CLASSIC EXPERIMENT 20.1

CELL BIOLOGY EMERGING FROM THE SEA: THE DISCOVERY OF CYCLINS

T. Evans et al., 1983, *Cell* **33**:391.

From the first cell divisions after fertilization to aberrant divisions that occur in cancers, biologists have long been interested in how cells control when they divide. The processes of cell division have been separated into stages known collectively as the *cell cycle*. While studying early development in marine invertebrates in the early 1980s, Joan Ruderman and Tim Hunt discovered the cyclins, key regulators of the cell cycle.

Background

The question of how an organism develops from a fertilized egg continues to drive a large body of scientific research. Whereas such research was classically the concern of embryologists, the developing understanding of gene expression in the 1980s brought new approaches to answer this question. One such approach was to examine the pattern of gene expression in both the oocyte and the newly fertilized egg. Ruderman and Hunt were among the biologists who took this approach to the study of early development.

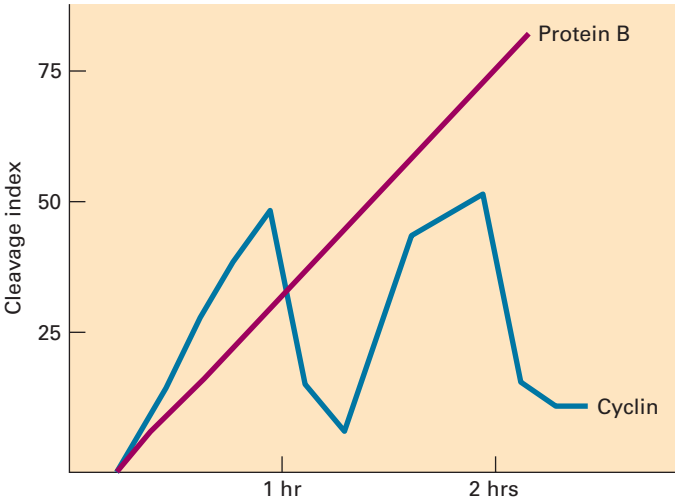
Biologists had well characterized the early development of a number of marine invertebrate systems. During the early stages of development, the embryonic cells grow synchronously, which allows an entire population of cells to be studied at the same stage of the cell cycle. Researchers had established that a large portion of the mRNA in the unfertilized oocyte is not translated. Upon fertilization, these maternal mRNA are rapidly translated. Previous studies had shown that when fertilized eggs are treated with drugs that inhibit protein synthesis, cell division could not take place. This suggested that the initial burst of protein synthesis from the maternal mRNA is required at the earliest stages of development. Ruderman and Hunt, while teaching a physiology course at the Marine Biological Lab in Woods Hole, Massachusetts, began a set of experiments designed to uncover the genes that were expressed at this point as well as the mechanism by which this burst of protein synthesis was controlled.

The Experiment

In a collaborative project, Ruderman and Hunt looked at regulation of gene expression in the fertilized egg of the surf clam *Spisula solidissima*. Whereas it was known that overall protein synthesis rapidly increased upon fertilization, they wanted to find out whether the proteins expressed in the earliest stage of development, the two-cell embryo, were different from those expressed in the unfertilized egg. When either oocytes or two-cell clam embryos are treated with radioactively labeled amino acids, the cell takes up the amino acids, which are subsequently incorporated into newly synthesized proteins. Using this technique, Ruderman and Hunt monitored the pattern of protein synthesis by breaking open the cells, separating the proteins using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then visualizing the radioactively labeled proteins by autoradiography. When they compared the pattern of protein synthesis in the oocyte with that in the two-cell embryo, they saw that three different proteins that were either not expressed or expressed at an extremely low level in the oocyte were highly expressed in the embryo. In a subsequent study, Ruderman examined the pattern of protein expression in the oocytes of the starfish *Asterias forbesi* as they mature. She again observed the increased expression of three proteins of similar size to those that she and Hunt had seen in surf clam embryos.

Soon afterward, in a third study, Hunt examined the changes in protein expression during the maturation and fertilization of sea urchin oocytes. This time he performed the experiment in a slightly different manner. Rather than treating the oocytes and embryo with radioactively labeled amino acids for a set time period, he labeled the cells continuously for more than 2 hours, removing samples for analysis at 10-minute intervals. Now, he could monitor the changes in protein expression throughout the early stages of development. As had been shown in other organisms, the pattern of protein synthesis was altered when the sea urchin oocyte was fertilized. Three proteins—represented by three prominent bands on an autoradiograph—were expressed in the embryos, but not in the oocytes. Interestingly, the intensity of one of these bands changed over time; the band was intense at the early time points, then barely visible after 85 minutes. It increased in intensity again between 95 and 105 minutes. The intensity of the band, representing the amount of the protein in the cell, appeared to be oscillating over time. This suggested that the protein had been quickly degraded and then synthesized again.

Because the time frame of the experiment coincided with early embryonic cell divisions, Hunt next asked whether the synthesis and destruction of the protein was correlated with progression of the cell cycle. He examined a portion of cells from each time point under a microscope, counting the number of cells dividing at each time



◄ **FIGURE Fast axonal transport was characterized by observing the movement of radioactively labeled proteins along the length of the axon.** This figure compares the changing levels of sea urchin cyclin (drawn in blue) with a control protein (drawn in purple) as early embryonic cells progress through the cell cycle. The overall level of cyclin increases over time, and then it is rapidly destroyed as the cells approach division. This pattern appears to repeat through each cell division. Meanwhile, the overall level of the control protein continues to increase throughout the time period of the experiment. [Adapted from T. Evans et al., 1983, Cell 33:391.]

point where samples had been taken for protein analysis. Hunt then correlated the amount of the protein present in the cell with the proportion of cells dividing at each time point. He noticed that the level of expression of one of the proteins was highest before the cell divided and lowest upon cell division (see Figure 1), suggesting a correlation with the stage of the cell cycle. When the same experiment was performed in the surf clam, Hunt saw that two of the proteins that he and Ruderman had described previously displayed the same pattern of synthesis and destruction. Hunt called these proteins *cyclins* to reflect their changing expression through the cell cycle.

Discussion

The discovery of the cyclins heralded an explosion of investigation into the cell cycle. It is now known that these proteins regulate the cell cycle by associating with cyclin-dependent kinases, which in turn regulate the activities of a variety of transcription and replication factors, as well as other proteins involved in the complex alterations in cell architecture and chromosome structure that occur during mitosis. In brief, cyclin-CDK complexes direct and regulate through the cell cycle. As with so many key regulators of cellular functions, it was soon shown that the cyclins discovered in sea urchins and surf clams are conserved in eukaryotes from yeast to man. Since the identification of the first cyclins, scientists have identified at least 15 other cyclins that regulate all phases of the cell cycle.

In addition to the basic research interest in these proteins, the cyclins' central role in cell division has made them a focal point in cancer research. Cyclins are involved in the regulation of several genes that are known to play prominent roles in tumor development. Scientists have shown that at least one cyclin, cyclin D1, is overexpressed in a number of tumors. The role of these proteins in both normal and aberrant cell division continues to be an active and exciting area of research today.