1 History and Context

1.1 From Cells to Their Nuclei

Until the last decades of the previous century, the history of the cell cycle is nearly the same as the history of the chromosome theory of heredity. And before chromosomes, we have to go back to when cells were first seen and then recognized as the unit of life. By the early seventeenth century, advances in lens grinding in the Netherlands allowed several individuals in Europe to experiment with and construct microscopes. Robert Hooke first used the word "cell" to describe what he saw when looking with his microscope at plant tissue. School children today have seen Hooke's famous drawings of cork sections. The drawings resemble a honeycomb, and they first appeared in Hooke's *Micrographia* in 1665. Hooke did not recognize that what he saw were the skeletal remains of all life's basic units. That realization did not happen until much later. In the decades after Hooke, numerous microscopists made a series of observations of cells. In 1719, looking at red cells from fish (which do not lose their nuclei as ours do), Antonie van Leeuwenhoek probably made the first drawings of the nucleus (1). The term "nucleus" was introduced in 1831 by Robert Brown (also of "Brownian" motion fame) when he used it to describe cells from orchids (1).

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The discovery of the nucleus was highly significant. Not only because the nucleus contains the chromosomes (which had not been discovered at the time), but also because it is a visible cellular landmark. A marker that scientists can look for and monitor. Cellular landmarks, morphological or molecular, have driven and continue to drive cell cycle research. But just because a landmark is there does not necessarily mean that it may also make certain things happen. An example is the nucleolus and its role in the generation of new cells. Rudolf Wagner clearly described the nucleolus within the nucleus in 1835, which he assumed to be the "germinative spot" for cell formation. The nucleolus has important roles, but not in "seeding" new cells.

1.1.1 The Cell Theory

Theodor Schwann and Matthias Schleiden are usually credited with the formulation of key tenets of the cell theory. That the cell is the fundamental unit of life, and that all plants and animals are made of cells, each cell having a nucleus and a nucleolus (2). But it was not clear to them how new cells were made. They favored the idea that intracellular or extracellular matter crystallizes somehow into new cells (1, 2). Schleiden thought that new nuclei form without any relationship to preexisting ones. In essence, the views of Schwann and Schleiden on how new cells are made fell squarely into the realm of the spontaneous generation of life from nonliving matter, which was a popular theory at the time. Until Louis Pasteur put an end to spontaneous generation with his unambiguous *col de cygnet* (swan neck flask) experiment in 1859.

Explicit descriptions of cell division and the concept of new cells arising from preexisting ones came from Robert Remak (1, 2). Remak reached these conclusions from observations in multiple contexts, from red blood cells of

chicken embryos to frog eggs immediately after fertilization (1). The splitting of eggs after fertilization had been observed by others before. But Remak's histological manipulations allowed him to visualize the membrane of the egg, and follow the origin of the embryonic cells from the fertilized egg. The continuity of all the cells in the embryo from one fertilized ancestor was now a settled issue. This placed cell division and the cell cycle as the basis of how multicellular organisms develop. Remak's data fit nicely with Pasteur's that there is no spontaneous generation of life. The notion that new cells arise only from cell division was opposed by many, including Rudolf Virchow. But Virchow changed his tune later. Virchow's famous aphorism *omnis cellula e cellula* (all cells arise only from preexisting cells) encapsulated succinctly and popularized a key part of the cell theory, a pillar of modern biology. Together with the other pillar of modern biology, Darwin's theory of evolution, we arrive at a stunning and profound conclusion: Starting with a single cell, all life that ever was, is, and will be on this planet results from cell division. Spend a moment to reflect on this. If you had any doubt that the topics we will discuss in this book are important, now is the time to put those doubts to rest.

1.1.2 Mitosis

By the mid-nineteenth century, the cell theory was established, and microscopy was getting better. Several individuals (including Remak) had documented distinct stages of nuclear division, including nuclear elongation in some cases, and nuclear dissolution in others. Wilhelm Hofmeister noticed that the nuclei of plant cells dissolved, but some nuclear material remained, in what he thought were coagulates, which then segregated into the nuclei of daughter cells. Although Hofmeister could not realize the biological role of what he was seeing, his descriptions

were remarkably close to the stages of mitosis we recognize today (1). Scientists kept looking and looking under the microscope. They also processed and stained the cells with various techniques and dyes -all searching for crisp landmarks. Walther Flemming experimented with basic dyes and fixatives. To this day, pathologists use similar methods to look at cells in tissues. Flemming found that a nuclear substance, which was presumably acidic and negatively charged, stained very strongly with basic, positively charged dyes. He used the term chromatin (from the Greek "colored") to describe that substance. Flemming used salamander cells, because they were big and had big nuclei. As always, choosing the right experimental system for a research objective can reap enormous rewards.

