Chapter 3

When: Stopwatches at Many Scales

"Dost thou love life? Then do not squander time, for that is the stuff life is made of." - Benjamin Franklin

Chapter Overview: In Which Various Stopwatches Are Used to Measure the Rate of Biological Processes

Just as biological structures exist over a wide range of spatial scales, biological processes take place over time scales ranging from much faster than microseconds to the time scales that characterize the history of Earth itself. Using the cell cycle of *E. coli* as a standard stopwatch, this chapter develops a feel for the rates at which different biological processes occur illustrating the great diversity of biological processes, mirroring the structural diversity seen in the previous chapter. With this "feeling for the numbers" in hand, we then explore several different views of the passage of biological time. In particular, we show how sometimes cells manage time by stringing together processes in succession while at other times they manipulate time (using enzymes to speed up reactions, for example) to alter the intrinsic rates of processes.

3.1 The Hierarchy of Temporal Scales

One of the defining features of living systems is that they are dynamic. The time scales associated with biological processes run from the nanosecond (and faster) scale of enzyme action to the more than 10^9 years that cover the evolutionary history of life itself. The inexorable march of biological time is revealed over many orders of magnitude difference in time scale, as illustrated in fig. 3.1. If we are to watch biological systems unfold with different stopwatches in hand, the resulting phenomena will be different - at very fast time scales we will see the molecular dance of different biochemical species as they interact and change

identity. At much slower scales we see the unfolding of the lives of individual cells. If we slow down our stopwatch even more, what we see is the trajectories of entire species. To some extent, there is a coupling between the temporal scales described in this chapter and the spatial scales described in the previous chapter; small things such as individual molecules tend to operate at fast rates, and large things such as elephants tend to move around at slow rates.

The aim of this chapter is to describe the time scales of biological phenomena from a number of different perspectives. In this section, we develop a feeling for biological time scales by examining the range of different time scales seen in molecular, cell, developmental and evolutionary biology. This discussion is extended by describing the experimental basis for what we know about time scales in biology. As in chap. 2, we once again invoke *E. coli* as our reference system, this time by using the cell cycle of our "reference cell" as the standard stopwatch. The remainder of the chapter is built around viewing time in biology from three distinct perspectives. In section 3.2, we show how the time scale of certain biological processes is dictated by how long it takes some particular procedure (such as replication) to occur. We will refer to this as *procedural time*. Section 3.3 explores time from a different angle. In this case, we consider a broad class of biological processes whose timing is of the "socks before shoes" variety. That is, processes are linked in a sequential string and in order for one process to begin, another must have finished. We will refer to this kind of time keep as *relative time*. Finally, section 3.4 reveals a third way of viewing time in biological processes, as a commodity to be manipulated. In this case in a process we will call *manipulated time*, we show how cells and organisms find ways to either speed up or slow down key processes such as replication and metabolism.

3.1.1 The Pageant of Biological Processes

Biological Processes Are Characterized By a Huge Diversity of Time Scales

A range of different processes associated with individual organisms, and their associated time scales, is shown in fig. 3.2 (we leave a discussion of evolutionary processes for the next section). Broadly speaking, the aim of this figure is to show a loose powers of ten representation of different biological processes in the same spirit as fig. 2.7 (pg. 70) showed a powers of ten representation of spatial scales. As we will see later in the chapter, an *absolute* measure of time in seconds or minutes is sometimes not the most useful way to think about the passage of time within cells. For example, embryonic development for humans takes drastically longer than for chickens, but the relative timing of common events is meaningfully compared. For the moment, our discussion of fig. 3.2 is intended to give a feeling for the numbers; how long do various key biological processes actually take in absolute terms as measured in seconds, minutes and hours?

We begin (fig. 3.2(A) and (B)) with some of the processes associated with

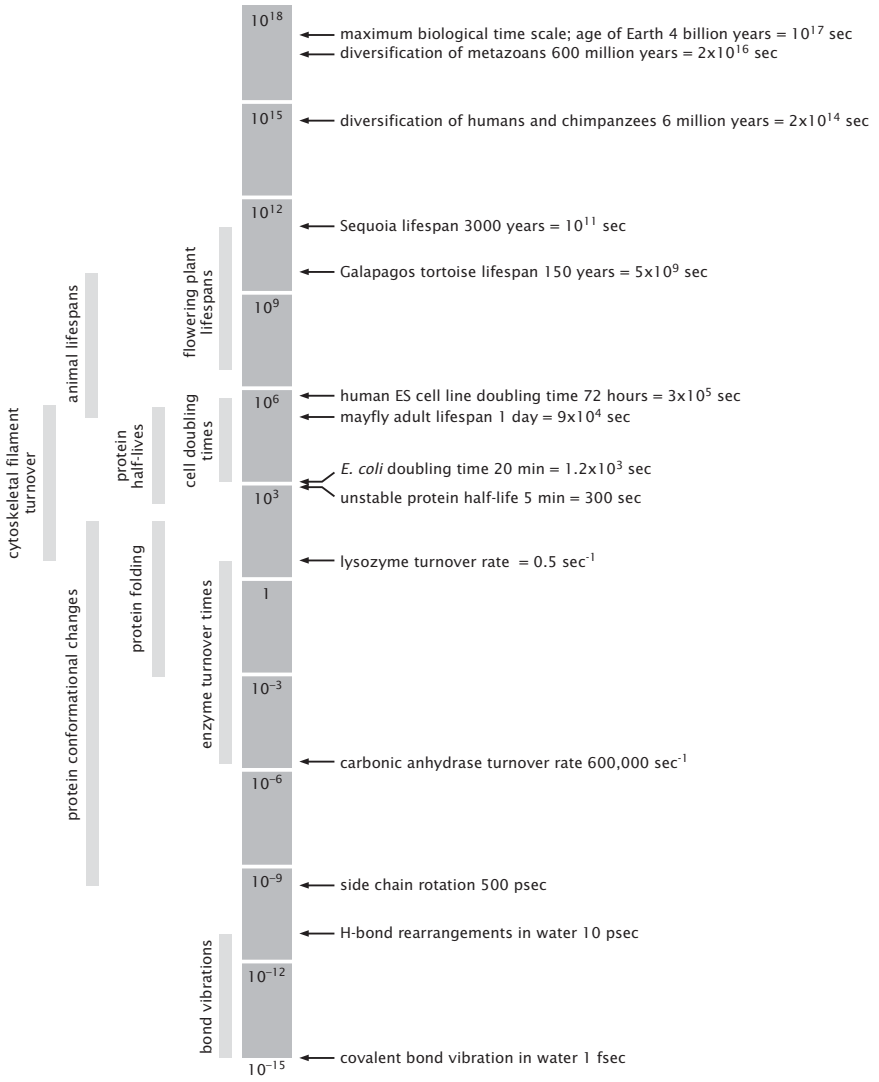

Figure 3.1: Gallery of biological time scales. Logarithmic scale showing range of times scales associated with various biological processes. The time scale is in seconds.

the development of the fruit fly *Drosophila melanogaster*. *Drosophila* has been one of the key workhorses of developmental biology, and much that we know about embryonic development was teased out of watching the processes which take place over the roughly ten days between fertilization of the egg and the emergence of a fully functioning fly. If we increase our temporal resolution by a factor of ten, we see the processes in the development of the fly embryo itself. Over the first ten hours or so after fertilization as shown in fig. 3.2(B), a single cell is turned into an organized collection of thousands of cells with particular spatial positions and functions. One of the most dramatic parts of this embryonic development is the process of gastrulation when the future gut forms as a result of a series of folding events in the embryo. This process is indicated schematically in fig. 3.2(B).

Individual cells have a natural developmental cycle as well. The *cell cycle* refers to the set of processes whereby a single cell, through the process of cell division, becomes two daughter cells. The time scales associated with the cell cycle of a bacterium such as *E. coli* are shown in fig. 3.2(C), with a characteristic scale of several thousand seconds. The lives of individual cells are fascinating and complex. If we are to dissect the activities of an individual cell as it goes about its business between cell divisions, we would find a host of processes taking place over a range of different time scales. If we stare down a microscope at a swimming bacterium for several seconds, we will notice episodes of directed motion, punctuated by rapid directional changes. Fig. 3.2(D) shows the time scales over which an individual bacterium such as *E. coli* exercises its random excursion during movement. If our stopwatch now runs a factor of ten faster we are now operating at the scale of deciseconds, a scale which characterizes the rate of amino acid incorporation during protein synthesis, a process represented in fig. 3.2(E). Macromolecular synthesis is one of the most important sets of processes which any cell must undertake to make a new cell. Another key part of the macromolecular synthesis required for cell division is the process of transcription, which is the intermediate step connecting the genetic material as contained in DNA and the readout of that message in the form of proteins. Transcription refers to the synthesis of messenger RNA molecules as faithful copies of the nucleotide sequence in the DNA, a polymerization process catalyzed by the enzyme RNA polymerase. The rate of incorporation by RNA polymerase of nucleotides onto the messenger RNA during transcription, as depicted schematically in fig. 3.2(F), happens roughly a few times as fast as does the rate of amino acid incorporation by ribosomes during protein synthesis.

In the moment to moment life of the cell, proteins do most of the work. Many proteins are able to operate at time scales much faster than the relatively stately machinery carrying out the central dogma operations. For example, a great number of biological processes are dictated by the passage of ions across ion channels, with a characteristic time scale of milliseconds as shown in fig. 3.2(G). A factor of thousand speed up of our stopwatch brings us to the world of enzyme kinetics at the microsecond time scale (fig. 3.2(H)) and faster. It is important to note that these time scales merely represent a general rule of thumb. For example, turnover rates for individual enzymes may range from 0.5 s^{-1} to 600,000

s^{-1}.

Before proceeding, one of the questions we wish to consider is how the time scales depicted in fig. 3.2 are actually known. As with much of our story, the stopwatches associated with each of the cartoons in that figure have been determined as the results of many kinds of complementary experiments.

- **Experiments Behind the Facts.** Broadly speaking, the experiments which characterize the dynamics of cells and the molecules that populate them are ultimately based on tracking transformations. We can divide these experiments into four broad categories that can be applied across all levels of spatial scale from molecular to ecological. These methods are summarized in fig. 3.3.

  1. Direct Observation. The first and most obvious way to characterize time in a biological process is simply to observe the process unfold and to record the absolute time at which transformation occurs. An example of this strategy is shown in fig. 3.3 which shows the motion of a neutrophil. Similarly, looking down a microscope at a mammalian cell in tissue culture it is possible to observe many of the steps in the cell cycle (see figs. 3.7 (pg. 133) and 3.15 (pg. 149)) unfolding over real time, including condensation of the chromosomes, alignment of the chromosomes through the action of the mitotic spindle, their segregation into daughter nuclei and finally cytokinesis when the cell is pinched into two fully formed daughter cells. Although this is easy to do for processes that take minutes to hours and occur over spatial scales that can be observed with the light microscope or the unaided human eye, it is extremely difficult to measure time simply by observation for events that are very fast, very slow, very small or very large. Over the past few decades there have been vast experimental improvements in direct or near-direct observation of single molecules such that this naturalistic approach to "observing a lot just by watching" can be applied all the way down to the molecular scale. We will see many examples of this approach throughout the book.

  2. Fixed time points. When events of interest cannot be directly observed, there are other ways to probe their duration. Rather than continuously observing an individual over time, one can draw individuals from a population at given time intervals and examine their properties at this series of fixed time points. For example, a bacterial population in a liquid culture started from a single cell will grow exponentially and then plateau and eventually die off over a period of several days. Rather than staring at the tube continuously for several days, the essential kinetics of this process can be measured simply by examining cell density at some fixed interval such as every hour as shown in fig. 3.3. Similarly, the events of embryonic development for useful model organisms such as flies and frogs unfold over a period of days to weeks. However, under a given set of environmental conditions, the sequence and timing of these events is stereotyped from one individual to another. Therefore the investigator can accurately

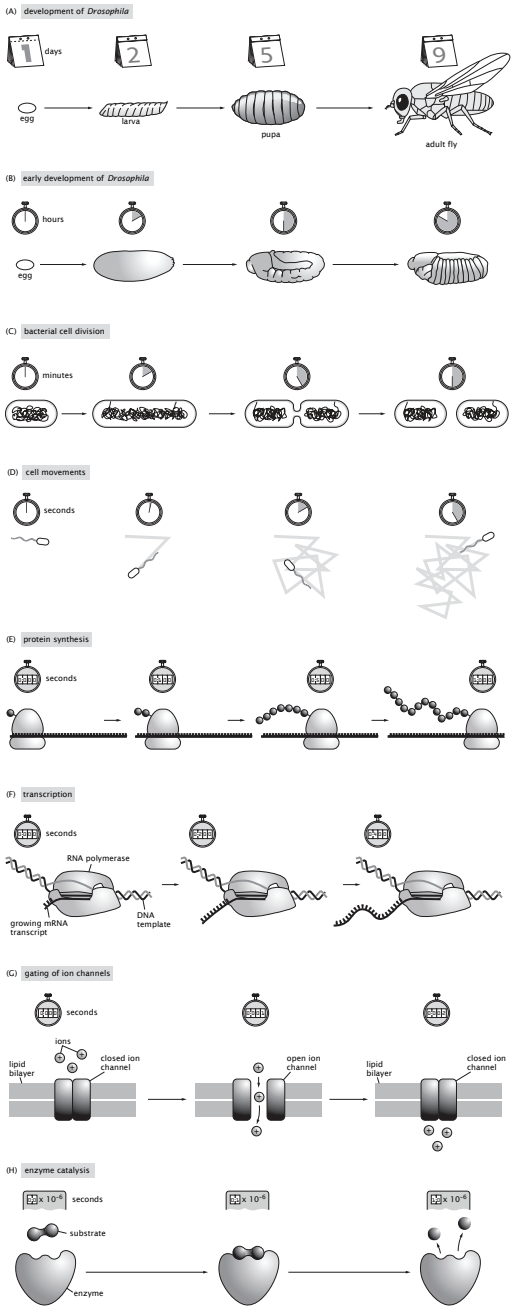

Figure 3.2: Hierarchy of biological time scales. Cartoon showing range of time scales associated with different biological processes.

describe the sequence of events in frog development by examining one dish of embryos an hour after fertilization, a second dish of embryos two hours after fertilization, etc. This is useful when the methods used to examine the embryos result in their death. For example, fixing them and staining for a particular protein of interest or preparing them for electron microscopic examination. At a much smaller spatial scale and faster time, the method of stop-flow kinetics enables investigators to follow enzymatic events by mixing together an enzyme and its substrate and then squirting the mixture into a denaturing acid bath after fixed intervals of time. These methods are all more indirect than direct observation, but in many cases are technically easier and different kinds of complementary information can be gleaned by comparing both for a single process.

3. Pulse-chase. Many biological processes operate in a continuous fashion. For example, bacteria constantly take in sugar from their medium for energy and to generate the molecular building blocks to synthesize new constituents. The process of glycolysis converts a molecule of glucose into two molecules of pyruvate. Because glucose is continuously taken up and pyruvate is continuously generated, it is extremely difficult to ask how long the conversion process takes. The set of methods used to tackle these kinds of problems are generally called pulse-chase experiments. In this particular example, a bacterial cell may be fed glucose tagged with radioactive carbon for a very brief period of time, for example one minute. This is followed by feeding with nonradioactive glucose. Cells can then be removed from the bacterial culture at various time intervals and their metabolites can be examined to see which contain the radioactive carbon. Over time, the amount of labeled glucose will decrease and the major radioactive species will pass through a series of intermediates until finally most of the radioactive carbon will be found in pyruvate. Thus, a pulse-chase experiment can be used to determine the order of intermediates in a metabolic pathway and also the amount of time it takes for the cell to perform each transformation. A classic example of this strategy to examine transport in neurons is shown in fig. 3.3. Essentially the same method is used by naturalists examining dispersion times of birds and other animals by tagging individuals with a band or radio-transmitter and releasing them back into their natural population to see where they are and when.

