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REVIEW

The energetics of subunit rotation in the ribosome

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Abstract

Protein synthesis in the cell is controlled by an elaborate sequence of conformational rearrangements in the ribosome. The composition of a ribosome varies by species, though they typically contain ∼50–100 RNA and protein molecules. While advances in structural techniques have revolutionized our understanding of long-lived conformational states, a vast range of transiently visited configurations can not be directly observed. In these cases, computational/simulation methods can be used to understand the mechanical properties of the ribosome. Insights from these approaches can then help guide next-generation experimental measurements. In this short review, we discuss theoretical strategies that have been deployed to quantitatively describe the energetics of collective rearrangements in the ribosome. We focus on efforts to probe large-scale subunit rotation events, which involve the coordinated displacement of large numbers of atoms (tens of thousands). These investigations are revealing how the molecular structure of the ribosome encodes the mechanical properties that control large-scale dynamics.

Keywords Energy landscape · Free-energy barriers · Molecular machine · Brownian ratchet

Introduction

The ribosome is one of the most important molecular machines in biology and biotechnology. In the cell, it is solely responsible for translating genetic information into proteins (Fig. 1a). The proteins produced by the ribosome then fulfill a countless number of cellular roles. With regard to therapeutics, mRNA technologies (including vaccines (Pardi et al. 2018)) depend on the ribosome to read artificial mRNA molecules, where the expressed proteins serve to modulate biological or pathogenic processes. Structurally, a ribosome may be described in terms of two subunits: the "small" (SSU) and "large" (LSU) subunits (Fig. 1b). After the ribosome binds an mRNA molecule, it then recruits aminoacyl-tRNA (aa-tRNA) molecules, in order to decode each frame of the mRNA and add amino acids to the growing peptide chain (Rodnina and Wintermeyer 2001). While peptide bond formation is localized to a well-defined catalytic center, the ribosome must undergo many large-scale conformational rearrangements in order to properly position and release each tRNA molecule (Korostelev and Noller 2007; Schmeing and Ramakrishnan 2009; Frank and Gonzalez 2010).

Over the last several decades, there have been astounding advances in the experimental investigation of ribosome structure and dynamics. Prior to the availability of atomic models, biochemical analysis (Green and Noller 1997; Rodnina and Wintermeyer 2001) provided a framework for studying the kinetics and architecture of the ribosome. This foundation allowed for crystallographic (Carter et al. 2000; Ban et al. 2000; Schluenzen et al. 2000; Yusupov et al. 2001) and cryo-EM studies (Valle et al. 2003) to isolate the ribosome in distinct structural states, which detailed the molecular interactions that impart stability. Subsequently, single-molecule techniques were able to measure low-dimensional (normally 1D) projections of conformational substeps (Blanchard et al. 2004; Cornish et al. 2008; Fei et al. 2008; Salsi et al. 2014; Ning et al. 2014). Together, this broad range of experimental efforts has produced an

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extensive collection of data on the properties of long-lived conformational states of the ribosome, as well as the interactions that contribute to stability at each stage of function.

The quantity of high-quality experimental data available for the ribosome makes it a model system for developing an understanding of the physical principles that govern biological dynamics. This potential to serve as a paradigm system has been recognized by many, and it has inspired the application of essentially every available computational technique. These efforts have included highly detailed quantum-mechanical calculations (Trobro and Aqvist 2008; Adamczyk and Warshel 2011), all-atom explicit-solvent simulations (Bock et al. 2013; Bock et al. 2015; Whitford et al. 2013), coarse-grained models (Tama et al. 2003; Wang et al. 2004; Trylska et al. 2004; Zhang et al. 2011; Kim et al. 2014; Hori et al. 2021), in addition to a range of custom models for studying large-scale rearrangements (Levi et al. 2019). In this short review, we will focus specifically on studies that have aimed to quantify the energetics of collective rearrangements, in particular subunit rotation dynamics. These domain motions involve coordinated displacements of large numbers of atoms (~20,000–60,000) over relatively large length scales (Fig. 1). In this context, we will organize the discussion around the study of three categories of dynamical processes: fluctuations about energetic minima, small-scale ($\sim 1k_BT$, $<$ 1nm) barrier-crossing events and rearrangements that involve large-scale ($>> 1k_BT$, $>$ 2nm) barriers. Together, these studies serve as examples for how to dissect the factors that control dynamics in molecular assemblies.

Fluctuations about energetic minima

Due to the immense size of the ribosome, most computational analyses have probed small-scale deviations from experimentally resolved conformations. Even though these approaches describe motions about free-energy minima, they have yielded notable insights into the relationship between structure and dynamics. In terms of techniques, the two most popular prediction/analysis strategies have been normal mode analysis (NMA) and principal component analysis (PCA). These methods are fundamentally distinct, yet they have provided similar descriptions of ribosome dynamics, suggesting that general physical principles may be gleaned. In particular, qualitatively similar global deformations (including rotary-like motions; Fig. 1c/d) are often predicted, even though the underlying theoretical models differ in spatial and energetic resolution. When a dynamical feature is robust to the choice of model, one may infer that the property/motion is encoded in the architecture of the assembly. In this case, the connectivity of interactions in the ribosome, which is determined by the secondary/tertiary/quaternary structure, is a dominant factor that controls the accessible large-scale rotary dynamics.

To appreciate the insights that can be provided by NMA and PCA, it is necessary to review the principles that underlie each technique. In NMA, one calculates a second-order expansion of the energy about a minimum. The resulting stiffness matrix (i.e., the Hessian) is then diagonalized, in order to identify collective modes that are energetically uncoupled (to second order). Since low-frequency modes are associated with smaller restoring forces, these modes provide larger contributions to the overall flexibility of the assembly. Low-frequency (low-energy) modes are often associated with collective rearrangements in multi-domain systems (Miyashita et al. 2003), where the majority of thermally driven fluctuations can typically be described by the first 10–20 modes (out of thousands) (Chacón et al. 2003). This property has enabled a broad range of coarse-grained/multi-scale elastic-network-based models to be applied to the study of multi-domain protein assemblies (Takada 2012; Flechsig and Mikhailov 2019). In contrast to NMA, it is possible to apply PCA to any simulated data set, regardless of the model details and simulation protocols. The defining aspect of PCA is that one diagonalizes a covariance matrix, in order to identify linearly uncorrelated fluctuations. There is an endless number of coordinates that may be used to construct a covariance matrix, such as Cartesian and internal (e.g., interatomic distances, dihedral angle) coordinates. Even though NMA and PCA both represent second-order descriptions of a system, they are independent approaches that can provide complementary insights into the dynamics of large-scale assemblies.

