How-To-Do-It

A Physical Model Illustrating Protein Synthesis on the Ribosome

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We encountered difficulty in teaching the steps in protein synthesis to college students at various levels. These difficulties stemmed from the usual method of describing the dynamics of protein synthesis by sketching a set of discrete states, such as those illustrated in a typical textbook (e.g., Lewin 1985). The textbook method of illustrating the addition of an amino acid to a nascent peptide chain requires students to grasp intermediate steps in the movement and relationships of the various components. If students were unable to integrate the steps into a coherent whole we found they had great difficulty understanding protein synthesis as well as other concepts incorporating stepwise polymerization such as nucleic acid synthesis.

As an aid to illustrating the steps in protein synthesis we devised a simple physical model of the basic components of protein synthesis: a 70s prokaryotic (80s eukaryotic) ribosome, a messenger RNA, transfer RNAs and amino acids. Our model does not include aminoacyl t-RNA synthetases, the initiation reaction, transfer factors, "G" factor, removal of f-Met from the nascent peptide, or termination factors. We have found that once the basic steps are outlined these other embellishments are relatively easy to add.

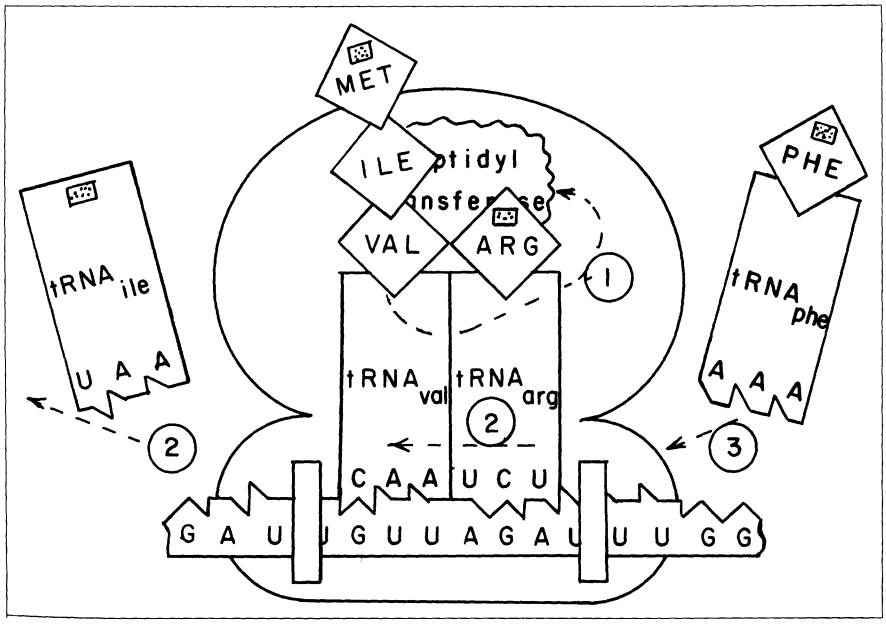

Figure 1. A drawing of the model in operation. Peptidyl-t-RNA$_{VAL}$ is shown in the "P" site and ARG-t-RNA$_{ARG}$ is in the "A" site. The previously discharged t-RNA$_{ILE}$ is to the left; the incoming PHE-t-RNA$_{PHE}$ is on the right. The m-RNA is truncated as drawn (see text for discussion). The next steps are 1) peptidyl transfer from the t-RNA$_{VAL}$ to ARG; 2) translocation of the peptidyl-tRNA$_{ARG}$ to the "P" site with concomitant expulsion of the discharged t-RNA$_{VAL}$ and 3) entry of PHE-tRNA$_{PHE}$ into the "A" site.

We have successfully used the model to teach in a non-majors course, in an introductory course, in a sophomore-level standard genetics course and in an upper level molecular genetics course. The model is useful both in lecture and as a "hands-on" tutorial available in a learning-center, a lab, or on reserve in the reading room.

Our first model was constructed of single-thickness cardboard and the components held together with paper clips. These construction materials proved to be of poor durability and were flimsy. Subsequent models were cut from foam-cored construction board and components secured by the use of Velcro "hook" and "loop" strips pinned and glued to the construction board pieces. It is the latter model which we describe here.

Figure 1 is a drawing of one of our models. The model can be any convenient size. This particular model has a

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70s (80s) "ribosome" which is about 50 cm by 50 cm, the "m-RNA" is about 4 cm by 1 m with "nucleotides" of about 2 cm each. The "t-RNAs" are each about 6 cm by 9 cm, and the "amino acids" are about 5 cm on a side.

