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Review

Ras-catalyzed hydrolysis of GTP: A new perspective from model studies

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ABSTRACT **Despite the biological and medical importance of signal transduction via Ras proteins and despite considerable kinetic and structural studies of wild-type and mutant Ras proteins, the mechanism of Ras-catalyzed GTP hydrolysis remains controversial. We take a different approach to this problem: the uncatalyzed hydrolysis of GTP is analyzed, and the understanding derived is applied to the Ras-catalyzed reaction. Evaluation of previous mechanistic proposals from this chemical perspective suggests that proton abstraction from the attacking water by a general base and stabilization of charge development on the γ-phosphoryl oxygen atoms would not be catalytic. Rather, this analysis focuses attention on the GDP leaving group, including the β-γ bridge oxygen of GTP, the atom that undergoes the largest change in charge in going from the ground state to the transition state. This leads to a new catalytic proposal in which a hydrogen bond from the backbone amide of Gly-13 to this bridge oxygen is strengthened in the transition state relative to the ground state, within an active site that provides a template complementary to the transition state. Strengthened transition state interactions of the active site lysine, Lys-16, with the β-nonbridging phosphoryl oxygens and a network of interactions that positions the nucleophilic water molecule and γ-phosphoryl group with respect to one another may also contribute to catalysis. It is speculated that a significant fraction of the GAP-activated GTPase activity of Ras arises from an additional interaction of the β-γ bridge oxygen with an Arg side chain that is provided in trans by GAP. The conclusions for Ras and related G proteins are expected to apply more widely to other enzymes that catalyze phosphoryl ($-PO_3^{2-}$) transfer, including kinases and phosphatases.**

Ras and other G proteins are active in signaling when GTP is bound. Hydrolysis of GTP to give bound GDP turns these signaling proteins off (1–3). For this reason, the mechanism of GTP hydrolysis by Ras and other G proteins has elicited much interest. Nevertheless, despite a wealth of structural, kinetic, and site-directed mutagenesis data, the catalytic mechanism of Ras remains controversial.

Crucial to an understanding of enzymatic catalysis is knowledge of the nature of the transition state of the reaction and how that transition state differs from the ground state; transition state theory defines catalysis as stabilization of a reaction's transition state relative to its ground state. An underlying feature common among the several distinct models presented for Ras catalysis is an explicit or implicit assumption that the transition state is associative in character. However, recent model studies have shown that the transition state for GTP hydrolysis in solution is dissociative rather than associative (4). This transition state information is used to evaluate previously proposed mechanisms from a chemical perspective. A new catalytic mechanism is then proposed, involving a hydrogen bond to the β-γ bridge oxygen of GTP. This proposal is consistent with pre-existing structural, spectral, and energetic data.

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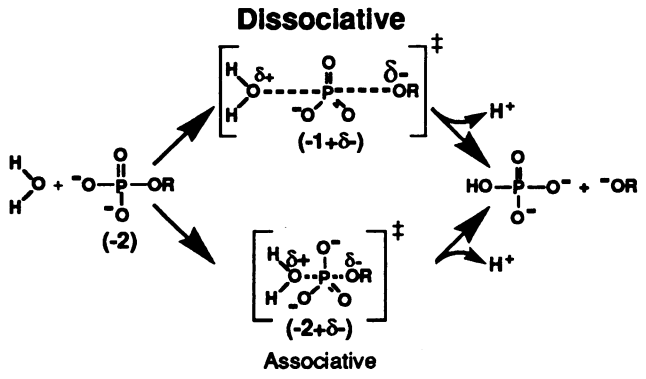
Background: The Nature of the Transition State for GTP Hydrolysis

There is a continuum of potential transition state structures for phosphoryl transfer reactions, ranging from dissociative to associative depending on the nature of the bonding (i.e., the electronic distribution; Scheme I).



Scheme I

A dissociative transition state is dominated by bond cleavage; in the extreme case, the bond to the outgoing leaving group is fully or nearly broken (Scheme I top, large δ−), and the bond to the incoming nucleophile is absent or barely formed (small δ+). To maintain conservation of charge, there must then be a *loss* of charge on the phosphoryl group (i.e., the $-PO_3$ group) being transferred. In contrast, in an associative transition state there is a large amount of bond formation to the incoming nucleophile (Scheme I bottom, large δ+) but only a small amount of bond cleavage to the outgoing leaving group (small δ−); thus, there is a *gain* in charge on the phosphoryl group being transferred. A wealth of physical organic data obtained over the past 40 years has implicated a dissociative, metaphosphate-like transition state in reactions of phosphate monoesters, acyl phosphates, and phosphorylated amines (5–10, †).

Abbreviations: GTP[γS], guanosine 5′-[γ-thio]triphosphate; GMP-PNP, guanylyl β,γ-imidodiphosphate; GMPPCP, guanylyl β,γ-methylenediphosphonate.

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†A transition state can be dissociative or metaphosphate-like in nature and yet occur in a second-order reaction that has some amount of association with an incoming nucleophile in the transition state. Thus, the absence of racemization of stereochemistry of phosphoryl groups in nonenzymatic and enzymatic reactions, although providing evi-

Recent linear free energy relationships have shown that transfer of the γ-phosphoryl group of GTP proceeds via a dissociative, metaphosphate-like transition state, analogous to the previously characterized transition states for reactions of other monosubstituted phosphoryl compounds (4). It has been suggested that metal ion coordination of the γ-phosphoryl oxygens in GTP·Mg could increase the susceptibility of the phosphorus to nucleophilic attack and therefore increase the associative character of the transition state (15–21). However, comparisons of linear free energy relationships for reactions of GTP and GTP·Mg strongly suggest that Mg^{2+} does not increase the associative character of the transition state (ref. 4; see also refs. 22 and 23). Furthermore, large changes in reactivity induce only small changes in the nature of the transition state for phosphoryl transfer, suggesting that these transition states are difficult to change (23–25).

