Ernst Schering Foundation Symposium Proceedings, Vol. 2, pp. 1–21
DOI 10.1007/2789_2006_001

Published Online: 16 May 2007

G-Proteins and GPCRs: From the Beginning

H.R. Bourne(✉)

UCSF, 600 16th Street, 94143-2140 San Francisco, USA
email: *bourne@cmp.ucsf.edu*

1 Prologue: GTP Enters the Picture 2
2 The Stimulatory Regulator of Adenylyl Cyclase 4
3 Rhodopsin and Transducin 6
4 Confusion, Error, Truth: Discovering the β-AR 9
5 DNA: Revolution and Revelation 12
References 16

Abstract. From the point of view of a participant observer, I tell the discovery stories of trimeric G-proteins and GPCRs, beginning in the 1970s. As in most such stories, formidable obstacles, confusion, and mistakes make eventual triumphs even more exciting. Because these pivotally important signaling molecules were discovered before the recombinant DNA revolution, today's well-trained molecular biologist may find it amazing that we learned anything at all.

Born three decades ago and now grown to robust maturity, trimeric G-proteins and G-protein-coupled receptors (GPCRs) continue to generate exciting advances in biology and drug discovery. Here I recount the story of their births, from the point of view of a participant observer. As in most discovery stories, formidable obstacles, confusion, and mistakes make eventual triumphs even more satisfying.

Two unrelated events—Sutherland's discovery of cAMP in the 1950s and the Vietnam war of the 1960s—brought me into the story. To avoid military service, I spent two years at the National Institutes of Health (NIH), in Bethesda, Maryland, where I learned to measure cAMP synthesis in fat cell extracts. In 1969 I moved to the University of California San Francisco (UCSF) as a research fellow, and began to study cAMP in human leukocytes, a choice that reflected widespread interest in cAMP as a second messenger, plus the fact that no one else west of the Mississippi river knew the adenylyl cyclase assay. Neither I nor my colleagues could have foreseen the delights cAMP would eventually bring.

Indeed, signaling research in the 1960s and 1970s would be almost unrecognizable to scientists trained after the recombinant DNA revolution of the 1980s. The cutting edge was hard-core biochemistry, but many experiments focused on bio-assays using animal tissues or enzyme assays in extracts. In multiple laboratories from 1964 to 1972, I never heard the words "genetics", "DNA", or "evolution" mentioned, much less used in planning an actual experiment. Today's molecular biologists will find it astonishing that we learned anything at all.

I shall tell the birth stories of G-proteins and GPCRs in more or less chronological order, emphasizing what investigators thought and imagined at the time and explicitly labeling explanations based on hindsight. A caveat is in order: more memoir than scholarly treatise, these stories necessarily reflect a personal point of view, replete with limitations of observer bias, faulty memory, and ignorant omission. Nonetheless, the message is as true as I can make it, even if some details are wrong.

1 Prologue: GTP Enters the Picture

In the early 1970s, Martin Rodbell's laboratory at the NIH was assaying adenylyl cyclase and binding of radioactive glucagon in liver membranes. Lutz Birnbaumer, who was responsible for many of the experiments, tells me (L. Birnbaumer, personal communication) that they were pleased when the EC50 for glucagon's activation of adenyl cyclase appeared identical to its Kd for binding to membrane sites. But Lutz reminded his colleagues that the cyclase assay contained Mg^{2+} and ATP, while the binding assay did not. Repeating the binding as-

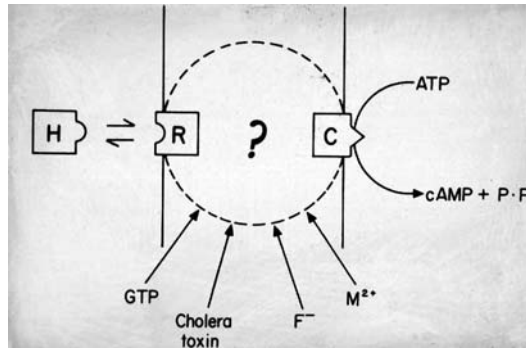

Fig. 1. The receptor and adenylyl cyclase in the early 1970s. The diagram is taken from a slide presented in seminars by the author in 1973–1975

says in the presence of Mg^{2+} and ATP produced a disconcerting result: the glucagon binding curve shifted to the right, with a higher Kd. Astutely, they tested other nucleotides: GTP shifted the binding curve more potently than ATP (Rodbell et al. 1971b). (The "pure" ATP they used turned out later to be contaminated by GTP.) A chemically pure synthetic ATP analog did not shift the binding curve, but did serve as an effective substrate for glucagon-stimulated cAMP synthesis, but only if GTP was added to the assay (Rodbell et al. 1971a).

These observations triggered fanciful speculations, but investigators were slow to realize that the evidence might point to a GTP-binding protein distinct from both receptor and adenylyl cyclase. Now we know that GTP reduced the receptor's affinity for glucagon by preventing the trimeric G-protein, Gs, from enhancing the GPCR's affinity for agonist: agonist affinity was reduced because GTP binding to Gs caused it to dissociate from the GPCR (De Lean et al. 1980; Ross and Gilman, 1980). At the time, however, many were not even convinced, despite accumulating evidence, that receptors and adenylyl cyclase were separate molecules (see Fig. 1). In 1975, Al Gilman's laboratory summarized their failed attempts to purify adenylyl cyclase in the title of a paper: "Frustration and adenylate cyclase" (Maguire et al. 1975).