While many aspects of ribosome dynamics will depend on the details of the model, most applications of NMA and PCA have predicted structural fluctuations that correlate with small subunit rotation (Fig. 1c), rotation of the small subunit head (Fig. 1d), or bending deformations of the stalk regions (Fig. 1b). Of these, the most pronounced motion has been distinct ratchet-like motion of the SSU relative to the LSU (Tama et al. 2003; Chacón et al. 2003; Wang et al. 2004; Tama and Brooks 2006; Kurkcuoglu et al. 2008; Kurkcuoglu et al. 2009; Matsumoto and Ishida 2009; Shasmal et al. 2010; Seo et al. 2014; Trylska et al. 2005; Whitford 2015). The presence of these fluctuations has been found to be independent of the tRNA binding state (i.e., tRNA-bound, or tRNA-free), and they are even predicted by models that do not include the ribosomal proteins (Kurkcuoglu et al. 2008). This robust character indicates that rotary-like fluctuations directly arise from the

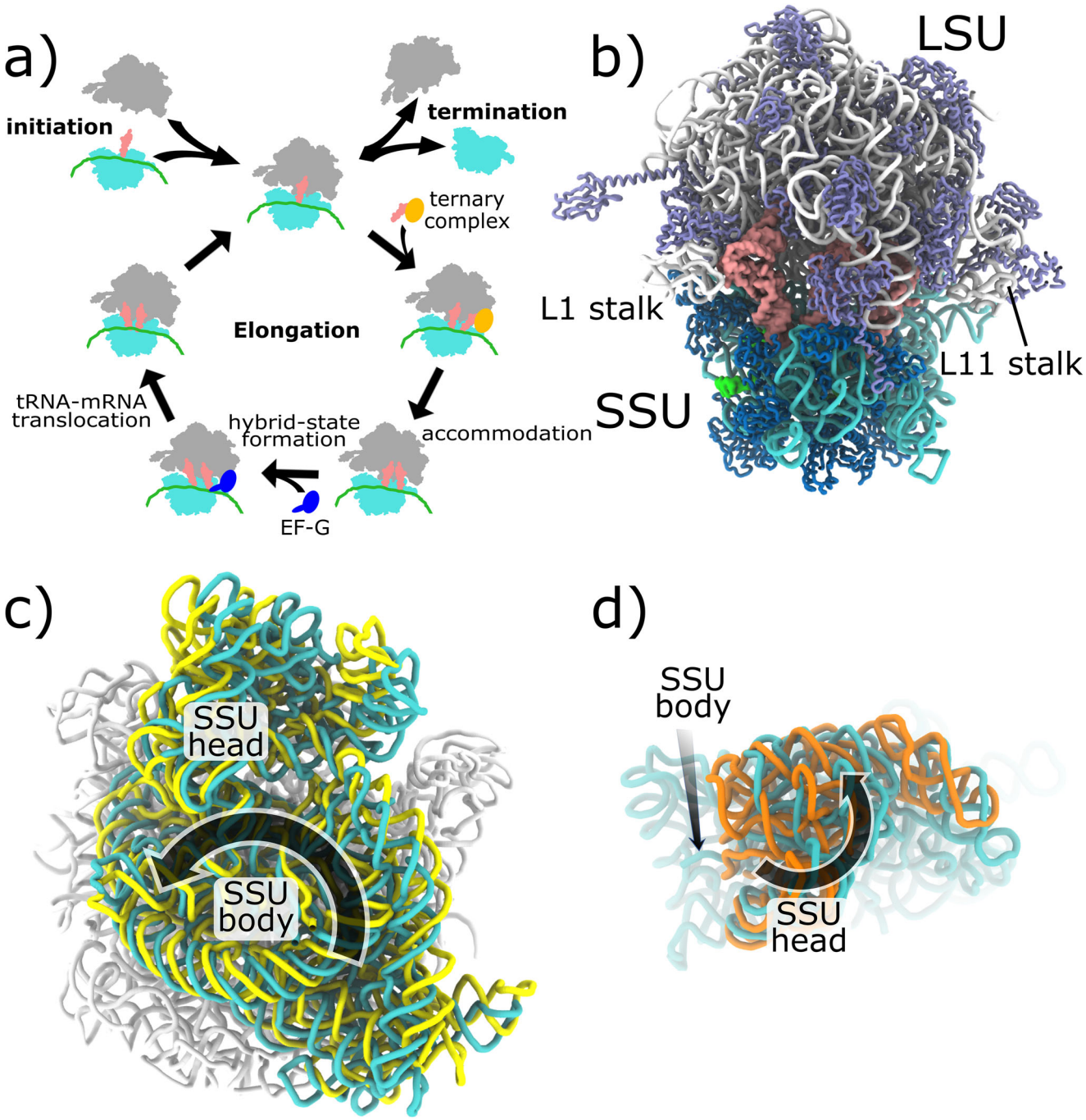

Fig. 1 Ribosome structure and subunit rotation. **a**) Simplified schematic of translation by the ribosome. During initiation, the ribosomal subunits and mRNA assemble. After initiation, each cycle of elongation involves delivery of tRNA molecules in the form of ternary complex. Delivery of an incoming tRNA molecule is followed by a sequence of steps, including tRNA accommodation, peptide bond formation, hybrid-state formation, translocation and tRNA release. Upon recognition of a stop codon, the elongation cycle terminates and the ribosome disassembles. **b**) Bacterial ribosome structure (PDB: 6QNR (Rozov et al. 2019)) showing the large subunit (LSU; RNA: white, protein: purple), small subunit (SSU; RNA: cyan, protein: blue) tRNA molecules (pink) and mRNA (green). The peripheral L11 and L1 stalk regions facilitate tRNA recruitment and release. **c**) During tRNA hybrid formation and translocation, the entire SSU reversibly interconverts between unrotated (cyan) and rotated (yellow) orientations (PDB: 4V9D (Dunkle et al. 2011)). Perspective rotated 90°, relative to panel **b**. **d**) In addition to SSU rotation, the SSU head undergoes intra-subunit rotation, relative to the body. The head transitions between unrotated (cyan; PDB: 4V9D (Dunkle et al. 2011)) and rotated (orange; PDB: 4V4Q (Schuwirth et al. 2005)) orientations. Perspective is the same as panel **b**. As described in the literature (Nguyen and Whitford 2016; Freitas et al. 2021), "tilting" is defined as any rotational motion that is orthogonal to those shown in panels **c** and **d**. All structures were visualized using VMD (Humphrey et al. 1996)

architecture of the ribosomal RNA. In most NMA and PCA studies, extended peripheral elements have also been found to exhibit large-scale motions that can be correlated with rotary rearrangements. These elements include the "beak" and "spur" of the SSU, as well as the L1 and L11 stalks of the LSU (Tama et al. 2003; Wang et al. 2004; Tama and Brooks 2006; Kurkcuoglu et al. 2008; Kurkcuoglu et al. 2009; Matsumoto and Ishida 2009; Shasmal et al. 2010; Seo et al. 2014; Trylska et al. 2005). In some cases, the normal modes can also provide signatures into the relationship between tRNA binding and ribosomal motions (Kurkcuoglu et al. 2008).

Despite the use of simplified energy functions in earlier applications of NMA and PCA, they have frequently predicted collective motions that are consistent with other theoretical and experimental techniques. For example, NMA analysis implicated tilt-like motions of the SSU body (Fig. 3b of Kurkcuoglu et al. (2008)). Here the term "tilt" is used to indicate any deviations of the body that are orthogonal to the experimentally resolved rotation, as described by Freitas et al. (2021). Interestingly, subsequent cryo-EM studies reported a similar type of rearrangement (referred to as "rolling") in eukaryotic ribosomes (Budkevich et al. 2015). Since the normal mode calculations were performed for a bacterial ribosome, the presence of these motions in a eukaryotic ribosome shows how higher-level organisms have evolved to amplify properties that are intrinsic to their bacterial counterparts. NMA also predicted the presence of SSU head rotation fluctuations (Kurkcuoglu et al. 2008), which is reminiscent of the so-called "swivel" rearrangement seen in structural studies (Ratje et al. 2010). In addition, NMA predicted an apparent opening of the head that is similar to later predictions of head tilting (Nguyen and Whitford 2016) during tRNA translocation.

