Heterotrimeric G_q proteins as therapeutic targets?

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Heterotrimeric G proteins are the core upstream elements that transduce and amplify the cellular signals from G protein–coupled receptors (GPCRs) to intracellular effectors. GPCRs are the largest family of membrane proteins encoded in the human genome and are the targets of about one-third of prescription medicines. However, to date, no single therapeutic agent exerts its effects via perturbing heterotrimeric G protein function, despite a plethora of evidence linking G protein malfunction to human disease. Several recent studies have brought to light that the G_q family–specific inhibitor FR900359 (FR) is unexpectedly efficacious in silencing the signaling of G_q oncoproteins, mutant G_q variants that mostly exist in the active state. These data not only raise the hope that researchers working in drug discovery may be able to potentially strike G_q oncoproteins from the list of undruggable targets, but also raise questions as to how FR achieves its therapeutic effect. Here, we place emphasis on these recent studies and explain why they expand our pharmacological armamentarium for targeting G_q protein oncogenes as well as broaden our mechanistic understanding of G_q protein oncogene function. We also highlight how this novel insight impacts the significance and utility of using $G_{(q)}$ proteins as targets in drug discovery efforts.

GTP/GDP exchange and the intrinsic activity of GTP-binding proteins constitute widespread regulatory mechanisms in cells. These are utilized by heterotrimeric $\alpha\beta\gamma$ G proteins, downstream effectors of G protein–coupled receptors (GPCRs),[2] to directly or indirectly regulate numerous physiological processes in mammals (1–6). Despite the discovery of G proteins about 40 years ago and their relevance for maintaining homeostasis in response to a myriad of extracellular cues, remarkably little effort has been devoted to development of selective and cell-permeable pharmacological agents for inhibition of members of this protein family (7–15). This is in stark contrast to the plethora of modulators currently available for pharmacological control of GPCRs (16, 17) and likely relates to the fact that perturbation of receptor function rather than their shared signaling cascades is a more specific approach to interfere with pathologies. However, such specific approaches may fail, if pathology is complex and involves dysregulation of more than one receptor and/or its associated signaling circuitry, as is the case in certain diseases of the lung (18–23) as well as various forms of pain (24–27) and cancer (28–35). Therefore, development of G protein–targeting pharmacological agents that are active in intact cells, on the level of an isolated organ and ideally also in the living organism, would offer unique opportunities to explore the biological consequences that arise from more broad inhibition of signaling components.

G proteins are grouped into four major families (G_q, G_i, G_s, and G_{12}) based on α subunit homology and function (1–6). Missense mutations to codons within almost all of these (G_q, G_i, and G_s) result in diverse pathological conditions, yet all but G_q are lacking effective pharmacological inhibitors (*i.e.* remain untapped from a drug development perspective) (1–6). Note that members of the $G\alpha_{i/o}$ family except for $G\alpha_z$ are effectively hindered from signal transmission by pertussis toxin through ADP-ribosylation of a C-terminal cysteine residue (36–38). However, cell-permeable small-molecule inhibitors specifically targeting the $G\alpha_{i/o}$ branch have yet to be identified. Therefore, this review will focus primarily on the more recent discoveries obtained with the G_q family–specific inhibitors FR900359 (FR) and YM254890 (YM) (Fig. 1) and will highlight the conceptual advances originating therefrom for basic biological research and drug discovery. Specifically, we will single out a subset of G_q protein activities, namely aberrant signaling in cancer, to advance the ideas on drug–G protein interaction for therapeutic advantage. Because much of today's progress in this field traces back to a resurgence of interest in G_q protein inhibitors, a brief historical perspective will also be included.

G protein signaling

The delicate balance between on and off states

To maintain organismal homeostasis, mammalian cells require an exquisite balance between G protein activation and deactivation. They achieve this by tight control over GDP/GTP exchange and GTP hydrolysis rates. Ligand-activated GPCRs act as guanine nucleotide exchange factors (GEFs) to stimulate GDP/GTP exchange on the G protein α subunit (Fig. 2). Upon GTP binding, $G\alpha$ changes its conformation, and this is followed by separation of the heterotrimer (the extent of physical separation may vary however (39–45)) into $G\alpha_{GTP}$ and a $G\beta\gamma$ dimer, each of which interacts with downstream effectors (Fig. 2) (1–6). GTP hydrolysis by the inherent GTPase activity, which is often supported by GTPase-activating proteins (GAPs), then terminates G signaling and allows $G\alpha_{GDP}$ to associate with $G\beta\gamma$ to return the G protein to the inactive

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[2] The abbreviations used are: GPCR, G protein–coupled receptor; FR, FR900359; YM, YM254890; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; UM, uveal melanoma; GDI, guanine nucleotide dissociation inhibitor.