Nevertheless, could the greater preponderance of positively charged side chains and metal ions in an enzymatic active site change the nature of the transition state from dissociative to associative? This proposal was recently tested with *Escherichia coli* alkaline phosphatase, which utilizes two active site zinc ions and an Arg side chain. The large dependence of rate on leaving group pK_a suggested a large amount of bond cleavage in a dissociative transition state (26). Heavy atom isotope effects similarly suggest a dissociative transition state for reactions of two protein phosphatases (27).

In summary, the simplest expectation is that the transition state for GTP hydrolysis at the active site of Ras is dissociative in character. Although not proven, this chemical perspective provides a logical starting point for a discussion of enzymatic catalysis. Any proposal of a predominantly associative transition state for phosphoryl transfer requires a concomitant proposal that some distinct feature of the enzyme changes the transition state.

Previous Mechanistic Proposals for the Ras GTPase Reaction

The change in charge distribution that occurs in proceeding from the ground state to the transition state for GTP hydrolysis is shown in Fig. 1. These changes are used in this section to analyze proposed mechanisms of catalysis by Ras and other G proteins. The purpose is not to survey all previously proposed mechanisms but rather to discuss the most common of these from a chemical perspective.

General Base Catalysis? It has been stated recently that: "The major unresolved problem [of Ras catalysis] is the nature of the general base activating the catalytic water molecule which acts as the nucleophile in the hydrolysis reaction" (28). However, in a dissociative transition state, there is little bond formation to the incoming water nucleophile and therefore little charge development (Scheme **I** and Fig. 1). This means that there would be little advantage from removal of a proton from the attacking water in the transition state. Thus, general base catalysis is not expected to be an important catalytic component (see refs. 23, 29, and 30).

The question of general base catalysis can be addressed quantitatively for the specific proposal that Gln-61 fills this role (31, 32). The hypothetical equilibrium of Reaction **1** depicts proton transfer between the attacking water in the GTPase transition state and the Gln side chain. If the P—O bond to the incoming water is ~0.1 formed in the dissociative

dence against the formation of a discrete free metaphosphate *intermediate*, says nothing about the nature of the *transition state* (7, 11–14). The nature of the transition state is described as "dissociative" for reactions in which there is a decrease in combined bond order to the incoming and departing groups relative to the reactant and "associative" for reactions in which there is an increase in combined bond order.

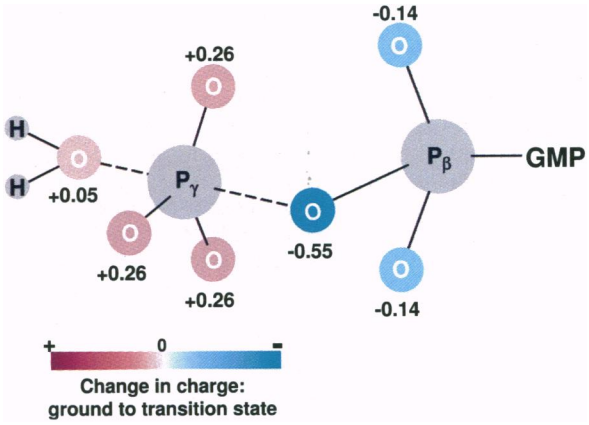
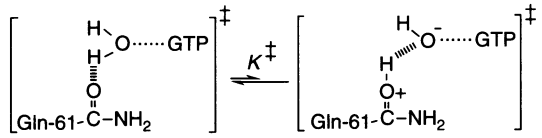


FIG. 1. Changes in charge in the nonenzymatic hydrolysis of GTP^{4-}. The transition state geometry is shown, with color intensity proportional to the charge development in the transition state relative to the ground state. The dark blue color of the β-γ bridge oxygen indicates that it undergoes the largest change in charge. The crude quantitative estimates were derived from linear free energy relationships (4) and are used solely for qualitative arguments in the text. This figure was adapted from ref. 4.

transition state (4), the $pK_a^{\ddagger}$ for loss of a proton from the nucleophilic water is expected to be ~14 in the transition



Reaction 1

state.§ The $pK_a^{\ddagger}$ of 14 is much higher than the $pK_a \approx -2$ for deprotonation of the acid form of the Gln side chain carbonyl [i.e., $Gln(C{=}OH^+)NH_2$] (33).

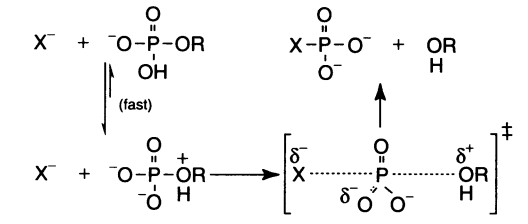
This means that the hypothetical equilibrium of Reaction **1** lies very far to the left ($K^{\ddagger}_{estimated} \approx 10^{-16}$) so that no significant proton transfer from the attacking water to Gln-61 is expected in a dissociative transition state (see also ref. 34).

Replacement of Gln-61 by Glu increases the rate of GTP hydrolysis by 20-fold, and this result has been considered evidence that Gln-61 acts as a general base (35). However, Glu would not be expected to abstract a proton in the transition state for the same reasons outlined above for Gln. Perhaps the migration of negative charge away from the attacking water and transferred phosphoryl group in going from the ground state to the transition state (Fig. 1, red coloration) is stabilized by positioning the negatively charged Glu near to the attacking water (4). The rate enhancement from the Glu substitution could also result from better positioning of the attacking water molecule with respect to the γ-phosphoryl group or from an increase in the strength of the hydrogen bond to the attacking water molecule in the transition state (see below).

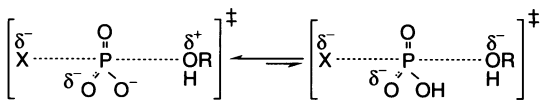
§This pK_a was *estimated* as follows. The value of ~0.1 bond formation is derived from a linear free energy relationship with a slope of $\beta_{nucleophile} \approx 0.1$ (4). $\beta_{nucleophile} = 0$ would correspond to a transition state in which the attacking water is electrostatically equivalent to free water (H_2O) and thus has a pK_a of 16; $\beta_{nucleophile} = 1$ would correspond to a transition state in which the attacking water is electrostatically equivalent to hydronium ion (H_2O—H^+), and thus has a pK_a of −2. The observed $\beta_{nucleophile}$ of 0.1 corresponds to electrostatic behavior 0.1 of the way from H_2O to H_2O—H^+ and thus gives an estimated $pK_a^{\ddagger} = 14$ {$= 16 - 0.1 \times [16 - (-2)]$}.

