**Data Analysis Problem**

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to accompany

*The Cell: A Molecular Approach,* Eighth Edition

Geoffrey M. Cooper

**19.4 The Role of Apaf-1 Protein in Hyperosmotic Stress**

This Data Analysis Problem does not appear in the textbook.

**Source:** Saikia, M., R. Jobava, M. Parisien, A. Putman, D. Krokowski, X.-H. Gao, B-J. Gnan, Y. Yuan, E. Jankonsky, Z. Feng, G-F. Hu, M. Pusztai-Carey, M. Gorla, N. B. V. Seput, T. Ran, M. Hatzoglon. 2014. Angiogenin-cleaved tRNA halves interact with cytochrome *c*, protecting cells from apoptosis during osmotic stress. *Mol. Cell. Biol.* 34: 2450–2463.

**Corresponding chapter(s) in the textbook:** Chapter 19

**Review the following terms before working on the problem:** Apaf-1 protein, cellular stress, osmosis, fibroblast, cell culture, sucrose, Western blot analysis, caspases, translation initiation factors, protein phosphorylation, tubulin

**Experiment**

The role of Apaf-1 protein in response to cellular stress induced by increased osmotic pressure outside the cells (hyperosmotic stress) was studied in this experiment.

Wild-type mouse embryonic fibroblasts (Apaf-1+/+ cells) and homozygous Apaf-1 knock-out fibroblasts (Apaf-1–/– cells) were cultured in hyperosmotic medium (brought up to 600 mOsmol/liter) for the time periods indicated in the figure. Protein extracts were prepared and subjected to Western blot analysis using anti-Apaf-1, anti-caspase-3, anti-eIF2α, and anti-tubulin antibodies, and an antibody specific for the phosphorylated form of eIF2 [labeled eIF2-P(S51) in the figure].

**Figure**



Source: Saikia, M., R. Jobava, M. Parisien, A. Putman, D. Krokowski, X.-H. Gao, B-J. Gnan, Y. Yuan, E. Jankonsky, Z. Feng, G-F. Hu, M. Pusztai-Carey, M. Gorla, N. B. V. Seput, T. Ran, M. Hatzoglon. 2014. Angiogenin-cleaved tRNA halves interact with cytochrome *c*, protecting cells from apoptosis during osmotic stress. *Mol. Cell. Biol.* 34: 2450–2463.

**Questions**

1. What was the goal of using anti-tubulin antibody?

2. How successful was the Apaf-1 gene targeting procedure?

3. Describe the pathway by which caspase-3 is regulated during hyperosmotic stress.

4. How is protein synthesis affected by hyperosmotic stress?

5. What is the biological response of Apaf-1+/+ and Apaf-1–/– fibroblasts to hyperosmotic stress?