Imagine you have a simple microscope, and you have figured out how to stain cells, with some nuclear substance being intensely stained. Assuming that it takes several hours to days for typical proliferating animal cells to divide, what would you expect to see? For the most part, not much. Each cell would be very similar to others and to itself, from its birth until it divides. Shortly before division, Flemming noticed that the colored nuclear substance was organized into threadlike structures (the Greek word for a thread is μίτος/mitos), which were then distributed into the daughter cells, in a process he called mitosis (Figure 1.1).

The nuclear threads/filaments were named chromosomes a few years later by Waldeyer. Unlike others that had also seen thread-like structures forming and segregating, Flemming was the first to discover their splitting during mitosis lengthways (1). Flemming imaged and documented the complete series of events during mitosis, a fundamental cellular process, in all its glory: first, chromosomes appear, becoming denser and more compact over time, while at the same time the nuclear membrane disappears in most animal and plant cells (prophase); second, the compact chromosomes reach an equilibrium position at an

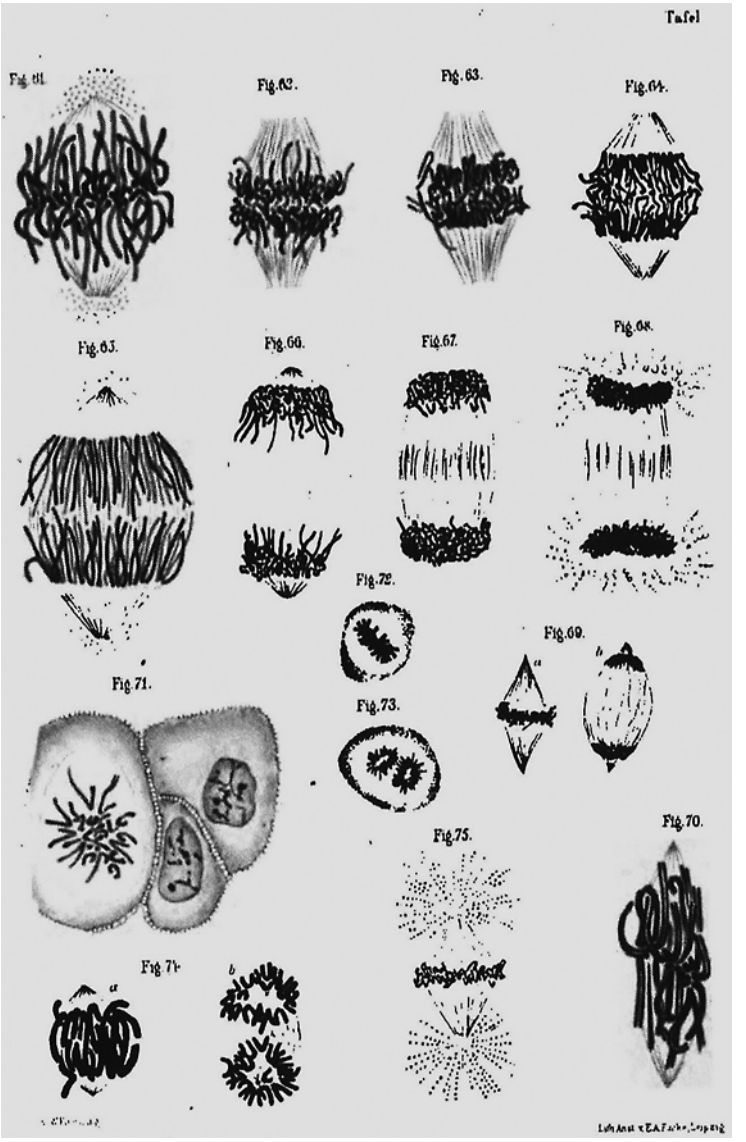


Figure 1.1 Illustration of mitotic cells.

Source: (3) Walther Flemming (1882), F.C.W. Vogel.

