

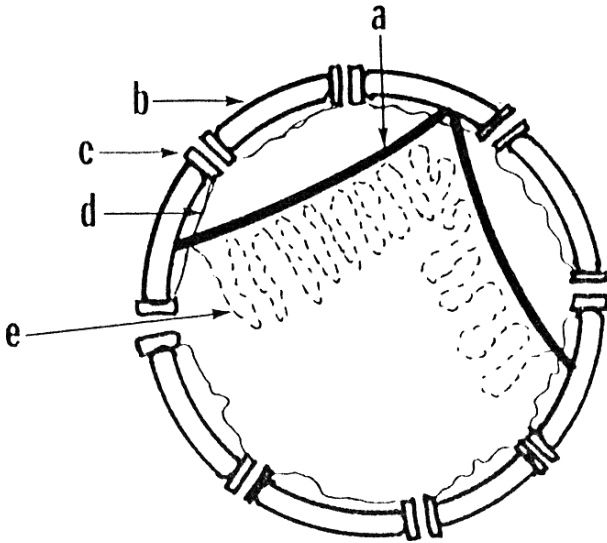
## Chapter 1

# HOW DO CELLS REGULATE THE POSITIONS AND AMOUNTS OF THEIR ORGANELLES?

All cells in the body are descended from undifferentiated cells of the early embryo called embryonic stem cells. These cells have a relatively simple, spherical shape and a balanced collection of all the organelles necessary to maintain a simple life. This is not to say that even the organelles of a simple cell are not impressive; indeed, they are enormously complex and highly organized. But a key to further differentiation of all cells is a reworking of the amounts and positions of organelles so that the cell can take on a specialized function. Each cell type has its own characteristic complement and ratio of organelles, in addition to its own shape and size of the nucleus. How is this accomplished? Only partial answers to this question are known at the moment.

### 1. Regulation of Nuclear Shape and Function

While the nuclei of all cells (except for germ cells) contain exactly the same number of chromosomes, the shapes of nuclei vary greatly between cells, and the functions of nuclear genes change tremendously, depending upon which cell type is being examined. For example, nuclei of muscle cells are characteristically oval-shaped and pale-staining (see Fig. 3.5), nuclei of neurons are large, round, and pale-staining and have prominent nucleoli (see Fig. 6.1), and nuclei of plasma cells are rounded and dark-staining (see Fig. 1.7). These distinctive nuclear characteristics are the most important features that allow cell biologists to distinguish between one cell type and another when looking through the microscope.



**Fig. 1.1.** A simplified diagram of a cell nucleus, showing (a) proteins of the nuclear matrix, (b) the outer nuclear membrane of the nuclear envelope, (c) a nuclear pore, (d) lamin proteins, and (e) chromatin.

What is responsible for this re-shaping and re-tasking of nuclei that occurs as cells differentiate? To answer this question, we must start with a review of the basic structure of the nucleus (Fig. 1.1).

The first structure we need to discuss is the nuclear envelope, which is peculiar because it consists of *two* unit membranes separated from each other by a space, the perinuclear cisterna. Most cellular organelles are bounded by only a single unit membrane; the one other exception to this rule is the mitochondrion.

The explanation for the two membranes of the mitochondrion is now well established: mitochondria arose in antiquity when a primitive cell engulfed bacteria, which survived to become organelles. The supporting evidence for this conclusion is pretty good:

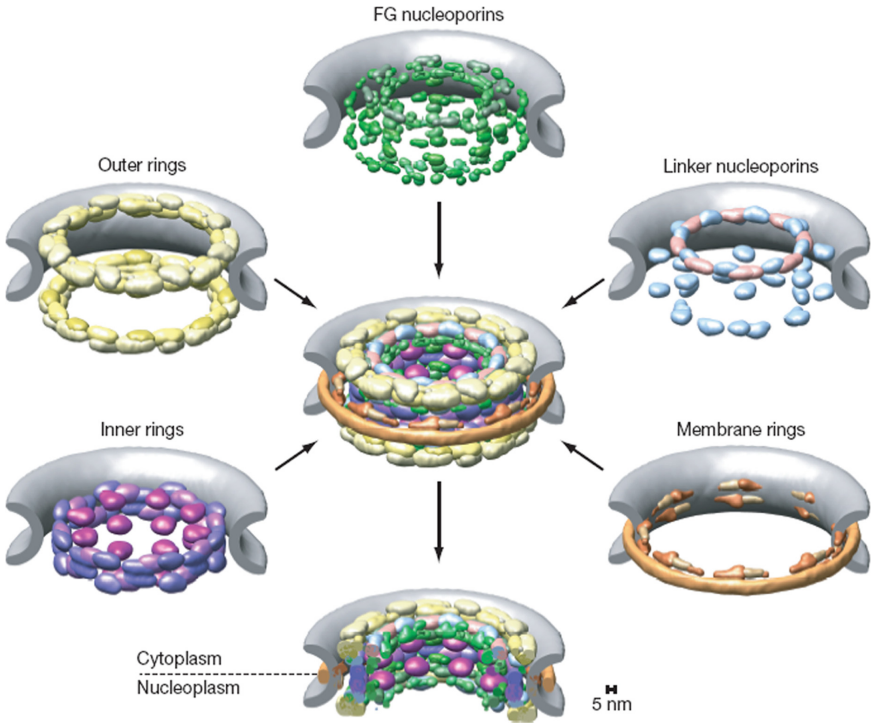
- mitochondria possess a small loop of DNA that resembles the DNA loops of bacteria, and which codes for 13 of the 80 mitochondrial proteins that regulate electron transport during ATP synthesis.