More recent mechanistic proposals have invoked the γ-phosphoryl group of GTP as a general base catalyst in the Ras and related GTPase reactions (refs. 20 and 35–38; see also ref. 39). However, protonation of the γ-phosphoryl group is expected to destabilize a dissociative transition state, not stabilize it. This is because protonation stabilizes electron density on the phosphoryl oxygen, whereas electron density is expected to be donated *from* the phosphoryl oxygen atoms to achieve the dissociative transition state (Scheme **I** and Fig. 1, red shading). The ~10^3-fold slower nonenzymatic reactions of phosphate diesters than phosphate monoesters provide experimental evidence for such an "anticatalytic" effect, with the alkyl group of the diester providing a model for protonation (40). The destabilization of the transition state from protonation of a γ-phosphoryl oxygen atom described above would hold whether the proton transfer to the γ-phosphoryl group were direct or involved an intermediary (20, 35–39, ¶).

Nonenzymatic reactions of phosphate monoester monoanions provide strong evidence that protonation of the transferred phosphoryl group in the transition state is not the preferred path for a dissociative reaction. In these reactions, the proton is completely or nearly completely transferred to the leaving group in the transition state (Reaction **2**) (6, 43–45). This indicates that the hypothetical transition state depicted on the left in Reaction **3**, with the proton on the leaving group, is lower in energy than that on the right, with the proton on the phosphoryl group. Reaction **2** is simply the reverse of the GTPase reaction, with ROH being water (R = H) and X-PO_3 being GTP (X = GDP; the forward and reverse reactions must proceed through the same transition state, according to the principle of microscopic reversibility). The mechanism in which a γ-phosphoryl oxygen acts as a general base and is protonated leads to a transition state resembling the higher energy species on the right in Reaction **3**, whereas the proton resides on the nucleophile or leaving group in the classical mechanism.



Reaction 2



Reaction 3

These observations do not prove that the γ-phosphoryl group does not act as a general base in enzymatic reactions, although this proposal is the antithesis of the mechanism followed in the dissociative nonenzymatic reaction.

Possible Roles for a General Base. A "general base" or hydrogen bond acceptor could aid the overall process of GTP hydrolysis, even if the Ras-catalyzed GTPase reaction proceeds via a dissociative transition state with no significant proton transfer.

(*i*) The final reaction product is not H_2O^+—$PO_3^=$. There must therefore be a pathway for loss of a proton from water, even if this loss occurs after the rate-limiting transition state and is therefore not involved in stabilizing this transition state, as suggested above (23, 29).

(*ii*) Even in a dissociative transition state, there is typically some nucleophilic participation.† Thus, hydrogen bonds from the water protons to Gln-61 and Thr-35 can be strengthened in the transition state. However, the increase in strength is expected to be small for the Ras reaction due to the small amount of nucleophilic participation in GTP hydrolysis and the correspondingly small increase in charge density on the water protons in the transition state (Fig. 1) (4).

(*iii*) A water molecule that appears correctly positioned for nucleophilic attack is observed in the x-ray crystal structure of Ras and related G proteins with bound GTP and GTP analogs (21, 32, 46, 47). Significant rate advantages from positioning of the attacking group have been observed in model phosphoryl transfer reactions that proceed via dissociative transition states (29). The ~10-fold decrease in the turnover rate upon mutation of Gln-61, which appears to interact with the nucleophilic water (Fig. 2) (48–50), is most simply accounted for by a disruption in positioning of the nucleophilic water with respect to the γ-phosphoryl group (32).

Stabilization of Charge Buildup on the γ-Phosphoryl Oxygen Atoms? It has been proposed that Arg-178 of the $G_{i\alpha}$ subunit of a trimeric G protein assists catalysis "by stabilizing the developing negative charge on the γ phosphate" (51). Although Ras lacks a homologous residue (see *Related Proposal for Activation of the Ras GTPase by GAPs*), such roles have been suggested for Lys-16 (21, 32, 46, 47, 52), Gln-61 (21), the backbone amide of Gly-60 (21), and the bound magnesium ion (21, 32, 52) of Ras (Fig. 2). Indeed, analogous proposals have been put forth for many kinases, phosphatases, and related enzymes (discussed in ref. 4). However, negative charge does not accumulate on the γ-phosphoryl group in a dissociative transition state (Fig. 1). Thus, these interactions are not predicted to provide electrostatic catalysis unless the enzyme changes the nature of the transition state from dissociative to associative.†‖ Although electrostatic interactions with the γ-phosphoryl oxygens are not predicted to be strengthened in a dissociative transition state and may even be somewhat weakened (22), such interactions may be present because they are important for binding and positioning the reactive phosphoryl group (4, 53). These interactions cannot be taken as evidence that the transition state is associative rather than dissociative.

Interactions with the Nonbridging β-Phosphoryl Oxygens? The increase in negative charge on the nonbridging β-phosphoryl oxygens in the transition state allows electrostatic interactions to be catalytic, although the charge change on these oxygens is less than that on the β-γ bridge oxygen (Fig. 1). There is ample precedent from nonenzymatic reactions for

¶The proposal that the minor tautomer of Gln [i.e., −C(OH)=NH] is formed by picking up a proton on the carbonyl oxygen from the attacking water and donating a proton to a γ-phosphoryl oxygen (37) has an additional problem. Formation of this tautomer is highly unfavorable ($\Delta G_{taut} \approx 10$ kcal/mol; 41, 42) so that the proton transfer or active site would need to provide an enormous amount of compensating transition state stabilization.

