PATHWAYS OF DISCOVERY

The Incredible Life and Times of Biological Cells

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One of the most important ideas in the history of biology is the cell theory, which proposes that all forms of life are composed of cells. Cells are the simplest units to exhibit the functions characteristic of life, and the field of cell biology has helped reveal how the immense variety of life-forms are organized and operate. In this essay, I trace the pathways that led to the ascent of the cell to its central role in biological understanding. Along the way, I describe discoveries that identified the cell as the fundamental structural and functional unit of life. I focus particularly on the mechanisms and controls of cell reproduction that ultimately allow growth, development, and evolution to occur. Finally, I will speculate about how future discoveries should provide further understanding of how cells function.

Finding Life's Pixel (*1*, *2*, *3*, *4*)

As is often the case in science, technology begets discovery. The invention of the microscope led to the discovery of the cell. Improvements in lenses in early 17th century Holland resulted in the construction of simple microscopes that were used to investigate insects and other small organisms. Using a compound microscope, Robert Hooke extended this work to examine sections of cork. He published drawings of what he saw in his 1665 book *Micrographia*, in which he clearly illustrated the walled cavities of what he termed “cells,” after the Latin *cella*, for small room or cubicle.

Within a few years, plant cells had been comprehensively characterized in two beautifully illustrated studies, one by Nehemiah Grew and one by Marcello Malpighi. Their work led to the view that plant tissues are mostly composed of aggregates of cells. A little later in the century, Malpighi, Anton van Leeuwenhoek, and Jan Swammerdam became the first scientists to recognize cells in animals. These microscopists described corpuscles in blood, but no one at this time proposed that solid animal tissues also were made up of cells. The oversight is understandable. Animal tissues were more difficult to preserve than plant tissues, and they presented a more fibrous appearance compared to the well-defined cellular geometry of plant tissues. Leeuwenhoek also discovered single-celled organisms, which he called animalcules, growing in extracts of plants. Improvements in microscopical observation led to better descriptions of cells, and in 1766 Abraham Trembley reported observing animalcules (the protozoan synhedra) reproducing; it probably was the first report of binary fission of a cell.

The gradual acceptance of the atomic nature of matter also helped in the development of the cell theory. The idea that all matter might be composed of indivisible subunits, or atoms, arose in the 5th century B.C. in Greece, but it took 2 millennia for atomism to become a serious scientific topic. By the 18th

century, it had become natural for biologists to think about fundamental subunits of living matter when interpreting the microscopic structures of plants and animals. A pivotal speculation was made by Lorenz Oken in 1805. He argued that multicellular plants and animals are assemblages of the tiny living “infusoria,” such as protozoa, that grow in animal and plant extracts. In succeeding years, microscopists generalized that idea by noting parallelisms between infusoria, on the one hand, and animal and plant cells, on the other.

These studies culminated in the cell theory. It was popularized by the botanist Matthias Schleiden and the zoologist Theodor Schwann, who in 1839 stated, “We have seen that all organisms are composed of essentially like parts, namely of cells.” In his 1858 book *Cellularpathologie*, Rudolf Virchow stated, “Every animal appears as a sum of vital units, each of which bears in itself the complete characteristics of life.” This discovery—that cells form the fundamental structural and functional units of all living organisms—was a landmark in the history of biology.

Cells Do It All: Heredity, Development, Disease, and Death (*1*, *4*)

Although Schleiden and Schwann correctly articulated the cell theory, they were confused about the formation of cells, thinking that they arose by processes akin to precipitation or crystallization. Others, particularly Barthelemy Dumortier working with plant cells and Robert Remak with animal cells, recognized that cells arose from preexisting cells by a process of binary fission. This view was well championed by Virchow, who popularized the phrase “*Omnis cellula e cellula*,” that is, all cells come from cells.

Research into cell reproduction began revealing the cell's wondrous complexity and machinelike features. At the heart of cellular reproduction is a system of heredity, which works by a molecular mechanism that ultimately explained Mendel's laws of inheritance. In the 1860s, Gregor Mendel had concluded that attributes of plants, such as seed shape and stem height, were determined by pairs of characters, one derived from the male plant and the other from the female. He thought these characters remained distinct in the hybrid plant and were passed on as discrete entities in further crosses. These conclusions became more scientifically acceptable only at the turn of the 20th century, when researchers found that the behavior of Mendel's characters mirrored the behavior of chromosomes at cell division.