- to produce energy, mitochondria expel hydrogen ions from their interior to the intermembrane space, and then allow them to trickle back into the interior via pores (ATP synthase molecules) that use the energy in this flow of ions to generate ATP. Many bacteria have analogous hydrogen-ion export systems.<sup>3,16</sup>

If this explanation applies to double-membraned mitochondria, does it also apply to the nucleus? Some investigators have speculated that the nucleus arose when a swarm of eubacteria engulfed another ancient organism called an archaebacterium.<sup>20</sup> Thus, the outer nuclear membrane represents the cell membrane of the surrounding organism and the inner nuclear membrane represents the membrane of the ancient, engulfed archaebacterium. The acquisition of definitive evidence for or against this theory has proven to be more difficult than for the theory of the origin of mitochondria.

The next nuclear component to be considered is the nuclear pore that forms a channel from the cytoplasm into the nucleus (Fig. 1.2). Each nuclear pore is composed of about 30 different types of proteins called *nucleoporins*. Approximately 16 copies of all these proteins are assembled to form 16 columns that correspond to 8 large structures that are visible in routine electron micrographs.<sup>2</sup> The nucleoporins regulate traffic into or out of the nucleus. Messenger RNA molecules traversing the pores are attached to proteins that are recognized by pore proteins and which are guided outwards. All proteins found within the nucleus are originally synthesized within the cytoplasm; these proteins contain a string of basic amino acids that constitute a Nuclear Localization Signal, and which are also recognized by nucleoporins and guided into the nucleus.

Nuclear pores are interconnected by a network of proteins that are most abundant along the inside of the inner nuclear membrane. These proteins, termed *lamins*, belong to a class of filamentous proteins called *intermediate filaments* (they are intermediate in diameter between thinner *actin* filaments and thicker *myosin* filaments). *Intermediate filaments*, unlike other elements of the cytoskeleton, are *non-labile*, that is, once they are formed, they are not readily disassembled. In addition to *lamin intermediate filaments*, all cells contain



**Fig. 1.2.** Diagram of a nuclear pore, showing how the different types of nucleoporins are assembled to form the entire structure. Reproduced from Ref. 2, with permission.

cytoplasmic *intermediate filaments*. These cytoplasmic filaments form tough, strong fibers that interconnect with each other at cell junctions and thus form a network of cables strung throughout the cell that give cells structural strength. Epithelial cells have intermediate filaments formed of *keratin*, connective tissue cells possess intermediate filaments formed from a related protein, *vimentin*, and muscle cells have filaments formed from *desmin* (see Ch. 2). Neurons have intermediate filaments called neurofilaments, and astrocytes possess intermediate filaments formed from *glial fibrillary acidic protein* (see Ch. 6).

There are four types of *lamin* proteins: A, B1, B2, and C. The nuclear meshwork of *lamins* appears to lend strength and stability to the nuclear envelope and plays a role in the overall shaping of the

nucleus. This can be shown by experiments involving developing sperm cells. Sperm cell nuclei are very much smaller and more arrow-like in shape than most nuclei, which is in accord with their function of swimming as streamlined rockets through fluids of the reproductive tract. They also possess a type of *B lamin* that is not found in any other cell. If an ordinary cell is forced to produce sperm cell *lamins*, its nucleus will become deformed to resemble the pointed morphology of a sperm cell nucleus.<sup>9</sup>

The types of *lamins* produced by other cells seem to correlate with how specialized the cell is. For example, the unspecialized (stem) cells of the early embryo produce *B-type lamins* but not A- or C-types. Once cells become more specialized, they synthesize *A-type lamins*.<sup>6</sup> Also, a mutation in *A-type lamins* leads to a rare but devastating syndrome called progeria, in which cell nuclei become more fragile and teenaged patients acquire the aged appearance and fragile skins and bones of elderly people.<sup>25</sup> It is likely that variations in nuclear shape between different cell types involve rearrangements in nuclear *lamin* proteins.

Another subset of proteins closely associated with *lamins* are proteins of the so-called nuclear matrix. The nuclear matrix forms a scaffold upon which the chromatin of chromosomes is organized. The nuclear matrix contains proteins like *topoisomerase*, which can cause bending of the DNA double helix, and anchoring proteins like *SATB* (*special AT-rich sequence binding protein*, which binds to DNA). The DNA of nuclear chromosomes is attached to the nuclear matrix at an estimated 30,000 to 80,000 spots (called matrix attachment regions or *MARs*). Each of these attachment spots is not always active; when activated, a *MAR* will cause a loop of chromatin to be pulled closer to the nuclear matrix. This appears to be one mechanism for the activation of cell-specific genes: mature lymphocytes possess more *MARs* than immature lymphocytes, and if the *MARs* are inactivated, the genes normally active in mature lymphocytes will not be transcribed.<sup>22</sup>

The chromatin attached to the nuclear matrix contains the DNA and the DNA-associated proteins found in each chromosome. Human cells contain 23 pairs of chromosomes, for a total of 46. Some

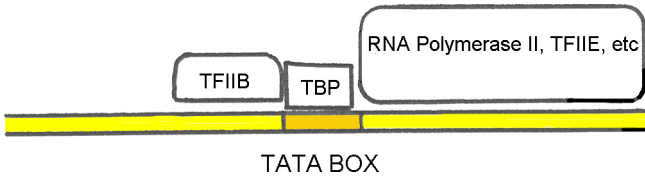
of the chromosomes are rather long and contain 1,000 to 2,000 genes; other shorter chromosomes contain only several hundred genes. Chromosomes are not randomly tangled together, but occupy discrete territories at specific spots along the nuclear envelope. Also, the gene-rich portions of chromosomes tend to be located at the center of the nucleus, whereas the gene-poor regions are found adjacent to the inner nuclear membrane.<sup>7</sup>

It may seem puzzling to speak of “gene-poor” and “gene-rich” regions of chromosomes. What are chromosomes if they are not linear arrays of genes? In fact, only a small amount (1.5%) of the DNA in chromosomes actually can be read, or transcribed, into messenger RNA molecules that code for proteins. The human genome contains only about 25,000 such gene sequences. The remainder of the DNA (almost one meter long, if all the chromosomes are laid end-to-end) contains highly repeated sequences that do not code for proteins at all.<sup>3</sup>

The DNA of each chromosome is basically composed of two intertwined strands of repeated sugar molecules (deoxyribose sugars), plus associated bases. In some ways, DNA is not really a remarkable molecule: cells commonly make a large variety of polymers of sugar molecules. For example, long chains of sugars called *glucose-amino-glycans* (*GAGs*) are commonly secreted by cartilage cells, mast cells, and epithelial cells. Other polymers of sugar (glycogen, starch) are used as a storage form of nutrients by cells and accumulate within the cell cytoplasm. What makes DNA unique is that the double helix of deoxyribose sugars contains within it sequences of bases (adenine, thymine, cytosine, and guanine, or A, T, C, and G) that form a code for the assembly of proteins.

