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Kinetic models of Ras-GTPases in molecular signaling networks.

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Outline

1. CeTICS: institutional status and mission.
2. Signaling networks: static maps and dynamic models.
3. Ras-GTPases molecular switches.

Center of Toxins, Immune-response and Cell Signaling

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FAPESP

Mission of CeTICS

CeTICS

Premise

Novel toxins **might lead to discovery of** ***molecular signaling networks*** **of potential therapeutic value**.

Objectives

1 - To discover, isolate and chemically characterize *novel toxins*.

2 - **To analyze systemic responses of cells and/or organisms to** ***novel toxins*** **aiming to uncover** ***molecular signaling networks*** **underlying complex biological phenomena.**

Interdisciplinary Group of Molecular Cell Biology, Bioinformatics and Computer Biology

Principal investigators: Hugo A. Armelin (Group leader) and Junior Barrera

Associate investigators: Marcelo S. Reis and Milton Y. Nishiyama-Jr

Postdoc fellows: Matheus H. Dias and Vincent Noël

PhD students: Cecília S. Fonseca and Eduardo Lopes

Present critical challenge

Transformation of

molecular static maps

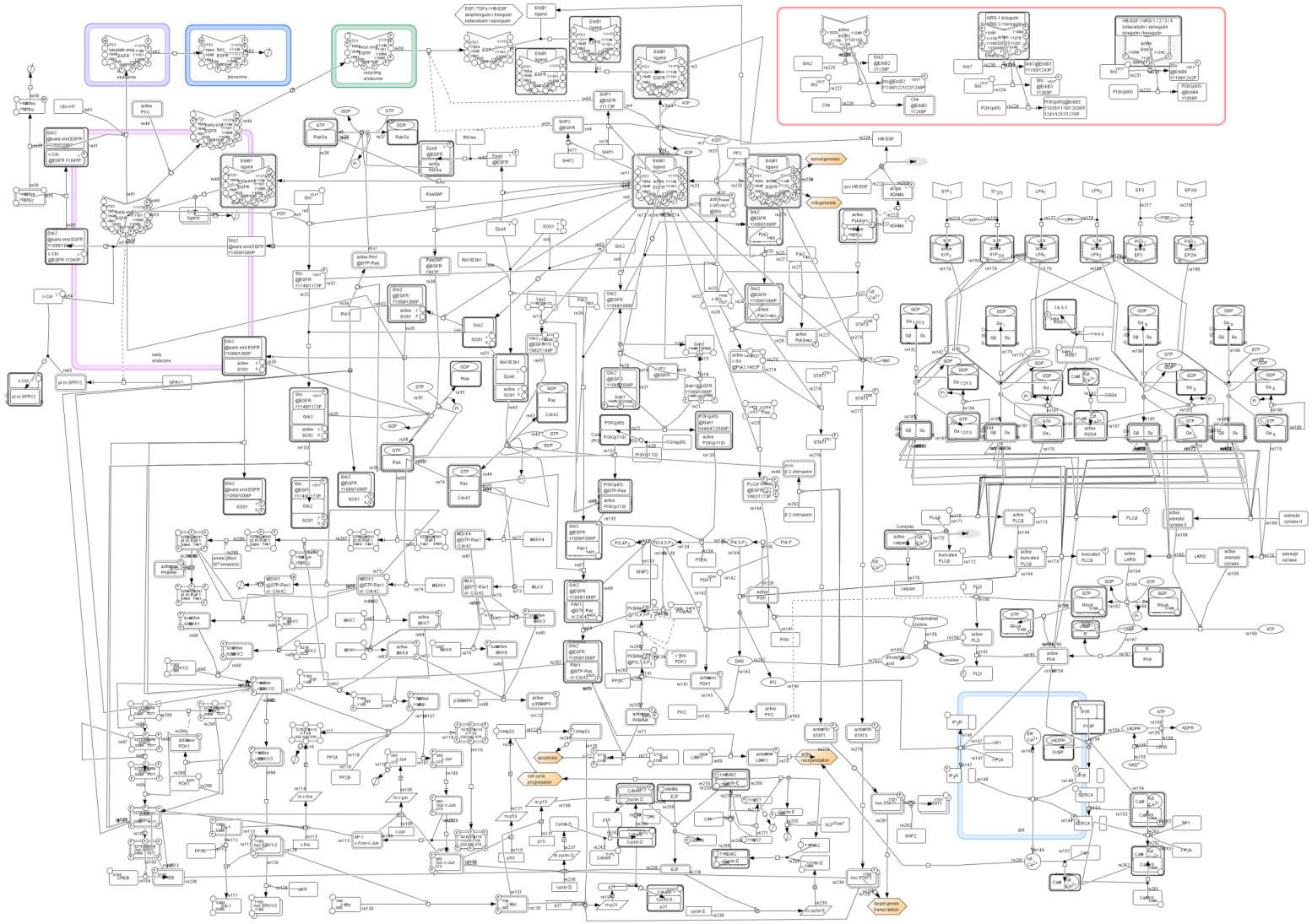
into functionally predictive **dynamic models** of