To highlight the degree to which rotary-like fluctuations of the subunits are robust, it is useful to survey the physical differences between models that have been applied. In the study of Tama et al., landmark points were used to denote pseudo-atomic positions (between 1000 and 2000 points per ribosome), which were assigned based on cryo-EM electron densities (Tama et al. 2003). While the landmark points were not associated with specific residues, they were distributed in a manner that preserved the distribution of atoms in the ribosome. While some models have applied a single bead per RNA or protein residue (Trylska et al. 2005), others have included all RNA and protein atoms in the absence of solvent (Whitford 2015). Block-level coarse graining has also been used, where nodes are aggregated to form dynamical units (Chacón et al. 2003; Tama et al. 2003; Hoffmann and Grudinin 2017). There have also been hybrid representations in which portions of the ribosome are treated as blocks, while more flexible elements (e.g., tRNA molecules) are described by residue-level coarse graining (Seo et al. 2014). At a more coarse level of resolution, entire ribosomal proteins have been represented as single pseudoparticles (Kurkcuoglu et al. 2008).

Small-scale rearrangements and diffusion

To complement studies with coarse-grained models, all-atom explicit-solvent simulations have been used to probe the energetics of subunit rotation. In principle, these models can provide a detailed characterization of the interactions that govern dynamics. However, there are some notable challenges, including the large scale of the system ($\sim$ 2 million atoms, when solvated), as well as outstanding questions regarding the accuracy of RNA and ion force fields when applied to assemblies (Tan et al. 2018). Due to the large size, alone, these simulations have historically been limited to microsecond scales (Arenz et al. 2016; Whitford et al. 2013), or shorter (Bock et al. 2013; Bock et al. 2015). Even though recent hardware advances promise to enable much longer timescales (Shaw et al. 2014), it will likely remain intractable to simulate spontaneous and complete rotation events, which may occur on the timescales of tens-to-hundreds of milliseconds. Nonetheless, shorter timescale simulations have been able to provide various insights into the energetics of rotation.

One strategy for studying rotation with explicit-solvent simulations is to perform short-timescale simulations at various points along a putative pathway. This approach was deployed in the studies of Bock et al. (2013, 2015), where $\sim$300-ns simulations were initiated from 13 different conformations that had been resolved in cryo-EM experiments of reverse translocation (Fischer et al. 2010). Even though each system remained near its initial configuration, the simulated dynamics was used to estimate rates for large-scale rearrangements (Bock et al. 2013) and to characterize interactions at the subunit interface (Bock et al. 2015). To quantify the kinetics, reaction coordinates were defined to describe collective displacements of the tRNA molecules and L1 stalk, in addition to rotation of the SSU body and head. The tRNA and stalk coordinates were based on PCA of the set of simulations. The coordinates for SSU body and head rotation involved defining pivot points and rotation axes based on non-linear least-squares fitting. With these metrics, kinetically proximal states were inferred by applying quasi-harmonic approximations (Fig. 2a) to each minimum (i.e., each cryo-EM state). With this, the study estimated the putative barrier heights (ΔG) and barrier-crossing attempt frequencies (C) between adjacent

