PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

GTPases: A Family of Molecular Switches and Clocks

Henry R. Bourne

Phil. Trans. R. Soc. Lond. B 1995 **349**, 283-289
doi: 10.1098/rstb.1995.0114

GTPases: a family of molecular switches and clocks

HENRY R. BOURNE

Departments of Pharmacology and Medicine and the Cardiovascular Research Institute, University of California, San Francisco, California 94143, U.S.A.

SUMMARY

Members of the GTPase superfamily share a core domain with a conserved three-dimensional structure and a common GTPase cycle, but perform a wide variety of regulatory tasks in eukaryotic cells. Evolution has created functional diversity from the conserved GTPase structure in two principal ways: (i) by combining in the product of a single gene the core GTPase domain attached to one or more additional folded domains; (ii) by building around a core GTPase an assembly of proteins encoded by different genes. Analysis of the patterns of conserved amino acid side chains on surfaces of Gα proteins reveals interfaces with other proteins in the G-protein signal linking device.

1. INTRODUCTION

Comprising perhaps a dozen families and hundreds of individual gene products, members of the superfamily of GTPases share the same three-dimensional (3-D) architecture, stretches of similar amino acid sequence, and similar or identical enzymatic mechanisms. They also share a general biological function, as timers of molecular events and as switches responsible for turning these events on and off. Because the functions of many of its members are well characterized, the GTPase superfamily provides an attractive target for investigators of molecular evolution.

Figure 1*a* schematically depicts features of the GTPase cycle and 3-D structure that are shared by all members of the superfamily (for reviews, see Bourne *et al.* 1990, 1991). As enzymes, most GTPases are remarkably inefficient, hydrolysing bound GTP at very slow rates, for example, once every 45 min or so for $p21^{ras}$, the product of a mammalian proto-oncogene. After hydrolysis, most GTPases retain GDP, a product of the reaction, for several additional minutes. Consequently, arrays of separate proteins regulate most GTPases at two points in the cycle: GTPase-activating proteins (GAPs) stimulate GTP hydrolysis, and guanine nucleotide-releasing proteins, or GNRPs, accelerate binding of substrate (GTP) by promoting release of bound GDP. Structurally, members of the GTPase superfamily share several short stretches of similar or identical amino acid sequence (black boxes in the primary structures of figure 1). Superfamily members also share a common GTPase mechanism and guanine nucleotide-binding site, as indicated by the similar effects in diverse GTPases of mutations that replace cognate amino acids. In crystals of proteins from three GTPase families (Cai *et al.* 1990; Coleman *et al.* 1994), these conserved amino acids form loops that cradle bound guanine nucleotide in an α/β 3-D structure that is also highly conserved.

The ability of GTPases to serve as switches and timers derives from the stereotyped conformational changes induced in these proteins by binding GTP and by converting bound GTP back to GDP. By shifting the positions of three or four conserved amino acids, negative charges on the γ-phosphate of GTP induce changes in the conformations of two structural elements, loop 2 and α helix 2 (L2 and α2, depicted schematically in figure 1). Although GTP-dependent conformational changes may be propagated to other parts of an individual GTPase, changes in the shape of L2 and α2 largely account for the very different abilities of G·GDP and G·GTP to associate with many proteins, including downstream effectors and gaps. In combination with the shared GTPase timing mechanism, this conservation of structure and switching function strongly implies that most of the vast array of GTPases in bacteria and eukaryotes evolved from a common precursor.

As examples of general themes in the evolution of families of regulatory proteins, I shall discuss three features of GTPase structure and function. First, addition to the core GTPase structure of additional folded domains that modulate or redirect function, as an example of a common way in which evolution modifies proteins to perform new functions. Second, the trimeric signaling G-proteins as an example of a signal linking device (SLD), an assembly of coevolved proteins capable of transducing many varied and complex signals by a common, stereotypical mechanism. Third, a hypothesis regarding evolution of surfaces of a protein that interact with other proteins, tested by analysis of the α subunit (Gα) of trimeric G-proteins.