metabolic reactions and molecular signaling networks

based on

low- and high-throughput quantitative omics data

plus biological knowledge of heuristic value.



Map of the EGF/EGFR signaling axis (Oda & Kitano, 2005)

Ras-GTPase Subfamily

Ras-GTPases paralogs.

A

_____G1_____ ___G2____ ___G3____

HRAS MTEYK**LVVVG**AGG**VGKS**ALTIQLIQNHFVDEYDP**T**IEDSYRKQVVIDGETCLLDIL**D**TA**G**QEEYSAMR

NRAS MTEYK**LVVVG**AGG**VGKS**ALTIQLIQNHFVDEYDP**T**IEDSYRKQVVIDGETCLLDIL**D**TA**G**QEEYSAMR

KRAS MTEYK**LVVVG**AGG**VGKS**ALTIQLIQNHFVDEYDP**T**IEDSYRKQVVIDGETCLLDIL**D**TA**G**QEEYSAMR

__G4____

HRAS DQYMRTGEGFLCVFAINNTKSFEDIHQYREQIKRVKDSDDVPMVLV**GNK**C**D**LAA

NRAS DQYMRTGEGFLCVFAINNSKSFADINLYREQIKRVKDSDDVPMVLV**GNK**C**D**LPT

KRAS DQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLV**GNK**C**D**LPS

___G5__

HRAS RTVESRQAQDLARSYGIPYI**ETSAK**TRQGVEDAFYTLVREIRQH^ESGPG**C**MS**C**K**C**VLS

NRAS RTVDTKQAHELAKSYGIPFI**ETSAK**TRQGVEDAFYTLVREIRQY^DGTQG**C**MGLP**C**VVM

KRAS RTVDTKQAQDLARSYGIPFI**ETSAK**TRQGVDDAFYTLVREIRKH^**KKKKKK**S**K**T**K**C**VIM

B

KRAS Splicing variants:

KRAS2B (C-terminal):KKKKKKSKTKCVIM***

KRAS2A (C-terminal):KTPGC*VKIKKCIIM***

**** Farnesylation site**

*** Palmitoylation site**

*** Three C-terminal residues eliminated by proteolysis.**

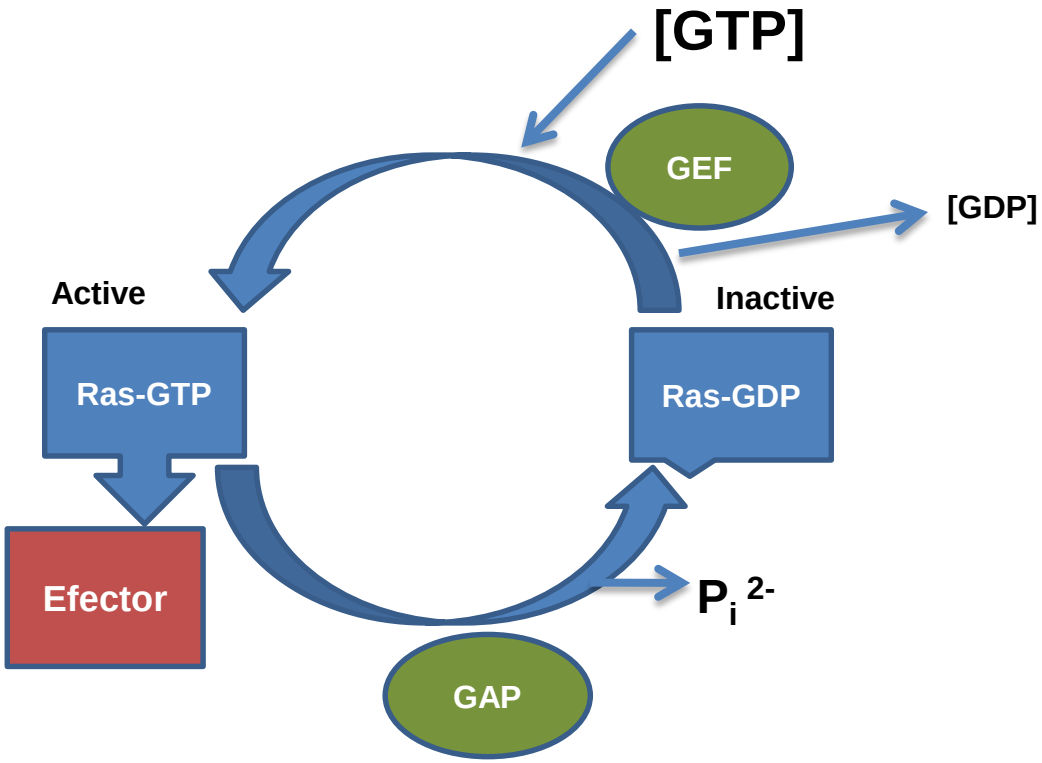
Ras superfamily of small monomeric GTPases

