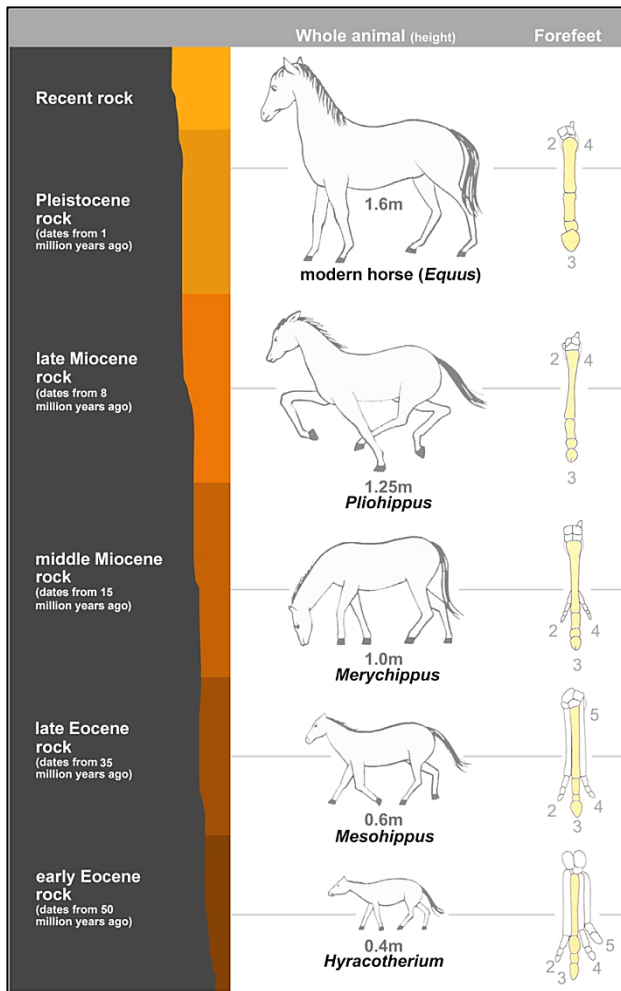


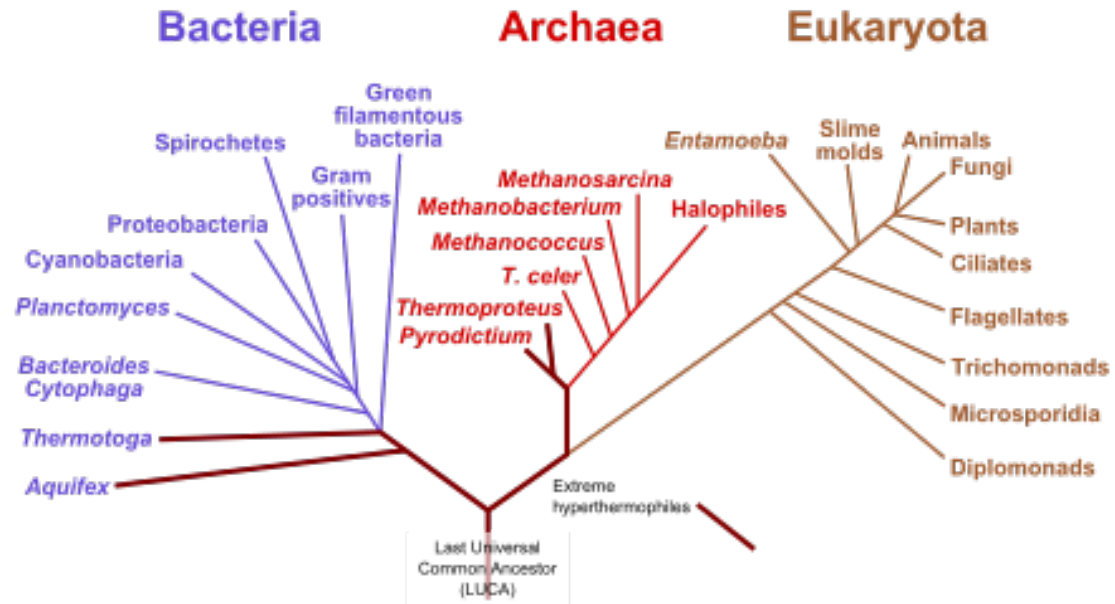
PRACTICAL EVOLUTION:
HARNESSING VARIATION
AND SELECTION
IN RATIONAL DRUG DISCOVERY

Robin Pals Rylaarsdam
Benedictine University, Lisle, IL

“Evolution” in hearts and minds



Phylogenetic Tree of Life



Useful principles of evolution

- Protein homologies
- Conservation of biochemical pathways
- Variation and selection

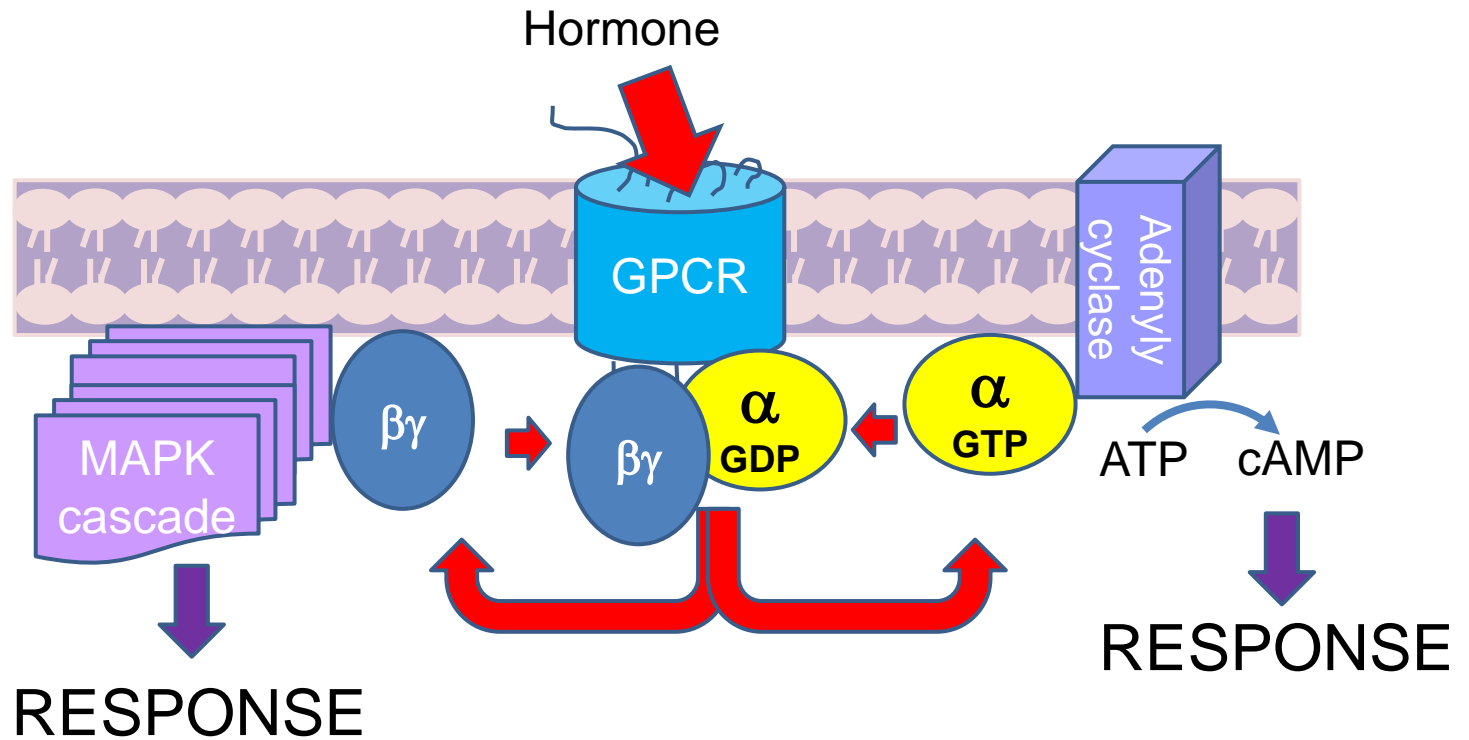
McCune-Albright Syndrome



- Mosaicism for constitutively active $G\alpha_s$
 - Precocious puberty,
 - Café-au-lait hyperpigmentation,
 - Polyostotic fibrous dysplasia of bone
 - Other hyperendocrinopathies
- 3 possible mutations in Gs protein:

