Frederick National Laboratory
for Cancer Research

Ras project overview

Frank McCormick

The Frederick National Laboratory is a federally funded research and development center operated by SAIC-Frederick, Inc., for the National Cancer Institute
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

RAS mutations in human cancer

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Pancreas	95%	KRAS
Colorectal	45%	KRAS
Lung	35%	KRAS
AML	30%	NRAS
Melanoma	15%	NRAS
Bladder Cancer	5%	HRAS

Ras pathway mutations in lung adenocarcinoma

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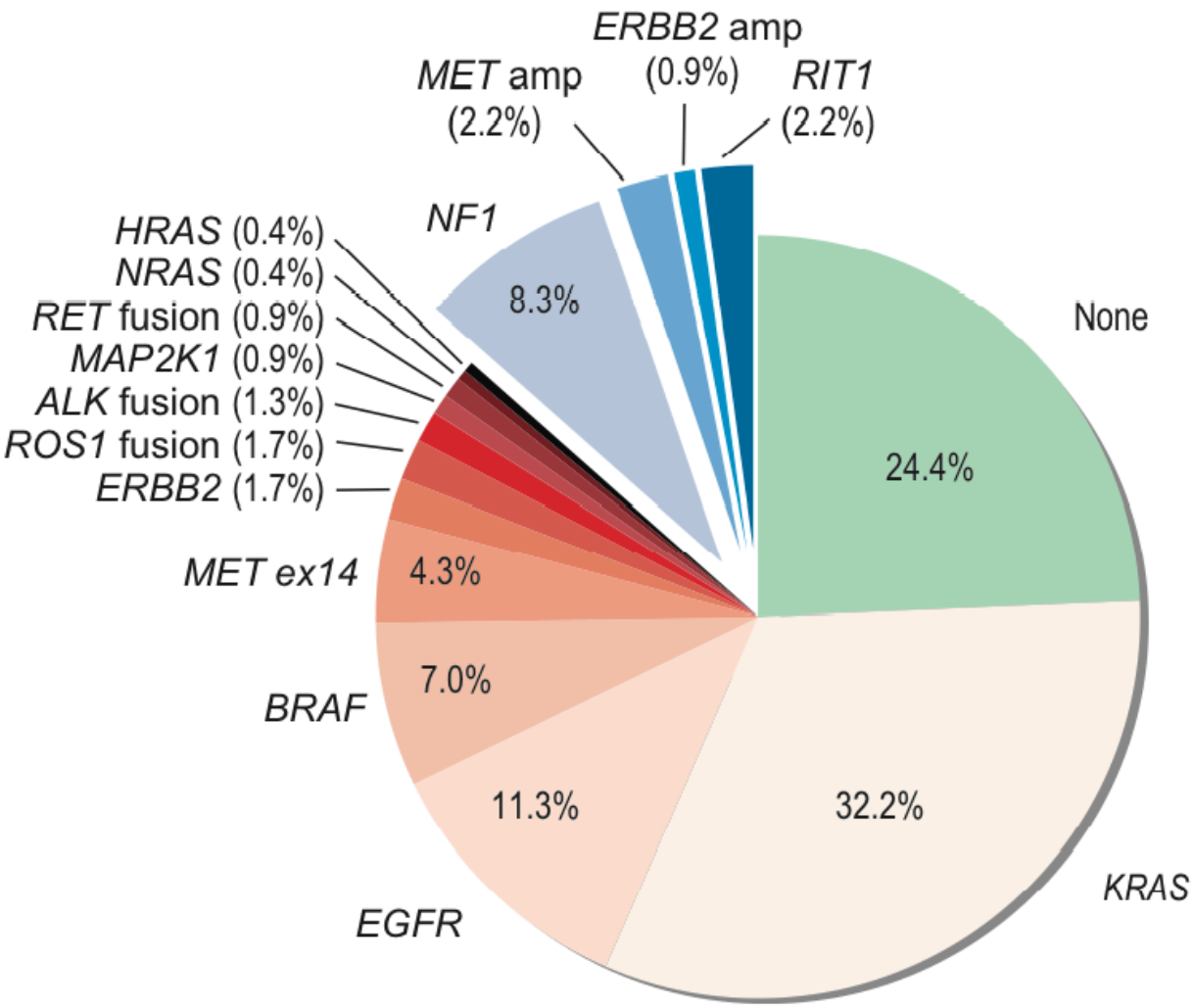

TCGA: Alice Berger, William Lee

Challenges to targeting Ras cancers

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No structures of mutant KRAS complexed with any effector or regulator

No structure of full length KRAS

Don’t know how Ras activates Raf kinase

No structure of full length Raf, free or Ras-bound

Unclear which KRAS cancers depend on KRAS in vivo

Unclear which effector pathways are critical in vivo

Opportunities to harness the immune system for KRAS cancers?

Parameters affecting oncogenic Ras activity

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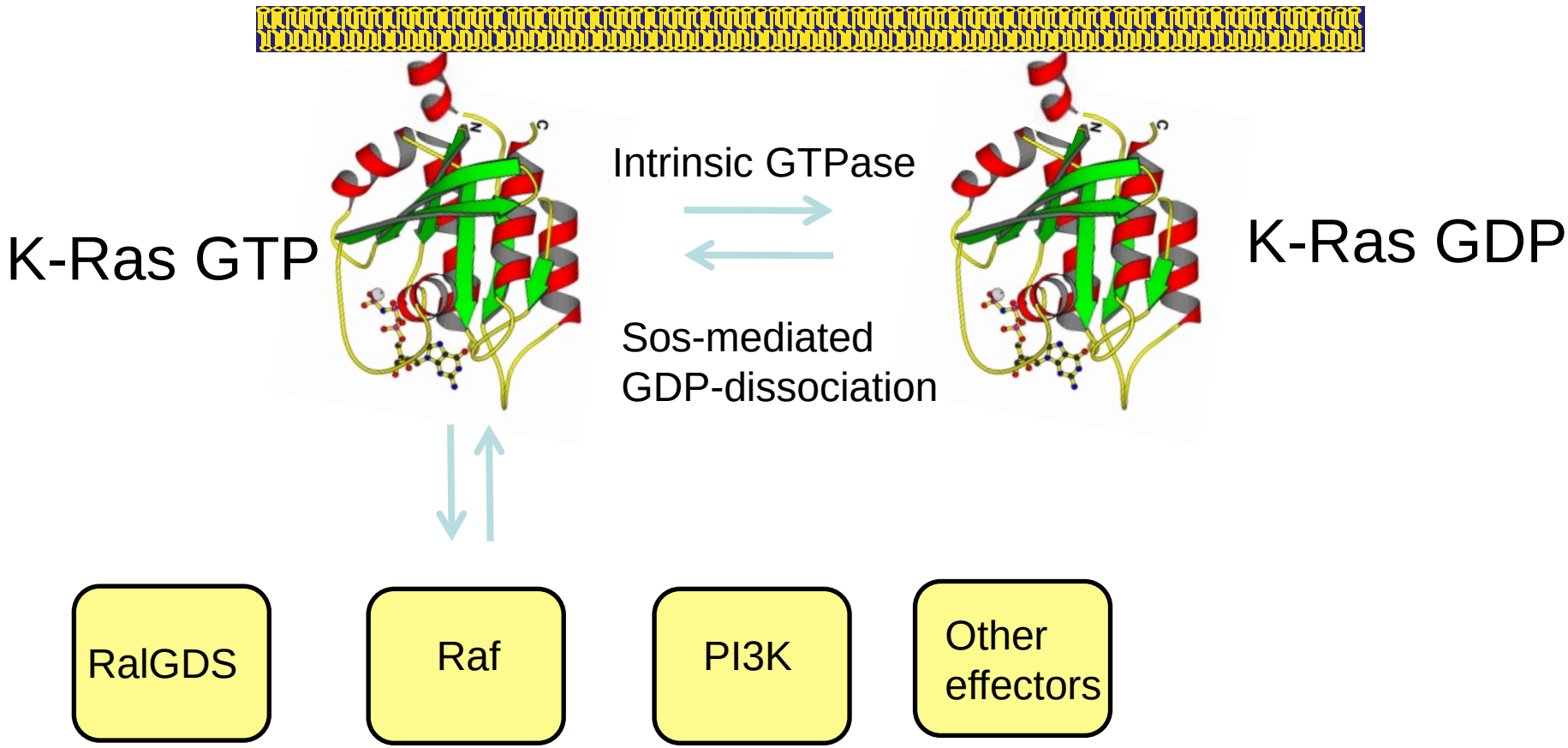

