

LECTURE #6: STRUCTURE AND REGULATION OF G PROTEINS

Assigned (Required) Reading is (1) - (4):

•General Background (in your Reader):

(1) Wennerberg K, Rossman KL, Der CJ (2005) The Ras superfamily at a glance. J. Cell Sci. 118: 843-846.

(2) Bos JL, Rehmann H, Wittinghofer A (2007) GEFs and GAPs: critical elements in the control of small G proteins. Cell 129: 865-877.

(3) Oldham WM, Hamm HE (2006) Structural basis of function in heterotrimeric G proteins. Quart. Rev. Biophys. 39: 117-166.

•Paper for Discussion Session (Friday, 12/7) (handed out in class):

Ballon DR, Flanary PL, Gladue DP, Konopka JB, Dohlman HG, Thorner J (2006) DEP-domain-mediated regulation of GPCR signaling responses. Cell 126: 1079-1093.

G protein structure and function (general references):

Castro AF, Rebhun JF, Quilliam LA. (2005) Measuring Ras-family GTP levels in vivo-running hot and cold. Methods 37: 190-196.

Vetter IR, Wittinghofer A (2001) The guanine nucleotide-binding switch in three dimensions. Science 294: 1299-1304.

Corbett KD, Alber T (2001) The many faces of Ras: recognition of small GTP-binding proteins. Trends Biochem Sci. 26: 710-716.

Sprang SR (2000) Conformational display: a role for switch polymorphism in the superfamily of regulatory GTPases. Sci STKE Sep 19, 2000; 2000(50):PE1. Review.

Sprang SR (1997) G protein mechanisms: insights from structural analysis. Annu. Rev. Biochem. 66: 639-678.

Bourne HR (1995) GTPases: a family of molecular switches and clocks. Philos Trans R Soc Lond B Biol Sci 349: 283-289.

Ras and its closest relatives:

Mitin N, Rossman KL, Der CJ (2005) Signaling interplay in Ras superfamily function. Curr. Biol. 15: R563-R574.

Campbell PM, Der CJ (2004) Oncogenic Ras and its role in tumor cell invasion and metastasis. Semin. Cancer Biol. 14: 105-114.

Bar-Sagi D (2001) A Ras by any other name. Mol Cell Biol. 21: 1441-1443.

Takai Y, Sasaki T, Matozaki T (2001) Small GTP-binding proteins. Physiol Rev. 81: 153-208.

Cullen PJ. (2001) Ras effectors: buying shares in Ras Plc. Curr Biol. 11: R342-R344.

Reuther GW, Der CJ (2000) The Ras branch of small GTPases: Ras family members don't fall far from the tree. Curr Opin Cell Biol. 12: 157-165.

Ayllon V, Rebollo A (2000) Ras-induced cellular events Mol. Membr. Biol. 17: 65-73.

Du X, Frei H, Kim SH (2000) The mechanism of GTP hydrolysis by Ras probed by Fourier transform infrared spectroscopy. J Biol Chem. 275: 8492-8500.

McCormick F. (1999) Signalling networks that cause cancer. Trends Cell Biol. 9: M53-M56.

Feig LA. (1999) Tools of the trade: use of dominant-inhibitory mutants of Ras-family GTPases. *Nature Cell Biol.* 1: E25-E27.

Rho proteins:

Hall A (2005) Rho GTPases and the control of cell behaviour. *Biochem. Soc. Trans.* 33: 891-895.

Jaffe AB, Hall A. (2005) Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol.* 21: 247-269.

Burridge K, Wennerberg K (2004) Rho and Rac take center stage. *Cell* 116: 167-179.

Ridley AJ. (2001) Rho proteins: linking signaling with membrane trafficking. *Traffic* 2: 303-310.

Fukata Y, Amano M, Kaibuchi K (2001) Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol Sci* 22: 32-39.