‖The catalytic proposal for Arg-178 of $G_{i\alpha}$ was derived in part from analysis of the $G_{i\alpha}$·GDP·AlF_4 complex (51). This complex has been considered a transition state analog because AlF_4^- is planar, analogous to the transferred phosphoryl group (PO_3) in a trigonal bipyramidal transition state. However, AlF_4^- has four fluoride substituents around the central atom instead of the three oxygen atoms of the transition state. Nevertheless, AlF_4^- binds strongly (54), and the crystal structure shows interactions with all four of the fluorides in a site presumably designed to bind the three phosphoryl oxygens. This suggests that the structure with AlF_4^- is rearranged relative to the actual transition state. The Arg that interacts with two fluorides in this complex could interact with one nonbridging phosphoryl oxygen and the β-γ bridging oxygen in the transition state, as has also been proposed (37) and is consistent with expectations from chemical precedent (see text).

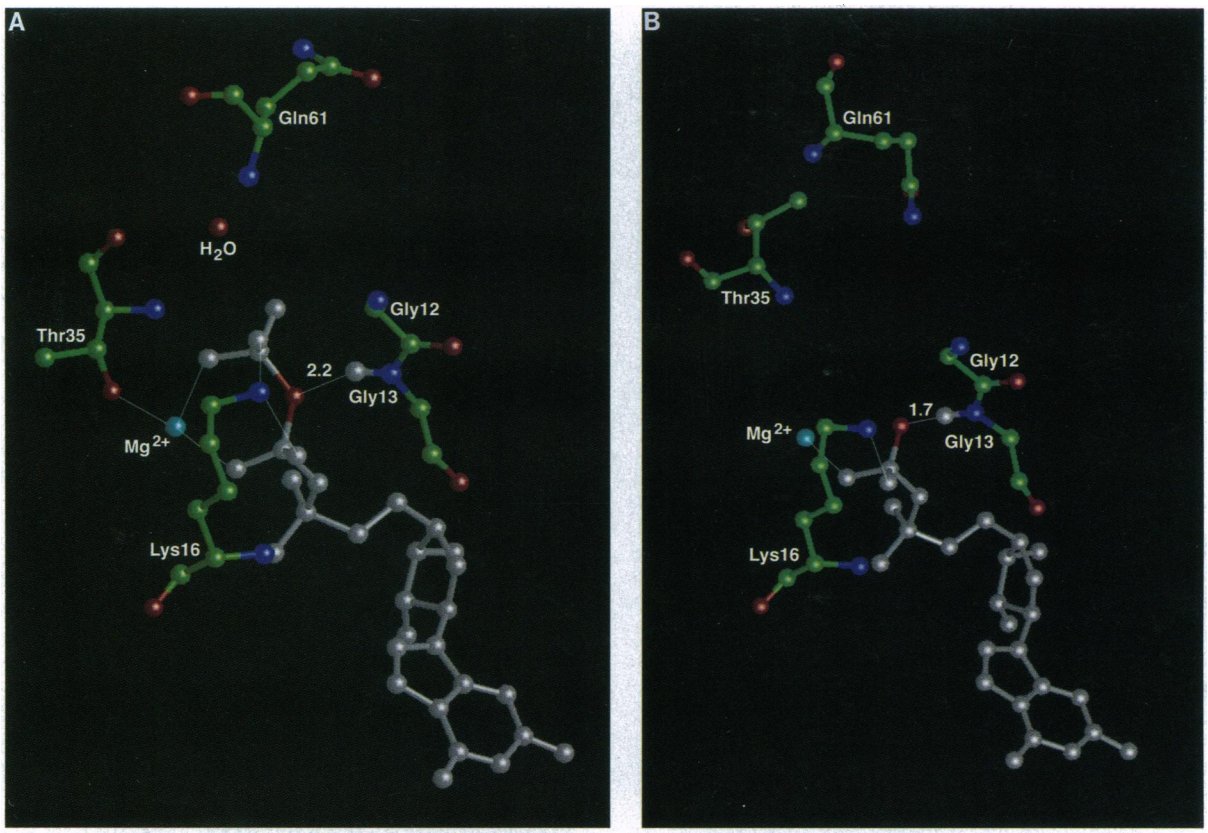


FIG. 2. X-ray crystal structure of the Ras active site with bound GMPPNP, a GTP analog (*A*), and with bound GDP (*B*). The β-γ bridge atom is shown in red, the rest of the GTP analog or GDP is in gray, the positioned water is in red, and the magnesium ion is in cyan. Active site amino acids are depicted with carbons in green, oxygens in red, and nitrogens in blue. The hydrogen atom of the Gly-13 backbone amide is shown in gray, and the distance (in ångstroms) between this hydrogen and the β-γ bridge atom is shown.

catalysis of phosphoryl transfer via interactions with the leaving group (see refs. 9, 22, and 55). The bound Mg^{2+} and Lys-16 have been suggested to play this role (Fig. 2) (32, 46, 52). Mg^{2+} is also bound in solution, so that no large catalytic enhancement is expected relative to the solution reaction in the absence of significant perturbations within the active site.

Catalysis via Stabilization of the GDP Leaving Group: A New Proposal

As an enzyme must preferentially stabilize a reaction's transition state relative to the substrate, one might anticipate a catalytic interaction with the atom that undergoes the largest change in charge upon reaching the transition state. For the GTPase reaction in solution, that atom is the β-γ bridge oxygen, as depicted in Fig. 1. In the GTP ground state, it is attached to two electron-withdrawing phosphoryl groups (56) and thus has a low electron density. However, in a dissociative transition state, this bond is nearly broken so that the charge will approach −0.67, the formal charge on the β-phosphoryl oxygens of the GDP^{3-} product (4).

Structural data support the possibility of such a catalytic interaction for Ras. The backbone amide of Gly-13 is positioned to donate a hydrogen bond to the β-γ bridge oxygen of GTP (Fig. 2*A*) (21, 32, 46). The analogous hydrogen bond is made to a β-phosphoryl oxygen when the GDP product is bound (Fig. 2*B*). Thus, it is also likely to be present in the transition state. We propose that the hydrogen bond from Gly-13 is catalytic. It is expected to be stronger in the transition state than the ground state because of the much higher electron density of the bridging oxygen in the transition state than in GTP.**

A backbone amide positioned to donate a hydrogen bond to the β-γ bridge oxygen is conserved among all of the small and large G proteins whose structures with bound GTP, GTP analog, or GDP have been solved. The N(H)··O heteroatom distances and N—H··O angles range from 2.7 to 3.5 Å and 131 to 171°, with average values of 3.1 Å and 147°, respectively (21, 32, 46, 51, 58–62, ††). These hydrogen bond distances and angles are similar to the values observed for hydrogen bonds donated by backbone amides in peptides and proteins of 2.9–3.1 Å and 150–160° (63). Furthermore, the residue whose amide donates the hydrogen is conserved in its position in the primary sequence: GXGXXGK(S/T) (61, 62). This sequence motif is observed in kinases and other enzymes that carry out related phosphoryl transfer reactions, where the analogous backbone amide/bridge oxygen hydrogen bond is also observed (not shown).

