GTP Hydrolysis in Protein Synthesis: Two for Tu?

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The question of the energy requirements for protein synthesis dates back 25 to 30 years, to the experiments that elucidated the genetic code. In these experiments, RNA homo- and copolymers were used as messenger RNA (mRNA) templates in vitro to direct incorporation of specific amino acids into polypeptides. After the code was established, protein synthesis was dissected by direct biochemical analysis, and quantitative estimates were made of the chemical energy consumption at each step. The deduced numbers, though stated explicitly or implicitly in textbooks, have not been universally accepted in the field. In this issue of *Science*, Weijland and Parmeggiani apply a clever new method to reinvestigate the stoichiometry of one source of chemical energy—hydrolysis of guanosine triphosphate (GTP)—and arrive at a number higher than that found in most textbooks (*1*).

Both adenosine triphosphate (ATP) and GTP are consumed during protein synthesis. One molecule of ATP is required for amino acid activation—the formation of an enzyme-bound aminoacyl adenylate, in which chemical potential energy is stored as a mixed phosphoanhydride bond (*2*). The enzyme-bound, activated amino acid then attaches to the 3' end of its cognate transfer RNA (tRNA); during this step, the chemical potential energy from ATP is stored in an aminoacyl ester linkage at one of the two ribose hydroxyl groups of the tRNA. The activation and transfer steps are catalyzed by aminoacyl-tRNA synthetases. It is these enzymes that interpret the genetic code, by coupling ATP hydrolysis to the attachment of specific amino acids to cognate tRNAs containing specific anticodons (*3*).

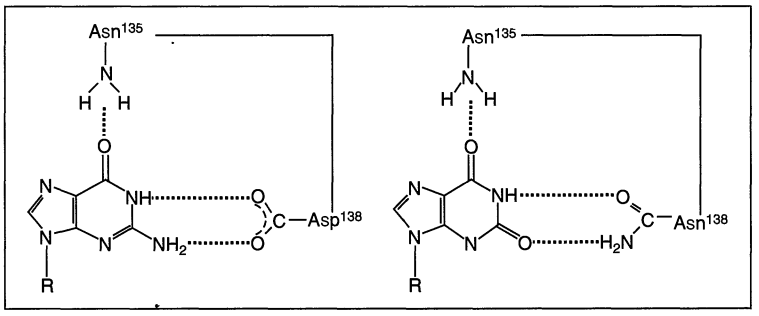
In principle, there is no need for additional energy consumption beyond the one molecule of ATP consumed in the aminoacylation reaction because the aminoacyl ester linkage is higher in energy than the peptide bond. However, early investigations established that additional energy is provided in the form of GTP hydrolysis. The stoichiometry (number of GTP molecules consumed per peptide bond) was initially believed to be one (*4*) and was later revised to two (*5*), the value that now appears in textbooks. But there have been reports that suggest a stoichiometry even greater than two (*6*, *7*), and the data of Weijland and Parmeggiani (*1*) strongly support this upward revision.

GTP is hydrolyzed during protein synthesis by the action of two members of the G-protein superfamily, the elongation factor Tu (EF-Tu) and the G-factor translocase. The translocase couples the hydrolysis of GTP to the translocation of a ribosome-bound peptidyl-tRNA from the A (acceptor) site to the P (peptidyl) site. The vacant A site is then filled by a new molecule of aminoacyl-tRNA which, after formation in the aminoacylation reaction, is bound to an EF-Tu•GTP complex. The release of aminoacyl-tRNA is accompanied by the hydrolysis of GTP to give an EF-Tu•GDP (guanosine diphosphate) complex that is cycled back to EF-Tu•GTP though a GDP-GTP exchange reaction catalyzed by elongation factor Ts. From simple summation of these two GTP-requiring partial reactions, a stoichiometry of two molecules of GTP hydrolyzed per peptide bond formed has generally been assumed to account for all of the factor-dependent hydrolysis of GTP during protein synthesis (*8*, *9*).

Weijland and Parmeggiani reinvestigated this stoichiometry by separating the ribosome-dependent hydrolysis of GTP by the translocase and ribosome-associated GTPases, which occurs even in the absence of protein synthesis, from the hydrolysis catalyzed by EF-Tu. This separation was achieved by using a mutant EF-Tu with changed substrate specificity. Earlier, Hwang and Miller (*10*) had taken advantage of the known structure of the EF-Tu•GDP complex to change the nucleotide specificity of EF-Tu from GTP to xanthosine triphosphate (XTP) by substituting an Asn residue for Asp at position 138 (figure). The interaction of the modified EF-Tu with XTP, in terms of binding and hydrolysis, is comparable to that of wild-type EF-Tu with GTP (*10*).