4. Product accumulation. The final type of experiment used to determine biological rates is exemplified by an assay with a purified enzyme where a colorless substrate is converted into a colored product over time. By measuring the concentration of the colored product as a function of time, the investigator can extrapolate the average turnover rate given the known concentration of enzymes in the test tube. Similar experiments where observation of the accumulation of a product can be used as a surrogate for rate measurements can also be performed in living cells. A particularly useful example is expressing the green fluorescent protein (GFP) down-

stream of a promoter of interest as shown in fig. 3.3. When the promoter is induced (i.e. by exposing the cells to some molecule that turns the gene of interest on) GFP begins to accumulate and the amount of fluorescence can be directly measured and converted into numbers of GFP molecules. Because GFP is remarkably stable, its accumulation can often represent a more accurate reporter for promoter activity than the promoter's natural product which may be subject to other layers of regulation including rapid degradation.

3.1.2 The Evolutionary Stopwatch

The general rule that all biological processes are dynamic and undergo change over time applies to molecules, cells, organisms and species. The evolutionary clock started more than three billion years ago with the appearance of the first cellular life forms on Earth. It is generally accepted that there were complex life-like processes occurring prior to the emergence of the first recognizable cells, though we cannot learn anything about what they were like either from the fossil record or comparative studies among organisms living today.

All of the astonishing diversity of cellular life currently existing on the planet ranging from archaea living in geothermal vents deep in the ocean to giant squid to redwood trees to the yeast that make beer were all descended from a universal common ancestor (probably a population of cells rather than an individual). This last universal common ancestor (LUCA) would have been clearly recognizable as a cell: it contained DNA as a genetic material, it transcribed its DNA into mRNA and translated mRNA into proteins using ribosomes. It also processed sugar to make energy through the process of glycolysis and contained a rudimentary cytoskeleton consisting of an actin-like molecule and a tubulin-like molecule. We can attribute all of these features to LUCA because they are universally shared among all existing branches of cellular life. However, the demonstrable differences between redwood trees and giant squids accumulated slowly over evolutionary time as individual cellular populations became genetically isolated from one another and underwent change and divergence to fill different ecological niches. As the planet Earth is constantly being reshaped and remodeled by the uncounted legion of organisms that inhabit it, environmental niches are always unstable and can be changed either by geological processes, global climate alterations or the actions of competing organisms.

We can fruitfully think of evolution as the process of change in the genetic information carried by a population of related organisms. Sometimes a single lineage can be seen as altering over time as its environment changes. More commonly, a single population will subdivide into populations that will become isolated and suffer different fates. Some will die off, some will remain similar to the ancestral population and some will undergo significant biochemical, morphological or behavioral alterations over time that are ultimately recognized as new species. These basic ideas were beautifully articulated by Charles Darwin in *The Origin of Species* and illustrated by the single figure in that book

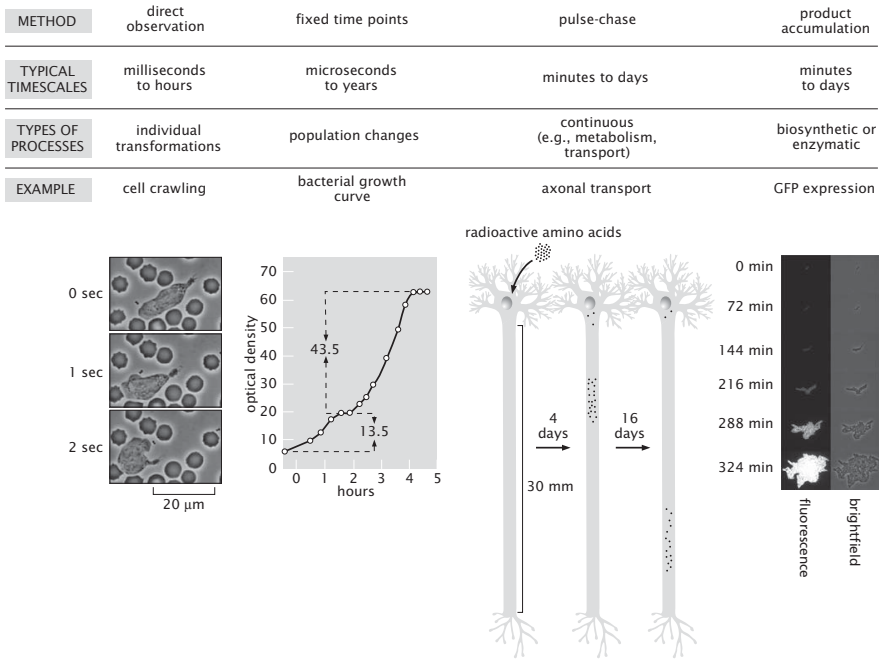

Figure 3.3: Experiments to measure the timing of biological processes. The figure summarizes four strategies for measuring biological rates. For *direct observation* the example shows three frames from a video sequence taken by David Rogers in the 1950s of a single white blood cell (neutrophil) pursuing a bacterium through a forest of red blood cells. The movement of the cell is sufficiently fast that it can be directly observed by the human eye. For *fixed time points*, the experiment shown is a classic performed by Monod who tracked the growth of *E. coli* in a single culture when two different nutrient sugars were mixed together. The bacteria initially consumed all of the available glucose and then their growth rate slowed as they switched over into a new metabolic mode enabling them to use lactose. For *pulse-chase*, labeling proteins at their point of synthesis in a neuron cell body with a pulse of radioactive amino acids followed by a chase of unlabeled amino acids was used to measure the rate of continuous axonal transport. *Product accumulation* is illustrated by the expression of GFP under a regulated promoter in a bacterial cell. The rate of gene transcription can be inferred by measuring the amount of GFP present as a function of time. (Growth curve adapted from B. Müller-Hill, The *lac* Operon: a short history of a genetic paradigm, New York, Walter de Gruyter, New York, 1996; fluorescent image series adapted from N. Rosenfeld *et al.*, *Science* 307:1962, 2005.)

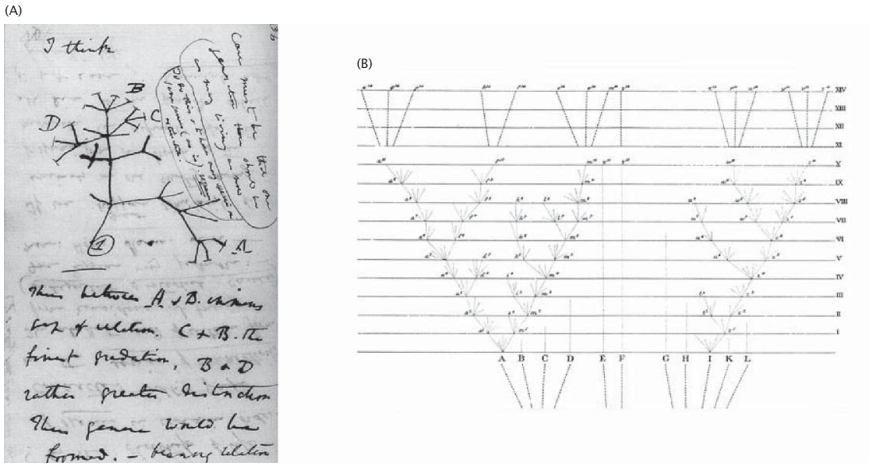

Figure 3.4: Two versions of Darwin's phylogenetic tree. (A) In his notebooks, Darwin drew the first version of what we now recognize as a common schematic demonstrating the relatedness of organisms. He introduced this speculative sketch with the words "I think" as his theory was beginning to take form. (B) In the final published version of *The Origin of Species*, the tree had assumed more detail showing the passage of time and explicitly indicating that most species have gone extinct. (Adapted from C. Darwin, On the Origin of Species, London, John Murray, 1859.)

reproduced as fig. 3.4.

How long does this evolutionary process take and how can we measure the passage of evolutionary time? It is unsatisfying to rely on the extrapolation of mutation rates measured in artificial laboratory experiments to the evolution of species over time in the real world. Real world conditions are much less stable or controlled than laboratory conditions, and furthermore the time scales of greatest interest for studying the evolution of species are much longer than can be achieved in the laboratory by even the most patient experimentalist. Traditionally, our understanding of evolutionary alterations depended upon two kinds of observations: comparisons among currently living species and examination of the fossil record. Information about the age of particular fossils can be inferred from identification of the geological strata in which they are found, and also by examining the proportions of different radioisotopes which decay at a regular rate and thereby provide information about when the rock was formed.

Comparison of living species to ascertain degree of relatedness was carried out for many hundreds of years before the modern theory of evolution was first described. It is immediately obvious that some organisms are more closely related than others. For example, horses and donkeys are clearly more similar to each other than either is to a dog, but horses and dogs are more similar to each other than either is to a squid. These obvious morphological differences

have been the basis of the science of systematics going back to Linnaeus in the 1700s. In the modern era of molecular genetics, we can more easily ascribe a universal metric for genetic similarity among organisms based on similarities and differences in DNA sequence. As a population evolves over time, its DNA complement will change by several mechanisms. First, small scale mutations or large scale rearrangements of its genome may occur (an illustration of the consequences of this kind of rearrangement is shown in fig. 3.5). Second, it may acquire new genes or even entire groups of genes by horizontal transfer from other organisms (horizontal transfer refers to cells passing genetic material to cells other than their own descendants). And third, it may simply lose large chunks of DNA. Thus different organisms contain different complements of genes as well as sequence differences between homologous copies of the same gene. The term homologous refers to descent from a common ancestor. For example, ribosomal RNAs are homologous in all cells. In chap. 18, we will give some examples of ribosomal RNA sequences and show how they can be used to build a universal phylogenetic tree (see fig. 18.15 on pg. 986). One example of a tree based on ribosomal RNA sequences that attempts to show the relatedness among all branches of existing life is shown in fig. 3.6.

Phylogenetic trees established by molecular methods tend to be in excellent agreement with analogous trees of similarity based on morphological or biochemical criteria as have been established by botanists, zoologist and microbiologists over the past several hundred years. We will examine statistical methods for constructing such trees in chap. 18.

What does any of this have to do with the determination of evolutionary time? In the laboratory, we can observe that certain types of changes in DNA sequences within a population happen frequently (for example, single point mutations changing a C to a T) while others happen more rarely (crossover events reversing the order of all the genes within a segment of a chromosome). We can even measure the time constants that characterize such events. If we assume that these kinds of mutational events happen with the same frequency in wild populations as they do in the laboratory, then we can estimate divergence times for organismal populations based on calculating how long on average it would take to achieve the observed number of sequence alterations given known rates of sequence alteration events. In a few cases, these time estimates can be anchored by reference to the fossil record. In reality, inferring evolutionary time from sequence similarity is fraught with peril because not all sequence alterations are equally likely to be randomly incorporated into the genetic heritage of a population of organisms. Some mutations will prove to be unfavorable for a given organism's lifestyle and individuals carrying those mutations will be eliminated from the population by natural selection. Other mutations will prove to be advantageous and organisms carrying those mutations will quickly outcompete other members of their species. These selection effects can make the sequence-determined evolutionary clock appear to run too slow or too fast. Biologists face challenges similar to those faced by astronomers. In the astronomical setting, continual refinements in cosmological distance scales based on various types of standard candle (light sources of known absolute intensity) have

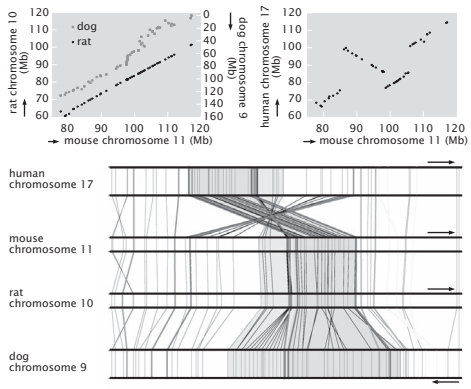

Figure 3.5: Inferring evolutionary relatedness by chromosome alignment. Equivalent regions of four chromosomes from human, mouse, rat and dog were compared to find the location of homologous genes. The graphs at top show the position of each gene in the rat, dog and human sequences as a function of their positions on the mouse sequence. Because little change has occurred in chromosomal structure between the mouse and the rat, the points representing the locations of homologous genes form a nearly perfectly straight line. On the equivalent chromosomal segment from the dog, the genes are again mostly in the same order, but the spacing between them has changed substantially. Comparing the human to the mouse, a large inversion can be detected. The same data is shown in a different form in the chart at the bottom. Each vertical line on the chromosome represents a particular gene and the diagonal lines between the chromosomes link up homologs between human and mouse, mouse and rat and rat and dog. (Adapted from G. Andelfinger *et al.*, *Genomics* 83:1053, 2004.)

Figure 3.6: Universal phylogenetic tree. This diagram shows the similarity among 16S ribosomal RNA sequence for representative organisms from all major branches of life on Earth. The ribosome is made up of different subunits which migrate at different rates during centrifugation. The "16S" label refers to the RNA content of one such subunit.

led to increasingly refined measurements of astronomical distance. Similarly, biologists have a number of different standard stopwatches that can be used to calibrate the flow of evolutionary time.

3.1.3 The Cell Cycle and the Standard Clock

The *E. coli* Cell Cycle Will Serve as Our Standard Stopwatch

In fig. 2.1 (pg. 58) we used the size of an *E. coli* cell as our standard measuring stick. Similarly, we now invoke the time scale of the *E. coli* cell cycle as our standard stopwatch. The goal of fig. 3.2 was to illustrate the variety of different processes that occur in cell biology and the time scales over which they are operative. As with our discussion of structural hierarchies, we once again use the trick of invoking *E. coli* as our reference, this time with the several thousand seconds of its cell cycle as our reference time scale.

As shown in fig. 3.2(C), the bacterial cell cycle will be defined as the time between the "birth" of a given cell resulting from division of a parental cell to the time of its own subsequent division. This cell cycle is characterized structurally by the segregation of the duplicated bacterial chromosome into two separate clumps and the construction of a new portion of the cell wall, or septum, that separates the original cell into two daughters. These processes are illustrated in

more detail in fig. 3.7. Because *E. coli* is a roughly cylindrical cell that maintains a nearly constant cross-sectional area as it grows longer, the total cell volume can be easily estimated simply from measuring the length and this also provides a guide as to the point in the cell cycle. As cell division proceeds, *E. coli* doubles in length and hence also doubles in volume. The time scale associated with the binary fission process of interest here is of order an hour (to within a factor of two), though division can take place in under 20 minutes under optimal growth conditions.

In the previous chapter, we argued that having a proper molecular inventory of a cell is a prerequisite to building models of many problems of biological interest. Here we argue that a similar "feeling for the numbers" is needed concerning biological time scales. How long does it take for an *E. coli* cell to copy its genome and is this rate consistent with the speed of the molecular machine (DNA polymerase) that does this copying? On what time scale do newly formed proteins in neurons reach the ends of their axons and can this be explained by diffusion? Often, the time scale associated with a given process will provide a clue about what physical mechanisms are in play. In addition, one of our biggest concerns in coming chapters will be to figure out under what conditions we are justified in using the ideas from equilibrium physics (as opposed to nonequilibrium physics). The answer to this question will be determined by whether or not there is a separation of time scales and the only way we can know that is by having a feeling for what time scales are operative in a given problem. To that end, we begin by taking stock of the processes that an *E. coli* cell must make to copy itself.

