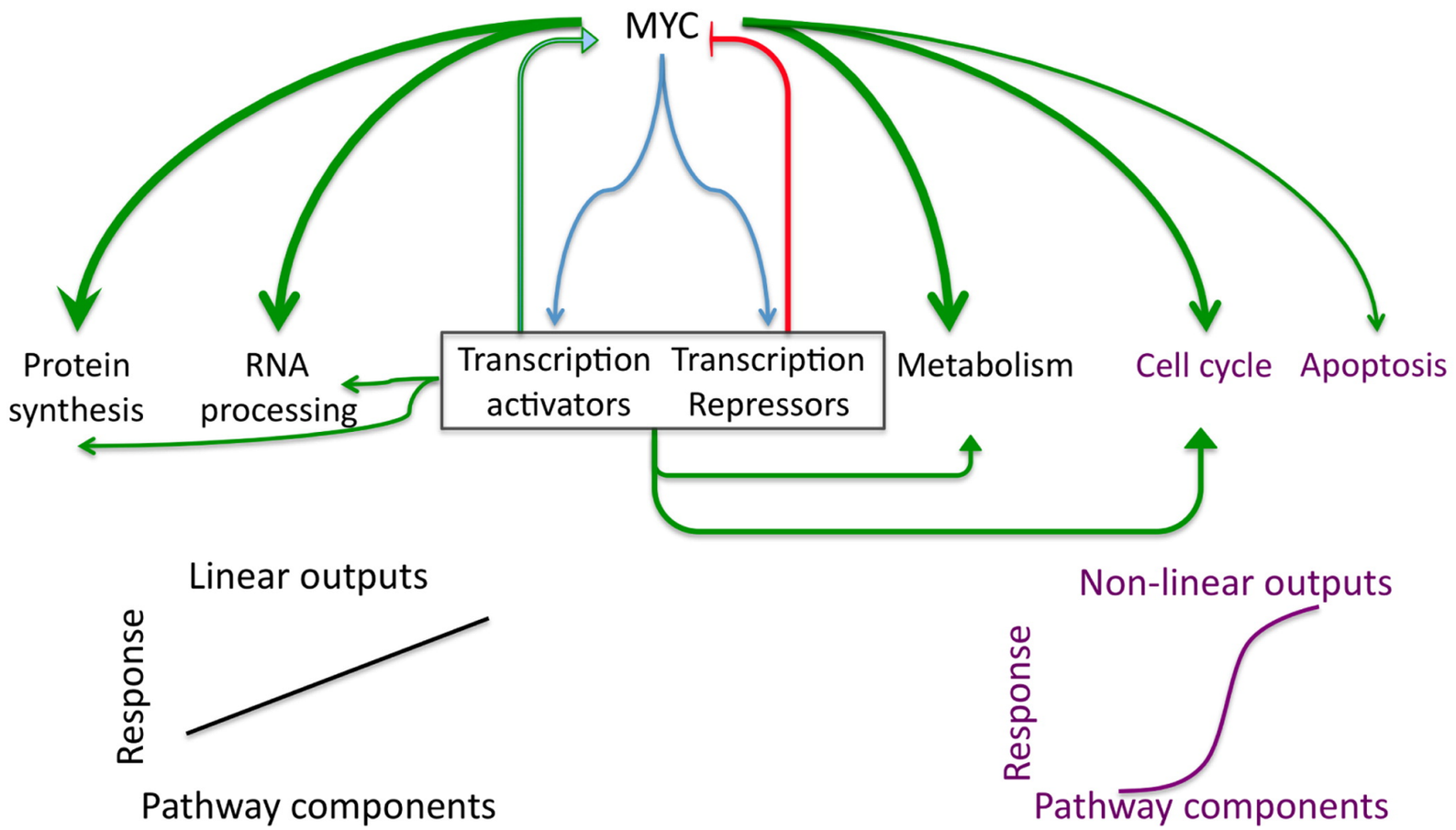
Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

Putting the pieces together

myc is a universal amplifier



Levens, D. et al. *Cell*. **2012**, 151, 1, 68–79.

An overview of common oncogenes

myc

myc as an oncogene

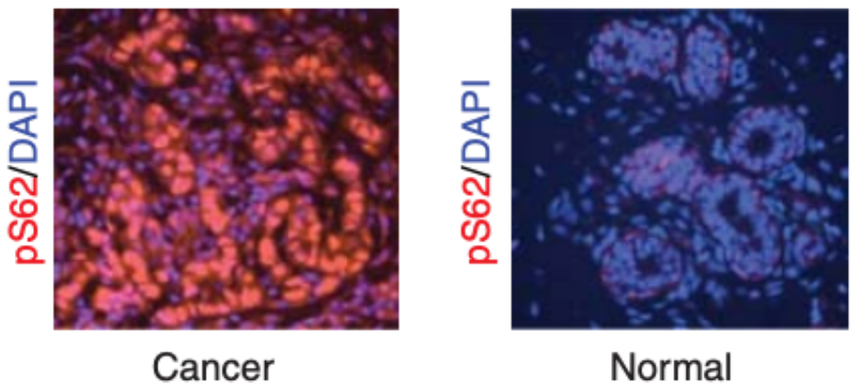
myc is a universal amplifier

normally tightly regulated for degradation

primarily via ubiquitination/E3 ligase

typically expressed as a “pulse” which then deteriorates

myc over-expression leads to uncontrolled cell growth



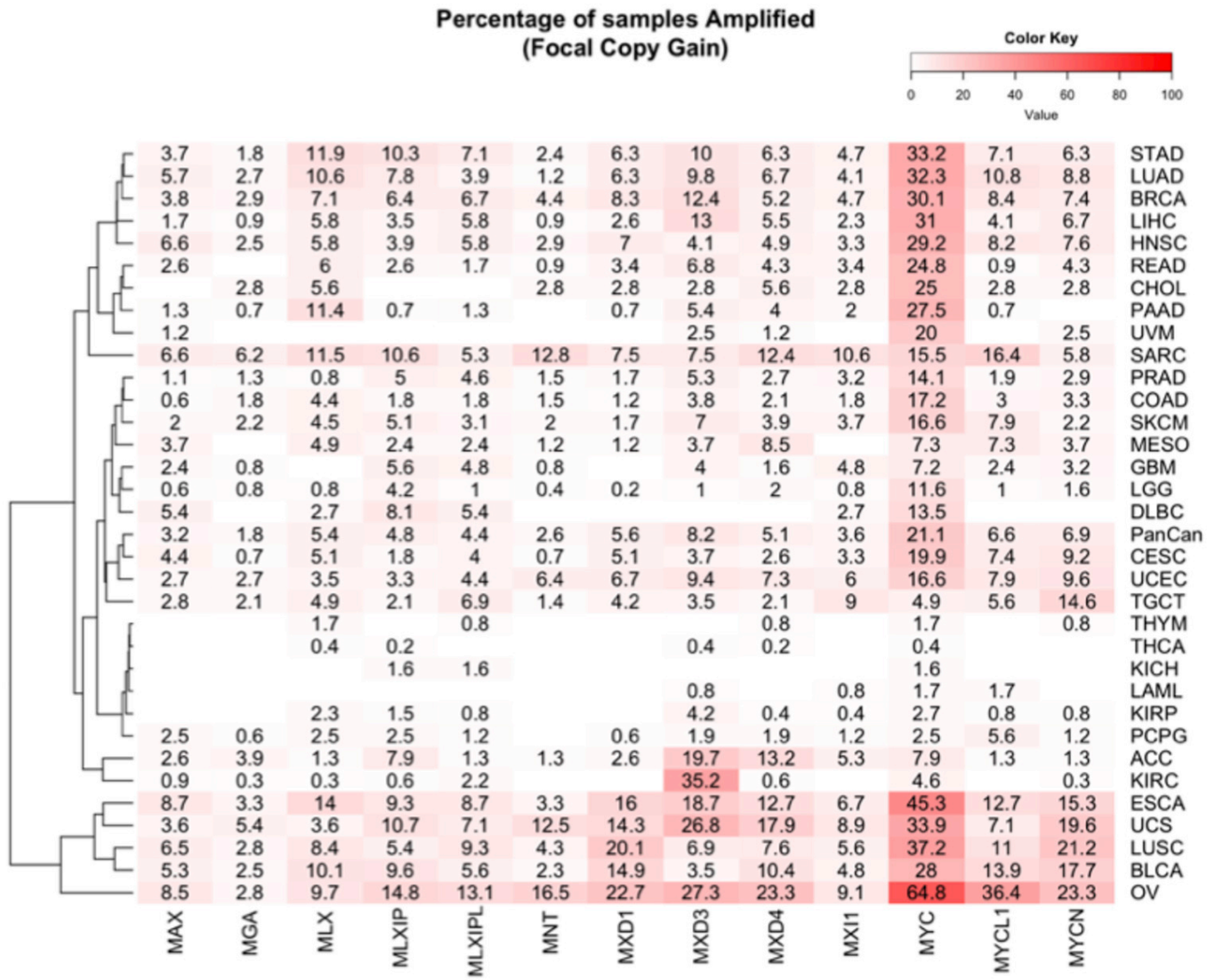
Farrel, A.; Sears, R. *Cold Spring Harb. Perspect. Med*. **2014**, 4 : a014365.

An overview of common oncogenes

myc

myc as an oncogene

myc amplification is a pan-cancer phenomenon



Schaub et al. *Cell Systems*. **2018**, 6, 282–300.

An overview of common oncogenes

myc

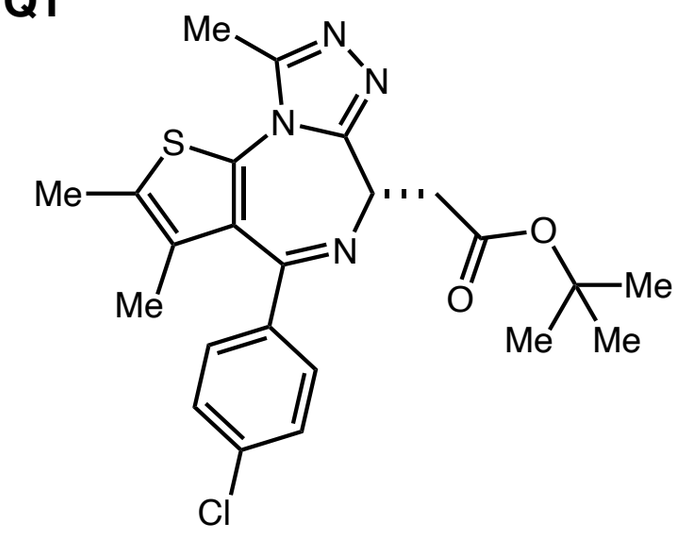
myc as a target for cancer therapy

to date, no direct myc inhibitors exist

↓

indirect myc targeting via BRD4 has been promising

JQ1



Misiades, C. et al. *Cell*. **2011**, 146, 904–917.

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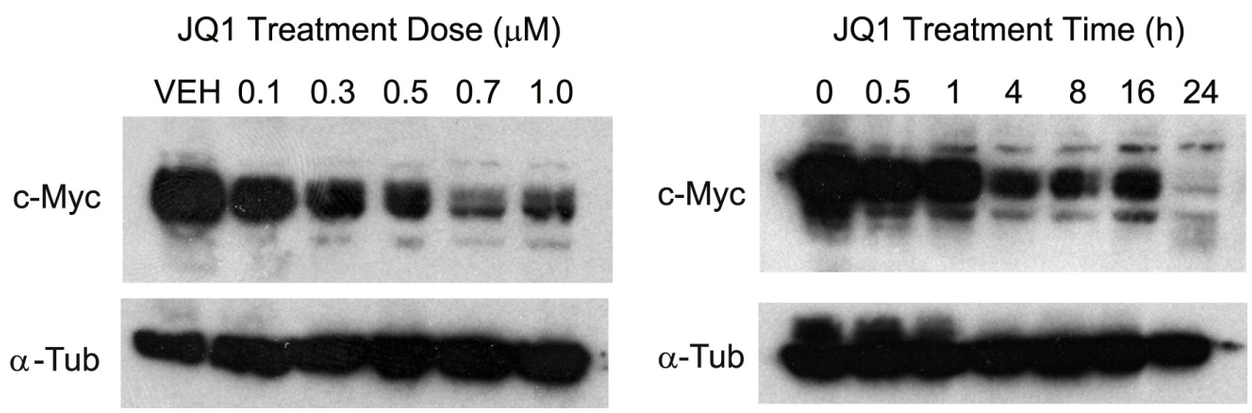


interest in myc targeting remains high

Misiades, C. et al. *Cell*. **2011**, 146, 904–917.

Outline

- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib
- Future directions/Outlook

An overview of common oncogenes

Ras

Discovery of Ras

viral origin - sarcoma rat virus studies

H-Ras and K-Ras genes discovered virtually at the same time

An overview of common oncogenes

Ras

Discovery of Ras

viral origin - sarcoma rat virus studies

Nucleotide Sequence of the p21 Transforming Protein of Harvey Murine Sarcoma Virus

RAVI DHAR, RONALD W. ELLIS, THOMAS Y. SHIH, STEPHEN OROSZLAN, BRUCE SHAPIRO, JACOB MAIZEL, DOUGLAS LOWY, AND EDWARD SCOLNICK Authors Info & Affiliations

SCIENCE • 3 Sep 1982 • Vol 217, Issue 4563 • pp. 934-936 • DOI: 10.1126/science.6287572

Nucleotide Sequence of the Oncogene Encoding the p21 Transforming Protein of Kirsten Murine Sarcoma Virus

NOBUO TSUCHIDA, TOM RYDER, AND , EIICHI OHTSUBO Authors Info & Affiliations

SCIENCE • 3 Sep 1982 • Vol 217, Issue 4563 • pp. 937-939 • DOI: 10.1126/science.6287573

Tsuchida, N. et al. *Science*. **1982**, 217, 4563.