Most cells contain a single nucleus that reproduces during mitosis and cell division. Elongated chromosomal threads, which Walther Flemming and Eduard Strasburger described in the 1880s, were observed to split lengthways before shortening and thickening as mitosis proceeds. The longitudinal halves then separate into the two daughter nuclei. About the same time as these observations were made, Edouard van Beneden showed that the chromosomes in a fertilized nematode egg are derived in

equal numbers from the egg and the sperm. That led August Weisman to propose that the heredity system is based on the chromosomes.*

Even as scientists were developing their first ideas about the mechanisms behind cell reproduction, they were discerning connections between such reproduction and the growth and development of organisms. Population growth of single-celled microbial organisms entails cell division, and by the late 19th century researchers understood that the growth of multicellular organisms is also based on cell division. In the 1860s, Rudolf Kölliker observed that the cleavage of early embryos reflects a series of cell divisions yielding cells that subsequently differentiate into specialized tissues. And by the 1880s, scientists had concluded that all multicellular organisms, regardless of their complexity, emerge from a single cell.

That cell reproduction is central to growth and development makes immediate sense. That the death of cells is also key for these processes is not so intuitive, yet data supporting this idea began accumulating during the last century. Pathologists had observed dying cells in certain healthy tissues, and the term "apoptosis" was coined in 1972 to describe the phenomenon (*5*). It was the discovery of the role cell death plays in the development of the nematode worm, however, that confirmed a positive function for apoptosis. The worm egg undergoes highly defined cell divisions that generate an adult comprised of a fixed total number of cells. Monitoring of cell lineages revealed that certain cells always undergo apoptosis at specific stages of development. Also, mutants were isolated in which apoptosis was suppressed; they ended up with more cells. These experiments led to the concept that there is a program of cell death. At specific developmental stages, the cell death pathway switches on in certain cells and a cascade of proteases is activated, leading to the lysis of these cells. In recent years, researchers have identified many genes underlying this built-in cell death program.

Cells that don't kill themselves for the greater good can go wrong in a thousand ways. In the mid-19th century, Remak and Virchow argued that the cells of diseased tissues are derived from normal tissues. The implication was that malfunctioning cells beget disease. This argument has been of great medical importance, because it focused attention on changes in cellular (and ultimately molecular) behavior as critical factors for understanding disease.

This strategy is well illustrated by the study of cancer. Early pathologists recognized that cancer arises in abnormally developing tissues in which cell reproduction has become uncontrolled. By the 1970s, researchers had shown that genetic alterations, such as chromosomal rearrangements and the presence of certain viral genes, are responsible for cells becoming cancerous (*6*). The identification in the 1970s of the *src* oncogene in the chicken Rous sarcoma virus, and later of its mammalian counterparts, was an important discovery. Many other oncogenes, which promote cell growth and division when activated, were subsequently discovered.

Tumor-suppressor genes, which normally restrain cell growth and division, also were identified. When these become inactivated in cells, those cells become cancerous. Other cancer-related genes participate in safety circuits, including ones that monitor gene damage and activate the cell death pathway if the genetic damage to individual cells is so great that it might induce cancer. The gene encoding p53, the most frequently mutated gene in human cancer, has become known as the "guardian of the genome," because it's required for proper surveillance of genomic damage. Cells lacking p53 survive even if they have suffered extensive gene damage (*7*). Cancer cells can also be defective in the cell death pathway; in these cells, apoptosis cannot be activated, so cells with genetic damage survive. If these surviving cells have damage to genes that control cell growth and division, they will selectively proliferate and dominate the tissue of origin. Eventually, they may undergo metastasis and spread to other parts of the body.

Round and Round She Goes: The Cell Cycle

Because cell reproduction is the basis for heredity, growth, and development, the cell needs to replicate and segregate all the genes so that the entire genome can properly pass on at each cell division. This is achieved by a series of events called the cell cycle.