For estimates in this book we will choose a standard for bacterial growth in a minimal defined medium with glucose as the sole carbon source. As mentioned previously, the rate of cell division can vary by more than tenfold depending upon nutrient availability and temperature, so we must define the terms under which we will proceed with our estimates. The choice of minimal media with glucose at 37 degrees Celsius is a practical one since many quantitative experiments have been performed under this condition. With sufficient aeration, *E. coli* in this medium typically double in the range of 40-50 minutes and we will use 3000 seconds as our canonical cell cycle time. In general, time scales for biological processes are much more variable than spatial scales, although it is true that rapidly growing *E. coli* are slightly larger than slowly growing *E. coli*. The difference in size may be an order of magnitude less than the difference in cycle time.

- **Estimate: Timing** ***E. coli*****.** In section 2.1.2 (pg.59), we sized up *E. coli* by giving a series of rough estimates of its parts list. We now borrow those estimates to gain an impression of the rates of various processes in the *E. coli* cell cycle. The simple idea behind these estimates is to take the total quantity of material that must be used to make a new cell and to divide by the time ($\approx$ 3000 seconds) of the cell cycle. When *E. coli* is grown on minimal media with glucose as the sole carbon source, six atoms of carbon are added to the cellular inventory for each molecule of glucose

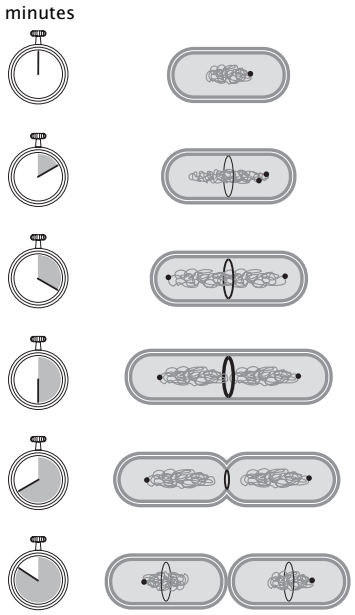

Figure 3.7: Schematic of an idealized bacterial cell cycle. A newborn cell shown at the top has a single chromosome with a single origin of replication marked by the dot. The cell cycle initiates with the duplication of the origin and DNA replication then proceeds in an orderly fashion around the circular chromosome. At the same time, a group of cell division proteins beginning with the tubulin analog FtsZ form a ring at the center of the cell that will dictate the future site of septum formation. As DNA replication proceeds and the cell elongates, the two origins become separated from each other with one traveling the entire length of the cell to take up residence at the opposite pole. As the septum begins to close down, the two chromosomal masses are physically separated into the two daughter cells where the cycle can begin anew.

taken up. In the previous chapter, we estimated that the number of carbon atoms it takes to double the material in a cell so that it can divide in two (just the construction material) is of order 10^{10}. For this estimate we ignored the material released as waste products and the reader will have the opportunity to estimate this contribution in the problems at the end of the chapter. We are also deliberately ignoring the glucose molecules that must be consumed to generate energy for the synthesis reactions - this topic will be taken up in chap. 5. At this point, we can estimate the rate of sugar uptake required simply to deliver the 10^{10} carbon molecules necessary for building the material of the new cell. 10^{10} carbons must be captured over 3000 seconds with 6 carbons per glucose molecule, giving an average rate of roughly 5×10^5 glucose molecules every second.

Of course, having the carbon present is not the same as the macromolecular synthesis required to make a new cell. One of the most important processes in the cell cycle is replication. Given that the complete *E. coli* genome is about 5×10^6 base pairs (bp) in size, we can estimate the required rate of replication as

$$\frac{dN_{bp}}{dt} \approx \frac{N_{bp}}{\tau_{cell}} \approx \frac{5 \times 10^6 \text{ bp}}{3000 \text{ s}} \approx 2000 \text{ bp/s}. \tag{3.1}$$

Note that we have rounded to the nearest thousand.

Similarly, the rate of protein synthesis can be estimated by recalling from the previous chapter that the total number of proteins in *E. coli* is roughly 3×10^6, implying a protein synthesis rate of

$$\frac{dN_{protein}}{dt} \approx \frac{N_{protein}}{\tau_{cell}} \approx \frac{3 \times 10^6 \text{ proteins}}{3000 \text{ s}} \approx 1000 \text{ proteins/s}. \tag{3.2}$$

A similar estimate can be performed for the rate of lipid synthesis resulting in

$$\frac{dN_{lipid}}{dt} \approx \frac{N_{lipid}}{\tau_{cell}} \approx \frac{5 \times 10^7 \text{ lipids}}{3000 \text{ s}} \approx 20,000 \text{ lipids/s}. \tag{3.3}$$

Yet another intriguing aspect of the mass budget associated with the cell cycle is the control of water content within the cell. Recalling our estimate from the previous chapter that an *E. coli* cell has roughly 2×10^{10} water molecules, results in the estimate that the rate of water uptake during the cell cycle is

$$\frac{dN_{H_2O}}{dt} \approx \frac{N_{H_2O}}{\tau_{cell}} \approx \frac{2 \times 10^{10} \text{ waters}}{3000 \text{ s}} \approx 7 \times 10^6 \text{ waters/s}. \tag{3.4}$$

This rate of water uptake can be considered slightly differently by working out the average mass flux across the cell membrane. The flux is defined as the amount of mass crossing unit area per unit time and in this instance is given by

$$j_{water} \approx \frac{dN_{H_2O}/dt}{A_{E.coli}} \approx \frac{7 \times 10^6 \text{ waters/s}}{6 \times 10^6 \text{ nm}^2} \approx 1 \text{ water/nm}^2 \text{ s}, \tag{3.5}$$

though we also note that this mass transport is mediated primarily by proteins which are distributed throughout the membrane.

We argue that each of these estimates tells us something about the nature of the machinery that mediates the processes of the cell. In remaining sections, these estimates will serve as our jumping off point for estimating the rate at which individual molecular machines carry out the processes of synthesis and transport needed to support metabolism and the cell cycle.

3.1.4 Three Views of Time in Biology

Modern humans have built much of the activity of our societies around an obsession with absolute time. This obsession is revealed by the propensity for events to occur at a certain time of day, for example, class starts at 9am, or scheduling our activity by measured blocks of time, for example, you must practice the piano for half an hour. It is not clear, however, that other organisms relate to time in this manner. In the remainder of the chapter we will discuss three different views of time that seem to be important to life and we will term them *procedural time*, *relative time* and *manipulated time*.

In the previous chapter we explored the question of why biological things are a certain size and the ultimate reason is the finite extent of the atoms that make up biological molecules. Here we are trying to understand why biological processes take a certain amount of time, a difficult task. For the most part, the size of things does not strongly depend on environment and external conditions, but the time scale of processes often does. For example, bacteria growing in left-over potato salad will replicate rapidly when the salad is left on a picnic table in full sun but much more slowly in a refrigerator. The fundamental reason for the difference in replication rates as a function of temperature can be attributed to the slowing of the many individual enzymatic steps that must take place for the cell to double in size and divide. In this sort of context, it appears that organisms pay attention to *procedural time* (an idea to be fleshed out in section 3.2) rather than absolute time: they do something for as long as it takes to get it done since there is some procedure such as DNA replication dictated by an enzymatic rate. A particularly interesting class of procedural time mechanisms are those that organisms use to build clocks that are extremely good at keeping track of absolute time without regard to perturbation by external conditions. One fascinating example of this that we will explore in more detail later in the chapter is the diurnal clock that enables an organism to perform different acts at different times of the day, even in the absence of external signals such as the rising and setting of the sun. For these clocks to work, organisms must have a way to convert procedural time into absolute time so as to ignore external conditions, including temperature.

Although calculating procedural time for a process of interest can often put a lower limit on how fast that process can occur, cells often seem to put as much effort into making sure that processes occur in the correct order as in making sure that they occur quickly. In the context of cell division, for example, it

would be disastrous for a cell to try to segregate its chromosomes into the two daughters until the process of DNA replication is complete. The result would be that at least one daughter would lack the full genetic complement of the mother cell. We will refer to processes where one must be complete before another can start under the category of *relative time* (i.e. before or after rather than how long). This topic will be explored in section 3.3.

Third, and perhaps most interestingly, it appears that living organisms are rarely content to accept time as it is. In some cases, they seem to be impatient, demanding that their life processes occur more quickly than permitted by the underlying chemical and physical mechanisms. Rate acceleration by enzyme catalysis is a prime example. In other cases, they seem to delay the intrinsic proceeding of events, freezing time in "suspended animation" as in formation of bacterial spores that can survive for hundreds or thousands of years, only to be reanimated when conditions become favorable. In section 3.4, we will argue that these processes are examples of what we will refer to as *manipulated time*.

3.2 Procedural Time

The underlying idea of measurements of procedural time is simply that the chemical and physical transformations characteristic of life do not happen instantaneously. Complex processes can be thought of as being built up from many small steps, each of which takes a finite amount of time. For many biological processes that are intrinsically repetitive such as the replication of DNA or the synthesis of proteins, the same step is used over and over again; addition of single nucleotides to a growing daughter strand or addition of single amino acids to a growing polypeptide chain. In this section on procedural time, we will begin by making some estimates about these processes of the central dogma as an example of the general issues of computing procedural time for multi-step biological processes. Then we will move on to the interesting special examples of clocks and oscillators where procedural times are calibrated so that cell cycles and diurnal cycles can follow the constant ticking of a reliable clock.

3.2.1 The Machines (or Processes) of the Central Dogma

The Central Dogma Describes the Processes Whereby the Genetic Information Is Expressed Chemically

One of the most important classes of processes in cellular life are those associated with the so-called Central Dogma of molecular biology. The suite of processes associated with the Central Dogma are those related to the polymerization of the polymer chains that make up the nucleic acids and proteins that are at the heart of cellular life. The fundamental processes of replication, transcription and translation and their linkages are shown in fig. 3.8. The basic message of this "dogma" in its least sophisticated form is that DNA leads to RNA which leads to proteins. From the standpoint of cellular timing, the

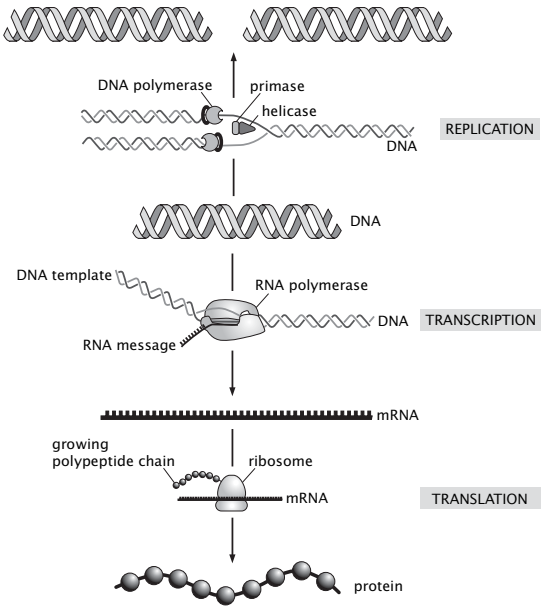

Figure 3.8: The processes of the central dogma. DNA is replicated to make a second copy of the genome. Transcription refers to the process when RNA polymerase makes a mRNA molecule. Translation refers to the synthesis of a polypeptide chain whose sequence is dictated by the arrangement of nucleotides on mRNA.

processes of the Central Dogma will serve as a prime example of procedural time. A typical circular bacterial genome, for example, is replicated by just two DNA polymerase complexes that take off in opposite directions from the origin of replication and each travel roughly halfway around the bacterial genome to meet on the opposite side. The time to replicate a bacterial genome is governed by the rate at which these polymerase motors travel along to copy the roughly 5×10^6 bp of the bacterial genome. Similarly, the time to synthesize a new protein is governed by the rate of incorporation of amino acids by the ribosome.

The Processes of the Central Dogma Are Carried Out By Sophisticated Molecular Machines

One of the primary processes shown in fig. 3.8 is the copying of the genome, also known as replication. DNA replication must take place before a cell divides. As shown in fig. 3.8, the process of DNA replication is mediated by a macromolecular complex (the replisome) which has a variety of intricate parts such as the enzyme DNA polymerase which incorporates new nucleotides onto the nascent DNA molecule, and helicases and primases that pry apart the DNA at the replication fork and prime the polymerization reaction, respectively.

The DNA molecule serves as a template in two different capacities. As described above, a given DNA molecule serves as a template for its own replication (DNA → DNA). However, in its second capacity as the carrier of the genetic material, a DNA molecule must also dictate the synthesis of proteins (the expression of its genes). The first stage in this process of gene expression is the synthesis of a messenger RNA molecule (DNA → RNA) with a nucleotide sequence complementary to the DNA strand from which it was copied, which will serve as the template for protein synthesis. This transcription process is carried out by a molecular machine called RNA polymerase that is shown schematically in fig. 3.8. In eukaryotes, transcription takes place in the nucleus while subsequent protein synthesis takes place in the cytoplasm so there must be an intermediate step of mRNA export.

Once the messenger molecule (mRNA) has been synthesized, the translation process can begin in earnest, (RNA → Protein). As already described in section 2.1.4 (pg. 67), translation is mediated by one of the most fascinating macromolecular assemblies, namely, the ribosome. The ribosome is the apparatus that speaks both of the two great polymer languages and in particular, forms a string of amino acids (a polypeptide chain) which are dictated by the codons (collections of three letters) on the mRNA molecule. The structure of the ribosome is indicated in cartoon form in fig. 3.8. As might be expected for a bilingual machine, the ribosome contains structural components of both RNA and protein. The two halves of the ribosome clamp a messenger RNA and then the ribosome moves processively down the length of the mRNA. As the ribosome moves along, successive triplets of nucleotides are brought into registry with active sites in the ribosomal machinery that align special RNA molecules (tRNA), charged with various amino acids, to recognize the complementary triplet codon. Subsequently, the ribosome catalyzes transfer of the correct amino acid from the tRNA onto a growing polypeptide chain and releases the now empty tRNA. This set of processes was indicated schematically in fig. 3.2(E). As shown in fig. 3.9, the nascent mRNA molecules in bacteria are immediately engaged by ribosomes so that protein translation can occur before transcription is even finished.

The timing of all three of these processes is dictated by the intrinsic rate at which these machines carry out their polymerization reactions. All of them can be thought of in the same framework as repetitions of N essentially identical reactions, each of which takes an average time Δt to perform (though clearly there are substantial fluctuations). We will now estimate total times for each of the three central processes of the central dogma.

- **Estimate: Timing the Machines of the Central Dogma.** The estimates concerning the mass budget of dividing cells from chap. 2 (pg.59) can be used as a springboard for contemplating the rates of the machines that mediate the processes of the central dogma. In our first estimate, we expand upon the estimate of the rate at which the genome of an *E. coli* cell is copied performed earlier in the chapter with the aim of learning more about the speed of the DNA replication complex. DNA replication

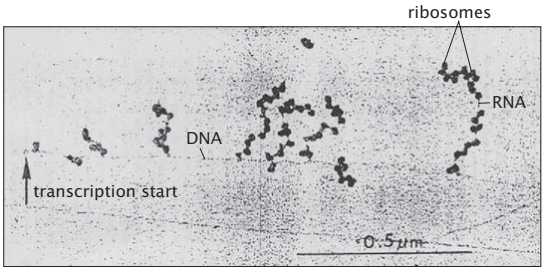

Figure 3.9: Electron microscopy image of simultaneous transcription and translation. The image shows bacterial DNA and its associated mRNA transcripts, each of which is occupied by ribosomes. (Adapted from O. L. Miller *et al.* , *Science* 169:392, 1970.)

in bacteria such as *E. coli* is undertaken by two replication complexes which travel in opposite directions away from the origin of replication on the circular chromosome.