Key insights into the mysterious relation between GTP and adenylyl cyclase came from Zvi Selinger's laboratory (Cassel et al. 1977;

Cassel and Selinger, 1976). He and his colleagues were intrigued by a report from the Rodbell laboratory (Londos et al. 1974) showing that a hydrolysis-resistant GTP analog, Gpp(NH)p, activated adenylyl cyclase on its own, and to an extent greater than GTP; moreover, Gpp(NH)p cooperated with hormones to further stimulate cAMP synthesis. If resistance to hydrolysis made GTP more effective, they reasoned that hormones might regulate GTPase activity. Soon the Selinger lab found that a GTPase activity in turkey erythrocyte membranes was stimulated by isoproterenol, and that this stimulation was blocked by propranolol. They proposed that cAMP synthesis depended on agonist-stimulated binding of GTP to a component of the adenylyl cyclase complex, that GTP hydrolysis terminated stimulation, and that continued cAMP synthesis required repeated agonist-stimulated cycles of GTP binding and hydrolysis. Their proposals were not greeted with enthusiasm. The *Journal of Biological Chemistry* rejected the first Selinger paper, which was deemed "prejudice not science", because "if anything, the hormone should inhibit GTP hydrolysis" (Z. Selinger, personal communication). In 1976, a respected senior investigator—perhaps a reviewer of the Selinger paper—admonished me to "be very cautious about accepting such a strange interpretation".

2 The Stimulatory Regulator of Adenylyl Cyclase

In 1972, I struck up a commute bus conversation with Gordon Tomkins, a UCSF faculty member. Gordon told me that somatic genetics—an entire field that was news to me—could furnish valuable clues to understanding hormone action. A postdoc in his laboratory had found that S49 mouse lymphoma cells die when exposed to a cAMP analog, and was beginning to isolate cAMP-resistant S49 variants (Daniel et al. 1973). cAMP resistance, Gordon suspected, resulted from mutation of a gene encoding a key protein in the cAMP response pathway. I jumped at the chance to join the project.

Soon I found myself working with Phil Coffino, an immensely talented postdoc in Gordon's lab. We isolated cAMP-resistant clones carrying mutations that inactivated protein kinase A (Bourne et al. 1975b; Coffino et al. 1975; Insel et al. 1975). Then we looked for an S49

clone lacking the β-adrenoceptor (β-AR). We imagined that such cells would die in the presence of cAMP analogs but resist killing by isoproterenol. To our surprise, clones that met these criteria also failed to die, or even to accumulate cAMP, in response to two additional stimulators of adenylyl cyclase, prostaglandin-E_1 and cholera toxin. We called these cells *cyc*$^-$, to indicate a deficiency of adenylyl cyclase (Bourne et al. 1975a), unaware that somatic genetics was hinting at existence of a protein we could not then imagine.

Later in 1974, Gordon received a postdoctoral application from a Cornell graduate student, Elliott Ross. Elliott's letter proposed to reconstitute hormone-sensitive adenylyl cyclase in *cyc*$^-$ membranes, using wild type S49 membranes as a source for purifying the component missing in *cyc*$^-$. Gordon promptly invited Elliott to join his lab, but it was not to be: a few months later, Gordon died after a brain operation, and Elliott joined Al Gilman's laboratory instead. We had sent *cyc*$^-$ cells to the Gilman laboratory as part of a separate collaboration, resulting in a paper (Insel et al. 1976) whose title revealed the meager state of our knowledge: "β-adrenergic receptors and adenylate cyclase: Products of separate genes?" (We got the right answer, all the while ignoring the fact that *cyc*$^-$ cells are not deficient in adenylyl cyclase.)

Much more important, with *cyc*$^-$ cells in hand Elliott could begin to tackle reconstitution of isoproterenol-stimulated adenylyl cyclase. It was not easy. Elliott and Al plowed through myriad detergent extractions and reconstitution strategies before they showed that *cyc*$^-$ can be persuaded—by addition of a membrane extract from wild type cells—to synthesize cAMP in response to isoproterenol (Ross and Gilman 1977a). Then came the critical observations: *cyc*$^-$ membranes do not lack adenylyl cyclase, and wild type extracts supplied to the reconstituted mixture an activity that was neither adenylyl cyclase nor the β-AR, both of which were already present in *cyc*$^-$; instead, the wild type extract supplied a new entity, whose thermal stability was increased by a GTP analog (Ross and Gilman 1977b).

By 1980 painstaking efforts in the Gilman laboratory had purified this entity, showing that the *cyc*$^-$ mutation inactivates a protein they named Gs, the stimulatory regulator of adenylyl cyclase (Ross and Gilman 1980). Discovery of the αβγ structure of Gs led rapidly to new insights, including the pathogenesis of three diseases. The ability of

whooping cough (pertussis) toxin to inhibit GTP-dependent hormonal inhibition of adenylyl cyclase and catalyze covalent modification of a Gα protein distinct from αs (Murayama and Ui 1983) led to discovery and purification of a second putative trimeric G-protein, which we now call Gi (reviewed in Gilman 1987). (Now we know that this effect of pertussis toxin inhibits Gi activation, thereby causing the bronchial irritability of whooping cough.) Gαs, the target of the *cyc*⁻ mutation, turned out to be the target of two diseases. Cholera is caused by a toxin that elevates cAMP in gut cells by covalently modifying αs, thereby turning off its GTPase activity and stabilizing it in its active form (Cassel and Pfeuffer 1978; Cassel and Selinger 1977; Johnson et al. 1978). Mutational inactivation of one αs allele causes the second disorder, pseudohypoparathyroidism, in which patients respond poorly to hormones that activate Gs-coupled receptors (Farfel et al. 1980).