KRAS for structural analysis

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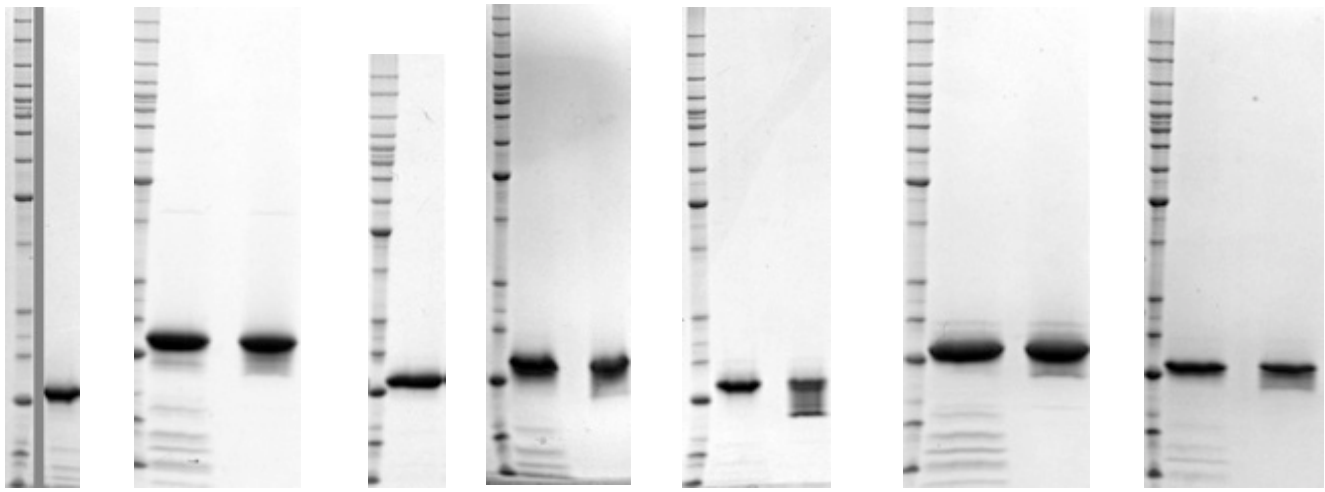
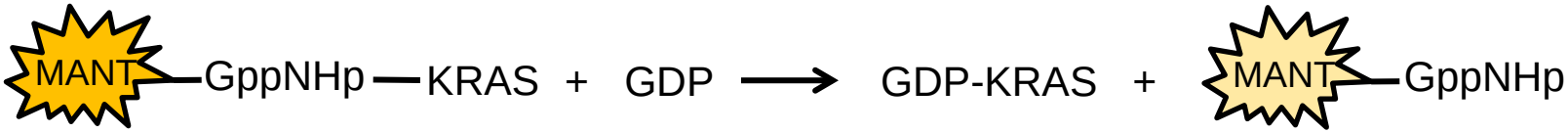
All proteins were expressed in *E. coli* as His6-MBP-tev-RAS, and purified by IMAC, TEV digestion, IMAC, SEC with only monomeric protein collected for the final samples



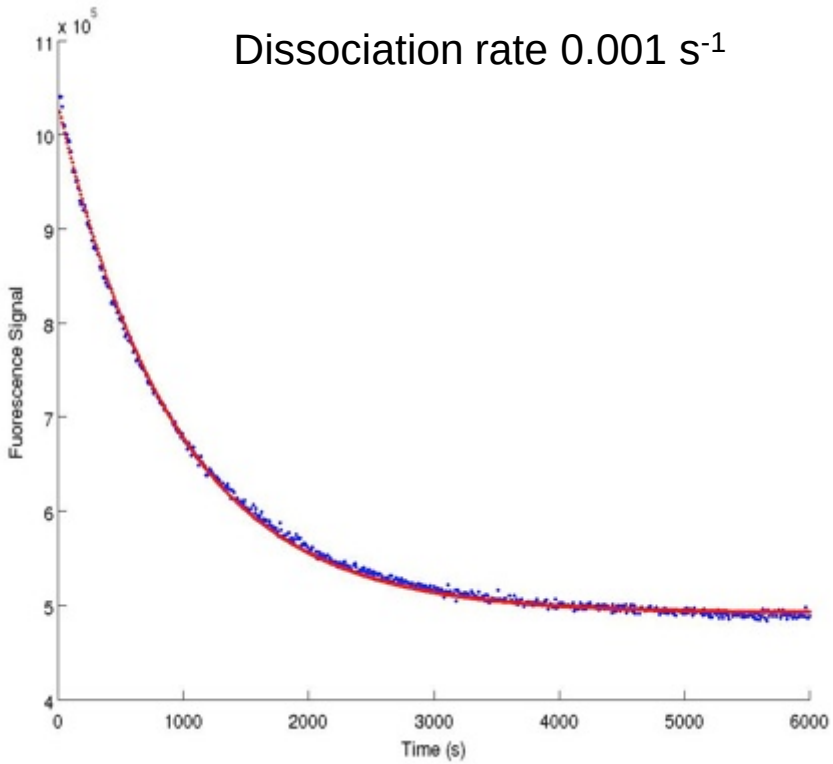
	WT	G12C	G12D	G12V	G13D	Q61L	Q61R
Lot #:	1NOV13	032014-2	1NOV13	040314-1	022814-1	032814-2	040114-1
	19.1 mg/ml	11.6 mg/ml	14 mg/ml	4.5 mg/ml	10 mg/ml	10.6 mg/ml	12 mg/ml