Given that the complete *E. coli* genome is about 5×10^6 base pairs (bp) in size and it is copied in the 3000 seconds of the cell cycle, we already found that the rate of DNA synthesis is roughly 2000 bp/s, or 1000 bp/s per DNA replication complex (replisome) since there are two of these complexes moving along the DNA simultaneously. Biochemical studies have found rates for the DNA polymerase complex in the 250-1000 bp/s range. As we have mentioned, *E. coli* are actually capable of dividing in much less than 3000 seconds, in fact, as little as 1000 seconds, although their DNA replication machinery cannot proceed any faster than this absolute speed limit. How do they pull it off? For now we will leave this as an open mystery and will return to the question in the final section of the chapter on manipulating time.

For a bacterial cell, transcription involves the synthesis of messenger RNA molecules with a typical length of roughly 1000 bases. Our reasoning is that the typical protein has a length of 300 amino acids, with 3 bases needed to specify each such amino acid. Both bulk and single-molecule studies have revealed that a characteristic transcription rate is tens of nucleotides per second. Using 40 nucleotides/s, we estimate the time to make a typical transcript is roughly 25 seconds.

Yet another process of great importance in the central dogma is protein synthesis by ribosomes. Recall from our estimates in the previous chapter that the number of proteins in a "typical" bacterial cell like *E. coli* is of order 3×10^6. This suggests, in turn, that there are of order 9×10^8 amino acids per *E. coli* cell which are produced over the roughly 3000 seconds of the cell cycle. We have made the assumption that each protein has 300 amino acids. This implies that the mean rate of amino acid incorporation

per second is given by

$$\frac{dN_{aa}}{dt} \approx \frac{9 \times 10^8 \text{ amino acids}}{3000 \text{ seconds}} \approx 3 \times 10^5 \text{ aa/s}. \tag{3.6}$$

The number of ribosomes at work on synthesizing these new proteins is roughly 20,000 which implies that the rate per ribosome is 15 aa/s, while the measured value is 25 amino acids incorporated per second. These numbers also imply that the mean time to synthesize a typical protein is roughly 20 seconds.

One of our conclusions is that the rate of protein synthesis by the ribosome is slower than the rate of mRNA synthesis by RNA polymerase. However, as shown in fig. 3.9, multiple ribosomes can simultaneously translate a single mRNA by proceeding in a linearly, orderly fashion and indeed, multiple RNA transcripts may exist in different degrees of completion, being transcribed from the same genetic locus. Thus when considering the net rates of processes in cells, the number of molecular players is clearly as important as the intrinsic rate.

3.2.2 Clocks and Oscillators

In the context of the central dogma, we have described measurements of procedural time for processes that essentially happen once and run to completion such as the synthesis of a protein molecule. However, many cellular processes run in repeated regular cycles. These cyclic or oscillatory processes frequently represent control systems where procedural times of some subprocess can be used to set the oscillation period. Two widely studied examples are the oscillators used to drive the cell division cycle and the mechanisms governing behavioral switches between daytime and nighttime which will be explored in detail below. These daily clocks are called circadian or diurnal oscillators. Other everyday oscillators run the beating of our hearts and the pattern of our breathing.

Developing Embryos Divide on a Regular Schedule Dictated by an Internal Clock

One of the best understood examples of an oscillatory clock used by cells is seen in the early embryonic cell cycle of many animals. The best studied example is the South African clawed frog, *Xenopus laevis*. After the giant egg (1mm) is fertilized, a cell division cycle proceeds roughly every twenty minutes until the egg has been cleaved into approximately four thousand similar sized cells. The regularity and synchrony of these cell divisions reflects an underlying oscillatory clock based on a clever manipulation of procedural time. The clock starts each cell division with the synthesis of a protein called cyclin. Cyclin is made from a relatively rare mRNA. As a result, the protein accumulates slowly. The biological function of cyclin is to activate a protein kinase, an enzyme which covalently attaches phosphate groups to amino acid sidechains on other proteins. This process, known as phosphorylation, is one of the key ways that some proteins are controlled after translation. Essentially, the protein is inactive

in the absence of its phosphate group. Kinase activation cannot begin until the cyclin protein has accumulated to a certain threshold level. After the kinase is activated, one of its targets is an enzyme which in turn catalyzes the destruction of the cyclin protein. All cyclin in the cell is quickly destroyed with a half-life of 90 seconds, resetting the clock to its zero position.

The regularity of this oscillatory clock depends upon several measurements of procedural time. First, accumulation of the cyclin protein to its threshold level depends upon the rate of ribosomal synthesis of that protein. Second, activation of the cyclin-dependent protein kinase kicks off a second procedural time measurement which reflects the length of time required by the kinase to encounter and phosphorylate its enzymatic substrates. Third, the degradation of the cyclin protein also requires a fixed, but brief amount of time. The sum of these three procedural times gives the total period of the clock. The outcome of these molecular events in terms of molecular concentrations is illustrated in fig. 3.10. Just as for all the examples of procedural time described above, the amount of absolute time in seconds, minutes or hours may change depending upon external conditions such as temperature.

This cyclin driven cell cycle oscillator is one example of a very general category of two-component oscillatory systems found throughout biology. A simplified idealized representation of such an oscillator is shown in fig. 3.11(A), while a more accurate representation of the real cell cycle control system from the yeast *S. cerevisae* is shown in fig. 3.11(B). The mathematics of these oscillators is explored in the problems at the end of the chapter.

Diurnal Clocks Allow Cells and Organisms to Be on Time Everyday

A second example of the use of procedural time to build a clock is when cells arrange a series of molecular processes in such a way that they can measure an absolute time. Unlike the cell cycle clock, it is critical that the diurnal clock not change its period when the temperature changes such as during the change of seasons. Many organisms perform some specific task at the same time everyday. A spectacular example is shown in fig. 3.12 where an animal alters the light sensitivity of its eyes in anticipation of sundown. While we might imagine that these kind of daily changes are triggered by, for example, the intensity of sunlight, it has been demonstrated for many organisms that they continue to perform their diurnal cycle even when kept in the dark. Direct observation of these cycles over long periods of time in cyanobacteria have demonstrated that they can operate with tight precision over a week time scale without any external cues about absolute time.

Different organisms use information about the time of day for vastly different purposes. Nevertheless, as illustrated in fig. 3.13, the molecular circuitry governing their circadian rhythms conserves certain common features. Generally, these systems include positive elements which activate transcription of so-called clock genes which drive rhythmic biological outputs as well as promoting the expression of negative elements that inhibit the activities of the positive elements. Phosphorylation of the negative elements leads to their degradation allowing them to restart the cycle. Although the circadian oscillators are capable of continuing to measure time in constant light or constant darkness, they

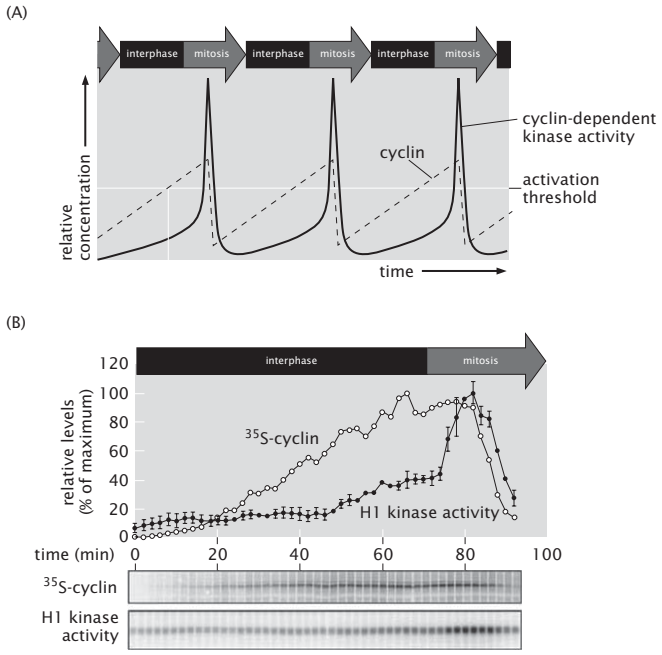

Figure 3.10: The oscillatory cell-cycle clock. This diagram shows the procedural events that underlie the regular oscillations of the cell-cycle clock in the *Xenopus laevis* embryo. (A) Cyclin protein concentration rises slowly over time until it reaches a threshold at which point it activates cyclin-dependent kinase. Cyclin-dependent kinase activity increases sharply at this threshold and in turn activates enzymes involved in cyclin protein degradation. Once the degradation machinery is turned on, cyclin protein levels quickly fall back to zero. Cyclin-dependent kinase activity also falls and the degradation machinery inactivates. This oscillatory cycle is repeated many times. (B) Actual data for a single cycle of the oscillation. (Adapted from J. R. Pomerening *et al.*, *Cell* 122:565, 2005.)

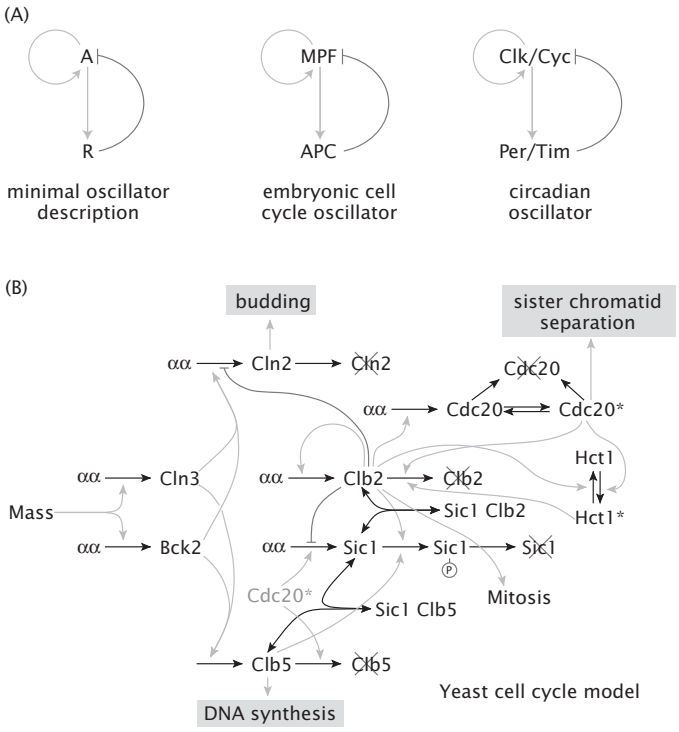

Figure 3.11: Logic diagrams for construction of cell cycle oscillators. (A) The minimal oscillator requires only two components. The first component activates the second component, for example by catalyzing its synthesis. The second component inhibits the first, for example by catalyzing its degradation. (B) A biochemically realistic representation of the cell cycle oscillator in yeast is outrageously more complicated. This is because the real oscillator must work under a wide variety of conditions, be insensitive to fluctuations in the concentrations or activities of its components, and be subject to multiple kinds of regulatory inputs. (Adapted from N. W. Ingolia and A. W. Murray, *Curr. Biol.*, 14:R771, 2004.)

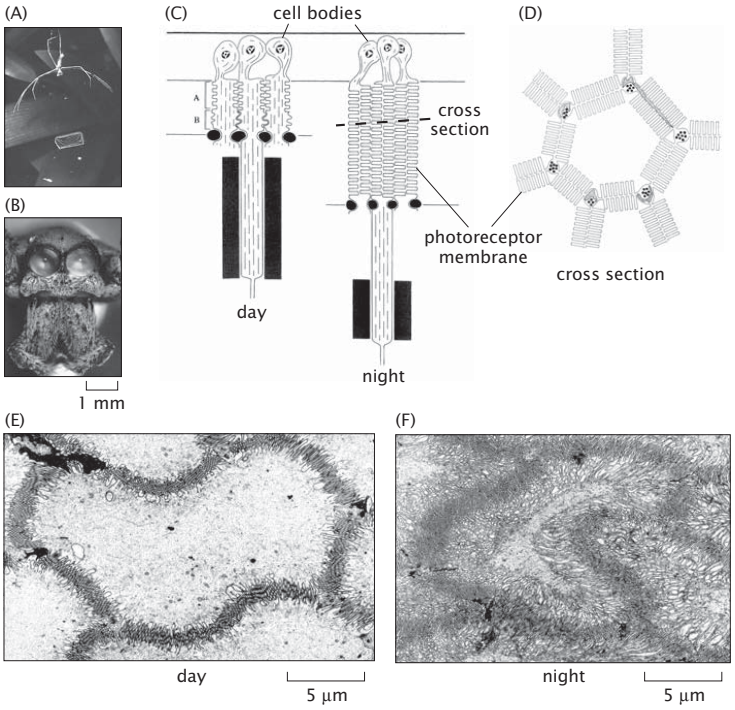

Figure 3.12: An extreme example of a structural change driven by the diurnal clock. (A) The net-casting spider, *Deinopis subrufa*, is a nocturnal hunter with an unusual strategy. It spins a small net which it holds with its legs and tosses to entangle unwary prey passing by.(B) In order to see the prey and know when to toss its net, the spider must have excellent night vision. Two of its eight eyes are extremely enlarged and exquisitely light sensitive. (C) The light sensitivity of the spider's eyes change by a factor of approximately one thousand between daytime and nighttime. During the day, the photoreceptor cell processes are short and fairly disorganized. At night, the total amount of membrane containing light sensors increases both by lengthening of the cells and by the construction of convoluted membrane folds, all packed with photoreceptor molecules. (D) In cross section, the photosensitive membranes of neighboring cells abut each other forming a regular tile-like pattern. (E) A cross section through the photoreceptor cells of a spider sacrificed during the day shows relatively modest thickening of the boundary membranes. (F) An equivalent section taken from a spider sacrificed at night shows a vast increase in the number and size of the membrane folds. At dawn, these membranes will all be degraded only to be resynthesized the following dusk. (A, adapted from *http://www.amonline.net.au/spiders/toolkit/hairy/see.htm*; B, adapted from *http://www.ski.org/Vision/Eyepage/netcasting.html* ; (C)-(F) adapted from A. D. Blest, *Proc. Roy. Soc. London*, B200:463, 1978.)

can nevertheless accept inputs from environmental signals such as the sun to reset their phase. Humans commonly experience the inefficiency of the phase resetting mechanisms as the phenomenon of jetlag.

The circadian oscillator known to function with the fewest components is the one from the photosynthetic cyanobacterium *Synechococcus elongatus*. This organism's clock requires just three proteins called KaiA, KaiB and KaiC and remarkably, it appears that neither gene transcription nor protein degradation is necessary for this clock to function. A purified mixture of just these three proteins together with ATP is capable of sustaining an oscillatory cycle of KaiC protein phosphorylation over periods of at least several days. KaiC is able to catalyze both its own phosphorylation and its own dephosphorylation. KaiA enhances KaiC auto-phoshorylation and KaiB inhibits the effect of KaiA. Fig. 3.14 shows the data supporting this remarkable finding.

3.3 Relative Time

The examples in the previous section on procedural time have emphasized the ways that cells set and measure the time that it takes to accomplish specific tasks. Some processes are rapid and others are slow because of intrinsic features or environmental circumstances. In the well-regulated life of the cell, it is frequently important that fast and slow processes not be permitted to run independently of one another, but instead be linked in a logical sequence that depends upon the cell's needs. In this context, we now turn to what we will call *relative time* which includes the governing mechanisms that ensure that related processes can be strung together in a "socks before shoes" fashion in which event A must be completed before event B can begin. Event C dutifully awaits the completion of event B before it begins, and so on.