5 Families: **Ras** Rho Ran Rab Arf

7 **Ras** subfamilies: **Ras** Ral Rit Rap Rheb Rad Rhot-Miro

Ras subfamily: **HRas** **KRas** **NRas**

All **Ras** superfamily members possess a G domain core, which provides latent **GTPase** and **G nucleotide binding** activities.



ON / OFF molecular switch.

wt-Kras/amplification-driven mouse Y1 adrenocortical tumor cell line

Y1 tumor cells display a surprising association of phenotypes*:

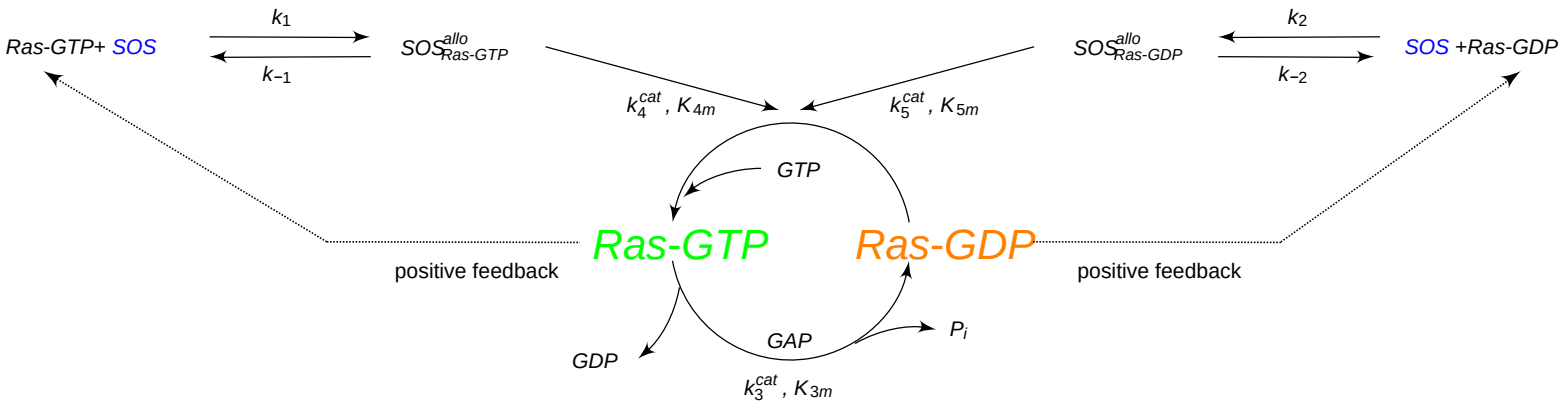
a) **high basal levels of [K-Ras-GTP]** and

b) **irreversible cell cycle arrest by FGF2**

Simulations with K-Ras kinetic models suggested a molecular basis for this unexpected phenotypic association, which was experimentally validated.