2. FUNCTIONS MEDIATED BY MULTIPLE DOMAINS

$p21^{ras}$ and many other members of the GTPase superfamily comprise only the core GTPase domain and undergo relatively simple cycles controlled by

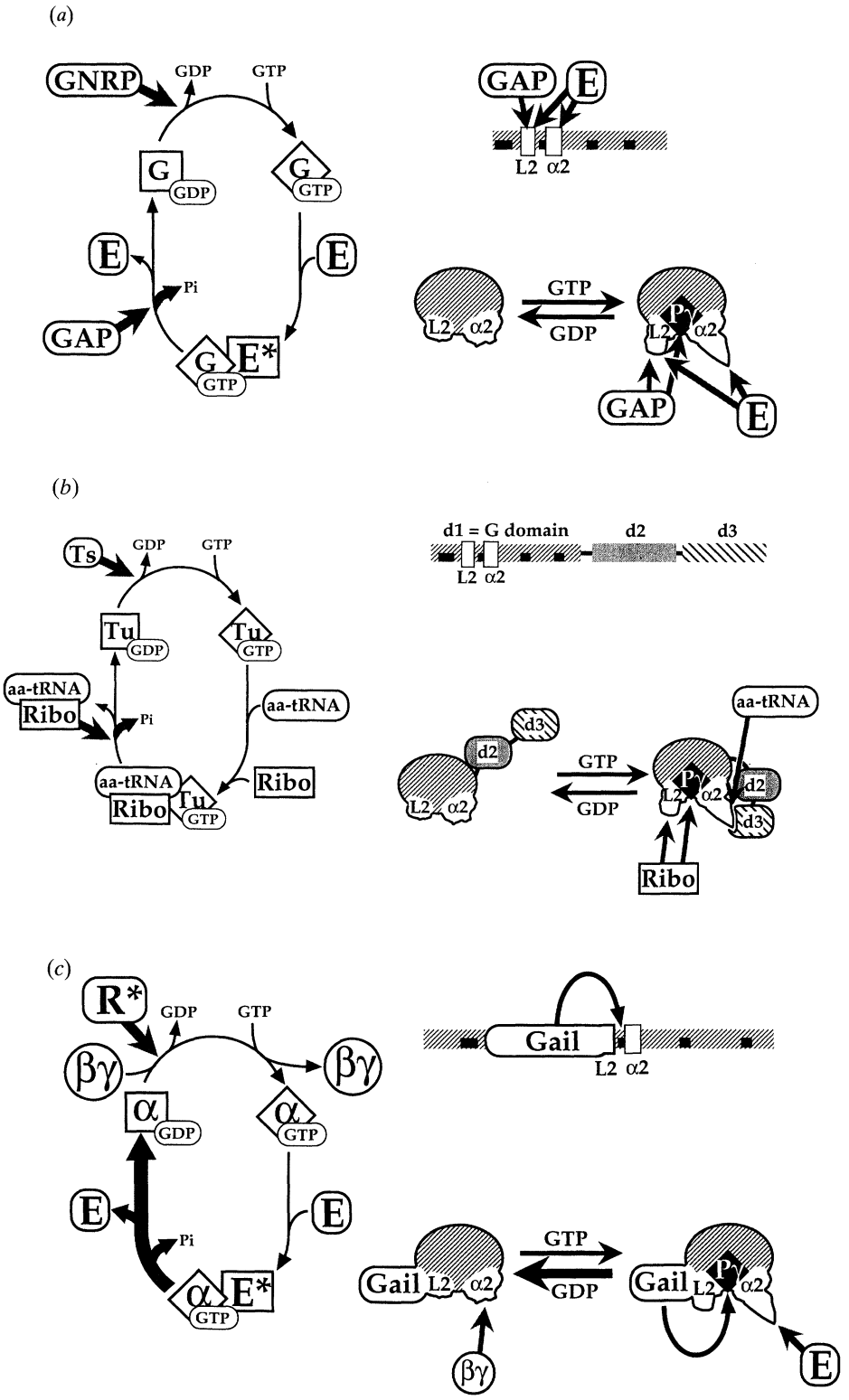

Figure 1. GTPase cycles, structural features and conformational changes in different GTPases. Each panel shows a characteristic GTPase cycle (left); a cartoon of primary structure (upper right), in which black bars indicate conserved amino acid sequences and white boxes indicate regions of GTP-induced conformational change; and a cartoon (lower right) schematically depicting the γ-phosphate of GTP that is the target of GAPs (Pγ, black diamond), as well as the effects of conformational change on associations with other proteins. (*a*) $p21^{ras}$ serves as a model of a simple, stripped-down GTPase, regulated by a GNRP and a GAP. In reality, $p21^{ras}$ is subject to control by multiple GNRPs and GAPs. (*b*) EF-Tu, a kinetic proofreader of the binding of the anticodon of aa-tRNA to an mRNA codon in ribosomal protein synthesis. Here the GNRP is EF-Ts and Ribo, the mRNA-programmed ribosome, serves as the GAP. (*c*) The α subunit of a trimeric signaling G-protein, which transmits messages from receptors to effector enzymes and ion channels. Here the ligand-activated receptor acts as a GNRP, and GTP-induced conformational change (in the α2 helix and elsewhere) reduces the affinity of α for binding the βγ complex and increases its affinity for binding

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

GNRPs and GAPs, as depicted in figure 1*a*. Other GTPases, embellished by additional, separately folded domains become compound molecular machines that perform more complex functions and dance through more complex cycles.