3.3.1 Checkpoints and the Cell Cycle

In our initial discussion of the eukaryotic cell division cycle in the context of clocks and oscillators, we used the example of early embryonic divisions in the frog *Xenopus laevis* and asserted that the underlying driver was a simple two-component oscillator. Once past the earliest stages of embryonic development, the cell cycle becomes much more complex and in particular, becomes sensitive to feedback control from the cell's environment. The points in the cell cycle which are subject to interruption by external signals are referred to as checkpoints. These checkpoints ensure, for example, that chromosomes are not segregated until the DNA replication process is complete.

The Eukaryotic Cell Cycle Consists of Four Phases Involving Molecular Synthesis and Organization

Fig. 3.15 shows the key features of the eukaryotic cell cycle with an emphasis on the regulatory checkpoints that ensure that all processes will occur in the correct order. There is not a single universal time scale for the eukaryotic cell

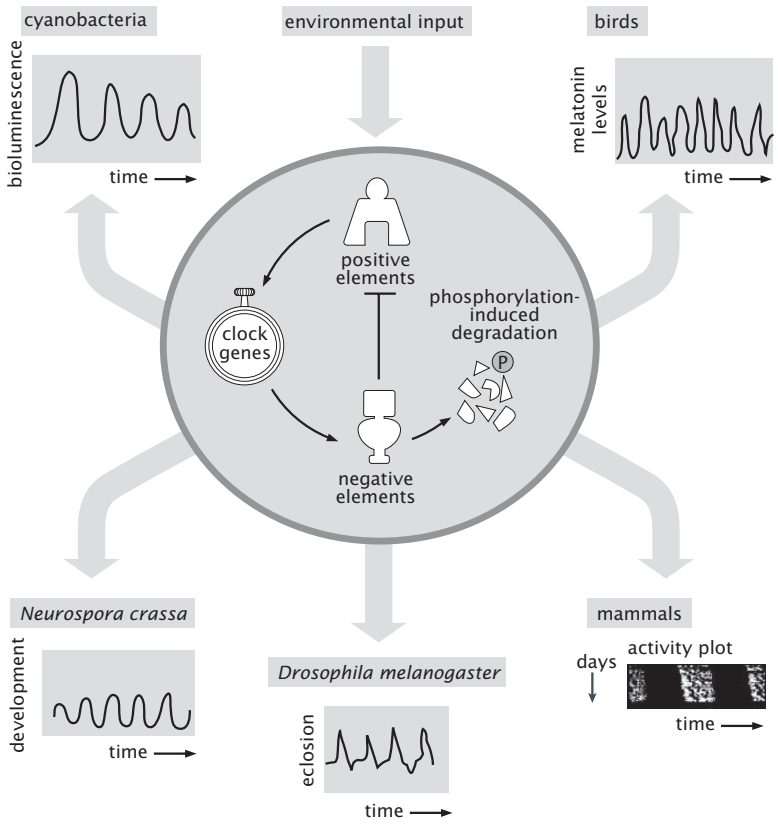

Figure 3.13: Schematic showing generic features of circadian clocks. Circadian clock mechanisms are autonomously driven oscillators that can be modulated by external inputs. Different organisms ranging from cyanobacteria to fungi to insects, birds and mammals use their circadian timers to regulate different kinds of biological outputs and also use very different kinds of protein components in the internal circuitry. (Adapted from D. Bell-Pedersen *et al.*, *Nat. Rev. Genetics* 6:544, 2005.)

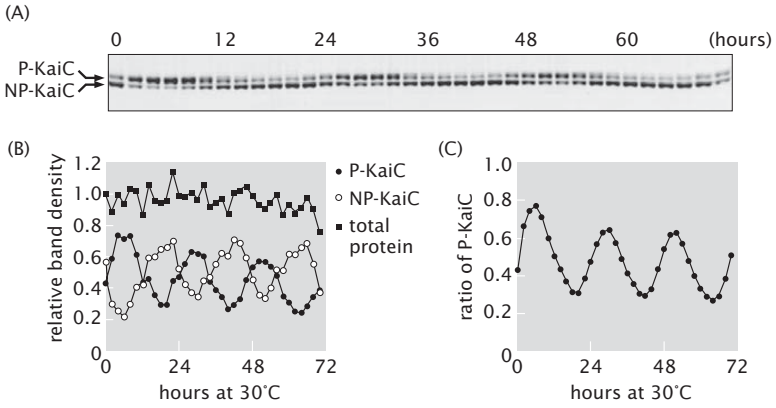

Figure 3.14: Reconstitution of the circadian oscillator. (A) In a mixture of purified KaiC protein together with KaiA, KaiB and ATP, the molecular weight of KaiC can be seen to shift up and down slightly over a twenty-four hour period. The upper band on this gel shows the phosphorylated form of KaiC protein and the lower band is the nonphosphorylated form. (B) Quantitation of the density of these two bands over time reveals that their concentration oscillates in a reciprocal manner such that the total amount of KaiC protein remains roughly constant. (C) The ratio of the amount of phosphorylated KaiC to total KaiC oscillates with a regular period of slightly under twenty-four hours. (Adapted from M. Nakajima *et al.*, *Science* 308:414, 2005.)

cycle, which can vary greatly from one cell type to the next. In the human body, some cells in the intestinal lining can divide in as little as 10-12 hours while others such as some tissue stem cells have cell cycles measured in days or weeks. The eukaryotic cell cycle is usually described in terms of four stages denoted as G_1, S, G_2 and M, with the M phase including the most recognized features, namely, nuclear division (mitosis) and cell division (cytokinesis), the two G phases (gap) as periods of growth and the S phase (synthesis) during which the nuclear DNA is replicated. Together, the phases other than the M phase constitute interphase. During interphase, the mass content of the cell increases as does its size.

If we use a cultured animal cell such as a fibroblast as our standard, G_1 is roughly 10 hours long and is characterized by a significant increase in cell mass and culminates in a checkpoint to insure sufficient cell size and appropriate environmental conditions to pass to the next stage. At this point, the cell examines itself for DNA damage. Any signs of damage such as double-strand breaks will trigger a checkpoint control that prevents the cell from initiating DNA replication until the damage is completely repaired. This checkpoint also ensures a critical aspect of the regulation of relative time for the cell's replication, specifically, that it must have grown to approximately twice its prior size before it is allowed to begin to divide. If this checkpoint is successfully passed then DNA replication can begin. In S phase, the eukaryotic DNA is replicated over a period of about 6 hours.

Following S phase and a shorter gap phase called G_2, another checkpoint verifies that every chromosome has been completely replicated before the initiation of assembly of the mitotic spindle, the microtubule-based apparatus that physically separates the chromosomes into the two daughter cells. This enforcement of relative time is particularly critical because if a cell were to try to segregate the chromosomes before replication was complete, then at least one of the daughters would inherit an incomplete copy of the genome. After passing this checkpoint, M phase begins. This relatively brief period of the cell cycle (of order one hour) involves most of the spectacular events of cell division that can be directly observed in a light microscope. Within M phase again, it is critical that events occur in the proper order. The bipolar mitotic spindle built from microtubules forms symmetric attachments to each pair of replicated sister chromosomes. When they have all been attached to the spindle, the chromosomes all suddenly and simultaneously release their sisters and are pulled to opposite poles. A spindle-assembly checkpoint ensures that every chromosome is properly attached before segregation begins. The molecular mechanisms governing the enforcement of relative time in the cell cycle involve protein phosphorylation and degradation events as well as gene transcription. In order to delve deeper into the principles governing the measurement and enforcement of relative time, we will now turn to a different example where gene transcription is the principal site of regulation.

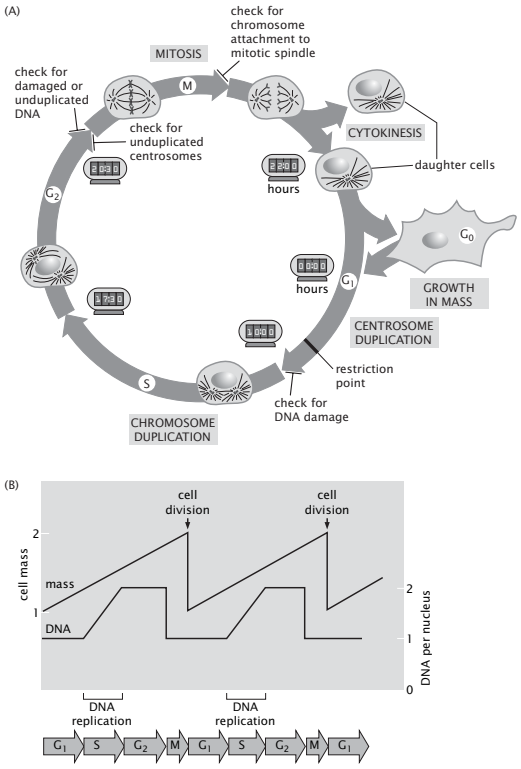

Figure 3.15: The eukaryotic cell cycle. (A) This cartoon shows some of the key elements of the process of cell division. (B) Time course of cell mass and DNA content over the course of the cell cycle. (A, adapted from T. D. Pollard and W. C. Earnshaw, Cell Biology, Philadelphia, Saunders, 2002; B, adapted from A. Murray and T. Hunt, The Cell Cycle, New York, Oxford University Press, 1993.)

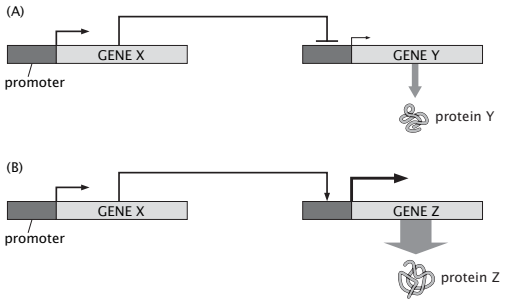

Figure 3.16: Network of interacting genes. Representation of a hypothetical genetic network where the output of the first gene represses or activates the expression of the second. (A) Repression process in which the output of gene X represses the expression of gene Y. (B) Activation process in which the output of gene X activates the expression of gene Z.

3.3.2 Measuring Relative Time

There is great regulatory complexity involved in orchestrating the cell cycle. That is, the time ordering of the expression of different genes follows a complex program with certain parts clearly following a progression in which some processes must await others before beginning. By measuring the pattern of gene expression, it becomes possible to explore the relative timing of events in the cell cycle. To get a better idea of how this might work, we need to examine how networks of genes are coupled together.

Genetic Networks Are Collections of Genes Whose Expression Is Interrelated

Sets of coupled genes are shown schematically in fig. 3.16. For simplicity, this diagram illustrates how the product of one gene can alter the expression of some other gene. Perhaps the simplest regulatory motif is direct negative control in which a specific protein binds to the promoter region on DNA of a particular gene and physically blocks binding of RNA polymerase and subsequent transcription. This protein is itself the result of some other gene which can in turn be subject to control by yet other proteins (or perhaps the output of the gene that it controls). The second broad class of regulatory motif is referred to as activation and results when a regulatory protein (a transcription factor called an activator) binds in the vicinity of the promoter and "recruits" RNA polymerase to its promoter.

One way to measure the extent of gene expression is using a technique known as a DNA microarray. The idea is that a surface is decorated in an ordered x-y

array with fragments of DNA, and the sequence of each spot on this array is different. To take a census of the current mRNA contents of a cell (which gives a snapshot of the current expression level of all genes), the cell is broken open and the mRNA contents are hybridized (i.e. they bind to their complementary strand via base pairing) to the DNA on the microarray. DNA molecules on the surface which are complementary to RNA molecules in the cell lysate will hybridize with their complementary fragments and those molecules that bind are labeled with fluorescent tags so that the fluorescence intensity can be used as a readout of the number of bound molecules. There is a bit more subtlety in the procedure than we describe since really the mRNA is turned into DNA first, but we focus on the concept of the measurement rather than its practical implementation. The intensity of the spots on the microarray report the extent to which each gene of interest was expressed. By repeating this measurement again and again at different time points, it is possible to profile the state of gene expression for a host of interesting genes at different times in the cell cycle. These measurements yield a map of the *relative* timing of different genes.

One of the key model systems for examining the bacterial cell cycle is *Caulobacter crescentus*. In this system, DNA microarray analysis was used to examine the timing of various processes during the course of the cell cycle. In a beautiful set of experiments roughly 20% of the *Caulobacter* genome was implicated in cell cycle control as a result of time varying mRNA concentrations which were slaved to the cell cycle itself. The idea of the experiment was to break open synchronized cells every fifteen minutes and to harvest their messenger RNA. Then by using a DNA microarray to find out which genes were being expressed at that moment, it was possible to put together a profile of which genes were expressed when. The outcome of this experiment is shown in fig. 3.17. What these experiments revealed is the relative timing of a series of events associated with the cell cycle, though our reason for showing these experiments here is to illustrate the *relative* timing of different events over the roughly 150 minutes of the *Caulobacter* cell cycle.

The Formation of the Bacterial Flagellum Is Intricately Organized in Space and Time

A higher resolution look at the relative timing of cellular events is offered by the macromolecular synthesis of one of the key organelles for cell motility, namely, the bacterial flagellum. Fig. 3.18 shows the various gene products (FlgK, MotB, etc.) that are involved in the formation of the bacterial flagellum. Essentially, each of these products corresponds to one of the protein building blocks associated with flagellar construction. Once the flagella are assembled, the cell propels itself around by spinning them. The dynamical question posed in the experiment is to what extent is the expression of the genes associated with these different building blocks orchestrated in time.

The idea of the experiment is to induce the growth of flagella in starved *E. coli* cells (which lack flagella) and to use a reporter gene, namely, a gene leading to the expression of green fluorescent protein, to report on when each of the different genes associated with the flagellar pathway are being expressed.

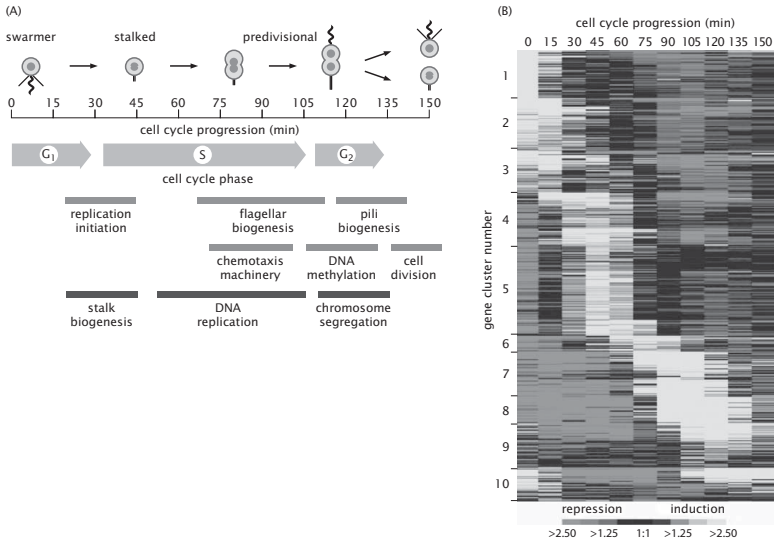

Figure 3.17: Gene expression during the cell cycle for *Caulobacter crescentus*. (A) The 150 minute cell cycle of *Caulobacter* is shown, highlighting some of the key morphological and metabolic events that take place during cell division. (B) The microarray data shows how different batteries of related genes are expressed in a precise order. The genes are organized by time of peak expression. Each row corresponds to the time history of expression for a given gene with time running from 0 to 150 minutes from left to right. From top to bottom the genes are organized into different clusters associated with different processes such as DNA replication and chromosome segregation, for example. (Adapted from M. T. Laub *et al.*, *Science* 290:2144, 2000.)

