Discussion Letter

Are guanine nucleotide binding proteins a distinct class of regulatory proteins?

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Proteins which bind guanine nucleotides are found in a diverse set of key regulatory positions. They are involved in hormone action, visual transduction, protein synthesis and microtubule assembly. In addition to their ability to bind guanine nucleotides these proteins possess several other common features. (i) They all have similar subunit composition, (ii) they can be ADP-ribosylated, (iii) their conformation changes depending on the nucleotide bound. These regulatory G-proteins have close functional homologies. Do they form a general class of regulatory proteins, like the protein kinases? Do they have a common evolutionary ancestry?

Guanine nucleotide binding protein *Protein synthesis* *Adenylate cyclase* *ADP-ribosylation* *Tubulin* *Visual transduction*

1. INTRODUCTION

Cells use energy from nucleotide triphosphate hydrolysis to control many systems. Those which most readily spring to mind involve ATP, either in the 'futile cycles' of intermediary metabolism, cyclic AMP production or phosphorylation of proteins.

In this article I want to draw attention to another, possibly general, strategy used by cells in their internal regulation. Energy from GTP is used to control the conformation of a diverse range of guanine nucleotide binding proteins. These proteins have a number of features in common which include GTP/GDP exchange, ADP-ribosylation, subunit size and allosteric action on other proteins. They form a class of proteins, present in a range of systems as diverse as visual transduction, protein synthesis and the cytoskeleton, which are functionally closely related and may have evolved from a common ancestor.

2. G-PROTEINS AND CONTROL

Apart from guanylate cyclases, with which I will not deal here, almost all proteins which utilize GTP for regulatory purposes have a number of common features:

(i) binding of GTP to the polypeptide changes its conformation such that it associates with other proteins;
(ii) hydrolysis of GTP releases phosphate and leaves GDP non-covalently bound to the polypeptide which then dissociates from the other proteins;
(iii) G-protein exerts its control through these protein:protein interactions.

Proteins having these properties (which I will refer to collectively as G-proteins) include:

(a) G-proteins of the hormone sensitive adenylate cyclase;
(b) G-protein of the retinal rod cell cyclic GMP phosphodiesterase;

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(c) a variety of prokaryotic and eukaryotic protein synthesis cofactors;
(d) the subunit dimer of microtubules, tubulin;
(e) a number of other, less well characterized proteins.

I will discuss each system in turn endeavouring to bring out the similarities and differences as I go along.

3. ADENYLATE CYCLASE

Many hormone receptors modulate the activity of adenylate cyclase on the cytoplasmic side of the plasma membrane, thereby influencing cytoplasmic cyclic AMP levels (review [1]). It is now accepted that the receptors do not act directly on adenylate cyclase but that the signal is carried from receptor to cyclase by a G-protein [2]. The hormone-bound receptor causes release of GDP and binding of GTP on the stimulatory G-protein (N_s). The G-protein in its $N_s \cdot$GTP form then activates adenylate cyclase. There is dispute as to exactly how these protein–protein interactions occur. Do the receptor, G-protein and cyclase form a ternary complex in the membrane [3] or does the $N_s \cdot$GTP diffuse between the receptor where it is formed and the cyclase which it activates [4]? After activation of the cyclase the G-protein hydrolyzes its bound GTP to leave $N_s \cdot$GDP. This is no longer capable of activating cyclase, which reverts to its inactive form. If sufficient activated receptor is still present the G-protein will once again become activated. Whether or not cyclase is activated thus depends on the relative rates of GTP hydrolysis and receptor catalyzed GDP/GTP exchange on the G-protein. GDP/GTP exchange is hormone regulated, whilst only cholera toxin is known to modulate hydrolysis in vivo. The total amount of activatable G-protein present relative to cyclase could also have a regulatory role.

Recently the G-proteins of the cyclase system have been isolated and characterized. The turkey erythrocyte G-protein has two subunits of M_r 35000 and 45000 [5], the larger of which binds GTP. However the rabbit liver protein has three subunits of M_r 52000, 45000 and 35000, the two largest binding GTP [6]. Activation of G-protein by non-hydrolyzable GTP analogues or fluoride causes subunit separation [7].

The advantage of a G-protein system of this sort is two-fold. It allows one receptor–ligand complex to activate many cyclase units and it permits a variety of different hormone receptors to act on cyclase at once, i.e., it amplifies and integrates the inputs. Therefore less total protein is needed on two counts:

(i) as one occupied receptor can catalyze GDP/GTP exchange on several G-proteins the signal is amplified so less receptor and hormone are needed;
(ii) the G-protein transduces the signal from many receptor types so each receptor does not have to have its own cyclase units. The amplification also allows a more rapid response as a small change in hormone concentration can cause a large change in cyclic AMP production.