Unusually downfield ^1H NMR chemical shifts also provide evidence for hydrogen bond donation from the Gly-13 amide proton. The amide proton of Gly-13 in the Ras·GDP complex

**Solution reactions of phosphate diesters, which proceed via a transition state with less dissociative character, also experience a large change in charge at the leaving group oxygen in going from the ground state to the transition state (23, 57). Thus, the hydrogen bond from the backbone amide of Gly-13 could be catalytic even if Ras were to change the nature of the transition state to one with more associative character.

††For Ras and EF-Tu with bound GMPPNP, the N(H)··N(P) distances were used. Backbone amide hydrogens are essentially fixed in the plane of the amide bond. This allowed their positions to be modeled with INSIGHTII (version 2.3.0, BioSym Technologies, San Diego) for the angle determinations.

is deshielded, as expected for participation in a hydrogen bond, with a chemical shift of $\delta^H = 10.4$; a chemical shift of $\delta^H = 9.2$ was suggested for this amide proton in the Ras·GTP[γS] complex (64–68). Analogous downfield amide proton resonances with bound GDP and GTP have also been suggested for EF-Tu, which is homologous to Ras (69).

The GTP analogs GMPPNP and GMPPCP appear to bind Ras significantly weaker than GTP (70). This is consistent with expected energetic effects: a hydrogen bond between Gly-13 and the bridge nitrogen of GMPPNP may be weaker than that to the bridge oxygen of GTP and the bridge methylene would be unable to participate in a hydrogen bond (46). In addition, GTP binds only ~10-fold stronger than GDP, despite several interactions with the γ-phosphoryl group (71, 72). A destabilizing interaction of the amide of Gly-13 and the β-γ bridge oxygen of GTP, relative to interactions in aqueous solution, could contribute to this difference (73, 74), although other explanations are also possible.

Although the catalytic advantage from increased hydrogen bond strength can in principle be large (e.g., ref. 75 and S. Shan and D.H., unpublished results), the overall catalysis by Ras is only ~10^3-fold (S.J.A. & D.H., unpublished results; ref. 72, §§). An enzyme such as Ras that requires a low intrinsic reaction rate for its biological function may not maximize the difference in ground versus transition state hydrogen bond strength. This could be accomplished by using a hydrogen bond donor such as a backbone amide that is weak relative to positively charged side chains, by leaving possible stabilizing hydrogen bonding interactions unfulfilled, or by forming an active site with a relatively high effective dielectric (refs. 74, 75, and 77 and S. Shan and D.H., unpublished results). Other enzymes might then maximize the rate enhancement by more precise positioning of the backbone amide hydrogen bond and/or by positioning a second, stronger hydrogen bond donor for interaction with the β-γ bridge oxygen (see below) and by altering the electrostatic environment of the active site.

Effect of Gly-13 and Gly-12 Mutations. Mutations that add a side chain at position 13 have little effect on the intrinsic GTPase activity (34), suggesting that a catalytic interaction with the backbone amide, if present, is not perturbed. Although no structures are available for these mutants, inspection of wild-type structures suggests that a side chain at position 13 would point away from the protein interior and into the solvent. These mutations do, however, perturb GAP activation, consistent with a nearby interaction with GAP (refs. 34 and 79; also see below).

A majority of the oncogenic mutations in Ras are located at position 12. These mutations have impaired intrinsic GTPase activity, and many are also insensitive to GAP activation (80, 81). In the three cases for which there is both structural and biochemical data, active site perturbations near the γ-phosphate and nucleophilic water molecule are observed. In the G12R mutant, the Arg-12 side chain is located in front of the γ-phosphate of GTP, displacing the proposed nucleophilic water molecule (48). When Val is located at position 12, the side chain has an orientation similar to Arg in the G12R structure. As a result, the hydrophobic side chain would be close to the negatively charged γ-phosphoryl group. This unfavorable interaction presumably results in movement of the phosphate binding loop and loop L4 away from the γ-phosphate. These changes also result in a 0.7-Å increase in the Gly-13 β-γ bridge oxygen hydrogen bond distance and a loss in the interactions of the γ-phosphoryl group and nucleophilic water with Gly-60 and Gln-61 of loop L4 (48). In the G12D structure, the negatively charged side chain Asp hydrogen bonds with Gln-61, preventing it from interacting with the nucleophilic water molecule or the γ-phosphoryl group (82).

In contrast, the nononcogenic mutant G12P has a GTPase activity similar to wild-type Ras (82). As expected, its crystal structure is almost identical to the wild type. The heteroatom distance for the hydrogen bond between the Gly-13 amide and the β-γ bridge atom is 3.2 Å, with an angle of 142°, compared with 3.1 Å and 148° for wild type (GMPPNP structures; refs. 32 and 82).

These observations are all consistent with a catalytic role of the backbone amide of Gly-13, but they do not provide a significant test of the proposal.

Related Proposal for Activation of the Ras GTPase by GAPs. GTPase Activating Proteins, or GAPs, stimulate the GTPase activity of Ras and related small G proteins, playing an important role in turning off signaling by Ras·GTP (83). Combining the catalytic insights described above with previous observations and proposals (84–87) leads to the following specific hypothesis about the origin of this activation: in addition to the hydrogen bond donated from Gly-13 of Ras to the β-γ bridge oxygen atom of GTP, an Arg residue of GAP also donates a hydrogen bond to this bridge oxygen, thereby further stabilizing the transition state.