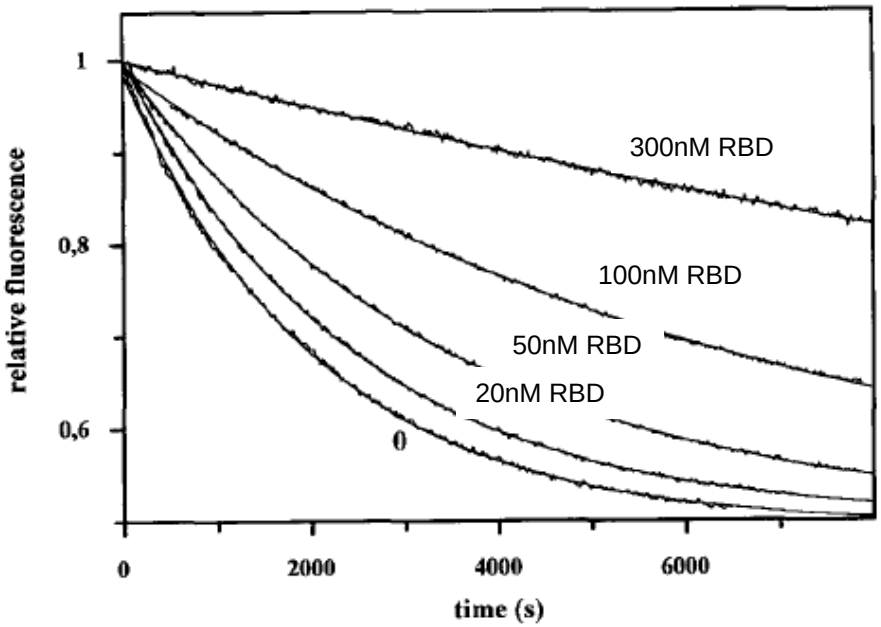
Storage buffer: 20 mM HEPES, pH 7.3
150 mM NaCl
2 mM TCEP
5 mM MgCl2
+ various GDP concentrations

KRAS characterization - Nucleotide dissociation



Dissociation rate 0.001 s^{-1}





Herman et al JBC 1995

Pat Alexander

Intrinsic GTP hydrolysis activity of K-Ras mutants

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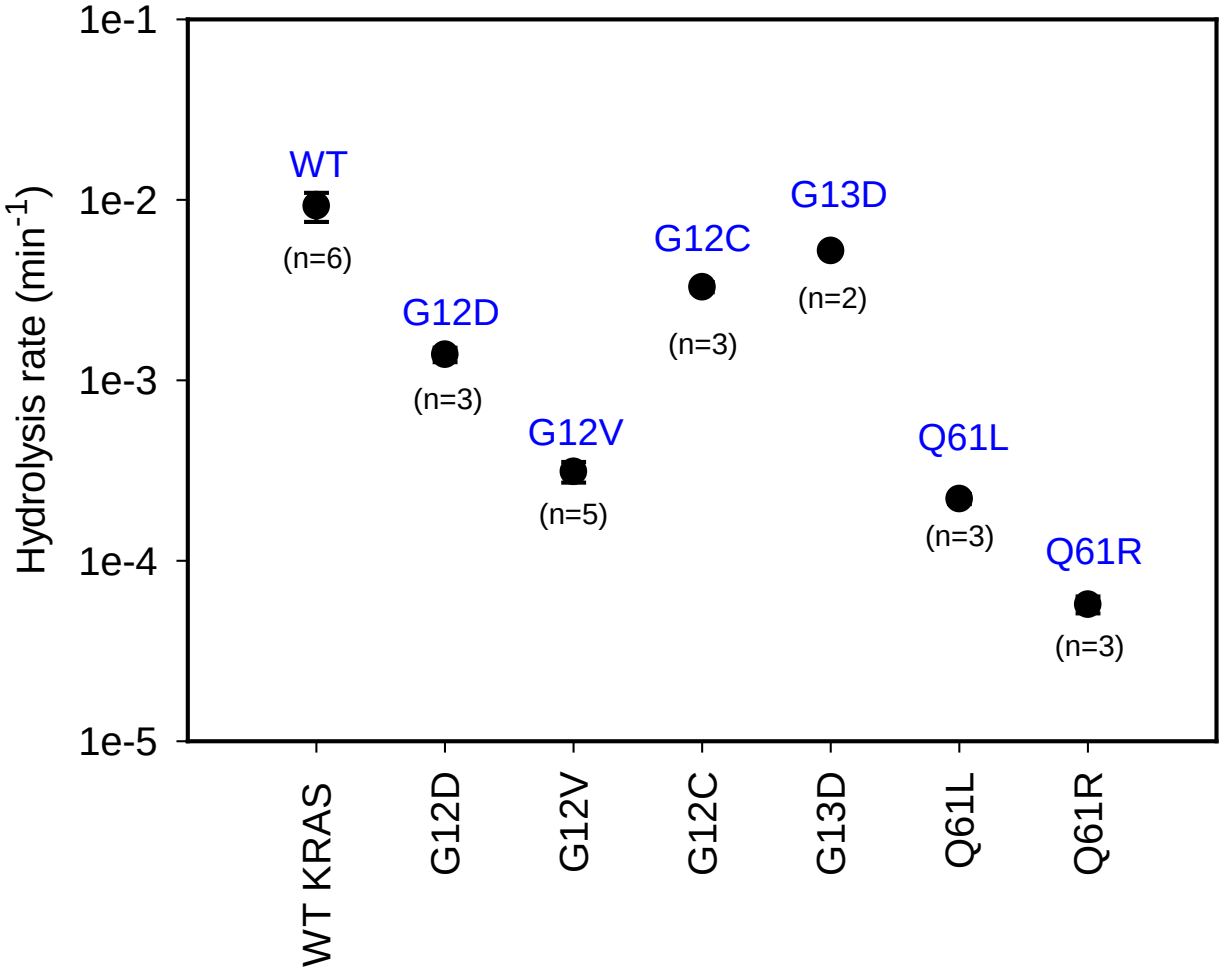

Structural determination of full length WT KRAS and oncogenic mutants

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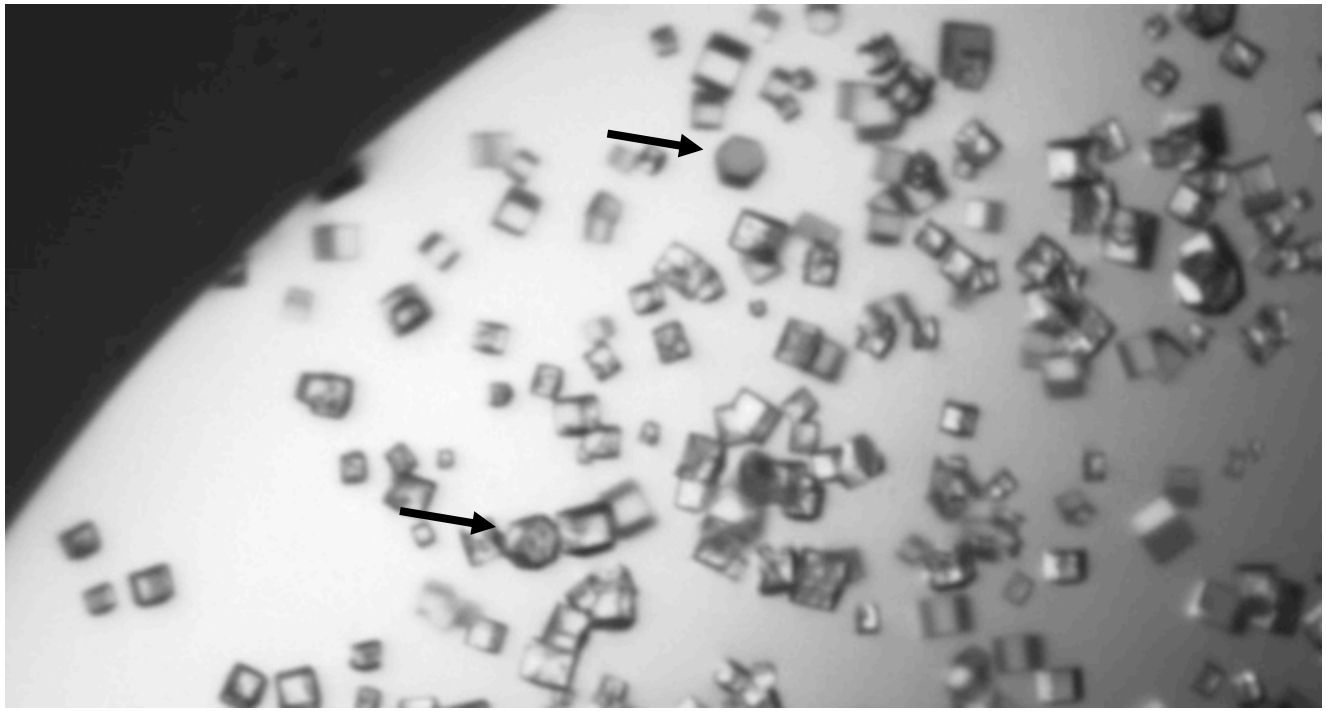
- **Crystals observed from in house screening of all 7 proteins (in GDP bound form)**