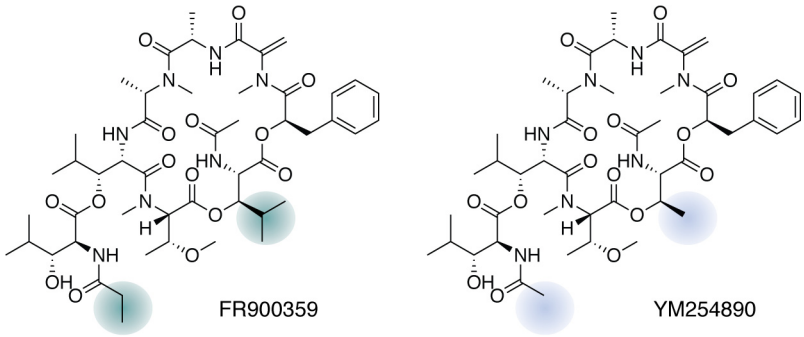

Figure 1. Chemical structures of G_q inhibitors FR and YM. *Colored areas* highlight the components of the amino acid building blocks that differ between FR and YM, accounting for the higher hydrophobicity of FR as well as for the distinct pharmacological features of the two inhibitors (123, 124).

state (Fig. 2) (1, 46–48). This activation-inactivation cycle suffices to explain why guanine nucleotide dissociation inhibitors (GDIs), such as FR and YM, are efficient terminators of G protein signaling; they block the rate-limiting step of the cycle, which is GDP release (Fig. 2) (11, 49). It also rationalizes why G protein activity may be elevated in cancer cells because (i) GPCRs and/or their activating ligands are present in excess, (ii) cancer cells may harbor constitutively active receptor variants, (iii) cancer cells may have activating mutations within the Gα protein itself (29–31, 35), or (iv) may be deficient in expression of GAPs as well as carry mutated versions of these effective terminators of G protein–dependent signaling (50–53). Unlike the conventional GPCR-targeted therapies that intervene with categories (i) and (ii), the therapeutic concept discussed in this review is also, and perhaps especially, effective for category (iii). GAPs, category (iv), are not within the scope of this review and interested readers may refer to several excellent reviews on this topic elsewhere (46, 47, 54–56).

When the balance is tipped toward the on state

It has been known for many years that activating point mutations in Gα proteins are important causative factors in several human cancers (31, 57). Of the four families of heterotrimeric G proteins, gain-of-function mutations were found in *GNAS* ($G\alpha_s$) (58–61), *GNAI2* ($G\alpha_{i2}$) (62), *GNAO1* ($G\alpha_{o1}$) (63, 64), and *GNAQ/GNA11* ($G\alpha_q$/$G\alpha_{11}$) (65–68) gene loci. Whereas *GNAS* and *GNAI* mutations occur in subsets of human endocrinopathies (57, 62, 69, 70), the first activating somatic *GNAO1* mutation was found in breast cancer (63). Within the *GNAQ* and *GNA11* genes, two particular codons are frequently mutated: arginine 183 and glutamine 209. Mutations at these two positions cause diminished GTPase function and so are linked to gain-of-signaling phenotypes (7, 9, 11, 31, 35, 71). Interestingly, both are also considered oncogenic driver mutations in ocular (uveal) melanoma (UM), an aggressive malignancy of the adult eye (72–76). Aside from mutationally activated Gα subunits, an additional recurrent hotspot mutation in UM was recently identified in the *CYSLTR2* gene, which codes for the G protein–coupled cysteinyl-leukotriene receptor type 2: $CysLTR2^{L129Q}$ (77). A hallmark feature of this mutant receptor is an overactive G_q signaling cascade coupled with impaired arrestin-mediated down-regulation, abolished responsiveness to its cognate endogenous ligands, and insensitivity to CysLTR2 antagonist/inverse agonist ligands (78). It follows that inhibitors of G_q function such as FR or YM should have therapeutic potential to suppress the aberrant activity of this signaling module originating on either the receptor or the G protein level. In other words, targeting a convergence point in signal transduction with a single agent might bring therapeutic benefit irrespective of the precise nature of the upstream activating oncoprotein.