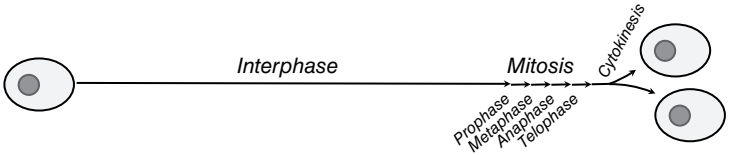


Figure 1.2 The duration of the interphase is much longer than the mitotic stages in the cell cycle. In most cells, interphase is devoid of any morphological landmarks.

"equator" position (metaphase); third, the chromosomes split lengthways and move toward the opposite poles of the cell (anaphase); fourth, the chromosomes arrive to the poles and the daughter nuclei appear, with reconstituted nuclear membrane around them (telophase).

Now we can divide the cell cycle into two phases. The relatively short phase of mitosis ("M" phase) starts when threads appear in the nucleus and ends when two nuclei appear (Figure 1.2). Mitosis is typically followed very quickly with cytokinesis when the cell's cytoplasm segregates to two daughter cells, each with a nucleus. The rest of the cell cycle, which in most cells lasts a lot longer than the M phase, is called the interphase (Figure 1.2). During interphase nothing much appears to be happening. The dramatic visual landmarks of mitosis are hard to miss and continue to guide research about when and how the nucleus divides. But it would be many decades later, in the middle of the twentieth century, when interphase landmarks were discovered. Until then, scientists followed what they had, the mysterious chromosomes.

1.1.3 The Chromosome Theory of Heredity

When the chromosomes were first seen, their role was unknown. That the chromosomes carry the genetic information would not be clearly formulated until 1903 from Walter Sutton, and at about the same time from

Theodor Boveri. The chromosome theory of heredity would be proven beyond doubt in the first decades of the twentieth century. Mendel's laws, which by 1900 were rediscovered, were abstract and could apply to any substance that carried the genetic information. As we will discuss later, the chromosomes do behave according to Mendel's laws. But what was the evidence that led to the idea that chromosomes carry the genetic information?

It was becoming more apparent that the nucleus, and not the cytoplasm, carried the genetic information. The unambiguous pieces of evidence for the role of the nucleus in carrying the genetic information came from fertilization experiments. Several individuals had already shown that the male and female parents make equal genetic contributions to the zygote, even though the egg is usually much bigger than the sperm. But these observations do not necessarily exclude cytoplasmic contributions to heredity. Until Eduard Strasburger showed in 1884 that during orchid fertilization, it is only the nucleus and not the cytoplasm (he coined the term) that is forced out at the end of the pollen tube into the embryo sac (1). Suppose both parents make equal genetic contributions to the zygote, but only the male parent's nucleus makes it into the embryo. In that case, the nucleus must carry the genetic information.

What nuclear component could be the carrier of genetic information? There was nothing better to look at than the chromosomes. If chromosomes carry the genetic information, then their continuity from generation to generation must be preserved. Again, the choice of the experimental system was critical. If you want to follow chromosomes, pick a system where you can track them easily. Edouard van Beneden chose *Ascaris* (a genus of roundworms) with only four (and sometimes just two), large, and easily distinguishable chromosomes, where he could follow the male and female-derived chromosomes in the zygote after fertilization. In 1883 he stated that each

parent's chromosomes never mix and maintain their identity in the zygote and possibly in all the nuclei derived from it as the embryo develops (1). Boveri used the same system, *Ascaris univalens*, and extended van Beneden's observations to show that the number, morphology, and identity of each chromosome is maintained, division after division. Although in interphase the chromosomes are not visible, they are not destroyed. William Sutton made similar observations by looking at grasshopper cells, noting that each of their 11 pairs of chromosomes was morphologically distinct (4). In diploid organisms, two sets of chromosomes are brought together at fertilization, and they are inherited by all the cells of the new individual. It is only in the germinal cells through meiosis that the haploid state is generated again from the diploid state (1). Both Sutton and Boveri noted that their data about the constancy of chromosomes fit very nicely with Mendel's rules of inheritance: Genetic traits are stable from generation to generation, come in pairs (in diploid organisms), with each pair separating in gametes (during meiosis), but each pair segregating in gametes independently of how the other pairs segregate (because different chromosome pairs orient at random during division). All the evidence pointed to the direction that chromosomes and their behavior constitute the physical basis of Mendel's laws. Still, however, the evidence was mostly indirect. What was needed to unambiguously test the Sutton–Boveri chromosome theory was evidence that specific genetic traits localize to specific chromosomes.

