requires remodeling of multiple, spatially distant structural components of the
machine. In order to function efficiently, therefore, molecular machines likely
must allow for multiple, mutually independent conformational rearrangements. Due to the significant technical challenges associated with
characterizing their structural dynamics, however, the questions of whether
and how large molecular machines coordinate such dynamics so as
to maximize the efficiency with which they perform their biological functions
remain exceptionally challenging to answer. Using a combination of structural
and phylogenetic analyses, molecular genetics, single-molecule fluorescence
resonance energy transfer, and in vitro biochemical assays, here we demon-
strate that the ribosome uses cooperative conformational changes to maximize
the efficiency with which it translocates and ejects its transfer RNA adaptors
during protein synthesis. Interpretation of our data within the context provided
by atomic-resolution ribosome structures and phylogenetic analyses of ribo-
somal RNA and ribosomal protein sequences leads us to propose a structure-
based model for the observed cooperativity. Our results demonstrate that large,
multi-component, molecular machines such as the ribosome can use networks
of cooperative conformational changes to facilitate mechanical processes
that would otherwise limit their catalytic rates.

1214-Plat
Rotational Motions of Domains in Elongation Factor G Detected by Single-
Molecule Polarized Fluorescence Microscopy
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During the elongation cycle of protein synthesis, translocation of tRNAs and
mRNA is catalyzed by the GTPase elongation factor G (EF-G) with high pre-
cision and speed. Conversion of the GTP to the GDP form of EF-G is consid-
ered essential for translocation, but the structural dynamics on the ribosome
have not been reported. We used single molecule polarized total internal reflec-
tion fluorescence (pHOME) microscopy to characterize tilting and rotational
fluctuations within specific domains of EF-G. When EF-G binds to the ribo-
some pre-translocation (PRE) complex, domains I and IV of EF-G undergo
small rotations (10-15°) in conjunction with translocation, whereas domain
III shows a much greater angular change, averaging 50°. Voiymycin (Vio),
which prevents translocation, reduces the rotational motions of domain III to
10-15° but has virtually no effect on the other domains. Spectromycin also
reduces domain III motions but less strongly than Vio. EF-G binding to ribosomal
initiation complexess lacking A-site tRNA gives a similar pattern of domain
rotations, but with shorter dwell times. In this case, the large rotation of domain
III is barely inhibited by Vio. Irrespective of completion of translocation or
presence of A-site tRNA, the initial 10-15° rotations of EF-G domains I, III
and IV in the ribosome EF-G complex indicate that the EF-G initially shifts
the minimum of the free energy profile in the direction of translocation, sug-
gesting that EF-G generates a force on the ribosome and/or the mRNA and
tRNAs. Near the end of translocation, domain III completes its rotation either
to push the mRNA and tRNAs (a working stroke) or to prevent reversal of
translocation driven by thermal fluctuations (a ratchet). Supported by NIH grant
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1215-Plat
Reframing Crystal Structures Against Cryo-EM Data using Molecular Dy-
namics Simulations to Obtain a Complete Atomatic Pathway of Transfer
RNA Translocation
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The movement of tRNAs during translocation is accompanied by large confor-
mational changes of the ribosome such as intersubunit rotations. Here, we pre-
sent a method to cover conformational changes of the ribosome that occur on
timescales not accessible to equilibrium Molecular Dynamics (MD) simulations
through the combination of X-ray crystallography, cryo-EM data and MD sim-
ulations. Cryo-electron microscopy (Cryo-EM) provides medium/low-resolution
density maps for many intermediate states of large molecular complexes. In
contrast, X-ray crystallography provides high-resolution structures, usually
limited to the stable states. To obtain pathways connecting the intermediate states
in tRNA translocation, we used molecular dynamics simulations from crystal structures
with an additional biasing potential. This biasing potential, which maximizes the cor-
relation between atomic model and cryo-EM map (Tama 2008), allows us to drive
the ribosome from one intermediate state to another, covering the full transloca-
tion pathway. This method of cryo-EM driven MD was implemented in the
high-throughput and highly parallel MD simulation package GROMACS.