It has recently become clear that this system is even more elegant. Not only do G-proteins mediate the switching-on of cyclase, but there is also a second GTP-utilizing function in many cell types which inhibits cyclase [8–10] in response to a different set of hormones. This system too can be activated by non-hydrolyzable GTP analogues, i.e., the inhibitory G-protein (N_i) can be turned on and hence cyclase inhibited. Thus the adenylate cyclase activating system can be adapted so that the response to a given excitatory stimulus need not be the same under all conditions.

The nature of N_i is not known. Is it a completely separate protein from N_s? Or is it perhaps another function of the same protein in response to a second class of receptors? A third possibility for the nature of N_i is that it is formed by substitution of an inhibitory GTP binding subunit for the stimulatory one on a common core polypeptide. This idea is supported by the observation that G-protein composition has been found to be quite variable depending upon from which cells it is isolated. Northup and co-workers [6] find the 3 subunits of rabbit liver G-protein to occur in unequal proportion and two of them bind GTP and are ADP-ribosylated. Whereas in turkey erythrocytes which have no inhibitory G-protein only two subunits, one binding GTP and ADP-ribose are seen. Yet both N_s and N_i contain M_r 35000 subunits, which have very similar proteolysis patterns [11]. An interesting characteristic of the G-proteins in this system is that they can be affected by bacterial toxins. Cholera toxin irrever-

sibly activates N_s by an ADP-ribosylation of the GTP binding subunit (review [12]). Cholera toxin and the islet activating protein from *Bordatella pertussis* block the effect of inhibitory hormones on cyclase by ADP-ribosylating a different protein of M_r 40000 [13].

Another feature of these G-proteins is that their effects are not only on adenylate cyclase. They also have a feedback control on receptor-ligand interaction. A number of groups have recently shown modulation of receptor-agonist affinity depending on the state of the G-protein [14]. This opens up the possibility that G-protein modulation could control either sensitivity of a particular cell type to a particular blood hormone concentration or desensitization of the system to prolonged hormone exposure.

Thus in the cyclase system N_s and N_i seem likely to be evolutionarily related as they have similar actions, sensitivities to effectors such as non-hydrolyzable GTP analogues, M_r of subunits and propensity to be ADP-ribosylated.

4. RETINAL ROD CELL CYCLIC GMP PHOSPHODIESTERASE

A closely analogous system to adenylate cyclase has been discovered in vertebrate retinal rod cells. Rhodopsin, the primary pigment for absorption of light, catalytically activates a G-protein which then activates a cyclic GMP phosphodiesterase (PDE) (review [15]). This set of reactions occurs on the intracellular disk membrane and may be involved in carrying the light signal across the cytoplasm to the plasma membrane in the form of a transient drop in cyclic GMP concentration. Alternatively modulation of cyclic GMP level may form part of the mechanism of adaptation of the cells to various background light intensities.

This system has the advantage that the G-protein is about 5% of the disk membrane protein and can be removed in low ionic strength medium [16] so it is readily isolatable. The G-protein has three subunits of M_r 39000, 36000 and 6000 [16]. It has been shown that the G-protein binds to bleached rhodopsin and that this causes GDP to be released [17]. If GTP is present it binds, leading to a change in the G-protein such that it dissociates from bleached rhodopsin. Indeed, it appears that the largest, GTP binding subunit (G_α) may separate from the rest ($G_{\beta\gamma}$) as may occur in N_s of adenylate cyclase. $G_\alpha \cdot$GTP then activates PDE, itself a 3 subunit protein of M_r 88000, 85000, 11000 [18]. Hydrolysis of GTP on the G-protein leads to reversal of this process and a return to the inactive state. Non-hydrolyzable GTP analogues irreversibly activate the G-protein here too. Originally it was generally assumed that G-protein binding to PDE leads to PDE activation but the 11000-M_r PDE_γ subunit is inhibitory to PDE activity and it has been suggested that the G-protein removes this subunit in vivo to activate PDE [19]. This receives support from two quarters. Firstly we showed that the PDE target size does not change substantially in response to light [20], consistent with the removal of a small inhibitory subunit. Secondly it has been shown that a 60000-M_r unit with PDE inhibiting activity after boiling is released from the disk membrane in response to light [21]. This unit, which copurifies with G_α, has the right size to be G_α bound to the heat stable PDE_γ. The rod cell G-protein has great similarities to those of adenylate cyclase. Cholera toxin irreversibly activates it [22]. The subunit composition and proteolysis patterns [11] are similar. Most convincingly functional exchange can occur between the G-proteins from rods and cyclase [23]. Exchange reconstitution experiments showed that frog rod cell G-protein will mediate adrenalin stimulation of cyclase on rat cerebral cortex and hypothalamic synaptic membranes and frog erythrocyte ghosts. Similarly illuminated rhodopsin will activate cyclase in a GTP-dependent manner. Also the heat stable inhibitor of PDE was capable of reducing cyclase activity in a way reversible by rod cell G-protein bound to guanyl-5′-ylimidodiphosphate. The cross-reactivity of the two systems is so striking that it may be that adenylate cyclase too could be activated by removal of a small inhibitory subunit.