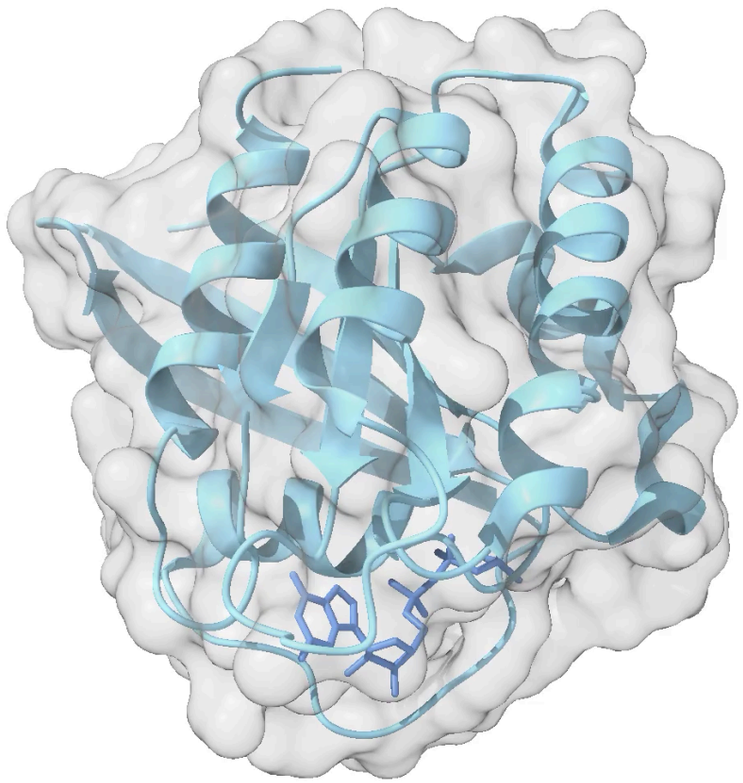
Scolnick, E. et al. *Science*. **1982**, 217, 4563.

An overview of common oncogenes

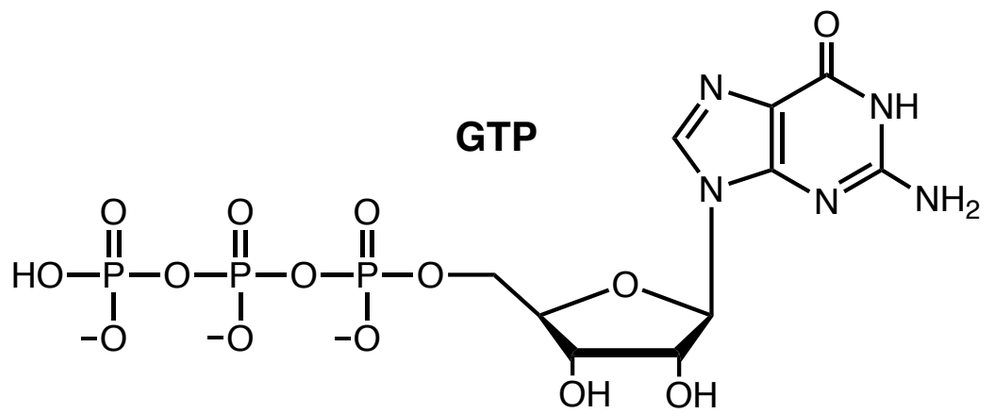
Ras

Ras protein - identity and function

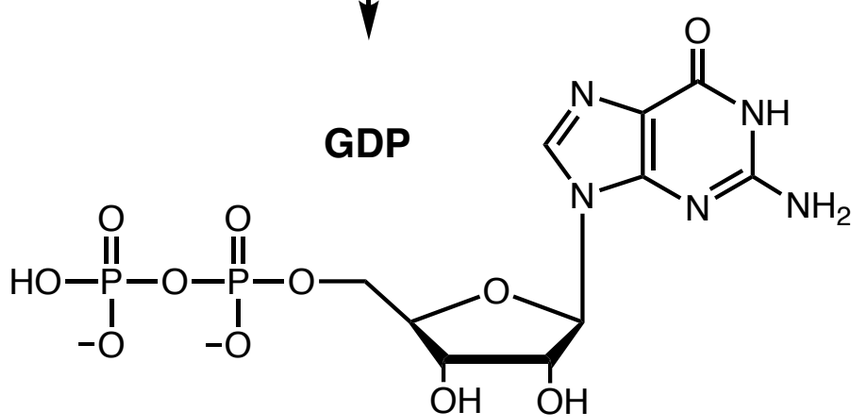
Ras



- GTPase - Guanosine triphosphate hydrolase



Ras



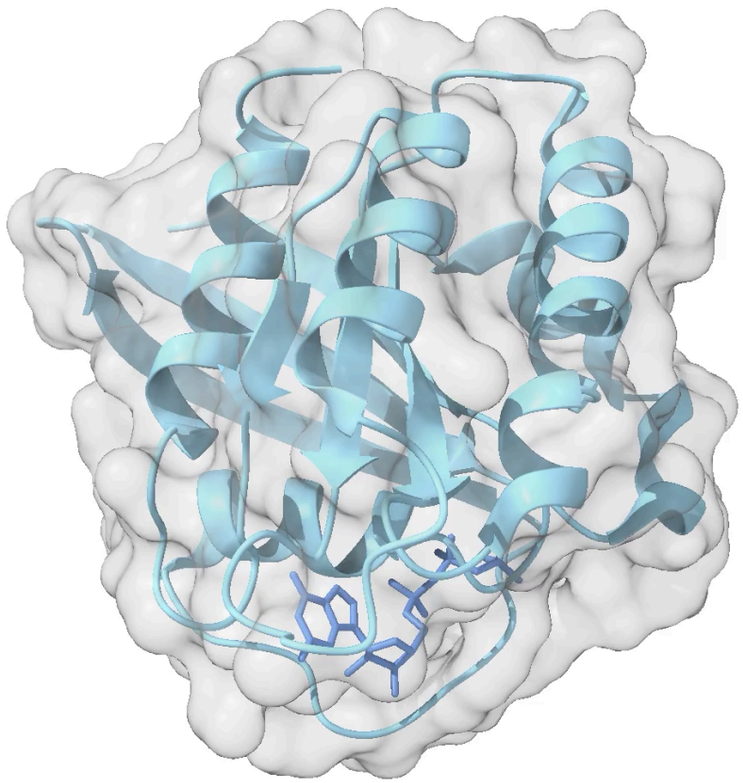
Downward, J. *Nature Reviews Cancer*. **2003**, 3, 11–22.

An overview of common oncogenes

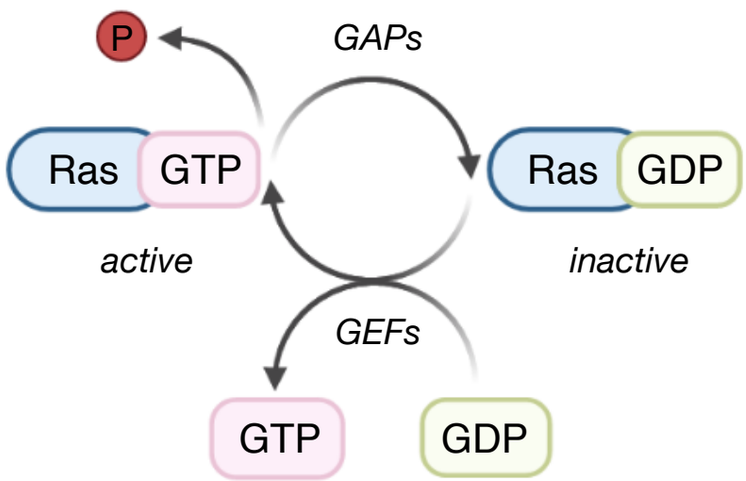
Ras

Ras protein - identity and function

Ras



- GTPase - Guanosine triphosphate hydrolase
- active when GTP bound - GDP inactivates Ras
- primary function is signal transduction



cell growth, proliferation

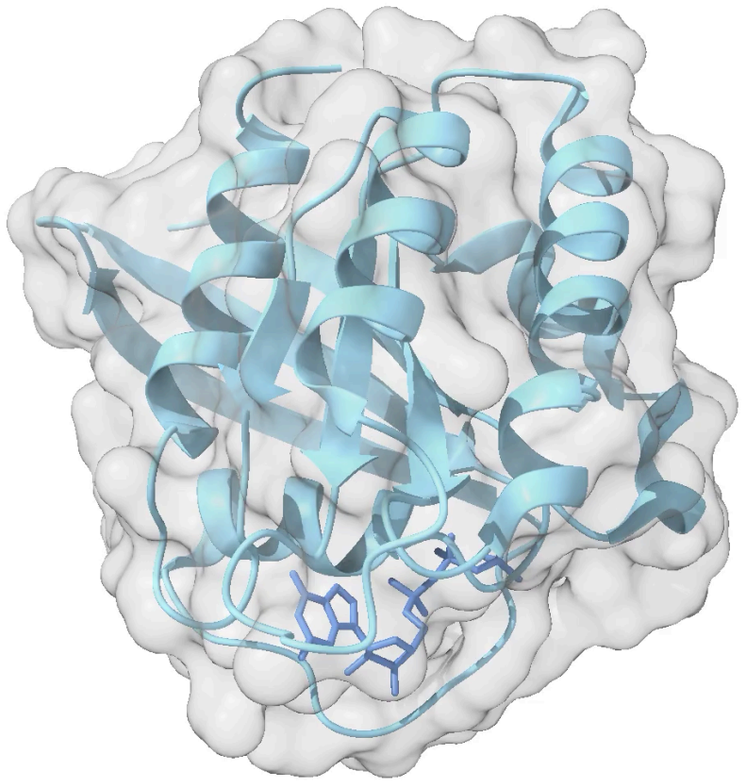
Downward, J. *Nature Reviews Cancer*. **2003**, 3, 11–22.

An overview of common oncogenes

Ras

Ras protein - identity and function

Ras



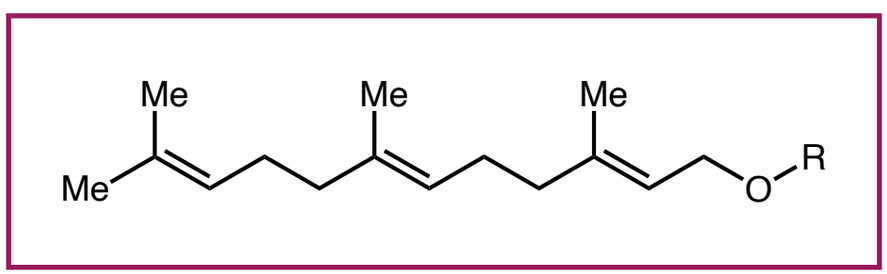
- GTPase - Guanosine triphosphate hydrolase
- active when GTP bound - GDP inactivates Ras
- primary function is signal transduction
- consists of 4 closely related members

Hypervariable Domain

HRAS	HKLRKLNPPDESGPGCMSCKCVLS
NRAS	YRMKKLNSSDDGTQGCMGLPCVVM
KRAS 4A	YRLKKISKEEKTPGCVKIKKCIIM
KRAS 4B	HKEKMSKDGKKKKKKSKTKCVIM

membrane targeting domain

farnesyl group

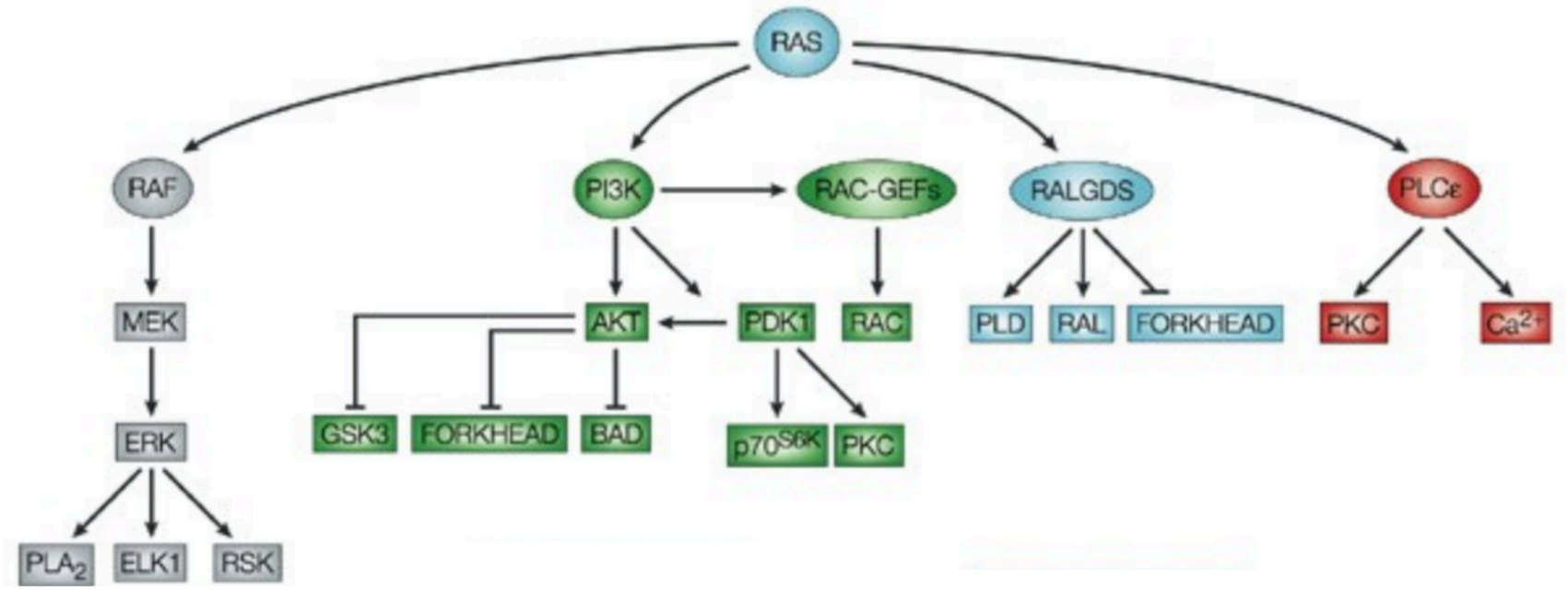


Downward, J. *Nature Reviews Cancer*. **2003**, 3, 11–22.

