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Identification of Functional Amino Acids in the G Protein Alpha-Subunit

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Principal Investigator: Gundersen, Robert E.
Organization: University of Maine
Title:
Identification of Functional Amino Acids in the G Protein Alpha-Subunit

Project Participants

Senior Personnel

Name: Gundersen, Robert
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Graduate Student

Name: McCarty, Chirstopher
Worked for more than 160 Hours: Yes
Contribution to Project:
Chris's M.S. degree project was to screen for additional functional mutations in Ga2 of Dictyostelium. This addressed Specific Aim B.

Name: Rauch, Steve
Worked for more than 160 Hours: Yes
Contribution to Project:
Steve is working towards his M.S. degree looking for Ga2 mutations that specifically block guanylyl cyclase activation. Part of Specific Aim A.

Undergraduate Student

Name: Gilbert, Amanda
Worked for more than 160 Hours: Yes
Contribution to Project:
Involved in screening for functional Ga2 mutation in Dictyostelium

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

Activities and Findings

Research and Education Activities:
The project has involved the isolation of nonfunctional Ga2 mutations through the use of simple developmental screening of Dictyostelium phenotypes. Aggregation-minus cells are collected and the Ga2 containing plasmid which has failed to rescue the wild-type phenotype is sequence to isolate the responsible Ga2 mutation. Results to date on roughly 15 characterized mutations reveal a wide variety of potential sites for inactivation of Ga2. These appear distributed throughout the protein contained in both of the structural domains of the a-subunit. No specific biochemical phenotype has yet been assinged to any of the Ga2 mutations.

Findings:

The G protein a-subunit is composed of two structural domains. The majority of a-subunit fucntion is thought to lie in the GTPase domain which is quite similar to the structure of the Ras superfamily of small G proteins. The Helical domain, unique to the hetrotrimer G proteins, has no assigned functions to date. Somewhat surprisingly, up to half of the mutations which result in a nonfunctional Ga2 are localized to the Helical Domain. Their biochemical role is still to be determined.

Training and Development:

Each of thestudents working on this project have been exposed to general cell and molecular techniques. These reange from cell luture techniques to plasmid preparation, cell trransformation via electroporation and working with and comparing DNA sequences.

Outreach Activities:

Journal Publications

Robert E. Gundersen, Chris McCarty, Steve Rauch, Kate Farnham-Daggett, Alison Prince and Jianxin You

, "Random mutagenesis of the G protein a-subunit: Isolation of nonfunctional mutations using Ga2 of Dictyostelium discoideum.", to be determined, p. , vol. , ( ). In preparation

Books or Other One-time Publications

Web/Internet Site

URL(s):

Description:

Other Specific Products

Contributions

Contributions within Discipline:

Contributions to Other Disciplines:

Contributions to Human Resource Development:

For this specific award two students will receive their M.S. degree as described. The undergraduate student earned research credits toward her degree while working on this project in my laboratory. Amanda continued on after graduation working towary a degree in Pharmacy. Chris McCarty has completely his M.S. degree and is currently deciding on a career in elementary eduacation in the sciences or to continue on in research to obtain his Ph.D. Steve Rauch will complete his M.S. degree in the near future and will persue a career as a research technician.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Organizational Partners