Looking at a random gathering of people, say in a classroom, there are many traits one could identify. However, most show a broad distribution among individuals (e.g., height, weight, coloration of hair or skin, etc.), which could complicate their study. But one trait is unmistakably distributed in a precise pattern: a one-to-one male-to-female ratio. Could gender be linked to a particular chromosome? Nettie Stevens answered precisely that, looking at cells

from *Tenebrio* beetles, which have a small Y chromosome that is easily distinguished from the larger X. Stevens saw that in *Tenebrio* (and as we know now in people too) the female was XX and the male XY (5). Somehow, Stevens' observation did not receive the attention it deserved. Thomas Hunt Morgan and his students developed *Drosophila* as a genetic system. They showed that specific mutant phenotypes are always associated with the transmission of particular chromosomes (6). Those triumphant and elegant *Drosophila* experiments have a special place in the history of genetics. They "sealed the deal" on the chromosome theory of heredity, and some are still standard laboratory exercises in undergraduate genetics courses.

Our brief overview of the cell and chromosome theories dealt with normal cells and organisms. The role of cell, nuclear, and chromosome division in disease was proposed early on, most notably from Theodor Boveri. In an astonishingly prophetic monograph published in 1914, accessible again in English (7), he described aberrant cell divisions associated with cancer, often leading to abnormal chromosomes in cancer cells. Many of Boveri's ideas were validated decades later, and they are now standard in cancer cell biology. Based on the evidence he had, Boveri proposed that "*The cells of even the most malignant tumors can be formed from normal tissue cells. The determinants of this abnormal behavior are to be found in the tumor cells themselves, not in their surroundings*." Indeed, every cell from a preexisting cell.

1.1.4 Deoxyribonucleic Acid (DNA)

With the chromosome theory in place, the frontier now shifted. To what chromosomes are made of and what exactly is the genetic material? To answer, it was biochemistry, not genetics, that was needed. In 1871, Friedrich Miescher was able to fractionate and purify a viscous,

phosphorus-rich nuclear extract he called "nuclein" (now known as a nucleic acid). As early as 1884, Oscar Hertwig proposed that nuclein carries the genetic information (1), but this was speculation, not proof. Once chromosomes were established to have genetic information, scientists purified and studied their chemistry. It turns out that in addition to nucleic acid (mostly DNA and some RNA), chromosomes have a lot of protein. The precise ratios vary among different cell types, but DNA is rarely even the majority in chromosomes. Generally, eukaryotic chromosomes are 30–50% DNA, 1–10% RNA, and 50–65% proteins (histones and nonhistones). Furthermore, looking at each of these fractions' composition, nucleic acids were found to be the least chemically diverse group compared to proteins. The genetic material must account for the incredible diversity of life. It is only natural that nearly all scientists until the middle of the twentieth century thought that biological diversity should be matched by a chemical one. Hence, they placed their bets on chromosomal proteins being the physical basis of heredity, not nucleic acids.

To do biochemistry, you have to work with nonliving matter, to fractionate and purify it. But cells are the unit of life and they are only generated through cell division. One cannot make a cell in a test tube from inanimate matter. So how can you do biochemistry to show that something is the genetic material? The door opened with Griffith in 1928, and his "transforming principle" experiment (8). He found that a substance from nonliving cells could be transferred to and change the traits of living cells. It is the genetic material (the genotype) that specifies a range of possible traits (the phenotype). Griffith's experiment showed that the genetic material need not be living itself. Oswald Avery and colleagues realized that they could separate the transforming but inanimate matter in different fractions, and repeat Griffith's experiment using the individual, purified fractions. The DNA fraction was necessary and

sufficient to change the phenotype (9). The experiment was simple, lucid, and of the utmost significance. It defined the physical basis of heredity. Yet, it did not receive the universal attention it deserved (including by the Nobel Prize committee). Some scientists did notice, including Maurice Wilkins, who was convinced to continue his work on DNA using X-ray crystallography. Wilkins would eventually share the Nobel Prize with Crick and Watson for determining the structure of DNA. Probably in one of the most famous sentences in the scientific literature, Watson and Crick noted at the end of their paper (10): "*it has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material*." What followed is history and well-covered elsewhere. What concerns us at this point is the pattern of DNA synthesis in the cell cycle. As it turns out, DNA synthesis is a key landmark in the cycle.