An overview of common oncogenes

Ras

Ras protein - major signalling functions



transcription, cell cycle progression	*survival, transcription, translation*	*transcription, vesicle transport, cell cycle*	*calcium signalling*

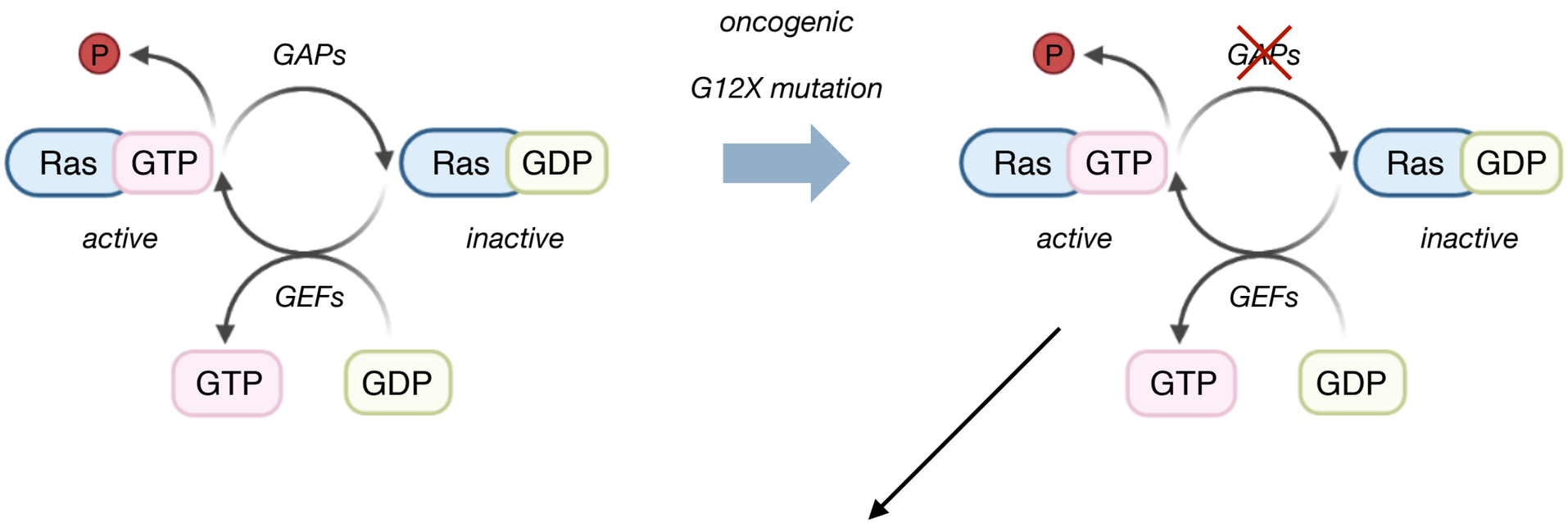
Ras signalling plays a crucial role in cell cycle progression

Downward, J. *Nature Reviews Cancer*. **2003**, 3, 11–22.

An overview of common oncogenes

Ras

Ras protein - oncogenic activity



constitutionally active Ras

Ras remains in its "on" state and signals for uncontrolled proliferation

most Ras driven cancers involve G12X mutations

Downward, J. *Nature Reviews Cancer*. **2003**, 3, 11–22.

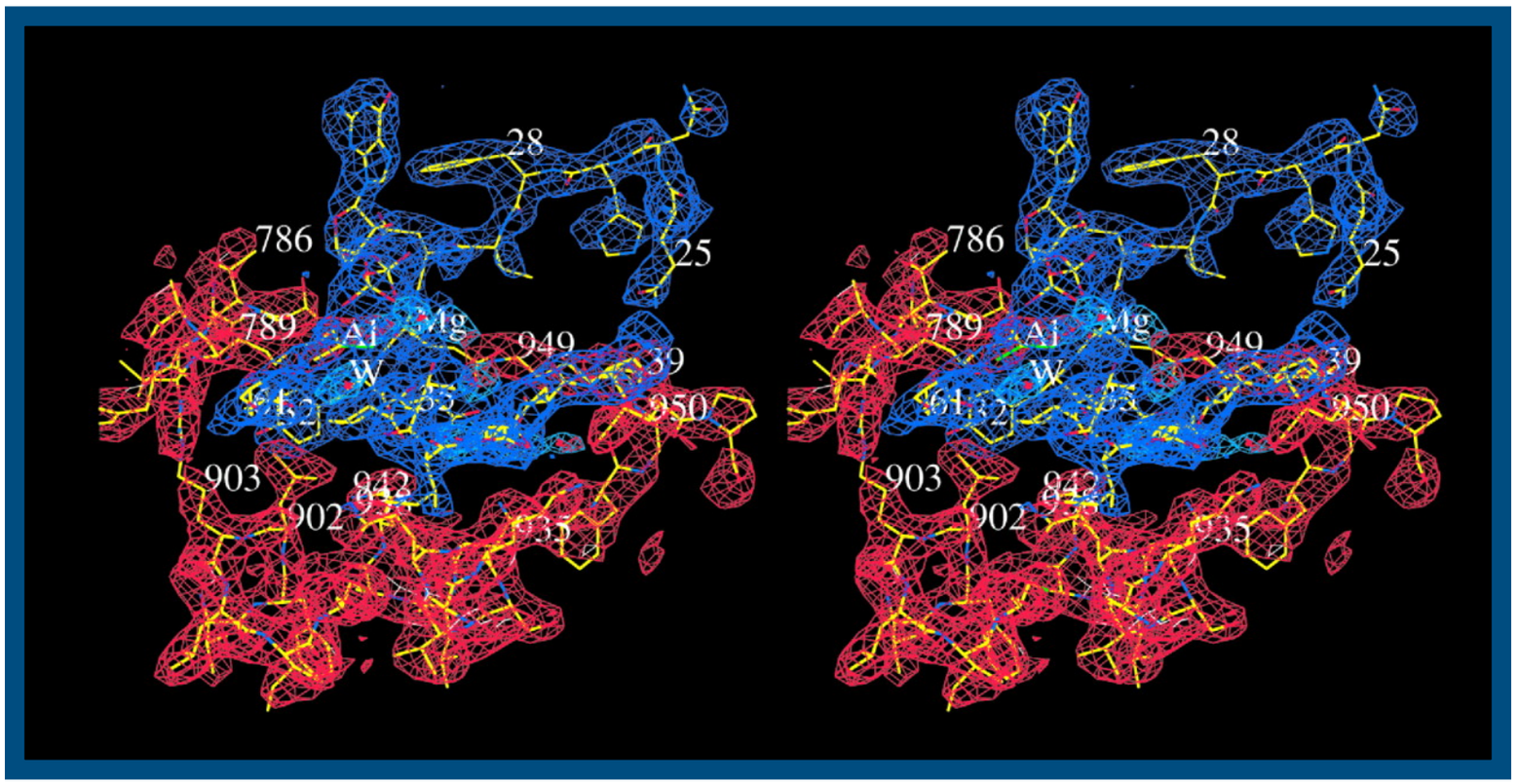
An overview of common oncogenes

Ras

Ras protein - oncogenic activity

1997 - Crystal structure of Ras-GDP & GAP interaction solved

H-Ras & GAP



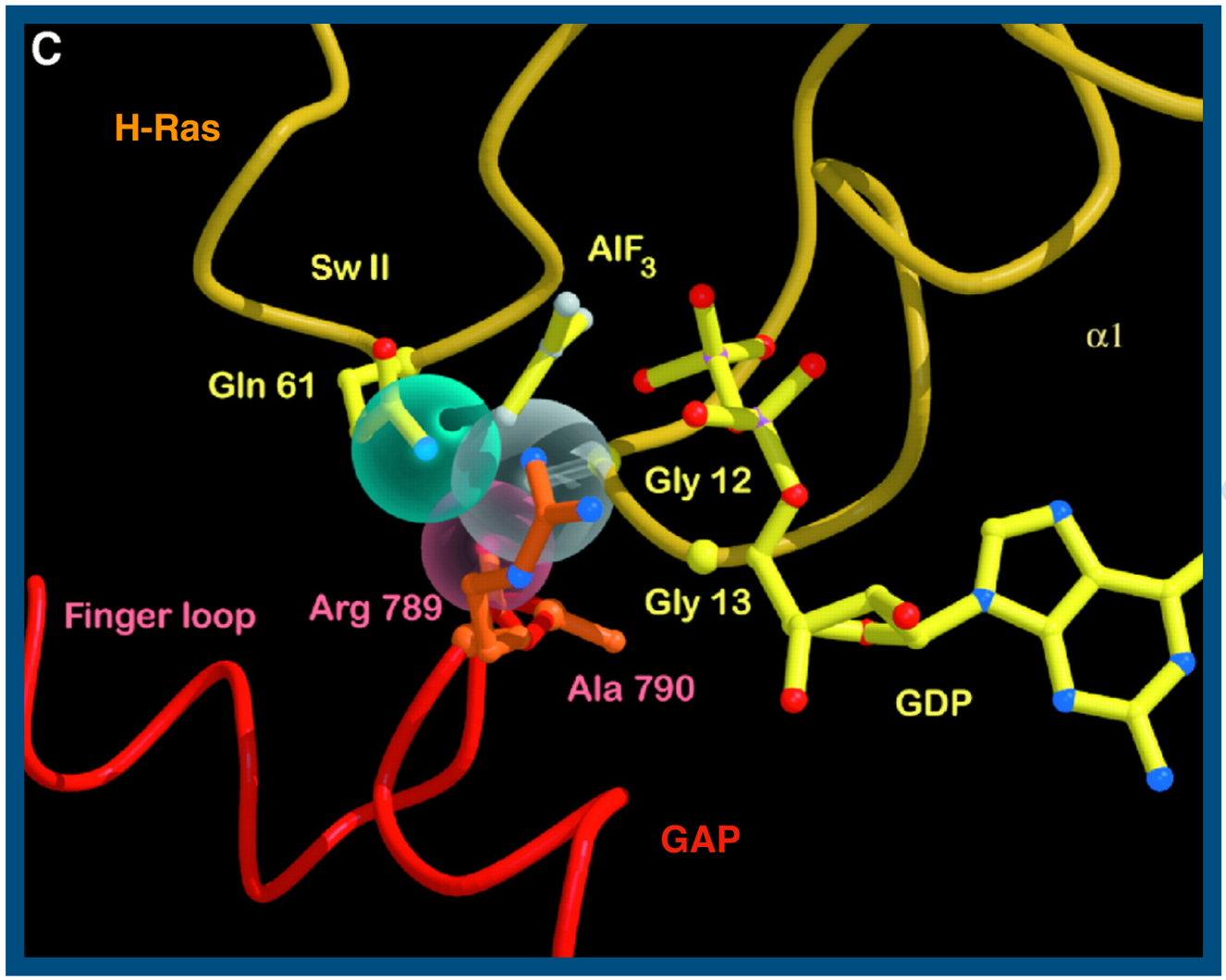
Wittinghofer, A. et al. *Science*. **1997**, 277, 5324, 333-8.