Activities and Findings: Any Outreach Activities

Any Book

Any Product

Contributions: To Any within Discipline

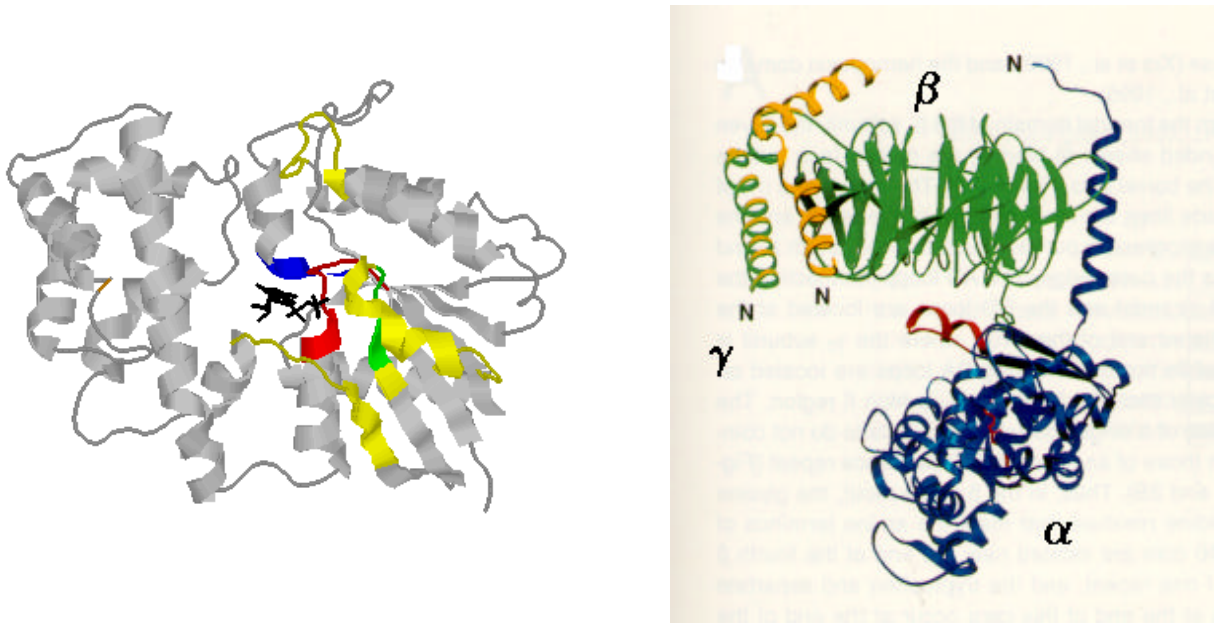
Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering

G Proteins (αβγ) are essential in a wide variety of cellular functions, such as sensory and hormonal responses, differentiation and movement. Crystallographic studies of the G protein α-subunit and the heterotrimer by several laboratories have revealed the structural basis for much of their function. The α-subunit (Fig. 1A) consists of two domains: a **GTPase domain** that is similar to the structure of ras and the **helical domain.** The GTPase domain contains the guanine nucleotide binding pocket built from consensus sequences found in all GTPases: a GTP binding motif, GXGXXGKS (red); a Mg^{2+}-binding domain, DXXG (green) and a guanine ring binding motif, NKXD (blue). The helical domain forms a lid to bury the guanine nucleotide within the protein, yet has no defined function. It has been shown to be the site of phosphorylation (S113, orange) in response to activation in the *Dictyostelium* G protein α-subunit, Gα2. α-subunit activation occurs via a ligand bound membrane receptor and induces the exchange of bound GDP for GTP and the release of the βγ complex. Structural analysis identified three structural changes in the α-subunit upon exchange of GTP for GDP defined as Switches I, II, and III (shown in yellow). Switches I and II are instrumental in binding the βγ complex (Fig. 1B) and presumably the α-subunit effectors as well as a protein that regulates α-subunit GTPase activity (the RGS protein). Mutational studies identified key sites on the α-subunit C-terminus for receptor binding.

A full understanding of the molecular mechanisms underlying the numerous functions played by the family of G protein α-subunits is still to be determined. We are using the essential function of Gα2 in *Dictyostelium* development to identify amino acid residues important to α-subunit function. Our approach uses a library of randomly mutagenized *ga2* cDNA with expression in a *ga2*-null cell line.



A. **B.**

Fig. 1 Crystal structures of Gαt (A) and Gαβγt (B).