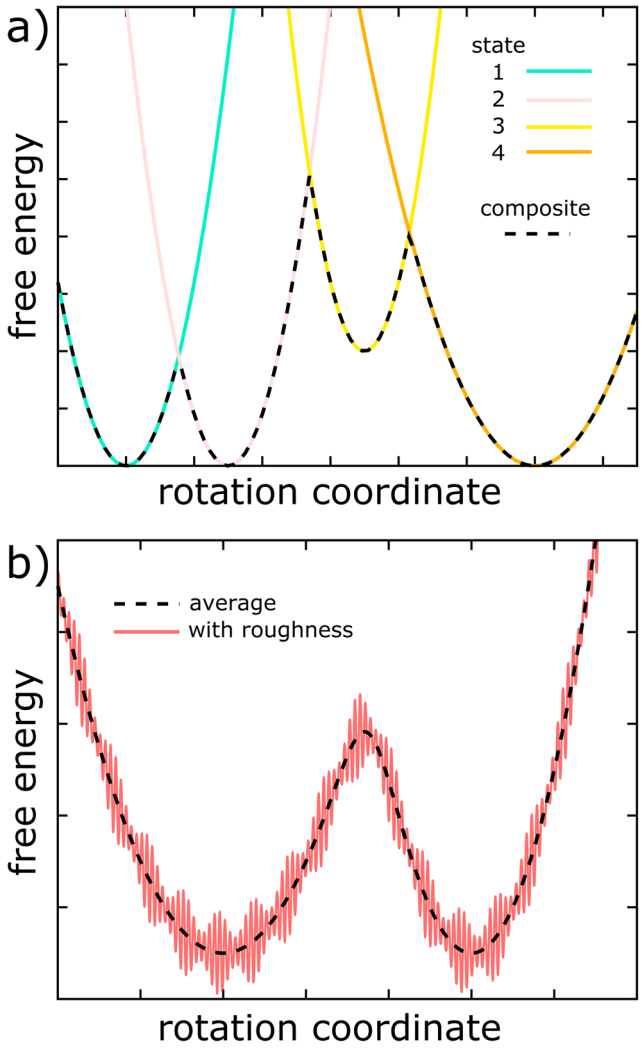

Fig. 2 Perspectives on the energy landscape of rotation. Various strategies have been applied to use explicit-solvent simulations to probe the energetics of rotation. **a**) In the study of Bock et al. (2013), simulations were performed for different states, based on cryo-EM data. To estimate barriers between states, a quasi-harmonic approach was used to generate a composite landscape (dashed). **b**) In the study of Whitford et al. (2013), explicit-solvent simulations were used to estimate the diffusion coefficients associated with head and body rotation. In this perspective, the short-scale roughness (red) dictates the diffusive properties (Bryngelson and Wolynes 1989), with which one may infer the corresponding experimental free-energy barriers (dashed)

states, from which the timescale of interconversion was estimated according to:

$$\langle \tau \rangle = \frac{1}{C} \exp(\Delta G / k_B T). \tag{1}$$

While the rates of individual substeps are still not precisely known, the global kinetics predicted by these calculations was compatible with known rates of tRNA translocation.

An alternate utility of explicit-solvent simulations is to provide estimates of the effective diffusion coefficients along putative rotation coordinates. When a suitable reaction coordinate is applied (ρ), one can relate the free-energy barrier with the mean first passage time (τ) through the relation (Szabo et al. 1980; Bryngelson and Wolynes 1989)

$$\langle \tau \rangle = \frac{1}{k} = \int_{\rho_{\text{initial}}}^{\rho_{\text{final}}} d\rho \int_{-\infty}^{\rho} d\rho' \frac{\exp[(G(\rho) - G(\rho'))/k_B T]}{D_\rho^{\text{eff}}(\rho)} \tag{2}$$

where k is the rate, ρ_{initial} and ρ_{final} are the values of the reaction coordinate at the endpoint conformations,[1] $G(\rho)$ is the free energy as a function of the reaction coordinate and D_ρ^{eff} is the effective diffusion coefficient along ρ. To facilitate the application of this relation to describe rotation-associated barriers, an explicit-solvent simulation was used to estimate effective diffusion coefficients for body and head rotation coordinates (Whitford et al. 2013). Since the simulation was relatively long ($\sim$ 1.3 μs), it was possible to identify large sets of atoms within the LSU, SSU head and SSU body that undergo motions for which a rigid-body approximation is suitable. That is, the simulated trajectory was used to identify "core" residues within each domain that undergo minimal internal deformations on the microsecond timescale. These core groups were then used in conjunction with available crystallographic structures to construct rotation measures that separate unrotated and rotated conformations. The effective diffusion coefficients along these collective coordinates were then estimated. In addition to connecting experimental rates with predicted free-energy barriers (Eq. 2), these calculations provide immediate insights into the short-length energetic roughness (Fig. 2b). Specifically, since the effective diffusion coefficient is inversely proportional to the roughness (Bryngelson and Wolynes 1989), these calculations help quantify the degree of short-scale roughness that is present on the free-energy landscape of the ribosome.

Large-scale barrier-crossing events

Biomolecular rates are often limited by large-scale rearrangements that require the system to overcome pronounced free-energy barriers. With 150,000–200,000 non-hydrogen atoms in a typical ribosome, only a few studies have been able to report free-energy barriers for such large-scale rearrangements. While barriers have been obtained using a class of simplified models, called structure-based (SMOG) models (Noel et al. 2016), these types of calculations remain challenging and limited in number. SMOG models utilize an intentionally simplified description of the landscape, which (perhaps surprisingly) allows for precise insights into the physical interactions that govern large-scale processes. In

[1] The bounds on the inner integral assume $\rho_{\text{initial}} < \rho_{\text{final}}$.

the first applications of these models to tRNA accommodation (Whitford et al. 2010) and translocation (Nguyen and Whitford 2016), non-equilibrium strategies were used to simulate spontaneous conformational transitions. While, due to the sampling protocols employed, free-energy barriers could not be calculated in those studies, the presence of kinetic pauses suggested the existence of sterically-induced barriers. That is, since the models are energetically "smooth" (i.e., only endpoint interactions are stabilizing), these phenomenological signatures most likely arise from the influence of molecular sterics. Over the last 10 years, these models have been used to simulate nearly every conformational step of the elongation cycle (Levi et al. 2019, 2020, Freitas et al. 2021). Of these studies, we will provide a detailed discussion of how structure-based models have been used to identify and quantify the free-energy barriers associated with subunit rotation in bacterial and eukaryotic systems.