One of several literature suggestions for the action of GAP is that it donates one or more catalytic residues (32, 84–87). Precedent for this comes from an intermolecular contact to the γ-phosphoryl group of a GTP analog in one crystal form of Ras (32). One candidate catalytic residue is an Arg, as trimeric G proteins have a conserved Arg that makes contacts within the active site but is absent in Ras (61, 88). The G_α subunits hydrolyze GTP ~100-fold faster than Ras, and mutation of this Arg residue of G_α lowers the GTPase activity to a level similar to that of Ras (84, 87, 89). Relating these observations to GAP, Arg-903 of GAP contributes to the ability of GAP to activate Ras, but it is not necessary for binding of GAP to Ras. This is consistent with a catalytic role for Arg-903 (85, 86).

Following the analysis of the nature of the GTPase transition state described above; the β-γ bridge oxygen is likely to be involved in a catalytic interaction. Indeed, an interaction of the β-γ bridge oxygen and the conserved Arg of transducin is observed in the crystal structure (Arg-174; N(H)··O bond distance is 2.95 Å, and the bond angle is 131° with bound GTP[γS]) (61). Finally, the structure of Ras with a bound GTP analog reveals that this bridging oxygen is exposed at the base of a crevice (Fig. 3). We therefore propose that an Arg residue donated from GAP is positioned in the Ras crevice and preferentially stabilizes the transition state by donating a hydrogen bond to the β-γ bridge oxygen. The Arg would presumably be anchored in position via an additional interaction with a nonbridging oxygen of the γ-phosphoryl group, although this interaction would not be strengthened in a dissociative transition state. Additional catalytic interactions are suggested by the larger effect of GAP on the Ras reaction than the effect of the active site Arg of the trimeric G proteins (10^5- versus 10^2-fold) (87, 90, 91).

Summary and Perspective

We have evaluated mechanistic proposals for Ras catalysis from a chemical perspective. The analysis suggests that several proposed mechanisms would not be catalytic if the transition state for the enzymatic reaction is dissociative, as observed in solution. This transition state is not easily changed in solution, and several enzymatic reactions appear to proceed via dissociative transition states. Thus, the simplest expectation is that a dissociative transition state will also be followed on Ras and related GTPases.

§§In order for a hydrogen bond to provide a catalytic *advantage*, the active site interaction must increase in strength in going from the ground to transition state more than the interaction with water in the corresponding solution reaction. Mechanisms that enzymes can employ to provide such an advantage have been extensively discussed (e.g., refs. 73–75 and 78).

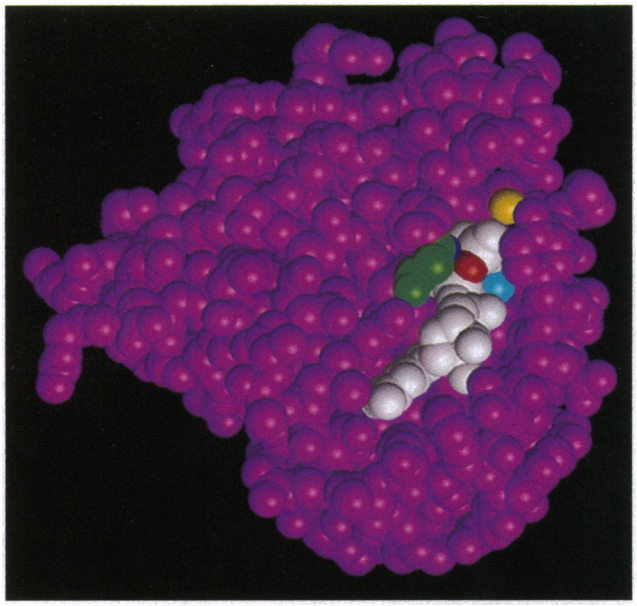


FIG. 3. Space filling model of Ras with the GTP analog GMPPNP bound (32). The β-γ bridge atom, shown in red, is accessible from solvent. The rest of the nucleotide is shown in white; Gly-13 is shown in green, with its amide hydrogen atom highlighted in blue and the rest of the protein in purple; the magnesium ion is shown in cyan, and the catalytic water is in yellow. This view is from the backside of Ras, relative to the view in Fig. 2.

This analysis has led to a new catalytic proposal, that the backbone amide of Gly-13 donates a hydrogen bond to the β-γ bridge oxygen of GTP that is strengthened in the transition state. Other interactions can also contribute to the modest catalysis of Ras. Interactions of the β-nonbridging oxygens with Lys-16 and Mg^{2+} may be strengthened in the transition state. Indeed, we cannot predict which interaction would undergo a larger increase in strength in the transition state: a hydrogen bond from the neutral Gly-13 donor to the β-γ bridge oxygen, which undergoes the largest change in charge, or a hydrogen bond from a stronger, positively charged Lys-16 donor to one or both of the β-phosphoryl oxygens, which undergo a smaller change in charge (4, 78, 92). Interactions that are not strengthened electrostatically in the transition state may also be catalytic. For example, hydrogen bonds between the nucleophilic water and Gln-61 and Thr-35 may help position the water with respect to the γ-phosphoryl group and lower the entropic barrier for reaction. More generally, active sites may be considered as transition state templates that provide electrostatic and geometrical complementarity and allow exploitation of the changes between the ground and transition states (e.g., refs. 54, 76, 77, and 93).

The principles outlined herein for G proteins are expected to hold more widely for other biological phosphoryl transfers. All enzymes catalyzing $-PO_3^{2-}$ transfer must deal with the same dissociative transition state, either stabilizing it or perturbing it by providing much greater stabilization to an alternative transition state.