Pharmacological inhibitors of G protein function: Focus on FR900359

Discovery of a cyclic depsipeptide with the code name FR900359 from a methanol extract of the evergreen plant *Ardisia crenata* dates back to 1988 (Fig. 3) (79). Along with the elucidation of its chemical structure, a preliminary description of biological effects was provided: FR inhibits platelet aggregation, decreases blood pressure, and is cytotoxic to cultured rat fibroblasts and myelocytic leukemia cells (data not shown in Ref. 79). Whereas all of the observed biological effects may be explained entirely by specific inhibition of G_q family proteins, it was not until 2010 that FR was rediscovered as "compound 362-63-08" in a plant extract library screen searching for inhibitors of the gut hormone cholecystokinin type 1 (CCK1, formerly CCK-A) receptor (Fig. 3) (10). The structural similarity of compound 362-63-08 with YM together with its *in vitro* selectivity profile led the authors to conclude that the screening hit 362-63-08 does not target the receptor itself but rather hinders CCK1 receptor signaling by specific inhibition of its signal transducing $G_{q/11}$ proteins (10). Selective inhibition by FR of G_q, G_{11}, and G_{14} over all other mammalian G proteins, its molecular mechanism of GDI action, and the potential to probe the G_q contribution to complex biological processes in physiology and disease were not addressed until 2015, when a comprehensive study provided *in vitro* and *ex vivo* characterization at a level of detail sufficient to reinvigorate the field of G_q protein inhibitors (11) (Fig. 3). Indeed, this very study impacted G protein inhibitor research in manifold beneficial ways: it (i) created scientific community awareness for the existence of a most valuable signal transduction inhibitor, (ii) triggered independent confirmatory studies to re-examine FR's selectivity profile (80–82), (iii) helped fuel the competitive efforts to identify the best-suited synthetic methodology for preparing the complex molecule by chemical synthesis (83–86), (iv) sparked broad interest for the application of FR and YM to explore the biological consequences that arise from specific G_q inhibition (7, 87–107), and (v) provided experimental evidence that G_q inhibition may

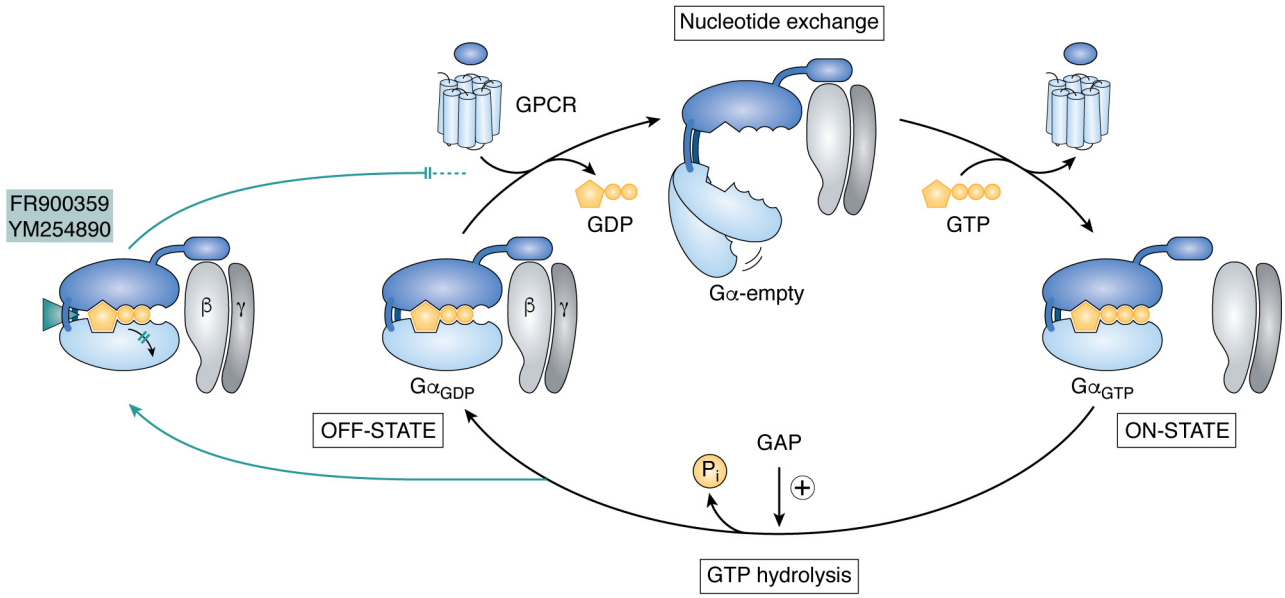

Figure 2. Schematic of the guanine nucleotide cycle and Gα signaling states. Heterotrimeric G protein signaling commences when ligand-activated GPCRs act as GEFs, causing the release of bound GDP and its replacement by GTP via a short-lived intermediate "empty pocket" state. Exchange of the bound nucleotide results in ternary complex disassembly, separation of Gα from Gβγ, and initiation of downstream signaling. Intrinsic GTP hydrolysis, which is accelerated by GAPs, then resets $G\alpha_{GDP}$ to form the inactive heterotrimer. FR and YM block G protein signaling by preventing GDP release. They freeze the heterotrimer in an inactive conformation by intercalating between the interdomain cleft at a site distinct from the nucleotide-binding pocket, thereby preventing domain separation (11, 49).

qualify as an effective postreceptor strategy to target oncogenic signaling in cancer cells with elevated G_q activity.