Recently a number of modulators of the rod cell system have been discovered. Increasing calcium over the physiological range reduces the light-sensitivity of PDE [24], however no direct effect of calcium on purified PDE can be shown. It is tempting to suggest that calcium might exert its effect at the level of the G-protein.

Two inhibitory roles of ATP on activated PDE can be resolved [25]. ATP-induced inhibition of PDE was originally interpreted as being due to phosphorylation of bleached rhodopsin which

prevents it from catalyzing GDP/GTP exchange. The novel inhibitory effect of ATP is faster than that due to phosphorylation of rhodopsin and leads to a sharp spike of PDE activity after a short flash of low intensity light. Again the basis of this effect is open to hypothesis, but it could be via the G-protein.

Not only does the G-protein affect PDE, there is also a marked effect of G-protein on rhodopsin itself [26,27] (analogous to the effects of N_s on hormone receptors). Thus there is the possibility that the G-protein could control rhodopsin phosphorylation or regeneration and thereby rod cell adaptation.

So the rod G-protein has remarkable similarity to the G-proteins of adenylate cyclase. It functions by a GTP hydrolysis cycle dependent on protein–protein interaction, it is activated by ADP-ribosylation, it has similar subunit structure and it can interact functionally with the cyclase system at a number of levels.

5. PROTEIN SYNTHESIS COFACTORS

5.1. *Initiation factors*

Both eukaryotic and prokaryotic initiation factor 2 bind GTP (review [28]). Eukaryotic Initiation Factor-2 (eIF-2) is responsible for presenting met-tRNAi to the 43 S ribosomal subunit during the initiation of protein synthesis. Rabbit reticulocyte eIF-2 probably has 3 subunits α, β and γ of M_r 38000, 45000 and 55000, respectively [29]. eIF-2_γ is responsible for binding met-tRNAi whilst eIF-$2_{\alpha\beta}$ is capable of binding guanine nucleotides. During initiation eIF-2 must have GTP bound but upon association of the 43 S ribosomal subunit with mRNA and 60 S subunit GTP is hydrolyzed and eIF-2·GDP is released. Evidence from several groups (see [30]) has shown that a second factor, now designated eIF-2B [31], catalytically stimulates exchange of GTP for GDP on the inactive eIF-2.

Here again we find the G-protein pattern of GDP/GTP exchange catalyzed by one protein and GTP hydrolysis occurring in conjunction with another.

eIF-2·met-tRNAi binding to 43 S can be rate limiting for protein synthesis. Being early in initiation this step is an ideal control point. As in the case of other G-proteins there is a suggestion that eIF-2_β may leave eIF-$2_{\alpha\gamma}$·GTP·met-tRNAi when it binds to 43 S [30]. Phosphorylation of eIF-2 prevents eIF-2B interaction thereby inhibiting protein synthesis by interfering with the necessary recycling of eIF-2. Two eIF-2 kinases are known, one regulating globin synthesis in reticulocytes, the other mediating host cell shut-off of protein synthesis during viral infection. eIF-2 has also been reported to be inhibited by ADP-ribosylation [31].

Similarities between eIF-2 and other G-proteins therefore include:

(i) M_r of the GTP binding α and β subunits;
(ii) possible separation of α and β subunits during activation;
(iii) protein catalyzed exchange of guanine nucleotides;
(iv) ADP-ribosylation;
(v) use of GTP hydrolysis to release the G-protein from its active complex with other proteins.

In prokaryotes IF-2 is also a GTP binding protein. It is a single polypeptide chain of about 100000 M_r. Binding GTP allows it to catalyze fmet-tRNAf binding to the 30 S ribosomal subunit. GTP is then hydrolyzed and IF-2 is released [32].