Figure 1*b* depicts one example, bacterial Elongation Factor Tu (EF-Tu). In addition to its core GTPase domain, EF-Tu contains two other folded domains, d2 and d3; these are attached in series to the carboxy terminus of the core domain. Upon binding GTP, the altered conformation of α2 in the GTPase domain disrupts its interaction with d2 and induces binding of d3 to the α2 helix of the core (Berchtold *et al.* 1993; Kjeldgaard *et al.* 1993). A 4.6 Å movement of a conserved glycine in the core domain, near the γ-phosphate of GTP, is transmitted into a 46 Å movement of the most distal part of d2. This GTP-induced change in EF-Tu makes it able to bind an amino-acyl tRNA (aa-tRNA), perhaps in a groove between the core GTPase domain and d3. In turn, by matching the mRNA codon on a programmed ribosome, the anticodon of the bound aa-tRNA binds the aa-tRNA·Tu·GTP complex to the ribosome. Although the aa-tRNA by itself does not speed up the slow GTPase activity of EF-Tu, it orients EF-Tu·GTP correctly on the ribosome, allowing the ribosome to act as a GAP and accelerate GTP hydrolysis 10^5-fold.

The extra domains of EF-Tu, together with the GAP-like function of the ribosome, impart to a simple GTP/GDP switch the ability to perform a highly sophisticated task, kinetic proofreading of the fidelity of protein synthesis (Thompson *et al.* 1986; Thompson 1988). Although the aa-tRNA·EF-Tu·GTP complex is quite long-lived, its binding to the programmed ribosome sets the GTPase timer, and the complex compares the time required for GTP hydrolysis *versus* the time required for dissociation of the anticodon from the mRNA codon. A correctly paired anticodon binds tightly to the mRNA codon, so that bound GTP will be hydrolysed before the codon and anticodon dissociate. As a result, EF-Tu·GDP simply dissociates from the complex of aa-tRNA and ribosome, and the elongation reaction continues, incorporating into the growing polypeptide an amino acid that is correctly matched to the codon. In contrast, an incorrectly matched anticodon dissociates from the mRNA codon before the GTP bound to EF-Tu is hydrolysed; in this case the entire aa-tRNA·EF-Tu·GTP complex simply dissociates from the ribosome, and the incorrect amino acid is not incorporated. The precise molecular reactions are more complex, too much so for discussion here (see Weijland & Parmeggiani 1993).

The α subunits (Gα) of trimeric G-proteins are also embellished by an extra domain, in addition to the core GTPase domain (see figure 1*c*). This ~120-residue extra domain, composed largely of a helices (Noel *et al.* 1993), acts as an intrinsic, built-in GAP for the Gα GTPase, prompting us to call the extra domain Gail, for 'GAP-like' (Markby *et al.* 1993). Purified Gα subunits hydrolyse GTP at a rate (~ 4 min^{-1}) that is certainly slow in comparison to most enzymes, but is much faster than GTP hydrolysis by EF-Tu in the absence of its extrinsic GAP, the ribosome. The different rates of GTP hydrolysis mesh with the very different functions of these two proteins. In the case of EF-Tu, kinetic proofreading requires not only that GTP be hydrolysed quickly, but also that the GTPase timer start only when the codon and anticodon become engaged, and not a moment before. In contrast, the function of GTP-induced conformational change in Gα is to preserve an 'active' conformation, capable of allosterically regulating an effector molecule for a period of time that does not depend upon the duration of the signal received from the activated receptor. Thus the Gα timer is set as soon as the GNRP – an activated cell surface receptor – promotes conversion of α·GDP to α·GTP. Hydrolysis of GTP is necessary to make sure that the transmitted signal eventually turns off, but a 10–20 s delay before GTP is hydrolysed serves to amplify the signal initiated by an activated receptor. In addition to the intrinsic GAP provided by Gail, GTP hydrolysis by certain Gα proteins can also be accelerated by extrinsic GAPs, including effectors (Arshavsky & Bownds 1992; Berstein *et al.* 1992).