The two major cell cycle events required for the replication and segregation of the genome are S-phase (for DNA synthesis) and M-phase (for mitosis) (*8*). During S-phase, the cell makes a copy of its chromosomal DNA. The process originally was identified by labeling studies and by making precise measurements of changes in cellular DNA content during the cell cycle (*9*, *10*). These experiments showed that DNA synthesis occurred only within a limited period early in the cycle. During M-phase, which occurs at the end of the cell cycle, the replicated chromosomes segregate into the two daughter cells formed at cell division. The periods between the birth of the cell and S-phase, and between the end of S-phase and M-phase, are respectively known as G_1 and G_2 (G stands for gap), leading to a four-phase cell cycle: G_1, S-phase, G_2, and M-phase.

There's an industrial park's worth of molecular machinery running the cell cycle. Consider DNA replication. Nothing revealed more about this process than the 1953 discovery of the structure of DNA (*11*). This revelation solved the twin problems of how DNA could be precisely replicated and how it could encode information. DNA's sequence of bases encodes the information; the unwinding of the two-stranded, base-paired structure yields a pair of single-stranded templates for the synthesis of exactly complementary strands.

With DNA's structure in hand, the search for the machinery behind cellular replication became easier. The discovery of an enzyme, DNA polymerase, which could synthesize a DNA strand complementary to a preexisting DNA template, provided the first key (*12*). Many other relevant enzymes were subsequently discovered, among them topoisomerases and helicases, which unwind DNA strands,

primases and DNA polymerases, which synthesize new DNA strands, and ligases, which link strands together (*13*). During a cell's S-phase, these enzymes assemble on specialized regions of DNA, called "origins of replication," from which replication of the DNA extends bidirectionally. The two trajectories of replication move apart and form "bubbles" of DNA, which ultimately fuse to complete the process.

Like any complex machinery, the replication machinery requires control. Before replication can begin, for example, initiating factors have to bind to the origins of replication. These factors include origin recognition complexes, which act as "landing pads" for initiator proteins. These, in turn, load other proteins required for DNA replication. Normally, this process is activated only once in each cell cycle so only one S-phase takes place. Otherwise, cells might copy their chromosomes too many times.

Proper DNA replication is the first major component of the cell cycle's reproductive machinery. The second operates during M-phase, mitosis, first described in the 19th century (*4*). Condensed replicated chromosomes, each consisting of two sister chromatids paired along their length, become visible at the beginning of mitosis. The chromosomes line up in the cell's middle and become associated with the mitotic spindle. The spindle extends between two structures, called centrosomes, located at opposite ends of the cell. The chromatids then move apart toward their respective sides of the cell, where they then segregate into two nuclei to be inherited by the newly forming daughter cells.

Central to this mechanism are tubulin polymers, which form the microtubules of the mitotic spindle. These microtubules exhibit dynamic instability—they undergo transitions between growth and shrinkage (*14*). The centrosomes stabilize the microtubules and thereby the spindle. Other growing microtubules emanating from the centrosomes "explore" the cell's interior until their ends become stabilized by association with the kinetochore, a structure at the centromere found on each chromatid. That process links each chromatid to a centrosome.

Stable linkage can occur only if the kinetochores on the respective sister chromatids are linked to the cell's two centrosomes. When this happens, each half of each paired chromatid becomes oriented toward opposite ends of the cell. Cohesion between sister chromatids subsequently is lost, allowing the chromatids to separate toward opposite centrosomes. The act of separation unfolds by the combined actions of microtubular shrinkage and motor proteins that impel the microtubules to slide over each other. This process links the replication of DNA at the molecular level to the separation of chromosomes at the cellular level and ensures the precise replication and segregation of the genome.

Checks and Balances

To work properly, this molecular machinery requires regulation. Our understanding of the cell cycle's control systems has emerged from two concepts. The first one, the idea of checkpoints, began with the

proposal that the cell cycle is a sequence of dependent steps, the initiation of later events occurring only after the successful completion of earlier ones (*8*, *15*). The notion of checkpoints was developed by studies with budding yeast (*16*). This work revealed how the cell "checks" at particular "points" whether early events in its cycle have been completed properly. If not, the cell sends signals that block later events. For example, an incomplete S-phase leads to the sending of a signal that prevents mitosis. This spares the cell from undergoing a potentially lethal mitosis with only partially replicated DNA.

