The Ribosome –

a Restless Molecular Machine

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The year 2000, well known as the year of the Y2K scare, as the threshold to
the new Millennium, and to specialists
as the annus mirabilis in the long quest of
X-ray crystallography for the structure of
the ribosome (Ban et al., 2000; Schluenzen
et al., 2000; Wimberly et al., 2000). This has
a fourth special significance to me because
of our discovery of the ratchet-like motion
of the ribosome (Frank and Agrawal, 2000).
Rajendra Agrawal and I found, by comparing
cryo-electron microscopy density maps of
the Escherichia coli ribosome with and
without elongation factor G bound, that the
two subunits rotate with respect to each
other, back and forth, once for every amino
acid that is added to a growing polypeptide
chain. Thus, a good-sized protein of 200
residues takes 200 back-and-forth rotations
to make, about 15 per second, consuming on
average a total of 13 seconds in the cell. We
hypothesized that this motion was part and
parcel of the complex mechanism required
to move mRNA and the tRNAs relative to the
ribosome, in a process called translocation.
In fact, such a ratchet-like relative motion
of the two subunits had been much earlier
proposed by Alexander Spirin (1968), well
before the means of visualization by electron
microscopy were available.

We could infer from sequence
comparisons and other earlier work that
eukaryotic and bacterial ribosomes must
be quite similar in structure and functional
principles, so it was not too much of a
flight of imagination to assume that the
same motion would be employed during the
protein synthesis of multicellular organisms,
as well, which include plants, animals, and
thus, humans. The realization that each
of the thousands of ribosomes in each cell
each living being on this planet the
exponent keeps compounding as I’m writing
this down!-performs this motion incessantly
was a mesmerizing idea. I do remember
driving through Vermont on the way from
Albany to the Gordon Conference in New
Hampshire, taking one of the rural undivided
highways flanked by the unspoiled green of
early summer, when I was suddenly overcome
with this mind-boggling idea: that every leaf
of every tree housed millions of these restless
molecular machines. (It was fortuitous that
I was alone in my car since I might have
sounded exceedingly stupid in my euphoria).
As I was driving on, the idea became even
more obsessive: some of the waving motions
of the leaves might not be due to the wind,
but to some global effect of a sporadic but
massive vector addition? Might the rotation
of the earth have been affected in a subtle
way, adding or subtracting a second over the
span of the past 3 ½ billion years that the
ribosome has been around?

Meanwhile a decade has gone by; we
have learned more about the ribosome’s
incessant motion, and are grappling to put it
into the conceptual framework of molecular
machines. First, from what I stated before,
it was not a surprise to find that eukaryotic
ribosomes are busy rotating back and
forth in a similar way (Spahn et al., 2004).
Next, an experimental corroboration of the
motion in the bacterial ribosome came from
fluorescence experiments by Harry Noller’s
group: an acceptor B attached to the large
subunit, and two nearby donors A,C attached
on either side of B to the small subunit are
expected to report anticorrelated distance
changes upon EF-G binding: when A-B is
large, B-C should be small, and vice-versa.
This behavior could indeed be observed in
the bulk FRET experiment in which EF-G was
added to a sample with translating ribosomes
(Ermolenko et al., 2007) (Fig.1). Single-
molecule FRET experiments allowed signals
to be picked up from single ribosomes, and
these not only confirmed the results of the
bulk study, but also showed that the motion
is a stochastic process (Cornish et al., 2008).

But the real surprise came from the
observation by Ermolenko et al. (2007) and in
single-molecule FRET observations (Cornish et
al., 2008; Fei et al., 2008; Kim et al., 2007) that
for the intersubunit motion and associated
movements of the L1 stalk and tRNAs to
depend, no EF-G is required at all, as long as
the concentration of magnesium ions is in
the range of physiological concentrations.
As all surprises in Science, this one offered
a new insight. After accommodation of
a new aminocarboxyl-tRNA into the A site
and subsequent transfer of the peptide
bond to the amino acid on the A site, the
ribosome — it now appears — is constantly
oscillating between the normal state (termed
Macrostate I) with the tRNAs in the classic
A/A, P/P positions and an intersubunit-
rotated state (Macrostate II) in which the tRNAs are allowed to slip into the A/P, P/E hybrid positions. These random oscillations of the pre-translocational ribosome are evidently driven by Brownian motion, and the conformation of the ribosome required for the binding of EF-G is being constantly visited even in a sample devoid of EF-G. The role of EF-G in binding to the ribosome, then, is to "nail down" the ribosomal conformation that productively leads to the next steps, namely GTPase activation, GTP hydrolysis and Pi release, accompanied by conformational changes in EF-G and the decoding center that unleash the final steps of translocation (Ratje et al., 2010; Taylor et al., 2007).

Two cryo-EM studies, on the footsteps of the FRET results, produced a beautiful confirmation of the existence of a heterogeneous mixture in a factor-free pre-translocational sample (Agirrezabala et al., 2008; Julian et al., 2008). Classification techniques had to be used to extract the two subpopulations, resulting in three-dimensional density maps of ribosomes bound with tRNAs in conformations either characterized as {Macrostate I, A/A, P/P} or {Macrostate II, A/P, P/E} (Fig. 2). More recent studies conducted with large datasets and refined methods of classification indicate that the passage from one state to the other goes through numerous intermediates (Agirrezabala et al., 2011; Connell et al., 2008; Fischer et al., 2010; Frank, 2010; Fu et al., 2011).

The new perspectives provided by the complementary experimental studies, mainly using smFRET and cryo-EM, have been articulated in recent reviews (Frank and Gonzalez, 2010; Munro et al., 2009). Although the collection of relevant experimental data is far from complete, the conceptual framework for analyzing the rich troves of information coming from the two experimental techniques and X-ray crystallography, as well as exploratory molecular dynamics simulations (Gumbart et al., 2011), has been set.

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**Fig. 2.** Cryo-EM study of a pre-translocational ribosome complex in the absence of EF-G. The sample proves to be heterogeneous, with part of the molecules in the classic state \{Macrostate I, A/A, P/P\} (panel A) and part in the intersubunit-rotated, hybrid state \{Macrostate II, A/P, P/E\} (panel B). Annotations: 30S: small subunit; bk: beak; h: head; sp: spur; 50S: large subunit; L1: L1 stalk; CP: central protuberance; L7/L12: L7/L12 stalk; A/A, P/P, A/P, P/E: tRNA positions. (From Agirrezabala et al., 2008; reproduced with permission by Cell Press).

**References**


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