WT KRAS4b hexagonal crystals diffracting to 3-3.5 Å

Bill Gillette

New Recruit!

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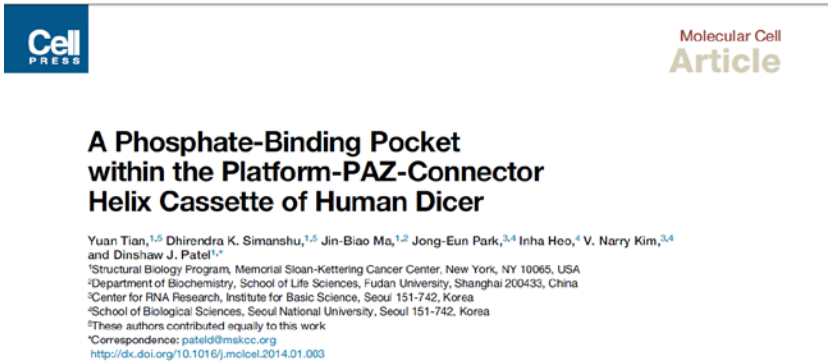
- **Dhirendra Simanshu**
  - Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.



Cell PRESS

Molecular Cell
Article

A Phosphate-Binding Pocket within the Platform-PAZ-Connector Helix Cassette of Human Dicer

Yuan Tian,[1,5] Dhirendra K. Simanshu,[1,5] Jin-Biao Ma,[1,2] Jong-Eun Park,[3,4] Inha Heo,[4] V. Narry Kim,[3,4] and Dinshaw J. Patel[1,*]
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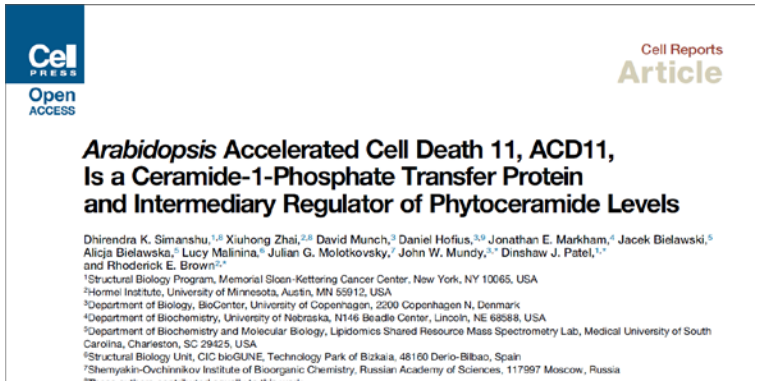
[4]School of Biological Sciences, Seoul National University, Seoul 151-742, Korea
[5]These authors contributed equally to this work
*Correspondence: pateld@mskcc.org
http://dx.doi.org/10.1016/j.molcel.2014.01.003



Cell PRESS
Open ACCESS

Cell Reports
Article

***Arabidopsis* Accelerated Cell Death 11, ACD11, Is a Ceramide-1-Phosphate Transfer Protein and Intermediary Regulator of Phytoceramide Levels**

Dhirendra K. Simanshu,[1,6] Xiuhong Zhai,[2,6] David Munch,[3] Daniel Hofius,[3,9] Jonathan E. Markham,[4] Jacek Bielawski,[5] Alicja Bielawska,[5] Lucy Malinina,[6] Julian G. Molotkovsky,[7] John W. Mundy,[3,*] Dinshaw J. Patel,[1,*] and Rhoderick E. Brown[2,*]
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[2]Hormel Institute, University of Minnesota, Austin, MN 55912, USA
[3]Department of Biology, BioCenter, University of Copenhagen, 2200 Copenhagen N, Denmark
[4]Department of Biochemistry, University of Nebraska, N146 Beadle Center, Lincoln, NE 68588, USA
[5]Department of Biochemistry and Molecular Biology, Lipidomics Shared Resource Mass Spectrometry Lab, Medical University of South Carolina, Charleston, SC 29425, USA
[6]Structural Biology Unit, CIC bioGUNE, Technology Park of Bizkaia, 48160 Derio-Bilbao, Spain
[7]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia

HTS assay development

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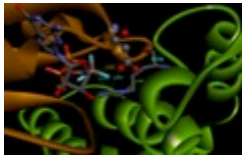
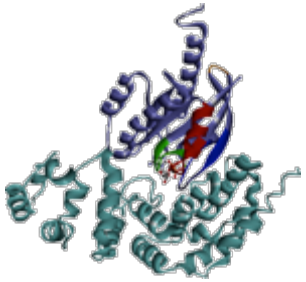
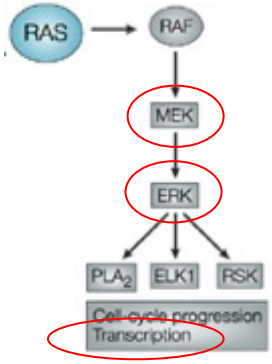
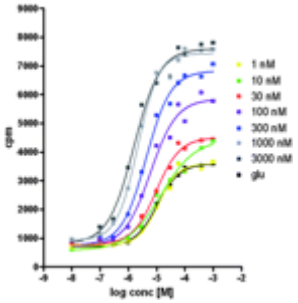
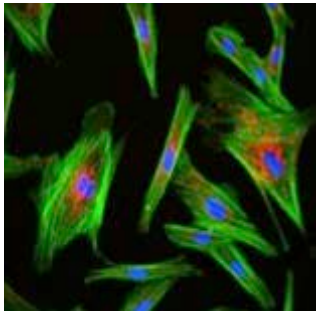






Molecular targeted assays	Phenotypic assays
Gap function	Proliferation
GTP hydrolysis	Cell death
Effector binding	RAS localization
Effector activation	