FR suppresses oncogenic signaling in melanoma cells with elevated G_q activity

The first signs for FR efficacy in cancer treatment were obtained when exposing a panel of skin melanoma cells to FR in cell culture (11). Interestingly, despite an intrinsically activated G_q cascade in a number of these lines, and despite potent suppression by FR of G_q-mediated inositol phosphate accumulation across all of these, proliferation, cell cycle progression, and mitogenic signaling were abolished in all but MZ7 cells. MZ7 cells harbor the constitutively active $G\alpha_q^{R183C}$ variant, considered susceptible to FR treatment (9, 83). These data provided the first hint that aberrant G_q activity *per se* does not suffice to instruct MZ7 cancer cells to proliferate. Apparently, an overactive G_q system is required but not sufficient to define the molecular subtype of melanoma that responds to FR treatment or else to forecast therapeutic efficacy of G_q-inhibiting agents. Given the rich mutational landscape of skin melanoma and the high frequency of mutations in the *BRAF*, *NRAS*, *CDK4*, *PTK2B*, and *ERBB4* genes (108, 109), along with the notion that MZ7 cells also harbor the constitutively active $BRAF^{V600E}$ allele, the findings argue that $BRAF^{V600E}$ but not $G\alpha11^{R183C}$ must act as the dominant oncogenic driver and that the occurrence of R183C may merely be a consequence of the general mutational burden in this melanoma cell line. Indeed, mitogenic signaling in MZ7 cells is completely blunted by the BRAF inhibitors vemurafenib and trametinib (11). Regardless, G_q inhibition with FR provided the proof of principle for a novel route to reprogram a range of skin melanoma cells—those that are instructed by G_q to proliferate—to a less aggressive phenotype (11). Because mutant $G\alpha_q$ or $G\alpha_{11}$ proteins are found in only 4% of skin melanoma but in 90% of uveal melanoma, it was not surprising to observe researchers turn to the study of FR in cell lines from uveal melanoma tumors: four independent studies on similar subject matter emerged within just a 6-month time frame (97, 100, 110, 111).

FR inhibition of uveal melanoma Gα oncoproteins: A mechanistic surprise?

Uveal melanoma is the most common cancer of the adult eye, originating from melanocytes in the choroid, iris, or ciliary body (72–76). The genetic signature and evolution of this particularly lethal form of melanoma is distinct from skin melanoma in that mutations within a G_q signaling module comprising the gene loci for *GNAQ*, *GNA11*, their downstream effector *PLCB4*, or the upstream activating *CYSLTR2* occur in a mutually exclusive fashion (65–67, 77, 112). Particularly predominant are gain-of-function mutations within the two highly homologous G protein α subunits, $G\alpha_q$ and $G\alpha_{11}$, at the recurrent hotspots Gln-209 and Arg-183 (65–67), with mutations at Gln-209 being 13 times more frequent than those at Arg-183 (67). Both mutation hotspots are located in the GTPase domain (Fig. 4*A*) and are catalytically important for the GTPase turn-off reaction by stabilizing the transition state for GTP hydrolysis. Gln-209 of $G\alpha_q$ and $G\alpha_{11}$ is analogous to Gln-204 within $G\alpha_i$, Gln-227 within $G\alpha_s$, and Gln-61 within the small GTPase Ras, the latter mutated in multiple human cancers (61, 113). If altered by mutation, $G\alpha_{q/11}$ deactivation is disturbed, driving inappropriate proliferative signaling, yet different in extent for each of the two hotspots: the Gln-209 mutations ($G\alpha_q^{Q209L/P}$ or $G\alpha_{11}^{Q209L/P}$) cripple the GTPase activity to create persistently active Gα subunits (as inferred from pioneering X-ray crystallographic studies with $G\alpha_i$ (114, 115) and recent biochemical investigations (71), whereas Arg-183 mutants ($G\alpha q^{R183C}$ or