### ***Regulation of DNA transcription***

How is the code on the DNA for a gene converted to a molecule of mRNA for transport out of the nucleus? Messenger RNA molecules are created by an enzyme, *RNA polymerase II*, that reads the code on a gene. This enzyme needs the assistance of many other proteins to accomplish this, however. Each gene sequence is preceded by a short

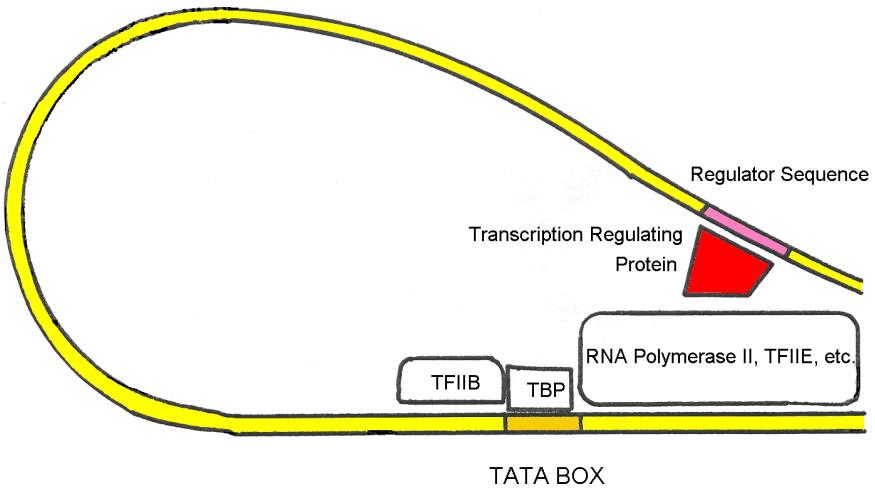


**Fig. 1.3.** Simplified diagram showing how accessory proteins link RNA polymerase II to the promoter region of a gene. Adapted from Ref. 37.

DNA sequence, 25 bases “upstream,” called the TATA box, which is located in the so-called promoter region of the gene and which is enriched in T and A nucleotides. A binding protein called the *TATA box binding protein* (*TBP*) first localizes to this TATA box and identifies where additional proteins should bind subsequently (Fig. 1.3). Accessory proteins for *RNA polymerase II*, called *Transcription Factors for Polymerase II* (*TFIIs*), perform functions like forcing open the DNA double helix so that the interior code can be read by *RNA polymerase*. With the aid of these proteins, RNA polymerase can read genes and produce a molecule of mRNA for export from the nucleus.

Even these proteins are not sufficient to ensure that a gene will be read. Additional DNA-binding proteins, which differ between one cell and another, are also needed. These DNA-binding proteins are called transcription regulating factors. They bind to DNA sequences called regulatory or enhancer sequences that are located hundreds or thousands of nucleotides distant from a gene on a particular chromosome. When transcription regulating proteins bind to enhancer regions, they cause the formation of a loop in the chromosome that brings the enhancer region close to the promoter region (Fig. 1.4). A complex of proteins binds to the enhancer region and helps stabilize the transcription factors located at the promoter. This action can enhance transcription by 1000-fold. Each cell in the body possesses its own mix of transcription regulating factors; this mix determines which genes will be transcribed for a muscle cell, a lymphocyte, a liver cell, etc.<sup>3</sup>

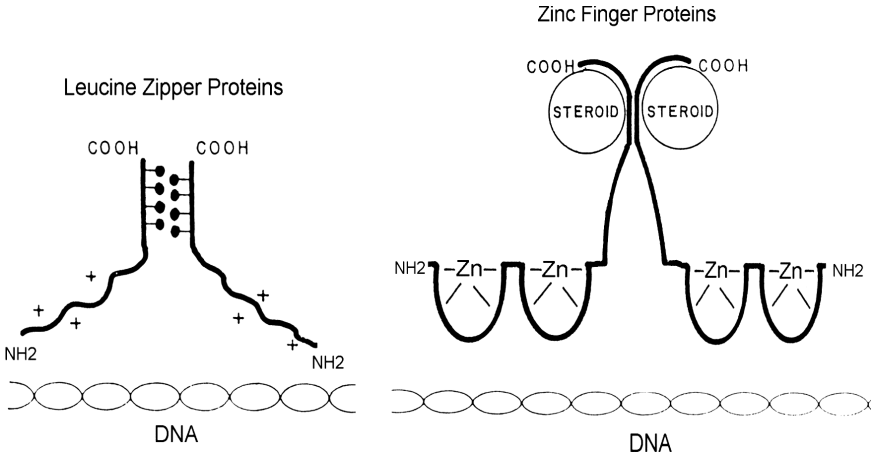
About 2000 different transcription regulating factors, amounting to almost 8% of the human genome, have been identified. Many of



**Fig. 1.4.** A simplified diagram showing how transcription regulating proteins, bound to a regulatory sequence on a chromosome, interacts with TFII proteins and RNA polymerase II at the promoter sequence of a gene (adapted from Ref. 37).

these proteins fall into a few basic categories. For example, some transcription regulating proteins belong to the so-called “*leucine zipper*” family.<sup>1</sup> Two of these proteins can bind to each other because they each have a stretch of peptides containing 4–5 leucine amino acids located at equal distances from each other. When two “*leucine zipper*” proteins approach each other, the complementary stretches of leucines interlock, much like the teeth of a zipper (Fig. 1.5).