KRAS:RBD binding is GTP dependent

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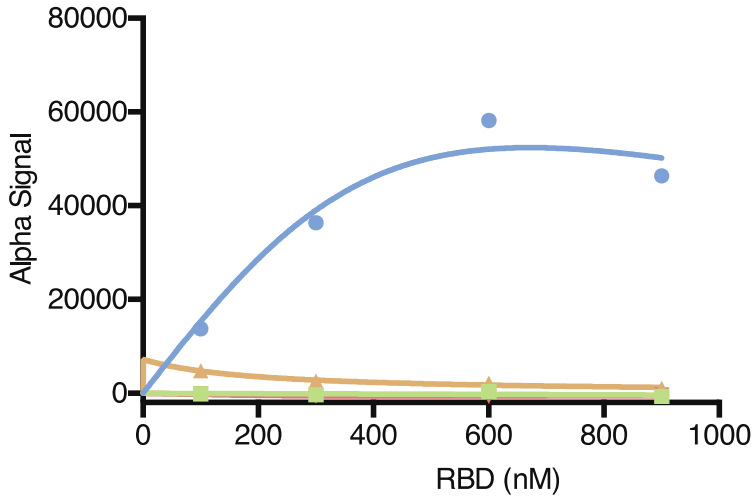
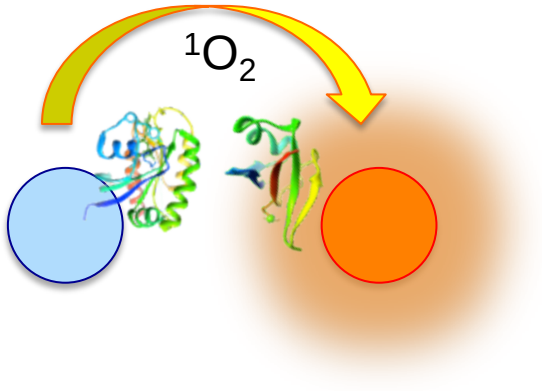


500 nM Avi-KRAS GTPγS
0 nM Avi-KRAS GTPγS
500 nM Avi-KRAS GDP
0 nM Avi-KRAS GDP



Avi-KRAS/GST-RBD

Maria Abreu-Blanco

Parameters affecting oncogenic Ras activity

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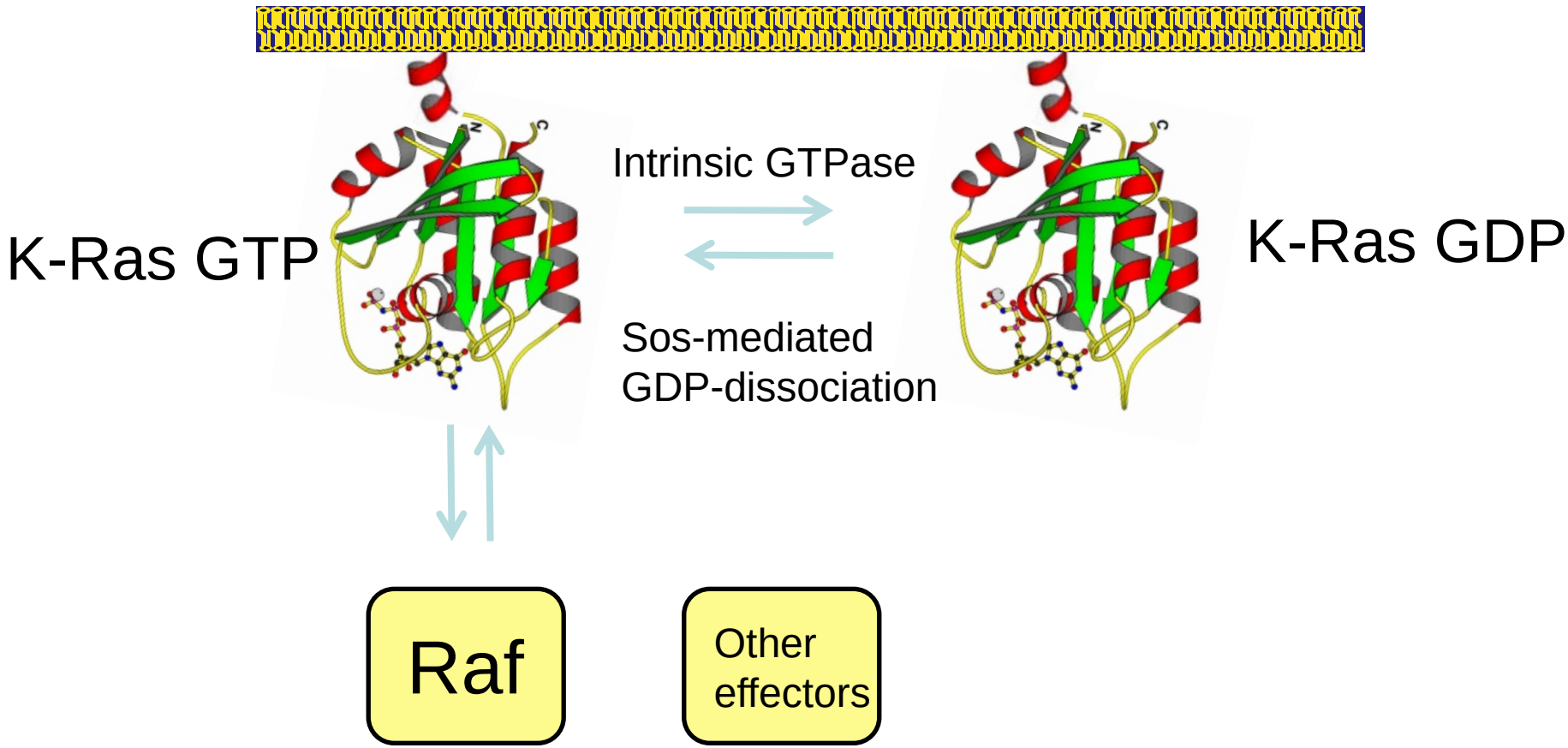
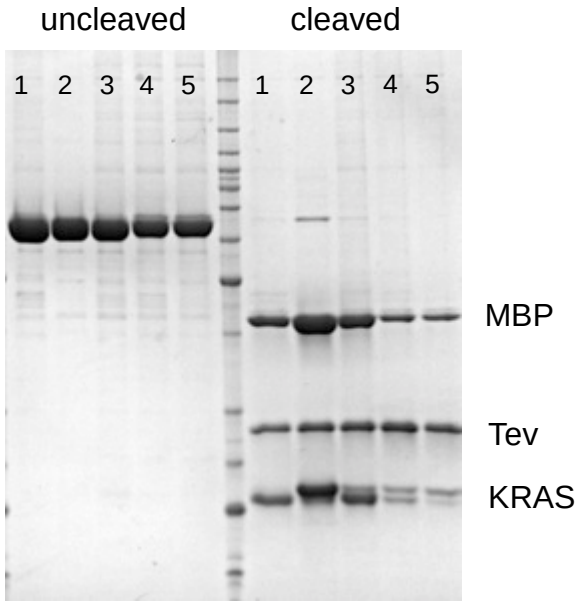
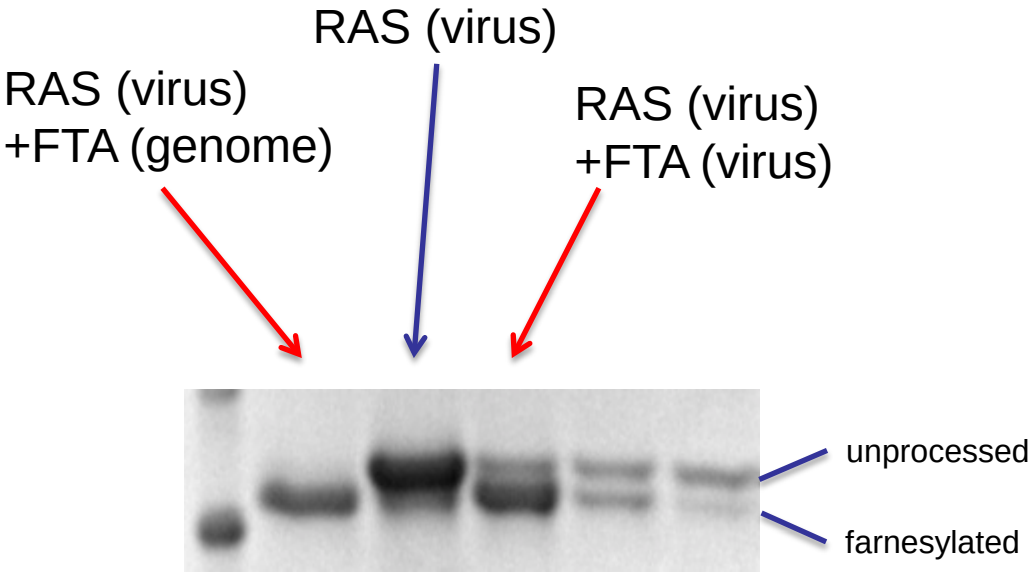