Various methods can monitor the synthesis of macromolecules in the cell. For example, feeding the cells with a labeled precursor and then following its incorporation in a macromolecule. The task becomes easy when the labeled precursor is uniquely incorporated into the macromolecule of interest. This is the case for tritiated thymidine in DNA. Around the same time as the Watson and Crick paper, these kinds of experiments showed that in animal and plant cells, DNA was synthesized during a fraction of the interphase and that it led to a precise doubling of DNA. The period of DNA synthesis is called the S phase. The interphase portions ("gaps") before and after S phase are termed G1, and G2, respectively (11). G1, S, and G2 are followed by mitosis, M. So now we have another landmark in the cell cycle. Unlike the morphological landmark of mitosis, however, the S phase is a molecular one. In most cases, one cannot tell if a cell is in the S phase by just looking at it. Nonetheless, with the proper molecular techniques, the start and finish of the S phase are

easily defined. Defining where G2 ends and M starts is not as straightforward. The appearance of compacted chromosomes defining the start of the M phase is a morphological landmark, open to different standards and techniques to visualize the chromosomes.

1.1.5 Cell Cycles Come in Many Flavors

The G1 → S → G2 → M description of the cell cycle phases is universally accepted and used. As we will discuss in detail later, each phase's duration in absolute terms and relative to each other can vary over a broad range across different cell types and for the same cells proliferating under different conditions. The canonical G1 → S → G2 → M scheme applies to most human somatic cells, with the cell cycle ending with cytokinesis (Figure 1.3). However, the canonical scheme is far from universal.

In the very rapid cell cycles of the embryo after fertilization, G1 and G2 may be absent. These cycles start from a very large fertilized egg, and the only tasks are to

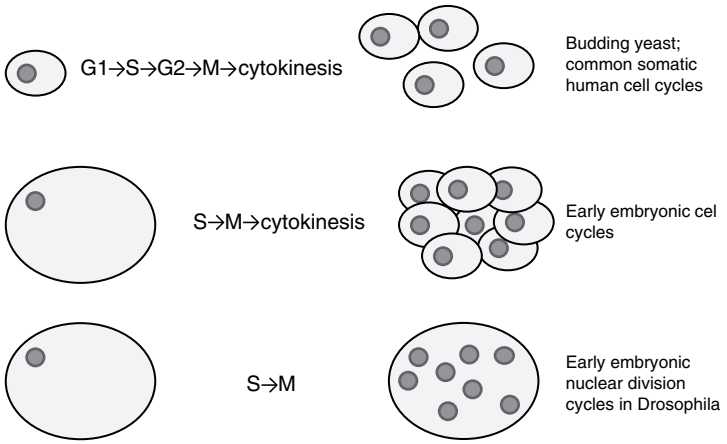


Figure 1.3 Embryonic cell cycles are usually devoid of gap phases.

duplicate and segregate the genome rapidly. There is no need to duplicate the cytoplasm because the egg is large enough to provide the necessary cytoplasm to as many as thousands of daughter cells. For example, embryonic *Xenopus* cell cycles starting from the huge fertilized egg are successive S → M → cytokinesis cycles (each lasting about 30 minutes), with a proportional reduction in cell size with every division. In *Drosophila* embryos, the scheme is pushed even further to a simple S → M (each lasting only eight minutes), with no time for cytokinesis (Figure 1.3). After about 13 such embryonic cycles, the result is a large cell with the same size as the fertilized egg, but with thousands of nuclei. At that point, the rapid divisions stop, and a cell membrane forms around each nucleus. Until then, the *Drosophila* embryonic cycles are technically *nuclear* division cycles, not *cellular* ones. After these embryonic divisions, subsequent cell cycles are slower and follow the canonical (i.e., G1 → S → G2 → M) scheme.

In most cases, S and M phases are temporally separated, with genome duplication (S) completed before its segregation (M). However, this temporal spacing may not hold in some rapid embryonic cycles, with a new S phase starting before the previous M phase is over. The timing of cytokinesis also varies among different cell types and organisms. If cytokinesis is fast, as it is in human cells, it usually happens quickly after telophase in the M phase. In fission yeast, cytokinesis is slow. It starts after M, but in rich nutrients, fission yeast enters the next S phase soon after M is completed (therefore lacking a G1 phase). As a result, cytokinesis ends even after S is completed, in the G2 before the next M. Why is this happening? It seems strange until one realizes that fission yeast spends most of its life cycle in the haploid state, with only one copy of each of its chromosomes. In rich nutrients, fission yeast has minimized or eliminated G1. Still, it spends a considerable amount of time in G2 with a duplicated

genome, which affords more DNA repair possibilities in case of damage.