Subsequently, the two interlocking proteins settle down on the DNA, aided by the positively charged amino acids at the DNA binding regions of the proteins that are attracted to the negatively charged phosphate molecules that form links between deoxyribose sugars of the DNA. Since the two proteins form mirror images of each other, they bind to a DNA sequence called a “palindrome.” In a palindromic sequence, each half of the sequence is also a mirror image of the other half, e.g., a sequence of nucleotides such as AATTGC-CGTTAA is a palindrome. Many transcription regulating proteins, such as the *C/EBP* family (see the passages in Chapter 4 that describe the generation of fat cells), belong to the leucine zipper family.



**Fig. 1.5.** Simplified diagrams of two types of transcription regulating proteins. Both leucine zipper proteins and zinc finger proteins form dimers that bind to palindromic DNA sequences. Adapted from Ref. 1.

Other transcription regulating proteins belong to the “*zinc finger*” family of proteins (Fig. 1.5). These proteins have regions of amino acids that bind zinc and are thrown into long loops, or *zinc fingers*. The shape of the loop, plus the positive charge associated with the zinc atom, allow the loops to interact with palindromic sequences of DNA. Important examples of this family of proteins are the receptors for steroid and thyroid hormones, which affect cell function by modulating transcription.<sup>16,18,37</sup>

Other families of transcription regulating proteins exist. Some proteins, for example, have two DNA-binding helices that are joined by a straight portion of the protein. These are termed *helix-turn-helix* proteins, and include the very important *homeotic* proteins that will be discussed in detail in Chapter 2. A final family is the so-called *STAT* family (for Signal Transduction and Activator of Transcription). This protein family will be examined in Chapter 8. The molecular structures of most of these transcription regulating proteins is becoming known in some detail.<sup>18</sup>

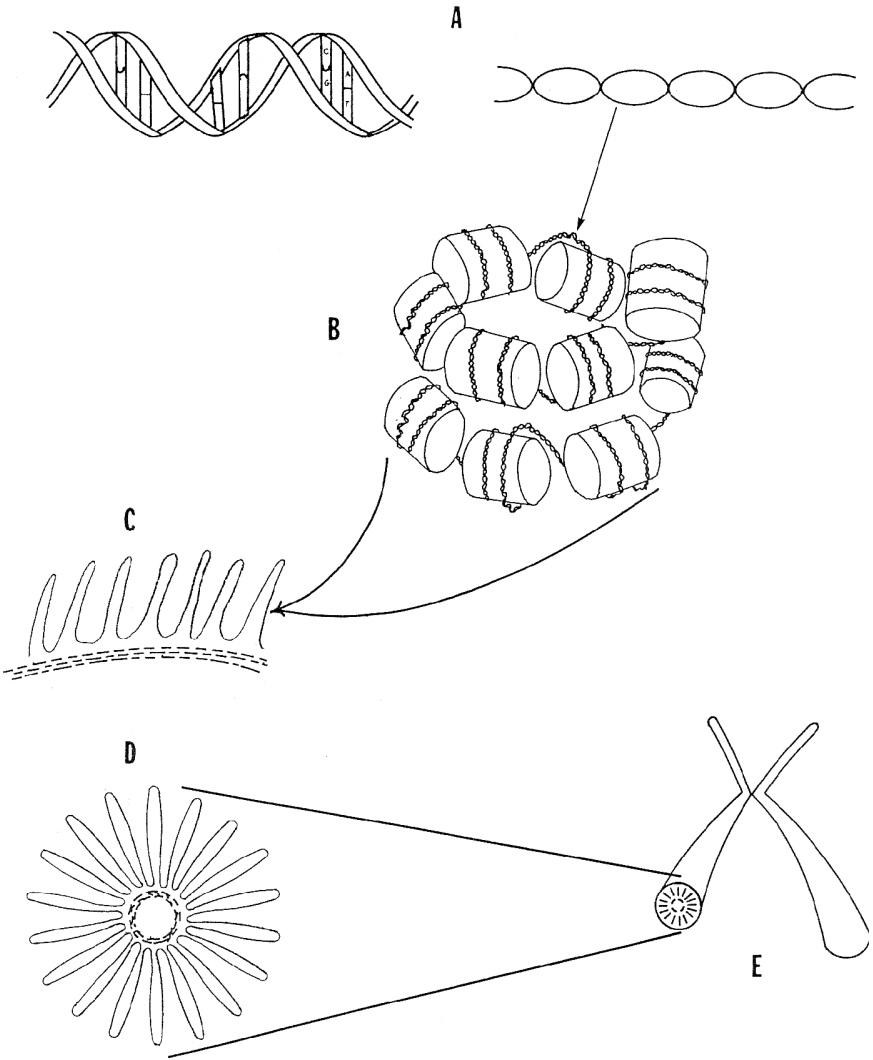
Considering the length of nuclear DNA and all the proteins associated with it, it almost seems surprising that a cell’s genetic material

would have room to fit inside a tiny cell nucleus. In fact, however, we must remember that even if a strand of chromosomal DNA is very long, it is also very delicate and thin. It has been calculated that if all of a cell's DNA were tightly compacted into a ball, it would occupy only 17% of the volume of the nucleus.<sup>7</sup> So there seems to be room enough, after all.

One thing about chromosome anatomy that *is* very remarkable is that a chromosome shrinks in length tremendously when it is compacted in preparation for cell division and mitosis. For example, chromosome number 22 shrinks in length from 1.5 centimeters to about 2 micrometers, a 10,000-fold reduction in length!<sup>3</sup> How is this accomplished?