Modified BV strain improves prenylation





- **Initial results are promising**
  - final protein is single species with good yield (>2 mg/l)
  - methylation not complete (50-60%); probably requires additional ICMT
  - scale-up and purification optimization in progress

Plans for structural and biophysical analysis of fully processed KRAS

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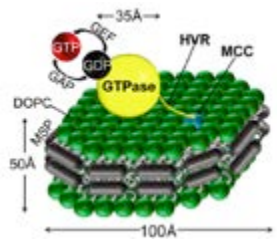
KRAS-lipid interactions

Prepare and QC nanodiscs

Prepare and QC liposomes



Crystallography

Cryo-EM high resolution structure (Subramaniam, NCI)

NMR structure KRAS-nanodisc (Altieri, NIST/UMD)

Quantitate KRAS-liposome interactions by SPR or FCS (Groves, LBL/UCB)

KRAS-membrane orientation by neutron reflectivity (Nanda, NIST)

Next generation assays using second harmonic generation (Salafsky, Biodesy)

Measure biochemical and biophysical parameters with and without effectors

Disrupting KRAS complexes

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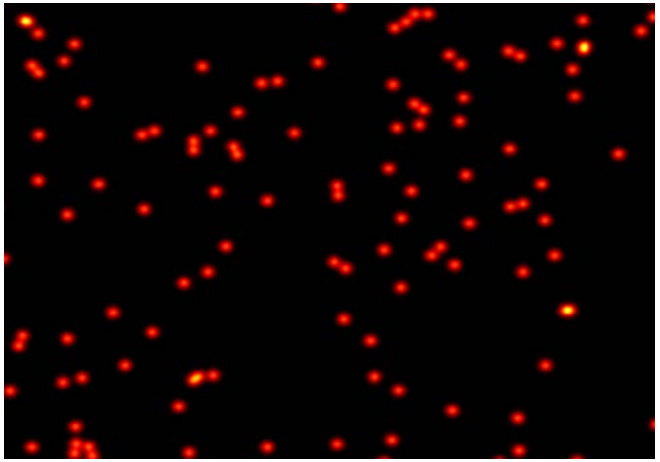
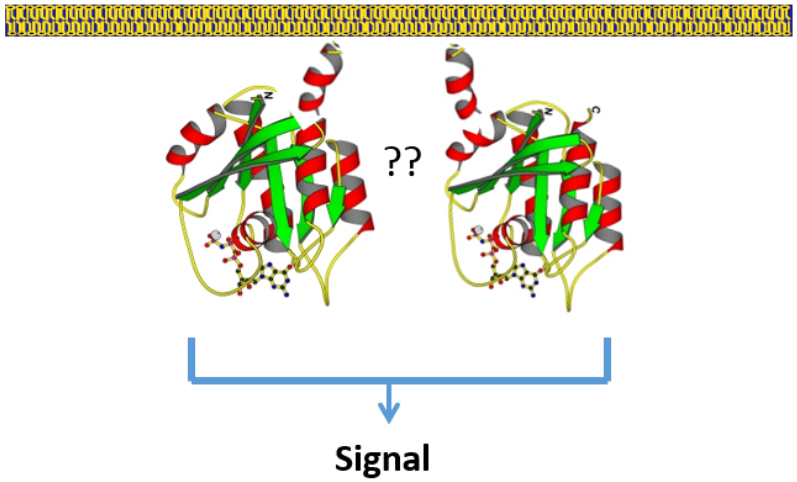
- Develop imaging methods to identify KRAS complexes in cells
- Develop screens for disrupting complexes





Novel cell line development in RAS-less MEFs

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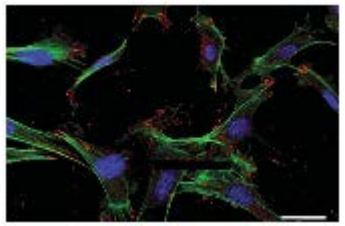

HRAS$^{-/-}$ NRAS$^{-/-}$ KRAS$^{lox/lox}$ MEFs

Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**. EMBO J. 2010

Rescue proliferation with normal RAS isoforms or mutant RAS isoforms

HRAS	NRAS	KRAS4A	KRAS4B
eGFP-WT	eGFP-WT	eGFP-WT	eGFP-WT
eGFP-G12V	WT	WT	WT
PAmCherry-WT	G12V	eGFP-mutant	eGFP-mutant
WT		G12V	G12V
G12V		G12D	**G12D**
		G12C	G12C
		G13D	G13D
		Q61L	Q61L
		Q61R	Q61R