***Costa et al, Cancer Res (2008)**

K-Ras-GTPase cycle module 1



$$\frac{d[SOS]}{dt} = -\ k_1[SOS][Ras\text{-}GTP] + k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}] - k_2[SOS][Ras\text{-}GDP] + k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}]$$

$$\frac{d[SOS^{allo}_{Ras\text{-}GTP}]}{dt} = +\ k_1[SOS][Ras\text{-}GTP] - k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}]$$

$$\frac{d[SOS^{allo}_{Ras\text{-}GDP}]}{dt} = +\ k_2[SOS][Ras\text{-}GDP] - k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}]$$

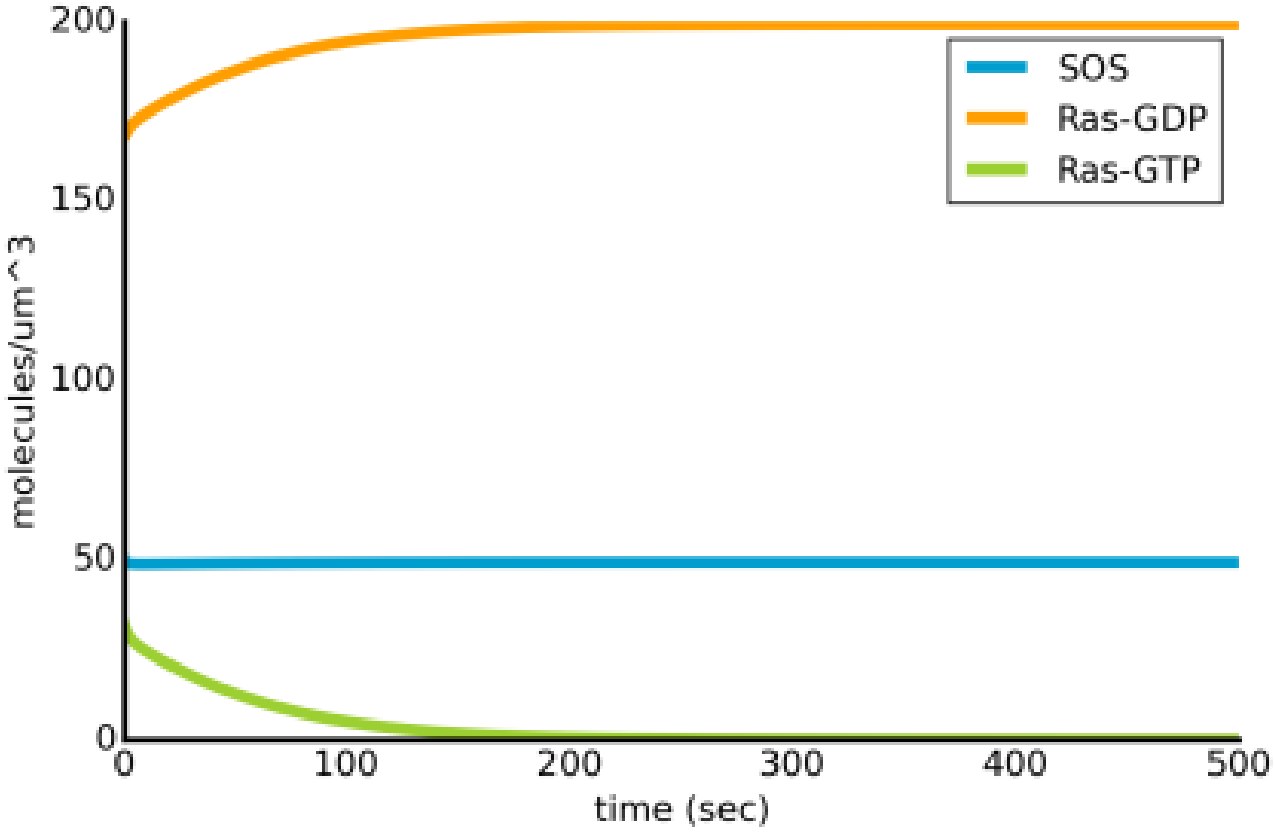
$$\frac{d[Ras\text{-}GDP]}{dt} = -\ \frac{k_4^{cat}[SOS^{allo}_{Ras\text{-}GTP}][Ras\text{-}GDP]}{K_{4_m} + [\ Ras\text{-}GDP]} - \frac{k_5^{cat}[SOS^{allo}_{Ras\text{-}GDP}][Ras\text{-}GDP]}{K_{5_m} + [\ Ras\text{-}GDP]} + \frac{k_3^{cat}[GAP][Ras\text{-}GTP]}{K_{3_m} + [\ Ras\text{-}GTP]} - k_2[SOS][Ras\text{-}GDP] + k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}]$$