3 Rhodopsin and Transducin

The extraordinary abundance of rhodopsin and transducin in retinal rod cells facilitated their initial discovery, and eventually made them the best-understood receptor-G-protein pair, at the levels of 3D structure, biochemical properties, and downstream signals. Rhodopsin was identified as a photosensitive pigment in the 1870s (reviewed in Hsia 1965), and in 1933 George Wald discovered retinal, rhodopsin's covalently bound ligand, and began to trace its light-induced chemical transformations (reviewed in Wald 1968). While the Gs and transducin stories evolved during the same time frame (Table 1), many aficionados of adenylyl cyclase and photoreception were barely aware of each other's findings until about 1980.

The transducin story began with three key findings: cGMP phosphodiesterase (PDE) was shown to be the light-activated effector (Bitensky et al. 1975); light increased the phosphodiesterase activity only in the presence of GTP and photoactivated rhodopsin (Yee and Liebman 1978); and light activated a GTPase activity in rod cell extracts (Wheeler and Bitensky 1977). Then Godchaux and Zimmerman (1979) purified from rod cell extracts a soluble guanine nucleotide binding protein that exhibited light-dependent stimulation of GTP-GDP exchange

Table 1 Trimeric G proteins and GPCRs: Key steps in discovery

	Adenylyl cyclase regulation	Phototransduction
Receptor identified	β-AR inferred from selectivity of agonists and antagonists (Ahlquist 1948)	1870s (reviewed in Hsia 1965)
Ligand identified	Epinephrine activates liver phosphorylase (Rall et al. 1957)	Retinal found by George Wald in 1933 (reviewed in Wald 1968)
Effector identified	Adenylyl cyclase (Sutherland and Rall 1960)	cGMP-PDE (Bitensky et al. 1975)
GTP	Regulates agonist binding and adenylyl cyclase activity (Rodbell et al. 1971a; Rodbell et al. 1971b)	Required for hν-stimulation of cGMP-PDE (Yee and Liebman 1978)
GTP hydrolysis	Stimulated by isoproterenol in turkey erythrocyte membranes (Cassel and Selinger 1976)	Activated by hν (Wheeler and Bitensky 1977)
Trimeric G protein purified	Gs α and β subunits (Northup et al. 1980)	Gt α and β (Godchaux and Zimmerman 1979); α, β, and γ (Fung et al. 1981)
Ligand binding	β-AR binds radioactive antagonists (Aurbach et al. 1974; Lefkowitz et al. 1974; Levitzki et al. 1974)	Retinal covalently attached to a lysine in rhodopsin (Collins, Morton, and Pitt 1950s; reviewed in Wald 1968)
Receptor phosphorylation	Desensitizes frog β-AR (Stadel et al. 1983)	hν-dependent (Kuhn and Dreyer 1972); role in adaptation (Kuhn et al. 1977)
Arrestin damps agonist signal	Retinal arrestin inhibits β-AR signaling (Benovic et al. 1987)	Retinal arrestin quenches hν-dependent cGMP-PDE activation (Wilden et al. 1986)
Receptor kinase	βARK (Benovic et al. 1986)	Rhodopsin kinase (Kuhn 1978; Shichi and Somers 1978)

Table 1 (continued)

	Adenylyl cyclase regulation	Phototransduction
Receptor purified	β-AR, by affinity chromatography (Benovic et al. 1984; Caron et al. 1979; Shorr et al. 1981)	1960s, many labs (reviewed in Hsia 1965)
Gα cDNA	αs (Harris et al. 1985)	αt (Lochrie et al. 1985; Medynski et al. 1985; Tanabe et al. 1985)
GPCR primary structure	β_2-AR cDNA (Dixon et al. 1986; Yarden et al. 1986)	Rhodopsin amino acid sequence (Ovchinnikov et al. 1982); cDNA (Nathans and Hogness 1983)

in the presence of membranes. They identified two polypeptide components of the soluble protein, which we now know as the α and β subunits of transducin, but did not mention adenylyl cyclase or GTP's role in its hormonal activation.

By 1981, reports from Lubert Stryer's laboratory made it impossible to ignore striking parallels between retinal phototransduction and hormone-stimulated cAMP synthesis. Lubert and his colleagues showed that the photon signal is enormously amplified: a single photon, activating a single rhodopsin, triggers binding of a hydrolysis-resistant GTP analog to 500 GTP-binding sites in rod cell extracts (Fung and Stryer, 1980). They then purified the GTP-binding protein, identified its α, β, and γ polypeptides, named it transducin (hereafter, Gt), and used it and rhodopsin to reconstitute light-stimulated binding and hydrolysis of GTP (Fung et al. 1981).