Most investigators feel that chromosomes are compacted for mitosis by a series of hierarchical coiling operations (Fig. 1.6). The DNA double helix itself forms the first level of coiling; the second level of coiling occurs when DNA becomes wrapped around roughly disc-shaped core particles composed of *histone* proteins. These core particles, in turn, form another coiled arrangement rather like the cord on a telephone. The larger chromatin coils are attached at intervals to proteins of the nuclear matrix. When the nuclear envelope breaks down during mitosis, it might be that the nuclear matrix proteins themselves can form an even larger coil. One could construct a model of a chromosome by stretching out part of a large “slinky” toy until it was straight and then fastening a telephone cord to the “slinky.” In the interphase condition, the slinky would remain straight and coils of chromatin would hang off from one side; in the mitotic condition, the slinky would snap back to its original coiled condition and smaller coils of chromatin would radiate away from the slinky “axis.” There is some evidence that this appealingly simple model has some similarity to reality, but it may not be strictly true in detail.<sup>17,29</sup>

One other variable that is noteworthy between the nuclei of different types of cells is that some cells have a prominent nucleolus, while others do not. The nucleolus represents the junction of genes for ribosomal RNA that are located on different chromosomes. When active, its function is to assemble new ribosomal subunits out of ribosomal RNA plus associated proteins. Cells that synthesize many



**Fig. 1.6.** A simplified model of a mitotic chromosome, showing the DNA double helix (a) wrapped around core particles made of histone proteins to form a coil (b), which is thrown into longer loops attached to the nuclear matrix (c). During mitosis, the nuclear envelope breaks down, allowing the nuclear matrix proteins to detach from the nuclear envelope and to form an axial core of the chromosome (d). Loops of chromatin are detectable in fully condensed, mitotic chromosomes.

proteins require lots of ribosomes, and thus tend to have enlarged, active nucleoli (see, for example, the large nucleoli present in oocytes and in nerve cells (Figs. 2.4 and 6.1)).

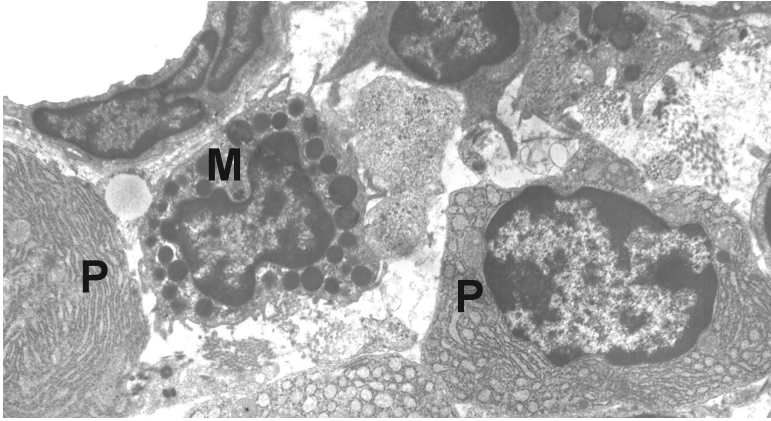
From the data reviewed above, it is clear that a cell biologist can infer a lot about a cell simply by looking at the morphology of its nucleus: a cell with dispersed chromatin and a large nucleolus will likely be transcribing many thousands of genes, whereas a cell with condensed, dark-staining chromatin probably transcribes only the minimal number of genes needed to keep the cell alive, plus a few genes for its particular specialty. The basic specializations of cells are the topics of the next chapter.

## 2. Regulation of the Endoplasmic Reticulum

When new functions of a cell arise, driven by changes in nuclear gene transcription, organelles of the cytoplasm must be adjusted to meet the new demands of the nucleus. The positions, activity, and volumes of each organelle may change, depending upon the specialized function of the cell. How do these changes come about? The differentiation of a plasma cell from a lymphocyte illustrates particularly well the challenges involved in answering this question.

Plasma cells are cells that secrete *immunoglobulins*, proteins (antibodies) that selectively bind to foreign molecules (antigens) that have invaded the body. When an antigen is presented in precisely the right way to a lymphocyte by a so-called antigen presenting cell, the lymphocyte is stimulated to differentiate into a plasma cell. The lymphocyte begins life as a small cell with a marginal amount of cytoplasm; as it turns into a plasma cell, the volume of the cytoplasm expands considerably and becomes filled with massive amounts of rough endoplasmic reticulum (rER) and Golgi stacks (Fig. 1.7).

This is appropriate for the function of the plasma cell. Plasma cells secrete prodigious amounts of protein (180,000 molecules per hour!) at about six times the rate of other secretory cells like fibroblasts.<sup>14</sup> In order to secrete so much protein, plasma cells must make appropriate adjustments to their protein-making machinery.



**Fig. 1.7.** A transmission electron micrograph showing two plasma cells (P) and a mast cell (M). The cytoplasm of the plasma cells is filled with masses of parallel stacks of rER. Courtesy of Dr. Lee Leak, Dept. Anatomy, Howard University College of Medicine.

The steps in synthesizing a protein are familiar elements of an introductory biology class:

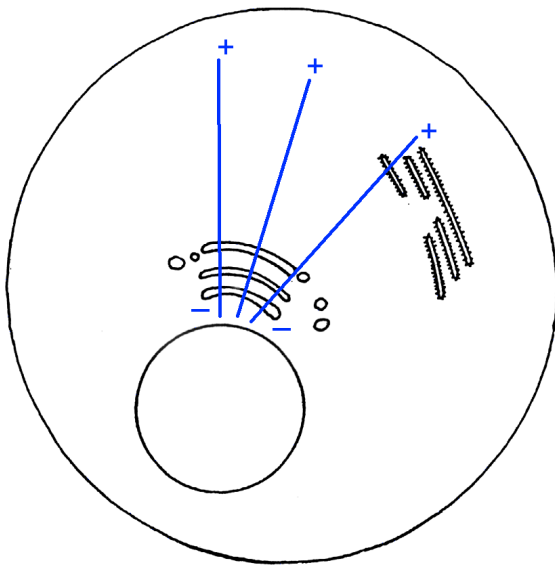
- (i) a molecule of messenger RNA (mRNA) is transcribed from the DNA in the nucleus,
- (ii) the mRNA is modified by editing and splicing and is then exported from the nucleus, where it binds to cytoplasmic ribosomes,
- (iii) ribosomes “read” the sequence of bases on the mRNA and, by binding the corresponding tRNA-amino acid complex, translate this sequence into a chain of amino acids, and
- (iv) if the resulting protein is destined for export from the cell, it has a “signal sequence” of amino acids on one end that is threaded through the membrane of the rER so that it winds up within the lumen (cisterna) of the rER.