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1. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56,** 779–827.
2. Bourne, H. R., Sanders, D. A. & McCormick, F. (1990) *Nature (London)* **348,** 125–132.
3. Lowy, D. R. & Williamson, B. M. (1993) *Annu. Rev. Biochem.* **62,** 851–891.
4. Admiraal, S. J. & Herschlag, D. (1995) *Chem. Biol.* **2,** 729–739.
5. Thatcher, G. R. J. & Kluger, R. (1989) *Adv. Phys. Org. Chem.* **25,** 99–265.
6. Kumamoto, J. & Westheimer, F. H. (1955) *J. Am. Chem. Soc.* **77,** 2515–2518.
7. Herschlag, D. & Jencks, W. P. (1989) *J. Am. Chem. Soc.* **111,** 7579–7586.
8. Butcher, W. W. & Westheimer, F. H. (1955) *J. Am. Chem. Soc.* **77,** 2420–2424.
9. Benkovic, S. J. & Schray, K. J., (1978) in *Transition States of Biochemical Processes*, ed. Gandour, R. D. (Plenum, New York), pp. 493–528.
10. Barnard, P. W. C., Bunton, C. A., Llewellyn, D. R., Oldham, K. G., Silver, B. L. & Vernon, C. A. (1955) *Chem. Ind. (London)* 760–763.
11. Buchwald, S. L., Friedman, J. M. & Knowles, J. R. (1984) *J. Am. Chem. Soc* **106,** 4911–4916.
12. Guthrie, R. D. & Jencks, W. P. (1989) *Acc. Chem. Res.* **22,** 343–349.
13. Jencks, W. P. (1981) *Chem. Soc. Rev.* **10,** 345–375.
14. Knowles, J. R. (1980) *Annu. Rev. Biochem.* **49,** 877–919.
15. Kirby, A. J. & Jencks, W. P. (1965) *J. Am. Chem. Soc.* **87,** 3209–3216.
16. Williams, A. & Naylor, R. A. (1971) *J. Chem. Soc. B* 1973–1979.
17. Benkovic, S. J. & Schray, K. J., (1973) in *The Enzymes,* ed. Boyer, P. D. (Academic, New York), pp. 201–238.
18. Hassett, A., Blattler, W. & Knowles, J. R. (1982) *Biochemistry* **21,** 6335–6339.
19. Mildvan, A. S. & Fry, D. C. (1987) *Adv. Enzymol.* **59,** 241–313.
20. Schweins, T., Langen, R. & Warshel, A. (1994) *Nat. Struct. Biol.* **1,** 476–484.
21. Prive, G. G., Milburn, M. V., Tong, L., DeVos, A. M., Yamaizumi, Z., Nishimura,. S. & Kim, S.-H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3649–3653.
22. Herschlag, D. & Jencks, W. P. (1987) *J. Am. Chem. Soc.* **109,** 4665–4674.
23. Herschlag, D. & Jencks, W. P. (1989) *J. Am. Chem. Soc.* **111,** 7587–7596.
24. Skoog, M. T. & Jencks, W. P. (1984) *J. Am. Chem. Soc.* **106,** 7597–7606.
25. Jencks, W. P. (1985) *Chem Rev.* **85,** 511–527.
26. Hollfelder, F. & Herschlag, D. (1995) *Biochemistry* **34,** 12255–12264.
27. Hengge, A. C., Sowa, G. A., Wu, L. & Zhang, Z.-Y. (1995) *Biochemistry* **34,** 13982–13987.
28. Hilgenfeld, R. (1995) *Nat. Struct. Biol.* **2,** 3–6.
29. Herschlag, D. & Jencks, W. P. (1990) *Biochemistry* **29,** 5172–5179.
30. Jencks, W. P. (1972) *J. Am. Chem. Soc.* **94,** 4731–4732.
31. Goody, R. S., Pai, E. F., Schlichting, I., Rensland, H., Scheidig, A., Franken, S. & Wittinghofer, A. (1992) *Philos. Trans. R. Soc. Lond. B* **336,** 3–11.
32. Pai, E. F., Krengel, U., Petsko, G. A., Goody, R. S., Kabsch, W. & Wittinghofer,. A. (1990) *EMBO J.* **9,** 2351–2359.
33. Lowry, T. H. & Richardson, K. S. (1987) *Mechanism and Theory in Organic Chemistry* (Harper & Row, New York), 3rd Ed., pp. 297–298.
34. Chung, H., Benson, D. & Schultz, P. G. (1993) *Science* **259,** 806–809.
35. Frech, M., Darden, T. A., Pedersen, L. G., Foley, C. K., Charifson, P. S., Anderson, M. W. & Wittinghofer, A. (1994) *Biochemistry* **33,** 3237–3244.
36. Schweins, T., Geyer, M., Scheffzek, K., Warshel, A., Kalbitzer, H. R. &. Wittinghofer, A. (1995) *Nat. Struct. Biol.* **2,** 36–44.
37. Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E. & Sigler, P. B. (1994) *Nature (London)* **372,** 276–279.
38. Langen, R., Schweins, T. & Warshel, A. (1992) *Biochemistry* **31,** 8691–8696.
39. Fisher, A. J., Smith, C. A., Thoden, J. B., Sutoh, K., Holden, H. M. & Rayment, I. (1995) *Biochemistry* **34,** 8960–8972.
40. Khan, S. A. & Kirby, A. J. (1970) *J. Chem. Soc. B* 1172–1182.
41. Fersht, A. R. (1971) *J. Am. Chem. Soc.* **93,** 3504–3515.
42. Schlegel, H. B., Gund, P. & Flunder, E. M. (1982) *J. Am. Chem. Soc.* **104,** 5347–5351.
43. Bunton, C. A., Llewellyn, D. R., Oldham, K. G. & Vernon, C. A. (1958) *J. Chem Soc.* 3574–3587.
44. Hengge, A. C., Edens, W. A. & Elsing, H. (1994) *J. Am. Chem. Soc.* **116,** 5045–5049.
45. Kirby, A. J. & Varvoglis, A. G. (1967) *J. Am. Chem. Soc.* **89,** 415–423.
46. Schlichting, I., Almo, S. C., Rapp, G., Wilson, K., Petratos, K., Lentfer, A., Wittinghofer, A., Kabsch, W., Pai, E. F., Petsko, G. A. & Goody, R. S. (1990) *Nature (London)* **345,** 309–315.
47. Milburn, M. V., Tong, L., DeVos, A. M., Brunger, A., Yamaizumi, Z., Nishimura, S. & Kim, S.-H. (1990) *Science* **247,** 939–945.
48. Krengel, U., Schlichting, I., Scherer, A., Schumann, R., Frech, M., John, J., Kabsch, W., Pai, E. F. & Wittinghofer, A. (1990) *Cell* **62,** 539–548.
49. John, J., Frech, M. & Wittinghofer, A. (1988) *J. Biol. Chem.* **263,** 11792–11799.
50. Der, C. J., Finkel, T. & Cooper, G. M. (1986) *Cell* **44,** 167–176.
51. Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G. & Sprang, S. R. (1994) *Science* **265,** 1405–1412.
52. Wittinghofer, A. & Pai, E. F. (1991) *Trends Biochem. Sci.* **16,** 382–387.
53. Herschlag, D. & Jencks, W. P. (1990) *J. Am. Chem. Soc.* **112,** 1942–1950.
54. Higashijima, T., Graziano, M. P., Suga, H., Kainosho, M. & Gilman, A. G. (1991) *J. Biol. Chem.* **266,** 3396–3401.
55. Cooperman, B. S., (1976) in *Metal Ions in Biological Systems*, ed., Sigel, H. (Dekker, New York), pp. 79–125.
56. Bourne, N. & Williams, A. (1984) *J. Org. Chem.* **49,** 1200–1204.
57. Williams, A. (1992) *Adv. Phys. Org. Chem.* **27,** 1–55.
58. la Cour, T. F. M., Nyborg, J., Thirup, S. & Clark, B. F. C. (1985) *EMBO J.* **4,** 2385–2388.