R201H, R201C, R201S

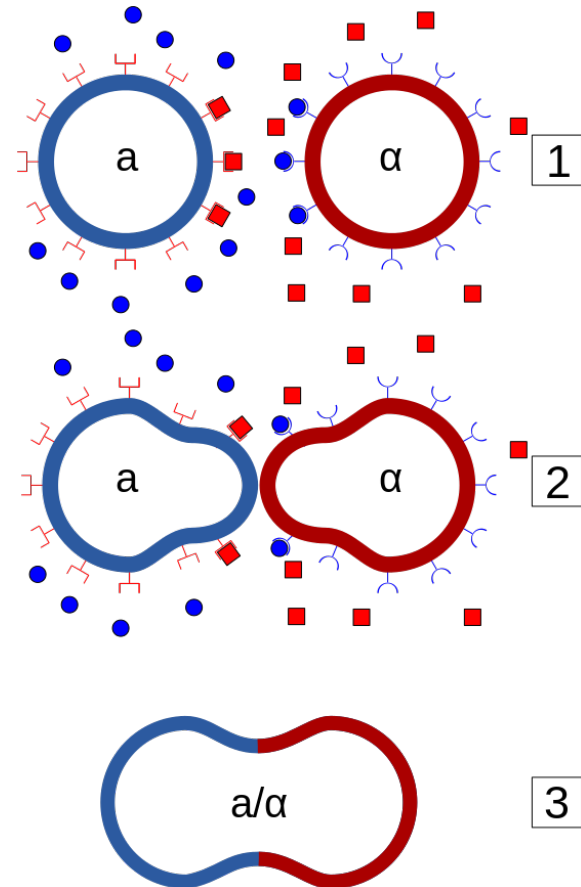
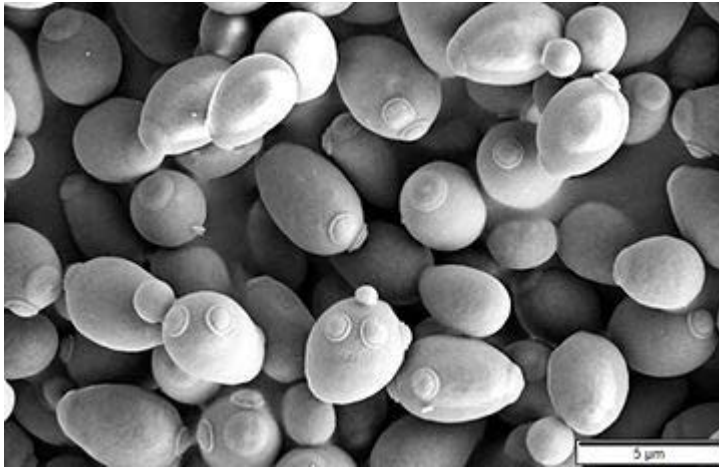
Normal Gs Signaling



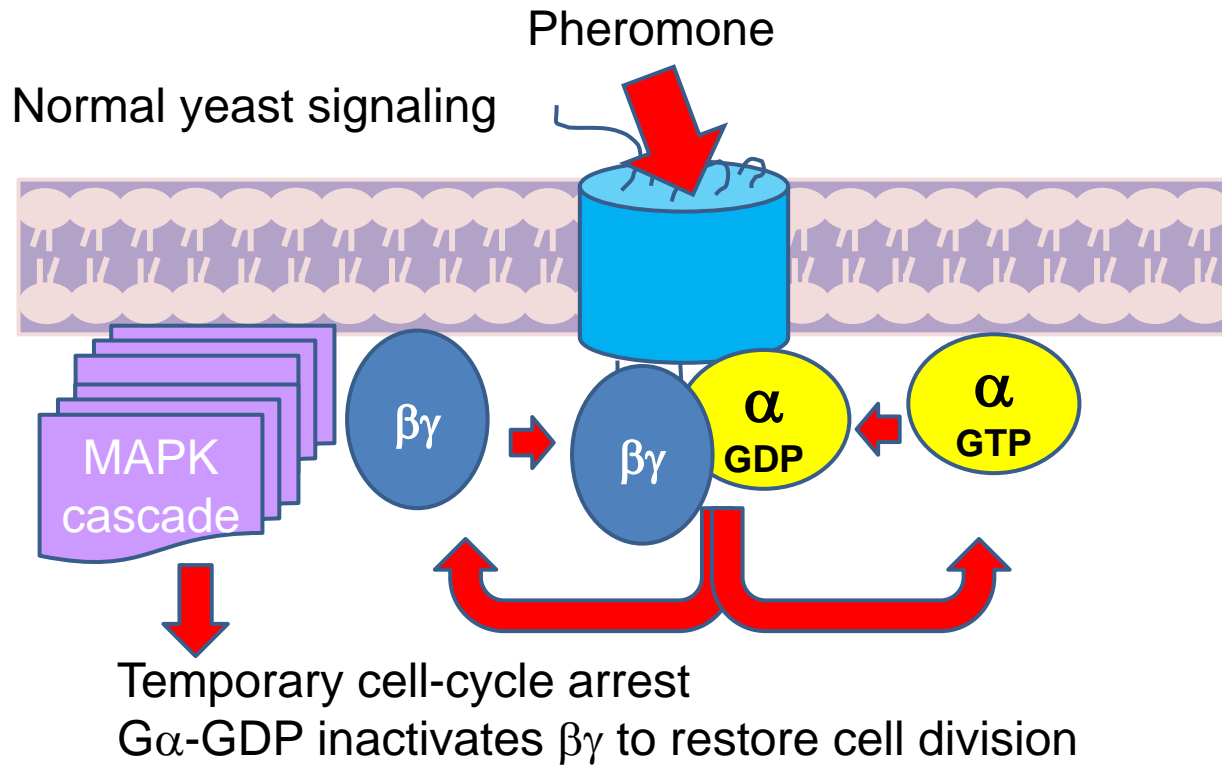
Useful principles of evolution

- Setting up a model system
 - ▣ Conservation of biochemical pathways
 - ▣ Protein homologies
- Finding drug targets
 - ▣ Variation and selection