In the primary structure of Gα, Gail is inserted in a position that corresponds to that of L2 in $p21^{ras}$ (see figure 1*c*). In the Gα 3-D structure, bound guanine nucleotide is sandwiched between Gail and the core domain, and the two domains are connected by short stretches of sequence, linkers 1 and 2. In biochemical experiments (Markby *et al.* 1993), the core GTPase domain of Gα by itself – like $p21^{ras}$ or EF-Tu – hydrolyses GTP very slowly; addition of recombinant Gail to the core domain brings GTPase activity of the complex up to a level (4 min^{-1}) comparable to that observed with the holoprotein. Gail appears to increase the basal GTPase of Gα by positioning the side chain of a conserved arginine residue in linker 2, near the γ-phosphate of bound GTP. A mutant Gail protein lacking this arginine could bind to the Gα core domain, but could not stimulate GTP hydrolysis (Markby *et al.* 1993). In current models of the GTPase reaction (Coleman *et al.* 1994; Sondek *et al.* 1994), based on 3-D structures of different Gα proteins, this conserved arginine helps to stabilize the pentavalent transition state of the GTPase reaction. Strikingly, the ability of a Ras-GAP to stimulate GTP hydrolysis by $p21^{ras}$ also depends upon an arginine residue (Brownbridge *et al.* 1993); perhaps ras-GAP and the built-in gap provided by Gail work by similar molecular mechanisms.

We imagine that the extra domains of EF-Tu and Gα arose by splicing together the gene for a primordial core GTPase with the exon(s) of another gene. In the case of Gα, intron-exon structures (Itoh *et al.* 1988; Kozasa *et al.* 1988; Matsuoka *et al.* 1990) are consistent

effectors. The α subunit's intrinsic GTPase is much faster than that of pure $p21^{ras}$ or pure EF-Tu (seconds, rather than minutes), because a separately folded GAP-like domain (Gail) introduces the side chain of a conserved arginine into the GTP binding site.

with this attractive notion, but – as is usually the case – do not prove it.

3. TRIMERIC G PROTEINS ARE A SIGNAL LINKING DEVICE

We no longer imagine that cells are regulated by discrete signaling pathways in which linear chains of signaling machines transmit a message from a cell-surface receptor to a set of responding enzymes. Instead, we now seek to explain key features of cell regulation – complexity, flexibility, adaptation and feedback, amplification, and modulation in both space and time – by unraveling complex signaling networks. Signal transmitters at the nodes of such networks do not simply receive one signal and transmit another. Instead, acting as signal linking devices (SLDs), they integrate multiple incoming signals and/or generate multiple signals going out. Recently discovered examples include receptor tyrosine kinases, cyclin-dependent protein kinases, multi-subunit ion channels, and protein complexes that regulate transcription.

Presumably evolution creates and sculpts signaling networks by modifying SLDs. Although a single polypeptide can serve as an SLD, many SLDs comprise protein products of several genes that appear to have evolved together during evolution. Thus SLDs tend to run in families in which each individual is composed of a different combination of proteins, several of which are identical or homologous (and sometimes interchangeable) with their cousins in a related SLD. Members of an SLD family use a more or less conserved quaternary structure and molecular mechanism to link diverse input and output signals. At a node of any signaling network, the specific array of inputs and outputs linked by a particular SLD depends upon the subset of specific proteins in the SLD.

Trimeric G-proteins may be considered a paradigmatic SLD. Each G-protein receives multiple stimulatory inputs communicated by cell-surface receptors for hormones, neurotransmitters, odorants, and photons. In turn the activated G-protein can activate multiple effector enzymes and ion channels, because GTP-dependent dissociation generates two potential signaling proteins, $\alpha\cdot$GTP and the $\beta\gamma$ complex (see figure 1*c*), each of which may be able to stimulate more than one kind of effector.

The proteins that provide inputs for an SLD or receive its outputs may or may not be structurally conserved. The receptors that activate G-proteins share similarities of primary structure and a seven-transmembrane-helix motif, suggesting that they derive from a common precursor (Strader *et al.* 1994). In vertebrates, the actual number of genes for this family of G-protein-coupled receptors ($\sim 10^3$) exceeds the number of extracellular stimuli. This is because many ligands, like acetylcholine, trigger activation of several different receptors, each of which activates a different subset of the available G-proteins. In contrast, G-protein effectors belong to several (probably less than a dozen) structurally and functionally distinct classes, including the adenylyl cyclases, phospholipase Cβs, families of K^+ and Ca^{2+} channels, and the cGMP phosphodiesterases. Each class contains effectors encoded by multiple different genes. For instance, genes for seven structurally similar adenylyl cyclases have been cloned; each adenylyl cyclase is itself an SLD capable of integrating a characteristic subset of inputs; these include intracellular Ca^{2+}, G$\beta\gamma$, and protein kinase C, in addition to $\alpha_s\cdot$GTP (Iyengar 1993).