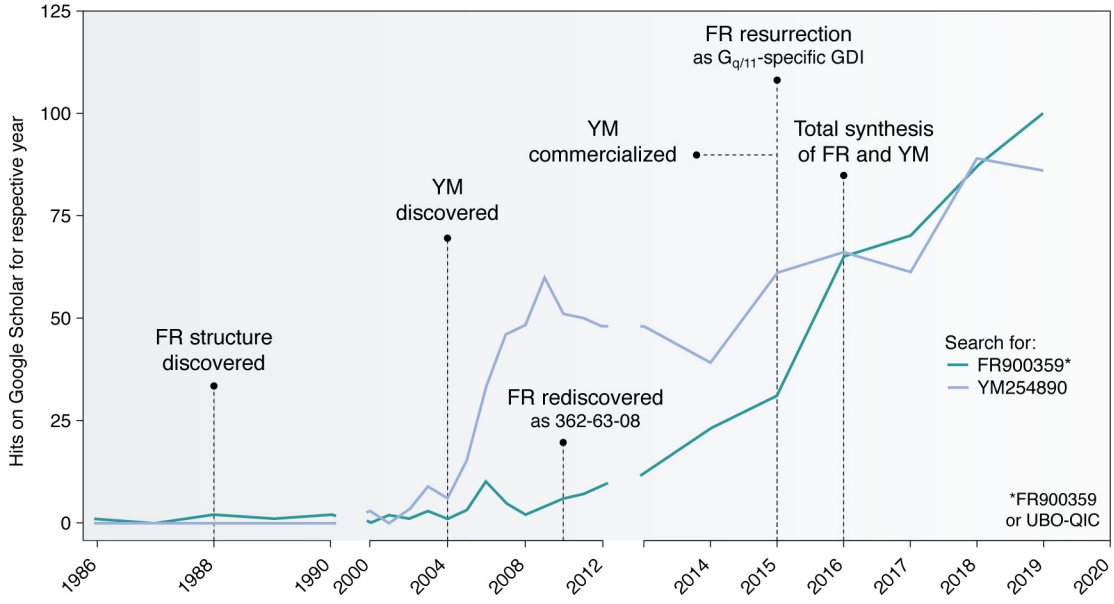

Figure 3. Google scholar hits for G_q inhibitors FR and YM. 1988: Isolation and structure elucidation of FR; biology and mechanism of action unknown (79). **2004:** Discovery of the structurally close analog YM (9) by Yamanouchi Pharmaceutical Co., later combined in a merger with Fujisawa to form Astellas Pharma, which chose to provide YM to the scientific community in a rather restrictive manner. Until commercialization (see below), YM was available for a small number of researchers only. **2010:** Rediscovery of FR, code-named "362-63-08," from a plant extract library as inhibitor of the G_q-coupled cholecystokinin CCK1 receptor (10). **2015:** Resurrection of FR by in-depth characterization of its *in vitro* specificity and mechanism of action by a concerted effort of members of the signal transduction community (11). **2016:** Commercialization of YM by Fujifilm Wako Chemicals, as well as total synthesis of YM and FR (83). Coincidentally, worldwide awareness of and interest in FR and YM has risen steeply. During a short period of time, FR was commercialized under the code name "UBO-QIC" (University of Bonn–G_q-inhibiting component), which indicated market potential and, in turn, encouraged commercialization of the competing molecule YM.

$G\alpha 11^{R183C}$) retain the capacity to hydrolyze GTP, albeit at a reduced catalytic rate (Fig. 4*A*) (116). Thus, both mutants differ in their oncogenic properties because R183C prefers GTP over GDP yet still responds to receptor stimulation, whereas Q209L/P is largely, if not entirely, uncoupled from activation by upstream acting GPCRs (9, 110, 116–118) (Fig. 4*A*). This mechanistic difference explains why $G\alpha_{q/11}{}^{Q209L/P}$ but not $G\alpha_{q/11}{}^{R183C}$ mutants were long considered unresponsive to inhibitors of receptor-mediated nucleotide exchange (so-called GDIs). FR and YM are precisely such GDIs, viewed as unsuited for manipulating the oncogenic signaling driven by GTPase-deficient $G\alpha_q$ proteins for experimental or therapeutic purposes. However, FR in particular has shown convincing efficacy against UM cancer cells as brought to focus by four independent studies (97, 100, 110, 111). How come?

Experimental efficacy of FR in UM cancer cells: Solving an apparent paradox

G protein signaling requires both activation and deactivation. In normal cells, deactivation is an intrinsic property of the $G\alpha$ subunit and is not rate-limiting (Fig. 4*B*). Mammalian $G\alpha$ proteins typically deactivate by hydrolyzing GTP to GDP at catalytic rates k_{cat} between 0.01 and 3.5 min^{-1} (116). Because GTP hydrolysis is faster than GDP release, the steady-state pool of activated $G\alpha$ subunits is tightly linked to the amount of agonist-occupied GPCRs (Fig. 4*B*). In this way, G protein signaling is largely controlled by and dependent on catalytic input from the upstream acting receptors. However, in $G\alpha_{q/11}{}^{Q209L/P}$ mutant cells, the inherent hydrolysis rate is far too slow to reset GDP-$G\alpha$ (Fig. 4*B*). It follows that the nucleotide state of $G\alpha_{q/11}{}^{Q209L/P}$ becomes more dependent on nucleotide affinity and concentration. Because GTP is in molar excess over GDP in living cells (119) and because GTP dissociates an order of magnitude slower than GDP (120), GTPase-deficient mutants predominantly exist in the GTP-bound state (Fig. 5). However, inhibitors of nucleotide dissociation may shift the nucleotide preference to enrich the fraction of inactive $G\alpha_{qGDP}$-$\beta\gamma$ heterotrimers over time (Fig. 5). Their onset of action will depend on the rate of nucleotide exchange and/or the rate of GTP hydrolysis in a given cellular environment. Let us pause for a moment to reiterate this point: For a GTPase-deficient G_q to become GDP-bound at a relatively fast pace, it must either exchange nucleotides in cells at rates much faster than those believed to occur in *in vitro experiments* and/or hydrolyze GTP better than predicted from *in vitro* studies. It may therefore be advisable to revisit the molecular details underlying these quintessential processes of nucleotide exchange and GTP hydrolysis in the living cell context. This does not only appear timely but may also be technically feasible, given the availability of CRISPR-Cas9 genome-edited cells depleted of multiple G protein α subunits (103). So far, only FR (and not YM) has shown efficacy in the UM context. It is conceivable that this efficacy is in keeping with the kinetic parameters recently determined for direct interaction between tritiated FR and G_q; unlike YM, FR dissociates from G_q with a remarkably slow off rate ($t_{½diss(FR)}$ ~92 min *versus* $t_{½diss}$(YM) ~4 min (124)), suggesting interaction in a pseudo-irreversible manner. Long G_q residence times may therefore be decisively advantageous for duration of action as well as experimental and therapeutic efficacy of G_q inhibitors in UM. Regardless of the kinetic differences, inhibitors of guanine nucleotide dissociation diminish the signaling of GTP-bound