$$\frac{d[Ras\text{-}GTP]}{dt} = +\ \frac{k_4^{cat}[SOS^{allo}_{Ras\text{-}GTP}][Ras\text{-}GDP]}{K_{4_m} + [\ Ras\text{-}GDP]} + \frac{k_5^{cat}[SOS^{allo}_{Ras\text{-}GDP}][Ras\text{-}GDP]}{K_{5_m} + [\ Ras\text{-}GDP]} + - \frac{k_3^{cat}[GAP][Ras\text{-}GTP]}{K_{3_m} + [\ Ras\text{-}GTP]} - k_1[SOS][Ras\text{-}GTP] + k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}]$$

(M.S.Reis, M.H.Dias, et al., unpublished)

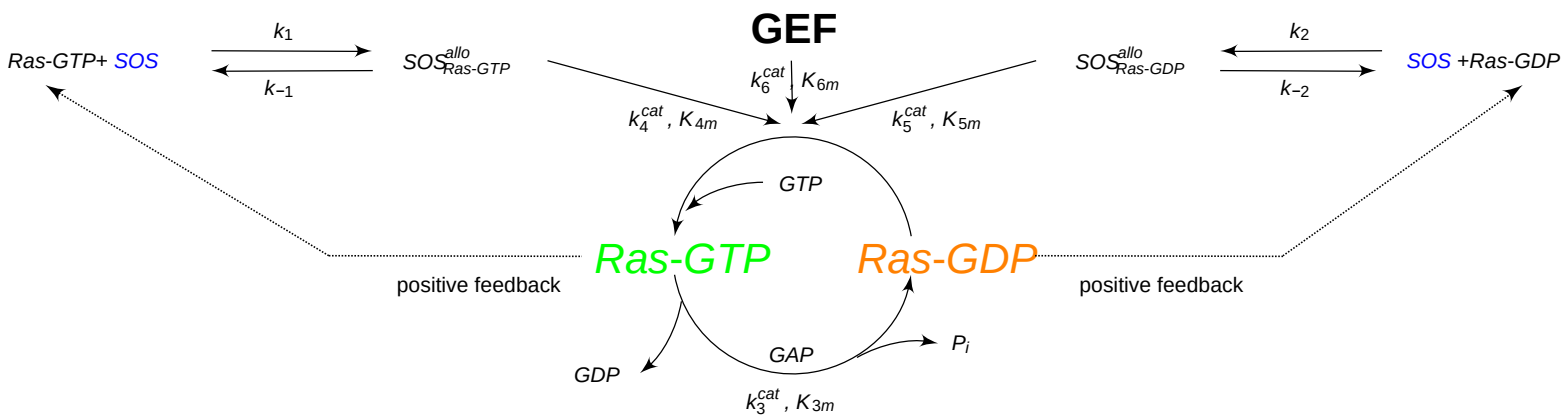
Computer simulation 1

Low [SOS]:



(M.S.Reis, M.H.Dias, et al., unpublished.)

K-Ras-GTPase cycle module 2



$$\frac{d[SOS]}{dt} = -\ k_1[SOS][Ras\text{-}GTP] + k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}] - k_2[SOS][Ras\text{-}GDP] + k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}]$$

$$\frac{d[SOS^{allo}_{Ras\text{-}GTP}]}{dt} = +\ k_1[SOS][Ras\text{-}GTP] - k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}]$$

$$\frac{d[SOS^{allo}_{Ras\text{-}GDP}]}{dt} = +\ k_2[SOS][Ras\text{-}GDP] - k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}]$$

$$\frac{d[Ras\text{-}GDP]}{dt} = -\ \frac{k_4^{cat}[SOS^{allo}_{Ras\text{-}GTP}][Ras\text{-}GDP]}{K_{4m} + [Ras\text{-}GDP]} - \frac{k_5^{cat}[SOS^{allo}_{Ras\text{-}GDP}][Ras\text{-}GDP]}{K_{5m} + [Ras\text{-}GDP]} +$$

$$+\ \frac{k_3^{cat}[GAP][Ras\text{-}GTP]}{K_{3m} + [Ras\text{-}GTP]} - k_2[SOS][Ras\text{-}GDP] + k_{-2}\ [SOS^{allo}_{Ras\text{-}GDP}] - \frac{\mathbf{k_6^{cat}[GEF][Ras\text{-}GDP]}}{\mathbf{K_{6m} + [Ras\text{-}GDP]}}$$