Although each member of the G-protein SLD family uses the same basic molecular mechanism for linking incoming to outgoing signals, specificity of the linkage depends on which polypeptides make up the G-protein heterotrimer. The mechanism (see figure 1*c*) uses activated receptors to promote guanine nucleotide exchange on Gα and the resulting GTP-dependent conformational change to produce $\alpha\cdot$GTP and free $\beta\gamma$. In vertebrates the structural diversity of different heterotrimers is large; to deploy a combinatorial subset of the available G-protein SLDs, a cell can choose from 16 Gα, 5 Gβ, and 7 Gγ genes (Gautam *et al.* 1990; Simon *et al.* 1991; von Weizsäcker *et al.* 1992). This subset apparently determines which of the potential G-protein nodes will function in the network of a particular cell.

In addition to its central mechanistic role, described above, each individual species of Gα also confers specificity in linking signals, by virtue of its ability to respond to a specific subset of receptors and to activate a specific subset of effector molecules. Although the role of Gα in determining signal specificity has been long recognized, investigators are now learning that the different β and γ polypeptides also confer specificity. Most cells express only a subset of the potentially available β and γ genes, and therefore a combinatorial subset of $\beta\gamma$ subunits. In principle, different combinations of β and γ could enhance the specificity of an individual G-protein SLD either by: (i) filtering receptor inputs; or (ii) by selecting among available effectors. The first possibility, which depends on the fact that receptors recognize and interact with the $\alpha\beta\gamma$ trimer rather than with Gα (or G$\beta\gamma$) alone, is supported by reasonably strong evidence (Fawzi *et al.* 1991; Kisselev & Gautam, 1993; Kleuss *et al.* 1992, 1993). In vitro experiments have shown that certain receptors can activate a specific Gα much more efficiently in the presence of specific $\beta\gamma$ complexes (Fawzi *et al.* 1991; Kisselev and Gautam, 1993). In an elegant series of antisense experiments in intact cultured cells, Kleuss *et al.* reported (Kleuss *et al.* 1992, 1993) that receptors for two different extracellular ligands required expression of different pairs of β and γ genes to trigger G_o-dependent inhibition of a Ca^{2+} channel. They inferred that each of the two receptors could interact with a G_o molecule only if it contained a unique $\beta\gamma$ complex composed of a specific β and a specific γ polypeptide.

Much weaker evidence supports the complementary possibility, that specific effectors respond selectively to specific complexes of α and γ. In biochemical experiments, most recombinant $\beta\gamma$ combinations regulate effectors at roughly equivalent concentrations (Tang & Gilman 1991; Wickman *et al.* 1994). It is nonetheless pertinent to point out that a number of $\beta\gamma$ combinations have not yet been tested, and that inferences

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

drawn from negative results of in vitro experiments may not be correct.

4. PROTEIN INTERACTION SURFACES OF Gα: TESTING AN EVOLUTIONARY HYPOTHESIS

Protein components of an SLD usually belong to multigene families, as is the case with the three G-protein polypeptides. From the ability of gene products in an SLD to interact with one another, we infer that the components of that SLD have coevolved. Similarly, because different members of an SLD family specifically link subsets of incoming and outgoing signals, we infer that the SLD components have also coevolved with families of proteins that generate or receive signals linked by that SLD. A concrete example: interaction surfaces of polypeptides like Gα, Gβ, and Gγ probably coevolved not only with one another but also with interacting surfaces on receptors and effectors.

To explain how such coevolution might occur, a general hypothesis would go something like this: gene duplication and random mutations provide a rich source of variation. The amino acid codon at each position in a gene is subject to many kinds of selective pressure, including the need to preserve stability of the encoded protein and its function(s) as a monomer, such as enzymatic activity or conformational change. In a protein component of an SLD, selective pressure must also be exerted on amino acid side chains at surfaces that interact with other components in the SLD and/or with incoming and outgoing signals (protein or otherwise). If this is true, each subfamily of a particular SLD component is likely to present conserved surfaces that have coevolved in tandem with complementary surfaces on parallel subfamilies of other proteins. Coevolution of such surfaces should leave unmistakable traces, in the form of patterns of conserved surface amino acids in complementary protein subfamilies.

