Peptidyl transferase

Uranya: If you talk to almost any contemporary molecular biologist or biochemist they will tell you that the answers lie in the *RNA World* point of view. I know you have already explained where you think the RNA World arises during geophysical chemical evolution but tell me again the key points.

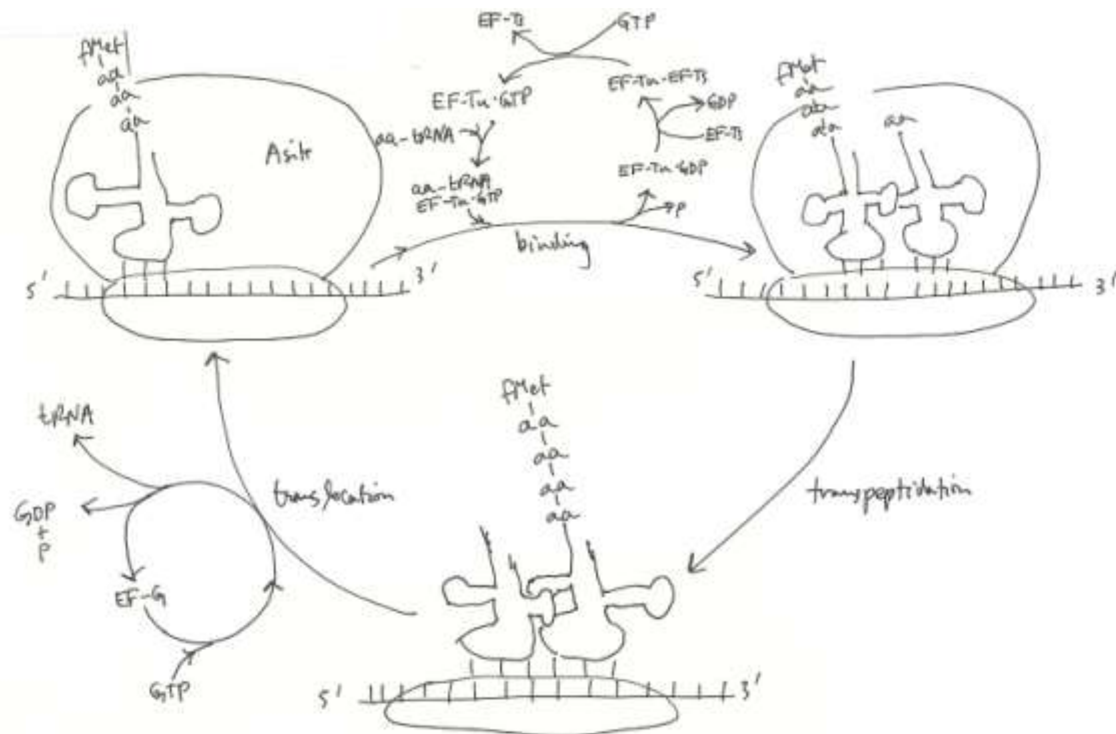
Reynard: Okay. The main objection is that the typical RNA World argument is given *out of context*. Let that idea sink in! Instead, it is assumed that RNA's of every length are readily available. Clever mechanisms are often constructed using this material. *In context*, there is a highly energized (UV, redox, thioester, P~P) microsphere world with dividing compartments that are impermeable to polymers, even of short length (6 to 9 units). Initially the *general context* is an energized racemic polypeptide world with membranes and microspheres and replication by division of these compartments. Furthermore, RNA requires mononucleotides, as its monomers, and these monomers are dehydration condensates of ribose, a purine or pyrimidine base and phosphate. *Mono*mer formation is thermodynamically *inhibited* and the presence of ribose needs to be explained. *Poly*nucleotide formation is both thermodynamically and kinetically *inhibited*. The inhibition is enormous! Only within the context of the energized micropheres, can any polynucleotides be considered possible, and these must be fairly short, say hexamers and heptamers (and smaller). A proto-ligase for polynucleotides would be one of the earliest acquisitions for the proto-genome. As explained earlier, the proto-ligase is a pure chirality polypeptide directly made from an pure chirality RNA gene (as such the proto-ligase is a linear polymer without branching). This sort of RNA World is spawned by polypeptides and does not entirely precede them, as is often assumed in the RNA World literature.