At this point, the two previously unrelated fields of investigation began to coalesce, each providing knowledge and insights to the other. Gs and Gt would quickly give rise to a larger family of trimeric G-proteins as well as a growing retinue of effectors and auxiliary regulators (for examples, see Table 1). Why then did I (and, I suspect, many of my colleagues) find family resemblances between Gs and Gt so surprising in 1980? One reason may be that laboratories focusing on different problems communicated less often with one another in 1980 than they do in the 21st century. More likely, we were simply not ready to imagine close parallels between disparate biological functions: why, after all, should cells in the liver and retina use nearly identical machinery to detect glucagon vs photons? Now such a revelation would not come as a surprise, because we have learned that evolution makes each new signaling machine by modifying and cobbling together parts of machines already in use somewhere else. For many of us, Gs and Gt furnished the first inkling of this principle.

4 Confusion, Error, Truth: Discovering the β-AR

By the early 1970s, investigators were beginning to transform putative hormone receptors into biochemical entities by binding radioactive agonist peptides to receptors in tissue extracts. In 1948, Ahlquist

had postulated the existence of two classes of catecholamine receptors, which he called α and β (Ahlquist 1948). Bob Lefkowitz and Gerry Aurbach, among others, saw a straightforward route to identifying these receptors: assess binding of ^{3}H-labelled catecholamines to particulate extracts of tissues with catecholamine-sensitive adenylyl cyclase activity. Unfortunately, a good idea may stir up confusion rather than shed light. The β-AR story unfolded much as predicted by the pioneer of scientific induction 400 years ago: ". . . truth will sooner come out from error than from confusion," wrote Francis Bacon in his *Novum Organum* (1620).

First came an era of confusion: in the early 1970s the Aurbach and Lefkowitz laboratories found plenty of ^{3}H-norepinephrine binding sites, with binding that was usually reversible and competed by nonradioactive catecholamine agonists (Bilezikian and Aurbach 1973a; Lefkowitz and Haber 1971); some reports even claimed receptor solubilization, affinity chromatography, and partial purification (Bilezikian and Aurbach 1973b; Lefkowitz 1973; Lefkowitz et al. 1972). These investigators also found disturbing mismatches between patterns of agonist binding and response: agents without agonist or antagonist activity, such as inactive optimal isomers of norepinephrine or dihydroxymandelic acid, efficiently competed against ^{3}H-norepinephrine for binding, while β-AR antagonists such as propranolol competed poorly, even at concentrations orders of magnitude greater than propranolol's IC50 (summarized in Lefkowitz 1974).

These discrepancies led to fanciful interpretations: perhaps the antagonist first associates with a necessary-but-not-sufficient "partial" binding site but does not activate the receptor unless it also interacts with one or more additional sites; the first site would be detected by binding of ^{3}H-norepinephrine, the second only by receptor activation (Bilezikian and Aurbach 1973a; Lefkowitz 1974). One review even suggested that perhaps there were "certain inherent limitations in relying solely on the criteria of specificity and affinity of binding for identification of receptors" (Lefkowitz 1974). The same review admitted, however, that "the data available . . . are not . . . sufficient to prove or disprove the hypothesis that these [binding] sites represent the β-adrenergic receptor binding sites." It was beginning to dawn on investigators that their confusion might reflect what Francis Bacon referred to as "error."

This recognition allowed truth to emerge from error. Maguire and co-workers (1974) showed that ascorbic acid and sodium metabisulfite prevented ^{3}H-norepinephrine binding, suggesting that the binding represented covalent attachment of oxidized radioactive products to macromolecules other than receptors. In the same year, three laboratories (Aurbach et al. 1974; Lefkowitz et al. 1974; Levitzki et al. 1974) reported that non-catechol β-AR antagonists bind to sites with specificities for competition by optical isomers, agonists, and other antagonists that match those expected for the real β-AR.

Reliable binding assays for β-ARs allowed their biochemical characterization and eventual purification. Because biochemistry can be hard, the new "truth" did not make further advances easy. Undaunted, Caron, Lefkowitz, and their colleagues eventually purified detergent-solubilized β-AR protein by affinity chromatography (Benovic et al. 1984; Caron et al. 1979; Shorr et al. 1981). Availability of pure receptor protein soon made it possible to reconstitute pure β-AR with Gs and adenylyl cyclase (Cerione et al. 1984; May et al. 1985) and to identify β-AR kinase (Benovic et al. 1986). Most important, the β-AR story was developing in the period when recombinant DNA technology was beginning to hit its stride. Pure receptors made it possible to probe genomic DNA libraries with nucleotide probes based on receptor peptides. Amino acid sequences of β_2-ARs from hamster and from turkey erythrocytes led to cloning receptor cDNAs from these animals and predictions of the receptors' very similar amino acid sequences (Dixon et al. 1986; Yarden et al. 1986).

Some of us still remember the enormous excitement generated by the obvious similarities between primary structures of rhodopsin (Nathans and Hogness 1983) and the β_2-AR (Dixon et al. 1986; Yarden et al. 1986). The seven homologous hydrophobic α helices heralded the birth of a GPCR superfamily. Our delighted surprise paralleled the surprise generated by the discoveries of Gs and Gt. Again we had failed to anticipate evolution's propensity to adapt a successful piece of machinery to new uses. Delight and surprise were even greater this time, because cDNA sequences of αs and αt had just been reported (see Table 1). For us, the Gα and GPCR primary structures were harbingers of a torrent of new discoveries, driven by the power of molecular biology.

5 DNA: Revolution and Revelation

Rather than attempt a comprehensive account of the dazzling post-DNA history of G-proteins and GPCRs, I shall end this essay with glancing sketches of a few examples from this history, and point out how they have altered our ways of posing and solving questions. Pre-DNA discoveries contained the essential seeds of a series of new general concepts (indicated below in italics). Without the DNA revolution, however, none of these would have reached its present level of explanatory power. Now each of these ideas is an essential item of an investigator's intellectual furniture, necessary for designing and interpreting almost every experiment.

