Cellular Logistics 4, e29391; June 2014; © 2014 Landes Bioscience

G Protein-coupled receptors

Multi-turnover GDP/GTP exchange catalysis on heterotrimeric G proteins

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Keywords: GTPases/G proteins, GEFs (guanine nucleotide exchange factors), GPCRs (G protein coupled receptors), GAPs (GTPase activating proteins), effectors, ARF, RAS, RAB

G protein-coupled receptors and heterotrimeric G proteins can diffuse laterally in the plasma membrane such that one receptor can catalyze the activation (GDP/GTP exchange) of multiple G proteins. In some cases, these processes are fast enough to support molecular signal amplification, where a single receptor maintains the activation of multiple G proteins at steady-state. Amplification in cells is probably highly regulated. It depends upon the identities of the G receptor and G protein - some do and some don't - and upon the activities of GTPase-activating proteins, membrane scaffolds, and other regulatory partners.

The idea that a single G protein-coupled receptor (GPCR) can sequentially activate multiple heterotrimeric G proteins on the surface of the plasma membranes derived from 2 separate experimental arguments made prior to 1990, and is now generally accepted. GPCRs catalyze GDP/GTP exchange to promote G protein activation, and the definition of a catalyst demands turnover of multiple substrate molecules. Massively catalytic G protein activation is best demonstrated in the mammalian photoreceptor membrane, where the majority of the membrane protein is the GPCR rhodopsin and the vast majority of GTP binding sites is the G_t (see essays by Arshavsky, Liebman, ...). In other cells, where both GPCR and its G protein targets may be well below 0.1% of plasma membrane protein, catalytic turnover by GPCRs remains hard to quantitate. In what membranes it occurs, which receptors and G proteins do it, if and how it is regulated, and which protein(s) is (are) the diffusing species are all variables.

The first suggestion that GPCRs and/or G proteins diffuse laterally and that a receptor can activate multiple G proteins came from the work of Levitzki and coworkers,[1-3] who used adenylyl cyclase activity as a surrogate measure of G_s activation; G_s was itself discovered at about the same time. They showed that covalent inactivation of β-adrenergic receptors in erythrocyte membranes progressively decreased the apparent first-order rate of activation of adenylyl cyclase by non-hydrolyzable GTP analogs at a fixed agonist concentration without decreasing maximal stimulation. Such inactivation also shifted the EC_{50} for agonist to higher concentrations. The number of catalytic turnovers for each receptor before cyclase was fully activated could be estimated from these data to be about 10. With the work of Selinger and coworkers,[4-7] who showed that β-adrenergic agonists promote the GDP/GTP exchange step in the receptor-stimulated GTPase cycle, the idea of *exchange catalysis* by receptors entered the review literature by 1980,[8] if not earlier. Even at this point, however, it was also clear that not all receptors recycle freely. Braun and Levitzki[9] showed that an adenosine receptor appeared by the above criteria to be tightly coupled to G_s, with no sign of multi-molecular turnover.

Accurate quantitation of catalytic turnover of G proteins by GPCRs is difficult in intact cells or isolated plasma membranes, but became possible when the purified proteins were reconstituted into phospholipid vesicles. In an early effort, Pedersen and Ross[10] found that a β-adrenergic receptor could catalyze nucleotide exchange on about 6 G_s molecules, and Asano et al.,[11] using more accurate quantitation, demonstrated at least 8 turnovers. A kinetic analysis of Asano's data similar to that performed by Levitzki and coworkers argued that a single receptor could turn over about 50 G_s molecules. Using a reconstituted system similar to that of Asano et al., Cerione et al.[12] also found that the α_2-adrenergic receptor could catalyze GDP/GTP exchange on about 7 molecules of G_i. Mirroring Braun, Senogles et al.[13] then found that the reconstituted D_2 dopamine receptor did not turn over multiple G_i molecules. Some receptors do; some don't. It is uncertain whether receptors that do or do not regulate multiple G protein molecules in phospholipid vesicles might behave differently in cells, although some co-fractionation studies have argued that these behaviors are consistent.

Given that a receptor can sequentially catalyze GDP/GTP exchange on multiple G proteins, at least in some cases, the more relevant physiologic question is whether it does so within the lifetime of the G protein's GTP-activated state. If it can, then a single agonist-liganded receptor can maintain the activation of multiple G proteins at steady-state, leading to molecular amplification of the signal. Catalytic amplification can

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Submitted: 05/05/2014; Accepted: 05/28/2014; Published Online: 06/04/2014
Citation: Ross EM. G Protein-coupled receptors: Multi-turnover GDP/GTP exchange catalysis on heterotrimeric G proteins. Cellular Logistics 2014; 4:e29391; http://dx.doi.org/10.4161/cl.29391

also increase agonist potency (decrease EC_{50}) because fewer agonist-bound receptors can activate the same number of G proteins.