With the recently demonstrated ability to simulate spontaneous subunit rotation events (Levi et al. 2017), it is now possible to identify ideal reaction coordinates for describing rotation dynamics, as well as to probe the mechanistic features that govern rotation kinetics. In the first simulations to report spontaneous reversible subunit rotation events, a customized coarse-grained structure-based model was developed, where each residue was represented by a single bead (Levi et al. 2017). In this case, the use of a coarse-grained representation was suitable, since structural models of the rotated and unrotated states did not suggest the presence of steric obstacles during rotation. Instead, visual inspection of the structures would suggest rotation likely involves shuffling of intersubunit "bridge" interactions. With this model, more than 300 spontaneous rotation/back-rotation events were simulated, and the trajectories demonstrated how two different single-molecule experiments could give rise to apparently contradictory descriptions of the dynamics. Specifically, this model indicated that the distance between labeled residues in one FRET strategy (Marshall et al. 2008) is only weakly coupled to rotation events, while a second strategy (Cornish et al. 2008) exhibits stronger correlations with rotation events. Since these models properly account for biomolecular flexibility, the analysis of FRET pairs demonstrated how molecular flexibility can yield differential apparent dynamics (Fig. 3a/b). Interestingly, even though one coordinate was more strongly correlated with rotation, subsequent analysis revealed that it is also incapable of precisely describing the rate-limiting free-energy barrier (Levi and Whitford 2019). This indicates that the potential of mean force as a function of this probe distance will underestimate the barrier (Fig. 3c), though the simulated data can be used to establish a quantitative bridge between the apparent dynamics in FRET experiments and the underlying free-energy barrier. That is, these calculations provide an explicit statistical relationship between experimentally accessible coordinates and an ideal (albeit experimentally inaccessible) coordinate. In this case, the superior coordinate was identified by applying an optimization strategy to refine a suitable metric that is based on subunit-subunit contacts. Surprisingly, this analysis also indicated the free-energy barrier is underestimated by coordinates that monitor domain orientations, relative to contact-based descriptions (Fig. 3c). Since previous explicit-solvent simulations focused on rotation metrics that measure domain orientations (Bock et al. 2013; Whitford et al. 2013), this analysis of reaction coordinates (Levi and Whitford 2019) suggests barriers obtained in those studies represent lower-bound estimates. As a final note, identifying and characterizing the contact-based coordinate made it possible to quantify the relative contribution of each bridge interactions to the free-energy barrier, and therefore the kinetics. These initial quantifications of the free-energy barriers associated with rotation provide a much-needed foundation, with which theoretical and experimental techniques for describing rotation may be further improved.

Building upon the study of subunit rotation in bacteria (Levi et al. 2017; Levi and Whitford 2019), our group was recently able to use an all-atom model to simulate spontaneous (reversible) subunit rotation in a eukaryotic ribosome (Freitas et al. 2021). As described above, even though rearrangements at the subunit interface are not expected to be associated with steric barriers, using an atomistic model allowed for a more precise representation of configurational entropy. With this approach, it was found that changes in configurational entropy can have a profound impact on the balance between rotated and unrotated states. Upon rotation, Extension Segment 31a (ES31a) docks with the small subunit and becomes slightly more ordered, while at the same time ES27b is released and enters a highly disordered ensemble (Fig. 3d). This balance of competing entropic contributions can then lead to a strong temperature dependence of the thermodynamic balance between rotation states. With these newly accessible approaches for studying the energetics of rotation, the field is positioned to continue extending these models to study any number of factors that contribute to eukaryotic translation.

Quantifying rotation energetics through cryo-EM

Alongside theoretical efforts, there has also been progress in developing experimental strategies for describing rotation energetics in the ribosome. With regard to directly estimating energetics, the application of cryo-EM techniques has made the most notable progress, to date. An