One such general concept is that of the *regulatory protein module*. The versatile R-G-E triad, comprising a GPCR, a trimeric G-protein, and an effector, is one of the best-studied regulatory modules in biology. The striking biochemical parallels between regulation of cAMP synthesis and phototransduction, in combination with similar primary structures of αs *v*s αt and of β-ARs vs rhodopsin, made R-G-E one of the very first of these modules. This module, we now know, is responsible for the mating dance of yeast and for detecting sensory cues and intercellular signals in flies, worms, mice, and humans. To see how far we have come, contrast the puzzle of hormone-sensitive adenylyl cyclase in the 1970s (Fig. 1) with the crystal-clear atomic structures of triad members solved two decades later: rhodopsin's transmembrane helices (Fig. 2a) and a complex of αs with adenylyl cyclase (Fig. 2b).

Like MAP kinase cascades, cytokine receptor signaling via JAK/STAT complexes, and many other modules, the R-G-E module is a set of evolutionarily conserved proteins that uses a common mechanism to transduce signals between different sets of inputs and outputs. From our standpoint in the 21st century, it may seem extraordinary that the concept of regulatory modules required a major shift in our way of looking at the world. In essence, we rediscovered evolution. Before DNA sequences came on the scene, scientists tended to imagine that *their* question and the molecule they hoped would answer it were essentially unique. In contrast, the R-G-E module showed us that duplication and divergence of GPCR and GTPase genes, combined by selection of useful gene products, had produced a module with interchangeable subunits

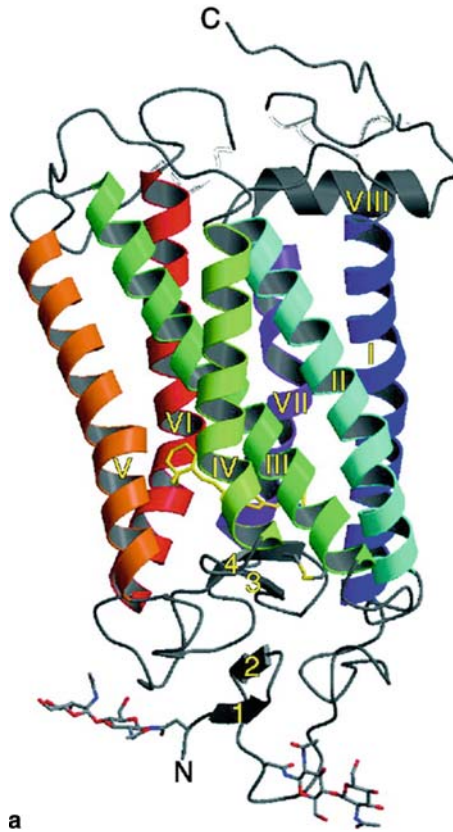

Fig. 2a,b. Atomic structures of a GPCR and a Gα-effector complex. **a** Rhodopsin, showing the seven transmembrane helices (*colored and numbered with Roman numerals*), loops connecting them (extracellular at *bottom*, cytoplasmic at *top*), and retinal (*yellow*). (Reprinted with permission from Fig. 2A of Palczewski et al. 2000, Science 289:739–745; copyright 2000 AAAS). **b** The α subunit of Gs (*left*) interacting with the catalytic domains of adenylyl cyclase (*right*). (Reprinted with permission from Fig. 4 of Tesmer et al. 1997; Science 278:1907–1916; copyright 1997 AAAS)

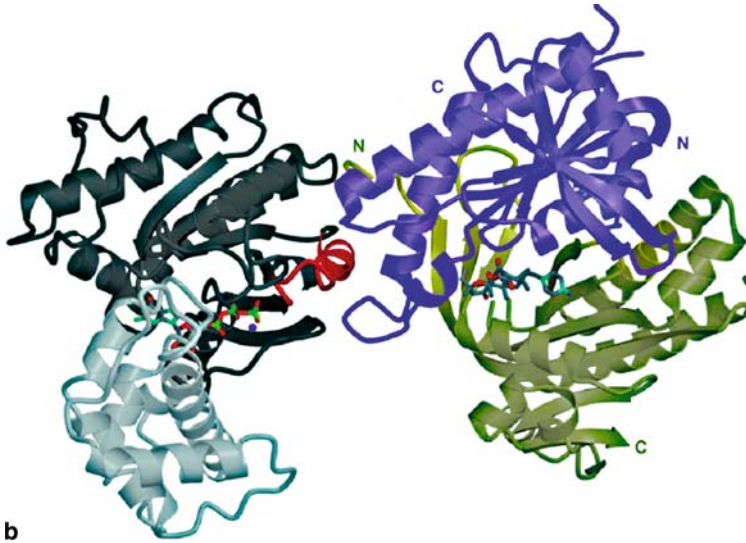

Fig. 2a,b. (continued)

that can selectively link large numbers of distinct inputs to different outputs.