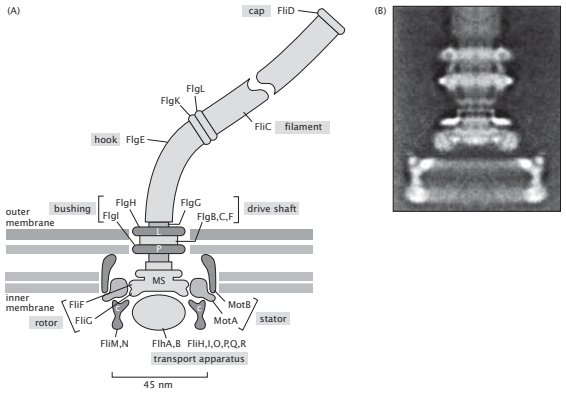

Figure 3.18: Molecular architecture of the bacterial flagellum. The schematic shows both the membrane-bound parts of the flagellar apparatus as well as the flagellum itself. The labels refer to the various gene products involved in the assembly of the flagellum. (Adapted from H. C. Berg, *Phys. Today* 53:24, 2000.)

This experiment permits us to peer directly into the dynamics of assembly of the bacterial flagellum which reveals a sequence of events that are locked into succession in exactly the sort of way that we argued is characteristic of relative time. Fig. 3.19 shows the results of this experiment. To deduce a time scale from this figure, we consider the band of expression shown for "Condition A" and note that the roughly 15 genes are turned on over a period of roughly 180 minutes. This implies an approximate delay time between each product of roughly 12 minutes. Note that the entire experiment was run over a period of roughly 10 hours, though induction of new flagella (the part of the experiment of interest here) begins after three or four rounds of cell division have occurred.

3.3.3 Killing the Cell: The Life Cycles of Viruses

Cells are not the only biological entities that care about relative timing. Once viruses have infected a host cell, they are like a ticking time bomb with an ever shortening fuse of early, middle and late genes. Once these genes have been expressed and their products assembled, as many as hundreds of new viruses emerge from the infected (and now defunct) cell to repeat the process elsewhere.

Viral Life Cycles Include a Series of Self-Assembly Processes

We have already described the cell cycle as a master process characterized by an enormous number of subprocesses. A more manageable example of an entire "life cycle" is that of viruses, which illustrate the intricate relative timing of biological processes. An example of the viral life cycle of a bacteriophage (introduced in the last chapter as a class of viruses that attack bacterial cells) is shown in fig. 3.20 which shows the key components in the life history of the

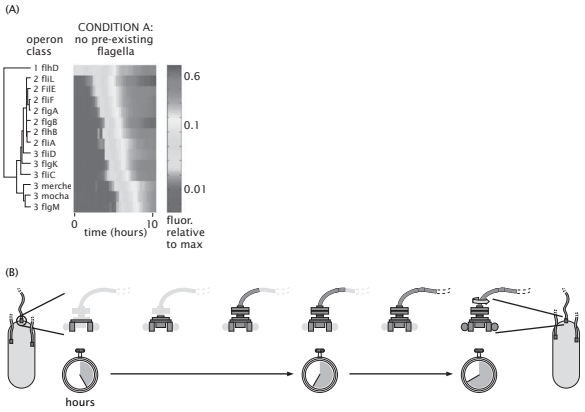

Figure 3.19: Timing of gene expression during the process of formation of the bacterial flagellum. (A) The extent of gene expression (as measured by fluorescence intensity) of each of the gene products as a function of time. (B) The cartoon show the timing of synthesis of different parts of the flagellum. The stopwatch times are chosen by assuming that the total time of the process takes roughly three hours and that flagellar synthesis begins roughly five hours after the beginning of the experiment. (Adapted from S. Kalir *et al.*, *Science* 292:2080, 2001.)

virus. The key stages in this life cycle are captured in kinetic verbs such as infect, transcribe, translate, assemble, package and lyse. Infection is the process of entry of the viral DNA into the host cell. Transcription and translation refer to the hijacking of the cellular machinery so as to produce viral building blocks (both nucleic acid and proteins). Assembly is the coming together of these building blocks to form the viral capsid. Packaging, in turn, is the part of the life cycle when the viral genome is enclosed within the capsid. Finally, lysis refers to the dissolution of the host cell and the emergence of a new generation of phage to go out and repeat their life cycle elsewhere.

As illustrated by the cartoon in fig. 3.20 and in particular, by our use of the stopwatch motifs, the time between the arrival of the virus at the bacterial surface and the destruction of that very same membrane during the lysis phase when the newly formed viruses are released seems very short at 30 minutes. Indeed, one of our charters in the chapters that follow will be to come to terms with the 30 minute characteristic time scale of the viral life cycle and the various processes that make it up. On the other hand, though the absolute units (30 minutes) are interesting, it is important to emphasize that this set of processes is locked together sequentially in a progression of relative timing events (as with the synthesis of the bacterial flagellar apparatus).

Because of their stunning structures and rich lifestyles, we now examine a second class of viruses, namely, RNA animal viruses such as HIV already in-

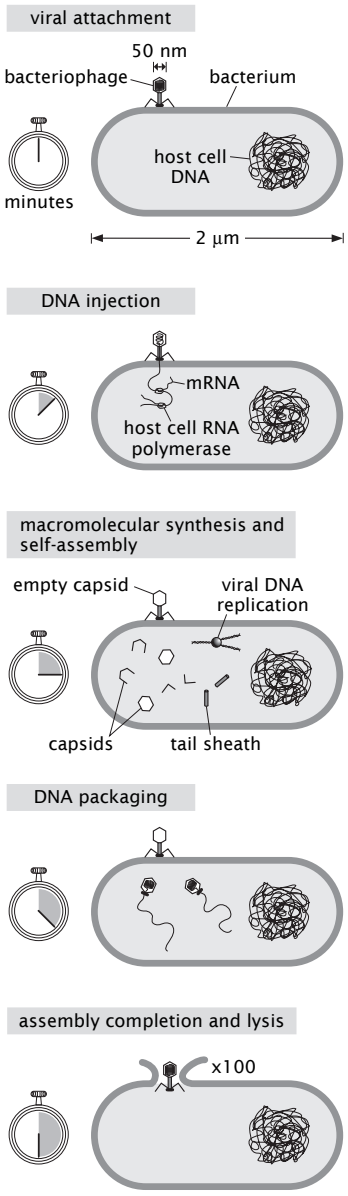

Figure 3.20: Timing the life cycle of a bacteriophage. The cartoon shows stages in the life cycle of a bacteriophage and roughly how long after infection these processes occur. Note that the head and the tail follow distinct assembly pathways and join only after they have separately assembled.

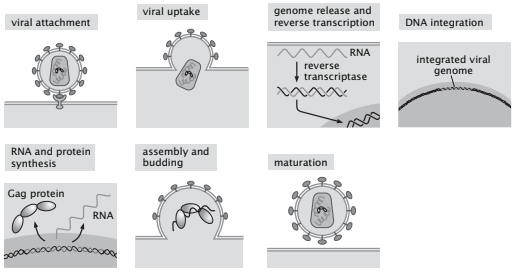

Figure 3.21: Stages in the life cycle of HIV. The timing of the events in the life cycle of HIV can be explored in Reddy and Yin (1999).

troduced from a structural perspective in section 2.2.4 (pg. 88). As shown in fig. 3.21, the infection process for these viruses is quite distinct from that in bacterial viruses. In particular, we note the presence of a membrane coat on the virus which allows the entire virus to attach to membrane-bound receptors on the victim cell. As a result of this interaction between the virus and the host cell, the virus is swallowed up by the cell which is under attack in a process of membrane fusion. Once the virus has entered the embattled cell, the genetic material (RNA) is released and reverse transcriptase creates a DNA molecule encoding the viral genome which is then delivered to the host nucleus and incorporated into its genome. After the genetic material has been delivered to the nucleus, a variety of synthesis processes are undertaken which result in copies of the viral RNA as well as fascinating polyproteins (the Gag proteins described in chap. 2) which are exported to the plasma membrane where they undergo an intricate process of self-assembly at the membrane of the infected cell. Once the newly formed virus is exported, it undergoes a maturation process resulting in new, fully infectious, viral particles. Each of these processes is locked in succession in a pageant of relatively timed events.

3.3.4 The Process of Development

As already demonstrated in section 2.3.3 (pg. 106), one of the most compelling, mysterious and visually pleasing processes in biology is the development of multicellular organisms. Like the cell cycle of individual cells, development depends upon a fixed, relative ordering of events. Perhaps the most studied organism from the developmental perspective is the fruit fly *Drosophila melanogaster*. The process of *Drosophila* development was already schematized in fig. 3.2(A) and (B). Embryonic development represents the disciplined outcome of an encounter between an egg and its partner sperm. In the hours that follow this encounter for the fruit fly, the nascent larva undergoes a series of nuclear divisions and migrations as shown in fig. 3.22. In particular, as the nuclei divide to the tenth

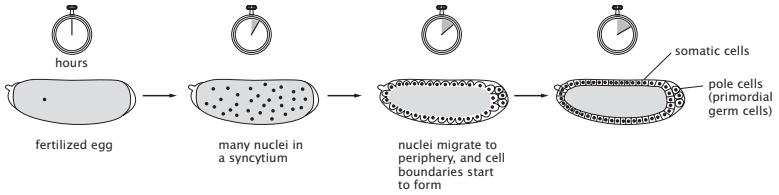

Figure 3.22: Early development of the *Drosophila* embryo. After fertilization, the single nucleus undergoes a series of eight rapid divisions producing 256 nuclei that all reside in a common cytoplasm. At this point the nuclei begin to migrate towards the surface of the embryo while continuing to divide. After reaching the surface, cell boundaries form by invaginations of the plasma membrane. At this early stage, the cells that are destined to give rise to sperm or eggs segregate themselves and cluster at the pole of the embryo. All of these events happen within roughly two hours.

generation (512 nuclei), they also start to collect near the surface of the developing larva forming the synctial blastoderm, a football shaped object with nearly all of the cells localized to the surface. At the 13th generation, the individual nuclei are enclosed by their own membranes to form the cellular blastoderm. The structural picture by the end of this process is a collection of roughly 5000 cells which occupy the surface of a football shaped object (roughly) which is 500 μm in length and roughly 200 μm in cross-sectional diameter.

Accompanying these latter stages of the developmental pathway is the beginning of a cellular dance in which orchestrated cell movements known as gastrulation lead to the visible emergence of the macroscopic structures associated with the nascent embryo. Snapshots from this process are shown in fig. 3.23 with a time scale associated with the process of gastrulation is on the order of hours. We already proclaimed the importance and beauty of the temporal organization of gene expression associated with the cell cycle. We now add to that compliment by noting that during the development of the *Drosophila* body plan, there is an ordered spatial pattern of expression of genes with colorful names such as *hunchback* and *giant*, which determine the spatial arrangement of different cells.

These developmental processes make their appearance here because they too serve as an example of relative time. In particular, an example of the time ordering is the cascade of genes associated with the segmentation of the fly body plan into its anterior and posterior parts as already introduced in section 2.3.3 (pg. 106). The long axis of the *Drosophila* embryo is subject to increasing structural refinement as a result of a cascade of genes known collectively as segmentation genes. This collection of genes acts in a cascade which is a code word for precisely the kind of sequential processes that are behind *relative time* as introduced in this section. The first set of genes in the cascade are known as the *gap* genes. These genes divide the embryo into three rough regions,

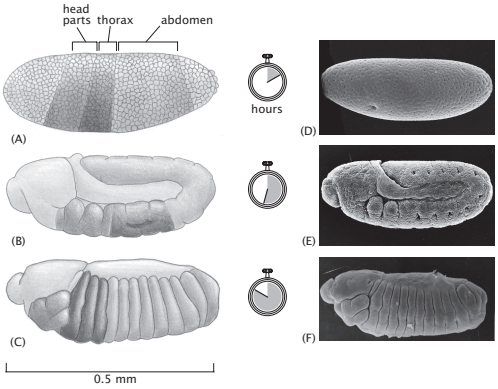

Figure 3.23: Early pattern formation in the *Drosophila* embryo. (A - C) show schematics of the shape of the *Drosophila* embryo at two hours, six hours and ten hours after fertilization, respectively. At two hours, no obvious structures have yet begun to form but the eventual fates of the cells that will form different parts of the animal's body have already been determined. By six hours, the embryo has undergone gastrulation and the body axis of the embryo has lengthened and curled back on itself to fit in the egg shell. By ten hours, the body axis has contracted and the separate segments of the animal's body plan have become clearly visible. On the right are scanning electron micrographs of embryos at each of these stages. Remarkably, all of this pattern formation takes place without any growth. (Adapted from B. Alberts *et al.*, Molecular Biology of the Cell, 4th ed. New York: Garland Science, 2002.)

the anterior, middle and posterior. The *gap* genes have as protein products transcription factors that control the next set of genes in the cascade which are known as *pair-rule* genes. The *pair-rule* genes begin to form the identifiable set of seven stripes of cells. Finally, the *segment polarity* genes are expressed in 14 stripes.

- **Estimate: Timing Development.** A simple estimate of the number of cells associated with a developing organism can be obtained by assuming perfect synchrony from one generation to the next,

$$\text{number of cells} \approx 2^N, \tag{3.7}$$

where N is the number of generations. Further, if we assume that the cell cycle is characterized by a time τ_{cc}, then the number of cells as a function of time can be written simply as

$$\text{number of cells} \approx 2^{t/\tau_{cc}}. \tag{3.8}$$

Interestingly, in the early stages of *Drosophila* development, since it is only nuclear division (and hence, largely DNA replication) which is taking place, the mean doubling time is eight minutes. Thus after 100 minutes, roughly 10 generations worth of nuclear division will have occurred with the formation of the approximately 1000 cells which form the syncytial blastoderm.

3.4 Manipulated Time

Sometimes the cell is not satisfied with the time scales offered by the intrinsic physical rates of processes and has to find a way to beat these speed limits. For example, in some cases the bare rates of biochemical reactions are prohibitively slow relative to characteristic cellular time scales and as a result, cells have tied their fate to enzymatic manipulation of the intrinsic rates. In a similar vein, diffusion as a means of intracellular transport is ineffective over large distances. In this case, cells have active transport mechanisms involving molecular motors and cytoskeletal filaments which can overcome the diffusive speed limit, or alternatively, the cell might resort to reduced dimensionality diffusion to beat the diffusive speed limit. There are even more tricky ways in which cells manipulate time such as in the case of beating the bacterial replication limit. These examples and others will serve as the basis of our discussion of the way cells manipulate time.

3.4.1 Chemical Kinetics and Enzyme Turnover

Some chemical reactions proceed much more slowly than necessary for them to be biologically useful. For example, the hydrolysis of the peptide bonds that make up proteins would take times measured in years in the absence of proteases,

which are the enzymes that cleave these bonds. Triose phosphate isomerase, one of the enzymes in the glycolysis pathway featured in fig. 2.22 (pg. 94), is responsible for a factor of 10^9 speed up in the glycolytic reaction it catalyzes. What these numbers show is that even if a given reaction is favorable in terms of free energy, the energy barrier to that reaction can make it prohibitively slow. As a result, cells have found ways to manipulate the timing of reactions using enzymes as catalysts. Indeed, the whole of biochemistry is in some ways a long tale of catalyzed reactions many of which take place on time scales much shorter than milliseconds, whereas, in the absence of these enzymes, they might not take place in a year! The individual players in the drama of glycolysis such as hexokinase, phosphofructokinase, triose phosphate isomerase and pyruvate kinase reveal their identity as enzymes with the ending *ase* in their names. Enzymes are usually denoted by the ending *ase* and are classified according to the reactions they catalyze.