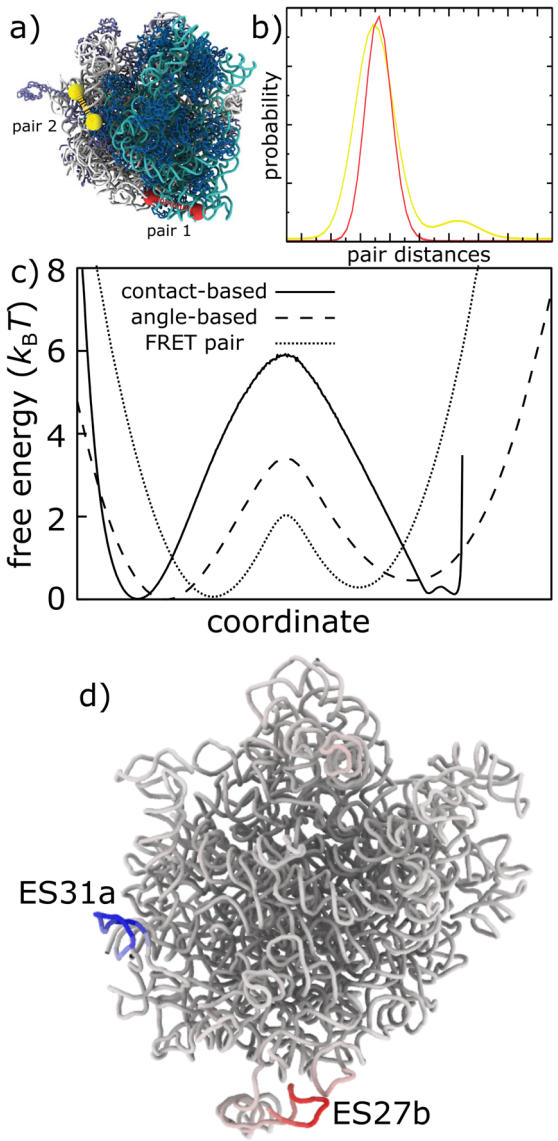

Fig. 3 Simulating SSU rotation in bacterial and eukaryotic ribosomes. **a**) Structure of the bacterial ribosome, with two sets of FRET pairs shown in red (pair 1 (Marshall et al. 2008)) and yellow (pair 2 (Cornish et al. 2008)). **b**) Probability distributions as functions of pair 1 (red) and pair 2 (yellow) inter-residue distances, calculated from simulations of the ribosome during rotation (Levi et al. 2017). Even when pair 1 is locked in a single state, pair 2 can interconvert reversibly between rotated and unrotated values. These phenomenologically distinct apparent dynamics are consistent with "contradictory" observations that have been reported experimentally (Marshall et al. 2008; Cornish et al. 2008). **c**) Free energy (potential of mean force) calculated as a function of the pair 2 distance, a collective rotation angle measure and an intersubunit contact-based metric. The largest barrier is obtained with the contact-based metric, and this coordinate is able to unambiguously identify the transition state associated with SSU rotation (Levi and Whitford 2019). **d**) LSU of a eukaryotic (yeast) ribosome, colored by the change in RMSF (by residue) upon forward rotation. RMSF was calculated from simulations of rotation with an all-atom structure-based model. Upon rotation, ES27b exhibits larger fluctuations, while ES31a becomes more ordered. Together, these changes in configurational entropy lead to a temperature-dependent free-energy landscape of rotation (Freitas et al. 2021)

early effort to quantify the free-energy landscape associated with ribosome functional dynamics was by Fischer et al. (2010). In that study, time-resolved cryo-EM was used to obtain snapshots of the ribosome during retro-translocation (reverse translocation). The obtained cryo-EM images were then classified into eight different sub-states using a hierarchial computational sorting process that could distinguish between signatures of SSU body rotation, SSU head rotation and tRNA position. Using these assigned states, the time-dependent populations were then used to provide an estimate of the free-energy landscape associated with SSU body and head.

The most rigorously obtained experimental estimates of the free-energy landscape of rotation was from Dashti et al. (2014). Through an analytical technique called manifold embedding (Frank and Ourmazd 2016), one can characterize the continuous conformational space based on cryo-EM images. This naturally allows one to calculate effective free-energy profiles along the cryo-EM-inspired coordinates, without prior knowledge of the dominant states. That is, the analysis of EM images provides a natural set of coordinates for describing variations in a collection of images (multidimentional manifold), where non-linear singular value decomposition is used to identify the dominant motions and reduce the dimensionality of the description ($\sim$ 5 degrees of freedom). With this approach, it has been possible to map the conformational changes during the elongation cycle in a yeast ribosome, and experimentally estimate the free-energy landscape.

Concluding remarks

With continual increases in computational capabilities and the availability of novel theoretical models, the ribosome is prime to become the model system for describing large-scale collective dynamics in biomolecular assemblies. After decades of effort and the application of many theoretical techniques, it is now clear how the structure of the ribosome can predispose it to undergo a range of experimentally observed rearrangements. In addition, recent advances are enabling simulations to provide precise and testable predictions. With regard to subunit rotation, the ability to simulate rotation events observed under equilibrium conditions is allowing the field to begin to quantify the relative contributions of biomolecular factors on global kinetics. In addition to quantifying the energetics associated with protein synthesis, these efforts may also help establish guiding principles for the development of engineered ribosomes, or synthetic molecular complexes. As an example, one could imagine future studies using this foundation to assist in the design of ribosomes that are more efficient at incorporating non-canonical amino acids.

While there has already been notable progress, it is our expectation that an iterative process of model development, prediction and experimental comparison will allow the field to establish a comprehensive description of the factors that control large-scale dynamics in the ribosome.

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Declarations

Consent for publication All authors consent to publication of the manuscript.

Conflict of interest The authors declare no competing interests.

References

Adamczyk AJ, Warshel A (2011) Converting structural information into an allosteric-energy-based picture for elongation factor tu activation by the ribosome. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.1105714108

Arenz S, Bock LV, Graf M et al (2016) A combined cryo-EM and molecular dynamics approach reveals the mechanism of ErmBL-mediated translation arrest. Nat Commun 7:12,026. https://doi.org/10.1038/ncomms12026

Ban N, Nissen P, Hansen J et al (2000) The complete atomic structure of the large ribosomal subunit at 2.4 angstrom resolution. Science 289(5481):905–920. http://doi.org/10.1126/science.289.5481.905

Blanchard SC, Kim HD, Gonzalez RL et al (2004) tRNA dynamics on the ribosome during translation. Proc Natl Acad Sci USA 101(35):12,893–8. https://doi.org/10.1073/pnas.0403884101

Bock LV, Blau C, Schröder GF et al (2013) Energy barriers and driving forces in tRNA translocation through the ribosome. Nat Struct Mol Biol 20:1390–1396. https://doi.org/10.1038/nsmb.2690

Bock LV, Blau C, Vaiana AC et al (2015) Dynamic contact network between ribosomal subunits enables rapid large-scale rotation during spontaneous translocation. Nucleic Acid Res 43(14):6747–6760. https:/doi.org/10.1093/nar/gkv649

Bryngelson JD, Wolynes PG (1989) Intermediates and barrier crossing in a random energy model (with applications to protein folding). J Phys Chem 93(19):6902–6915. https://doi.org/10.1021/j100356a007