In a series of in vitro experiments, Weijland and Parmeggiani showed that the poly(U)-dependent synthesis of polyphenylalanine requires two molecules of XTP per peptide bond, which, when added to the single molecule of GTP hydrolyzed by the translocase, gave a total of three GTPs (*1*). The two molecules of XTP are required for translational accuracy. This observation represents a specific example of a general idea developed by Hopfield (*11*) and by Ninio (*12*), who suggested that energy consumption is required to achieve high specificity in biological processes such as translation and replication. In this case, XTP hydrolysis is coupled to poly(U)-dependent binding of phenylalanyl-tRNA ($tRNA^{Phe}$) to the ribosomal A site and increases the likelihood of interaction between an exactly complementary mRNA codon and tRNA anticodon.

Hydrogen bonding between three complementary bases is not in itself sufficiently stable or specific to prevent "incorrect" pairings that contain single base mismatches. Although the GAA anticodon of $tRNA^{Phe}$ can form an antiparallel pair with the UUU triplet of poly(U) (with the third base of the mRNA codon in a wobble G-U base pair with the first base of the $tRNA^{Phe}$ anticodon), competition for pairing can occur with, for example, the leucine tRNA isoacceptor (designated $tRNA_2^{Leu}$), which has a GAG anticodon that forms two G-U wobble base pairs with the UUU triplet. Weijland and Parmeggiani showed that, with Phe-$tRNA^{Phe}$, two molecules of XTP are hydrolyzed with EF-Tu–dependent binding of aminoacyl-tRNA to the ribosomal A site, whereas with Leu-$tRNA_2^{Leu}$, seven molecules of XTP are consumed (*1*). The extra hydrolysis may result in destabilization and rejection of the mismatched $tRNA_2^{Leu}$.



Altering the nucleotide binding specificity of EF-Tu. The NH_2-terminal domain of EF-Tu is an α-β structure (*17–19*) that is topologically identical with the corresponding domain of human $p21^{Ha\text{-}ras}$, another member of the G-protein superfamily (*20*). The carboxyl side chain of Asp^{138} in native EF-Tu (**left**) is hydrogen-bonded to the ring N-1 and the exocylic 2-amino group of the guanine ring, and the 6-keto oxygen is bonded to the side chain amide NH_2 of Asn^{135}. An Asp^{138}→Asn substitution changes the specificity of EF-Tu from GTP to XTP (**right**), by stabilization of the bound xanthine ring through hydrogen bonding of the ring N-1 and exocylic 2-keto group to the side chain amide of Asn^{138}; this alteration simultaneously removes the potential for hydrogen bonding to the exocyclic 2-amino group of the guanine ring. [Figure courtesy of B. Henderson]

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Although the EF-Tu–mediated, energy-dependent rejection of mismatched codon-anticodon pairs has been long investigated, the present work is the first time that a rationally designed mutant has been exploited to look specifically at the energy consumption associated with EF-Tu. The relationship of the stoichiometry of GTP hydrolysis to the nature of the EF-Tu•GTP•aminoacyl-tRNA complex is unknown. It is assumed in most textbooks and research articles that one molecule of EF-Tu forms a ternary complex with GTP and aminoacyl-tRNA. This numerology makes it difficult to construct a mechanism that requires two molecules of GTP hydrolyzed for each EF-Tu–mediated event. But Ehrenberg and co-workers (*7*) have presented data for two molecules of EF-Tu•GTP bound per aminoacyl-tRNA and argue that earlier measurements were done under nonphysiological conditions. These authors also present kinetic evidence for the hydrolysis of two molecules of GTP with each EF-Tu cycle. If the higher order complex (one aminoacyl-tRNA:two EF-Tu•GTP) is confirmed, then binding of aminoacyl-tRNA may occur across the EF-Tu•GTP dimer interface and communication between the subunits on the ribosome may trigger GTP hydrolysis that is sensitive to the precision of the codon-anticodon interaction. Although synergistic interactions between mutant species of EF-Tu support the concept of a higher order complex of EF-Tu (*13–15*), recent evidence inconsistent with such a complex has also been presented (*16*). Thus, the stoichiometry of EF-Tu•GTP and aminoacyl-tRNA remains unresolved, yet this information is needed to interpret the latest stoichiometry of EF-Tu–mediated GTP hydrolysis associated with peptide bond formation.

In summary, the work of Weijland and Parmeggiani supports the notion that a total of three molecules of GTP is required for each chain elongation step of protein synthesis. But the long history of the field and the steady upward revision of the stoichiometry from one to two to three suggest that further investigations, perhaps with specificity-changing mutants of the translocase, may be needed before the field is prepared to accept any more numbers.

References and Notes

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21. I thank M. Sprinzl for helpful comments.