U: So you see the RNA world arising eventually ? You have already argued that chirality was selected at the same time RNA became important in the mechanism, and that RNA-gene replication and propagation within dividing microspheres is the *birth of genetics*.

R: Yes indeed. In the broader context of energy and microspheres the emergence of the RNA World is much richer. At the same time we understand the origin of the *cell* and of *genetics*. It is the *propagation of an encapsulated genome* that we recognize as *life*.

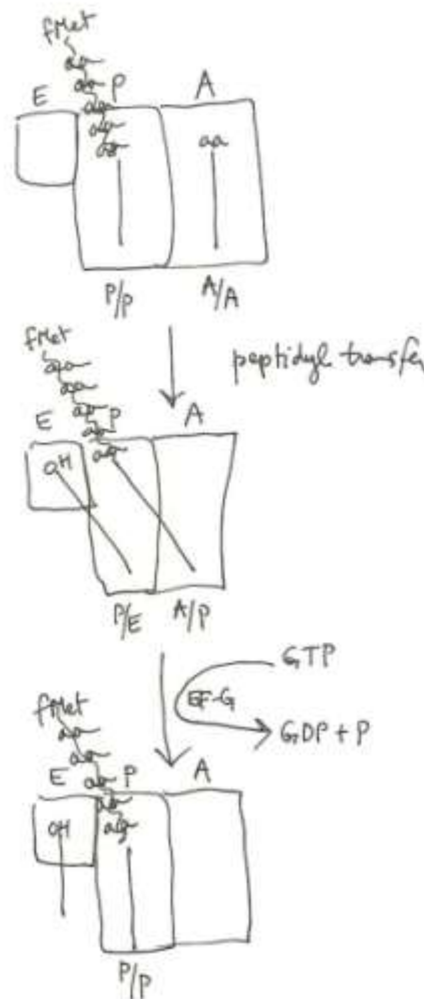
Considerations of these issues within a limited context can invite fundamental errors that are avoided if a broad enough context is constructed instead. I will give an example that has to do with the *peptidyl transferase* activity of the 23S rRNA component of the big ribosomal subunit. This activity is a prime example of *ribozyme* catalysis. However, the mechanism of its function is still unknown, inspite of the fact the during the early 2000’s it was asserted in the primary literature and in reviews that the mechanism had been found. Catalysis by a particular adenine base (#2451) was invoked. Eventually, mutant experiements threw this idea into disrepute. Ironically, during those earlier years *you* were held in disrepute if *you* questioned the adenine 2451 mechanism. This is fairly typical of how science really works at the people level. Let me present an alternative mechanism suggested by the foregoing. If you look inside the Short Cut titled [*Polymer biosynthesis*] you will find the following excerpt:

The 50S sununit contains three sites used during elongation of the polypeptide, the E site, the P site and the A site. The $fMet-tRNA_f^{Met}-mRNA-ribosome$ complex has $fMet-tRNA_f^{Met}$ in the ribosome’s P site while the empty A site is ready to accept an incoming aminoacyl-tRNA that will be recognized by codon anti-codon base pairing. A binary complex of GTP with elongation factor EF-Tu combines with the aa-tRNA. As this complex binds the ribosome, GTP is hydrolyzed to GDP and P and the aa-tRNA is bound to the A site by codon anti-codon base pairing. EF-Tu-GDP and P are released. EF-Tu-GDP has GDP displaced by EF-Ts, which in turn is displaced by GTP, regenerating EF-Tu-GTP.