The basis of enzyme action is depicted in fig. 3.24. For concreteness, we consider an isomerization reaction where a molecule starts out in some high energy state A and we interest ourselves in the transitions to the lower energy state B. For the molecule schematized in the figure, the energy associated with the conformation has an electrostatic contribution and an "internal" contribution. As seen in fig. 3.24(A), the internal contribution has two minima, while the electrostatic contribution to the free energy is a monotonically decreasing function. In the presence of the enzyme, the height of the barrier is suppressed because the charges on the molecule of interest have an especially favorable interaction with the enzyme at the transition state.

The key point about the reaction rate is that, as shown in the figure, it depends upon the energy barrier separating the two states according to

$$\Gamma_{A\to B} \propto e^{-E_{barrier}/k_BT}, \tag{3.9}$$

where $\Gamma_{A\to B}$ is the transition rate with units of s^{-1}. Even though the energy of state B might be substantially lower than state A, the transitions can be exceedingly slow because of large barrier heights (i.e. $E_{barrier} >> k_BT$). The presence of an enzyme does not alter the end states or their energies, but it suppresses the barrier between the two states.

3.4.2 Beating the Diffusive Speed Limit

A second example of the way in which cells manipulate time is offered by the question of transport and trafficking. Organelles, proteins, nucleic acids, etc. are often produced in one part of the cell only to be transported to another part where they are needed. For example, the messenger RNA molecules produced in the nucleus need to make their way to the ribosomes which are found on the endoplasmic reticulum. One physical process that can move material around is passive diffusion.

Diffusion Is the Random Motion of Microscopic Particles in Solution

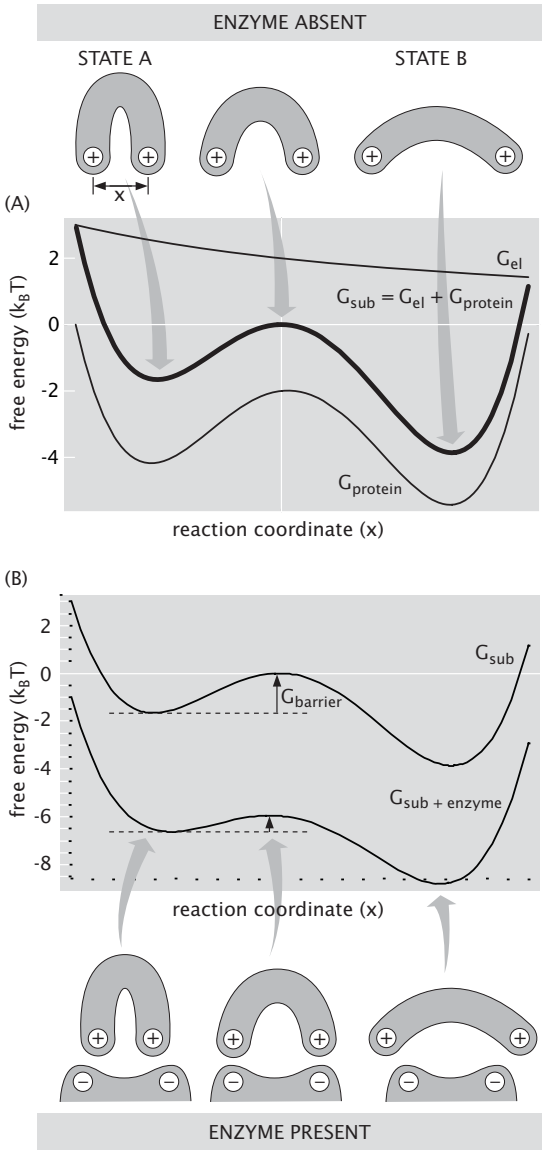

Figure 3.24: Enzymes and biochemical rates. (A) The electrostatic and internal contributions to the overall free energy. The sum of these two contributions gives the total conformational free energy (in the absence of the enzyme). (B) A simple one-dimensional representation of the energy landscape for a biochemical reaction in the absence and presence of an enzyme to catalyze the reaction. The presence of the enzyme lowers the energy barrier through favorable charge interactions at the transition state and increases the rate of the reaction.

Ions, molecules, macromolecular assemblies and even organelles, wander around aimlessly as a result of diffusion. Diffusion refers to the random motions suffered by microscopic particles in solution, and is sometimes referred to as Brownian motion in honor of the systematic investigations made by Robert Brown in the 1820s. Brown noticed the random jiggling of pollen particles suspended in solution, even for systems that are ostensibly in equilibrium and have no energy source. Indeed, so determined was he to find out whether or not this was some effect intrinsic to living organisms, he even examined exotic suspensions using materials such as the dust from the Sphinx and found the jiggling there too. The effects of Brownian motion are palpable for particles in solution which are micron size and smaller, exactly the length scales that matter to cells. Diffusion results from the fact that in the cell (and for microscopic particles in solution), deterministic forces are on nearly an equal footing with thermal forces, an idea to be fleshed out in section 5.1.1 (pg. 241) and illustrated graphically in fig. 5.1 (pg. 243). Thermal forces result from the random collisions between particles that can be attributed to the underlying jiggling of atoms and molecules. This fascinating topic will dominate the discussion of chap. 13.

- **Estimate: The Thermal Energy Scale.** One way to quantify the relative importance of the energy scale of a given process and thermal energies is by measuring the energy of interest in k_BT units. At room temperature, the thermal energy scale is

$$k_BT = 1.38 \times 10^{-23} \text{ J/K} \times 300 \text{ K} \approx 4.1 \times 10^{-21} \text{ J} = 4.1 \text{ pN nm}. \quad (3.10)$$

  One way to see the importance of this energy scale is revealed by eqn. 3.9 (and will also be revealed by the Boltzmann distribution (to be described in chap. 6) that says that the probability of a state with energy E_i is proportional to e^{-E_i/k_BT}). These expressions show that when the energy is comparable to k_BT, barriers will be small (and probabilities of microstates high). The numerical value ($k_BT \approx 4$ pN nm) is especially telling since many of the key molecular motors relevant to biology act with piconewton forces over nanometer distances, implying a competition between deterministic and thermal forces. This discussion tells us that for many problems of biological interest, thermal forces are on nearly an equal footing with deterministic forces arising from specific force generation.

Diffusion Times Depend Upon the Length Scale

One simple and important biological example of diffusion is the motion of proteins bound to DNA which can be described as one-dimensional diffusion along the DNA molecule. For example, it is thought that regulatory proteins (transcription factors) that control gene expression find their specific binding sites on DNA partly through this kind of one-dimensional diffusive motion. Another example is provided by the arrival of ligands to their specific receptors. The basic picture is that of molecules being battered about and every now and

then ending up by chance in the same place at the same time. To get a feeling for the numbers, it is convenient to consider one of the key equations that presides over the subject of diffusion, namely,

$$t_{\text{diffusion}} = \frac{x^2}{D}, \tag{3.11}$$

where D is the diffusion constant. This equation tells us that the time scale for a diffusing particle to travel a distance x scales as the square of that distance.

- **Estimate: Getting Proteins from Here to There.** For molecules and assemblies that move passively within the cell, the time scale can be estimated using eqn. 3.11. For a protein with a 5 nm diameter the diffusion constant in water is roughly 100 μ m^2/s; this estimate can be obtained from the Stokes-Einstein equation (to be discussed in more detail in chap. 12) which gives the diffusion constant of a sphere of radius R moving through a fluid of viscosity η at temperature T, as $D = k_B T/6\pi\eta R$. The time scale for such a typical protein to diffuse a distance of our standard ruler (i.e. across an *E. coli*) is

$$t_{\text{E. coli}} \approx \frac{L^2_{\text{E. coli}}}{D} \approx \frac{1\ \mu\text{m}^2}{100\ \mu\text{m}^2/s} \approx 0.01\ s. \tag{3.12}$$

  This should be contrasted with the time scale required for diffusion to transport molecules from one extremity of a neuron to the other as shown in fig. 3.3. In particular, the diffusion time for the squid giant axon which has a length of the order of 10 cm is $t_{\text{diffusion}} \approx 10^8$ s! The key conclusion to take away from such an estimate is the impossibly long time scales associated with diffusion over large distances. Nature's solution to this conundrum is to exploit *active* transport mechanisms in which ATP is consumed in order for motor molecules to carry out directed motion.

Another way of coming to terms with diffusion is suggested by thinking about concentration. In fig. 2.4 (pg. 65), we described physical interpretations of concentration in terms of number of molecules per *E. coli* cell and in terms of mean molecular spacings. We can also think of concentrations in terms of diffusion times by using eqn. 3.11. In particular, this equation can be used to estimate a characteristic time scale for reactants to find each other as a function of the concentration as shown in fig. 3.25.

Molecular Motors Move Cargo Over Large Distances in a Directed Way

In many instances, diffusion is too slow to be of any use for intracellular transport. To beat the diffusive speed limit, cells manipulate time with a sophisticated array of molecular machines (usually proteins) that result in directed transport. These processes are collectively powered by the consumption of some energy source (usually ATP). Broadly construed, the subject of active transport

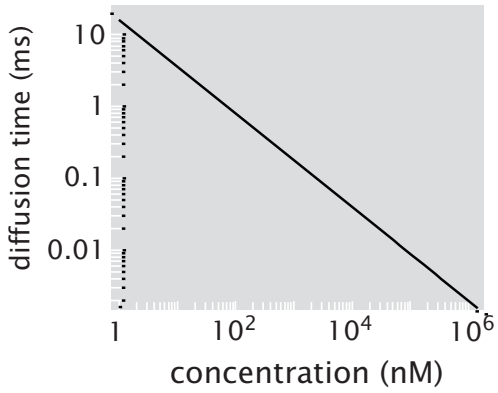

Figure 3.25: Concentration measured in diffusion times. The characteristic time scale for diffusion-mediated molecular encounters will depend upon the concentration of the diffusing species.

allows us to classify a wide variety of molecules as *molecular motors*, the main subject of chap. 16 (pg. 821). We have already seen the existence of such motors in a number of different contexts, with both DNA polymerase and RNA polymerase introduced earlier in the chapter satisfying the definition of active transport.

Concretely, the class of motors of interest here are those that mediate transport of molecules from one place in the cell to another. Often, such transport takes place as vesicular traffic, with the cargo enclosed in a vesicle (a flexible spherical shell made up of lipid molecules in the form of a lipid bilayer) which is in turn attached to some molecular motor. These molecular motors travel in a directed fashion on the cytoskeletal network which traverses the cell. For example, traffic on microtubules runs in both directions as a result of two translational motors, kinesin and dynein. Molecular motor mediated transport on actin filaments is shown in cartoon form in fig. 3.26. Note that this cartoon is meant to indicate a rough idea of the relative proportions of the motors and the actin filaments on which they move and to convey the overall structural features, such as two heads, of the motors themselves. In addition, fig. 3.26(B) shows a time trace of the position of a fluorescently labeled myosin motor which illustrates the discrete steps of the motor, also permitting a measurement of the mean velocity.

- **Estimate: Getting Proteins from Here to There, Part 2.** We have already noted that biological motility is based in large measure on diffusion. On the other hand, there are a host of processes that cannot wait the time required for diffusion. In particular, recall that our estimate for the diffusion time for a typical protein to traverse an axon was a whopping 10^8 seconds, or roughly three years. For comparison we can estimate the transport time for kinesin moving on a microtubule over the same distance.

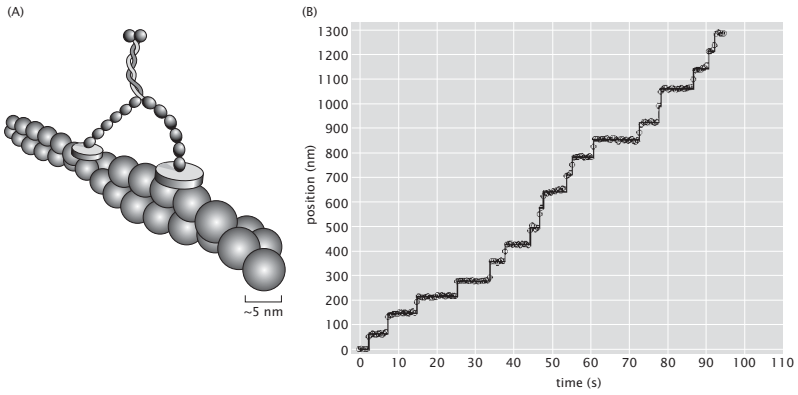

Figure 3.26: Motion of myosin on an actin filament. (A) Schematic of the motor on an actin filament. Note that the step size is determined by the periodicity of the filament. (B) Position as a function of time for the motor myosin V as measured using single-molecule techniques. (Adapted from A. Yildiz *et al.*, *Science* 300:2061, 2003.)

As the typical speed of kinesin in a living cell is 1 μm/s, the time for it to transport a protein over a distance of 10 cm is 10^5 seconds, or just over a day.

To see these ideas play out more concretely, we can return to fig. 3.3(C) (pg. 127). The classic experiment highlighted there traces the time evolution of radioactively labeled proteins in a neuron. The figure shows that the radiolabeled proteins travel roughly 18 mm in 12 days, which translates into a mean speed of roughly 20 nm/s. Observed axonal transport speeds for single motors are a factor of ten or more larger, but we can learn something from this as well. In particular, motors are not perfectly "processive" - that is, they fall off of their cytoskeletal tracks occasionally and this has the effect of reducing their mean speed. Observed motor velocities are reported, on the other hand, on the basis of tracking individual motors during one of their processive trajectories.

Membrane Bound Proteins Transport Molecules From One Side of a Membrane to the Other

Another way in which cells manipulate transport rates is by selectively and transiently altering the permeability of cell membranes through protein channels and pumps. Many ionic species are effectively unable to permeate (at least on short time scales) biological membranes. What this means is that concentration gradients can be maintained across these membranes until or if these protein channels open which then permits a flow of ions down their concentration gradient. In fancier cases such as indicated schematically in fig. 3.27,

ions can be pumped up a concentration gradient through mechanisms involving ATP hydrolysis. In fig. 3.2(G) we showed the process of ion transport across a membrane with a characteristic time of microseconds.

- **Estimate: Ion Transport Rates in Ion Channels.** An ion channel embedded in the cell membrane can be thought of as a tube with a diameter of approximately $d = 0.5$ nm (size of hydrated ion) and a length $l = 5$ nm (width of the lipid bilayer). With these numbers in hand, and a typical value of the diffusion constant for small ions (eg. sodium), $D \approx 2000\ \mu\text{m}^2/\text{s}$, we can estimate the flux of ions through the channel, assuming that their motion is purely diffusive.

