**Data Analysis Problem**

by Marianna Pap and József Szeberényi

to accompany

*The Cell: A Molecular Approach,* Eighth Edition

Geoffrey M. Cooper

**12.1 The Synthesis and Posttranslational Fate of a**

**Bee Venom Protein**

This Data Analysis Problem is also found on page 422 of the textbook.

**Source:** Ibrahimi, I. 1987. Signal recognition particle causes a transient arrest in the biosynthesis of prepromelittin and mediates its translocation across mammalian endoplasmic reticulum. *J. Cell Biol.* 104: 61–66.

**Level of difficulty:** High

**Corresponding chapter(s) in the textbook:** Chapter 12

**Review the following terms before working on the problem:** secretion, protein synthesis, posttranslational protein processing, *in vitro* translation, mRNA, [3H]proline/[35S]methionine labeling, microsomal fraction, endoplasmic reticulum, signal recognition particle (SRP), SRP receptor, proteases, SDS-polyacrylamide gel electrophoresis, autoradiography

**Experiment**

Promelittin (PM) is a small secretory protein, a predominant component of queen bee venom. Its synthesis and posttranslational metabolism was studied in an *in vitro* translation system containing total bee venom gland mRNA, [3H]proline, [35S]methionine, and other molecules required for protein synthesis. Supplements were added to this system as follows:

Sample 1: Rough ER vesicles (rough microsomes, RM)

Sample 2: No additional components

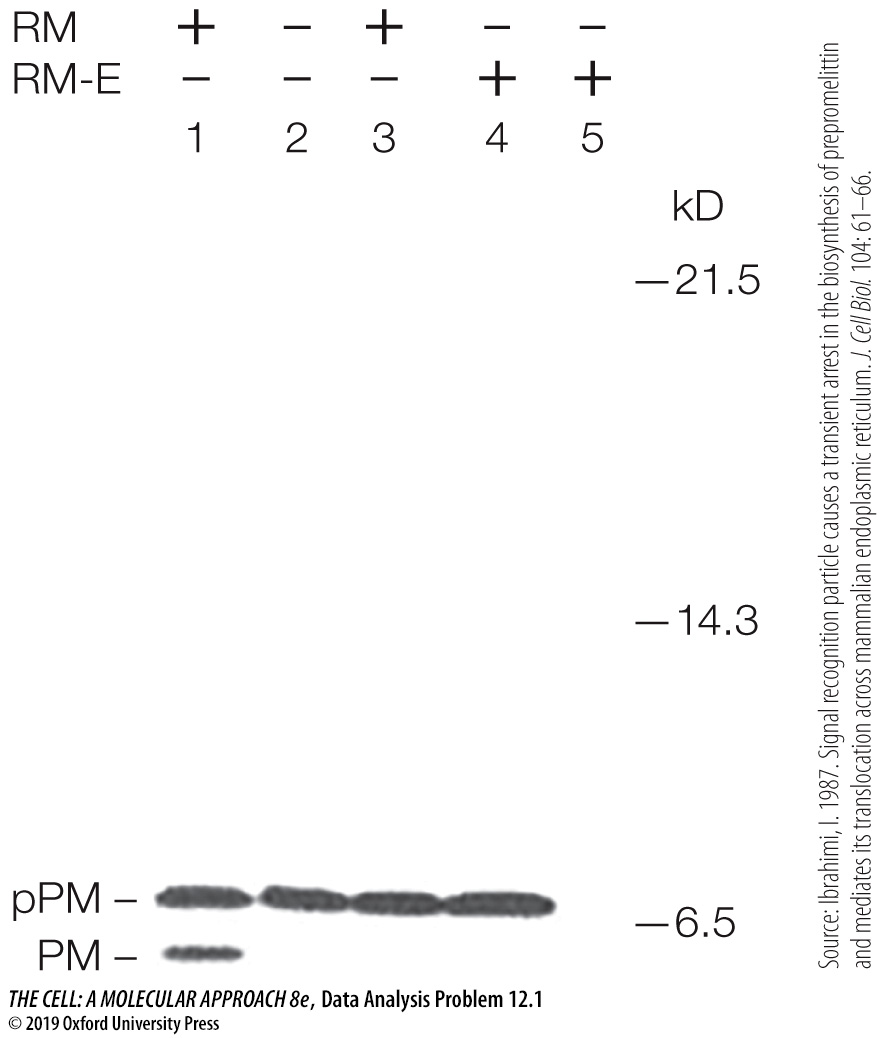
Sample 3: Cycloheximide (a protein synthesis inhibitor) was added at the end of translation, followed by the addition of rough ER vesicles (RM) and another 30 minute incubation.

Sample 4: Rough ER vesicles (RM-E) treated with N-ethylmaleimide (an inhibitor of SRP receptor) were added at the beginning of translation.

Sample 5: Same as sample 4, except this sample was digested with proteinase K after translation

The samples were subjected to SDS polyacrylamide gel electrophoresis followed by autoradiography.

**Figure**



**Questions**

1. Explain how the bands pPM and PM were resolved and suggest a possible relationship between them.

2. What conclusion can be drawn from comparing samples 1 and 2?

3. What conclusion can be drawn from comparing samples 1 and 3?

4. What conclusion can be drawn from comparing samples 1 and 4?

5. What conclusion can be drawn from comparing samples 4 and 5?