Budkevich TV, Giesebrecht J, Behrmann E et al (2015) Rolling: a Eukaryotic-Specific Ribosome Rearrangement. Cell 158(1):121–131. https://doi.org/10.1016/j.cell.2014.04.044.Regulation

Carter A, Clemons W, Brodersen D et al (2000) Functional insights from the structure of the 30s ribosomal subunit and its interactions with antibiotics. Nature 407(6802):340–348. https:/doi.org/10.1038/35030019

Chacón P, Tama F, Wriggers W (2003) Mega-Dalton biomolecular motion captured from electron microscopy reconstructions. J Mol Biol 326(2):485–492. https://doi.org/10.1016/S0022-2836(02)01426-2

Cornish PV, Ermolenko DN, Noller HF et al (2008) Spontaneous intersubunit rotation in single ribosomes. Mol Cell 30(5):578–88. https://doi.org/10.1016/j.molcel.2008.05.004

Dashti A, Schwander P, Langlois R et al (2014) Trajectories of the ribosome as a Brownian nanomachine. Proc Natl Acad Sci USA 111(49):17,492–17,497. https://doi.org/10.1073/pnas.1419276111

Dunkle J, Wang L, Feldman MB et al (2011) Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. Science 332(6032):981–4. https://doi.org/10.1126/science.1202692

Fei J, Kosuri P, Macdougall D et al (2008) Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. Mol Cell 30(3):348–359. https://doi.org/10.1016/j.molcel.2008.03.012

Fischer N, Konevega AL, Wintermeyer W et al (2010) Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy. Nature 466(7304):329–33. https://doi.org/10.1038/nature09206

Flechsig H, Mikhailov AS (2019) Simple mechanics of protein machines. J R Soc Interface 16(155). https://doi.org/10.1098/rsif.2019.0244

Frank J, Gonzalez RLJr (2010) Structure and dynamics of a processive brownian motor: The translating ribosome. Annu Rev Biochem 79(1):381–412. https://doi.org/10.1146/annurev-biochem-060408-173330

Frank J, Ourmazd A (2016) Continuous changes in structure mapped by manifold embedding of single-particle data in cryo-em. Methods 100:61–67. https://doi.org/ 10.1016/j.ymeth.2016.02.007

Freitas FC, Fuchs G, de OliveiraRJ, et al (2021) The dynamics of subunit rotation in a eukaryotic ribosome. Biophysica 1(2):204–221. https://doi.org/10.3390/biophysica1020016, https://www.mdpi.com/2673-4125/1/2/16

Green R, Noller HF (1997) Ribosomes and translation. Annu Rev Biochem 66:679–716. https://doi.org/10.1146/annurev.biochem.66.1.679

Hoffmann A, Grudinin S (2017) NOLB: Nonlinear rigid block normal-mode analysis method. J Chem Theory Comput. https://doi.org/10.1021/acs.jctc.7b00197

Hori N, Denesyuk NA, Thirumalai D (2021) Shape changes and cooperativity in the folding of the central domain of the 16S ribosomal RNA. Proc Natl Acad Sci USA 118(10). https://doi.org/10.1073/pnas.2020837118

Humphrey W, Dalke A, Schulten K (1996) VMD: Visual molecular dynamics. J Mol Graph 14(1):33–38. https://doi.org/10.1016/0263-7855(96)00018-5

Kim H, Abeysirigunawarden SC, Chen K et al (2014) Protein-guided RNA dynamics during early ribosome assembly. Nature 506(7488):334–338. https://doi.org/10.1038/nature13039

Korostelev A, Noller HF (2007) The ribosome in focus: new structures bring new insights. Trends Biochem Sci 32(9):434–41. https://doi.org/10.1016/j.tibs.2007.08.002

Kurkcuoglu O, Doruker P, Sen TZ et al (2008) The ribosome structure controls and directs mRNA entry, translocation and exit dynamics. Phys Biol. https://doi.org/10.1088/1478-3975/5/4/046005

Kurkcuoglu O, Turgut OT, Cansu S et al (2009) Focused functional dynamics of supramolecules by use of a mixed-resolution elastic network model. Biophys J. https://doi.org/10.1016/j.bpj.2009.06.009

Levi M, Whitford PC (2019) Dissecting the energetics of subunit rotation in the ribosome. J Phys Chem B 123:2812–2923. https://doi.org/10.1021/acs.jpcb.9b00178

Levi M, Nguyen K, Dukaye L et al (2017) Quantifying the relationship between single-molecule probes and subunit rotation in the ribosome. Biophys J 113(12):2777–2786. https://doi.org/10.1016/j.bpj.2017.10.021

Levi M, Noel JK, Whitford PC (2019) Studying ribosome dynamics with simplified models. Methods 162-163:128–140. https://doi.org/10.1016/j.ymeth.2019.03.023

Levi M, Walak K, Wang A et al (2020) A steric gate controls P/E hybrid-state formation of tRNA on the ribosome. Nat Commun 11:5706. https://doi.org/10.1038/s41467-020-19450-0

Marshall R, Dorywalska M, Puglisi J (2008) Irreversible chemical steps control intersubunit dynamics during translation. Proc Nat Acad Sci USA 105(40):15,364–9. https://doi.org/10.1073/pnas.0805299105

Matsumoto A, Ishida H (2009) Global conformational changes of ribosome observed by normal mode fitting for 3D Cryo-EM structures. Structure 17(12):1605–1613. https://doi.org/10.1016/j.str.2009.09.017

Miyashita O, Onuchic JN, Wolynes PG (2003) Nonlinear elasticity, proteinquakes, and the energy landscapes of functional transitions in proteins. Proc. Natl. Acad. Sci USA 100:12570–12575.