On the other hand, budding yeast prefers to proliferate in the diploid state, and its cell cycle shows a typical G1 phase. However, budding yeast has tiny chromosomes, challenging to see, and it does not have a clearly defined G2 phase. Despite these eccentricities, both yeasts have been and continue to be prime cell cycle model systems because of their unique experimental properties, which will become apparent in the next chapter when we discuss their cell growth pattern.

Regardless of the different ways the cell cycle phases are arranged in the above examples, the genome complement is maintained in all cases. It is accurately duplicated and segregated in subsequent generations. What happens if there is no M or S phase, or when they exist with a twist? We will try to answer these questions briefly before ending this chapter.

Several cell types go through successive S phases with no M phase, from insect larvae to our liver cells. These endoreduplication cycles increase the copy number of the genome, which likely enables overproduction of gene products. In the extreme case of salivary gland cells in *Drosophila* and other insects, ten rounds of endoreduplication yield cells with 1024 copies of each chromosome! Furthermore, these copies align with each other, in perfect register, making them easily visible. Such polytene chromosomes are an excellent cytogenetic resource, with clear, visible markers along each chromosome (Figure 1.4). In many cases, they have enabled fly researchers to localize mutations physically.

Sexually reproducing diploid organisms start from the zygote with two nearly identical copies of each chromosome, one from each parent. The vast majority of the progeny, in somatic cells, is generated by the canonical mitotic cell cycles we described. Each chromosome from

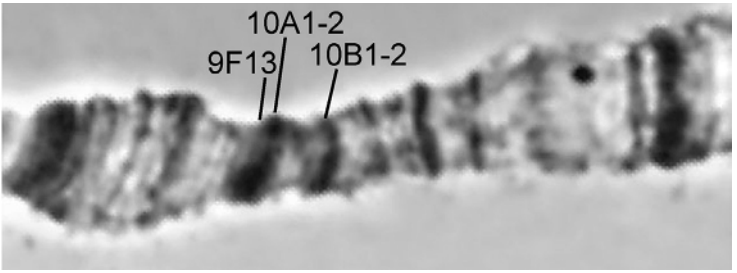


Figure 1.4 Polytene chromosomes from the salivary gland of a fruit fly larva. These chromosomes show many distinct, darkly staining bands (indicated by the labels in this image), providing clear physical chromosomal landmarks.

Source: (12) Vatolina et al. (2011), PUBLIC LIBRARY OF SCIENCE (PLOS), CC BY.

both parents is duplicated in the S phase and represented by two sister double-stranded DNA molecules, or chromatids, in the G2 phase. The sister chromatids of each of the parental chromosomes then segregate lengthways in the M phase. Hence, at the end of a mitotic cell cycle, each daughter cell receives an exact diploid set of chromosomes. But in the germline, how do these organisms generate their haploid gametes? The key difference is that in the first meiotic division, it is not the sister chromatids that segregate lengthways after DNA replication. Instead, the homologs from each parent align together in metaphase. They rarely do so in the mitotic cell cycle. In anaphase, the two identical sister chromatids of each chromosome do not separate, but the homologs from each parent do. Hence, although the products of the first meiotic division (a.k.a. reduction division) have the same genome content as the original cell, they do not carry two homologs from each parent anymore. Just a duplicated homolog from one parent only. Which parent contributes each homolog is random, depending on how the duplicated homologs oriented themselves in metaphase, accounting for Mendel's law of independent assortment of different traits. This first meiotic division is

beautiful and extremely important for sexually reproducing organisms like ourselves. The forces that align homologs but keep sister chromatids together are special and not seen in the typical mitotic cell cycles. The second meiotic division is just like a mitotic cell cycle, but without an S phase. Then, the sister chromatids separate in anaphase, giving rise to gametes with a haploid genome content and each unduplicated chromosome originating from one parent, but not both.

As spectacular and important meiosis is, we will not cover it in more detail. Likewise, we will not discuss some unusual and exotic eukaryotic cell cycles, for example, among protists. Lastly, there are several key differences in prokaryotic vs. eukaryotic cell cycles, perhaps most notably the initiation of multiple DNA replication rounds in one cell cycle. In rich nutrients, bacteria take advantage of the fact that they can double their cytoplasm faster than they can duplicate their genome. This is a short book, and we will not discuss much about bacterial cell cycles. Nonetheless, we will close with the observation that as more studies are reported, the differences in the mechanisms of cell division between prokaryotes and eukaryotes are getting less pronounced, not more.