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Miller, Tennyson, and Sutcliffe and many others has played a great role in understanding the H_3^+ spectrum in the laboratory and in space (*10*). Polyansky *et al.* (*3*) have demonstrated the power of this new method in approximately doubling the energy range for which assignments of the quantum states of H_2O have been made. This has led to an understanding of the spectral lines in the figure and a great many more in other frequency regions. It also revealed new spectroscopic features of hot water, such as strong rotational difference transitions induced by Fermi resonance and large splittings of normally nearly degenerate levels.

Over a longer term, the following two points come to mind. (i) The discovery of water on the sun, together with the recent observation of H_2O on many objects based on data from the Infrared Space Observatory satellite (*11*), is a part of the ongoing development of molecular astrophysics. Molecules are found everywhere, and it is now accepted that half of visible interstellar matter in this galaxy is in the form of molecules (*12*). This interplay of astronomy and chemistry will no doubt increase. (ii) The major remaining approximation in calculations of the sort that Polyansky *et al.* have done is the Born-Oppenheimer separation of electronic and nuclear motion. We can hope that full quantum mechanical treatment of the whole system without this approximation may now be possible, at least for simple molecules like H_3^+, with development of even more powerful computers.

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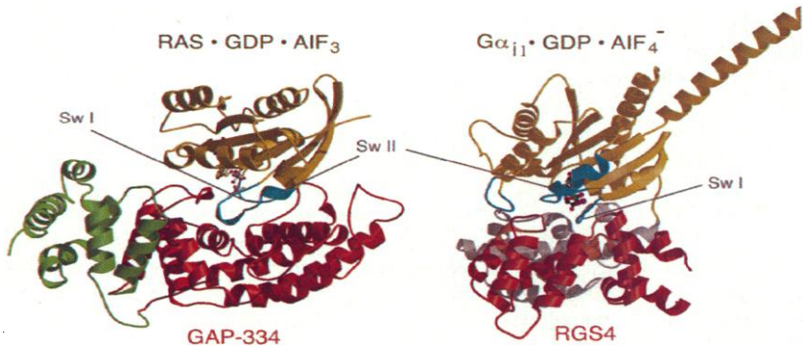
PROTEIN STRUCTURE

GAP into the Breach

Stephen R. Sprang

Regulatory pathways inside cells often make use of guanosine triphosphatases (GTPases) to turn cellular functions on and off. The signals transduced by GTPases such as Ras and heterotrimeric GTP-binding protein α subunits (Gα) (*1*) are transient. In the guanosine triphosphate (GTP)–bound state, these enzymes interact with downstream effectors but dissociate upon GTP hydrolysis. If Ras and Gα were efficient enzymes, the signals would be too short-lived to be effective; consequently they have evolved low catalytic rates. Ras, for example, hydrolyzes GTP with a rate constant of only 0.02 min^{-1}. These signals are switched off by GTPase-activating proteins (GAPs), which accelerate the rate of Ras-catalyzed GTP hydrolysis by a factor of 10^5 (*2*, *3*). Efforts to understand how GAPs function have now culminated in the crystal structure of the complex between Ras and the active, 334-residue fragment of $p120^{GAP}$, reported on page 333 of this issue by Scheffzek, Wittinghofer, and their colleagues (*4*). In this complex, guanosine diphosphate (GDP), aluminum trifluoride, and Mg^{2+} are bound to Ras and mimic the transition state for GTP hydrolysis.



The structures of Ras-GAP and $G\alpha_{i1}$-RGS. (**Left**) The complex between GAP-334 (red and green) bound to Ras•GDP•Mg^{2+}•AlF_3 (mustard yellow), with switch I and II segments in cyan. (**Right**) Shown in a similar orientation is the complex between RGS4 (red) and $G\alpha_{i1}$•GDP•Mg^{2+}•AlF_4^- (*15*) (yellow, helical domain in charcoal), with switch I and II in cyan.

GAPs are not unique to the Ras family. In light of recent biochemical data, genetic studies of yeast mating pheromone regulation can be seen to have long pointed to the existence of GAPs for heterotrimeric G proteins (*5*). These regulators of G protein signaling, or RGS, accelerate Gα–catalyzed GTP hydrolysis nearly 100-fold (*6*). RGS proteins and Ras-GAPs are completely unrelated (though both are α-helical proteins) and do not act on each other's substrates. The degree of stimulation afforded by RGS4 is at least three orders of magnitude less than that effected by GAP. On the other hand, the intrinsic rate of GTP hydrolysis catalyzed by Gα subunits (about 3 min^{-1}) is correspondingly higher than that of Ras. When stimulated by GAP and RGS4, respectively, the catalytic rates of Ras and $G\alpha_{i1}$ are within an order of magnitude of each other. As had been suspected (*7*, *8*), and the structures now demonstrate, GAP provides a catalytic residue, Arg^{789}, that is lacking in Ras but is intrinsic to Gα. This could account for at least two orders of magnitude of the total rate acceleration. However, the source of the remaining stimulatory power of GAP, and all of the acceleration provided by RGS proteins, is less easily understood.

To understand how GAP (or RGS4) causes rate acceleration, consider the conserved features in the active sites of Ras and Gα (*9*). In Ras, GTP is held between two flexible segments of chain, known as switch I and switch II. There are also inflexible elements that recognize the purine ring and the α and β phosphate groups, so there is no danger of nucleotide escaping from the active site. However, it is the switch regions that provide the essential catalytic residues. In Gα subunits, switch I is preceded by an α-helical

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domain that is not present in Ras family proteins. The two switch regions are so called because they change dramatically in conformation upon hydrolysis of GTP (*10*). This conformational change reduces the affinity of Ras and Gα for their effectors.