RAS dependent proliferation screening strategies

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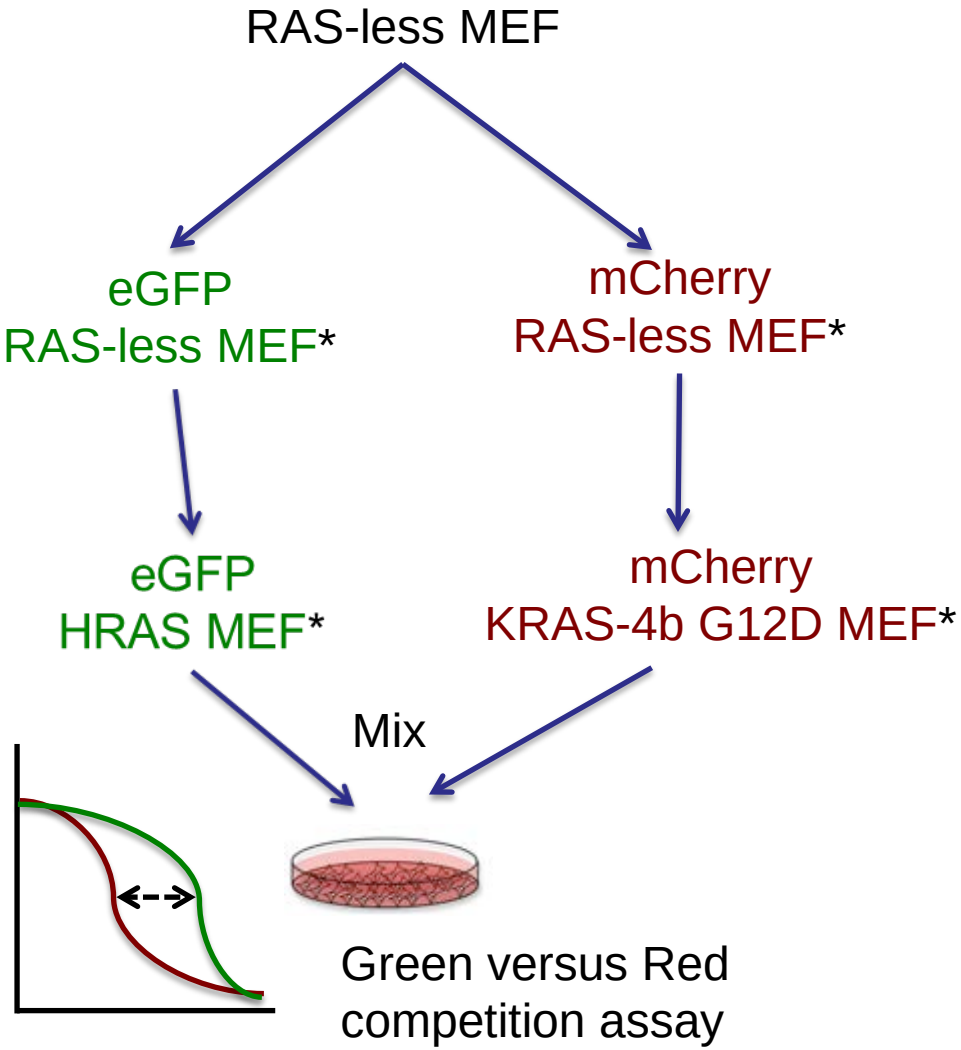
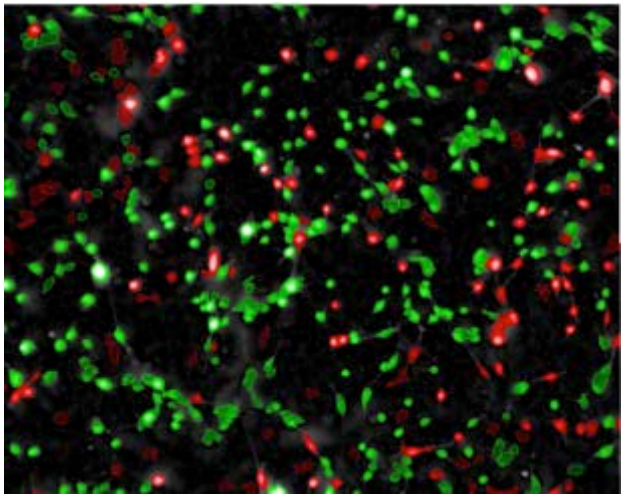


Measure proliferation of red vs green cells

- Total Fluorescence
- Use high content imaging to report actual cell counts



Oncogenic KRAS Effector Network

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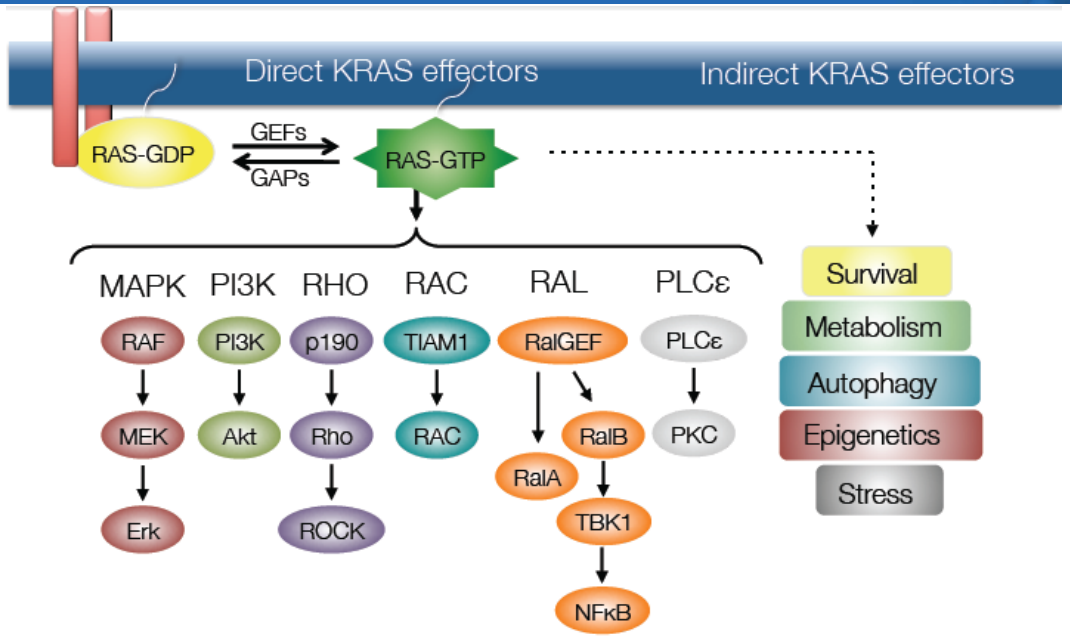


MAPK	PI3K	RHO	RAC	RAL	Metabolism	Stress
ARAF	PIK3CA	RHOA	RAC1	RALA	HK1	MAPK8
BRAF	PIK3CB	RHOB	RAC2	RALB	HK2	MAPK9
RAF1	PIK3CD	RHOC	RAC3	TBK1	HK3	MAPK10
MAP2K1	AKT1	ROCK1	PAK1	RALBP1	LDHA	MAPK14
MAP2K2	AKT2	ROCK2	PAK2	RALGDS	LDHB	MAPK11
MAPK1	AKT3		PAK3	RGL1	PDK1	MAPK12
MAPK3			TIAM1	RGL2	PDK2	MAPK13
RPS6KA1			TIAM2	RGL3	GLS	
RPS6KA3				RELA	HIF1A	Epigenetics
RPS6KA2				RELB	EPAS1	DNMT1
RPS6KA6				IKBKB	Autophagy	DNMT3A
RPS6KA5				CHUK	ATG5	DNMT3B
RPS6KA4				NFKB1	ATG7	
KSR1					BECN1	
KSR2						

RAS: KRAS, HRAS, NRAS

Current Assay Parameters

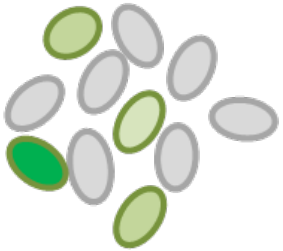
Competitive Growth
Proliferation Rate
Reactive Oxygen Species
Apoptosis
Cell Size