Methods

A library of randomly mutated gα2 cDNA was generated using altered PCR conditions and the PCR product was cloned into the expression plasmid, pJK1. Sequence analysis of the library revealed 0 to 4 amino acids altered per ga2 sequence. The library was transformed into the gα2-null cell line, MYC2 by electroporation. Preliminary screening was performed on SM/5 agar plates following selection in G418. Clones with abnormal or aggregation-minus phenotypes were selected for further analysis. Secondary screens are performed on non-nutrient agar (DB) plates and clones are checked for protein expression on immunoblots using Gα2-specific antiserum. Table 1. Summarizes results of the screening to date.

Table 1: Phenotypic screen of Gα2 Mutants

# of clones screened (on bacterial lawns)	# of clones agg-minus (on DB)	# of clones agg-minus expressing Gα2 protein	# agg-minus clones
2130	397	110 of 167 screened	85 of 114 screened

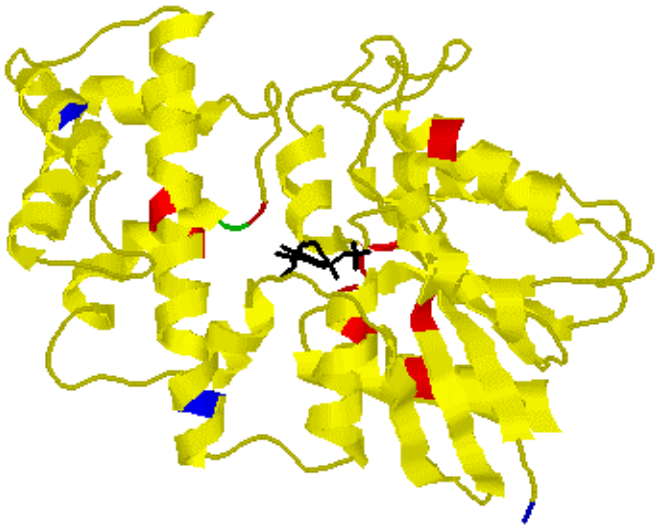
Identified Gα2 Mutations

To identify the gα2 mutation responsible for the mutant phenotype, pMC34 is isolated from the selected clones and sequenced using gα2-specific primers. To date fifteen single point mutations of Gα2 have been identified that result in a abnormal or aggregation-minus phenotype. Each of the fifteen mutations are summarized in Table 2 as to their identity, location within the α-subunit structure based on homology to Gαt (see also Fig. 2) and the degree of conservation of the mutated amino acid. The majority of mutated amino acids are well conserved among the α-subunits. Two (G43D, I47T) lie in the GTP binding domain and D204G is part of the Mg^{2+} binding motif. Two others (E24V and E190G) are likely involved in βγ binding. E190 is highly conserved and the heterotrimer crystal structure identifies it as a contact site with the β-subunit. D154V and N272D are both at the only site of noncovalent interdomain contact and may play a key role in GDP release. C327R is also significant since this area of the protein has been suggested to be important in relaying the signal from the ligand-bound receptor to the α-subunit. Others of special interest are those mutations located in the helical domain, these should contribute to identifying a function for this domain. Finally F338Y may be part of a new domain involved in α-subunit regulation by caveolins. (See below)

Table 2: Gα2 Single Amino Acid Changes that Result in a Mutant Phenotype

Mutation	Location	Level of Conservation
E24V	amino terminus	not conserved
G43D	α1, GTPase domain	highly conserved
I47T	α1, GTPase domain	conserved hydrophobic
R64G	αA, helical domain	not conserved
N74D	αA, helical domain	highly conserved
L76P	αA, helical domain	conserved hydrophobic
I104S	αB, helical domain	not conserved
D154V	DE loop, helical domain	highly conserved
S155P	DE loop, helical domain	moderately conserved
E190G	Switch 1, GTPase domain	highly conserved
D204G	β3, GTPase domain	highly conserved
E249G	a3, GTPase domain	highly conserved
N272D	β5αG, GTPase domain	highly conserved
C327R	β6α5 loop, GTPase domain	highly conserved
F338Y	α5, GTPase domain	moderately conserved