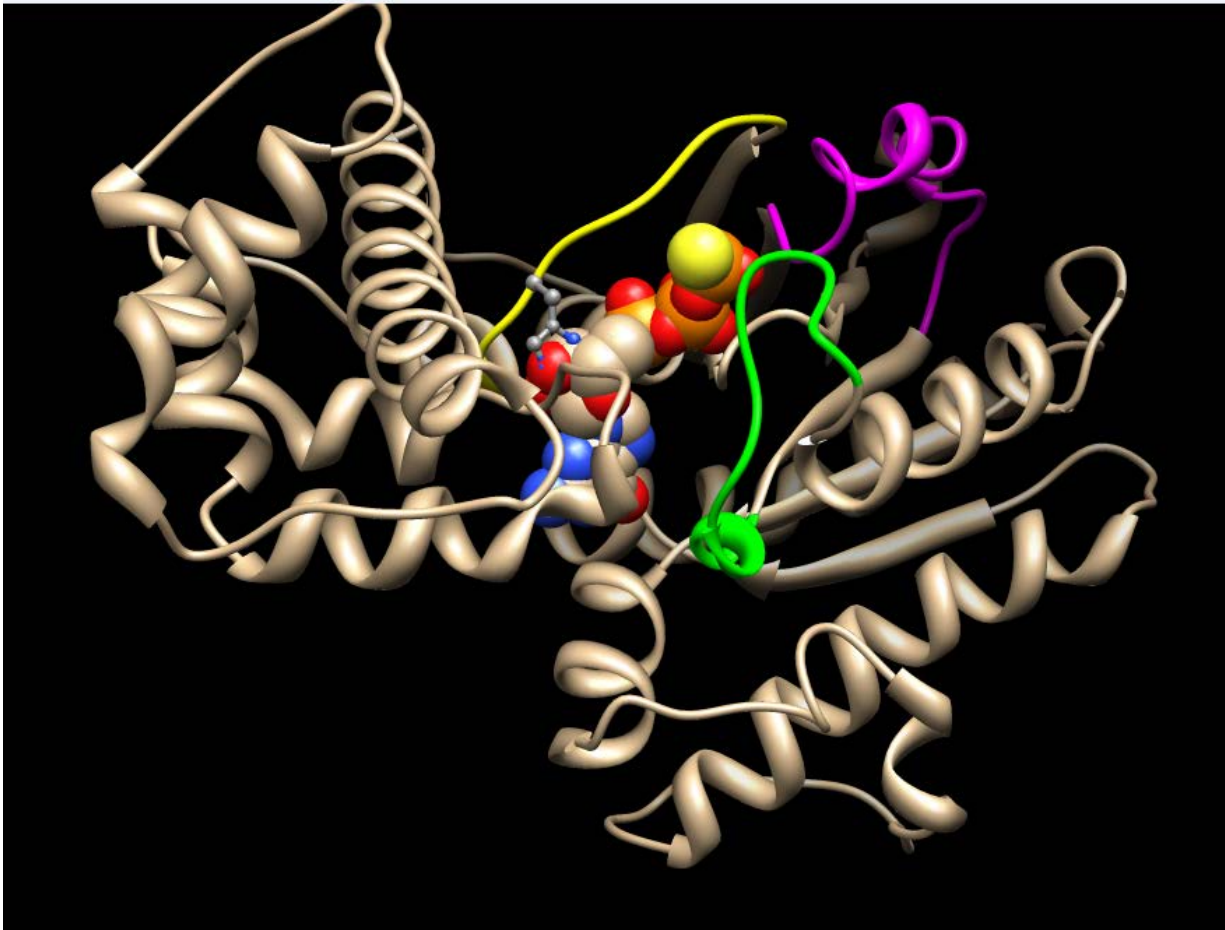
Homologous biochemical pathways – proteins are conserved between yeast and humans



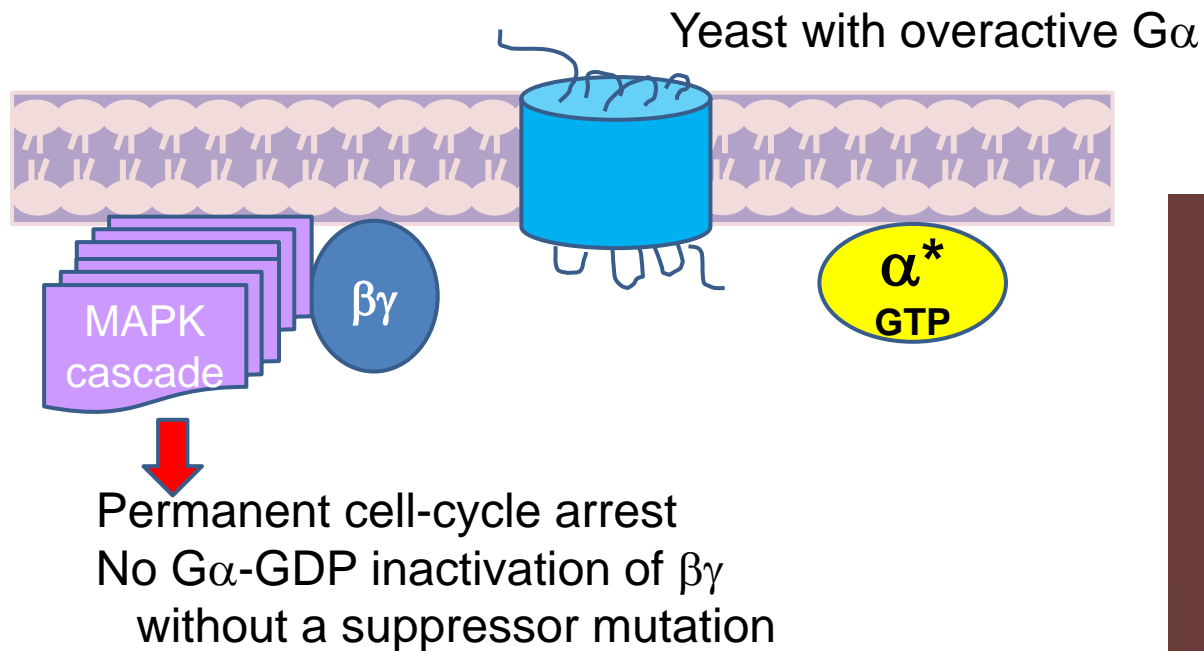
Homologous biochemical pathways – proteins are conserved between yeast and humans



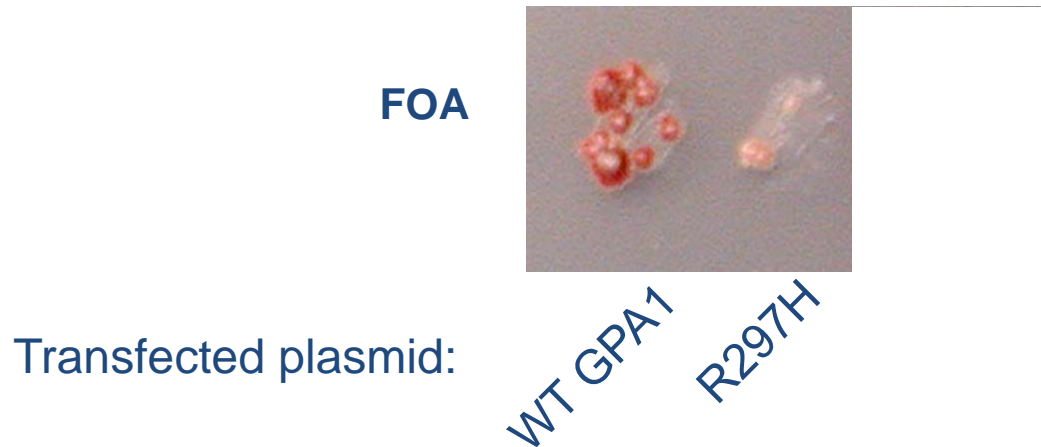
Common sequence, common structure



Homologous mutation in yeast $G\alpha$ causes same biochemical defect as in human protein



The MAS mutation activates yeast $G\alpha$ like the human gene



Arch Physiol Biochem. 2006 Jul;112(3):166-73.

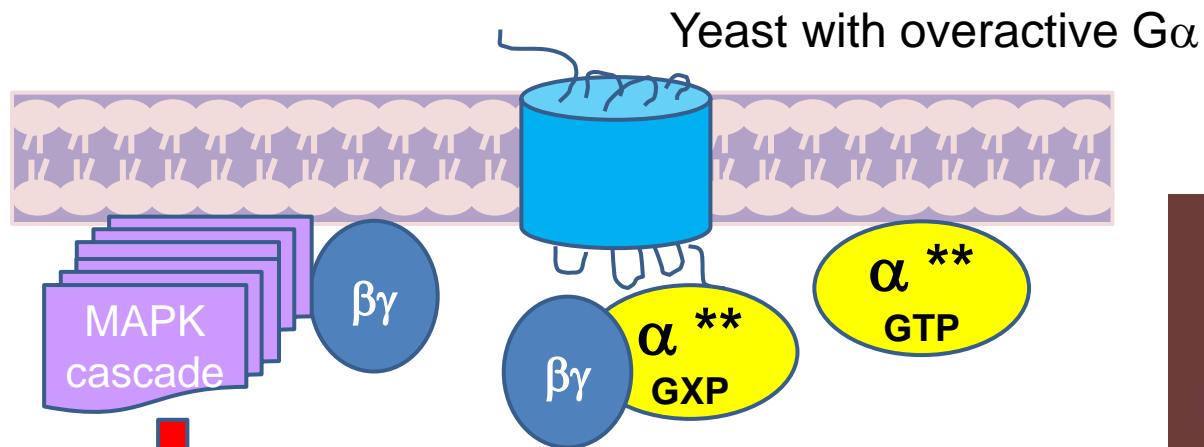
Identification of a second-site suppressor mutation of the GTPase defect associated with McCune-Albright syndrome: a model using the yeast heterotrimeric G protein, GPA1.