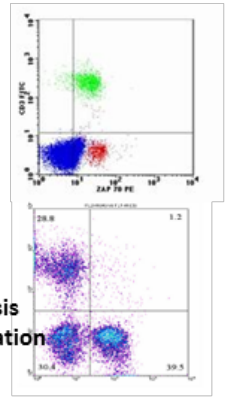
Assay Workflow

Cells transduced with eGFP

Potent multi-siRNA node KD

eGFP KD: Surrogate for transfection efficiency + siRNA node lethality





ROS
Apoptosis
Proliferation
Cell size

Multi-parameter analysis

Tina Yuan UCSF, Ji Luo NCI
Scott Lowe CSHL, Cyril Benes, Harvard/MGH

Mapping the surface of KRAS cancer cells

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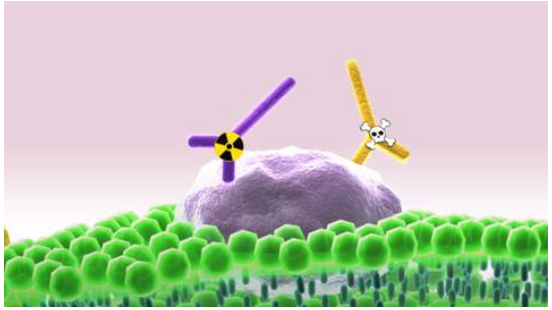
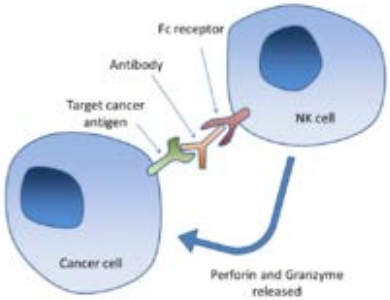
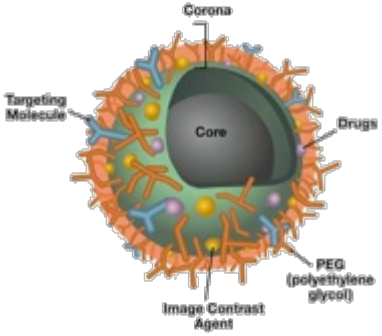
- **Objectives and rationale**
  - Survey the surface of KRAS-driven cells to generate a list of proteins differentially associated with KRAS phenotype
  - These KRAS associated cell surface determinants could represent new targets for
    - antibody-mediated attack
    - immune based therapy
    - nanoparticle delivery







Differences between K-Ras 4A and 4B?

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Lung Adenocarcinoma: Top Mutant Genes
Sample GSEA Enrichment Overall Result of 4b/4a Ratio High Samples

(less 4A)

GS	SIZE	SOURCE	ES	NES	NOM p-val	FDR q-val	FWER p-val	Tag %	Gene %	Signal	FDR (median)	glob.p.val
TP53	86	TP53	31.556	0.07889	0	1	0.001	0.628	0.444	0.702	NA	1
MAP2K1	6	MAP2K1	21.262	0.053155	0.002	1	0.308	0.833	0.181	0.707	NA	1
PIK3CB	7	PIK3CB	19.125	0.047812	0.006	1	0.462	0.857	0.316	0.611	1	1
APC	10	APC	18.841	0.047103	0.007	1	0.469	0.6	0.158	0.537	1	1
PIK3CG	7	PIK3CG	18.83	0.047075	0.009	1	0.47	0.714	0.181	0.61	1	1
BRD4	5	BRD4	18.362	0.045905	0.004	1	0.472	0.8	0.181	0.675	1	1
CACNA1E	32	CACNA1E	18.143	0.045358	0.028	1	0.474	0.438	0.216	0.422	1	1
RXRG	8	RXRG	17.862	0.044655	0.017	1	0.475	0.875	0.404	0.548	1	1
LAMB4	15	LAMB4	17.303	0.043258	0.017	1	0.475	0.8	0.474	0.462	1	1
PLCG2	8	PLCG2	16.809	0.042023	0.025	1	0.475	0.625	0.181	0.537	1	1
NOTCH1	7	NOTCH1	16.764	0.04191	0.02	1	0.475	0.714	0.24	0.566	1	1
AKAP9	18	AKAP9	16.635	0.041588	0.028	1	0.475	0.833	0.55	0.419	1	1
GRIN2A	20	GRIN2A	16.395	0.040987	0.033	1	0.475	0.55	0.287	0.444	1	1
GNAS	6	GNAS	16.114	0.040285	0.022	1	0.475	0.833	0.339	0.571	1	1
CAMTA1	7	CAMTA1	16.056	0.04014	0.02	1	0.475	0.571	0.117	0.526	1	1
HIP1	6	HIP1	14.683	0.036708	0.065	1	0.475	0.667	0.216	0.541	1	1
MMP2	11	MMP2	14.683	0.036708	0.064	1	0.475	1	0.673	0.35	1	1

Nom p-val <0.05

Differences between K-Ras 4A and 4B?

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LUAD Top Mutant Genes

Sample GSEA Enrichment Overall Result of 4b/4a Ratio Low Samples

(more 4A)

GS	SIZE	SOURCE	ES	NES	NOM p-val	FDR q-val	FWER p-va	Tag %	Gene %	Signal	FDR (median)	glob.p.val
RAPGEF2	9	RAPGEF2	-19.092	-0.04773	0.006	0.016242	0.502	0.889	0.421	0.543	0.017168	0
KIAA1549	8	KIAA1549	-18.609	-0.04652	0.009	0.020767	0.517	1	0.515	0.509	0.019048	0
CACNA1S	6	CACNA1S	-17.925	-0.04481	0.011	0.029191	0.521	1	0.456	0.564	0.028037	0
KRAS	46	KRAS	-17.21	-0.04303	0.036	0.040497	0.524	0.587	0.427	0.46	0.038835	0
CTNNB1	8	CTNNB1	-16.809	-0.04202	0.023	0.048646	0.525	0.625	0.187	0.533	0.046949	0
NOTCH2	8	NOTCH2	-16.089	-0.04022	0.036	0.067327	0.525	0.875	0.456	0.499	0.065986	0
LAMB3	6	LAMB3	-15.923	-0.03981	0.035	0.072138	0.525	0.833	0.351	0.561	0.070707	0
FLNB	6	FLNB	-15.446	-0.03862	0.038	0.086906	0.525	0.667	0.199	0.554	0.08547	0
PTCH1	7	PTCH1	-15.406	-0.03852	0.044	0.088862	0.525	0.857	0.427	0.512	0.087168	0
PRKCA	6	PRKCA	-14.779	-0.03695	0.055	0.11303	0.525	0.833	0.386	0.53	0.11211	0
TAOK3	5	TAOK3	-14.335	-0.03584	0.061	0.13422	0.525	0.6	0.123	0.542	0.13274	0
LAMB2	8	LAMB2	-14.234	-0.03559	0.082	0.13821	0.525	0.75	0.38	0.488	0.13636	0
CACNA1I	6	CACNA1I	-14.111	-0.03528	0.056	0.1462	0.525	0.667	0.24	0.525	0.14545	0
PDGFA	5	PDGFA	-14.023	-0.03506	0.063	0.14993	0.525	0.8	0.333	0.549	0.14864	0
WRN	10	WRN	-13.932	-0.03483	0.071	0.15438	0.525	0.9	0.579	0.402	0.15385	0
SOS1	8	SOS1	-13.846	-0.03462	0.077	0.16096	0.525	0.5	0.14	0.451	0.16087	0

Nom p-val <0.05

NCI Ras Program web site…

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A Message from Dr. Frank McCormick

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September 22, 2014 by Dr. Frank McCormick

Welcome to the NCI RAS Program on Cancer.gov.

Since I published my first paper on RAS a long while ago, our knowledge of RAS and its partners in illegitimate signaling has grown tremendously, but as you are aware we have yet to convert that knowledge into any drugs that directly target the RAS protein. We have struggled to integrate what we know of how RAS signals with the heterogeneity seemingly inherent in solid tumors and the subtle differences in the behavior of different *RAS* mutants, among many, many aspects of the RAS problem.

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Are All RAS Proteins Created Equal in Cancer?

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September 22, 2014 by Channing Der

The three human *RAS* genes encode four highly related RAS proteins (82-90% sequence identity), with alternative gene splicing accounting for the expression of the highly related K-RAS4A and K-RAS4B proteins (90% identity). There is an emerging perception that the roles and functions of specific RAS proteins in cancer are distinct and, consequently, distinct anti-RAS strategies will be needed for effective