Other checkpoint controls monitor for DNA damage and block S-phase or mitosis until repairs are made. Researchers have found that damaging DNA or blocking its replication in yeast cells prevents mitosis and cell division. They also have found mutant yeast cells that allow cell division to proceed despite such problems. It turns out that these mutants express genes that act in checkpoint pathways, which monitor DNA damage and replication (*7*). One example of such a gene in mammalian cells is *p53*.

The second major concept to deepen our understanding of cell cycle control is that of rate-limiting steps. This concept emerged from studies of fission yeast cells and frog oocytes. The discovery of yeast mutants that prematurely rush cells into mitosis led to a proposal that these mutants had altered versions of genes that regulate the timing of mitosis (*17*). Complementary work with frogs identified protein factors that hasten frog oocytes into M-phase, suggesting that these factors were also rate-limiting controls for cell cycle progression (*18*). The proteins encoded by the genes identified in yeast, and the protein factors identified in frogs, turned out to be the same—cyclin-dependent kinases, or CDKs (*19*).

CDKs act as the "cell cycle engine," driving cells through S-phase and mitosis (*7*). They are enzymes comprising a catalytic kinase subunit and a regulatory cyclin subunit (*20*). The task of CDKs is to phosphorylate, and thereby control, other proteins required for the onset of S-phase and mitosis. CDK activity is itself regulated by such factors as inhibitors and the availability of cyclins. As cells proceed through a cycle, relevant CDK activities take place; different activities promote the cell through G_1, initiate S-phase, and initiate mitosis. For cells to exit mitosis and enter the G_1 phase of the next cycle, CDK activity must drop. This happens when the cyclin subunit of CDK degrades.

Besides pacing the cell cycle, CDKs have a hand in checkpoint controls (*7*). For example, the signaling pathway that blocks mitosis if DNA is damaged or if replication is incomplete probably operates in many cell types by preventing an increase in CDK activity. Once these flaws are rectified, CDK activity increases and mitosis can follow. CDK activity has yet another regulatory role: preventing a further S-phase in G_2 cells until mitosis is finished. Probable targets for this control are the initiator proteins. Blocking them from loading the proteins required for DNA replication would prevent an S-phase.

Not all checkpoint controls monitor for DNA damage or replication flaws. Genome stability also requires faithful chromosome transmission at mitosis; a checkpoint for this process stops mitotic progression if the spindle is not properly assembled or if the chromosomes are not properly attached to the spindle (*7*). The likely role for this checkpoint control is to verify whether microtubules are securely attached to the kinetochores and, if not, to maintain the cohesion between sister chromatids so they cannot separate. This delays mitotic progression until all of the sister chromatids become both properly associated with the spindle and linked to the appropriate centrosomes.

This remarkable set of cell cycle and checkpoint controls helps ensure that cells proceed smoothly through their cycles. Without them, the genome could not be precisely replicated and segregated to provide each newly divided cell with a full complement of genes. The payoff is genomic stability.

Discovering Life's Chemistry (*2*, *21*, *22*, *23*, *24*)

Combining these characteristics of cell reproduction with Darwin's theory of evolution by natural selection yields a framework for defining life. This view, first articulated by Hermann Muller in the 1920s, argues that living things have properties allowing them to undergo natural selection and thus to evolve. First, living organisms must be able to reproduce. Second, they must have a heredity system that can pass on the information defining the properties of the organism. Third, the heredity system must involve some variability, which also can be passed on. This variability provides the biological differences on which natural selection operates.

These properties of organisms are also characteristics of cells. Cells reproduce; they possess a heredity system based on genes; and replication errors or DNA damage elicits genetic changes that will be inherited during cell division. These properties allow cells to evolve, and because cell reproduction is the basis for all biological reproduction, it follows that these same cellular properties are the basis of the evolution of all living organisms. That holds even for exotic life-forms that may harbor non-DNA-based heredity systems. Speculative possibilities include replicating clay particles as primitive life-forms and organisms in other solar systems (*25*).