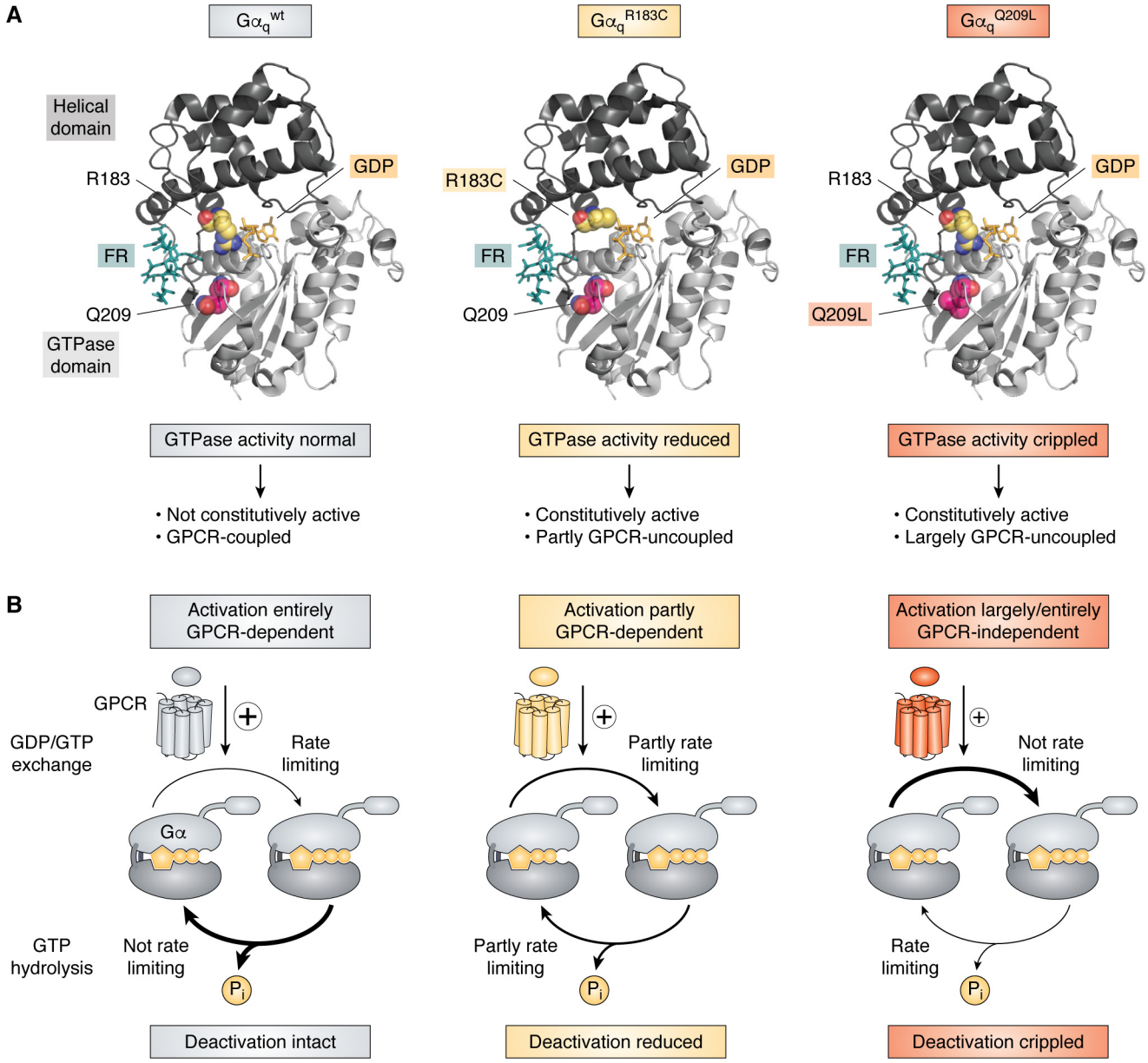

Figure 4. Tertiary structure and signaling phenotypes of WT $G\alpha_q$ and GTPase-inactivating mutations R183C and Q209L. *A*, *ribbon drawings* of WT and mutant $G\alpha_q$ subunits bound to GDP and FR and based on the atomic coordinates of the $G\alpha_{i/q}$-YM-inhibitor complex crystal structure (Protein Data Bank entry 3AH8 (49)). The GTPase-inactivating mutational hotspots R183C and Q209L are shown as *space-filling models*, and G_q inhibitor FR is illustrated as a *stick model* located in the interdomain cleft between the GTPase and the helical domain. *B*, schematic showing intrinsic properties of WT and mutationally activated $G\alpha_q$ oncoproteins. *Curved arrows* indicate rates of nucleotide exchange (*top*) or GTP hydrolysis (*bottom*), with *thin arrows* depicting rate-limiting reactions and *thick arrows* representing non-rate-limiting reactions: for $G\alpha_q^{wt}$, for example, nucleotide exchange is rate-limiting, but GTP hydrolysis is not, placing $G\alpha_q^{wt}$ under upstream control of a GEF, the GPCR.