Transpeptidation occurs in which the fMet carboxyl forms an amide linkage with the new aa's amino group. EF-Tu is needed to help the new aa-tRNA bind at a rate sufficient to support cell growth. It is present in the cell with ~ 100,000 copies per cell, which is approximately the number of tRNA's. Consequently, almost all the cell's tRNA's are bound to EF-Tu's. But EF-Tu does not bind either $fMet - tRNA_f^{Met}$ or $Met - tRNA_m^{Met}$. The transpeptidation reaction appears to be catalyzed by the 50S subunit's 23S rRNA. This is an example of *ribozyme* activity (Science **289**, 11Aug., 2000). A ribozyme is an RNA that catalyzes a reaction. The repertoire of known ribozyme activities, while impressive, is very much smaller than the repertoire of protein catalysts. Because RNA can serve as a repository of genetic imformation much like DNA does and because it has some ribozyme activities, some researchers have proposed that the origin of life went through a stage called the *RNA world* before there was gene directed protein biosynthesis.

Once the peptide synthesis has commensed, elongation takes place. This process brings into play the E site.



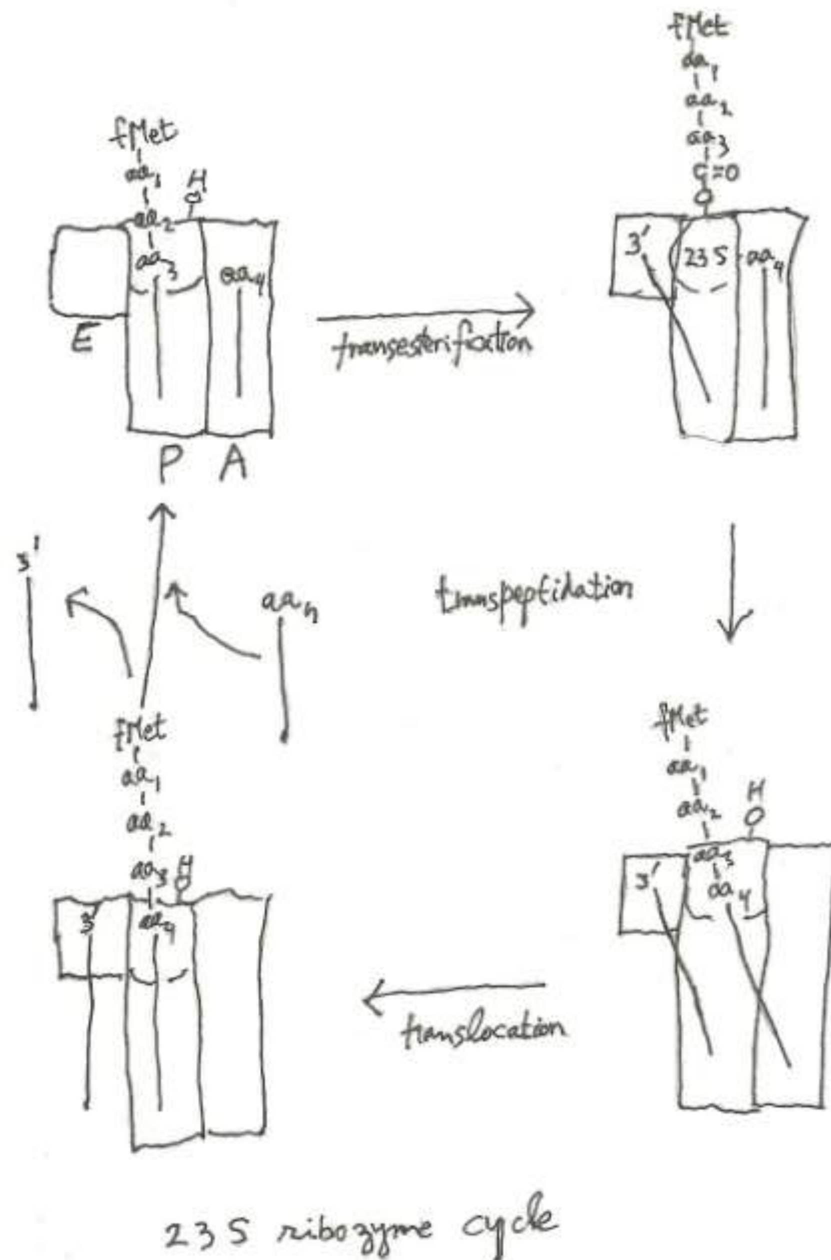
A growing peptide is in the P site and a new aa-tRNA has arrived at the A site. Each is attached to the mRNA by base pairing. A transpeptidation reaction takes the growing polypetide's