59. Kjeldgaard, M., Nissen, P., Thirup, S. & Nyborg, J. (1993) *Structure* **1,** 35–50.
60. Tong, L., DeVos, A. M., Milburn, M. V. & Kim, S. H. (1991) *J. Mol. Biol.* **217,** 503–516.
61. Noel, J. P., Hamm, H. E. & Sigler, P. B. (1993) *Nature (London)* **366,** 654–663.
62. Lambright, D. G., Noel, J. P., Hamm, H. E. & Sigler, P. B. (1994) *Nature (London)* **369,** 621–628.
63. Baker, E. N. & Hubbard, R. E. (1984) *Prog. Biophys. Mol. Biol.* **44,** 97–179.
64. Campbell-Burk, S., Papastavros, M. Z., McCormick, F. & Redfield, A. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 817–820.
65. Campbell-Burk, S. (1989) *Biochemistry* **28,** 9478–9484.
66. Miller, A.-F., Papastavros, M. Z. & Redfield, A. G. (1992) *Biochemistry* **31,** 10208–10216.
67. Miller, A. F., Halkides, C. J. & Redfield, A. G. (1993) *Biochemistry* **32,** 7367–7376.
68. Redfield, A. G. & Papastavros, M. Z. (1990) *Biochemistry* **29,** 3509–3514.
69. Limmer, S., Reiser, C. O. A., Schirmer, N. K., Grillenbeck, N. W. & Sprinzl, M. (1992) *Biochemistry* **31,** 2970–2977.
70. John, J., Schlichting, I., Schiltz, E., Rosch, P. & Wittinghofer, A. (1989) *J. Biol. Chem.* **264,** 13086–13092.
71. John, J., Sohmen, R., Feuerstein, J., Linke, R., Wittinghofer, A. & Goody, R. S. (1990) *Biochemistry* **29,** 6058–6065.
72. Neal, S. E., Eccleston, J. F., Hall, A. & Webb, M. R. (1988) *J. Biol. Chem.* **263,** 19718–19722.
73. Narlikar, G. J., Gopalakrishnan, V., McConnell, T. S., Usman, N. & Herschlag, D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3668–3672.
74. Herschlag, D., Eckstein, F. & Cech, T. R. (1993) *Biochemistry* **32,** 8312–8321.
75. Shan, S., Loh, S. & Herschlag, D. (1996) *Science* **272,** 97–101.
76. Wolfenden, R. (1972) *Acc. Chem. Res.* **5,** 10–18.
77. Stahl, N. & Jencks, W. P. (1986) *J. Am. Chem. Soc.* **108,** 4196–4205.
78. Jencks, W. P. (1975) *Adv. Enzymol.* **43,** 219–410.
79. McCormick, F. (1990) *Oncogene* **5,** 1281–1283.
80. Seeburg, P. H., Colby, W. W., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **312,** 71–75.
81. Trahey, M. & McCormick, F. (1987) *Science* **238,** 542–545.
82. Franken, S. M., Scheidig, A. J., Krengel, U., Rensland, H., Lautwein, A., Geyer, M., Scheffzek, K., Goody, R. S., Kalbitzer, R., Pai, E. F. & Wittinghofer, A. (1993) *Biochemistry* **32,** 8411–8420.
83. Boguski, M. S. & McCormick, F. (1993) *Nature (London)* **366,** 643–654.
84. McCormick, F. (1989) *Nature (London)* **340,** 678–679.
85. Brownbridge, G. G., Lowe, P. N., Moore, K. J. M., Skinner, R. H. & Webb, M. R. (1993) *J. Biol. Chem.* **268,** 10914–10919.
86. Skinner, R. H., Bradley, S., Brown, A. L., Johnson, N. J. E., Rhodes, S., Stammers, D. K. & Lowe, P. N. (1991) *J. Biol. Chem.* **266,** 14163–14166.
87. Freissmuth, M. & Gilman, A. G. (1989) *J. Biol. Chem.* **264,** 21907–21914.
88. Valencia, A., Chardin, P., Wittinghofer, A. & Sander, C. (1991) *Biochemistry* **30,** 4637–4648.
89. Landis, C. A., Masters, S. B., Spada, A., Pace, A. M., Bourne, H. R. & Vallar, L. (1989) *Nature (London)* **340,** 692–696.
90. Gideon, P., John, J., Frech, M., Lautwein, A., Clark, R., Scheffler, J. E. &. Wittinghofer, A. (1992) *Mol. Cell. Biol.* **12,** 2050–2056.
91. Linder, M. E., Ewald, D. A., Miller, R. J. & Gilman, A. G. (1990) *J. Biol. Chem* **265,** 8243–8251.
92. Hine, J. (1972) *J. Am. Chem. Soc.* **108,** 5766–5771.
93. Lienhard, G. E. (1973) *Science* **180,** 149–154.