With its known 3-D structure, conserved conformational change, and well characterized and specific interactions with other proteins, the family of Gα proteins seems an ideal target for testing this hypothesis. Such a test was performed by Olivier Lichtarge in the laboratory of Fred Cohen at the University of California, San Francisco, in collaboration with my laboratory; a detailed account will be published elsewhere. Lichtarge devised a general analytical procedure, called *f*unctional *i*somorphic *s*equence *h*omology (FISH) analysis. As applied to Gα, the first step was to compare aligned amino acid sequences of all known Gα proteins. Most of the sequences (60 of 88 examined) could be grouped in five distinct classes, each defined by a high proportion of identical amino acids (~ 75% or better). Each class formed a functionally distinct subfamily (two or more distinct genes in multiple vertebrate species) characterized by ability to regulate a specific class of effector. The classes included: α_s (adenylyl cyclase); α_q (phospholipase Cb); α_o (Ca^{2+} channels); α_i (K^+ channels); and α_t (cGMP phosphodiesterases).

In the second step of FISH analysis, alignments of the

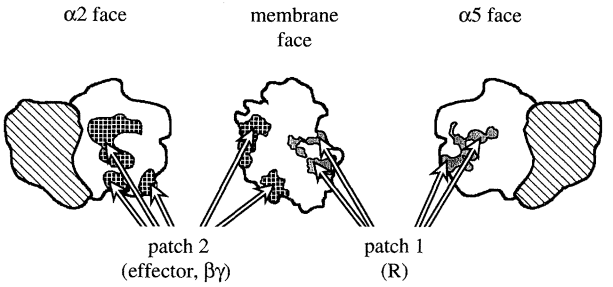

Figure 2. Patches of conserved residues on the surface of Gα. The diagram shows three faces of Gα, a two-domain protein with a core GTPase domain (white) and a 'Gail' domain (diagonal lines) that acts as a built-in gap (see text). The α 2 face (left) probably interacts with effectors and βγ, and the α 5 face (right) with receptors (R). The third face (middle) probably faces the membrane; in addition to small parts of patches 1 and 2, it includes the C-terminal tail of Gα, which is known to interact with receptor (R).

60 grouped sequences were searched for positions that exhibited either of two kinds of conservation of structure: (i) the same amino acid is encoded at that position in all members of all five classes; (ii) amino acids at that position are identical within each class but not between classes. Because we sought to analyse Gα surfaces, the list of identical residues was culled to remove all residues that were not at least 30% exposed in the 3-D structure of $\alpha_t \cdot$GDP (Lambright *et al.* 1994) or $\alpha \cdot$GTP (Noel *et al.* 1993), as well as residues that touch the bound guanine nucleotide (the latter are probably conserved for reasons unrelated to interfaces with other proteins). Finally, the two types of conserved positions were mapped onto the 3-D structure of $\alpha_t \cdot$GTP (see figure 2).

The results were a gratifying surprise. First, the proportion of conserved surface positions was small. Only 14 surface positions showed the same amino acid in all Gα proteins, and 14 more were conserved in a class-specific fashion. Together these two numbers added up to less than 18% of the 158 total surface positions in the protein, very much less than the 75% overall identity of amino acid sequence within each of the five subfamilies, and considerably lower than the average degree of identity (~ 45%) between members of two different subfamilies. These discrepancies reflect the fact that amino acid side chains on a protein's surface are more freely mutable in the course of evolution than are those directed toward the protein's interior, presumably because the latter are constrained to 'fit' other side chains to preserve the protein's stability and monomeric function.

Although small in number, the conserved surface positions were not distributed randomly over the protein's surface. Instead, they clustered into two more or less circumscribed patches, located on opposite sides of the core GTPase domain (see figure 2). Patch 1, located on the same face of Gα as the α 5 helix, bends around to cover a portion of the protein's membrane face as well. Patch 2 is found on the same face of Gα as is the α 2 helix. In addition, identical and class-specific positions were found mingled with one another in each patch, exactly as we would expect if: (i) both sets of conserved residues perform functions (protein-protein

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

association) that are mechanistically conserved among Gα subunits; and (ii) class-specific variations help to determine the specificity of these associations.

Our initial hypothesis about coevolution of SLD proteins and their input and output signals predicted that key interacting surfaces should be structurally conserved within functionally defined subfamilies. To test this prediction, we compared patches 1 and 2 with surface features of Gα already biochemically or genetically identified as belonging to surfaces that interact with receptors, Gβγ, and effectors. Indeed, many of the positions in patch 1 have already been identified as part of a receptor-interacting Gα surface (reviewed in Conklin & Bourne 1993). Patch 2 overlaps to a large extent with the 'switch regions' that assume different main-chain conformations in the GDP- versus the GTP-bound conformations of Gα. For this reason, we suspect that patch 2 indicates a surface that contains overlapping regions of contact with Gβγ and with effectors, because these proteins associate with different conformations of Gα . In addition, although most positions in patch 2 have not been directly tested, several have previously been identified as contact points for Gβγ and/or effectors (reviewed in Conklin & Bourne 1993).