Bishop AL, Hall A. (2000) Rho GTPases and their effector proteins. *Biochem J.* 348: 241-255.

Symons M, Settleman J. (2000) Rho family GTPases: more than simple switches. *Trends Cell Biol.* 10: 415-419.

Bokoch GM. (2000) Regulation of cell function by Rho family GTPases. *Immunol Res.* 21: 139-148.

Schmitz AA, Govek EE, Bottner B, Van Aelst L. (2000) Rho GTPases: signaling, migration, and invasion. *Exp Cell Res.* 261: 1-12.

Rac and Cdc42:

Hoffman GR, Cerione RA. (2002) Signaling to the Rho GTPases: networking with the DH domain. *FEBS Lett.* 513: 85-91.

Buchwald G, Hostinova E, Rudolph MG, Kraemer A, Sickmann A, Meyer HE, Scheffzek K, Wittinghofer A (2001) Conformational switch and role of phosphorylation in PAK activation. *Mol Cell Biol* 21: :5179-5189.

Erickson JW, Cerione RA (2001) Multiple roles for Cdc42 in cell regulation. *Curr Opin Cell Biol.* 13: 153-157.

Hoffman GR, Cerione RA. (2000) Flipping the switch: the structural basis for signaling through the CRIB motif. *Cell* 102: 403-406.

Symons M. (2000) Adhesion signaling: PAK meets Rac on solid ground. *Curr Biol.* 10: R535-R537.

Johnson DI. (1999) Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiol Mol Biol Rev.* 63: 54-105.

Structures of small G proteins:

Mossessova E, Corpina RA, Goldberg J (2003) Crystal structure of ARF1*Sec7 complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. *Mol. Cell* 12: 1403-1411.

Merithew E, Hatherly S, Dumas JJ, Lawe DC, Heller-Harrison R, Lambright DG (2001) Structural plasticity of an invariant hydrophobic triad in the switch regions of Rab GTPases is a determinant of effector recognition. *J Biol Chem.* 276: 13982-13988.

Amor JC, Horton JR, Zhu X, Wang Y, Sullards C, Ringe D, Cheng X, Kahn RA (2001) Structures of Yeast Arf2 and Arl1. Distinct roles for the N-terminus in the structure of Arf family GTPases. *J. Biol. Chem* 276: 42477-42484.

Spoerner M, Herrmann C, Vetter IR, Kalbitzer HR, Wittinghofer A (2001) Dynamic properties of the Ras switch I region and its importance for binding to effectors. *Proc Natl Acad Sci USA* 98: 4944-4949.

Renault L, Kuhlmann J, Henkel A, Wittinghofer A (2001) Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). *Cell* 105: 245-255.

Stroupe C, Brunger AT (2000) Crystal structures of a Rab protein in its inactive and active conformations. *J Mol Biol.* 304: 585-598.

Esters H, Alexandrov K, Constantinescu AT, Goody RS, Scheidig AJ. (2000) High-resolution crystal structure of *S. cerevisiae* Ypt51(DeltaC15)-GppNHp, a small GTP-binding protein involved in regulation of endocytosis. *J Mol Biol* 298: 111-121.

Chook YM, Blobel G (1999) Structure of the nuclear transport complex karyopherin-beta2-Ran x GppNHp. *Nature* 399: 230-237.

Rudolph MG, Wittinghofer A, Vetter IR. (1999) Nucleotide binding to the G12V-mutant of Cdc42 investigated by X-ray diffraction and fluorescence spectroscopy: two different nucleotide states in one crystal. *Protein Sci* 8: 778-787.

Goldberg J (1998) Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell* 95: 237-248.

GEFs:

Schmidt A, Hall A (2002) Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev.* 16: 1587-1609.

Wells CD, Gutowski S, Bollag G, Sternweis PC (2001) Identification of potential mechanisms for regulation of p115 RhoGEF through analysis of endogenous and mutant forms of the exchange factor. *J Biol Chem.* 276: 28897-28905.