An overview of common oncogenes

Ras

Ras protein - oncogenic activity

Crystal structure elucidates catalytic region of Ras



Ras

residues 10-16
phosphate binding region
hydrolysis

G12X, G13X mutations

residues 60-76
switch II region
structural rearrangment

Q61 mutations

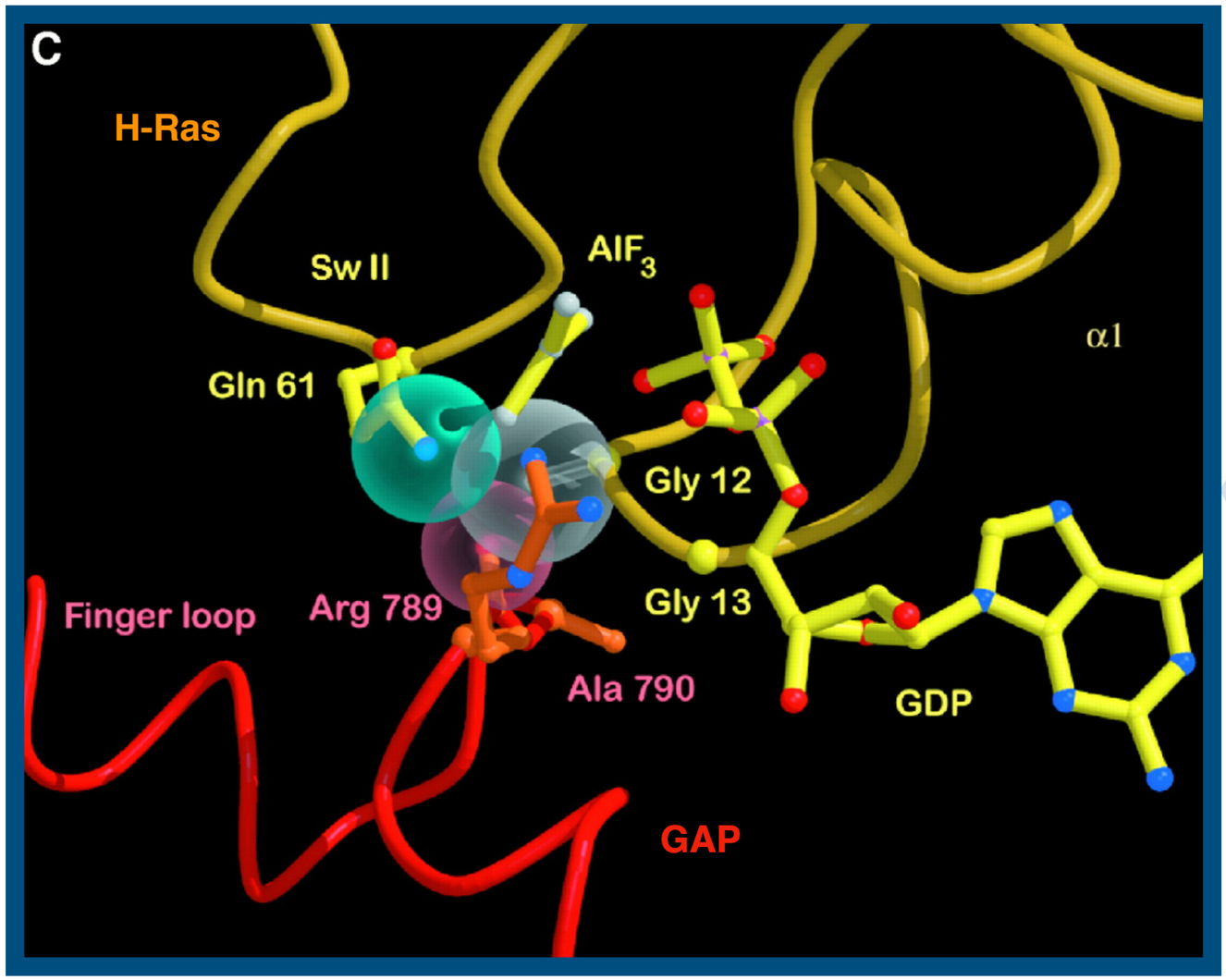
Wittinghofer, A. et al. *Science*. **1997**, 277, 5324, 333-8.

An overview of common oncogenes

Ras

Ras protein - oncogenic activity

Crystal structure reveals mechanism of G12X mutations



Ras

residues 10-16
phosphate binding region
hydrolysis

G12X, G13X mutations

↓

van der Waals contact between G12 on Ras and Arg 789 on GAP

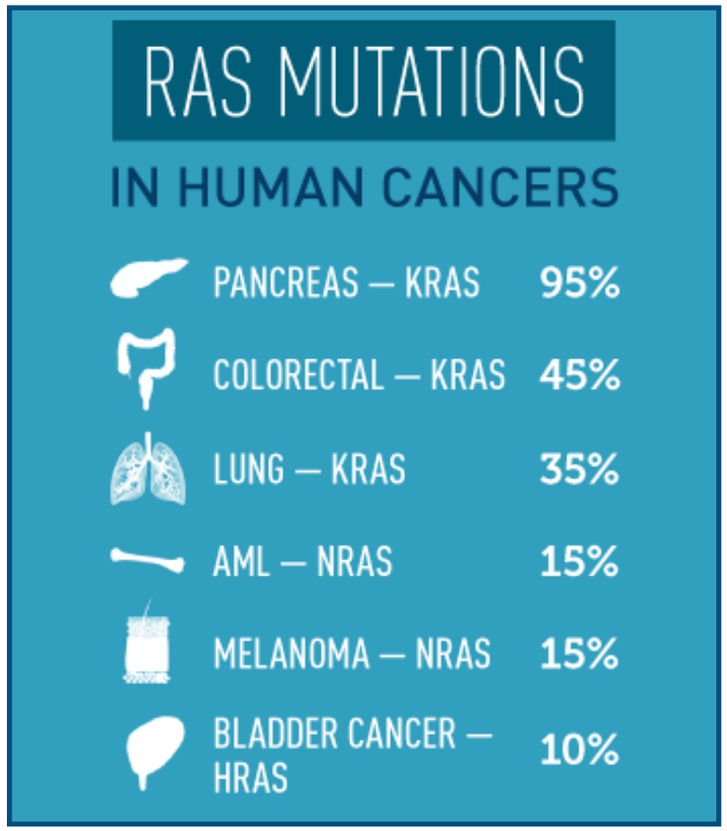
Wittinghofer, A. et al. *Science*. **1997**, 277, 5324, 333-8.

An overview of common oncogenes

Ras

Ras protein mutations are common in many cancers

More than 30% of all human cancers are driven by Ras family mutations



*"Ras oncogenes are the **worst** oncogenes"*

Dr. Frank McCormick, M.D.

NIH. *The Ras Initiative*. **2016**. Web.

Outline

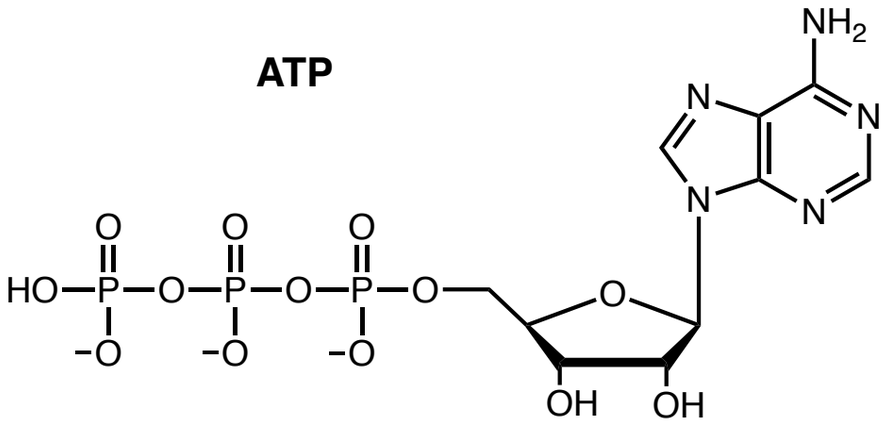
- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib
- Future directions/Outlook

The “Death Star” of Cancer

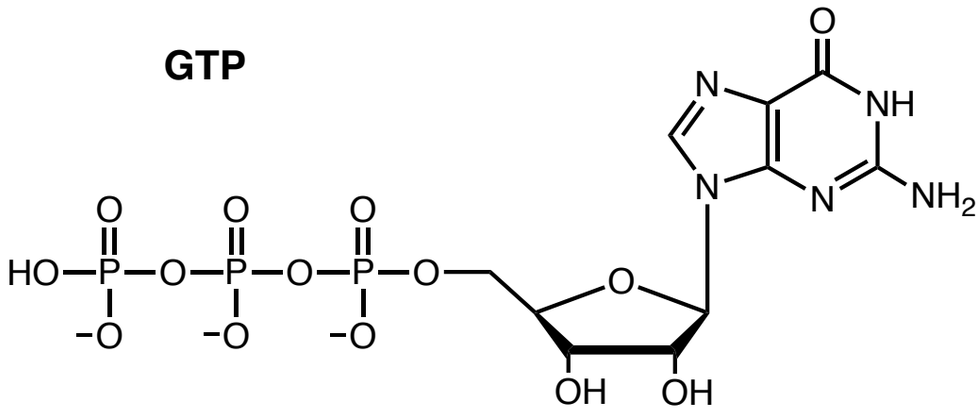
Ras as a drug target

Direct Ras targeting: outcompete GTP

ATP



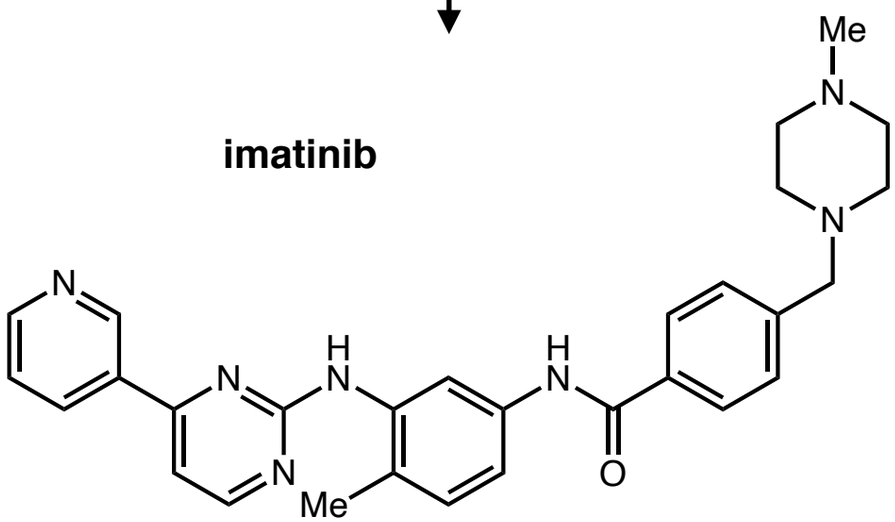
GTP



tyrosine kinase inhibitors block ATP binding site

can the same approach work for the GTP binding site?

imatinib



early optimism for this approach

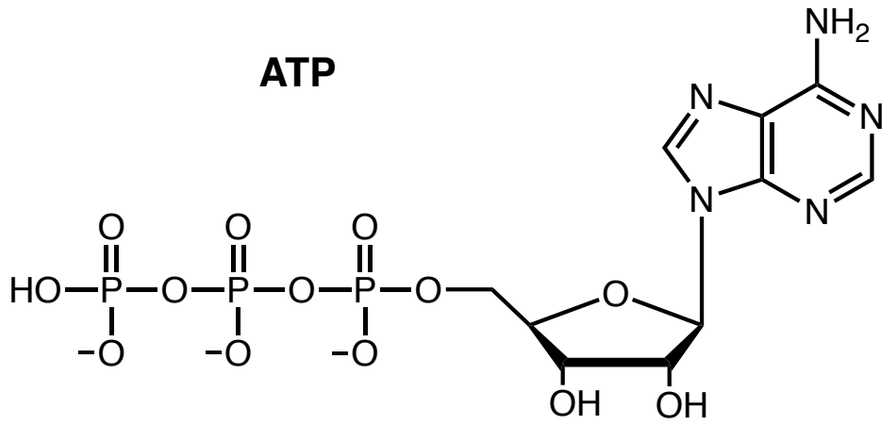
Der, C. et al. *Nature Reviews Drug Discovery*. **2014**, 13, 828-851.

The “Death Star” of Cancer

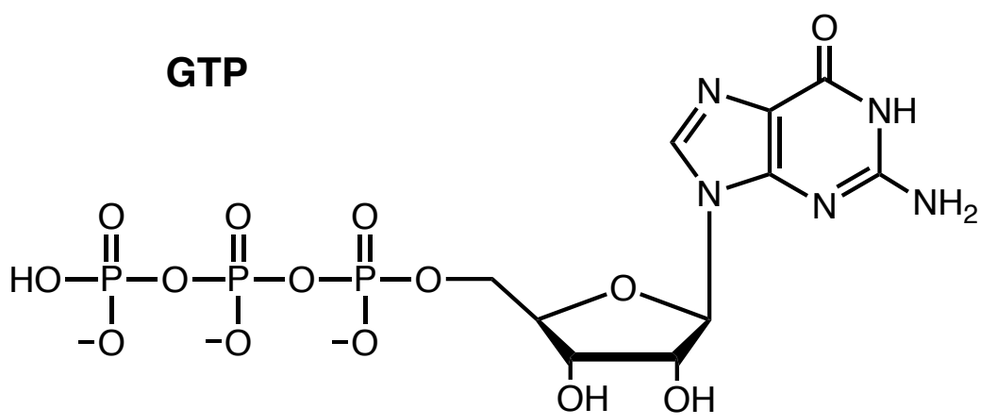
Ras as a drug target

Direct Ras targeting: outcompete GTP

ATP



GTP



tyrosine kinase inhibitors block ATP binding site

can the same approach work for the GTP binding site?

ATP binding affinity: low micromolar

$K_d \approx 5 * 10^{-6}$ M

GTP binding affinity: picomolar

$K_d \approx 20 * 10^{-12}$ M

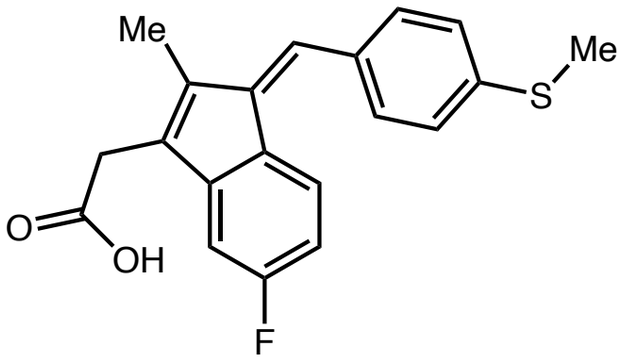
Der, C. et al. *Nature Reviews Drug Discovery*. **2014**, 13, 828-851.

The “Death Star” of Cancer

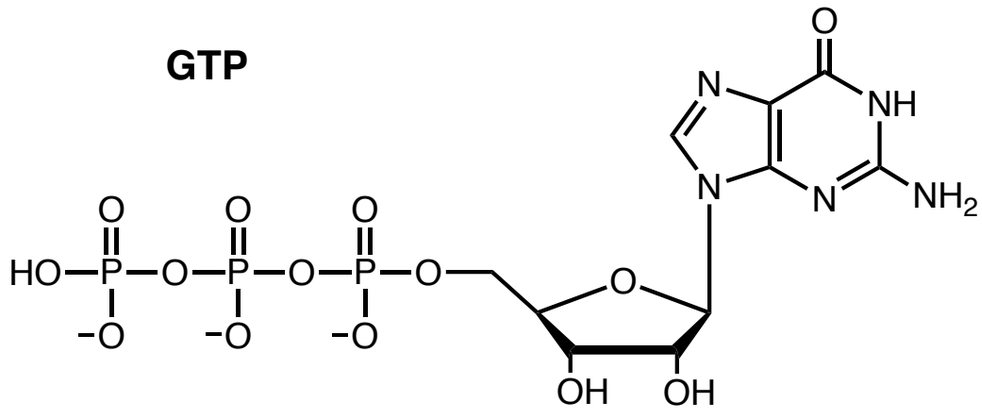
Ras as a drug target

Direct Ras targeting: outcompete GTP

sulinac sulfide



GTP



can the same approach work for the GTP binding site?