From this point, the completed protein will be packaged into membrane-bound vesicles that bud off from the rER and move to the Golgi stacks so that the protein can be further modified (addition of carbohydrate molecules, for example). Finally, vesicles will

then leave the Golgi stacks to fuse with the plasma membrane and deliver their protein contents into the environment outside of the cell.

How is traffic between these organelles coordinated for secretion of proteins? The positions of the rER and Golgi stacks, plus the traffic between them, are regulated by the array of microtubules that sprout from the centrosome, near the nucleus (Fig. 1.8). Microtubules, hollow structures that resemble miniature drinking straws, grow from the centrosome, so that their “plus” ends wind up near the cell membrane and their “minus” ends remain at the centrosome. Microtubules function as a “railroad track” system that delivers membrane-bound structures to specific destinations within a cell. The membranous organelles travel along these “railroad tracks” with the aid of so-called motor proteins that act as tiny locomotive engines and move their cargoes down the tracks.

Golgi membranes acquire *dynein* motor proteins that drag the Golgi stacks down towards the centrosome by traveling towards the



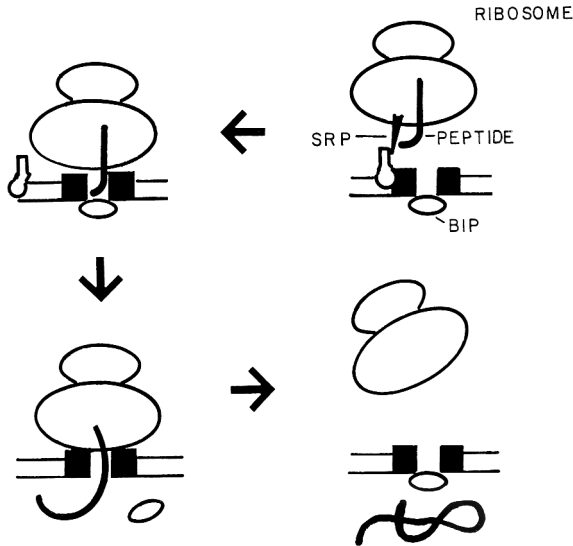
**Fig. 1.8.** Microtubules (blue lines) radiate from the centrosome and organize Golgi stacks in a position near the nucleus.

“minus” ends of microtubules. This can be demonstrated experimentally: if Golgi membranes are isolated from disrupted cells and then injected into a healthy cell, they will immediately attach to microtubules and migrate to the centrosome. So, the reason why the Golgi apparatus is located close to the nucleus is becoming clear. Likewise, vesicles that bud off of the rER and which contain secretory proteins also acquire *dynein* motors that automatically deliver their contents to the Golgi stacks for further modifications. Membranes of the rER appear to travel in the opposite direction upon microtubules and thus wind up in parts of the cell farther from the Golgi apparatus.<sup>33</sup> If microtubules are experimentally disrupted in cells, the Golgi membranes become dispersed all over the cell, whereas the rER membranes collapse towards the cell nucleus.

If microtubules play a major role in determining the positioning and trafficking between the Golgi and rER, what anchors the microtubules to a position near the nucleus? This fundamental question has been answered in cells from roundworms (*Caenorhabditis elegans*). A protein called *Zyg12* attaches the cloud of proteins surrounding the centrioles (centrosome) to the nuclear envelope.<sup>15</sup> So it appears that a hierarchy of binding proteins and motor proteins determine the positions of many cellular organelles.

When plasma cells secrete massive amounts of immunoglobulins, it becomes necessary to increase the size of their rER by almost four-fold compared to the rER of lymphocytes. In a certain sense, this is unsurprising: if the genes for immunoglobulins are activated and large numbers of mRNA molecules are generated for translation into protein, it is appropriate to up-regulate rER and Golgi membranes. But how do these organelles “know” that there is a greater need for them and what could cause such a remarkable growth of the rER to produce the masses seen in plasma cells? The answer to this question is somewhat complicated and involves an event called the unfolded protein response.<sup>13,19,36,38</sup> This response takes place when the rate of the synthesis of a protein exceeds the rate at which the protein can be processed. There are several steps in the response.

First, when an mRNA molecule binds to a ribosome that settles down onto the rough ER, a growing polypeptide chain (in this case,



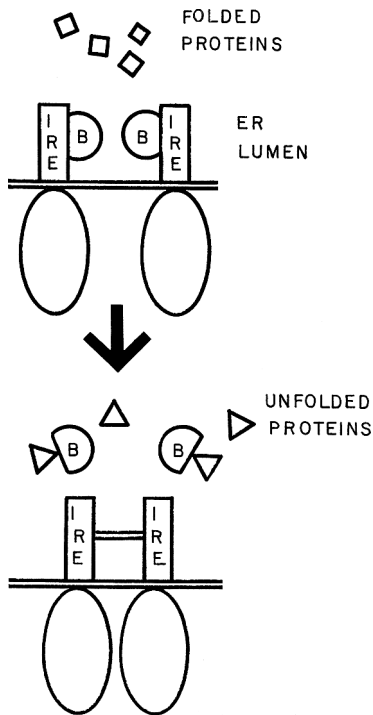
**Fig. 1.9.** Diagram of a translocon pore, which threads a new protein into the rER and which is blocked at one end by the *BIP* protein. Initially, the signal sequence in a growing polypeptide chain is bound to the signal recognition particle (SRP) and then drawn into the translocon pore. After complete synthesis of the polypeptide chain, the signal sequence is cleaved off, the protein folds into the proper shape, *BIP* once again attaches to the translocon pore, and the ribosome detaches from the cytoplasmic surface of the rough endoplasmic reticulum (modified from Ref. 13).

an immunoglobulin) emerges from the ribosome and is fed into the cisterna of the rER via a membrane pore called the translocon (Fig. 1.9). The translocon is composed of a protein called *Sec61* that forms a channel through the membrane. Once the newly synthesized immunoglobulin is injected into the rER, the translocon is re-sealed on the inside by another protein called *BIP* (this strange name comes from the fact that the protein was discovered due to its ability to *Bind Immunoglobulin Proteins*). *BIP* does not only serve to seal up the translocon pore, but also has the ability to bind to hydrophobic portions of proteins that have not folded properly within the rER. Other so-called chaperone proteins will then facilitate the proper folding of rER proteins. This normally works just fine.

### The unfolded protein response

If too much mRNA message is directed towards the rER and too much protein accumulates within the rER, the proteins overwhelm the ability of chaperone proteins to help fold them. Hence, misfolded proteins accumulate within the rER and saturate binding sites on the BIP protein. When this happens, the BIP protein dissociates from a membrane protein called *inositol requiring enzyme 1* (*IRE1*).

This minor event actually has a galvanizing effect on cell function. When the *IRE1* proteins form dimers of 2 proteins each, the proteins then can autophosphorylate each other and activate their function. The *IRE1* protein dimers then act as a peculiar type of enzyme that



**Fig. 1.10.** When *BIP* (B in the figure) binds an excess of misfolded proteins, it is released from the rER membrane, permitting the dimerization of *IRE1* proteins (modified from Ref. 19).

specifically splices out a 26 nucleotide long intron loop out of the mRNA for another protein called the *X-box binding protein (XBP)*. Introns are extraneous portions of the mRNA sequence that are usually removed in the nucleus prior to the export of the mRNA. Removal of the intron allows the mRNA for *XBP* to finally be translated into an active protein. But this *XBP* protein is only a single protein. How could it be responsible for such dramatic effects upon a cell's structure?

The basis for the power of the *XBP* is that it is a DNA-binding transcription regulating factor. When it binds to DNA, it binds to a type of DNA sequence called the X-box and stimulates the transcription of at least two dozen genes for proteins that are required for assembling the rER (these include *Sec61*, *signal sequence receptor*, *signal peptide protease*, *signal recognition particle*, a number of chaperone proteins, etc.). This leads to the synthesis of all the components of the rER and an expansion of the rER that is needed for the secretion of so much protein.

This sequence of events explains how, when the rER fills up with too much protein, it sends a signal to the nucleus to generate more rER.

The *XBP* protein is not the only one required for the transformation of a lymphocyte into a plasma cell. Another protein with the unusual name of *BLIMP (B-lymphocyte induced maturation protein)* is also needed for B-lymphocytes to turn into plasma cells. *BLIMP* is another transcription regulating protein (a zinc finger protein) that suppresses the activation of about 250 genes that are active in lymphocytes and which code for things such as B cell membrane receptors. So, *BLIMP* is turned on to suppress the features of B-lymphocytes, and then *XBP* is turned on to activate the features of plasma cells.<sup>26</sup>

This whole process is not only useful in explaining the transformation of lymphocytes into plasma cells, but very likely applies to other cell transformations as well. For example, the pancreas begins its existence as a modest sprouting of ducts and tubules from the lining of the primitive gut. Duct cells are simple, undistinguished cells with a very simple morphology. But when they mature into pancreatic acinar cells and begin secreting pancreatic enzymes, they too acquire massive amounts of rER, probably by the same mechanism as described above. So, an examination of the process governing plasma cell formation provides a useful insight into the specialized features of other cells.

This scheme shows how the volume of the rER might be adjusted, but it provides no information about how the structure of the rER is formed. Both smooth and rough endoplasmic reticulum membranes form either tubules or flattened, parallel sheets; neither configuration is natural for lipid bilayers, which spontaneously form only spherical vesicles when dispersed in water. Something must be forcing the ER membranes into the configurations seen in cells, but what is it? A number of intrinsic ER proteins (*reticulon* or *DPI* proteins) appear to be good candidates for supplying such a force: when these proteins are mixed with lipids, they form hollow tubules.<sup>11,27,31</sup> Similarly, proteins called *Golgins* or *CLASP* proteins may be responsible for the architecture of the Golgi apparatus.<sup>5</sup>

### 3. Regulation of Mitochondrial Number

Many cells of the body (e.g., cartilage cells, fibroblasts) live quiet lives within masses of extracellular material. Such cells have low requirements for energy, do not intensely utilize the energy-rich compound, *ATP*, and possess few mitochondria. Other cells, such as kidney cells that spend huge amounts of energy to recover molecules from the urine or muscle cells that steadily contract, require lots of *ATP* and possess many mitochondria to produce it. How are numbers of mitochondria adjusted to meet the energy requirements of a cell?

This question can be studied in a number of ways. One approach, for example, is to expose a cell to *thyroxine*. Two to three days after an injection of *thyroxine*, tissues from a rat will display about a two-fold increase in oxidative metabolism, primarily due to increased numbers of mitochondria. In confirmation of this, the mRNAs for mitochondrial proteins such as *Cytochrome C* and the *adenine nucleotide transporter* that imports the precursor for *ATP* into mitochondria are all increased by *thyroxine* administration. How do these events come to be?

*Thyroxine* acts by entering the cell nucleus and binding to *thyroxine receptor proteins*. Activated *thyroxine receptors* then bind to specific DNA sequences in the enhancer regions of genes called *thyroxine response elements*. Once in place, the *thyroxine receptor* also binds to another protein called the *thyroxine receptor associated protein*,

which has the ability to activate *RNA polymerase* and influence transcription of the coding region of the gene for a protein. At least 54 genes are known to be influenced by *thyroxine*. So it would seem logical that *thyroxine* could simply turn on the genes required for mitochondrial biogenesis. However, here we come to a roadblock on our way to a solution: genes coding for mitochondrial components such as *Cytochrome C* or *adenosine transporter* *lack thyroxine* response elements!<sup>35,37</sup> So how could they be turned on by *thyroxine*?