Muller's definition of life is attractive, especially to geneticists. But it is not useful for explaining cellular functions, such as metabolism and growth. Making sense of these requires looking into the chemistry going on within cells.

During the second half of the 19th century, studies of fermentation showed that living cells promote specific chemical reactions. Humans had enjoyed the fermentation of crushed fruits and seeds for millennia, but it was Antoine Lavoisier who in the 18th century first recognized that fermentation was due to chemistry. Subsequent microscopic examination of ferments led Theodor Schwann and Charles Cagniard-Latour to propose in 1835 that the force driving fermentation was associated with a living

organism, the yeast microbe. Louis Pasteur developed this idea by arguing that different microbes carried out specific fermentations, yielding different chemical substances. By 1858 he had concluded that fermentation was a physiological act that gives rise to multiple chemical products necessary for the life of the cell.

The next major insight about biological chemistry was the discovery that enzymes are behind it all. In 1860 Moritz Traube speculated that substances promoting fermentation reactions reside within cells and that their action is analogous to the soluble ferments known to exist outside cells. Within 2 years, Pierre Berthelot had provided experimental support for this speculation. From macerated yeast, he obtained a soluble ferment, called invertase, which could chemically degrade sucrose. About 30 years earlier, Jöns Berzelius had proposed the concept of catalysis, presciently providing the theoretical background necessary to appreciate Berthelot's experiments. Berzelius postulated the existence of catalytic substances, which behave like heat in promoting chemical transformations. He even suggested that thousands of different catalytic processes occur in animals and plants.

The stage was set for the key advance made in 1897 by the Buchner brothers, Eduard and Hans. They showed that a filtrate of a yeast extract contained a catalytic substance (zymase), which could promote in vitro the chemical reactions characteristic of fermentation. This discovery became the cornerstone of biochemistry. It led to the view that enzymes are protein catalysts that generate the compounds inside cells. Metabolism and cell growth, therefore, eventually were reinterpreted in the context of chemical reactions orchestrated by catalytic enzymes whose properties are specified by their associated genes.

Cells have to be innovative to allow such a myriad of chemical reactions to unfold within them even though many different conditions—including differing pHs and ionic environments—are required for the different enzymes to work well. Furthermore, for the cell's more complex chemical syntheses and molecular actions, several enzymes have to work in tandem to carry out a sequence of activities. For this, cells have to maintain a range of distinct microenvironments within different cellular compartments. This is a central principle underlying cellular organization.

To achieve this organization, the cell first has to maintain the general conditions necessary for life's chemistry; it must be insulated from the local environment. The cell does this with its outer lipid membrane, which is equipped with pumps and transporters that manage the movement of molecules into and out of the cell. Internal compartmentalization is the key to maintaining different microenvironments, and there are a number of mechanisms that generate these. First, enzymes have charged and precisely shaped surfaces, which establish a local environment conducive to specific chemical reactions. At a higher level of organization, enzymes link together to carry out sequential reactions. An example is the process of metabolic channeling. The product from one enzymatic reaction in a metabolic pathway becomes the starting material for the next enzyme in the pathway.

Similarly complex processes can be carried out by molecular machines consisting of the appropriate enzymes held in place within scaffolds made of protein and RNA. Such arrangements can undergo reconfigurations that promote sequential reactions. Examples are ribosomes, which carry out protein syntheses, and the complex of enzymes underlying DNA replication. Organelles such as the Golgi apparatus, the mitochondrion, and the nucleus embody yet a higher level of spatial organization. These are lipid-bound compartments, often with pumps and transporters, which maintain different microenvironments within the cell.

Spatial organization is a major cellular strategy for managing chemistry. Another is temporal organization. Examples here are DNA replication and mitosis. Both involve chromosomes but at different times during the cell cycle. DNA replication requires the chromosomes to be decondensed to allow access for enzymes; but mitosis requires the chromosomes to be condensed to foster their movement. The cell's solution is to separate the two processes temporally: DNA replication occurs early in the cell cycle and mitosis occurs later.