The DNA revolution also created the closely related idea of *protein families*. Growing families and subfamilies of GPCRs and G-proteins brought to light hundreds of targets for intensive research in hormone action, vision, olfaction, neurobiology, immune responses, and embryonic development. A bevy of intriguing orphan GPCRs stands ready to join their ranks. Conserved regions of primary structure in other protein families revealed families of auxiliary proteins (e.g., RGS and Goloco) that interact with the R-G-E module. Gα subunits share sequence and three-dimensional architecture with a huge superfamily of GTPase switches, which also includes bacterial elongation factors, Ras, a host of other small GTPases, and many others. Evolution found that a good switch is worth conserving.

By linking R-G-E modules to other regulatory proteins (PDEs, kinases, phosphatases, ion channels, and more), AKAPs and other scaf-

folds create *higher-order protein complexes*, which in turn harness specific stimuli to an enormous variety of responses. In 1980, allostery and covalent modification were recognized as the principal modes of signal transduction. To them we now add a third, just as essential: *regulated proximity of proteins and signaling modules*.

From pheromone receptors in yeast to rhodopsin and chemokine receptors in vertebrates, GPCR activation triggers densely complex regulatory circuits, replete with positive and negative feedback loops. We can now begin to trace and manipulate such *cellular signaling networks* in space and time, using recombinant fluorescent probes, mRNA arrays, RNAi, the polymerase chain reaction, genomic sequences of many animals, and a host of other new tools. Without these it would be impossible to measure—or even to conceive—physiologically crucial temporal or spatial changes in the interactions of GPCRs, arrestins, or effector substrates and products (e.g., PIP2 or PIP3) with one another.

Discoveries at the atomic level include the conserved architecture and molecular mechanism of the conformational switch common to small GTPases and Gα subunits; interactions of G-protein subunits with effectors and other regulators; and how one GPCR ligand, 11-*cis*-retinal, nestles within the seven-helix bundle of its receptor, rhodopsin (Fig. 2a). All but the last of these discoveries depended on modifying and expressing recombinant genes. As a result, *regulation at the level of conformational change* (aka allostery) is no longer confined to a few molecules such as hemoglobin and conceptual models of other molecules; instead, documented conformational change regularly generates testable hypotheses and experiments.

Although I have focused on G-protein and GPCR research, every discovery I mention has myriad counterparts in virtually every field of present-day biomedical research. Consequently, molecular biology's rapidly expanding toolbox and the new ideas it generates have dramatically altered our laboratories, how we interact with each other, and our goals and expectations. Laboratories are larger and depend on much more powerful technology. Even the disposable plastic tips of today's ubiquitous pipette-man would have amazed experimenters who depended on individually calibrated glass lambda pipettes, operated by sucking on a rubber tube and meticulously washed with acid after each use. For the average investigator, scientific communication is faster, and

critically important research papers and seminars more frequent. In the early 1970s, one meeting per year was often more than enough. Now we are much more frequently thrilled (or disconcerted) by a new finding directly pertinent to the question we are asking, and suddenly find ourselves learning a new technology or immersed in a whole new field.

For researchers today, these exciting changes have produced two especially wide-ranging consequences. First, we justifiably expect our research to produce more rapid and far-ranging discoveries. We complain mightily, of course, about funding, bureaucracy, competition, failed experiments, and threatening social or political developments, just as we did in the 1970s. More significantly, we now feel reasonably sure that tomorrow we will understand more than we do today.

The second consequence is closely related to these changed expectations and even more crucial: investigators now expect their discoveries to prove relevant and even genuinely useful in the world outside the laboratory. As compared to the days when G-proteins and GPCRs were born, individual scientists and ideas travel much more rapidly and efficiently between basic and clinical science, and between academia and the pharmaceutical industry.

Although expectations do not tell us what the future will bring, I find it encouraging to look back to the birth of our field. The questions scientists posed in 1970 led eventually to today's discoveries, and more questions, none of which any of us could have imagined in our wildest dreams.

Acknowledgements. I thank Thomas Sakmar, Lutz Birnbaumer, Paul Insel, Zvi Selinger, and Elliott Ross for commenting on parts of the manuscript. Mistakes, however, are clearly my own.

References

Ahlquist R (1948) A study of the adrenergic receptors. Am J Physiol 153:586–600

Aurbach GD, Fedak SA, Woodard CJ, Palmer JS, Hauser D, Troxler F (1974) Beta-adrenergic receptor: stereospecific interaction of iodinated beta-blocking agent with high affinity site. Science 186:1223–1224

Benovic JL, Kuhn H, Weyand I, Codina J, Caron MG, Lefkowitz RJ (1987) Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). Proc Natl Acad Sci USA 84:8879–8882

Benovic JL, Shorr RG, Caron MG, Lefkowitz RJ (1984) The mammalian beta 2-adrenergic receptor: purification and characterization. Biochemistry 23: 4510–4518

Benovic JL, Strasser RH, Caron MG, Lefkowitz RJ (1986) Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. Proc Natl Acad Sci USA 83:2797–2801

Bilezikian JP, Aurbach GD (1973a) A beta-adrenergic receptor of the turkey erythrocyte. I. Binding of catecholamine and relationship to adenylate cyclase activity. J Biol Chem 248:5577–5583

Bilezikian JP, Aurbach GD (1973b) A beta-adrenergic receptor of the turkey erythrocyte. II. Characterization and solubilization of the receptor. J Biol Chem 248:5584–5589

Bitensky MW, Miki N, Keirns JJ, Keirns M, Baraban JM, Freeman J, Wheeler MA, Lacy J, Marcus FR (1975) Activation of photoreceptor disk membrane phosphodiesterase by light and ATP. Adv Cyclic Nucleotide Res 5:213–240