Even with catalytic turnover, though, amplification will only occur if the rate of receptor-catalyzed GDP/GTP exchange is faster than the rate of hydrolysis of G protein-bound GTP. Rate constants for G protein deactivation, with or without stimulation by GTPase-activating proteins (GAPs), have not been measured directly in living cells or in intact membranes other than the photoreceptor disc. The deactivation rate constant for G_s and G_i in cells is estimated to be ~0.05–0.1 s^{-1}, based on the rates of signal termination,[14,15] and the rates of hydrolysis of GTP bound to purified $G\alpha_i$ and $G\alpha_s$, are similar to this value. Other G proteins are slower: ~0.02 s^{-1} for $G\alpha_q$[16] and ~0.002 s^{-1} for $G\alpha_z$,[17] $G\alpha_{12}$ and $G\alpha_{13}$.[18] GAPs can increase these rates as much as 2000-fold.[19,20] Somewhat faster rates of G protein activation and deactivation in cells have been estimated according to effector activation/deactivation rates (above) or with fluorescent G protein activation biosensors.[21,22] While they provide a beautiful insight into the time course of signaling, on/off kinetics has not clarified the amplification question. To do this would require stoichiometric information that is not available from ensemble fluorescence data. In general, stoichiometric relationships among the protein components of plasma membrane signaling networks are poorly understood. They are central to issues of molecular amplification, and they also dictate whether these networks act as first-order (linear) or non-first-order (nonlinear) transducers.

Beyond the not-so-simple activation/deactivation kinetic issues are the questions of cellular constraints on receptor and G protein diffusion. Clearly some receptors and G proteins are highly constrained in their motion. Constraints include stable binding to scaffolding proteins on the molecular scale (1–2 nm) and membrane domain restrictions on the 100–1000 nm scale. Other mechanistic constraints may also arise. For example, we have proposed that one way in which G protein modules use GAPs to accelerate response rates is to promote long-term (10 s *vs* 0.1 s) receptor-G protein binding.[20,23] This mechanism overcomes inhibition by the GAP, but at the cost of molecular amplification.