Fukuhara S, Chikumi H, Gutkind JS (2001) RGS-containing RhoGEFs: the missing link between transforming G proteins and Rho? *Oncogene* 20: 1661-1668.

Gulli MP, Peter M (2001) Temporal and spatial regulation of Rho-type guanine-nucleotide exchange factors: the yeast perspective. *Genes Dev.* 15: 365-379.

Bollag G, Crompton AM, Pevery-Mitchell D, Habets GG, Symons M. (2000) Activation of Rac1 by human Tiam1. *Methods Enzymol.* 325: 51-61.

Jackson CL, Casanova JE. (2000) Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. *Trends Cell Biol* 10: 60-67.

Donaldson JG, Jackson CL. (2000) Regulators and effectors of the ARF GTPases. *Curr Opin Cell Biol.* 12: 475-482.

Cherfils J, Chardin P (1999) GEFs: structural basis for their activation of small GTP-binding proteins. *Trends Biochem Sci.* 24: 306-311.

Sprang SR, Coleman DE (1998) Invasion of the nucleotide snatchers: structural insights into the mechanism of G protein GEFs. *Cell* 95: 155-158.

GAPs:

Cai SL, Tee AR, Short JD, Bergeron JM, Kim J, Shen J, Guo R, Johnson CL, Kiguchi K, Walker CL. (2006) Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *J. Cell Biol.* 173: 279-289.

Li Y, Inoki K, Guan KL. (2004) Biochemical and functional characterizations of small GTPase Rheb and TSC2 GAP activity. *Mol. Cell. Biol.* 24: 7965-7975.

Donovan S, Shannon KM, Bollag G (2002) GTPase activating proteins: critical regulators of intracellular signaling. *Biochim. Biophys Acta* 1602: 23-45.

Hiatt KK, Ingram DA, Zhang Y, Bollag G, Clapp DW (2001) Neurofibromin GTPase-activating protein-related domains restore normal growth in *Nf1*^{-/-} cells. *J. Biol. Chem.* 276: 7240-7245.

Stam JC, Collard JG. (1999) The DH protein family, exchange factors for Rho-like GTPases. *Prog Mol Subcell Biol.* 22: 51-83.

Scheffzek K, Ahmadian MR, Wittinghofer A (1998) GTPase-activating proteins: helping hands to complement an active site. *Trends Biochem. Sci.* 23: 257-262.

McCormick F. (1998) Going for the GAP. *Curr Biol.* 8: R673-R674.

Gamblin SJ, Smerdon SJ (1998) GTPase-activating proteins and their complexes. *Curr Opin Struct Biol.* 8: 195-201.

Whitehead IP, Campbell S, Rossman KL, Der CJ. (1997) Dbl family proteins. *Biochim Biophys Acta.* 1332: F1-F23.

GDI:

Grizot S, Faure J, Fieschi F, Vignais PV, Dagher MC, Pebay-Peyroula E (2001) Crystal structure of the Rac1-RhoGDI complex involved in nadph oxidase activation. *Biochemistry* 40: 10007-10013.

Erdman RA, Maltese WA (2001) Different Rab GTPases associate preferentially with alpha or beta GDP-dissociation inhibitors. *Biochem Biophys Res Commun.* 282: 4-9.

Hoffman GR, Nassar N, Cerione RA. (2000) Structure of the Rho family GTP-binding protein Cdc42 in complex with the multifunctional regulator RhoGDI. *Cell* 100: 345-356.

Keep, N.H., M. Barnes, I. Barsukov, R. Badii, L.Y. Lian, A.W. Segal, P.C. Moody and G.C. Roberts. (1997) A modulator of rho family G proteins, rhoGDI, binds these G proteins via an immunoglobulin-like domain and a flexible N-terminal arm. *Structure* 5: 623-633.

