Aquaporin Increases Membrane Permeability to Water
(Textbook Figure 6.13)

Introduction
Although water is a small molecule, its rate of diffusion through the plasma membrane is limited by the fact that water is polar, while the interior of the plasma membrane is largely composed of nonpolar hydrocarbon chains of fatty acids. In some cell types, such as kidney tubules and red blood cells, water movement across the membrane is much too rapid to be accounted for by simple diffusion through the lipid bilayer. For over a century, biologists had proposed that there must be a water channel in the membrane to allow for this rapid diffusion. At Johns Hopkins University, Peter Agre isolated a water channel protein by accident. A medical specialist in rheumatic diseases (conditions in which people often have adverse reactions to their own molecules), Agre was studying the Rh antigen, a membrane protein on the surface of red blood cells that can differ between people. Whenever he tried to purify the Rh antigen, there was another membrane protein that came along with it. This “extra” protein was also prominent in the plasma membranes of kidney cells. The protein’s amino acid sequence indicated that it was an integral protein that spanned the membrane and formed a channel, and Agre called it CHIP28 (“channel-forming integral protein, 28,000 daltons molecular weight”). The properties and tissue location of CHIP28 indicated that it might be a water channel, so Agre set out to prove it experimentally. He did this by inserting the channel into cells that did not express it, and then seeing whether they had increased water permeability. The experimental demonstration of aquaporins led to Agre being awarded the Nobel Prize in Chemistry in 2003.

Original Paper
Analyze the Data

Question 1
The oocytes (immature egg cells) of the frog, *Xenopus laevis* are not permeable to water to a significant extent and do not express CHIP28 protein on their plasma membrane. In the experiment, CHIP28 protein was expressed in the oocytes by injection of the mammalian mRNA for this protein into the frog egg cells. At various time intervals after injection of either a buffer without mRNA (0 ng) or CHIP28 mRNA (10 ng), the proteins of the oocyte membrane were separated by gel electrophoresis and stained specifically for CHIP28 protein, or a minor variant of it (glyCHIP). A photo of the gel is shown below.

a. What data show that the appearance of CHIP28 was dependent on the presence of CHIP28 mRNA?
b. What are the CHIP28 and glyCHIP bands at the far left lane of the gel?
c. What time after injection would you suggest for further experiments on oocytes to test their water permeability?

Question 2
At a suitable time after mRNA injection (See question 1), water permeability was tested in oocytes by incubating them in a hypotonic solution and measuring cell volume. Here are the results, where closed circles are cells injected with CHIP28 mRNA and open circles are cells injected with buffer without mRNA; after time X in the upper curve, intact oocyte cells were not visible.
a. Why did the cells increase in volume?
b. What happened at time X?
c. Calculate the relative rates (volume increase per minute) of swelling in the control and experimental curves. What does this indicate about the effectiveness of CHIP28?