Structural evidence for "Intermediate" along the activation pathway of Heterotrimeric G-proteins

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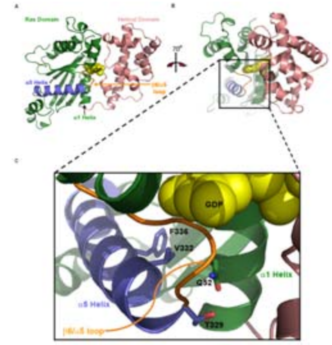
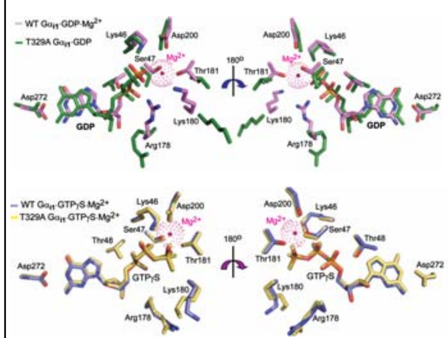
Abstract

Heptahelical G protein-coupled receptors (GPCRs) couple to heterotrimeric G proteins to relay extracellular signals to intracellular signaling networks, but the molecular mechanism underlying GDP release by the G protein α-subunit is not well understood. Amino acid substitutions in the conserved α5 helix of Gi, which extends from the C-terminal region to the nucleotide-binding pocket, cause dramatic increases in basal (receptor-independent) GDP release rates. For example mutant Gαi1-T329A shows an 18-fold increase in basal GDP release rate, and when expressed in culture it causes a significant decrease in forskolinstimulated cAMP accumulation. The crystal structure of Gαi1-T329A•GDP shows substantial conformational rearrangement of the switch I region and additional striking alterations of side chains lining the catalytic pocket that disrupt the Mg^{+2} coordination sphere and dislodge bound Mg^{+2}. We propose a "sequential release" mechanism whereby a transient conformational change in the α5 helix alters switch I to induce GDP release. Interestingly, this mechanistic model for heterotrimeric G protein activation is similar to that suggested for the activation of the plant small G protein Rop4 by RopGEF8.

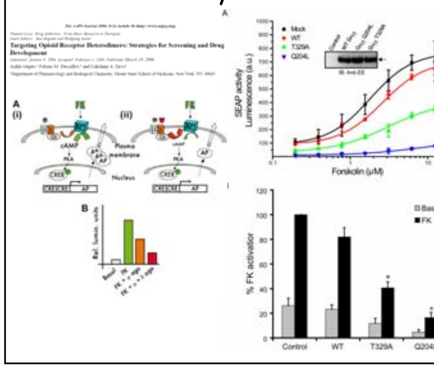
α5 helix point mutants



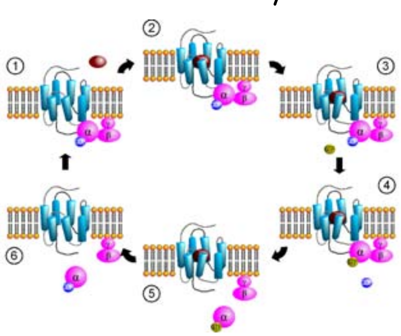
Changes in the Catalytic Pocket



In vivo activity of the mutant

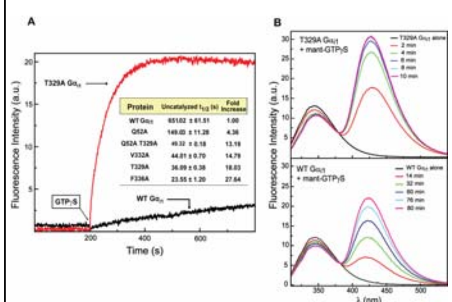


Activation cycle



http://upload.wikimedia.org/wikipedia/commons/6/6d/GPCR-Zyklus.png

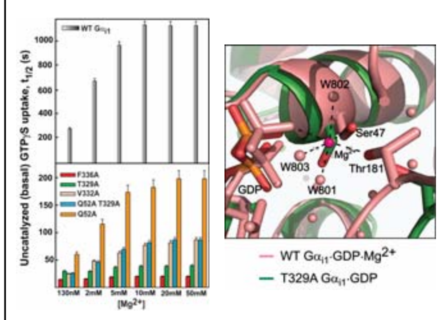
Monitoring Activation



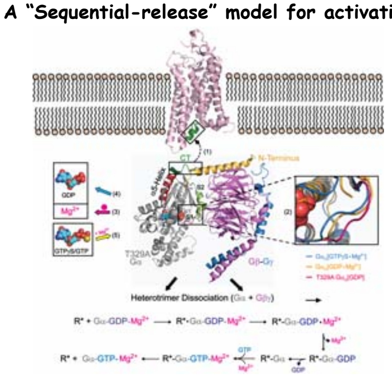
Protein	Uncatalyzed $t_{1/2}$ (s)	Fold Increase
WT Gαi1	651.02 ± 61.51	1.00
Q52A	149.03 ± 11.28	4.36
Q52A T329A	49.32 ± 0.18	13.19
V332A	44.01 ± 0.70	14.79
T329A	36.09 ± 0.38	18.03
F336A	23.55 ± 1.20	27.64

Basal rates of activation much faster for the mutant

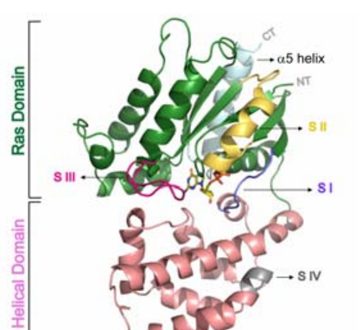
Loss of Mg^{2+} & Catalytic Waters



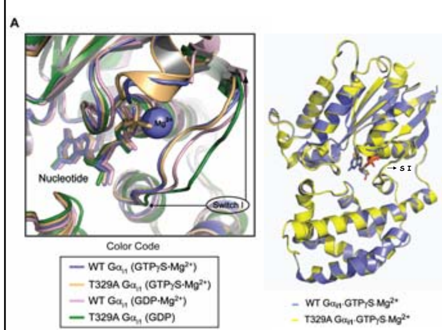
A "Sequential-release" model for activation



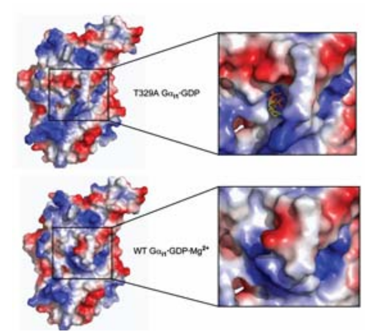
Structure of G protein α-subunit



Changes in Switch I conformation



Electrostatic Potential Surface



References

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