GTP is hydrolyzed by in-line attack of its γ phosphate by a nucleophilic water molecule. There appears to be no enzymic base to abstract a proton from this attacking water molecule, but a glutamine residue (Gln^{61} in Ras and Gln^{204} in $G\alpha_{i1}$) perched at the amino-terminus of switch II is essential for catalysis in both Gα and Ras. Mutations of this residue promote a constitutively active, GTP-bound state and are therefore transforming. Three-dimensional structures of Ras bound to a nonhydrolyzable GTP analog show that the switch II helix is flexible and Gln^{61} poorly ordered (*11*). In Gα, but not in Ras, mutation of a conserved arginine residue in switch I (Arg^{178} in $G\alpha_{i1}$) also impairs GTP hydrolysis. In the transition state for GTP hydrolysis, the γ phosphate is pentacoordinate. The attacking water (or hydroxyl group) and an oxygen atom of the β phosphate leaving group are transaxial ligands. The crystal structures of $G\alpha_{i1}$ and $G\alpha_t$ containing GDP and the square planar AlF_4^- that mimics the trigonal γ phosphate, demonstrate that Glu^{204} and Arg^{178} have distinct roles in stabilizing the transition state for GTP hydrolysis (*12*, *13*). However, both residues must be reoriented in order to stabilize the transition state. This barrier, together with the absence of an enzymic base, may explain the weak catalytic properties of Gα. Ras suffers further in that it lacks a catalytic arginine and cannot itself bind to AlF_4^- (*14*).

When GAP binds to Ras (*4*), it lands on the switches (see the left panel of the figure) and measurably reduces their flexibility. Precisely the same is true of the RGS4·$G\alpha_{i1}$ interaction (right panel) (*15*). RGS4 offers an asparagine residue (Asn^{128}) to stabilize the conformation of Gln^{204}. Likewise, GAP uses a main-chain carbonyl group to orient the corresponding Gln^{61}. Yet GAP goes a step further by inserting Arg^{789} into the active site of Ras, thereby mimicking, almost identically, the position and function of Arg^{178} in $G\alpha_{i1}$ [see figure 5C of (*4*)]. Because this residue is poised to stabilize developing charge on the γ phosphate rather than the leaving group, Ras·GAP most likely stabilizes an associative transition state.

Nevertheless, it is unlikely that the contribution of Arg^{789} accounts for all of the rate acceleration provided by GAP. RGS4 stimulates Gα even though it appears to provide no catalytic residues in the transition state complex (although Asn^{128} may assist catalysis in the ground state). Loss of flexibility in the Ras and Gα switches suggests that GAP and RGS4 stabilize conformations of $G\alpha_{i1}$ and Ras that are most complementary to the transition state. Both Ras and $G\alpha_{i1}$ bind to their stimulatory factors more tightly in the transition state than in the ground state (*6*, *14*). A larger view of catalysis (*16*) might suggest that GAPs and RGS couple the energy of substrate binding to transition state stabilization. How such coupling is achieved might be revealed in future structures of the ground state Ras·GAP complex. In the meantime, the structure at hand unites Ras and its elusive partner, arrested at the moment of catalysis.

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NEUROSCIENCE

How Does the Brain Organize Memories?

Howard Eichenbaum

Cognitive neuroscientists agree that there are multiple forms of memory, each mediated by distinct brain pathways (*1*, *2*). There is not such ready agreement, however, as to the critical distinctions among types of memory and the contributions of specific anatomical structures to each. On page 377 of this issue, Vargha-Khadem *et al.* (*3*) address both of these issues by analyzing the memory deficits in three individuals who had sustained brain lesions very early in life. Their results show that the hippocampus, a structure located within the medial temporal lobe of the brain and long associated with memory function (*4*), is critical for everyday episodic memory (our record of personal events), but is not necessary for semantic memory (our lifetime accumulation of universal factual knowledge). Although the hippocampus has been argued to function in episodic memory before (*5*), these new case studies offer a particularly impressive example that can be attributed to selective focal hippocampal damage early in life.

Striking as the findings are, they are also consistent with the possibility that both types of learning are impaired in these cases. Directly comparing new episodic and semantic learning in the laboratory turns out to be quite difficult, because normal subjects can take advantage of their episodic memory to recall new semantic material. This problem in separating performance of the two types of memory has led some to eschew the episodic-semantic distinction, focusing instead on amnesics' characteristic failure in conscious recollection of both events and facts. Such a deficit in so-called declarative memory is contrasted with fully spared acquisition of biases, skills, and habits expressed unconsciously through changes in performance speed or choice (*6*). Using this account, the seemingly selective deficit in these amnesics' memory for unique episodes, as well as their forgetting of a story or drawing, can be attributed to a partially compromised declarative capacity doing especially poorly on any type of complex material experienced only once.

Recognizing this interpretive stand-off, Vargha-Khadem *et al.* turned to nonconventional tests modeled after measures that in animals distinguish the memory functions of the hippocampus itself from that of the immediately surrounding parahippocampal cortical region (see the figure). Monkeys and rats with selective hippocampal damage do surprisingly well at stimulus recognition and stimulus association learning, but have severe deficits after parahippocampal damage (*7*). Likewise, the individuals with hippocampal lesions showed intact recognition memory in similar tests with words and faces, and even normal learning of verbal or face associations, as contrasted with the reports of more extensive impairment in these measures in a patient with identified

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