$G\alpha$ in an indirect manner, clearly illustrating their dual value to blunt signaling not only of WT GTPases but also of mutationally activated GTPase-deficient oncogenes.

Heterotrimeric $G\alpha$ subunits as drug targets?

Inhibition of $G\alpha_{GTP}$ and, thereby, its downstream signaling repertoire, may be relevant to treat pathologies that are driven by overactive G proteins as is the case in various types of human cancers (31, 35, 57). Provided that targeting of heterotrimeric G proteins in a subfamily- or even isoform-specific manner will be expanded beyond the $G_{q/11}$ branch, the issue of ubiquitous $G\alpha$ expression will still remain a perceived safety concern for potential medications. One possibility to overcome systemic toxicity is local drug application. For FR treatment of ocular melanoma, this may be achieved by local delivery directly into the eye just as established for a number of clinically used intravitreal therapeutics. Topical application, for the avoidance of systemic adverse effects, has already proven successful for FR inhibition of G_q-GPCR signaling in the airways using various *in vivo* models for acute and chronic lung diseases (94). Whereas the pulmonary administration route of an FR aerosol effectively suppressed G_q signaling, as evidenced by remarkable bronchodilation, systemic side effects that would directly result from G_q inhibition, such as blood pressure or heart rate alterations, were

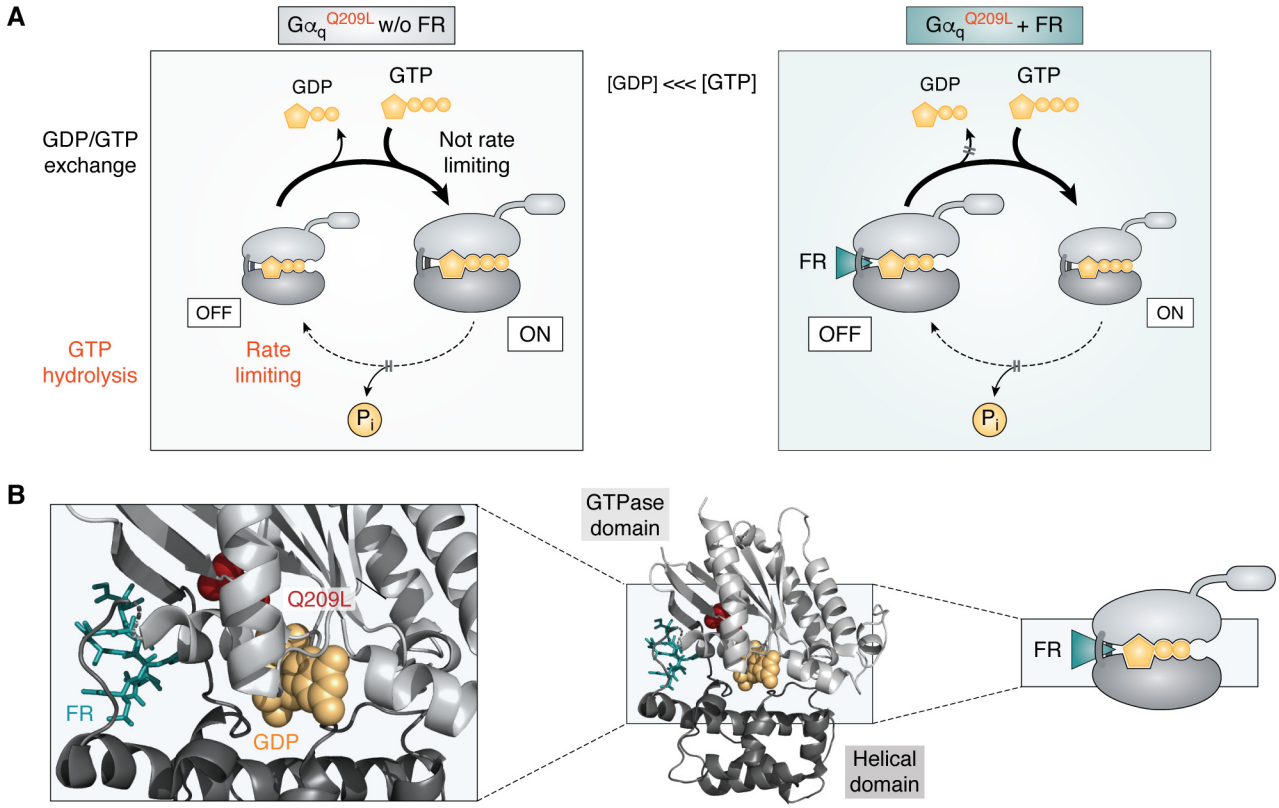

Figure 5. Schematic for FR inhibition of oncogenic $G\alpha_{q/11}$ GTPases. *A*, oncogenic Gln-209 mutations result in functional activation of $G\alpha_{q/11}$ family proteins by impairing GTP hydrolysis. With diminished regulation by GTPase activity (GTP hydrolysis is rate-limiting), the nucleotide state of mutant G_q becomes more dependent on nucleotide affinity and concentration. Because GTP is in higher abundance than GDP in cells, $G\alpha_{GTP}$ freed from its $G\beta\gamma$ binding partner is the major nucleotide-bound form of GTPase-deficient Gln-209 mutants. Inhibitors of nucleotide dissociation, such as FR, shift the equilibrium toward $G\alpha_{GDP}$-$\beta\gamma$ heterotrimers over time, thereby enriching the fraction of G proteins in a signaling-incompetent state. Subversion of the nucleotide preference of Gln-209 mutants to favor GDP over GTP is an allosteric mechanism whereby FR gains control over aberrant signaling of oncogenic GTPase-deficient $G\alpha_{q/11}$ proteins. *B*, schematic, overall structural fold and detailed view of the heterotrimeric G protein $G\alpha_q$ subunit (Protein Data Bank entry 3AH8) in its inactive, GDP-bound form; Q209L is visualized with a *space-filling model*. FR does not directly interact with Gln-209 but allosterically stabilizes the GDP-bound fraction of the oncoprotein, a conformation that cannot be maintained when $G\alpha_q$ is GTP-bound (49).

not detected (94). Long-term toxicity studies will be required to assess whether FR accumulates in certain cells, tissues, or organs to judge its potential to be administered to humans.