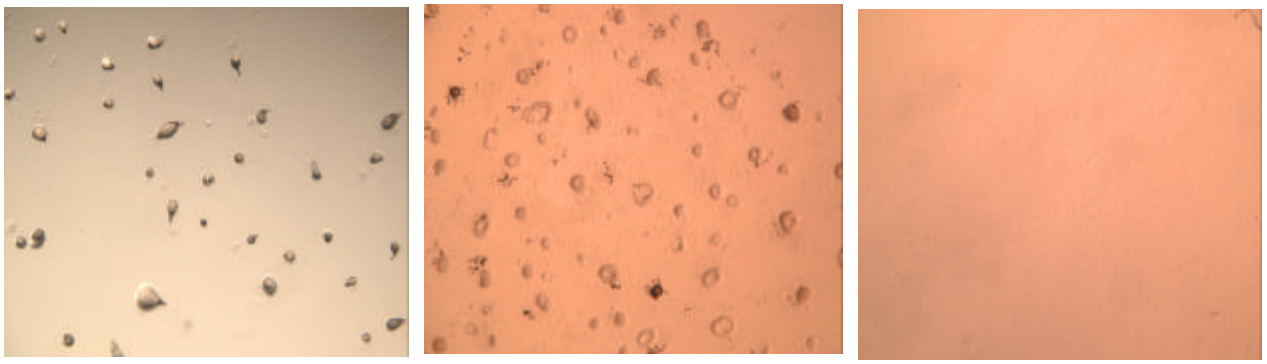
Fig. 2 Gα2 non-aggregating point mutations mapped to the crystal structure of Gαt.



E190G and F338Y

Two of the mutations in Gα2 isolated in a recent screen; E190G and F338Y, result in abnormal phenotypes. The phenotype for each on DB plates is shown below at 23 hours of starvation (Fig. 3). E190G is somewhat successful at aggregation and forming mounds but numerous cells are not involved. Culmination to normal fruiting bodies is rarely seen though small stalk-like structures are observed after two days. Very recent experiments show that E190G does not aggregate when plates are placed at 30°C suggesting it is a temperature-sensitive mutation. F338Y is aggregation-minus.

Fig. 3 Developmental phenotypes of cell line on DB agar plates after 23 hr at 22°C.



Ax3 23 hr E190G 23 hr F338Y 23 hr

Biochemical Responses

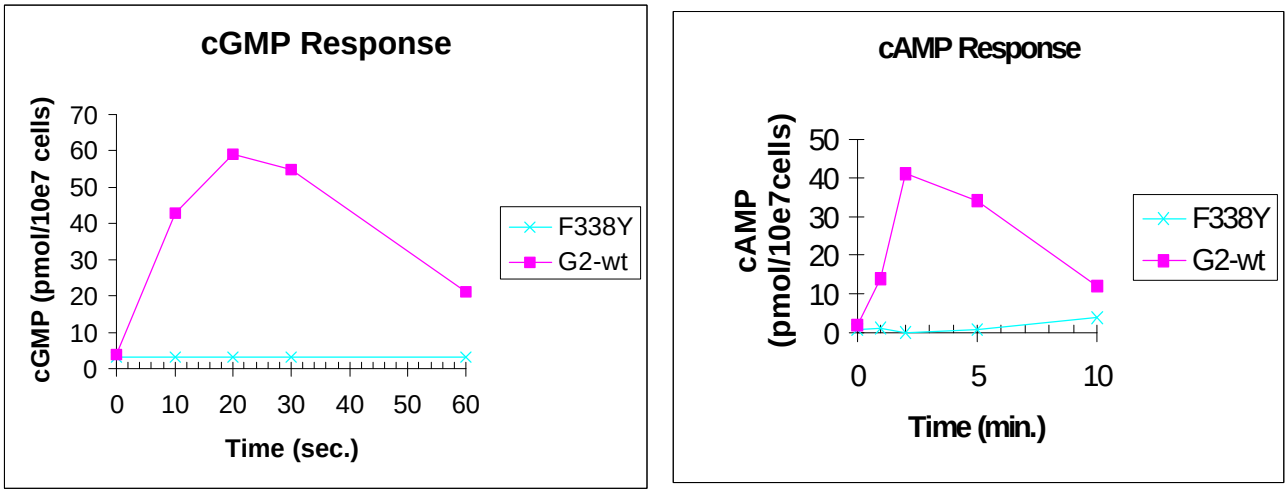
We have just recently initiated assays of E190G and both adenylyl and guanylyl cyclase activation appear to be normal when using cells starved at 22°C for 5 hours in shaking culture with cAMP pulses. These assays now will be performed on cells at 30°C which is expected to result in no effector activation.