Nguyen K, Whitford PC (2016) Steric interactions lead to collective tilting motion in the ribosome during mRNA-tRNA translocation. Nat Commun 7:10,586–10,586. https://doi.org/10.1038/ncomms10586

Ning W, Fei J, Gonzalez RL (2014) The ribosome uses cooperative conformational changes to maximize and regulate the efficiency of translation. Proc Natl Acad Sci USA 111(33):12,073–12,078. https://doi.org/10.1073/pnas.1401864111

Noel JK, Levi M, Raghunathan M et al (2016) SMOG 2: A versatile software package for generating structure-based models. PLoS Comput Biol 12(3):e1004,794. https://doi.org/10.1371/journal.pcbi.1004794

Pardi N, Hogan MJ, Porter FW et al (2018) mRNA vaccines-a new era in vaccinology. Nat Rev Drug Discov 17(4):261–279. https://doi.org/10.1038/nrd.2017.243

Ratje A, Loerke J, Mikolajka A et al (2010) Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. Nature 468(7324):713–716. https://doi.org/10.1038/nature09547

Rodnina MV, Wintermeyer W (2001) Fidelity of aminoacyl-tRNA selection on the ribosome: kinetic and structural mechanisms. Annu Rev Biochem 70:415–435. https://doi.org/10.1146/annurev.biochem.70.1.415

Rozov A, Khusainov I, El Omari K et al (2019) Importance of potassium ions for ribosome structure and function revealed by long-wavelength X-ray diffraction. Nat Commun 10(1):2519. https://doi.org/10.1038/s41467-019-10409-4

Salsi E, Farah E, Dann J et al (2014) Following movement of domain IV of elongation factor G during ribosomal translocation. Proc Natl Acad Sci USA 111(42):15,060–15,065. https://doi.org/10.1073/pnas.1410873111

Schluenzen F, Tocilj A, Zarivach R et al (2000) Structure of functionally activated small ribosomal subunit at 3.3 angstrom resolution. Cell 102(5):615–623. https://doi.org/10.1016/s0092-8674(00)00084-2

Schmeing TM, Ramakrishnan V (2009) What recent ribosome structures have revealed about the mechanism of translation. Nature 461(7268):1234–42. https://doi.org/10.1038/nature08403

Schuwirth BS, Borovinskaya MA, Hau CW et al (2005) Structures of the bacterial ribosome atv 3.5 å resolution. Science 310(5749):827–834. https://doi.org/10.1126/science.1117230

Seo S, Jang Y, Qian P et al (2014) Efficient prediction of protein conformational pathways based on the hybrid elastic network model. J Mol Graph Model 47:25–36. https://doi.org/10.1016/j.jmgm.2013.10.009

Shasmal M, Chakraborty B, Sengupta J (2010) Intrinsic molecular properties of the protein-protein bridge facilitate ratchet-like motion of the ribosome. Biochem Biophys Res Commun 399(2):192–197. https://doi.org/10.1016/j.bbrc.2010.07.053

Shaw DE, Grossman J, Bank JA et al (2014) Anton 2: Raising the bar for performance and programmability in a special-purpose molecular dynamics supercomputer. In: SC '14: Proceedings of the international conference for high performance computing, networking, storage and analysis, pp 41–53. https://doi.org/10.1109/SC.2014.9

Szabo A, Schulten K, Schulten Z (1980) First passage time approach to diffusion controlled reactions. J Chem Phys 72(8):4350–4357. https://doi.org/10.1063/1.439715

Takada S (2012) Coarse-grained molecular simulations of large biomolecules. Curr Opin Struct Biol 22(2):130–137. https://doi.org/10.1016/j.sbi.2012.01.010

Tama F, Brooks CL (2006) Symmetry, form, and shape: Guiding principles for robustness in macromolecular machines. https://doi.org/10.1146/annurev.biophys.35.040405.102010

Tama F, Valle M, Frank J et al (2003) Dynamic reorganization of the functionally active ribosome explored by normal mode analysis and cryo-electron microscopy. Proc Natl Acad Sci USA 100(16):9319–23. https://doi.org/10.1073/pnas.1632476100

Tan D, Piana S, Dirks RM et al (2018) RNA force field with accuracy comparable to state-of-the-art protein force fields. Proc Natl Acad Sci U.S.A 115(7):E1346–E1355. https://doi.org/10.1073/pnas.1713027115

Trobro S, Aqvist J (2008) Role of ribosomal protein L27 in peptidyl transfer. Biochemistry 47(17):4898–906. https://doi.org/10.1021/bi8001874

Trylska J, Konecny R, Tama F et al (2004) Ribosome motions modulate electrostatic properties. Biopolymers 74(6):423–431. https://doi.org/10.1002/bip.20093

Trylska J, Tozzini V, McCammon JA (2005) Exploring global motions and correlations in the ribosome. Biophys J 89(3):1455–1463. https://doi.org/10.1529/biophysj.104.058495

Valle M, Zavialov A, Sengupta J et al (2003) Locking and unlocking of ribosomal motions. Cell 114(1):123–134. https://doi.org/10.1016/s0092-8674(03)00476-8

Wang Y, Rader A, Bahar I et al (2004) Global ribosome motions revealed with elastic network model. J Struct Biol 147(3):302–314. https://doi.org/10.1016/j.jsb.2004.01.005

Whitford PC (2015) The ribosome's energy landscape: Recent insights from computation. Biophys Rev 7(3):301–310. https://doi.org/10.1007/s12551-014-0155-1

Whitford PC, Geggier P, Altman RB et al (2010) Accommodation of aminoacyl-tRNA into the ribosome involves reversible excursions along multiple pathways. RNA 16:1196–1204. https://doi.org/10.1261/rna.2035410

Whitford PC, Blanchard SC, Cate JHD et al (2013) Connecting the kinetics and energy landscape of tRNA translocation on the ribosome. PLoS Comput Biol 9(3):e1003,003

Yusupov MM, Yusupova GZ, Baucom A et al (2001) Crystal structure of the ribosome at 5.5 å, resolution. Science 292(5518):883–896. https://doi.org/10.1126/science.1060089

Zhang Z, Sanbonmatsu KY, Voth GA (2011) Key intermolecular interactions in the E. coli 70S ribosome revealed by coarse-grained analysis. J Amer Chem Soc 133(42):16,828–16,838. https://doi.org/10.1021/ja2028487