For the past century, ever more of life's functions have been interpreted in terms of cellular chemistry. As Jacques Loeb argued in his 1912 essay collection, *The Mechanistic Conception of Life*, the cell is like a chemical machine.

Enforcing Discipline (*24*)

It takes regulation and coordination to run all of the chemistry going on inside cells. Analyses of enzyme and gene activity, as well as the rise of cybernetics during the mid-20th century, put a spotlight on the concept of feedback control as a strategy for such regulation. The general idea was that the output of a process has regulatory consequence on the process itself. The simplest examples are quantitative, in which products generated by a metabolic pathway or by gene activity feed back to an earlier stage of the process, thereby slowing it down or speeding it up. The idea of feedback can also be applied in quality-control contexts. Consider the proofreading mechanism associated with the enzyme complexes that replicate DNA (*13*). This complex corrects errors of base-pair insertion that occur during the syntheses.

Such regulatory activities are closely associated physically with the process being regulated. That's not so when there is a need to coordinate activities that unfold at different cellular locations or at different times in a cell's history. Here, regulation requires a communication system that extends over substantial distances and times. One strategy is to send molecular signals from the cell's outer membrane to the nucleus, a process that affects gene expression. Other signals emerging from cellular checkpoint controls, which register problems during S-phase, serve as “reminders” to delay M-phase later in the cell cycle.

This kind of higher level organization distinguishes the chemistry of life from that of nonliving systems. It also highlights two features of cells: the ability to assume spatial organization and the ability to process information through signaling networks. Direct molecular interactions can lead to low-level spatial organization, such as the construction of cellular machines like ribosomes. But much of the cell's larger spatial organization requires more. How this extended organization is achieved remains unknown. However, chemical physicists and theoretical biologists who study the spatiotemporal organization of complex chemical reaction systems, such as the Belousov-Zhabotinsky reaction, have been finding clues. The network of chemical reactions underlying this remarkable system leads to changes in the concentrations of intermediates that oscillate in time and that generate standing waves. This results in patterns of chemicals within the reaction vessel. A primary goal for biologists is to explain how chemical reactions bring about analogous organization on cellular scales.

One interesting approach is to focus on the generation of dynamic chemical structures that require energy input for their maintenance and that involve fluxes of molecular components through the structures. Changing the kinetics of these processes can lead to structures of different sizes and shapes, features that presumably could be regulated in response to cellular needs. These ideas are more than metaphors for thinking about cellular organization; scientists are applying them to help explain the generation of structure and behavior, such as mitotic spindles (*26*) and cell cycle regulation (*27*).

The other feature of cells pertinent to higher level organization is the processing of information through signaling networks. These are not always linear pathways; they can be complex, with a variety of inputs and outputs, with bifurcation and amplification steps, and with some components acting at more than one step in a pathway and with others acting in parallel. The overall significance of such complex networks for signaling behavior is yet to be fully appreciated. One intriguing issue is the potential for information to be carried within the dynamic aspects of signaling, as is seen in the Morse code, which encodes messages in temporal patterns. Another is the role of threshold levels, in which high-intensity and low-intensity signals each send different messages. Modelers of interconnected networks have shown that ordered signaling patterns can emerge from relatively simple wiring diagrams and rules of operation (*28*). Exciting discoveries are in store for those who investigate how signaling pathways operate in the real space and real time of the cell.

The cell is the simplest unit to exhibit life's functions. We now have both the molecular tools and the conceptual frameworks to undertake a concerted program to understand how cells operate. The genome projects will anchor that foundation by identifying all the genes required for a cell to function, yet researchers will still have to work out how the relevant gene products act and interact to generate cellular organization. Particularly worthwhile will be investigations into those physiochemical properties that allow order to be generated on the extended scales within the cell, and studying the ways in which complex information networks operate. There are many different ways in which all the genes in the cell could function in concert, but only certain regulatory and organizational states are likely to be effective and stable, and therefore compatible with life. Uncovering the rules that govern

these states and the reasons why they exist are worthy research goals. The coming years will be exciting ones during which new ideas and theories will help us fully understand cells and thereby life itself.