It is best to cut out all pieces before assembling to allow proper location of supports and Velcro(R) attachment strips. We use standard white construction glue and 1.5-2 cm straight pins.

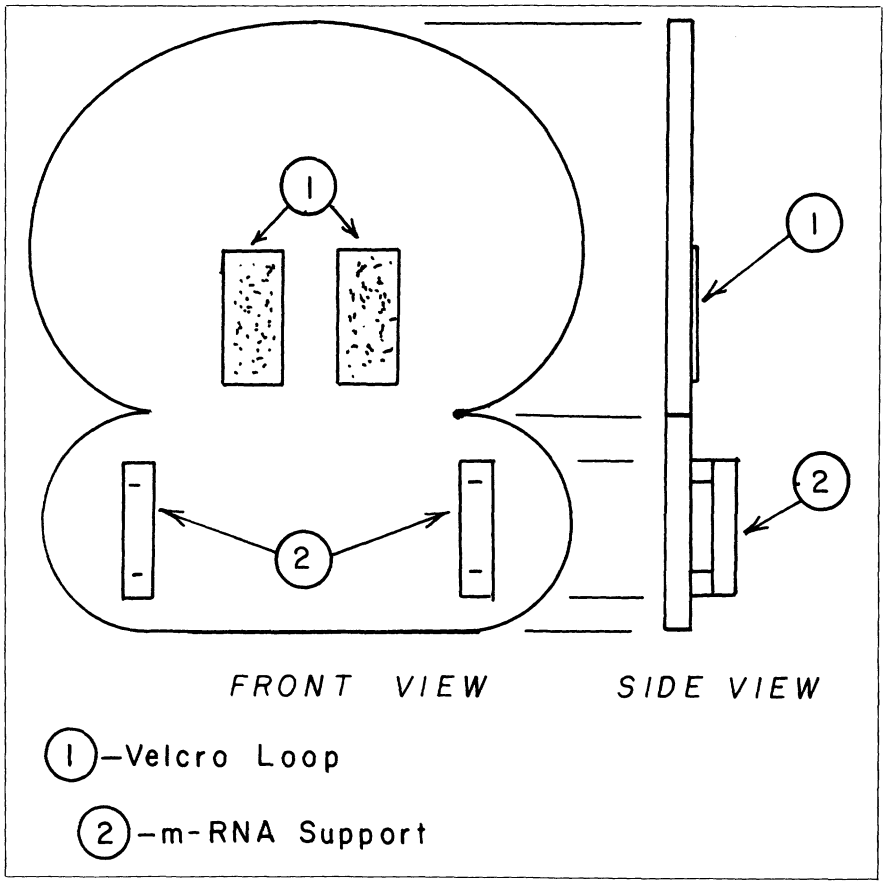

Figure 2. Construction details of the Ribosome. Pins (not shown) are sometimes used at the corners of each Velcro strip to reinforce the glue, as these strips are subject to the greatest stresses as the model is operated.

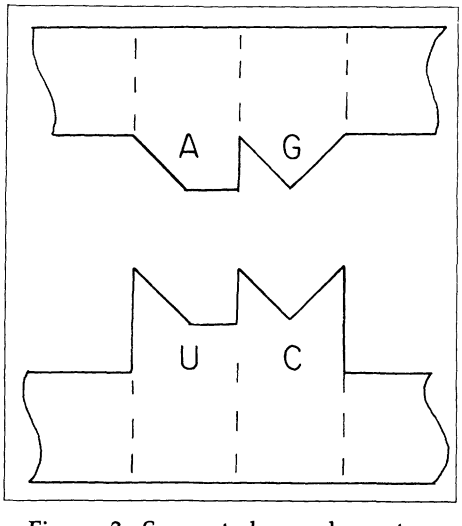

Figure 3. Suggested complementary shapes for the individual bases. The shapes shown form unique complementary surfaces. Simpler shapes can be used with a loss in uniqueness but not necessarily in function.

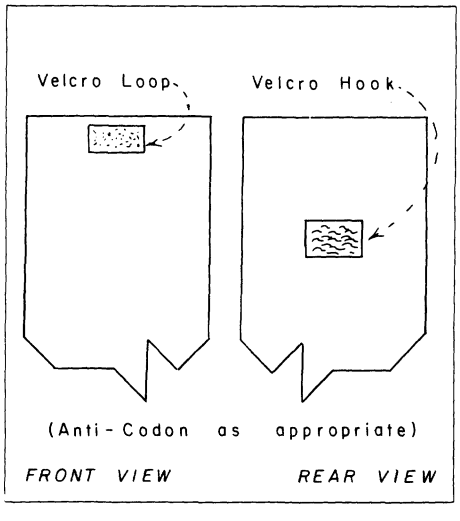

Figure 4. Construction details of a t-RNA.

The construction details of the "ribosome" are shown in Figure 2. The overall shape is bilobate to mimic the two sub-units. Two mechanical supports for the "m-RNA" are shown, along with two pieces of Velcro "loop" tape, which are glued and pinned in place at the "A" and "P" sites to allow binding of the "t-RNAs".

The "m-RNA" can be of varying complexity; part of one is shown in Figure 1. Our "m-RNAs" have nucleotides cut to complementary shapes as suggested in Figure 3. We always include an "AUG" initiator codon and one of the three terminator codons. There is, of course, no "t-RNA" for the terminator codon but we do not attempt to model termination factors, we simply talk about these factors at the appropriate time. In some models we simply label the nucleotides upstream of the initiator as "leader" and downstream of the terminator as "tail." Other models have the "AUG" embedded in a sequence of nucleotides to demonstrate the selection of reading frame and treat the terminator in a similar manner. We do not attempt to model features such as the Shine-Dalgrano sequence. Our "m-RNA" usually codes for about 5 amino acids beyond the methyonyl "AUG."

Details of a typical "t-RNA" are shown in Figure 4. The Velcro "loop" on the front upper side accepts the "amino acid"; the Velcro "hook" on the rear is for attachment to the Velcro "loop" at the "A" and "P" sites on the ribosome. The "anti-codon" nucleotides are cut as appropriate.

"Amino acids" are simple squares of construction material as shown in Figure 5. A small piece of Velcro "hook" on the lower rear and a piece of Velcro "loop" on the front mimic the carboxy and amino terminii, respectively, and allow attachment to the corresponding "t-RNA" as well as subsequent "polymerization."

Suggestions for labeling the various components are shown in Figure 6. We usually label the "A" and "P" sites on the ribosome and indicate the location of the peptidyl transferase and the m-RNA binding site on the small sub-unit. The "t-RNAs" are labeled "t-RNA_{AA}" to show that they carry the code for the amino acid. Each base in the anti-codon is identified by a single letter abbreviation. Amino acids are labeled by the triplet code, as indicated by "AA" in the figure.

The operation of the model is shown in Figure 1. Initiation is mimicked by "finding" the "AUG" with the (f-met)-"Methyonyl-t-RNA$_{met}$." The charged t-RNA is moved to the "P" site, and the next "AA-t-RNA$_{AA}$" is entered into the "A" site with appropriate commentary about accessory factors and the name of the "acceptor" site. Next, with commentary about peptidyl transferase and allosteric interactions between the various components, the "met" is removed from the "t-RNA$_{met}$" and transferred to the second amino acid.

The discharged "T-RNA$_{met}$" is removed from the "P" site. This is an appropriate time to discuss the reusability of transfer RNA, and to stress the recharging of the t-RNA by aminoacyl-t-RNA synthetase.

Translocation is mimicked (with appropriate tearing and remaking of Velcro closures) by moving the "dipeptide-tRNA$_{AA}$—m-RNA" complex from the "A" site to the "P" site. This is an appropriate time to discuss the name of the "peptide" site, as the dipeptide in the site makes its name suddenly very obvious to the student.

"AA#3-t-RNA$_{AA3}$" is then entered into the empty "A" site, and the cycle repeated. Most students grasp the basic mechanics of protein synthesis following the addition of two or three amino acids. The model is then offered on an after-class basis to those who do not grasp the mechanics from the in-class demonstration.

Following the demonstration, the steps of protein synthesis as illustrated in textbooks make more sense to most students. It is an easy matter to embellish the various reactions, accessory factors and the processes of initiation and termination in detail appropriate to the level of the class.

Acknowledgments

Thanks to C. Budd, T. Budd, M. Guccione and K. McKnight for help, criticism and encouragement.

References

Lewin, B. (1985). *Genes* (2nd ed.). New York: John Wiley and Sons.

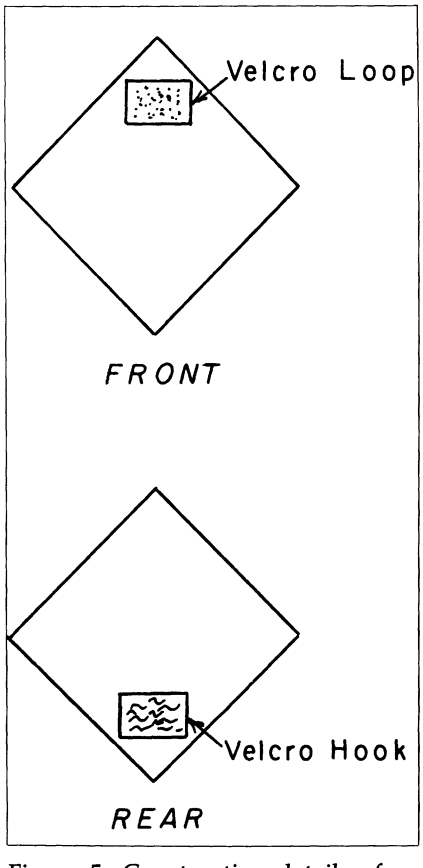

Figure 5. Construction details of an amino acid.

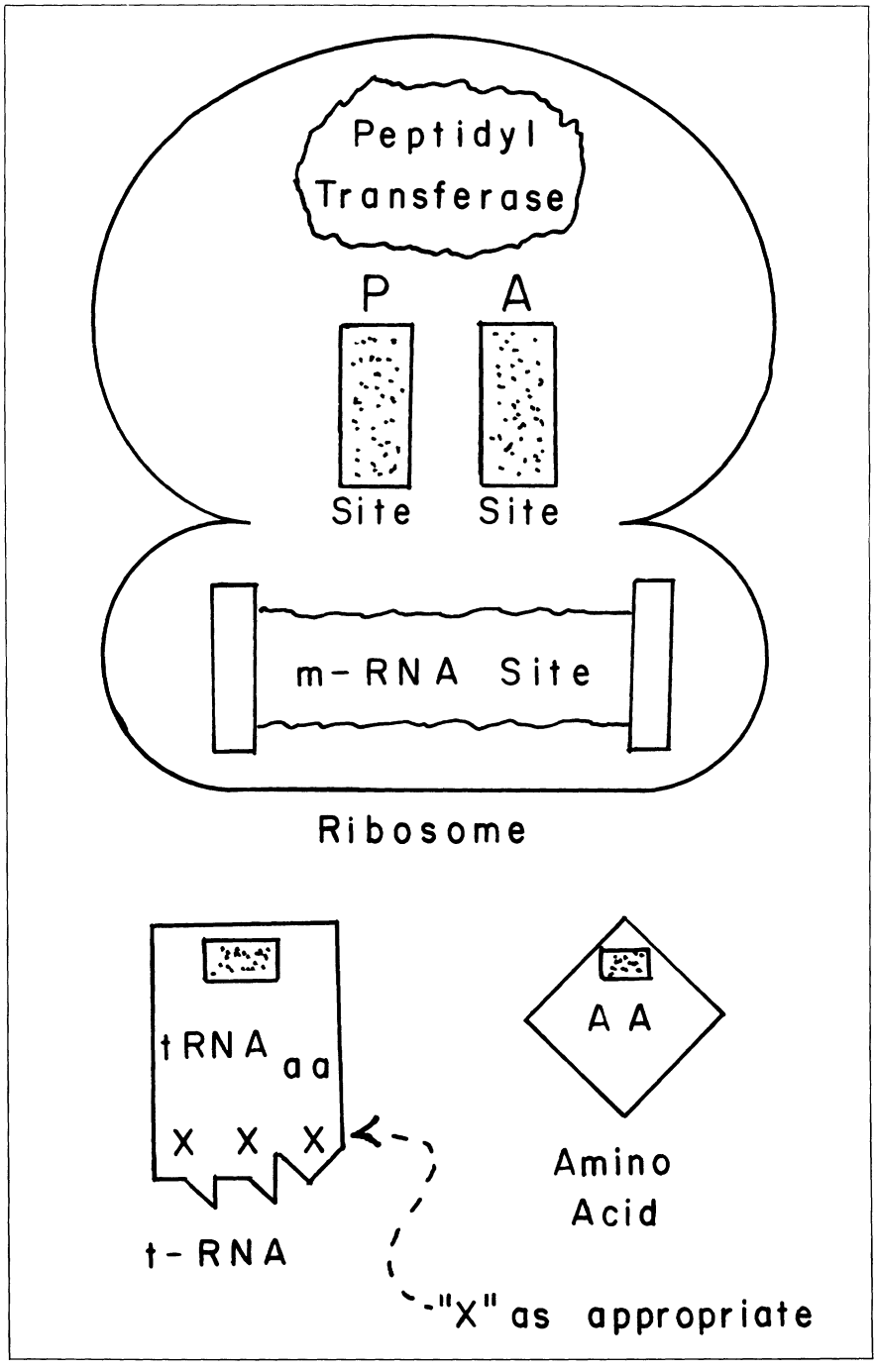

Figure 6. Suggested labeling of components, not including bases of m-RNA.