requires remodeling of multiple, spatially distant structural components of the
machine. In order to function efficiently, therefore, molecular machines likely
must allow for non-sequential, 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 (TIRF) microscopy to characterize tilting and rotational
fluctuations within specific domains of EF-G. When EF-G binds to the ribo-
somal 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 30°. Viomycin (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 complexes 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-
gestng 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
GM080376 to YEG and BSC and AHA fellowship 12POST8910014 to CC.

1216-Plat
The Effect of Codon Translation Rates on Cotranslational Protein Folding
Mechanisms of Arbitrary Complexity
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Bacterial cells use the naturally occurring variability in the rate at which
different codons are translated to guide the folding of nascent proteins into or-
dered, biologically-active structures during their synthesis by the ribosome.
Predicting how codon translation rates effect cotranslational protein folding
mechanisms is therefore of fundamental biological interest. Here we demon-
strate that cotranslational folding mechanisms sampling an arbitrarily large
number of states can be accurately modeled by treating this problem using
the Markov chain formalism. This allows a general equation to be derived
that describes the probability that a nascent protein is in any one of these
conformational or thermodynamic states as a function of translation rates of in-
dividual codons in an mRNA molecules’ open reading frame, which we show is
accurate in modeling molecular dynamics simulations of cotranslational
folding. Using this framework we demonstrate that there exists scenarios in
which, contrary to conventional wisdom, fast-translation codons can actually
increase the amount of cotranslational folding that occurs. This approach can
be applied to the cotranslational folding of cytosolic and membrane proteins,
and possibly the processing of nascent chains by auxiliary factors such as
chaperones and enzymes.

1217-Plat
Protein Synthesis by Ribosomes: Mapping In Vitro onto In Vivo Rates
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All living cells rely on ribosomes, powerful nanomachines that synthesize
proteins by translating the information encoded in mRNA molecules. Over
the past two decades, the various substeps of the translation process have been
studied in much detail using in vitro systems, but it is often questioned to
what extend these results can be applied to real living cells. Although in
vitro translation should proceed via essentially the same steps as in vitro, the
average protein synthesis rate turns out to be much faster. This difference in
synthesis rate has led to a long and controversial debate because - in contrast
to in vitro systems - it has not been possible to study individual conformational
transitions of the translating ribosome in vivo. Here, we address this long-
lasting and unresolved puzzle and introduce a general computational scheme,
which enables us to map in vitro onto in vivo rates. Using new experimental
data on in vitro translation at 20°C and 37°C, we first derive a complete set
of in vitro rates for the individual substeps of translation. We then use our
scheme, together with available in vivo data, to determine the corresponding
in vivo rates. As a result, we obtain a comprehensive description of in vitro
and in vivo translation for various experimental and growth conditions. This
description allows us to predict codon- and mRNA-specific translation rates,
which can be used, e.g., to investigate translational pauses and ribosome traffic.

Symposium: Molecular Basis of Voltage Dependence

1218-Sym
Thermodynamic Analysis of Voltage-Sensing Mechanisms
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Voltage-gated ion channels (VGIC) form a large superfamily of ion channels
and the activation of these channels underlie electrical and chemical signaling
in a variety of cell types. Structure-function studies are widely used to deduce
the energetic effects of a mutation by measuring macroscopic currents and
fitting their voltage-dependence to a Boltzmann function. However, in absence
of detailed kinetic models, this approach introduces serious errors in free-
ergy estimates because of the inherent assumption that the channel activation
is a two-state process. We recently developed analytical tools that allows us to
calculate the free energies required for activation of voltage-dependent pro-
cesses without any prior knowledge of the underlying gating scheme. Our
method involves measurement of conjugate displacement associated with the
flow of ions across the cell membrane and application of the fluctuation
theorem. In the case of voltage-gated ion channels, gating charge movement is the
conjugate displacement and the force is voltage across the membrane. We show that by measuring the median
electric voltage of charge transfer, VM, and the total gating charge per channel, we can
calculate the chemical free energy difference between the resting and activated
state of the channels. These free-energy measurements can be extended to other
members of the VGIC superfamily to obtain a measure of interaction energies