In principle, FISH analysis could be profitably performed with any protein family that participates in an SLD, providing that multiple similar but divergent amino acid sequences are available, functional constraints can be applied to group the sequences, and a 3-D structure is known. Examples include the cyclin-dependent protein kinases, families of small GTPases, and even conserved motifs, such as SH2 domains and the zinc finger domains of transcriptional regulators.

5. PERSPECTIVE

The 3-D structure and enzymatic function of the core domain are remarkably well conserved throughout the GTPase superfamily, even when evolution asks its members to perform a wide variety of regulatory tasks. One evolutionary strategy for endowing a conserved structure with diverse functions, apparently, is to construct multi-domain proteins around a conserved core, as with EF-Tu and the a subunit of trimeric G-proteins. A parallel strategy, exemplified by protein oligomers like the Gαβγ SLD, is to assemble products of different genes around a core structure. It is fairly easy to collect and describe examples of such strategies, but harder to imagine how they grew out of variation and natural selection, and more difficult still to devise rigorous tests for our hypotheses. As recounted here, FISH analysis represents a beginning approach to testing one fairly simple hypothesis.

Here we have touched on only a few of the questions we may want to ask about evolution of GTPases. To what degree do the enzymatic mechanisms of GDP/GTP exchange or GTP hydrolysis vary in different branches of the GTPase superfamily, and how did these variations evolve? Can we devise a FISH-like procedure to analyse patterns of conserved amino acid side chains within GTPases? Could such a procedure test (or reveal) possible mechanisms of conformational change, or predict a conformation not yet seen in crystals, such as the transient empty state of the guanine nucleotide binding site in the exchange reaction?

The GTPase superfamily will surely offer a host of intriguing targets for students of molecular evolution. For this fledgling science, these are early days, indeed.

REFERENCES

Arshavsky, V. Y. & Bownds, M. D. 1992 Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. *Nature, Lond.* **357**, 416–417.

Berchtold, H., Reshetnikova, L., Reiser, C. O. A., Schirmer, N. K., Sprinzl, M. & Hilgenfeld, R. 1993 Crystal structure of active elongation factor Tu reveals major domain rearrangements. *Nature, Lond.* **365**, 126–132.

Berstein, G., Blank, J. L., Jhon, D. Y., Exton, J. H., Rhee, S. G. & Ross, E. M. 1992 Phospholipase C-β1 is a GTPase Activating Protein (GAP) for $G_{q/11}$, its physiologic regulator. *Cell* **70**, 411–418.

Bourne, H. R., Sanders, D. A. & McCormick, F. 1990 The GTPase superfamily. A conserved switch for diverse cell functions. *Nature, Lond.* **348**, 125–132.

Bourne, H. R., Sanders, D. A. & McCormick, F. 1991 The GTPase superfamily: Conserved structure and molecular mechanism. *Nature, Lond.* **349**, 117–127.

Brownbridge, G. G., Lowe, P. N., Moore, K. J. M., Skinner, R. H. & Webb, M. R. 1993 Interaction of GTPase activating proteins (GAPs) with $p21^{ras}$ measured by a novel fluorescence anisotropy method. Essential role of arginine-903 of GAP in activation of GTP hydrolysis on $p21^{ras}$. *J. biol. Chem.* **268**, 10914–10919.

Cai, H., Szeberényi, J. & Cooper, G. M. 1990 Effect of a dominant inhibitory Ha-*ras* mutation on mitogenic signal transduction in NIH 3T3 cells. *Molec. Cell. Biol.* **10**, 5314–5323.

Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G. & Sprang, S. R. 1994 Structure of active conformations of $G_i\alpha_1$ and the mechanism of GTP hydrolysis. *Science, Wash.* **265**, 1405–1412.

Conklin, B. R. & Bourne, H. R. 1993 Structural elements of Gα subunits that interact with Gβγ, receptors, and effectors. *Cell* **73**, 631–641.

Fawzi, A. B., Fay, D. S., Murphy, E. A., Tamir, H., Erdos, J. J. & Northup, J. K. 1991 Rhodopsin and the retinal G-protein distinguish among G–protein beta gamma subunit forms. *J. biol. Chem.* **266**, 12194–12200.