Bourne HR, Coffino P, Tomkins GM (1975a) Selection of a variant lymphoma cell deficient in adenylate cyclase. Science 187:750–752

Bourne HR, Coffino P, Tomkins GM (1975b) Somatic genetic analysis of cyclic AMP action: characterization of unresponsive mutants. J Cell Physiol 85:611–620

Caron MG, Srinivasan Y, Pitha J, Kociolek K, Lefkowitz RJ (1979) Affinity chromatography of the beta-adrenergic receptor. J Biol Chem 254:2923–2927

Cassel D, Levkovitz H, Selinger Z (1977) The regulatory GTPase cycle of turkey erythrocyte adenylate cyclase. J Cyclic Nucleotide Res 3:393–406

Cassel D, Pfeuffer T (1978) Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. Proc Natl Acad Sci USA 75:2669–2673

Cassel D, Selinger Z (1976) Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes. Biochim Biophys Acta 452:538–551

Cassel D, Selinger Z (1977) Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. Proc Natl Acad Sci USA 74:3307–3311

Cerione RA, Sibley DR, Codina J, Benovic JL, Winslow J, Neer EJ, Birnbaumer L, Caron MG, Lefkowitz RJ (1984) Reconstitution of a hormone-sensitive adenylate cyclase system. The pure beta-adrenergic receptor and guanine nucleotide regulatory protein confer hormone responsiveness on the resolved catalytic unit. J Biol Chem 259:9979–9982

Coffino P, Bourne HR, Tomkins GM (1975) Somatic genetic analysis of cyclic AMP action: selection of unresponsive mutants. J Cell Physiol 85:603–610

Daniel V, Litwack G, Tomkins GM (1973) Induction of cytolysis of cultured lymphoma cells by adenosine 3′:5′-cyclic monophosphate and the isolation of resistant variants. Proc Natl Acad Sci USA 70:76–79

De Lean A, Stadel JM, Lefkowitz RJ (1980) A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. J Biol Chem 255:7108–7117

Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE et al. (1986) Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature 321:75–79

Farfel Z, Brickman AS, Kaslow HR, Brothers VM, Bourne HR (1980) Defect of receptor-cyclase coupling protein in pseudohypoparathyroidism. New Engl J Med 303:237–242

Fung B, Stryer L (1980) Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. Proc Natl Acad Sci USA 77:2500–2504

Fung BK, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc Natl Acad Sci USA 78:152–156

Gilman AG (1987) G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56:615–649

Godchaux W, Zimmerman WF (1979) Membrane-dependent guanine nucleotide binding and GTPase activities of soluble protein from bovine rod cell outer segments. J Biol Chem 254:7874–7884

Harris BA, Robishaw JD, Mumby SM, Gilman AG (1985) Molecular cloning of complementary DNA for the alpha subunit of the G protein that stimulates adenylate cyclase. Science 229:1274–1277

Hsia Y (1965) Photochemistry of vision. In: Graham CH (ed) Vision and visual perception. Wiley, New York

Insel PA, Bourne HR, Coffino P, Tomkins GM (1975) Cyclic AMP-dependent protein kinase: pivotal role in regulation of enzyme induction and growth. Science 190:896–898

Insel PA, Maguire ME, Gilman AG, Bourne HR, Coffino P, Melmon KL (1976) Beta adrenergic receptors and adenylate cyclase: products of separate genes? Mol Pharmacol 12:1062–1069

Johnson GL, Kaslow HR, Bourne HR (1978) Genetic evidence that cholera toxin substrates are regulatory components of adenylate cyclase. J Biol Chem 253:7120–7123

Kuhn H (1978) Light-regulated binding of rhodopsin kinase and other proteins to cattle photoreceptor membranes. Biochemistry 17:4389–4395

Kuhn H, Dreyer WJ (1972) Light dependent phosphorylation of rhodopsin by ATP. FEBS Lett 20:1–6

Kuhn H, McDowell JH, Leser KH, Bader S (1977) Phosphorylation of rhodopsin as a possible mechanism of adaptation. Biophys Struct Mech 3:175–180

Lefkowitz RJ (1973) Isolated hormone receptors: physiologic and clinical implications. N Engl J Med 288:1061–1066

Lefkowitz RJ (1974) Commentary. Molecular pharmacology of beta-adrenergic receptors: a status report. Biochem Pharmacol 23:2069–2076

Lefkowitz RJ, Haber E (1971) A fraction of the ventricular myocardium that has the specificity of the cardiac beta-adrenergic receptor. Proc Natl Acad Sci USA 68:1773–1777

Lefkowitz RJ, Haber E, O'Hara D (1972) Identification of the cardiac beta-adrenergic receptor protein: solubilization and purification by affinity chromatography. Proc Natl Acad Sci USA 69:2828–2832

Lefkowitz RJ, Mukherjee C, Coverstone M, Caron MG (1974) Stereospecific (3H)(minus)-alprenolol binding sites, beta-adrenergic receptors and adenylate cyclase. Biochem Biophys Res Commun 60:703–709

Levitzki A, Atlas D, Steer ML (1974) The binding characteristics and number of beta-adrenergic receptors on the turkey erythrocyte. Proc Natl Acad Sci USA 71:2773–2776

Lochrie MA, Hurley JB, Simon MI (1985) Sequence of the alpha subunit of photoreceptor G protein: homologies between transducin, ras, and elongation factors. Science 228:96–99