Ooms LS¹, Koster MJ, Mitchell JR, Pals-Rvlaarsdam R.

Useful principles of evolution

- Setting up a model system
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 - ▣ Variation and selection

Homologous mutation in yeast $G\alpha$ causes same biochemical defect as in human protein

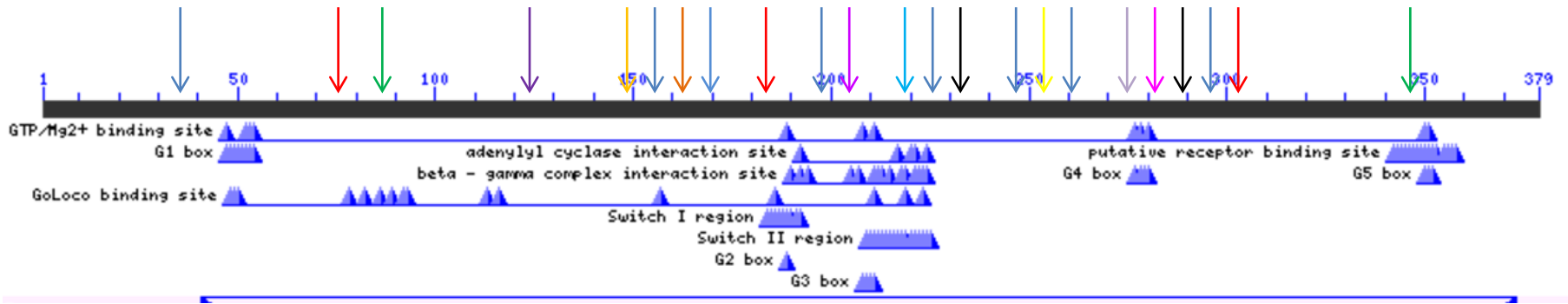


Permanent cell-cycle arrest
No $G\alpha$ -GDP inactivation of $\beta\gamma$
without a suppressor mutation

Inactivation of $\beta\gamma$
Restoration of cell cycle progression
Colony formation

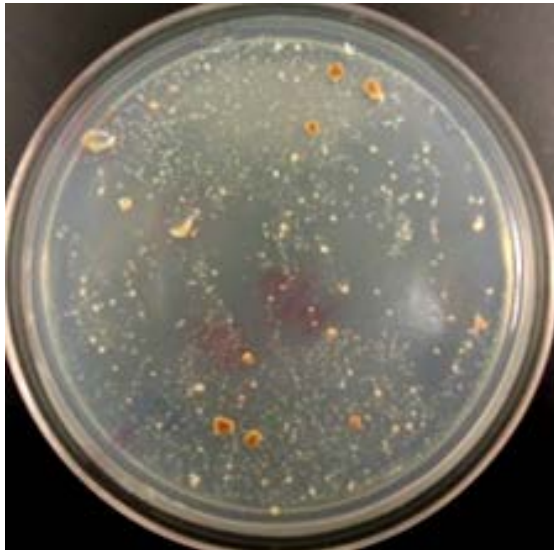


Variation

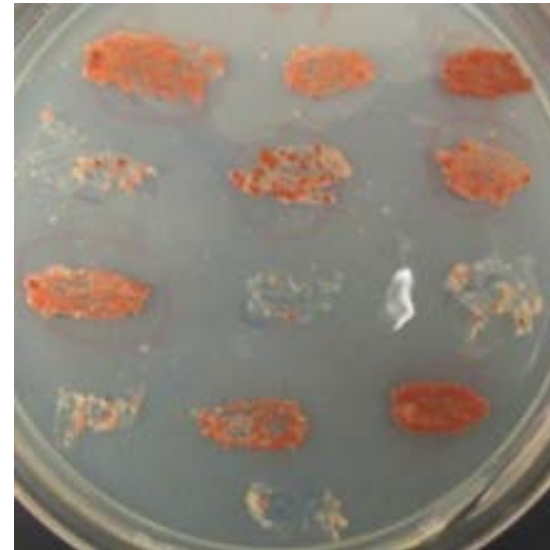


Randomly mutated library screened for intragenic suppressor mutations of an “MAS allele”