If catalytic amplification occurs at the initial receptor-G protein step in the signaling pathway, we have to know the absolute rate constants for actual nucleotide exchange and GTP hydrolysis, and we don't for any system I know of. One can envision double-label, single-molecule approaches to the problem even though the technology is not now available. I predict that the question of molecular amplification in cells will depend on such optical techniques perfected to give us both kinetic and stoichiometric data, as well as molecular-scale location. Imaging technology is pushing toward this goal.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Tolkovsky AM, Braun S, Levitzki A. Kinetics of interaction between β-receptors, GTP protein, and the catalytic unit of turkey erythrocyte adenylate cyclase. Proc Natl Acad Sci U S A 1982; 79:213-7; PMID:6281756; http://dx.doi.org/10.1073/pnas.79.2.21
2. Tolkovsky AM, Levitzki A. Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. Biochemistry 1978; 17:3795-810; PMID:212105; http://dx.doi.org/10.1021/bi00611a02
3. Hanski E, Rimon G, Levitzki A. Adenylate cyclase activation by the β-adrenergic receptors as a diffusion-controlled process. Biochemistry 1979; 18:846-53; PMID:217426; http://dx.doi.org/10.1021/bi00572a01
4. Cassel D, Selinger Z. Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes. Biochim Biophys Acta 1976; 452:538-51; PMID:188466; http://dx.doi.org/10.1016/0005-2744(76)90206-
5. Cassel D, Selinger Z. Catecholamine-induced release of [^{3}H]-Gpp(NH)p from turkey erythrocyte adenylate cyclase. J Cyclic Nucleotide Res 1977; 3:11-22; PMID:84528
6. Cassel D, Selinger Z. Mechanism of adenylate cyclase activation through the β-adrenergic receptor: catecholamine-induced displacement of bound GDP by GTP. Proc Natl Acad Sci U S A 1978; 75:4155-9; PMID:212737; http://dx.doi.org/10.1073/pnas.75.9.415
7. Cassel D, Levkovitz H, Selinger Z. The regulatory GTPase cycle of turkey erythrocyte adenylate cyclase. J Cyclic Nucleotide Res 1977; 3:393-406; PMID:20361
8. Ross EM, Gilman AG. Biochemical properties of hormone-sensitive adenylate cyclase. Annu Rev Biochem 1980; 49:533-64; PMID:6105841; http://dx.doi.org/10.1146/annurev.bi.49.070180.00253
9. Braun S, Levitzki A. Adenosine receptor permanently coupled to turkey erythrocyte adenylate cyclase. Biochemistry 1979; 18:2134-8; PMID:435473; http://dx.doi.org/10.1021/bi00577a04
10. Pedersen SE, Ross EM. Functional reconstitution of β-adrenergic receptors and the stimulatory GTP-binding protein of adenylate cyclase. Proc Natl Acad Sci U S A 1982; 79:7228-32; PMID:6296825; http://dx.doi.org/10.1073/pnas.79.23.722
11. Asano T, Ross EM. Catecholamine-stimulated guanosine 5′-O-(3-thiotriphosphate) binding to the stimulatory GTP-binding protein of adenylate cyclase: kinetic analysis in reconstituted phospholipid vesicles. Biochemistry 1984; 23:5467-71; PMID:6095900; http://dx.doi.org/10.1021/bi00318a01
12. Cerione RA, Regan JW, Nakata H, Codina J, Benovic JL, Gierschik P, Somers RL, Spiegel AM, Birnbaumer L, Lefkowitz RJ, et al. Functional reconstitution of the α_2-adrenergic receptor with guanine nucleotide regulatory proteins in phospholipid vesicles. J Biol Chem 1986; 261:3901-9; PMID:300530
13. Senogles SE, Benovic JL, Amlaiky N, Unson C, Milligan G, Vinitsky R, Spiegel AM, Caron MG. The D_2-dopamine receptor of anterior pituitary is functionally associated with a pertussis toxin-sensitive guanine nucleotide binding protein. J Biol Chem 1987; 262:4860-7; PMID:310432
14. Cassel D, Eckstein F, Lowe M, Selinger Z. Determination of the turn-off reaction for the hormone-activated adenylate cyclase. J Biol Chem 1979; 254:9835-8; PMID:48957
15. Doupnik CA, Davidson N, Lester HA, Kofuji P. RGS proteins reconstitute the rapid gating kinetics of gbetagamma-activated inwardly rectifying K+ channels. Proc Natl Acad Sci U S A 1997; 94:10461-6; PMID:9294233; http://dx.doi.org/10.1073/pnas.94.19.1046
16. Berstein G, Blank JL, Jhon D-Y, Exton JH, Rhee SG, Ross EM. Phospholipase C-β 1 is a GTPase-activating protein for $G_{q/11}$, its physiologic regulator. Cell 1992; 70:411-8; PMID:1322796; http://dx.doi.org/10.1016/0092-8674(92)90165->17.
Casey PJ, Fong HKW, Simon MI, Gilman AG. G_z, a guanine nucleotide-binding protein with unique biochemical properties. J Biol Chem 1990; 265:2383-90; PMID:210532
18. Kozasa T, Jiang X, Hart MJ, Sternweis PM, Singer WD, Gilman AG, Bollag G, Sternweis PC. p115 RhoGEF is a GTPase activating protein for $G\alpha_{12}$ and $G\alpha_{13}$. Science 1998; 280:2109-11; PMID:9641915; http://dx.doi.org/10.1126/science.280.5372.210
19. Mukhopadhyay S, Ross EM. Rapid GTP binding and hydrolysis by $G(_q)$ promoted by receptor and GTPase-activating proteins. Proc Natl Acad Sci U S A 1999; 96:9539-44; PMID:10449728; http://dx.doi.org/10.1073/pnas.96.17.953
20. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 2000; 69:795-827; PMID:10966476; http://dx.doi.org/10.1146/annurev.biochem.69.1.79
21. Lohse MJ, Hoffmann C, Nikolaev VO, Vilardaga J-P, Bünemann M. (2007). Kinetic analysis of G protein-coupled receptor signaling using fluorescence resonance energy transfer in living cells. In Advances in Protein Chemistry: Mechanisms and Pathways of Heterotrimeric G Protein Signaling, Stephen RS, ed. (Academic Press), pp. 167-188
22. Lohse MJ, Nuber S, Hoffmann C. Fluorescence/bioluminescence resonance energy transfer techniques to study G-protein-coupled receptor activation and signaling. Pharmacol Rev 2012; 64:299-336; PMID:22407612; http://dx.doi.org/10.1124/pr.110.00430
23. Ross EM. Coordinating speed and amplitude in G-protein signaling. Curr Biol 2008; 18:R777-83; PMID:18786383; http://dx.doi.org/10.1016/j.cub.2008.07.03