- metabolite that binds to Ras site
- inhibits Ras-Raf interaction
- no successful clinical trials
- requires high micromolar concentrations → cellular GTP concentration is 0.5 mM

GTP binding affinity: picomolar

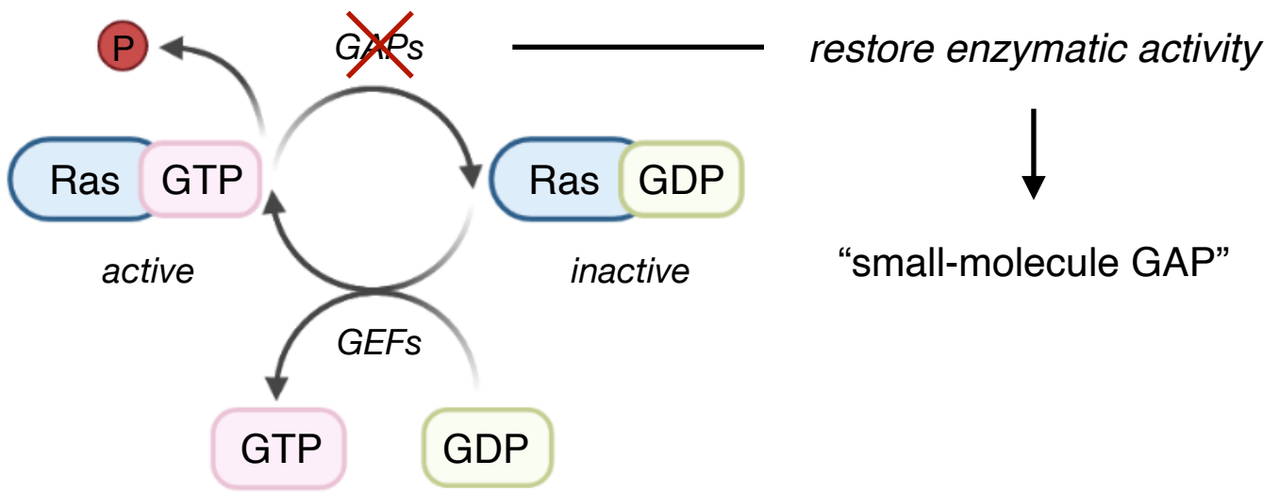
$K_d \approx 20 * 10^{-12}$ M

Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. *Cancer Cell*. **2014**, 25, 272–281.

The "Death Star" of Cancer

Ras as a drug target

Direct Ras targeting: restore GTPase activity



- compounds were screened throughout the 1980s for this purpose
- **no successful candidates identified**
- G12X blockage of GAP interaction discouraged small molecule efforts

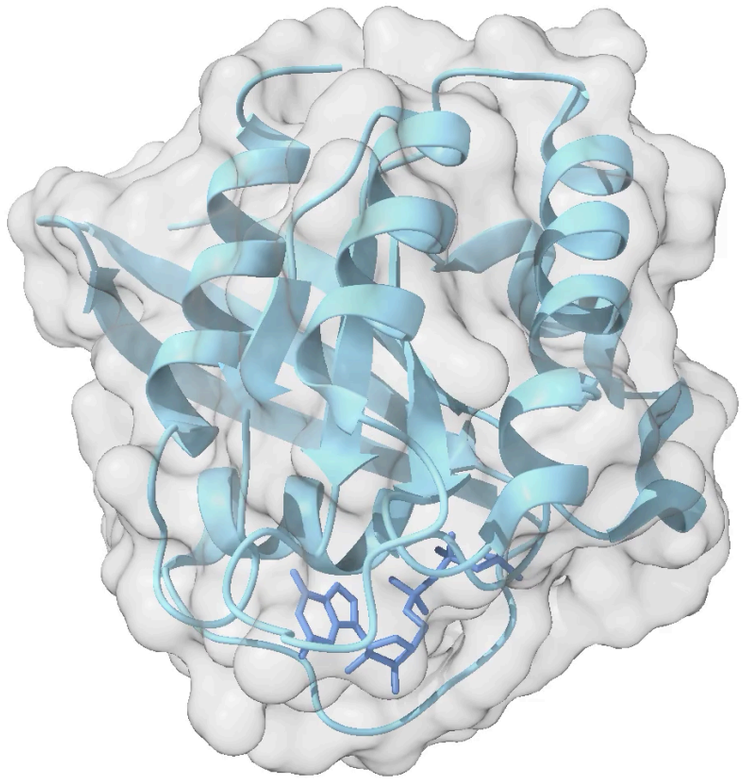
Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. *Cancer Cell*. **2014**, 25, 272–281.

The “Death Star” of Cancer

Ras as a drug target

Direct Ras targeting: “Death Star”

Ras



- Ras is spherical in shape
- No obvious binding grooves/pockets (except GTP)
- **Efforts moved towards indirect targeting**

Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. *Cancer Cell*. **2014**, 25, 272–281.

The "Death Star" of Cancer

Ras as a drug target

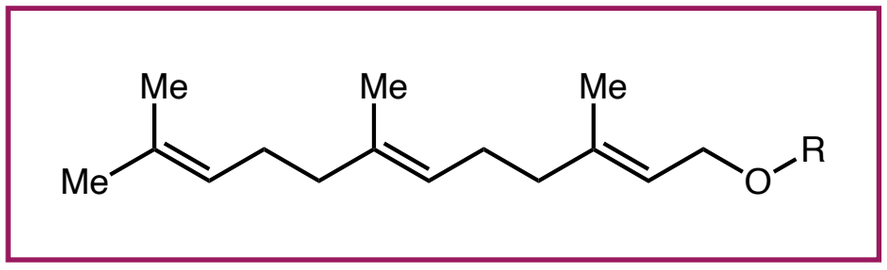
Indirect Ras targeting: farnesyltransferase inhibitors

Hypervariable Domain

HRAS	HKLRKLNPPDESGPGCMSCKCVLS
NRAS	YRMKKLNSSDDGTQGCMGLPCVVM
KRAS 4A	YRLKKISKEEKTPGCVKIKKCIIM
KRAS 4B	HKEKMSKDGKKKKKKSKTKCVIM

membrane targeting domain

farnesyl group



- farnesylation is key to Ras localization
- blocking modification blocks Raf interaction
- search for FTIs - high initial optimism
- initial efforts involved peptidomemetics

"CAAX - like motif"

compete with native farnesyl diphosphate

Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. *Cancer Cell*. **2014**, 25, 272–281.

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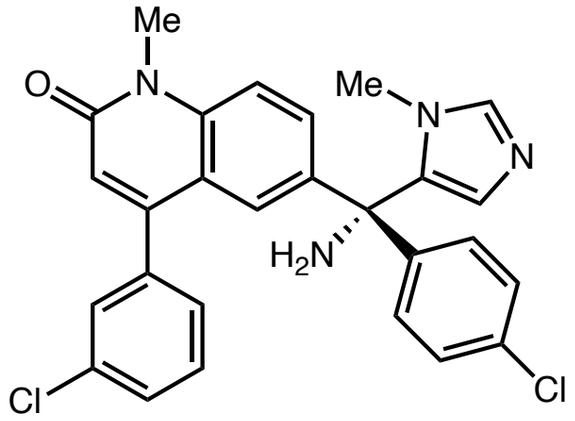
membrane targeting domain

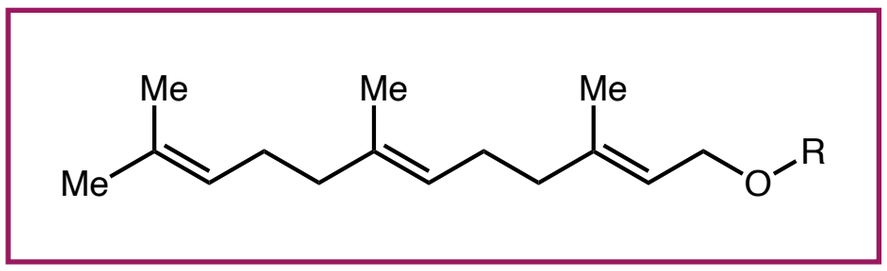
Tipifarnib



→ drugs like Tipifarnib blocked farnesylation

none have ever made it past phase III trials

farnesyl group



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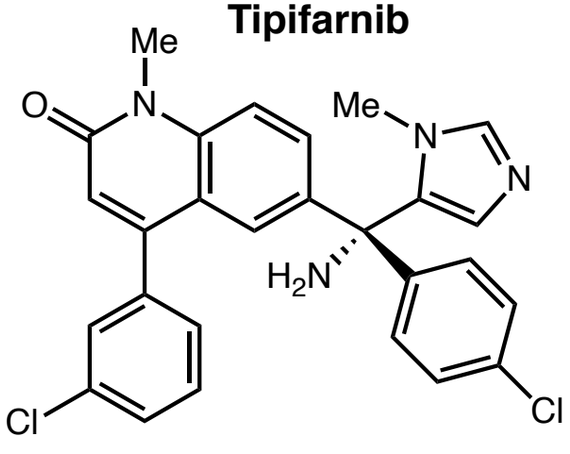
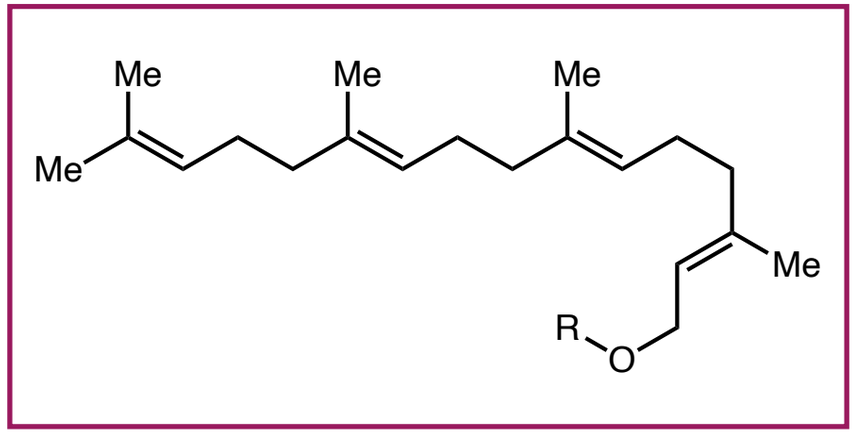
membrane targeting domain

- farnesyl inhibition successful
- KRas, NRas have built-in backups
- geranylgeranylation in absence of farnsenylation

Tipifarnib



geranylgeranyl group



Whyte, D. et al. *Cell Biology and Metabolism*. **1997**, 272, 22, 14459-14464.

The "Death Star" of Cancer

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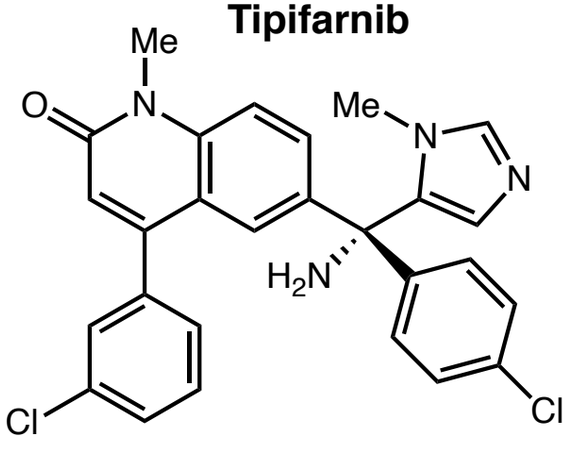
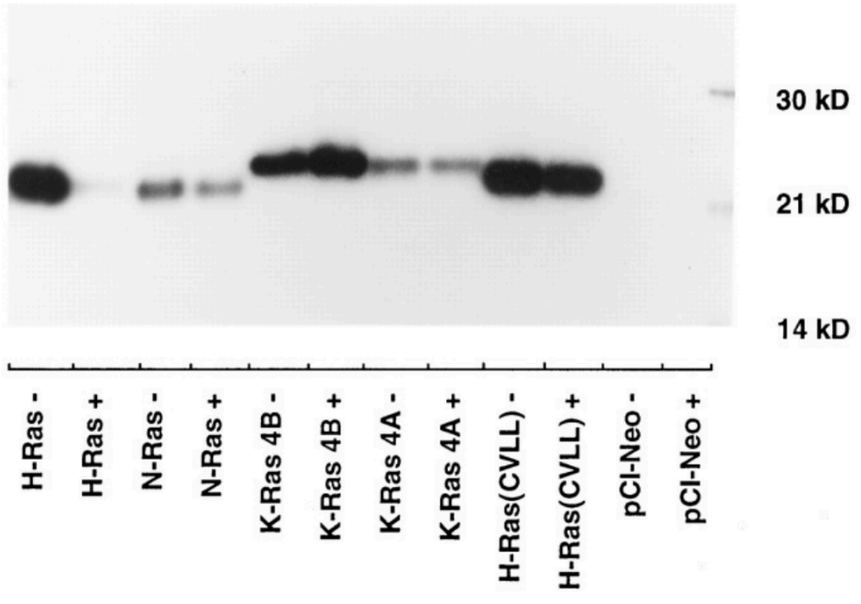
Tipifarnib



prenylation still occurs for KRas and NRas



Whyte, D. et al. *Cell Biology and Metabolism*. **1997**, 272, 22, 14459-14464.