Gautam, N., Northup, J., Tamir, H. & Simon, M. I. 1990 G protein diversity is increased by associations with a variety of γ subunits. *Proc. natn Acad. Sci. U.S.A.* **87**, 7973–7977.

Itoh, H., Toyama, R., Kozasa, T., Tsukamoto, T., Matsuoka, M. & Kaziro, Y. 1988 Presence of three distinct molecular species of G_i protein α subunit. Structure of rat cDNAs and human genomic DNAs. *J. biol. Chem.* **263**, 6656–6664.

Iyengar, R. 1993 Molecular and functional diversity of mammalian G_s-stimulated adenylyl cyclases. *FASEB J.* **7**, 768–775.

Kisselev, O. & Gautam, N. 1993 Specific interaction with rhodopsin is dependent on the gamma subunit type in a G protein. *J. biol. Chem.* **268**, 24519–24522.

Kjeldgaard, M., Nissen, P., Thirup, S. & Nyborg, J. 1993 The crystal structure of elongation factor EF-Tu from *Thermus aquaticus* in the GTP conformation. *Structure* **1**, 35–49.

Kleuss, C., Scherübl, H., Hescheler, J., Schultz, G. & Wittig,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

B. 1992 Different β-subunits determine G-protein interaction with transmembrane receptors. *Nature*, *Lond.* **358**, 424–426.

Kleuss, C., Scherübl, H., Hescheler, J., Schultz, G. & Wittig, B. 1993 Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. *Science*, *Wash.* **259**, 832–834.

Kozasa, T., Itoh, H., Tsukamoto, T. & Kaziro, Y. 1988 Isolation and characterization of the human $G_s\alpha$ gene. *Proc. natn Acad. Sci. U.S.A.* **85**, 2081–2085.

Lambright, D. G., Noel, J. P., Hamm, H. E. & Sigler, P. B. 1994 The 1.8 Å crystal structure of transducin α-GDP: Structural determinants for activation of a heterotrimeric G-protein α subunit. *Nature*, *Lond.* **369**, 621–628.

Markby, D. W., Onrust, R. & Bourne, H. R. 1993 Separate GTP binding and GTPase activating domains of a Gα subunit. *Science*, *Wash.* **262**, 1895–1901.

Matsuoka, M., Itoh, H. & Kaziro, Y. 1990 Characterization of the human gene for $G_x\alpha$, a pertussis toxin-insensitive regulatory GTP-binding protein. *J. biol. Chem.* **265**, 13215–13220.

Noel, J. P., Hamm, H. E. & Sigler, P. B. 1993 The 2.2 Å crystal structure of transducin α-GTPγS. *Nature*, *Lond.* **366**, 654–663.

Simon, M. I., Strathmann, M. P. & Gautam, N. 1991 Diversity of G proteins in signal transduction. *Science*, *Wash.* **252**, 802–808.

Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E. & Sigler, P. B. 1994 GTPase mechanism of G proteins from the 1.7-Å crystal structure of transducin α-GDP-AlF_4^-. *Nature*, *Lond.* **372**, 276–279.

Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D. & Dixon, R. A. F. 1994 Structure and function of G protein-coupled receptors. *A. Rev. Biochem.* **63**, 101–132.

Tang, W.-J. & Gilman, A. G. 1991 Type-specific regulation of adenylyl cyclase by G protein βγ subunits. *Science*, *Wash.* **254**, 1500–1503.

Thompson, R. C. 1988 EFTu provides an internal kinetic standard for translational accuracy. *Trends biochem. Sci.* **13**, 91–93.

Thompson, R. C., Dix, D. B. & Karim, A. M. 1986 The reaction of ribosomes with elongation factor Tu-GTP complexes. Aminoacyl-tRNA-independent reactions in the elongation cycle determine the accuracy of protein synthesis. *J. biol. Chem.* **261**, 4868–4874.

von Weizsäcker, E., Strathman, M. P. & Simon, M. I. 1992 Diversity among the beta subunits of heterotrimeric GTP-binding proteins: Characterization of a novel beta-subunit cDNA. *Biochem. biophys. Res. Comm.* **183**, 350–356.

Weijland, A. & Parmeggiani, A. 1993 Toward a model for the interaction between elongation factor Tu and the ribosome. *Science*, *Wash.* **259**, 1311–1314.

Wickman, K. D., Iûiguez-Lluhi, J. A., Davenport, P. A. *et al.* 1994 Recombinant G-protein βγ-subunits activate the muscarinic-gated atrial potassium channel. *Nature*, *Lond.* **368**, 255–257.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES