

CHAPTER 16

DINTZIS: PROTEINS ARE ASSEMBLED FROM ONE END

In 1963, Howard M. Dintzis demonstrated that proteins are assembled in a linear fashion, starting from the N-terminal end. He analyzed the alpha-hemoglobin proteins found in mature red blood cells. Using radioactive labeling along with electrophoresis, he was able to “watch” the proteins being made.

FORMATION OF PEPTIDE BONDS

With the isolation of tRNA, determinations of its structure, and elucidation of how it is charged by the amino acyl tRNA synthetase, the key elements in the translation of the genetic code had all become understood. The only question remaining was the formation of the bonds between adjacent amino acids—held in position by binding of their tRNAs to the mRNA. This final step in protein synthesis is not a simple one, however, as implied by the complex structure of the ribosome.

The most straightforward hypothesis to describe the overall process of protein polypeptide synthesis is that each of the various charged tRNA molecules makes its way to the appropriate codon, where the peptide bonds are formed as adjacent positions are filled, with the ribosomes helping to align the charged tRNA molecule. This model implies that, unlike DNA synthesis, the polymerization would occur simultaneously all along the chain, rather than proceeding from one end or from fixed internal initiating points.

POLYPEPTIDE FORMATION HYPOTHESIS

It is possible to distinguish between these two models of polypeptide chain formation if the process of synthesis and its intermediates can be studied. The first hypothesis of random tRNA binding predicts a random assortment of new protein fragments (peptides) as intermediates, while the second hypothesis of sequential synthesis predicts a single new fragment of variable length, depending on the time expired since initiation of synthesis. In principle, one could add a ^{14}C label to active cells, wait just a few moments, and then harvest the cells and isolate the proteins. Newly-finished proteins would carry ^{14}C label on the amino acids added last. In the first case, such labeled amino acids should appear scattered throughout the protein, while in the second case the labeled amino acids should be clustered in one or just a few proteins. This was the nature of Howard M. Dintzis's 1963 experiment, and his results were unmistakable: proteins are put together in serial sequence starting from the N-terminal end.

Recall that Sydney Brenner *et al.* established the direction of mRNA translation as being 5' to 3'. The three polarities of information in gene expression are therefore:

DNA	5' 3'
mRNA	5' 3'
protein	NH ₂ COOH

EXPERIMENTAL HURDLES

The key experimental problem in examining the intermediates of protein synthesis is that most cells are simultaneously producing many different proteins. If ^{14}C amino acids precursors are added and *several* labeled peptide fragments result, how can they be distinguished from one another? Are they newly-added amino acids scattered throughout a protein's length or are they a single labeled fragment for each protein

(but a different one for each of the many different proteins, which have different amino acid sequences)? The way to surmount this analytical problem, of course, was to look at the synthesis of a single protein, without “interference” from other proteins. It is in understanding the importance of this issue that Dintzis’s experiment stands out as a particularly lucid and powerful one.

To avoid the confusion introduced by simultaneous synthesis, Dintzis chose to work on mature rabbit reticulocytes (red blood cells). Reticulocytes are quite remarkable cells in that early in their development they stop the synthesis of almost all proteins—all but the two (α and β) polypeptide chains of hemoglobin, which they produce in large amounts. It is easy to isolate and purify hemoglobin from red blood cells (RBCs), and the α and β chains can be readily separated from one another. In this system, Dintzis was able to examine the pattern of protein synthesis in terms of a single polypeptide, the β -chain of hemoglobin (β -Hb).