Heterotrimeric G Proteins:

Holinstat M, Oldham WM, Hamm HE (2006) G-protein-coupled receptors— evolving views on physiological signalling; the Keystone Symposium on "G-protein-coupled receptors: evolving concepts and new techniques." *EMBO Rep.* 7: 866-869.

Siderovski DP, Willard FS. (2005) The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. *Int. J. Biol. Sci.* 1: 51-66.

Iyengar R (2005) Structure of G protein-coupled receptors and G proteins. *Sci STKE.* 2005 Mar 22; 2005(276):tr10.

Marinissen MJ, Gutkind JS (2001) G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci.* 22: 368-376.

Offermanns S (2001) In vivo functions of heterotrimeric G-proteins: studies in $G\alpha$ -deficient mice. *Oncogene* 20: 1635-1642.

Rondard P, Iiri T, Srinivasan S, Meng E, Fujita T, Bourne HR (2001) Mutant G protein alpha subunit activated by $G\beta\gamma$: a model for receptor activation? *Proc Natl Acad Sci USA* 98: 6150-6155.

Li, Y., P.M. Sternweis, S. Charnecki, T.F. Smith, A.G. Gilman, E.J. Neer, T. Kozasa (1998) Sites for $G\alpha$ binding on the G protein beta subunit overlap with sites for regulation of phospholipase $C\beta$ and adenylyl cyclase. *J. Biol. Chem.* 273: 16265-16272.

Schoneberg T, Schultz G, Gudermann T (1999) Structural basis of G protein-coupled receptor function. *Mol Cell Endocrinol.* 151: 181-193.

Gudermann T, Kalkbrenner F, Dippel E, Laugwitz KL, Schultz G. (1997) Specificity and complexity of receptor-G-protein interaction. *Adv Second Messenger Phosphoprotein Res.* 31: 253-262.

Hamm HE (1998) The many faces of G protein signaling. *J Biol Chem.* 273: 669-672.

Clapham DE, Neer EJ (1997) G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol.* 37: 167-203.

Structures of $G\alpha\beta\gamma$ heterotrimers:

Offermanns S (2007) Conditional mutagenesis of G-protein coupled receptors and G-proteins. *Handb Exp Pharmacol.* 178: 491-509.

Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE (2003) Insights into G protein structure, function, and regulation. *Endocr Rev.* 24: 765-781.

Lambright, D.G., J. Sondek, A. Bohm, N.P. Skiba, H.E. Hamm and P.B. Sigler (1996) The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature* 379: 311-319.

Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA, Gilman AG, Sprang SR (1995) The structure of the G protein heterotrimer $G_i\alpha 1\beta 1\gamma 2$. *Cell.* 83: 1047-1058.

Galpha targets:

Hart MJ, Roscoe W, Bollag G. (2000) Activation of Rho GEF activity by G alpha 13. *Methods Enzymol.* 325: 61-71.

Dessauer, CW; Tesmer, JJG; Sprang, SR; Gilman, AG (1998) Identification of a $G(i)\alpha$ binding site on type V adenylyl cyclase. *J. Biol. Chem.* 273: 25831-25839.

Jiang, Y; Ma, W; Wan, Y; Kozasa, T; and others (1998) The G protein $G\alpha 12$ stimulates Bruton's tyrosine kinase and a rasGAP through a conserved PH/BM domain. *Nature* 395: 808-813.

Yan, SZ; Huang, ZH; Rao, VD; Hurley, JH et al. (1997) Three discrete regions of mammalian adenylyl cyclase form a site for $G(s)\alpha$ activation. *J. Biol. Chem.* 272: 18849-18854.

Gbeta/gamma targets and diversity in coupling:

Harashima T, Heitman J (2002) The $G\alpha$ protein Gpa2 controls yeast differentiation by interacting with kelch repeat proteins that mimic $G\beta$ subunits. *Mol Cell.* 10: 163-173.