  To make this estimate, we invoke an approximate version of Fick's law (to be described in detail in chap. 8) which says that the flux (number of molecules crossing unit area per unit time) is proportional to the difference in concentration and inversely proportional to the distance between the two "reservoirs". Mathematically, this can be written as

  $$J_{\text{ion}} \approx D\frac{\Delta c}{l}, \tag{3.13}$$

  where Δc is the difference in ion concentration across the cell membrane. For typical mammalian cells the concentration difference for sodium or potassium is $\Delta c \approx 100$ mM (or, $\approx 6 \times 10^{-2}$ molecules/nm^3) and the distance across the membrane is $l \approx 5$ nm resulting in

  $$J_{\text{ion}} \approx 2{\times}10^9\ \frac{\text{nm}^2}{\text{s}} \times \frac{6 \times 10^{-2}\ \text{molecules/nm}^3}{5\ \text{nm}} \approx 2{\times}10^7\ \text{nm}^{-2}\ \text{s}^{-1}, \tag{3.14}$$

  where we have used a diffusion constant of $D = 2{\times}10^9\ \text{nm}^2/\text{s}$ appropriate for an ion. Given the cross-sectional area of a typical channel $A_{\text{channel}} = d^2\pi/4 \approx 0.2\ \text{nm}^2$, the number of ions traversing the membrane per second is estimated to be

  $$\frac{dN_{\text{ion}}}{dt} = J_{\text{ion}}A_{\text{channel}} \approx 2 \times 10^7\ \text{nm}^{-2}\text{s}^{-1} \times 0.2\ \text{nm}^2 = 4 \times 10^6\ \text{s}^{-1}. \tag{3.15}$$

  This estimate does remarkably well at giving a sense of the time scales associated with ion transport across ion channels.

Enzymes, molecular motors and ion channels (and pumps) are all ways in which the cell uses proteins to circumvent the intrinsic rates of different physical or chemical processes.

3.4.3 Beating the Replication Limit

The most fundamental process of cellular life is to form two new cells. A minimal requirement for this to take place is that an individual cell must duplicate its genetic information. Replication of the genetic material proceeds through the

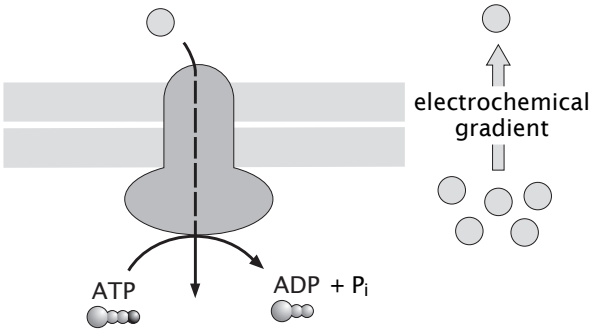

Figure 3.27: Active transport across membranes. Molecular pumps consume energy in the form of ATP hydrolysis and use the liberated free energy to pump molecules against their concentration gradient.

action of DNA polymerase, an enyzme which copies the DNA sequence information from one DNA strand into a complementary strand. Like all biochemical reactions, this requires a certain amount of time which we estimated earlier in the chapter. Why should any cell accept this speed limit on its primary directive of replication? When we calculated the replication time for the *E. coli* chromosome, we concluded that two replication forks operating at top speed would be sufficient to replicate the chromosome in approximately the 3000 second division time that we stipulated for a bacterium growing in a minimally defined medium with glucose as the sole carbon source under a continuous supply of oxygen. While this set of conditions is in many ways convenient for the human experimentalist, it is by no means ideal for the bacterium. If instead of only supplying glucose we add a rich soup of amino acids, *E. coli* will grow much faster with a doubling time of order twenty minutes (1200 seconds).

How can the bacterium double more quickly than its chromosome can replicate? For *E. coli* and other fast growing bacteria, the answer is a simple and elegant manipulation of the procedural time limit imposed by the DNA replication apparatus. The bacterium begins replicating its chromosome a second time before the first replication is complete. In a rapidly growing *E. coli* there may be between four and eight copies of the chromosomal DNA close to the replication origin, even though there may be only one copy of the chromosome close to the replication terminus. In other words, the bacterium has started replicating its daughter's, grandaughter's or even great grandaughter's chromosome before its own replication is complete. The newborn *E. coli* cell is thus essentially already pregnant with partially replicated chromosomes preparing for the next one or two generations.

As we have noted above, the genome size for bacteria tends to be substantially smaller than the genome size for eukaryotic cells. Nonetheless, eukaryotic

cells are still capable of replicating at a remarkably fast rate. For example, early embryonic cells of the South African clawed frog *Xenopus laevis* can divide every 30 minutes. Despite the fact that its genome (3100MB) is more than six hundred-fold larger than the genome of *E. coli*, two mechanical changes enable rapid replication of the *Xenopus* genome (and the genome of other eukaryotes). First, the genome is subdivided into multiple linear chromosomes rather than a single circular chromosome. Second, and more importantly, replication is initiated simultaneously from many different origins sprinkled throughout the chromosome as opposed to the single origin of bacterial chromosomes. This parallel processing for the copying of genomic information enables the task to be completed more rapidly than would be dictated by the procedural time limit imposed by a single molecule of DNA polymerase.

3.4.4 Eggs and Spores: Planning for the Next Generation

We have been considering the processes of cell growth and division as though they are tightly coupled with one another. In some cases, however, organisms may separate the processes of growth and division so that they occur over different spans of time. The most dramatic example of this is in the growth of giant egg cells followed by extremely rapid division of early embryos after fertilization. For example, in the frog *Xenopus laevis*, each individual egg cell is enormous - up to 1mm across - and grows gradually within the body of the female over a period of three months. Following fertilization, cell division occurs without growth so that a tadpole hatches after 36 hours that has the same mass as the egg from which it is derived.

Even for organisms where cell growth normally is coupled to cell division, there are several mechanisms whereby cells may choose to postpone either growth or division if conditions are unfavorable. For example, many cells ranging from bacteria through fungi to protozoans such as *Dictyostelium* are capable of creating spores. Spores are nearly metabolically inert and serve as a storage form for the genomic information of the species that can survive periods of drought or low nutrient availability. This is a mechanism by which an organism can effectively exist in suspended animation waiting for however much time is needed to pass until conditions become favorable again. When fortune finally favors the spore, it can germinate releasing a rapidly growing cell. The maximum survival time of spores is unknown. However, there are *Bacillus* spores that were put into storage by Louis Pasteur in the late 1800s that appear to be fully viable today and it is generally accepted that some spores may be able to survive for thousands of years. Some controversial reports even suggest that viable bacterial spores can be recovered from bodies trapped in amber over at least a few million years. The seeds of flowering plants perform a similar role, though they are typically not as hearty as spores.

Although animals do not form true spores, several do have forms that permit long term survival under starvation conditions. The most familiar examples are hibernation of large animals such as bears which can survive an entire winter season without eating. Smaller animals perform similar tricks. These include dauer

(German for "enduring") form larvae of several worms. The most impressive example of "suspended animation" among animals is presented by the tardigrade or water bear, a particularly adorable segmented metazoan that rarely grows larger than 1mm. The tardigrade normally lives in water, but when it is dried out it, it slows its metabolism and alters its body shape, extruding almost all of its water, to form a dried out form called a tun. Tardigrade tuns can be scattered by wind and can survive extreme highs and lows of temperature and pressure. When the tun falls into a favorable environment like a pond, the animal will reanimate. Each of these examples shows how organisms have evolved mechanisms that are completely indifferent to the absolute passage of time.

3.5 Summary and Conclusions

Because life processes are associated with constant change, it is important to understand how long these processes take. In the case of the diurnal clock, organisms are able to measure the passage of time with great regularity to determine their daily behaviors. For most other kinds of biological processes, times are not absolute. In this chapter, we explored several different views of time in biological systems starting with the straightforward assignment of procedural time as measured by the amount of time it takes to complete some process. In complex biological systems, processes that occur at intrinsically different rates may be linked together such that one must be completed before another can begin. Examples of this kind of measurement of relative time are found in the regulation of the cell cycle, the assembly of complex structures such as the bacterial flagellum, etc. Finally, we briefly explored some of the ways that organisms manipulate biological processes to proceed faster or slower than the normal intrinsic rates. Armed with these varying views of time, we will use time as a dimension in our estimates and modeling throughout the remainder of the book. Time will particularly take center stage in Part III, Life in Motion, where dynamic processes will be revealed in all their glory.

3.6 Problems

1. **Protein synthesis and degradation in the *E. coli* cell cycle.**

   Improve the estimates for the protein synthesis rates of *E. coli* during a cell cycle from sections 3.1.3 (pg. 131) and 3.2.1 (pg. 136) by including the effect of protein degradation. For simplicity, assume that all proteins are degraded at the same rate with a half life of 60 minutes and work out the number of ribosomes needed to produce the protein content of a new bacterium given that part of the synthesis goes to replacing degraded proteins. Compare to the results from the estimate in section 2.1.2.

2. **DNA replication rates**

Assuming that fig. 3.28 (pg. 171) is a representative sample of the replication process:
(a) Estimate the fraction of the total fly DNA shown in the micrograph. Note that the fly DNA is about 1.8×10^8 nucleotide pairs in size.
(b) Estimate the number of DNA polymerase molecules in a eukaryotic cell like this one from the fly *D. melanogaster*.
(c) There are eight forks in the micrograph. Estimate the lengths of the DNA strands between replication forks 4 and 5, counting up from the bottom of the figure. If a replication fork moves at a speed of 100 bp/s, how long will it take for forks 4 and 5 to collide.
(d) Given the mean spacing of the bubbles, estimate how long it will take to replicate the entire fly genome.

3. RNA polymerase and ribosomes.

(a) If RNA polymerase subunits β and β' together constitute approximately 0.5% of the total mass of protein in an *E. coli* cell, how many RNA polymerase molecules are there per cell assuming each β and β' subunit within the cell is found in a complete RNA polymerase molecule? The subunits have a mass of 150 kDa each. (Adapted from problem 4.1 of Schleif (1993)).
(b) Rifampin is an antibiotic used to treat *Mycobacterium* infections such as tuberculosis. It inhibits the initiation of transcription, but not the elongation of RNA transcripts. The time evolution of an *E. coli* rRNA operon after addition of rifampin is shown in fig. 3.29(A-C). Use the figure to estimate the rate of transcript elongation. Use the beginning of the "Christmas-tree" morphology on the left of fig. 3.29(A) as the start point for transcription.
(c) Using the calculated elongation rate estimate the frequency of initiation off of the rRNA operon. These genes are amongst the most transcribed in *E. coli*.
(d) As we saw in the chapter, a typical *E. coli* cell with a division time of 3000 s contains roughly 20,000 ribosomes. Assuming there is no ribosome degradation, how many RNA polymerase molecules must be synthesizing rRNA at any instant? What percentage of the RNA polymerase molecules in *E. coli* are involved in transcribing rRNA genes?

4. The sugar budget in minimal medium.

In rapidly dividing bacteria, the cell can divide in times as short as 1200 seconds. Make a careful estimate of the number of sugars (glucose) needed to provide the carbon for constructing the macromolecules of the cell during one cell cycle of a bacterium. Use this result to work out the number of carbon atoms that need to be taken into the cell each second to sustain this growth rate.

5. Metabolism of budding yeast.

(a) Make a careful estimate of the number of sugars (glucose) needed to provide the carbon for constructing the macromolecules in yeast during one cell cycle.
(b) In a famous demonstration experiment, yeast cells are grown in a tube that is attached to a balloon. As these cells go through their busy metabolic

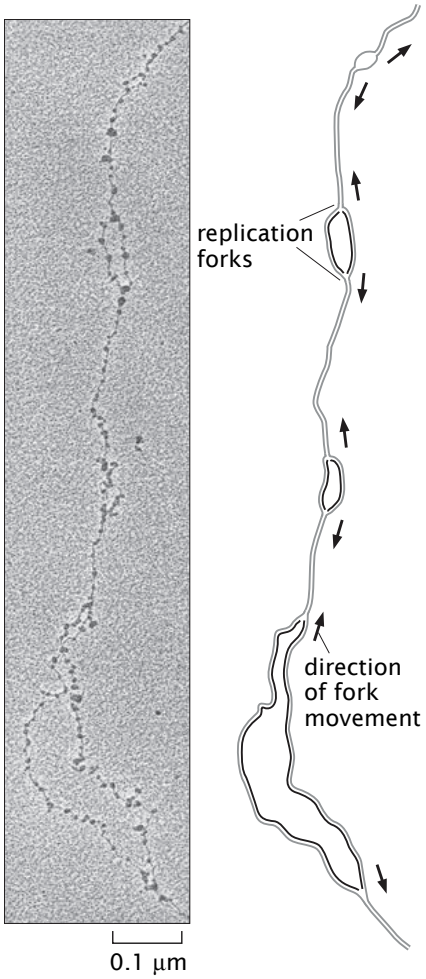

Figure 3.28: Replication forks in *D. melanogaster*. Replication forks move away in both directions from replication origins. (Adapted from B. Alberts *et al.*, Molecular Biology of the Cell, 4th ed. New York: Garland Science, 2002.)

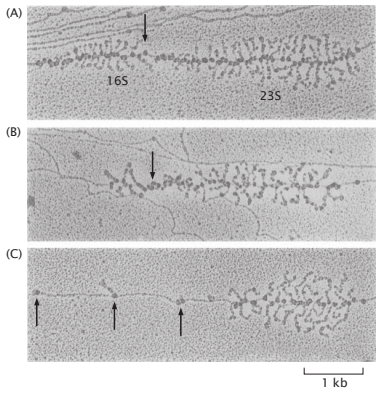

Figure 3.29: Effect of rifampin on transcription initiation. Electron micrographs of *E. coli* rRNA operons (A) before adding rifampin, (B) 40 s after addition of rifampin and (C) 70 s after exposure. No new transcripts have been initiated, but those already initiated are carrying on elongation. RNA polymerase molecules that have not been affected by the antibiotic are marked by the arrows. (Adapted from L. S. Gotta *et al.*, *J. Bacteriol.*, 20:6647, 1991.)

paces, they produce gases that inflate the balloon. A 5 mL culture of yeast at a density of **HG** grown in minimal media containing glucose as the carbon source will yield a rate of volume increase of the balloon of **HG/RP**. Assuming that **HG** CO_2 molecules get produced per glucose molecule, estimate the metabolic output on a per cell basis.

6. Metabolic rates.

Assume that 1 kg of bacteria burn oxygen at a rate of 0.02 mole/s. This oxygen gets into the bacterium by diffusion through its surface at a rate given by $\Phi = 4\pi DRc_0$, where $D = 2\ \mu\text{m}^2/\text{ms}$ is the diffusion constant for oxygen in water, $c_0 = 0.2\ \text{mole}/\text{m}^3$ is the oxygen concentration and R is the radius of the bacteria, which we assume to be spherical.
(a) Show that the amount of oxygen that diffuses into the bacterium is greater than the amount used by the bacterium in metabolism. For simplicity, assume that the bacterium is a sphere.
(b) What conditions does (a) impose on the radius R for the bacterial cell? Compare it to the size of *E. coli*.

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D. O. Morgan, **The Cell Cycle**, New Science Press Ltd, London: England, 2007. Morgan's book is full of interesting insights into the cell cycle.

E. Bier, **The Coiled Spring: How Life Begins**, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000. This is the best introduction to developmental biology that we are aware of. This book is a wonderful example of the seductive powers of development.

S. B. Carroll, **Endless Forms Most Beautiful**, W.W. Norton and Company, New York, New York, 2005. One of us (RP) read this book twice in the first few weeks after it hit the shelves. From the perspective of the present chapter, this book illustrates the connection between developmental and evolutionary time scales.

F. Neidhardt, "Bacterial Growth: Constant Obsession with dN/dt", J. Bacteriol., **181**, 7405 (1999). Bacterial growth curves are one of the simplest and most enlightening tools for peering into the inner workings of cells. Neidhardt's ode to growth curves is both entertaining and educational.

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