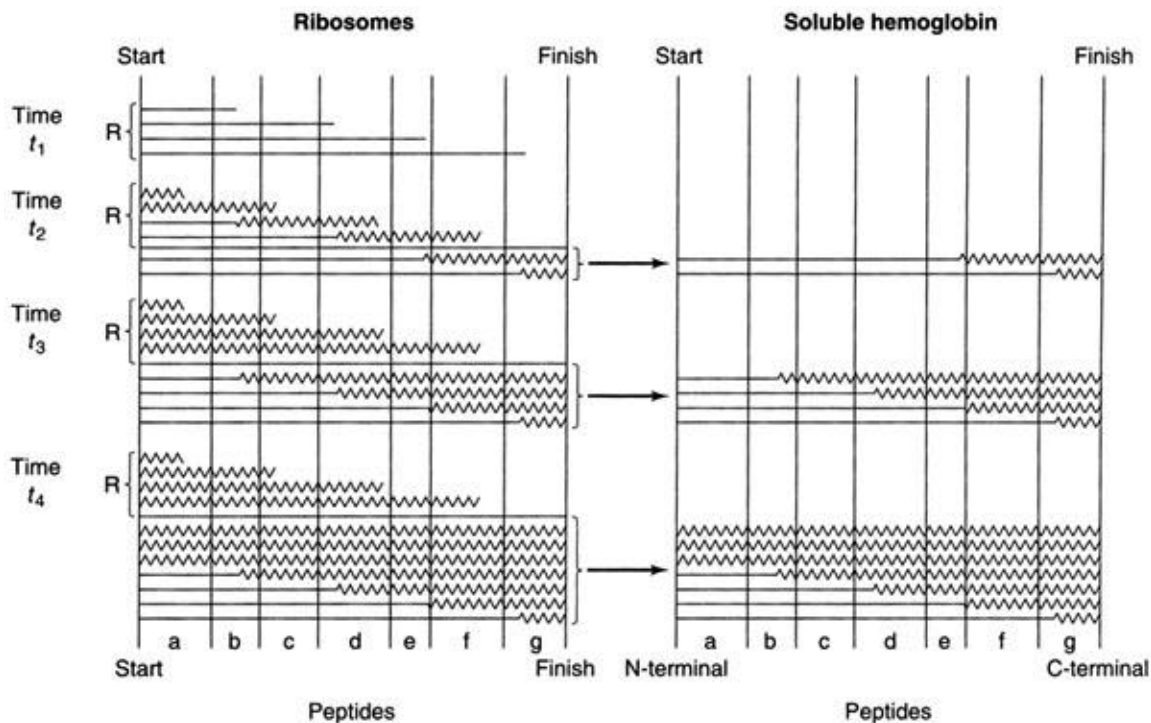


Figure 16.1

Dintzis’s experiment. The left side of this diagram shows the patterns of unlabeled (straight lines) and labeled (wavy lines) nascent polypeptide chains present on the ribosomes at t_1 and at progressively later times, t_2 , t_3 , and t_4 . As time progresses, the completed hemoglobin molecules contain more and more labeled peptides. The first to be seen occupy the last portion of the protein to be made, the C-terminal end. The last to appear represent the initial portion of the protein, the N-terminal end. Synthesis is thus in the $N \rightarrow C$ direction.

FINGERPRINTING HEMOGLOBIN MOLECULES

The β -Hb system had another great experimental virtue: this protein had been the subject of extensive structural investigations by Vernon M. Ingram and others, so that techniques existed for fragmenting the β -Hb proteins, a critical requirement of Dintzis’s experimental approach. In 1956, Ingram had developed a system of *fingerprinting* hemoglobin molecules, fragmenting them in specific ways so that variation (due to sickle-cell anemia or other causes) could be attributed to the appropriate fragment.

Ingram's fingerprinting technique was performed by purifying hemoglobin from red blood cells, fragmenting hemoglobin protein into peptides with the enzyme trypsin, separating the fragments (based on their respective charges) by electrophoresis, and staining his results. In this way he produced a "fingerprint" of the protein, as the different amino acids would migrate to different locations on the electrophoretic gradient based on their charges.

DINTZIS'S EXPERIMENT

Dintzis expanded Ingram's protein fingerprinting technique with a radioactive label (figure 16.1). He added ^{14}C -labeled amino acids to mature reticulocytes, which are always involved in synthesizing hemoglobin. At first, no label was apparent in the hemoglobin isolated immediately from the cells because newly-made proteins remain bound to their ribosomes until they are completed (partially synthesized "nascent" chains are not recovered as free fragments). At varying times, Dintzis removed cells and extracted the α -Hb. After a few minutes he started to obtain cells containing radioactive α -Hb. These represented completed Hb molecules in which the last few amino acids were added after the ^{14}C pulse and so then were radioactive. The longer Dintzis incubated the cells prior to extraction, the more strongly-labeled was the hemoglobin he obtained.

After he had extracted the hemoglobin, Dintzis fingerprinted each of the fragments to ascertain the distribution of the ^{14}C label. Here the power of using Ingram's well-characterized system was evident. Dintzis was able to identify the ^{14}C labeled spot as that of one corresponding to the C-terminal peptide, that fragment of the protein occurring at the end where the free carboxyl (COOH) group exists (not involved in a peptide bond because it is at the end of the chain). This result established that the C-terminal peptide of α -Hb was always made last. Longer incubation times yielded α -Hb fingerprints with progressively more ^{14}C labeled spots. By 60 minutes of incubation, all spots contained ^{14}C label.

Here Ingram's result again provided the key. Each of the α -Hb peptides could be assigned a number, depending on how far the known amino acid region of each peptide was from the N-terminal end of the overall protein amino acid sequence (for example, the peptide labeled first would be assigned the final number, as it is farthest from the amino terminal end, being the carboxy-terminal peptide). Dintzis was then able to directly ascertain the pattern of synthesis from the changing distribution of label among the peptides.

Under a random tRNA binding hypothesis, no sequence-correlated pattern would be expected, but rather a random order of labeling. But Dintzis found just the opposite: label appeared first in peptide #1, then in #5, then in #9, etc. The first peptide to be made therefore was the N-terminal one, and synthesis proceeded in an orderly way down the chain toward the C-terminal end.