Sondek J, Siderovski DP (2001) $G\gamma$ -like (GGL) domains: new frontiers in G-protein signaling and beta-propeller scaffolding. *Biochem Pharmacol.* 61 1329-1337.

Smith TF, Gaitatzes C, Saxena K, Neer EJ. (1999) The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci.* 24: 181-185.

Clapham DE, Neer EJ (1997) G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol.* 37: 167-203.

RGS proteins:

Kehrl JH, Sinnarajah S (2002) RGS2: a multifunctional regulator of G-protein signaling. *Int J Biochem Cell Biol.* 34: 432-438.

Ross EM, Wilkie TM (2000) GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu. Rev. Biochem.* 69: 795-827.

De Vries L, Zheng B, Fischer T, Elenko E, Farquhar MG (2000) The regulator of G protein signaling family. *Annu Rev Pharmacol Toxicol.* 40: 235-271.

Dohlman HG, Thorner J (1997) RGS proteins and signaling by heterotrimeric G proteins. *J Biol Chem.* 272: 3871-3874.

AGS proteins and GoLoco motifs:

- Willard FS, Kimple RJ, Siderovski DP. (2004) Return of the GDI: the GoLoco motif in cell division. *Annu. Rev. Biochem.* 73: 925-951.
- Afshar K, Willard FS, Colombo K, Johnston CA, McCudden CR, Siderovski DP, Gonczy P (2004) RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of *C. elegans* embryos. *Cell* 119: :219-230.
- Colombo K, Grill SW, Kimple RJ, Willard FS, Siderovski DP, Gonczy P (2003) Role of GoLoco domain proteins in translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. *Science* 300: 1957-1961.
- Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski D (2002) Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. *Natur* 416: 878-881.
- Kimple RJ, De Vries L, Tronchere H, Behe CI, Morris RA, Gist Farquhar M, Siderovski DP (2001) RGS12 and RGS14 GoLoco motifs are G α i interaction sites with guanine nucleotide dissociation inhibitor activity. *J. Biol. Chem.* 276: 29275-29281.
- Cismowski MJ, Takesono A, Bernard ML, Duzic E, Lanier SM (2001) Receptor-independent activators of heterotrimeric G-proteins. *Life Sci.* 68: 2301-2308.
- De Vries L, Fischer T, Tronchere H, Brothers GM, Strockbine B, Siderovski DP, Farquhar MG (2000) Activator of G protein signaling 3 is a guanine dissociation inhibitor for G α i subunits. *Proc. Natl. Acad. Sci. USA* 97: 14364-14369.
- Cismowski MJ, Ma C, Ribas C, Xie X, Spruyt M, Lizano JS, Lanier SM, Duzic E (2000) Activation of heterotrimeric G-protein signaling by a ras-related protein. Implications for signal integration. *J. Biol. Chem.* 275: 23421-23424.
- Takesono A, Cismowski MJ, Ribas C, Bernard M, Chung P, Hazard S 3rd, Duzic E, Lanier SM (1999) Receptor-independent activators of heterotrimeric G-protein signaling pathways. *J. Biol. Chem.* 274: 33202-33205.

Post-translational modifications of G proteins:

- Linder ME, Deschenes RJ (2003) New insights into the mechanisms of protein palmitoylation. *Biochemistry* 42: 4311-4320.
- Chen CA, Manning DR (2001) Regulation of G proteins by covalent modification. *Oncogene* 20: 1643-52.
- Volkert M, Wagner M, Peters C, Waldmann H (2001) The chemical biology of Ras lipidation. *Biol. Chem.* 382: 1133-1145.
- Manahan CL, Patnana M, Blumer KJ, Linder ME (2000) Dual lipid modification motifs in G(alpha) and G(gamma) subunits are required for full activity of the pheromone response pathway in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 11: 957-968.
- Fu HW, Casey PJ. (1999) Enzymology and biology of CaaX protein prenylation. *Recent Prog Horm Res.* 54: 315-342