Library: 7800 unique mutants in Gpa1 R297H
55,000 clones screened



424 colonies grew on FOA

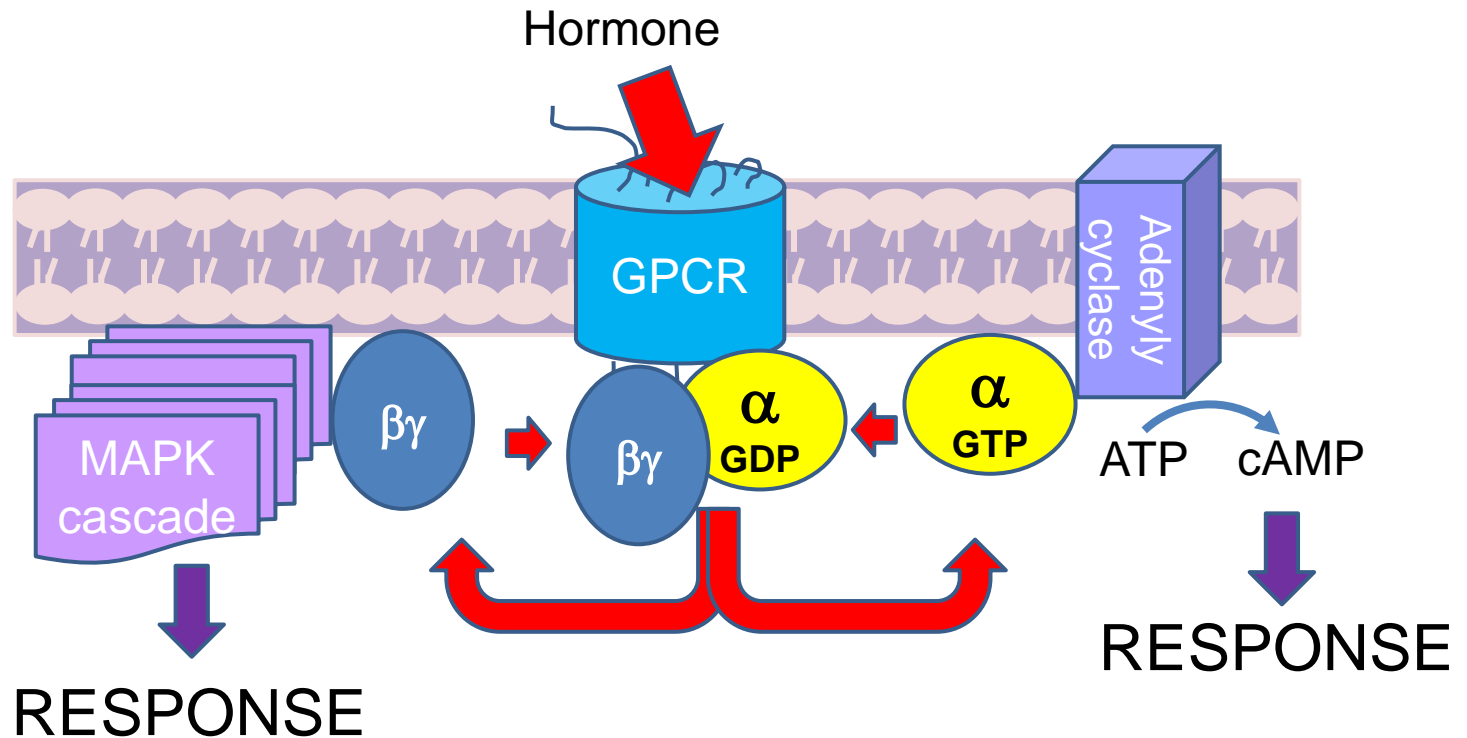


39 Clones remained FOA^R,
plasmids were sequenced

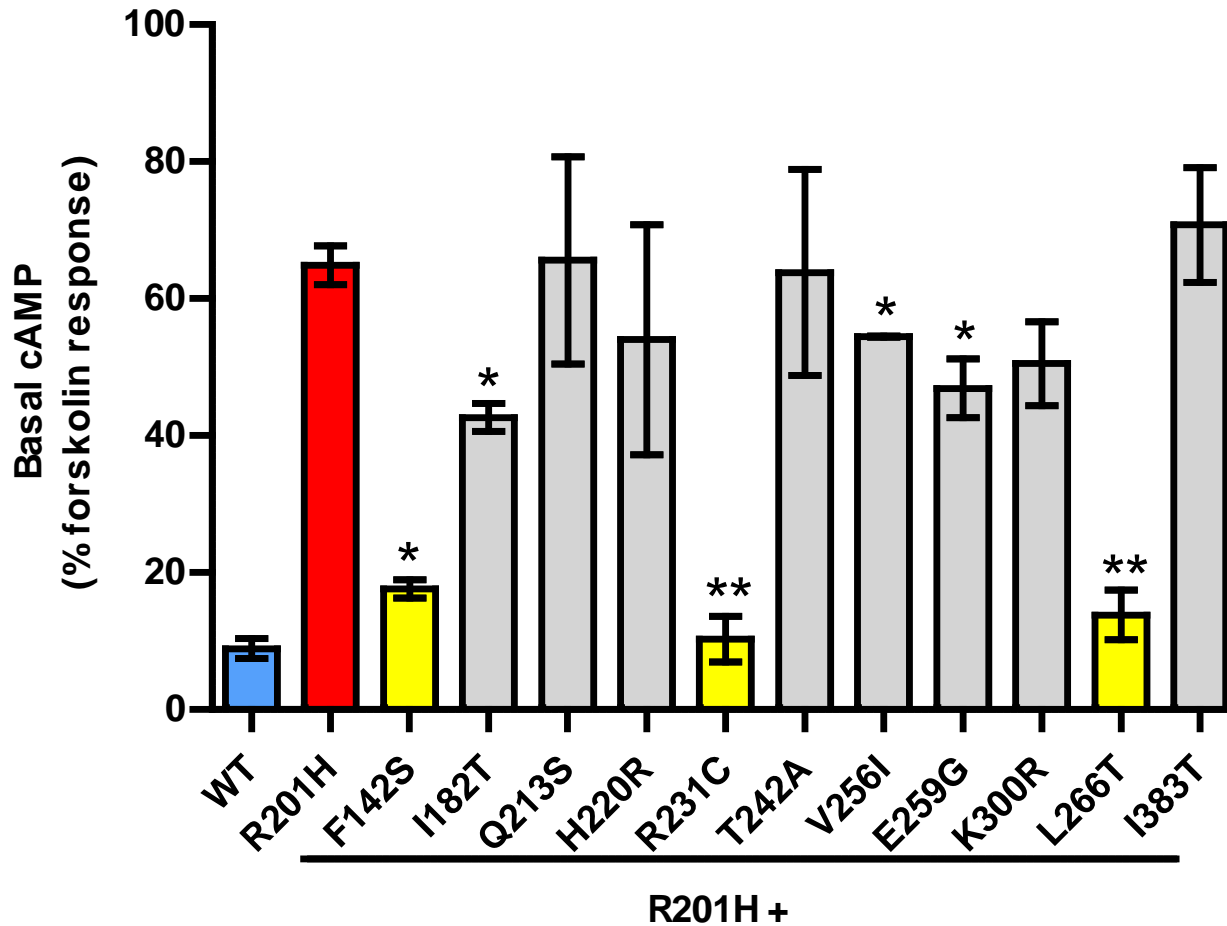
14/39 suppressor mutation sites are conserved between Gs and the yeast GPA1

YEAST GPA1 Gene			HUMAN Gs alpha gene	
Original AA	Mutation Location	Mutated to	Original AA	Mutation Location
Isoleucine	237	Serine	Phenylalanine	142
Isoleucine	278	Threonine	Isoleucine	182
Glutamic Acid	279	Glycine	Aspartic Acid	183
Isoleucine	293	Valine	Leucine	197
Asparagine	309	Serine	Glutamine	213
Lysine	316	Arginine	Histidine	220
Lysine	316	Asparagine	Histidine	220
Arginine	327	Cysteine	Arginine	231
Threonine	388	Alanine	Threonine	242
Methionine	352	Isoleucine	Valine	256
Glutamic Acid	355	Glycine	Glutamic Acid	259
Methionine	362	Threonine	Leucine	266
Lysine	396	Arginine	Lysine	300
Isoleucine	461	Threonine	Isoleucine	383

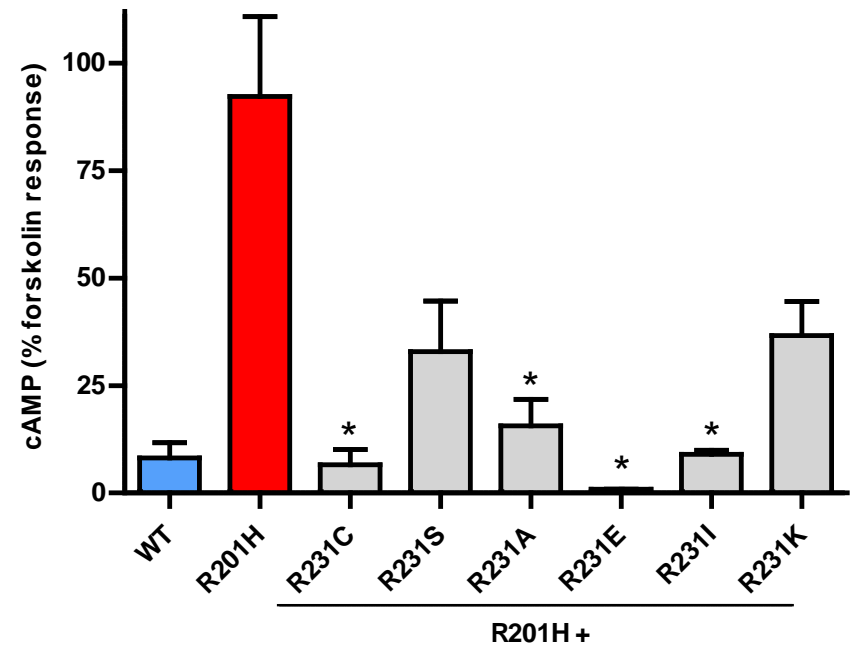
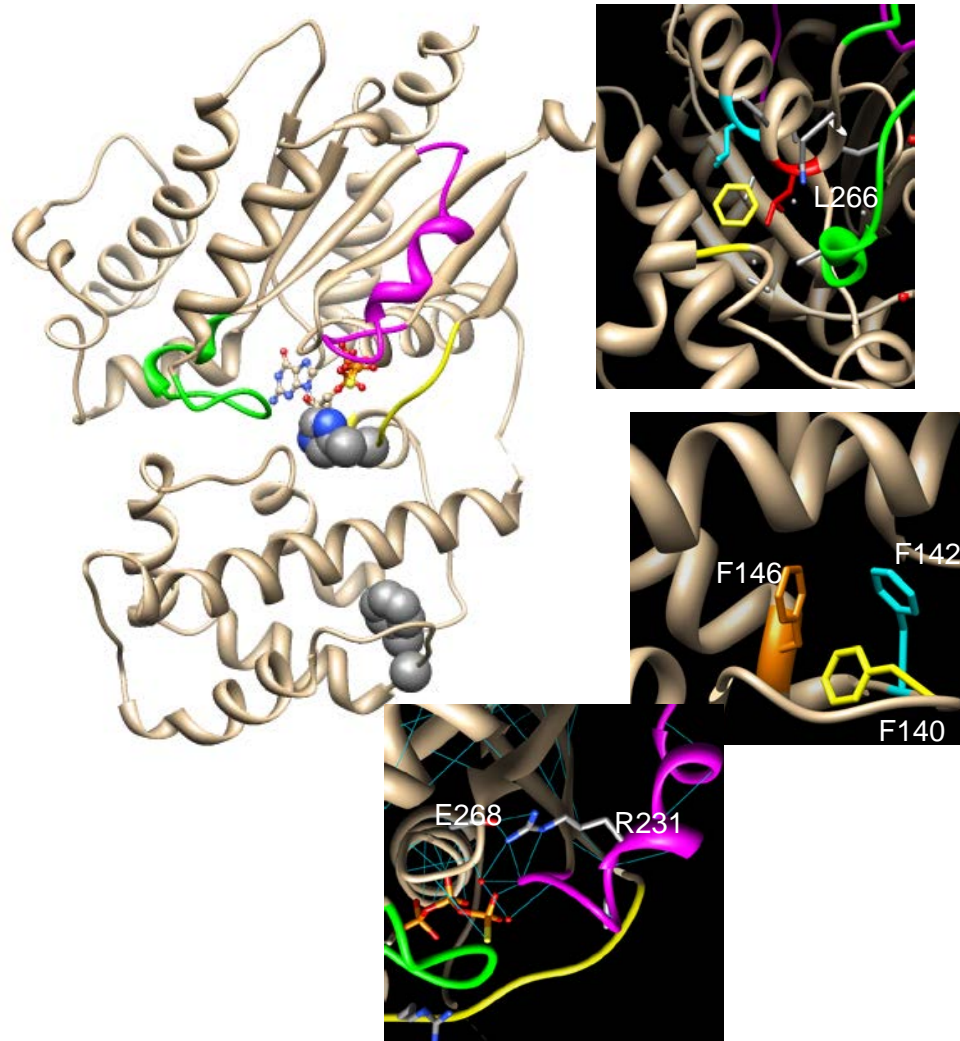
Do the mutations that suppress the yeast G-protein also suppress the human G-protein?