The aggregation-minus F338Y does not respond to cAMP stimulation; neither guanylyl cyclase nor adenylyl cyclase are activated (Fig. 4). The lack of effector activation appears due to an uncoupling of the mutated Gα2 from the cAMP receptor. GTPγS inhibition of caMP binding assays reveal no high affinity cAMP binding sites.

GTPγS Inhibition of cAMP Binding

Cell line	% inhibition of cAMP binding
Ax3	72
MYC2	32
F338Y	30

Fig. 4 *In vivo* activation of guanylyl and adenylyl cyclases in $G\alpha2^{F338Y}$



Discussion

The F338Y mutation is interesting as a result of two publications in 1997. One by Onrust *et al*. identifies the α5 helix of the GTPase domain as an important site for receptor coupling. This helix in conjunction with the β6α5 loop (TCAT) may be responsible for GDP stimulated release caused by ligand-bound receptor. The second paper by Couet *et al*. defines a consensus caveolin binding domain and identifies this domain in the G protein a-subunits. The domain consists of three to four phenylalanines (or Ile, Val) forming a hydrophobic pocket. This domain is present in the GTPase domain sequence comprising β2 through β3 which is directly between Switch I and Switch II. In the crystal structure the a5 helix lies across the hydrophobic pocket formed by this domain and F338 is one of several hydrophobic residues facing the hydrophobic domain (Fig. 5). Analysis of the F338Y mutation along with others in the caveolin-binding sequence and the α5 helix may be important in discovering the mechanism for relay of the activation signal between the receptor and the G protein α-subunit. The interaction of these two domains also suggests a role for caveolin in this process.

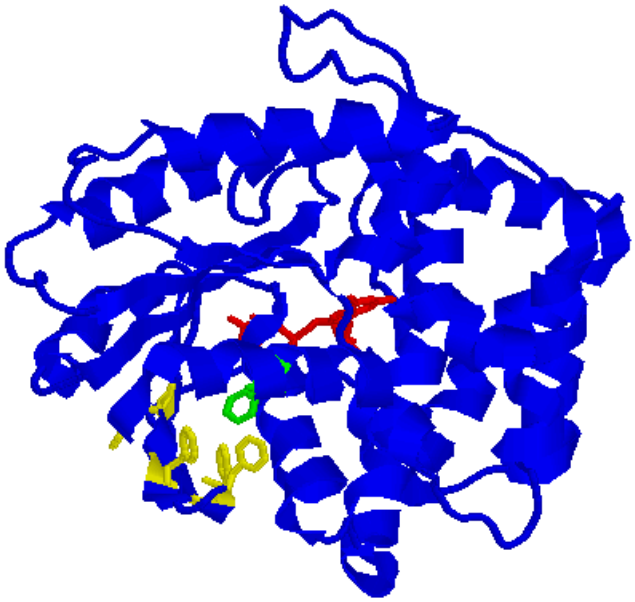


Fig. 5 Location of F338 (green) and the caveolin-binding domain (yellow) within the G protein α-subunit

References

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J. Couet, S. LI, T. Okamoto, T. Ikezu, M. Lisanti Identification of peptide and protein ligands for the caveolin-scaffolding domain. J. Biol. Chem. 272;6526-6533 (1997)