Response to Comment on "The Mechanism for Activation of GTP Hydrolysis on the Ribosome"

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Our report of the crystal structure of elongation factor Tu (EF-Tu) and aminoacyl–transfer RNA bound to the ribosome with a guanosine triphosphate (GTP) analog included a proposed mechanism of GTP hydrolysis by EF-Tu involving histidine-84. Liljas *et al*. summarize experimental evidence against this mechanism and propose a substrate-assisted catalytic model. However, these experiments and the model are also problematic. Further study is required to definitively determine the mechanism of GTP hydrolysis by EF-Tu.

Our study (*1*) described the structure of a cognate ternary complex of elongation factor Tu (EF-Tu), transfer RNA (tRNA), and a guanosine triphosphate (GTP) analog (β-γ-methyleneguanosine 5′-triphosphate, GDPCP) bound to the ribosome, thus mimicking the state of decoding just before GTP hydrolysis by EF-Tu. The structure suggests how codon recognition in the decoding center results in conformational rearrangements that activate GTP hydrolysis by EF-Tu. Furthermore, the structure identified a critical interaction between 23*S* ribosomal RNA (rRNA) residue A2662 and the conserved His^{84} of EF-Tu that reorients His^{84} into the GTPase center to catalyze GTP hydrolysis. The discussion section of our paper included a tentative model for the catalytic mechanism of the GTP hydrolysis reaction itself. The proposal had its basis in the conformation of the GTPase center and included His^{84} acting as a general base.

Liljas *et al*. (*2*) suggest that this mechanism is unlikely for a variety of reasons. They argue that the local environment of His^{84} between two charged phosphates (A2662 of the 23*S* rRNA and the γ-phosphate of GTP) would elevate its pK_a (where K_a is the acid dissociation constant) such that it is protonated and unable to act as the general base. In support, they cite a study that concludes that the rate of GTP hydrolysis for EF-Tu is independent of pH between 6.5 and 8.5 (*3*). However, these experiments used 2′(3′)-*O*-(*N*-Methylanthraniloyl)-guanosine 5′-[γ-thio]triphosphate (mant-GTP-γ-S) in place of GTP, which alters a functional group directly involved in the reaction and has a hydrolysis rate that is a factor of ~60,000 slower than GTP (*3*, *4*). GTP-γ-S is known to alter the arrangement of enzyme active sites (*5*), and the pH independence observed may not necessarily hold true for the native GTP. Furthermore, the interaction of His^{84} with the phosphate of A2662 and the possible perturbation of its pK_a would presumably not occur until His^{84} is repositioned into the GTPase center during GTPase activation. At this point, an increase in the pK_a of His^{84} would only make it a better base and thus more efficient at abstracting a proton from the catalytic water as we had proposed. Liljas *et al*. also point out that, although the substitution of Ala for His^{84} reduces the rate by six orders of magnitude (*3*), substitution with Gln (H84Q) results in only a modest impairment (*6*–*8*). However, the magnitude of the H84Q impairment is unclear: Previous reports have investigated either the rates of poly-Phe synthesis (*7*), where GTP hydrolysis is not normally rate-limiting, or the extremely slow rates of GTP hydrolysis catalyzed by *Thermus thermophilus* EF-Tu on *Escherichia coli* ribosomes (*8*). Understanding the catalytic role of a Gln^{84} substitution in EF-Tu will require a more direct measurement of the catalytic rate, preferably in conjunction with a structure of the mutant complex.

Next, Liljas *et al*. point out that the hydrolysis reaction in cellular GTPases, such as Ras, proceeds through a substrate-assisted catalytic mechanism (*9*). It is possible that translational GTPases use a similar strategy, but the proposed analogy with Ras has several problems. For example, Liljas *et al*. argue that His^{84} must be protonated and donating a hydrogen bond to the nucleophilic water, in part because the water hydrogens are interacting with the carbonyl oxygen of Thr^{62} and the γ-phosphate of GTP. However, these interacting partners result in a highly unfavorable geometry for the water molecule (*1*), with the caveats that the bond angles of GDPCP differ from those of GTP and that the structure was determined to relatively modest resolution (3.2 Å). Furthermore, in cellular GTPases such as Ras or Ran, the organization of the active site is extremely similar, with the exception that His^{84} is replaced by the unprotonated carbonyl oxygen of Gln^{61} (Ras numbering) (*10*). If His^{84} were playing the role of Gln^{61}, it would, at least in the ground state, be required to function as a hydrogen bond acceptor and therefore be unprotonated.

Although a common mechanism with cellular GTPases may be aesthetically pleasing, it is not clear that the His^{84} in EF-Tu is directly analogous to the Gln^{61} in Ras/Ran. For example, His^{84} cannot possibly perform the dual hydrogen bond donor and acceptor role of Gln^{61} in the mechanism cited by Liljas *et al*. for Ras (*9*). Indeed, a Q61H mutation in Ras decreases catalysis rates by six orders of magnitude (*9*), suggesting that the roles of His and Gln are not interchangeable. The absolute conservation of His^{84} in translational GTPases, which can differ radically outside the G domain (*11*), suggests that this histidine is catalytically important in this particular class of GTPases. Accordingly, in some other GTPases that contain residues other then Gln at this position (*12*), such as the Rap and SRP proteins, GTP hydrolysis is catalyzed by entirely different mechanisms (*13*, *14*).

Lastly, Liljas *et al*. suggest that His^{84} plays the role of both Gln^{61} and the arginine finger in Ras/Ran, which is not present in all GTPases (*10*). However, His^{84} is over 6 Å away from the bridging phosphate oxygen, where the arginine finger localizes in Ras/Ran. Arginine fingers can also interact with a nonbridging oxygen of the γ-phosphate, but His^{84} would have to change conformation to accomplish this, which would likely disrupt its interaction with the nucleophilic water and weaken the interaction between His^{84} and the phosphate of A2662 of the sarcin-ricin loop.

Instead of the roles proposed by Liljas *et al*., it is possible that the contribution of His^{84} to catalysis is entirely indirect, similar to the role suggested for Gln^{61} in some cellular GTPases (*15*).

Thus although Liljas *et al*. have raised legitimate concerns, we continue to feel that the precise mechanism of catalysis and the contribution of His^{84} will need, as we stated, "to be assessed by [additional] biochemical experiments" (*1*) and further structural studies. However, the main focus of our paper, the role of the ribosome in organizing the GTPase center into a catalytically competent arrangement, in particular the reorientation of His^{84} by A2662 of the sarcin-ricin loop of 23*S* rRNA, is still likely to be a universal feature for all translational GTPases.

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