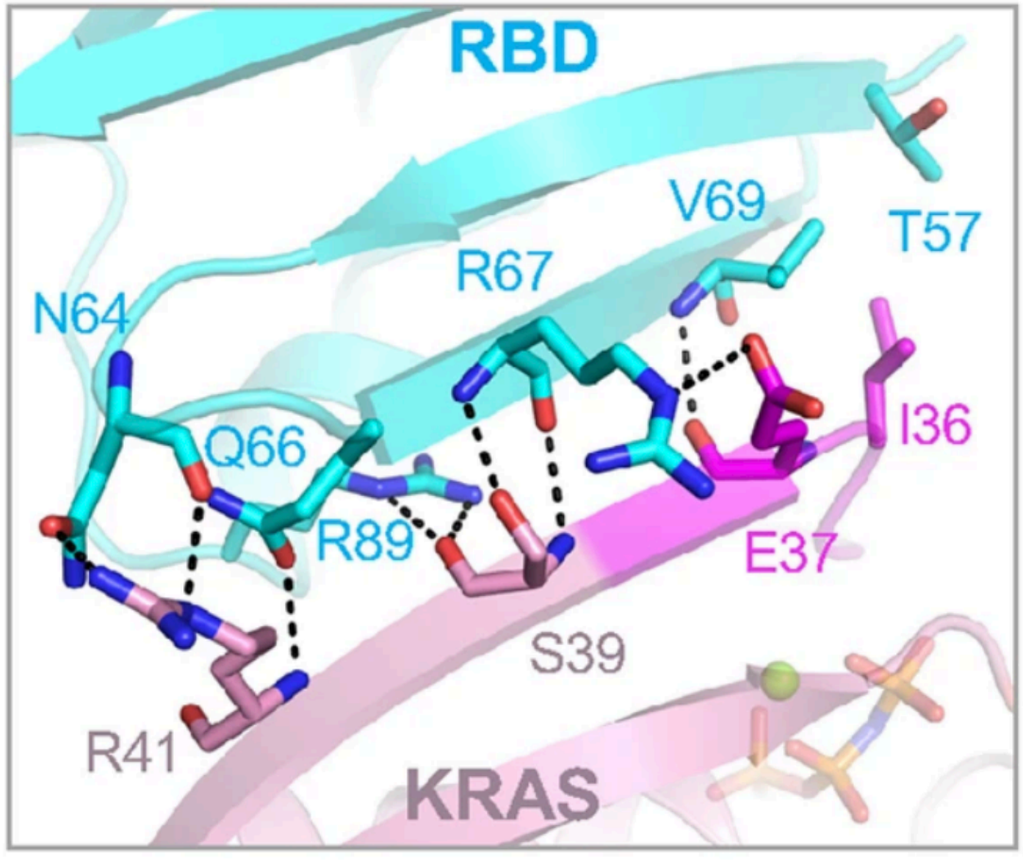
The “Death Star” of Cancer

Ras as a drug target

Indirect Ras targeting: other approaches

Late 1990s: targeting mutant Ras directly seems impossible with current tools

- attention was focused towards downstream targeting
- c-Raf known to bind Ras directly, attractive target for therapy



Ras-Raf binding interaction difficult to disrupt

- two antiparallel beta sheets

McCormick, F. et al. *Nature Communications*. **2021**, 12, 1176.

The “Death Star” of Cancer

Ras as a drug target

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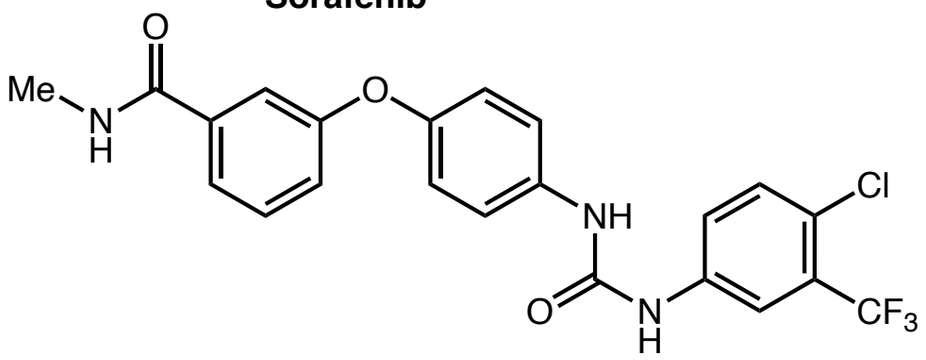
- attention was focused towards downstream targeting
- c-Raf known to bind Ras directly, attractive target for therapy
- Sorafenib - approved c-Raf inhibitor developed

Sorafenib



lack of efficacy
in Ras driven cancers

McCormick, F. et al. *Nature Communications*. **2021**, 12, 1176.

The “Death Star” of Cancer

Ras as a drug target

Indirect Ras targeting: other approaches

Late 1990s: targeting mutant Ras directly seems impossible with current tools

other targets

MEK/ERK

PI3K

feedback mechanisms activate “uphill” signaling

Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. *Cancer Cell*. **2014**, 25, 272–281.

The “Death Star” of Cancer

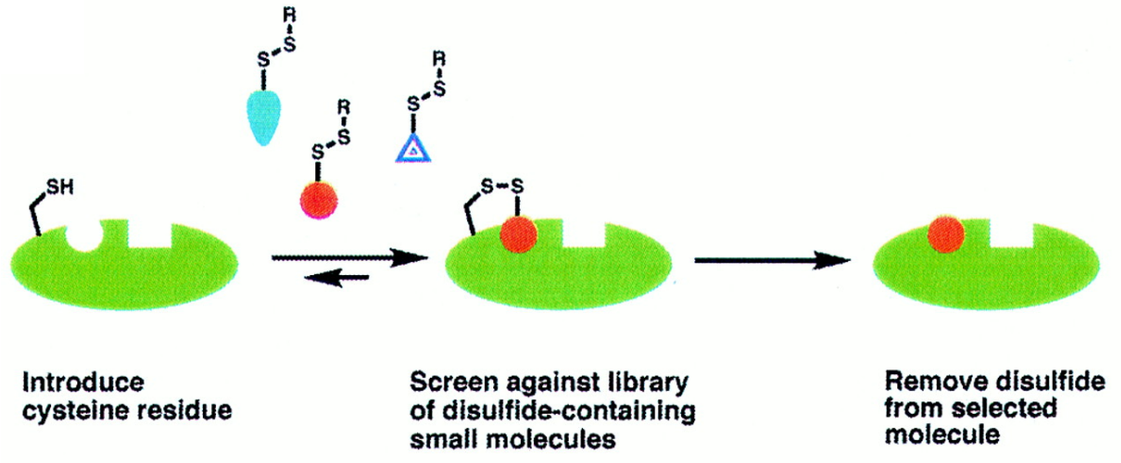
Shooting down the Death Star

The breakthrough: G12C inhibitors

2013 - discovery of the switch II pocket

- cystine tethering based fragment screen - over 480 compounds

Kevan Shokat



Shokat, K. et al. *Nature*. **2013**, 503, 548–551.

The “Death Star” of Cancer

Ras as a drug target

The breakthrough: G12C inhibitors

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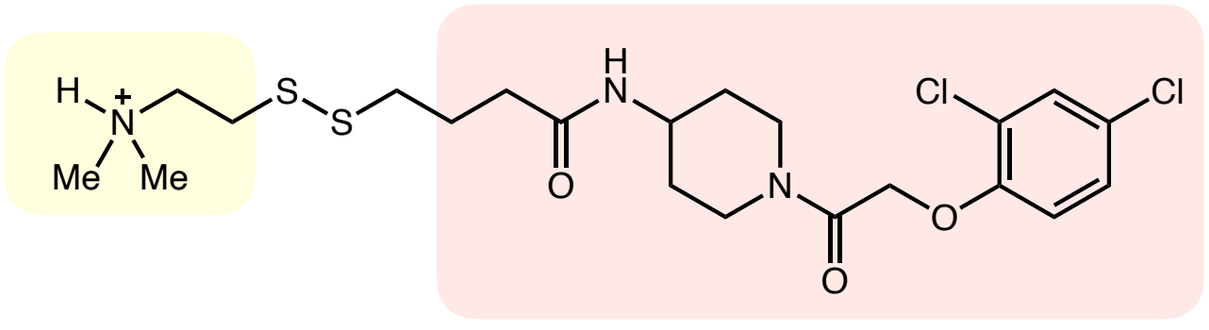
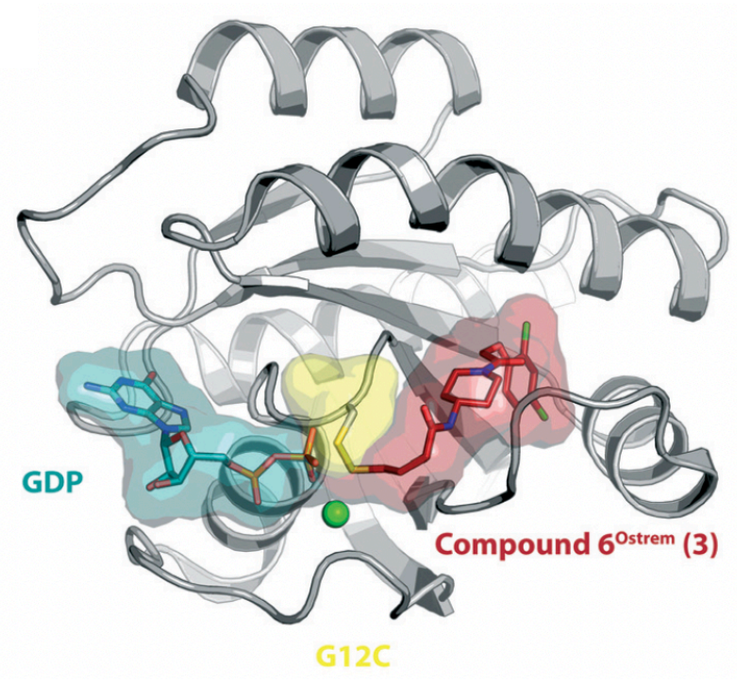
- discovery of previously unknown switch II binding pocket

Kevan Shokat

Compound 6





Shokat, K. et al. *Nature*. **2013**, 503, 548–551.

Rauh, D. et al. *RSC Med. Chem*. **2020**, 11, 760–770.

The “Death Star” of Cancer

Ras as a drug target

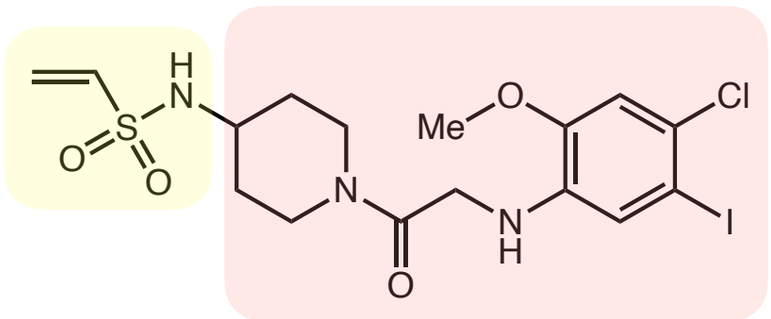
The breakthrough: G12C inhibitors

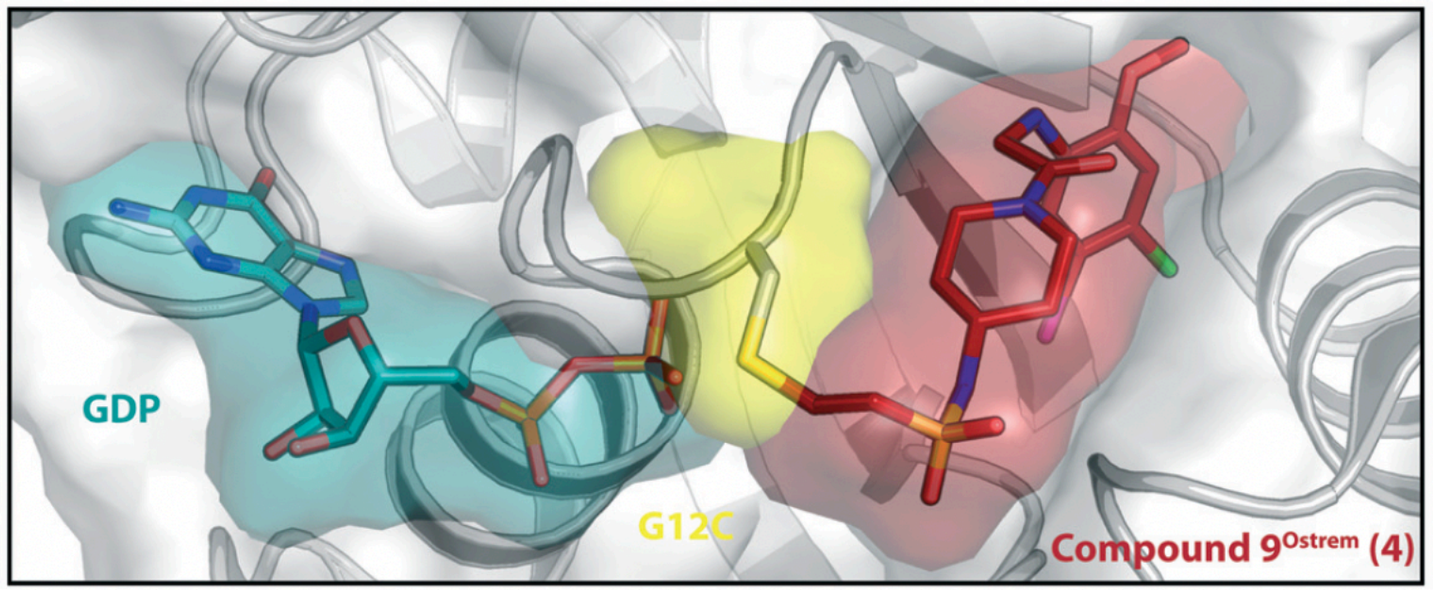
2013 - discovery of the switch II pocket