Londos C, Salomon Y, Lin MC, Harwood JP, Schramm M, Wolff J, Rodbell M (1974) 5′-Guanylylimidodiphosphate, a potent activator of adenylate cyclase systems in eukaryotic cells. Proc Natl Acad Sci USA 71:3087–3090

Maguire ME, Goldmann PH, Gilman AG (1974) The reaction of [3H]norepinephrine with particulate fractions of cells responsive to catecholamines. Mol Pharmacol 10:563–581

Maguire ME, Sturgill TW, Gilman AG (1975) Frustration and adenylate cyclase. Metabolism 24:287–299

May DC, Ross EM, Gilman AG, Smigel MD (1985) Reconstitution of catecholamine-stimulated adenylate cyclase activity using three purified proteins. J Biol Chem 260:15829–15833

Medynski DC, Sullivan K, Smith D, Van Dop C, Chang FH, Fung BK, Seeburg PH, Bourne HR (1985) Amino acid sequence of the alpha subunit of transducin deduced from the cDNA sequence. Proc Natl Acad Sci USA 82:4311–4315

Murayama T, Ui M (1983) Loss of the inhibitory function of the guanine nucleotide regulatory component of adenylate cyclase due to its ADP ribosylation by islet-activating protein, pertussis toxin, in adipocyte membranes. J Biol Chem 258:3319–3326

Nathans J, Hogness DS (1983) Isolation, sequence analysis, and intron-exon arrangement of the gene encoding bovine rhodopsin. Cell 34:807–814

Northup JK, Sternweis PC, Smigel MD, Schleifer LS, Ross EM, Gilman AG (1980) Purification of the regulatory component of adenylate cyclase. Proc Natl Acad Sci USA 77:6516–6520

Ovchinnikov YA, Abdulaev NG, Feigina MY, Artamonov ID, Zolotarev AS, Miroshnikov AL, Martynov VL, Kostina MB, Kudelin AB, Bogachuk AS (1982) The complete amino acid sequence of visual rhodopsin. Bioorg Khim 8:1424–1427

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M (2000) Crystal structure of rhodopsin: A G protein-coupled receptor. Science 289:739–745

Rall TW, Sutherland EW, Berthet J (1957) The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J Biol Chem 224:463–475

Rodbell M, Birnbaumer L, Pohl SL, Krans HMJ (1971a) The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanyl nucleotides in glucagon action. J Biol Chem 246:1877–1882

Rodbell M, Krans HMJ, Pohl SL, Birnbaumer L (1971b) The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Effects of guanyl nucleotides on binding of ^{125}I-glucagon. J Biol Chem 246:1872–1876

Ross EM, Gilman AG (1977a) Reconstitution of catecholamine-sensitive adenylate cyclase activity: interactions of solubilized components with receptor-replete membranes. Proc Natl Acad Sci USA 74:3715–3719

Ross EM, Gilman AG (1977b) Resolution of some components of adenylate cyclase necessary for catalytic activity. J Biol Chem 252:6966–6969

Ross EM, Gilman AG (1980) Biochemical properties of hormone-sensitive adenylate cyclase. Annu Rev Biochem 49:533–564

Shichi H, Somers RL (1978) Light-dependent phosphorylation of rhodopsin. Purification and properties of rhodopsin kinase. J Biol Chem 253:7040–7046

Shorr RG, Lefkowitz RJ, Caron MG (1981) Purification of the beta-adrenergic receptor. Identification of the hormone binding subunit. J Biol Chem 256:5820–5826

Stadel JM, Nambi P, Shorr RG, Sawyer DF, Caron MG, Lefkowitz RJ (1983) Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase is associated with phosphorylation of the beta-adrenergic receptor. Proc Natl Acad Sci USA 80:3173–3177

Sutherland EW, Rall TW (1960) Formation of adenosine-3,5-phosphate (cyclic adenylate) and its relation to the action of several neurohormones or hormones. Acta Endocrinol (Copenh) 34(Suppl 50):171–174

Tanabe T, Nukada T, Nishikawa Y, Sugimoto K, Suzuki H, Takahashi H, Noda M, Haga T, Ichiyama A, Kangawa K et al. (1985) Primary structure of the alpha-subunit of transducin and its relationship to ras proteins. Nature 315:242–245

Tesmer JJ, Sunahara RK, Gilman AG, Sprang SR (1997) Crystal structure of the catalytic domains of adenylyl cyclase in a complex with Gsalpha. GTPgammaS. Science 278:1907–1916

Wald G (1968) The molecular basis of visual excitation. Nature 219:800–807

Wheeler GL, Bitensky MW (1977) A light-activated GTPase in vertebrate photoreceptors: regulation of light-activated cyclic GMP phosphodiesterase. Proc Natl Acad Sci USA 74:4238–4242

Wilden U, Hall SW, Kuhn H (1986) Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. Proc Natl Acad Sci USA 83:1174–1178

Yarden Y, Rodriguez H, Wong SK, Brandt DR, May DC, Burnier J, Harkins RN, Chen EY, Ramachandran J, Ullrich A et al. (1986) The avian beta-adrenergic receptor: primary structure and membrane topology. Proc Natl Acad Sci USA 83:6795–6799

Yee R, Liebman PA (1978) Light-activated phosphodiesterase of the rod outer segment. Kinetics and parameters of activation and deactivation. J Biol Chem 253:8902–8909