Homology is not identity, but it works sometimes!



Examining the variations at each site that can suppress the MAS mutation



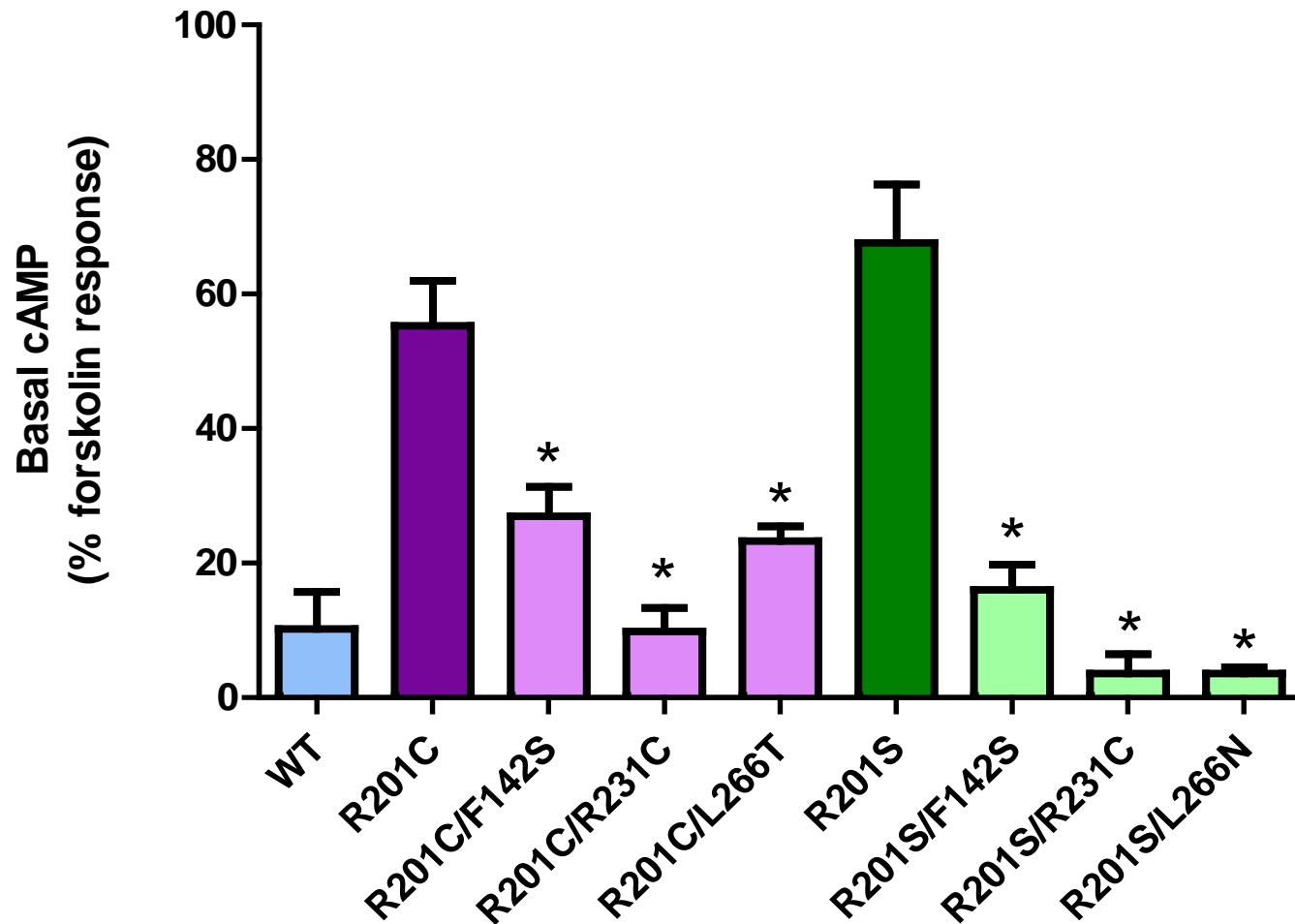
McCune-Albright Syndrome



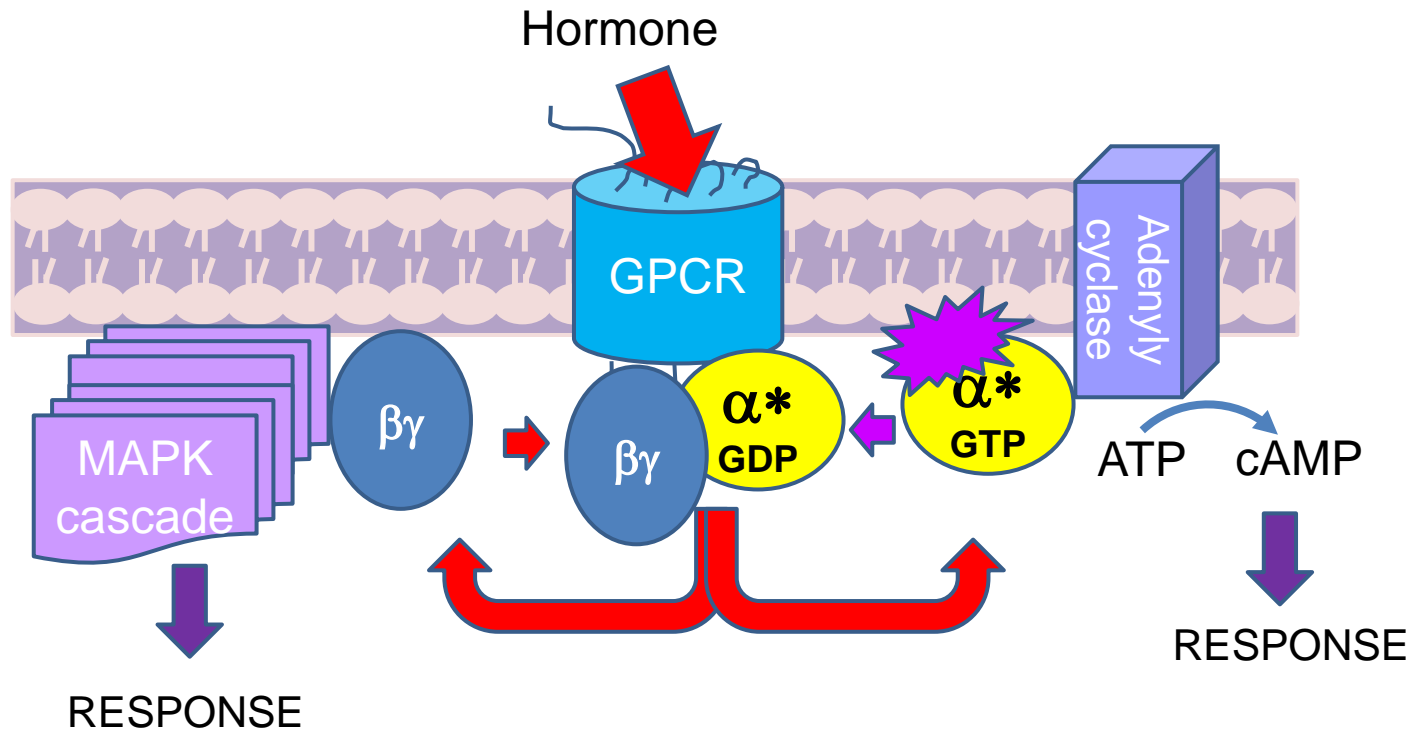
- Mosaicism for constitutively active $G\alpha_s$
 - Precocious puberty,
 - Café-au-lait hyperpigmentation,