- cystine tethering based fragment screen - over 480 compounds
- discovery of previously unknown switch II binding pocket
- carbon-based electrophile compounds for irreversible binding

Kevan Shokat

Compound 9





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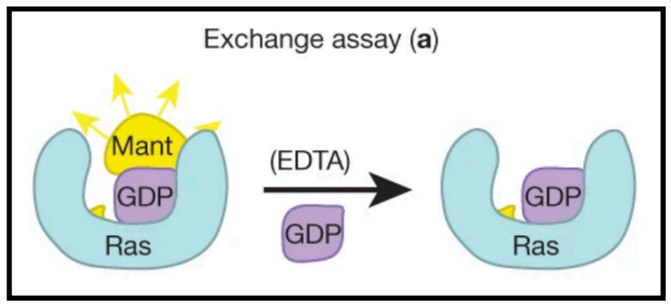

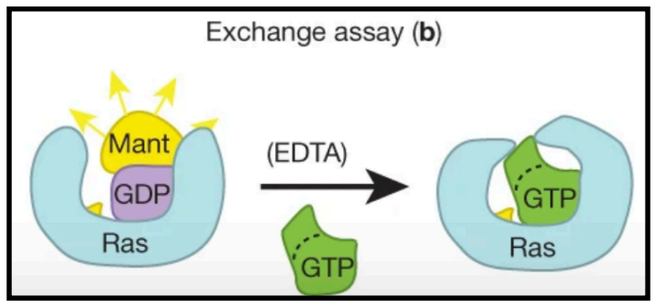

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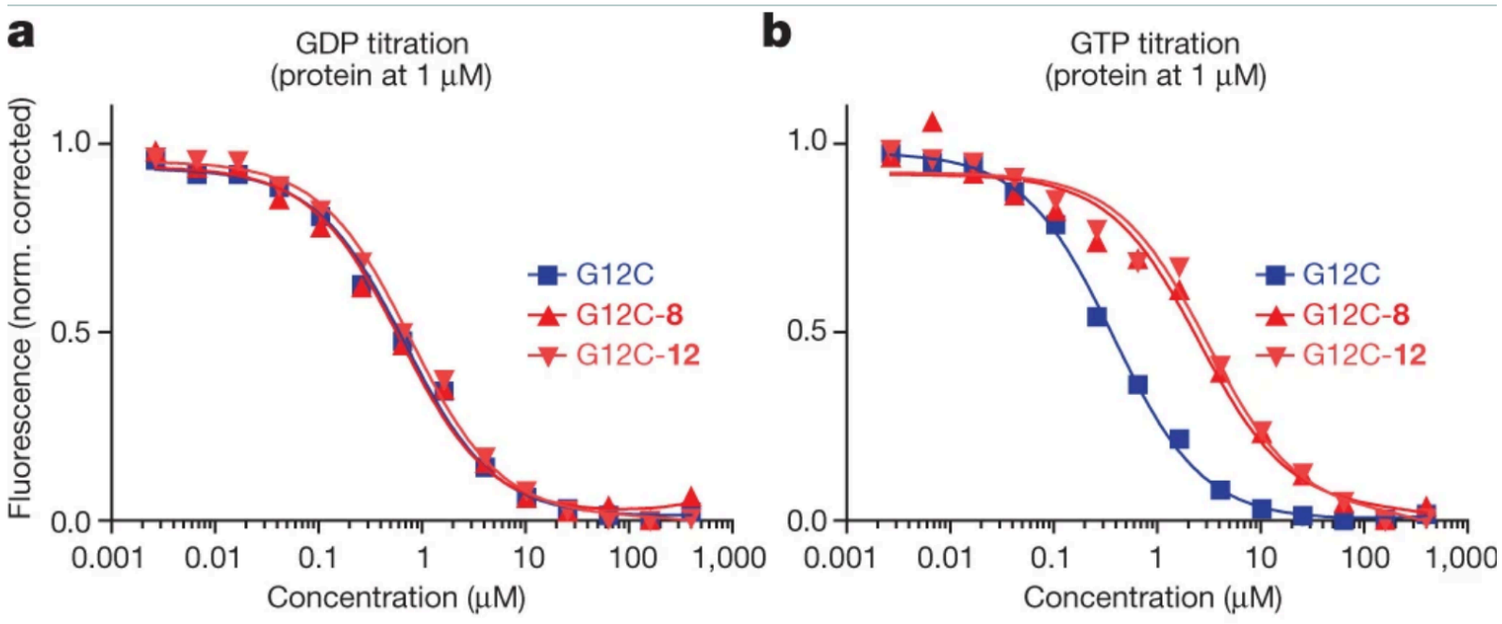

electrophilic G12C inhibitors change nucleotide preference to GDP

Shokat, K. et al. *Nature*. **2013**, 503, 548–551.

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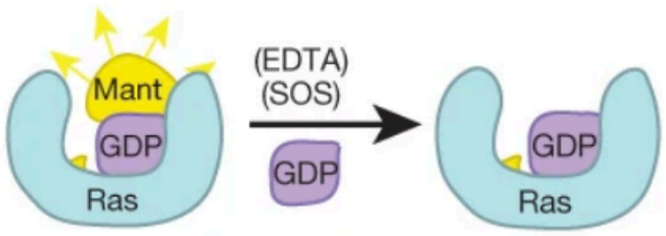

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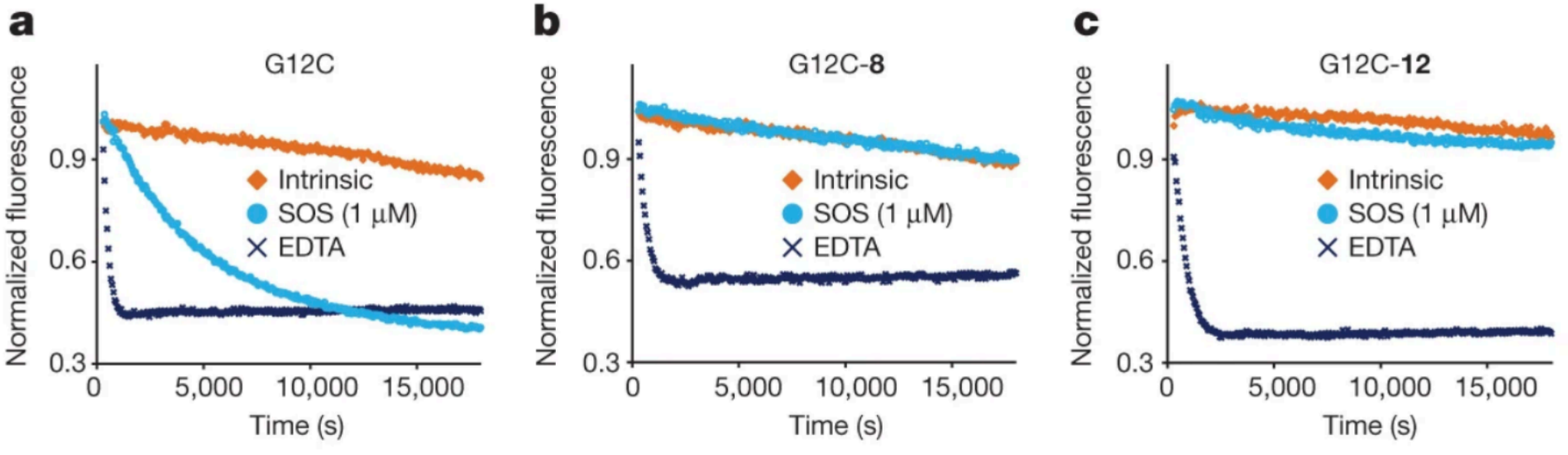

electrophilic G12C inhibitors prevent SOS/GEF GDP removal

Shokat, K. et al. *Nature*. **2013**, 503, 548–551.

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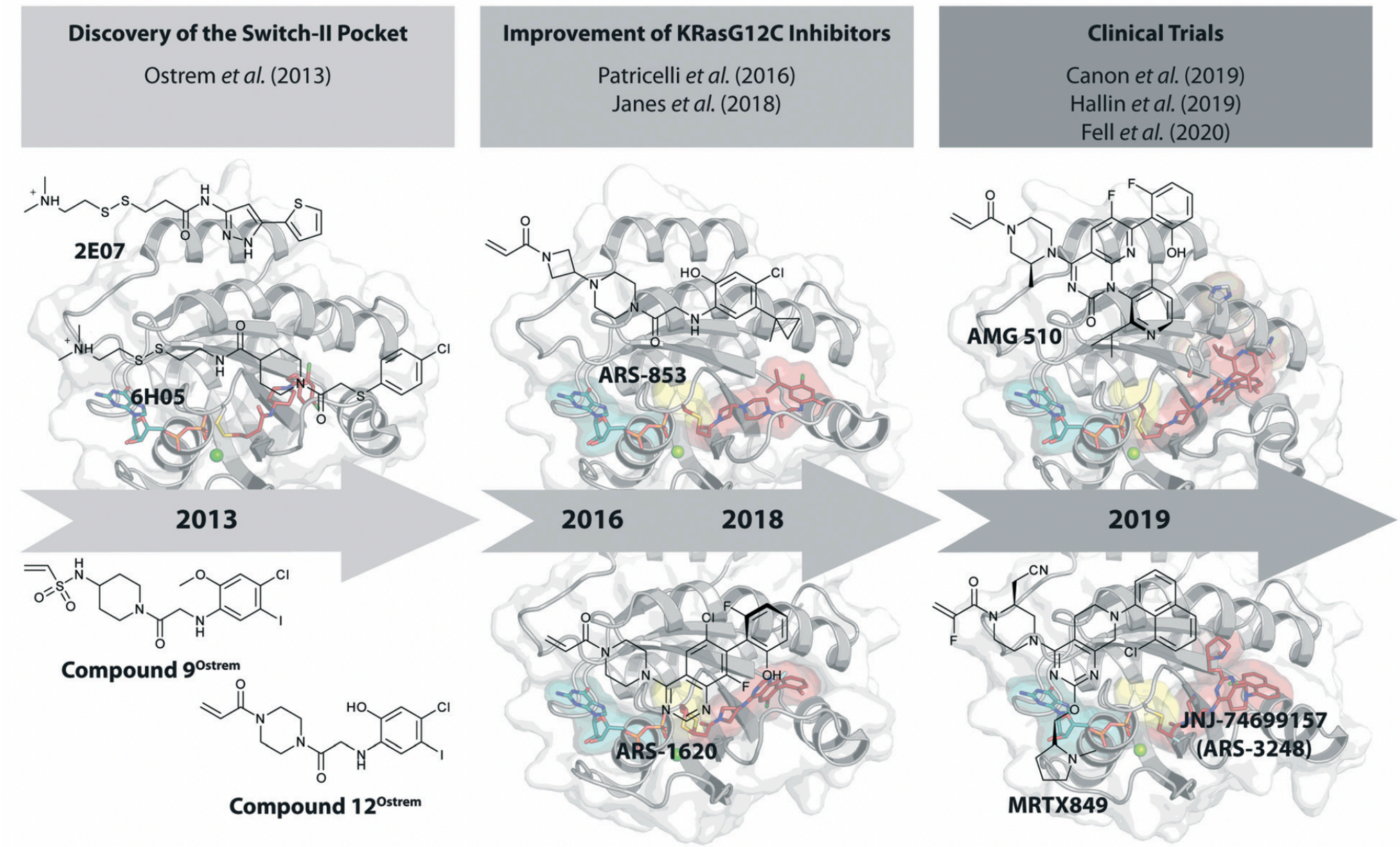
The "Death Star" of Cancer

Ras as a drug target

The race is on

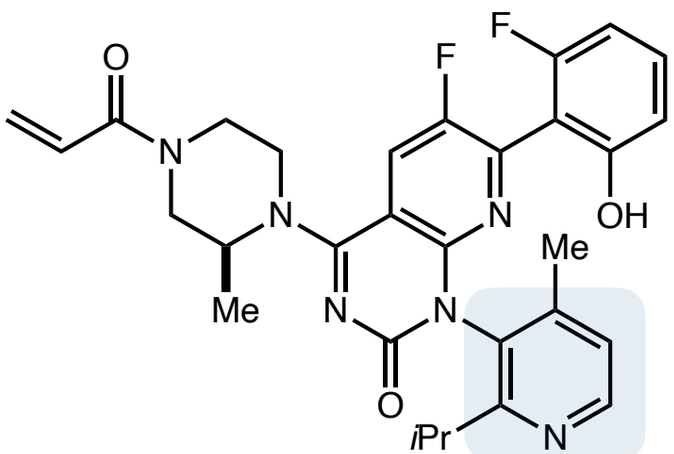


Rauh, D. et al. *RSC Med. Chem.* **2020**, 11, 760–770.