carboxyl group that is intially attached to the tRNA and joins it to the free amino group of the aa-tRNA in the A site. The terminal –OH group of the ribose at the 3’ end of the tRNA of the P site moves to the E site. The elongated polypeptide attached to the A site tRNA moves to the P site. Elongation factor EF-G binds and hydrolyzes GTP to GDP and P while shifting the mRNA so that the elongated polypeptide’s attached tRNA is now entirely in the P site and the freed tRNA is entirely in the E site from which it detaches. The ribosome is now ready for another aa-tRNA to arrive and elongate the polypeptide by one more residue.

U: Yes, I remember this.

R: These transpeptidation steps are somewhat ambiguous as stated. Once the aa-tRNA is in the A site, does the peptidyl-tRNA move its 3’ end over to the aa in the 3’ A site as intimated above? Or does the amino group at the 3’ end of the A site attack the peptidyl linkage in the P site as suggested in the adenine2451 catalysis (ribozyme) mechanism? Either of these possibilities seems to bring bulky groups on top of each other and would be stereochemically difficult. Instead, imagine that the first step is a transesterification of the growing peptide from the 3’ P site onto a 2’-OH group of a 23S rRNA ribose, perhaps the one attached to the adenine residue #2451 . Next, the freed 3’ end of the tRNA in the P site moves its empty 3’ end to the E site, a stereochemically easy step. Now the 3’ aa end of the tRNA in the A site can easily move its 3’ end into the vacant 3’ end of the P site. A third step, transpeptidation, occurs linking the growing polypeptide to the amino group that is now at the 3’ end of the P site. The translocation steps for the mRNA are the same as in the excerpt above. Release of the uncharged tRNA and binding of a charged one regenerates the initial state but with a polymer a unit monomer longer.

Experiments with *deoxy-ribose* mutants could support or eliminate this model. This idea is depicted in the figure below. The upper right-hand corner shows the state after transesterification and movement of the P-tRNA 3’ end to the E site has occurred. The lower right-hand corner shows that the A-tRNA 3’ end has moved to the 3’ end of the P site and transpeptidation has taken place. Translocation of the mRNA (not depicted) and recharging with aa-tRNA^{aa}’s finish the cycle.



The transesterification step is energy neutral, i.e. the high energy ester connecting the polypeptide and the tRNA becomes an equally high energy ester connecting the polypeptide and ribose 2'-OH . The transpeptidation step is downhill in Gibbs free energy. This is making polymers at the expense of phosphate energy.

[The reader should view this as a "chalk talk" by Reynard on a classroom blackboard in front of Uranya. That is the reason all of the material in the *Mysterium Tremendum* site is illustrated with simple hand drawings. The informality associated with chalk talks is reflected in the the drawings on the

blackboard. We need not attempt to amaze the reader with *high tech figures* since the primary focus here is with *ideas.*]

Even if this mechanism for *proto-peptidyl transferase* acitivity is eventually shown to be invalid, it demonstrates that detailed plausible modeling can be done. For the problem of the origin of the aaRS's, however, it would already be an achievement to have even one plausible model.