5.2. *Elongation factors*

Elongation factors in both prokaryotes and eukaryotes [33] have been found to utilize GTP. In prokaryotes EF-Tu presents aminoacyl-tRNA to the ribosome in a ternary complex with GTP. EF-Tu is released after GTP hydrolysis. GDP is removed and GTP bound to EF-Tu through the catalytic action of another protein, EF-Ts [34]. EF-Tu and EF-Ts are single polypeptides of M_r 42000 and 28000, respectively, in *E. coli*.

In eukaryotes this function is performed by analogous proteins EF-1_α and EF-1_β. EF-1_α binds GTP releasing GDP in the presence of EF-1_β. EF-1_α·GTP binds aminoacyl-tRNA and will only hydrolyze GTP in the presence of ribosomes and mRNA, thereby releasing free EF-1_α·GDP.

The clear similarity between this system and eIF-2/2B or IF-2 needs little comment as the process is identical but for the substrate.

The translocation step of elongation is catalyzed by another protein, known as EF-G in prokaryotes and EF-2 in eukaryotes. Both these proteins bind GTP and again this is hydrolyzed during the interaction of the protein with the ribosome.

However, translocation can occur without GTP hydrolysis so the hydrolysis must be required for some later step, possibly complex release.

EF-2 can be ADP-ribosylated by diphtheria toxin (review [35]) in a manner analogous to the action of cholera toxin on other G-proteins [36]. ADP-ribosylation leads to inhibition of function although GTP can still be bound [37]. Thus it is acting by a similar route to cholera toxin, that of GTPase inhibition.

It is important to note the differences of these G-proteins from others. No proteins have been found to be required for GDP/GTP exchange on free EF-G or EF-2, and the M_r of these cofactors is large (EF-G = 80000, EF-2 = 100000).

5.3. *Termination factors*

Eukaryotic protein synthesis requires a protein known as releasing factor (RF) and GTP. RF from reticulocyte lysates has two subunits of M_r about 56000. This system is not as well characterized as initiation and elongation but appears to be similar to them. GTP hydrolysis is required for complete termination and non-hydrolyzable analogues inhibit the reaction after binding of RF to the ribosome–mRNA complex [38].

In prokaryotes 3 releasing factors (RF-1, -2 and -3) are required. One of them (RF-3) acts to catalyze the action of the other two, but as yet the involvement of GTP in the system is unclear. By analogy one might hypothesize that RF-3 catalyzes GDP/GTP exchange on either RF-1 or RF-2.

Overall GTP is extensively used in protein synthesis. The proteins which bind GTP all conform to a common pattern. They have different binding affinities for a site on the ribosome depending on their guanine nucleotide bound state. eIF-2 and EF-1_α in eukaryotes and IF-2 and EF-Tu in prokaryotes have similar roles in bringing aminoacyl-tRNA to the ribosome. GTP binding allows aminoacyl-tRNA binding which in turn leads to a conformational change such that the ternary complex can bind to the ribosome. Hydrolysis of GTP again causes a change such that aminoacyl-tRNA is transferred to the ribosome and the cofactor protein is released. EF-G and EF-2 catalyze ribosomal translocation and use GTP to control their binding to the ribosome. EF-G and EF-Tu have been shown to have substantial sequence homologies in the first 140 amino acids [39]. The protein synthesis G-proteins appear to fall into two major categories, those which require other polypeptides to catalyze GDP/GTP exchange (eIF-2, EF-1_α, EF-Tu and RF) and those which do not (IF-2, EF-G and EF-2). It may be significant that those not requiring additional polypeptides are also those which have an M_r approximately double that of the generality of G-proteins. Could a gene fusion have occurred such that the catalytic and GTP binding units became permanently linked?

6. TUBULIN

Microtubules, major cytoskeletal elements of many eukaryotic cells, are composed of aggregated dimers of tubulin α and β forms in a helical array with 13 dimers per turn, α and β tubulin are closely related proteins (>40% homology in primary structure [40]). Both have M_r 50000 and both bind GTP. Only the β subunit allows GTP exchange with its medium on a reasonable time scale [41]. The utilization of GTP by tubulin has been reviewed [42]. GTP provides the driving force for the treadmilling of microtubules through conformational changes in the $\alpha\beta$ dimers. This means that the GTPase function of tubulin is intimately related to the control of microtubule assembly/disassembly. $\alpha\beta$ dimers can only bind to a microtubule in a specific orientation so each tubule has polarity. Different net binding constants for dimers at either end of the tubule lead to the designation of one end as an associating and the other as a dissociating end. Binding of dimers occurs 100-times faster if GTP is bound to the exchangeable site [43]. One hypothesis for treadmilling [44] is that after binding, when the dimer is totally surrounded in the tubule structure by other dimers, the GTP is hydrolyzed leaving non-exchangeable GDP bound. Only if such a GDP·dimer reaches an end of the tubule does the dimer become able to dissociate from the tubule.