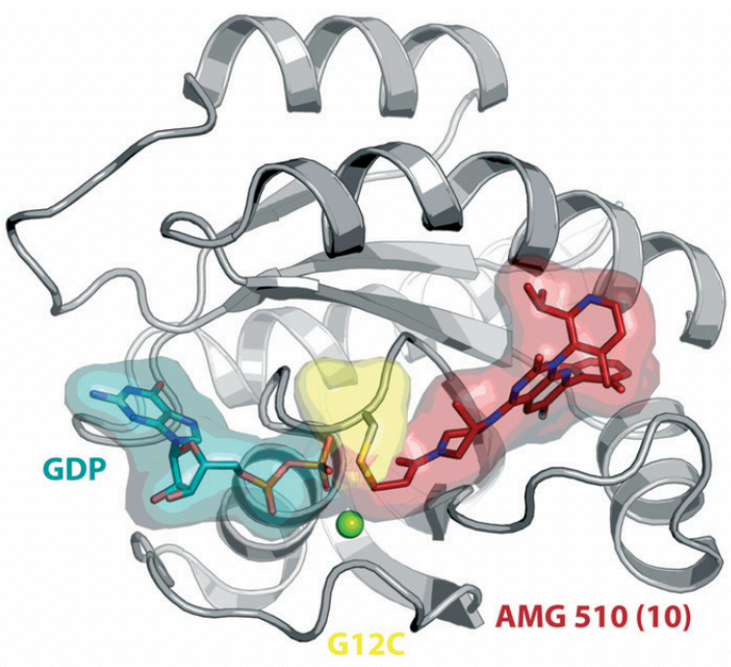
The “Death Star” of Cancer

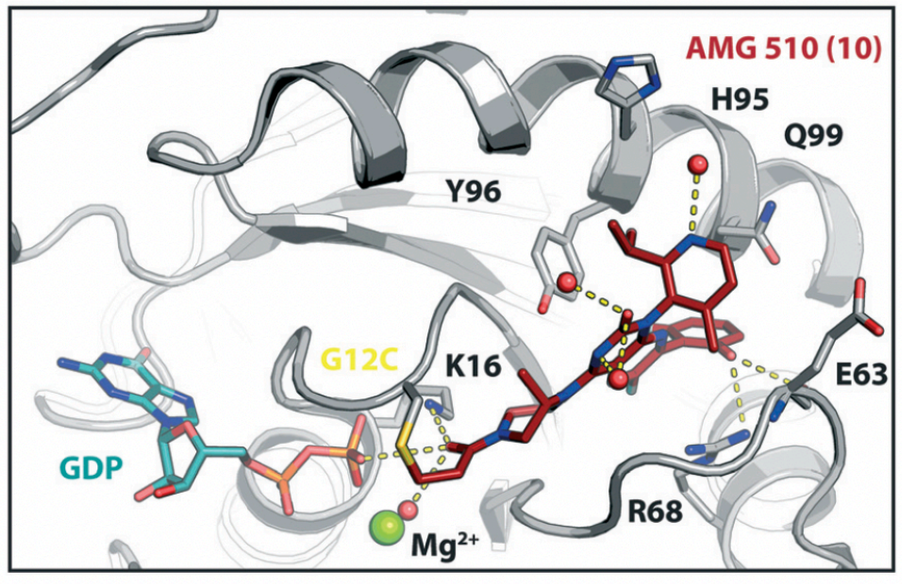
Ras as a drug target

AMG 510 - the magic bullet



- developed by Amgen - various binding optimisations
- His95 binding groove - 25 ligand-protein van der Waals contacts
- 10 fold potency improvement over previous best candidate





Lipford, J.R. et al. *Nature*. **2019**, 575, 217–223.

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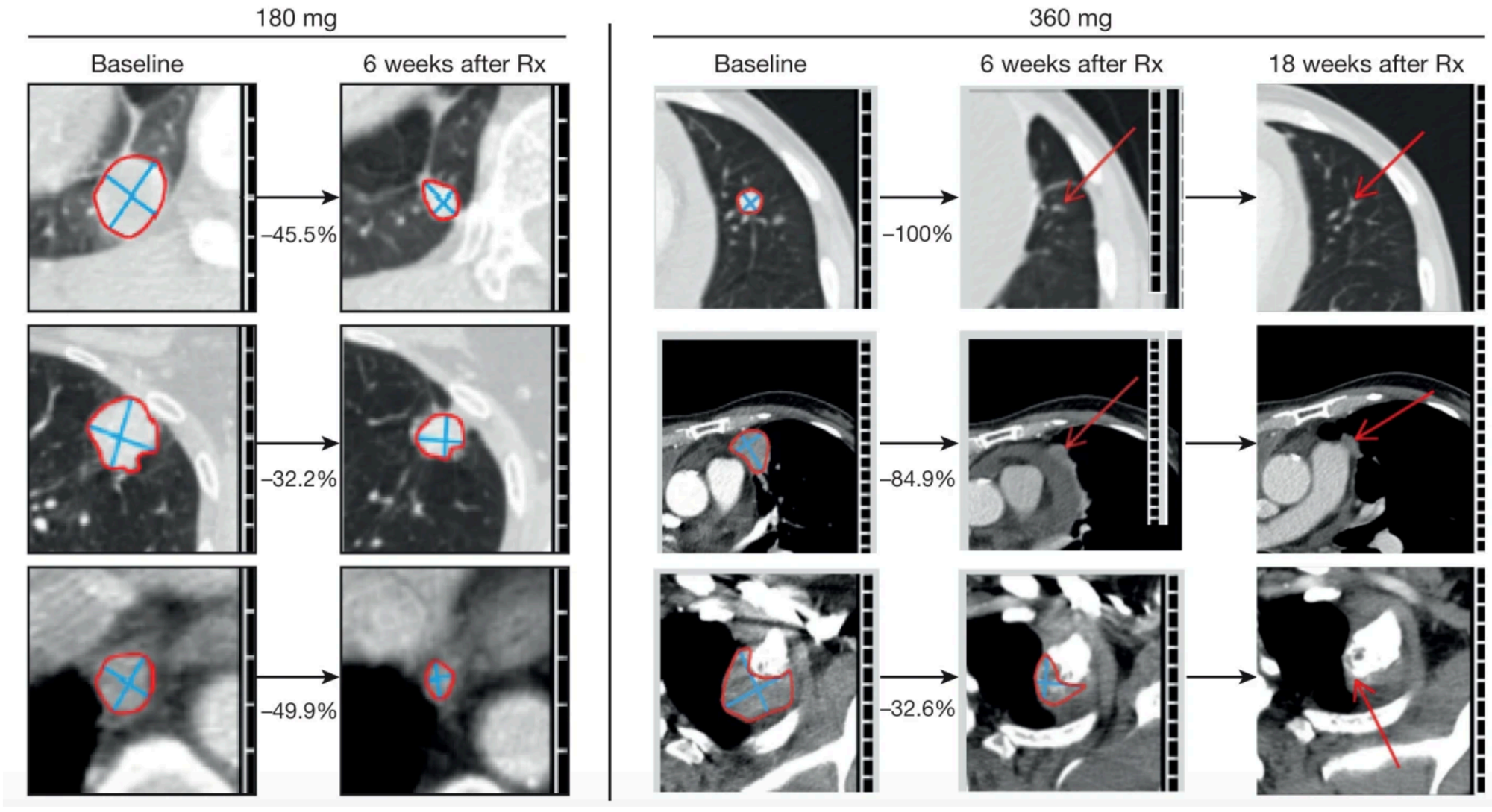
The “Death Star” of Cancer

Ras as a drug target

AMG 510 - clinical trials



greatly reduced carcinoma lesion size

Lipford, J.R. et al. *Nature*. **2019**, 575, 217–223.

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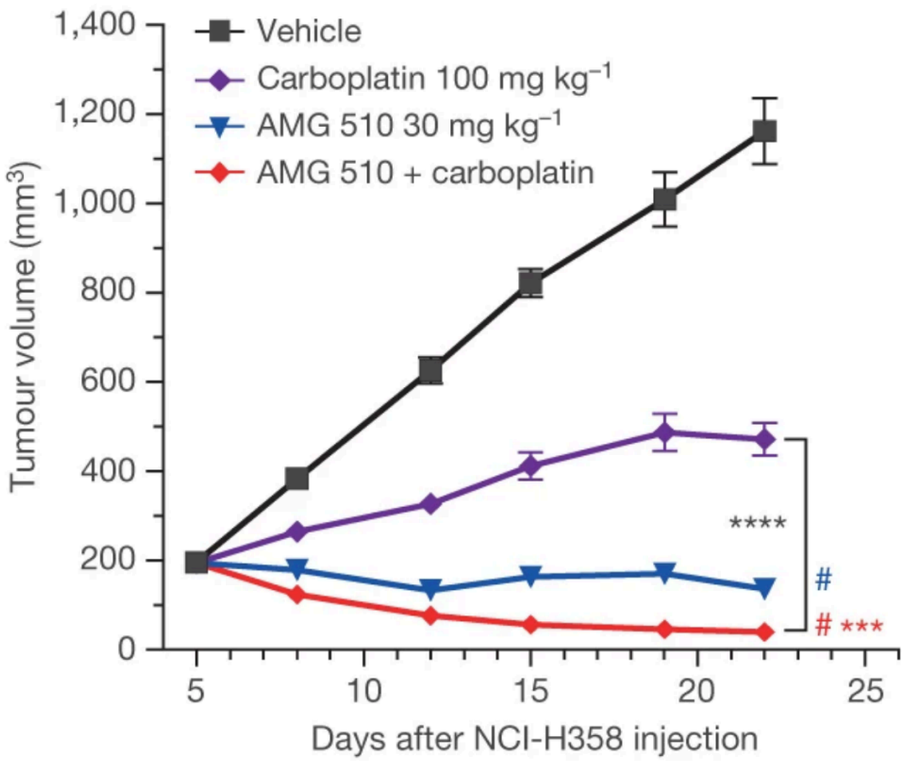
The "Death Star" of Cancer

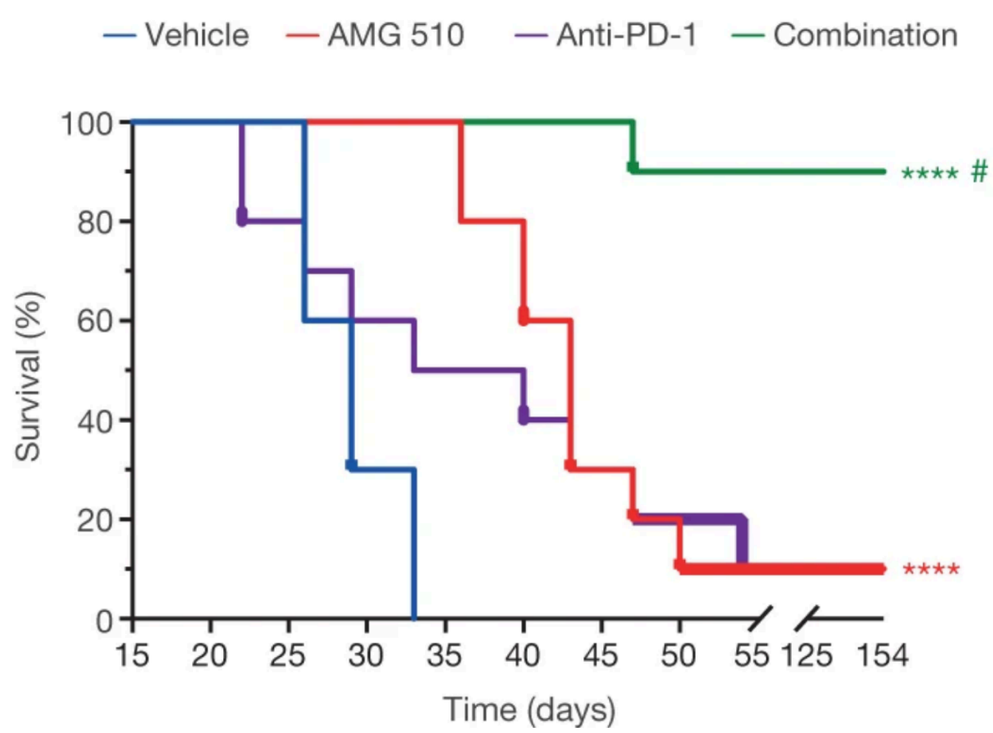
Ras as a drug target

AMG 510 - synergy with other therapies

chemotherapy



immunotherapy



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The “Death Star” of Cancer

Ras as a drug target

AMG 510 - FDA approval: Sotorasib

FDA NEWS RELEASE

FDA Approves First Targeted Therapy for Lung Cancer Mutation Previously Considered Resistant to Drug Therapy

May 28, 2021

“Sotorasib is the first KRas inhibitor to show an overall survival benefit.”

Amgen. Press Release. June 4, 2021. Web.

FDA. Press Release. May 28, 2021. Web.

Outline

- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib

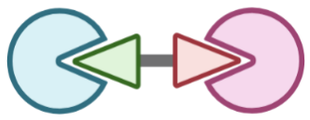
- Future directions/Outlook

Future Strategies for Oncogene targeting

Outlook

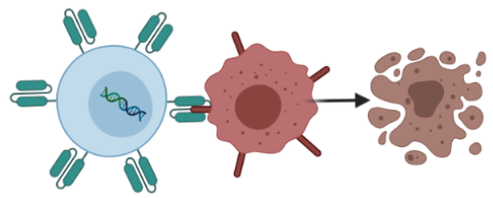
new strategies are being investigated to target oncogenes

new small molecules



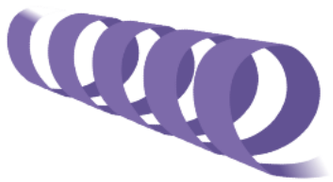
PROTACs

CAR-T



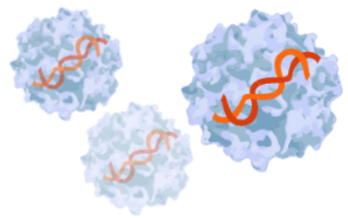
peptide-targeting

peptides/biologics



Omomyc

gene therapy



AAVs

Questions?

- Common oncogenes and their functions
  - *src*
  - *myc*
  - *Ras*
- The first KRAS treatment - sotorasib
- Future directions/Outlook