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Review

Modeling the mechanisms of biological GTP hydrolysis

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ABSTRACT

Enzymes that hydrolyze GTP are currently in the spotlight, due to their molecular switch mechanism that controls many cellular processes. One of the best-known classes of these enzymes are small GTPases such as members of the Ras superfamily, which catalyze the hydrolysis of the γ-phosphate bond in GTP. In addition, the availability of an increasing number of crystal structures of translational GTPases such as EF-Tu and EF-G have made it possible to probe the molecular details of GTP hydrolysis on the ribosome. However, despite a wealth of biochemical, structural and computational data, the way in which GTP hydrolysis is activated and regulated is still a controversial topic and well-designed simulations can play an important role in resolving and rationalizing the experimental data. In this review, we discuss the contributions of computational biology to our understanding of GTP hydrolysis on the ribosome and in small GTPases.

Introduction

GTPases are conserved regulators of cell motility, polarity, adhesion, cytoskeletal organization, proliferation and apoptosis [1–3]. They form a large family of hydrolytic enzymes that can be classified into a number of distinct subgroups: heterotrimeric G-proteins (involved in hormonal and sensory signals), translational GTPases (involved in ribosomal protein synthesis), members of the SPR/SR family (involved in translocating peptides into the endoplasmic reticulum), tubulins and cytoskeletal motor GTPases, and monomeric GTPases such as the Ras superfamily (which are responsible for signal transduction cascades and motility) [4]. The primary biochemical function of these enzymes is to catalyze the conversion of GTP to GDP and inorganic phosphate (P_i) [5].

The most extensively studied class of small GTPases are by far the members of the Ras superfamily [6]. Small GTPases are 20–30 kD_a proteins that function as molecular switches in numerous cellular functions [7]. These are, in turn, divided into five subfamilies (Ras, Rho, Rab, Arf and Ran) that share a common fold. In GTPases such as Ras, GTP binding and hydrolysis typically leads to conformational transitions, such that these enzymes display a GDP bound "OFF" state, an open state, and a GTP bound "ON" state [8]. "ON" and "OFF" state regulation can be controlled by mechanisms such as switches (Ras and homologs), clocks (heterotrimeric G-proteins and subunits) and sensors (elongation factors such as EF-Tu and EF-G). In some G-proteins such as the Ras proteins and trGTPases such as EF-Tu,[1] this activation is also regulated by guanine nucleotide exchange factors (GEFs) [9,10], which activate the enzyme by facilitating the exchange of GDP to GTP. Specifically, GEFs catalyze the release of the bound GDP, which is replaced by abundant cellular GTP [11] (Fig. 1). In the activated state G-proteins (also known as guanine nucleotide-binding proteins – GNBPs) interact with and activate downstream targets (effectors), which in turn trigger cellular responses [12,13]. GTP hydrolysis returns GNBPs to their inactive state, thereby terminating downstream signaling. The switch between the "OFF" and "ON" states is activated by the binding of GTPase-activating proteins (GAPs) [8,11]. The active and inactive forms differ in the presence or absence of the γ-phosphate on the nucleotide, which is reflected in considerable conformational differences in regions that contact this terminal phosphate in the GTP-bound form [14].

In parallel to the ongoing interest in Ras GTPases, the recent availability of an increasing number of crystal structures of translational GTPases such as elongation factors thermo unstable (EF-Tu) and G (EF-G) [15–24] has led to an explosion of interest in trying to understand the mechanisms of GTP hydrolysis on the ribosome [20,25–38]. Specifically, translation can be roughly divided into four phases: (i) initiation, where the ribosome binds to the messenger RNA, (ii) elongation cycles, where new amino

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[1] *Abbreviations used:* EF-Tu, elongation factors thermo unstable; GEFs, guanine nucleotide exchange factors; GAPs, GTPase-activating proteins; RRF, ribosome recycling factor; RF3, release factor 3; SRL, sarcin-ricin loop; WT, wild type.

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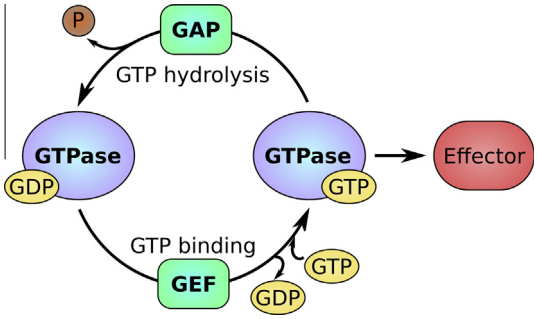

Fig. 1. The GTP → GDP cycle of small GTPases such as Ras. GDP-bound Ras (in its "OFF" state) is activated by GEF (guanine nucleotide-exchange factor), which facilitates the conversion of GDP to GTP (yielding the "ON" state of Ras). Complexation with GTPase-activating protein (GAP) in turn activates Ras for GTP hydrolysis.

acids are incorporated into the nascent polypeptide chain, (iii) termination, where the newly synthesized polypeptide is released from the ribosome, and, finally, (iv) recycling, where the ribosomal subunits dissociate and become ready to re-initiate the cycle by binding to a new mRNA. GTP hydrolysis is an essential part of all the steps mentioned above and these biologically crucial GTP hydrolysis reactions are catalyzed by a family of auxiliary proteins factors, referred to as the translational GTPases (trGTPases) [4,30,31,38]. Of these enzymes, the most mechanistically studied is EF-Tu [20,25–29,31–35,37]. Its biological role lies in the correct delivery of aminoacyl-tRNA at the A (aminoacyl) site of the ribosome. EF-Tu forms a ternary complex with aminoacyl-tRNAs and GTP that bind to the ribosome [39] (Fig. 2). Correct codon–anticodon recognition between the tRNA and the mRNA leads to a significant increase in the GTPase activity of EF-Tu by a factor of 10^5 [40]. Inorganic phosphate is released slowly after the hydrolysis [41] and release of the EF-Tu:GDP complex from the ribosome results in tRNA accommodation, which enables the correct conformation for the peptide bond formation reaction to take place [42,43].