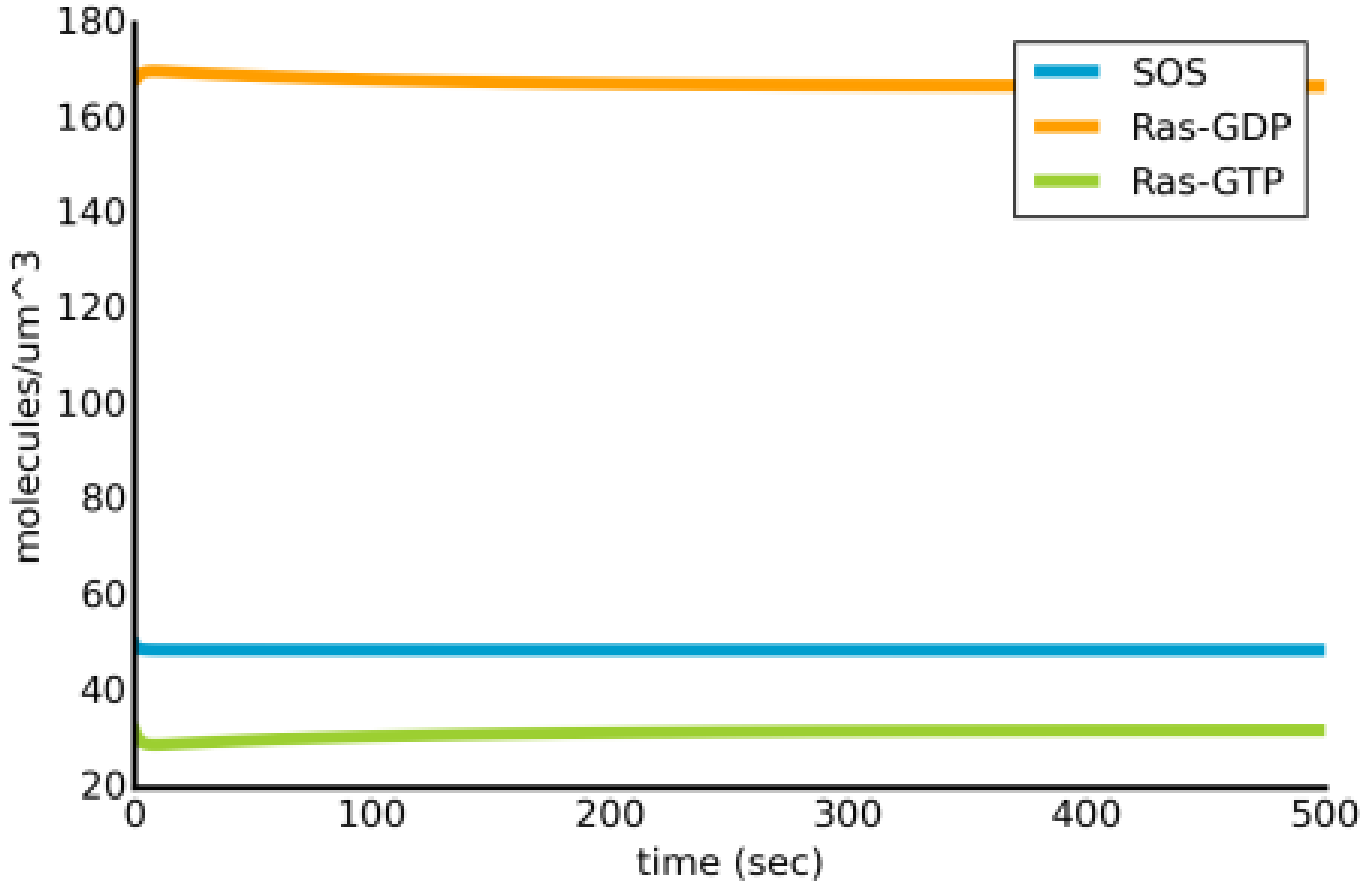
$$\frac{d[Ras\text{-}GTP]}{dt} = +\ \frac{k_4^{cat}[SOS^{allo}_{Ras\text{-}GTP}][Ras\text{-}GDP]}{K_{4m} + [Ras\text{-}GDP]} + \frac{k_5^{cat}[SOS^{allo}_{Ras\text{-}GDP}][Ras\text{-}GDP]}{K_{5m} + [Ras\text{-}GDP]} +$$