In the current absence of precision pharmacological targeting for mutationally activated Gα proteins, one can only speculate about possible advantages of targeted $G\alpha_{GTP}$ therapeutics. Such a strategy does spring to mind as an attempt to preferentially diminish the aberrant Gα activity in cancer cells only, akin to therapies targeting mutationally activated $BRAF^{V600E}$ in metastatic melanoma. Yet, mutation-specific inhibitors for active Gα have not been reported to date, and, moreover, $G\alpha_{GTP}$ antagonizing agents will likely also block the signaling of WT GTPases in that only a low dosage might afford a therapeutic window for targeted (preferential) inhibition of the oncogenic over the WT $G\alpha_{GTP}$ pool. In light of these considerations and the current absence of X-ray structural information on GTPase-deficient $G\alpha_q$, the recent successes to target mutationally activated G_q with FR in uveal melanoma must be viewed as a considerable breakthrough (97, 110, 111). Guanine nucleotide dissociation inhibitors of heterotrimeric G proteins such as FR may therefore evolve to be cornerstones of "anti-$G\alpha_{GTP}$ therapies," given their proven capacity to shift the nucleotide preference of Gα proteins toward the GDP-bound inactive state (Fig. 5). If combined with tissue- or cell-specific targeting, such as antibody-drug conjugates, systemic side effects may be kept at a minimum or even be spared. In such a scenario, concomitant inhibition of both mutationally activated and WT Gα may even be of advantage to harm the aberrant cells.

Conclusions and outlook

It has been known for decades that GTPase-inactivating point mutations in Gα proteins are important causative factors in many human cancers. However, there have been few attempts to establish approaches for inhibition of Gα oncoproteins (97, 100, 110, 111). One possible daunting challenge may have been that G protein–targeted pharmacological agents must enter the cell to exert their desired biological effect. However, molecules like FR or YM are beginning to bring this goal within reach. As far as pharmacological strategies are concerned, direct competition with GTP binding, in analogy to kinase inhibitors that compete with ATP binding, has not been seriously considered. This is because of the extremely high affinities of GTP and GDP for their nucleotide-binding pockets along with their micromolar abundance in cells, meaning that nucleotide binding to the catalytic site is very hard to overcome by any competitive inhibitor (121). What other strategies do come to mind to hinder constitutively active Gα proteins from

aberrant signaling? Pharmacological reactivation of deficient Gα-GTPase activity may be a way to go (122), but conceivably very hard to implement. Thus, in the current absence of pharmacological agents to directly antagonize persistently active Gα, targeting nucleotide exchange, for long viewed ineffective for this purpose, appears particularly straightforward. In this respect, the re-emergence of FR, a highly specific $G\alpha_q$-directed inhibitor of GDP/GTP exchange and cellular signaling, has not only revitalized the idea of targeting G protein oncogenes but also provided proof of principle *in vitro* (97, 100, 110, 111) and *in vivo* (110) that this is indeed experimentally feasible.

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