Another rather unique translational GTPase that facilitates translocation (*i.e.* relocation of the tRNAs from the A and P (peptidyl) sites to the P and E (exit) sites respectively, as well as the relative movement of mRNA to the ribosome by three bases) is EF-G [44–46]. This enzyme behaves both as a molecular switch and as a motor protein [47]. Its binding to the ribosome after the new peptide bond has been formed induces an inter-subunit rotation that repositions the bound tRNAs into hybrid A/P and P/E sites [48–50]. GTP hydrolysis takes place very quickly after binding [51], followed by the completion of the translocation. The inorganic phosphoric group remains bound and its release is linked to the completion of the translocation [52]. Another unique characteristic of this elongation factor is the absence of a GEF, as the affinities for GTP and GDP are similar and the exchange happens spontaneously [53]. EF-G also participates in the recycling phase, facilitating the dissociation of the ribosome recycling factor (RRF) from the ribosome [54]. Release factor 3 (RF3) is a GTPase participating in the termination. GTP hydrolysis promotes the dissociation of RF1 and RF2 [55].

Note that there are many global similarities between the active sites of both translational GTPases such as EF-Tu and EF-G [56], as well as regulatory GTPases such as Ras [57–59] (Fig. 3). There has been substantial experimental and (increasingly) computational work on these systems, and yet the mechanisms of GTP hydrolysis, in particular by translational GTPases on the ribosome, remain controversial [20,29,32,37,60]. In this review, we will provide an overview of the basic challenges with studying phosphoryl transfer, as well as the specific challenges in interpreting experimental and computational data on GTP hydrolysis in biological systems. We will present popular current mechanistic proposals and highlight the role of theory in enhancing our molecular understanding of GTP hydrolysis on the ribosome and in related biological systems.

Challenges in elucidating the mechanisms of GTP hydrolysis in biological systems

Phosphoryl transfer plays a critical role in signaling, protein synthesis and energy transduction, making it one of the most important classes of chemical reactions in biology [61]. As a result of this, many enzymes (phosphatases, kinases, mutases) have evolved to catalyze this class of reactions [62], and they operate *via* a range of different mechanisms and preferred environmental conditions such as low or high pH. Additionally, some of these enzymes utilize direct attack by water, others employ an enzyme-derived nucleophile and others still use metal ions as a catalytic tool [61,63]. Phosphorylation and dephosphorylation of a protein by kinases and phosphatases can affect the function of a protein in many ways: (i) by increasing or decreasing its biological activity, (ii) by stabilizing it or marking it for breakdown, (iii) by facilitating or inhibiting movement between subcellular compartments, or (iv) by initiating or disrupting protein–protein interactions [64]. Due to the wide range of different mechanisms that can be used in such enzymes, a comprehensive picture of the mechanism of enzyme-catalyzed phosphoryl transfer remains elusive.

One of the biggest controversies in the study of phosphoryl transfer reactions has been to distinguish between the precise molecular mechanisms involved and the nature of the corresponding transition states [61,63,65,66]. Specifically, the availability of low-lying d-orbitals on the phosphorus atom opens the door to a range of mechanistic possibilities, such that the mechanisms of phosphate hydrolysis may occur by a range of different mechanistic pathways (Fig. 4). In a fully associative mechanism ($A_N + D_N$, Fig. 4A), nucleophilic attack occurs prior to the departure of the leaving group, and the reaction proceeds *via* inversion of configuration at the phosphorus atom. In contrast, in a dissociative pathway ($D_N + A_N$, Fig. 4B), leaving group departure precedes nucleophilic attack and the reaction proceeds *via* a metaphosphate intermediate. In addition to the aforementioned stepwise pathways which proceed with intermediate formation, the reaction can also proceed *via* a concerted S_N2-like A_ND_N pathway (Fig. 4C), in which bond formation to the nucleophile and bond cleavage to the leaving group occur in a single transition state. Such a transition state can be dissociative or associative in nature, depending on the degree of bond formation to the incoming nucleophile and bond cleavage to the departing leaving group.

There have been extensive experimental studies on the hydrolysis of highly charged phosphate monoester dianions, as well as ATP and GTP hydrolysis in aqueous solution [67,68]. The experimental data would largely suggest a loose, dissociative transition state, based on a steep leaving group dependence of −1.23 in the linear free energy relationship [69], experimentally measured kinetic isotope effects [70], and a small, negative activation entropy [71]. However, quantum chemical calculations and careful theoretical analysis have suggested that the interpretation of the experimental observables is not unambiguous, as multiple different pathways can give rise to the same experimental observables [66,72]. Additionally, we recently demonstrated that in the case of phosphate monoester dianion hydrolysis, while there is a clear leaving-group dependent mechanistic preference between tighter (more associative) and looser (more dissociative) transition states, the competition between the two pathways is very close, suggesting that an enzyme could in principle use either as a solution to the