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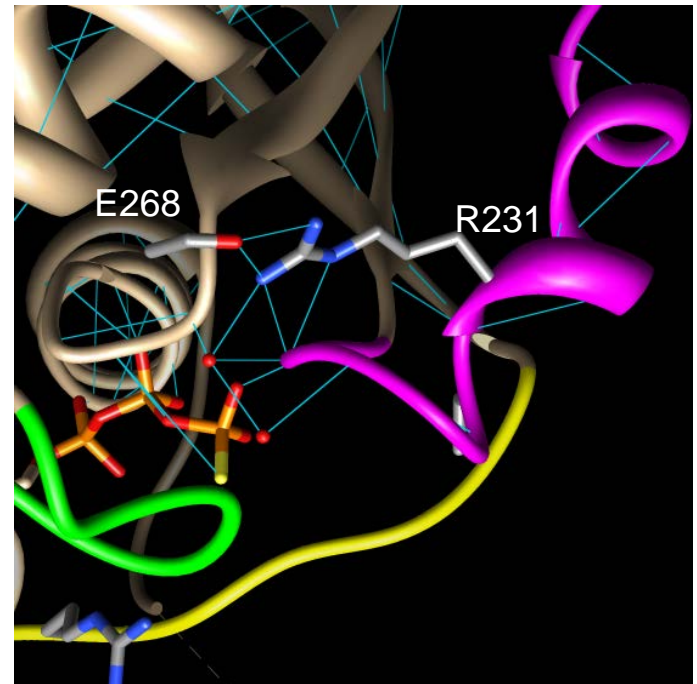
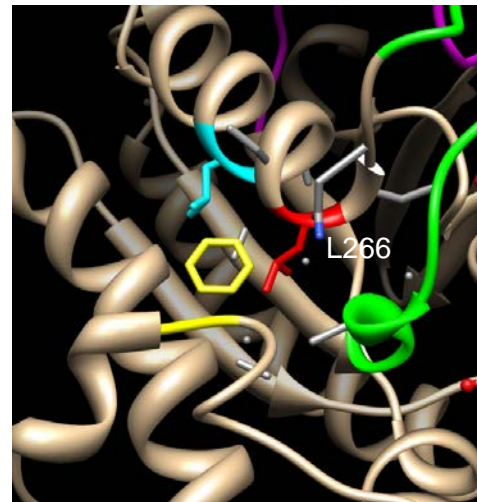
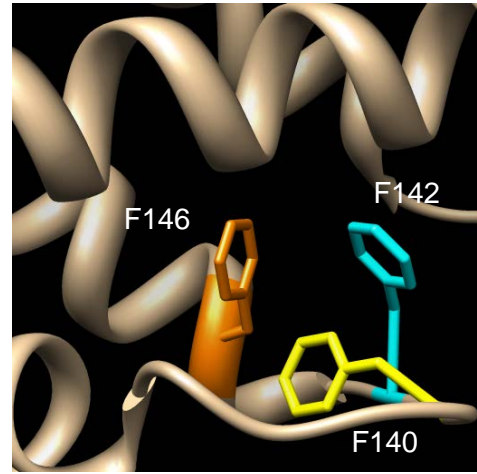
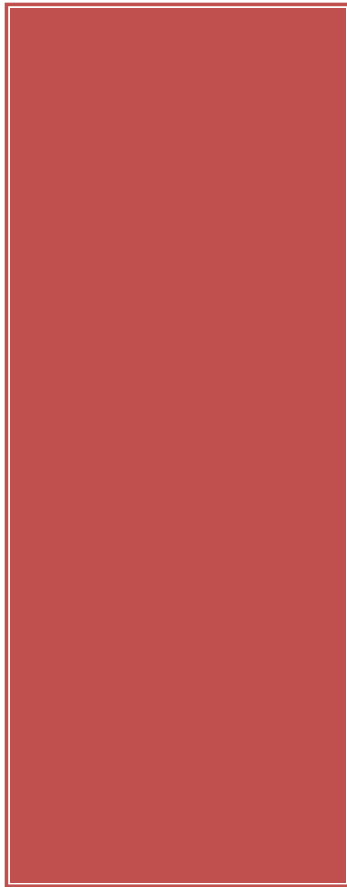
Other MAS alleles are suppressed by changes at these three sites



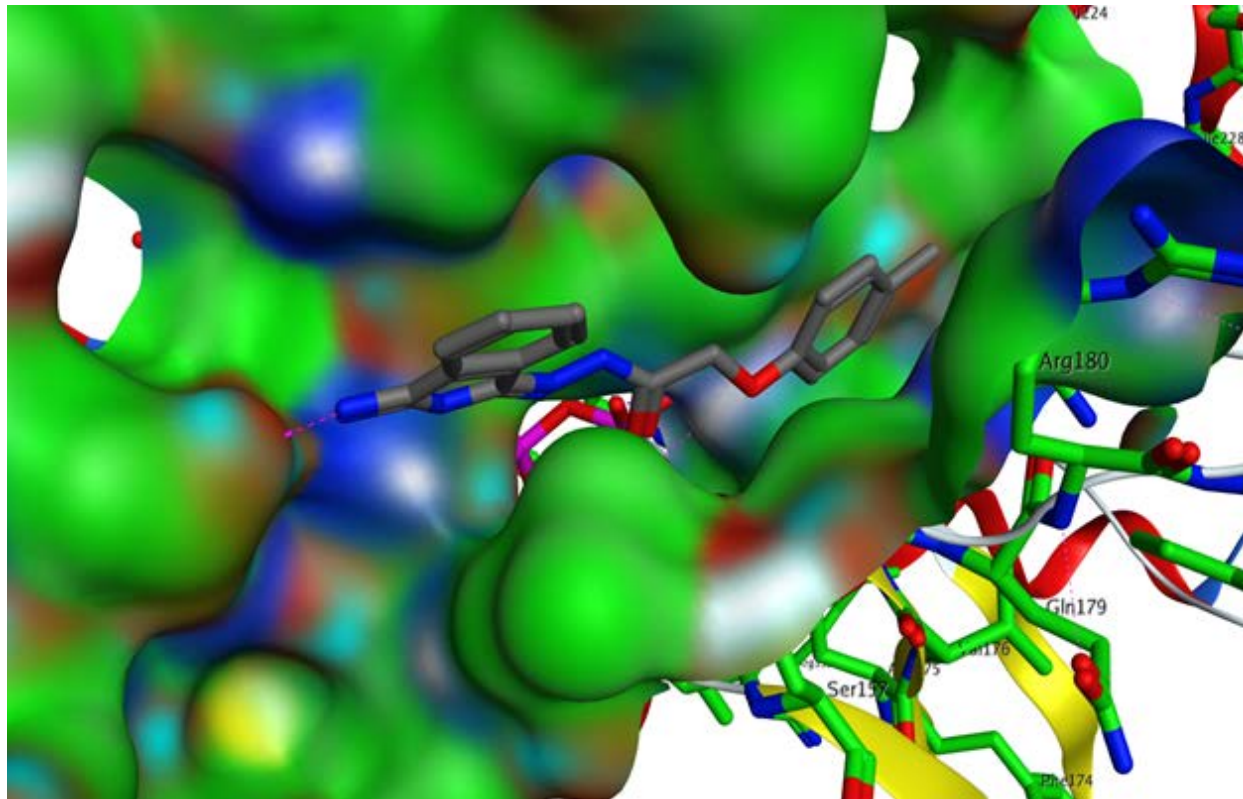
Cells carrying the MAS mutation have constitutively active signaling