$$-\ \frac{k_3^{cat}[GAP][Ras\text{-}GTP]}{K_{3m} + [Ras\text{-}GTP]} - k_1[SOS][Ras\text{-}GTP] + k_{-1}\ [SOS^{allo}_{Ras\text{-}GTP}] + \frac{\mathbf{k_6^{cat}[GEF][Ras\text{-}GDP]}}{\mathbf{K_{6m} + [Ras\text{-}GDP]}}$$

(M.S.Reis, M.H.Dias, et al., unpublished)

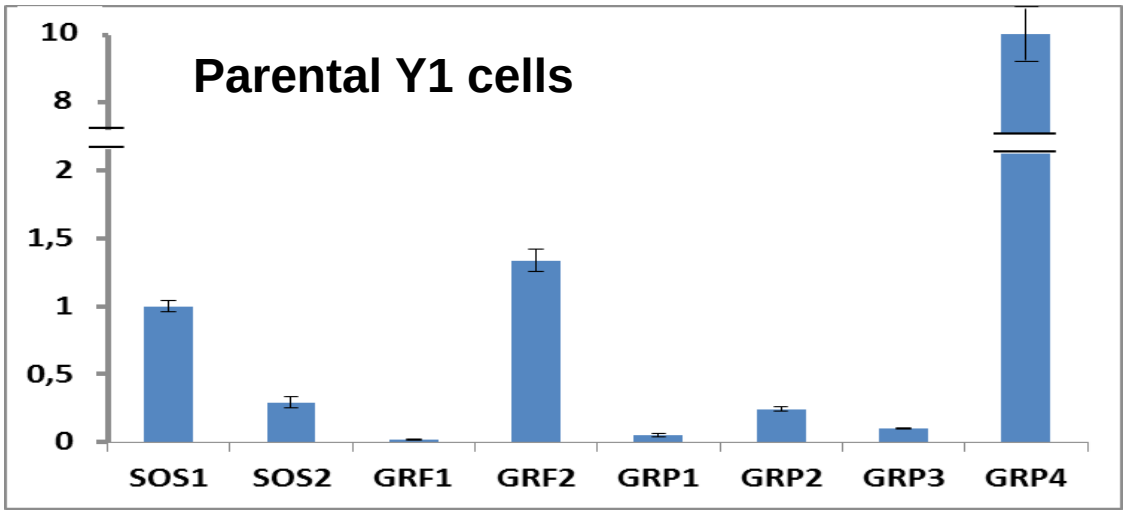
Computer simulation 2

Low [SOS]:



(M.S.Reis, M.H.Dias, et al., unpublished.)

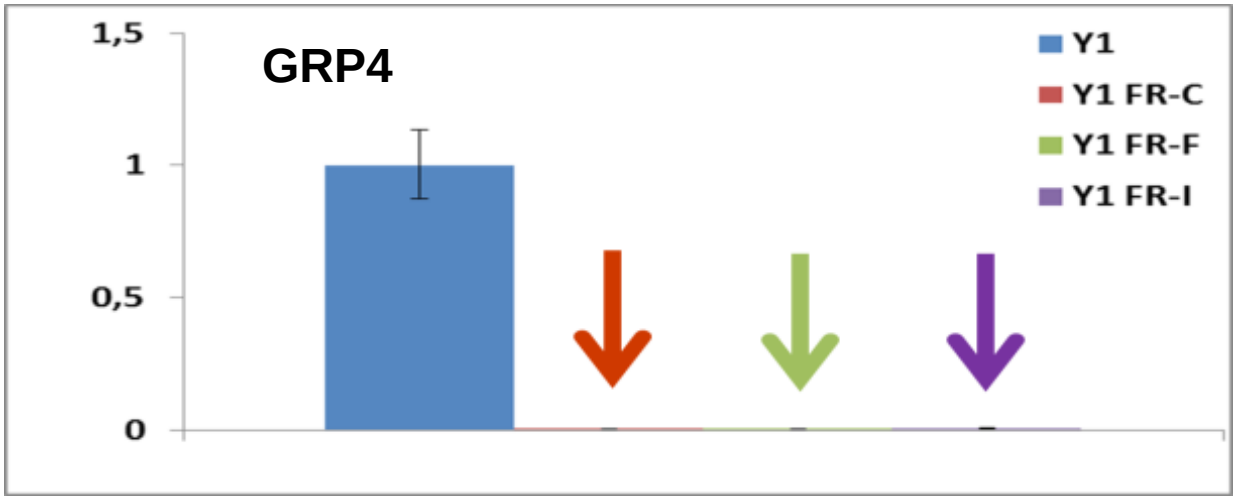
Expression of RasGEF families in Y1 cells