The binding of GTP can thus be thought to provide energy for the association of dimers whilst GTP hydrolysis, once inside the tubule, changes the properties of the dimer so that it can readily dissociate if not held in position by other dimers. Assembly of microtubules is strongly influenced by microtubule associated proteins (MAP's). If treadmilling does occur in vivo the observation that

phosphorylation of MAP's increases the tubulin flux rate may indicate how treadmilling can be used as a sensitive control system.

7. NOVEL G-PROTEINS

Recently a number of new proposals have been made concerning GTP binding proteins in cells.

One of the most interesting is the discovery [45] of GTP effects on insulin action. The authors propose an inhibitory G-protein (N_i) which transduces the inhibitory effect of insulin on adenylate cyclase in liver. They also find stimulation by insulin of a cyclic AMP phosphodiesterase on the plasma membrane of hepatocytes [46]. Cholera toxin also causes PDE activation which suggests that a G-protein may be involved. These conclusions, if substantiated by other workers, imply that part of the effect of insulin on cells is mediated through GTP binding protein(s). They also make clearer still the link between the G-protein in retinal rod cells which activates cyclic GMP PDE and those involved with adenylate cyclase.

Again concerning hormones, an effect of guanine nucleotides on α_1-adrenoreceptors has been found [47]. As the current view of adrenergic action does not implicate α_1-receptors in modulation of adenylate cyclase it is possible that this result is pointing towards a new G-protein involved in the α_1-response.

Another protein which specifically binds guanine nucleotides is the oncogene product of the related Harvey and Kirsten murine sarcoma viruses (v-Ha-ras and v-Ki-ras). These gene products are phosphoproteins of M_r 21000 called p21, which can transform cells [48]. Host cells contain homologous genes (c-Ha-ras1 and c-Ha-ras2 in rat) which produce a p21 with no known function apart from its ability to bind GTP [49]. Immunological evidence suggests that guanine nucleotide binding causes a conformational change in p21. Like hormonally controlled G-proteins p21 from v-Ha-ras has been localized on the inner surface of the plasma membrane [50]. Although it is possible that p21 may induce transformation by a route similar to $p60^{src}$ there is still no evidence that phosphorylation is integral to p21's malignant transforming ability.

These considerations lead to the totally hypothetical notion that G-proteins might form an important component of an integrated system in cells involved in regulation over the whole time scale of biochemical events; from millisecond control in the eye to permanent effects on cell growth.

8. CONCLUSION

Despite the diversity of function in terms of specialized role, kinetics and mechanism of action in the systems discussed above I hope the similarities of each of the systems and their common characteristics, outlined at the beginning, have become clear.

Obviously the G-proteins of adenylate cyclase and rod cell PDE have a much closer homology than the others, however the similarity in (i) the sizes of the GTP binding subunits which are all in the 35000–50000 M_r range (with the exception of the large prokaryotic protein synthesis cofactors which are around double the size at 80000–100000 M_r), (ii) the tendency of the GTP binding subunit to be associated with another, similar or slightly smaller subunit, (iii) the propensity towards ADP-ribosylation of the GTP binding subunits by bacterial toxins, which may reflect a real cellular mechanism of control. ADP-ribosylation invariably inhibits GTPase activity and (iv) the common mechanism of action in using energy from GTP binding and hydrolysis to control protein–protein interactions. Binding causing association of G-protein with other proteins and hydrolysis leading to G-protein release suggests that these species can be thought of as a unified class of regulatory, transducing proteins.

Nucleotide triphosphate hydrolysis provides energy in many situations. It is interesting to note that in almost all it is the binding of NTP which yields energy for the crucial step. The hydrolysis of the NTP seems necessary only for turning-off the system or recycling the NTP-binding protein.

Whether these proteins are indeed evolutionarily related will have to await sequence information. The amino acid sequences of tubulin α and β [40] and of EF-Tu and EF-G [39] are now available but the homology appears to be slight. Bearing in mind that these proteins are very separate evolutionarily (one solely eukaryotic, the other prokaryotic) perhaps this is not surprising.

It seems likely that the membrane-bound G-proteins, the protein synthesizing G-proteins and

the tubulins are of divergent origin within their groups but whether all G-proteins are in fact of any common ancestry (i.e., have arisen by divergent evolution from a common GTP binding protein) or whether there is some intrinsic facet of GTP metabolism in cells which causes it to evolve a role in regulatory protein–protein interactions convergently is not clear.

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