Choosing a target site



Finding molecules that bind at R231



Welcome to ZINC, a free database of commercially-available compounds for virtual screening. ZINC contains over 35 million purchasable compounds in ready-to-dock, 3D formats. ZINC is provided by the [Shoichet Laboratory](#) in the Department of Pharmaceutical Chemistry at the University of California, San Francisco (UCSF). To cite ZINC, please reference: Irwin, Sterling, Mysinger, Bolstad and Coleman, *J. Chem. Inf. Model.* 2012 DOI: [10.1021/ci3001277](https://doi.org/10.1021/ci3001277). The original publication is Irwin and Shoichet, *J. Chem. Inf. Model.* 2005;45(1):177-82 [PDF](#), [DOI](#). We thank [NIGMS](#) for financial support (GM71896).

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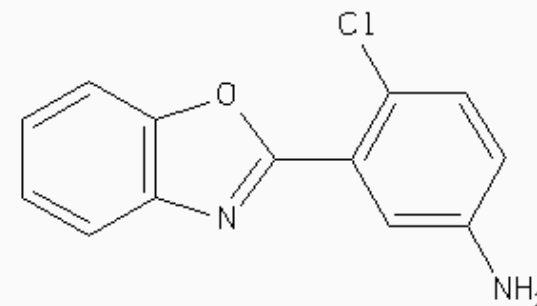
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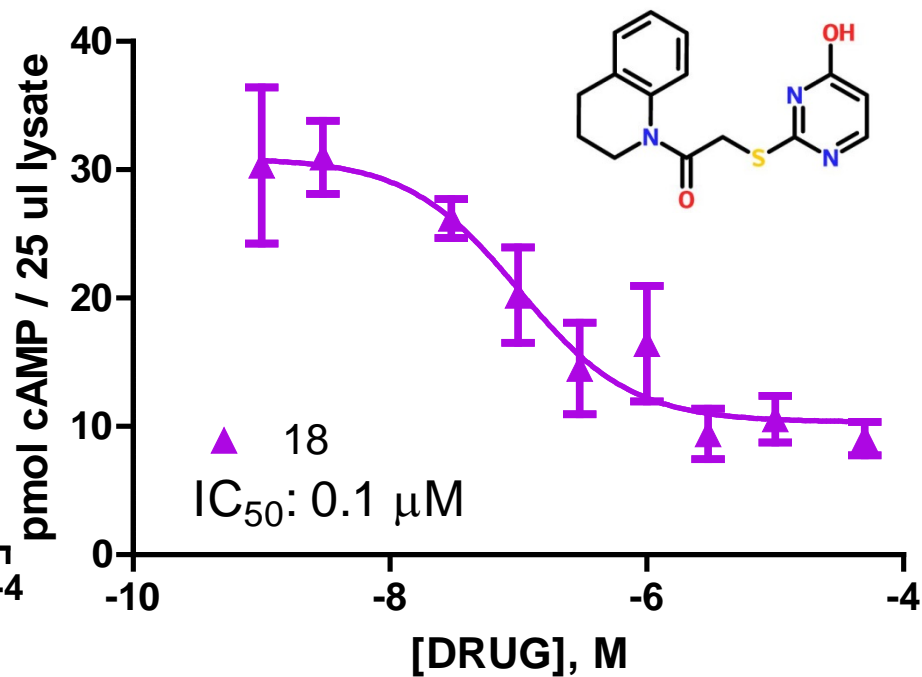
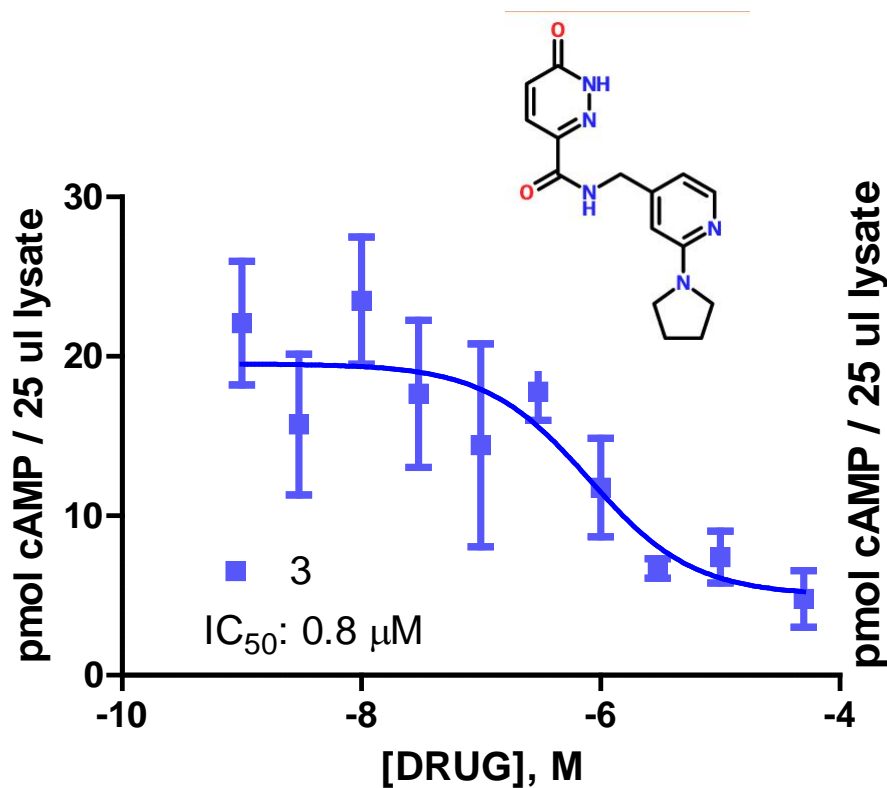
Molecule of the Week

[122974](#)

10-Special Subsets in ZINC

#	Name	Size	Last Updated	Selection Criteria	Source
1	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
2	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
3	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
4	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
5	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
6	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
7	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
8	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
9	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL
10	ChEMBL	1,128,000	2012-01-04	ChEMBL	ChEMBL

Dose-Dependent Relationships of Drugs 3 and 18



Conclusions:

Protein
homologies

Homologous
biochemical
pathways

Variation and
selection
processes
applied to
drug design

Evolution is
used to heal
disease

- McCune-Albright syndrome can be modeled at the cellular level using yeast
- Several intragenic suppressors of R201H activity were identified, these mutations alone do not abolish Gs signaling.
- The intragenic suppressors were able to suppress R201C and R201S mutations
- We have identified two molecules that inhibit Gs-R201H in a dose-dependent manner

Pilot studies

Matthew Koster, MD
Joshua Mitchell, MD
Laura Ooms, Ph.D.
Dahlia Sultan, PharmD
Brittany Swen, DPT
Regina Herrera, DDS

Yeast library construction & screen

Daniela Janevska, Ph.D.
Eraj Din, MD
Jennifer Haick, Ph.D.
Rebecca Alvarez

Site-directed mutagenesis

Kyle Turcic, MD
Alison Dufour, MS
Raquel Tobar-Rubin, PharmD
Julie Carroll, MD

Drug screens

Julie Carroll, MD
Renee Habbal, DDS
Evan Jenkins, MD
Joe Cruz
Matthew Raub
Morgan Schumacher, MD

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- Dr. Eric Walters, Rosalind Franklin University