RasGEF families:

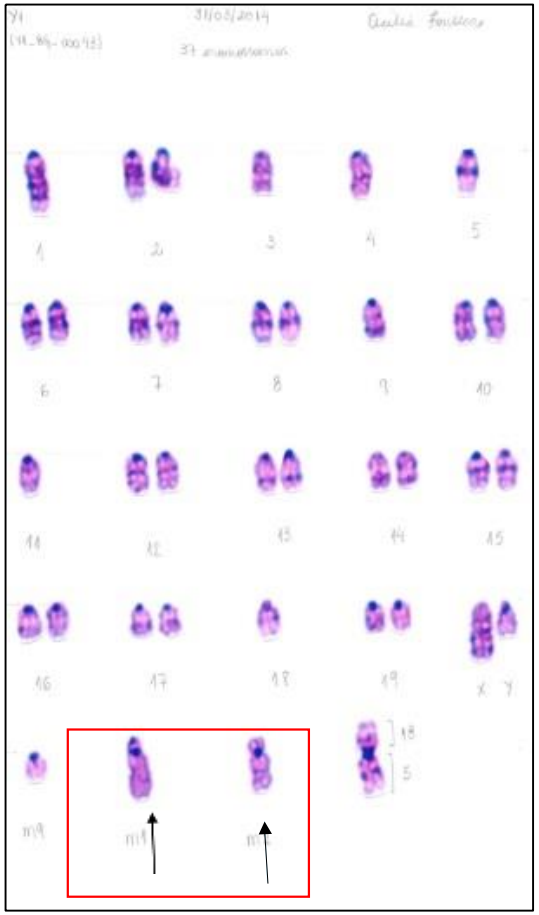
1. SOS
2. GRF
3. GRP

qRT-PCR

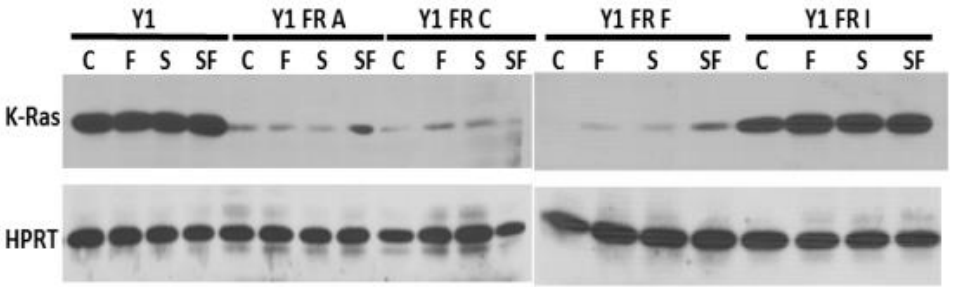


(M.S.Reis, M.H.Dias et al., unpublished)

FGF2-Resistant Y1 sublines (Y1FRs)



		Cell lineage		
		Parental Y1	Parental Y1-D1G	FGF2-resistant Sub-lines
Marker Chromosomes	M1 (HSR1)	94% (n= 67)	100% (n= 68)	0% (n= 158)
	M2 (HSR2)	98.5% (n= 68)	100% (n= 69)	94.1% (n= 170)
	M9	75.8% (n=33)	93.3% (n= 45)	82.4% (n= 17)
	Fusion 5-18	52.8% (n= 53)	98.2% (n= 55)	92.3% (n= 156)



(C.S.Fonseca, M.H.Dias et al., manuscript in preparation, 2016)

Thank you