The answer to this problem appears to be that *thyroxine* activates the gene that codes for a protein called *nuclear respiratory factor-1* (*NRF-1*). This is a DNA-binding protein that regulates the activity of over 400 genes, many of which control overall cell metabolism.<sup>16</sup> Among the genes activated by *NRF-1* are the genes required for mitochondrial components. So, *thyroxine* may partly act upon mitochondria *indirectly*, by stimulating the appearance of a transcription regulating protein that controls their number.

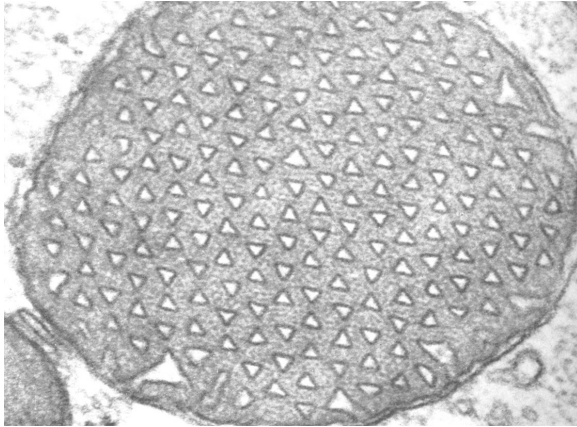
Once mitochondrial proteins are synthesized in the cytoplasm, how do they reach mitochondria? Each mitochondrial protein has a signal sequence at its N-terminal end that is recognized by translocator complexes of proteins that are present in the outer and inner mitochondrial membranes. These feed the required proteins into the interior of the mitochondrion.<sup>3,16</sup>

*Thyroxine* is not the only stimulus that causes the appearance of new mitochondria within cells. Mitochondrial genesis can also be regulated by a specialized protein that serves as a “fuel sensor” within cells. This protein is called *AMP-activated protein kinase* (*AMPK*). Simply put, this is an enzyme that becomes active when energy in a cell becomes depleted and the *AMP/ATP* ratio increases (e.g., as seen during starvation or fasting). In response, this enzyme can suppress the synthesis of fatty acids, proteins, and glycogen, and increase glucose uptake and glycolysis to replenish the energy supplies of a cell. In addition, *AMPK* also activates *NRF-1*, and so can stimulate the genesis of new mitochondria. Thus, there are several mechanisms that transform the energy economy of a cell and regulate mitochondrial number. This explains a number of remarkable events in cells. For example, repeated exercise can transform mitochondria-poor white muscle fibers

into mitochondria-rich red muscle fibers (the type of muscle cells seen in “dark meat” of postural muscles). This is due to a stimulation of *AMPK* by exercise and energy depletion of the muscles.<sup>10,16,24</sup>

Once new mitochondria are formed, they can be directed to the parts of a cell that have the greatest need for energy. If living cells are viewed under a microscope, it is easy to see that mitochondria are in continual movement. This is because they travel along the network of microtubules in cells, just as other organelles do. The motor proteins that move mitochondria in this way all require *ATP*. If they drag a mitochondrion into an energy-poor region of the cell, the motor proteins “run out of gas” and strand the mitochondrion in this area. This is actually a good thing, because the immobile mitochondrion will then release enough *ATP* in the region to both replenish local energy stores and also to get moving along the microtubules once more. The proteins responsible for this ingenious scheme have recently been identified: the motor protein *kinesin* binds to an adaptor protein called *milton*, which in turn regulates the movement of mitochondria by binding to a protein of the outer mitochondrial membrane called *Miro*.<sup>32</sup>

Mitochondria do not only differ in number between types of cells, they also differ in morphology. The inner mitochondrial membrane of all mitochondria is folded into structures called cristae. However, all cristae are not created alike. In most cells, cristae appear as flattened, shelf-like folds within mitochondria. In cells that make steroid hormones, the cristae take on a tubular shape. In some astrocytes in the brain, cristae form perfect triangles or prisms in cross-section (Fig. 1.11)!<sup>8</sup> No one knows why these morphological changes take place, or how they are related to the specialized functions of mitochondria in specialized cells. Recently, some mitochondrion-specific proteins have been shown to be involved in these transformations. Enzymes found on the outer mitochondrial membrane called *mitofusin* or *OPA1* regulate the fusion and fission of mitochondria. Proteins within mitochondria such as *mitofilin* appear to regulate the structure of cristae.<sup>12</sup> More work needs to be done to explain how these proteins are involved in changes in mitochondrial structure.



**Fig. 1.11.** Electron micrograph of an astrocyte mitochondrion, showing prismatic cristae. Reprinted from Ref. 8, with permission.

The mechanisms described above do not always operate smoothly. One extreme example of a dysfunction of these systems is found in oxyphil cells of the parathyroid gland. These cells, apparently derived from the hormone-secreting chief cells of the gland, become almost entirely filled with hundreds of mitochondria that leave little room for other organelles. No explanation has yet been found for why these cells fail to utilize the mechanisms described above to regulate their numbers of mitochondria.

#### **4. Control of Centrioles and Cilia**

Centrioles are very peculiar cell organelles. They are composed of dimers of *tubulin*, like most cytoplasmic microtubules, but instead of forming single, long, hollow tubules, the *tubulin* proteins are combined to form long triplets of tubules that are fused together. Nine of these triplets form the core of a centriole (Fig. 1.12). Each barrel-shaped centriole is positioned rigidly at a 90-degree angle relative to its nearby “daughter” centriole and the pair of centrioles is in turn surrounded by a cloud of proteins called the pericentriolar matrix that makes up the remainder of the centrosome.

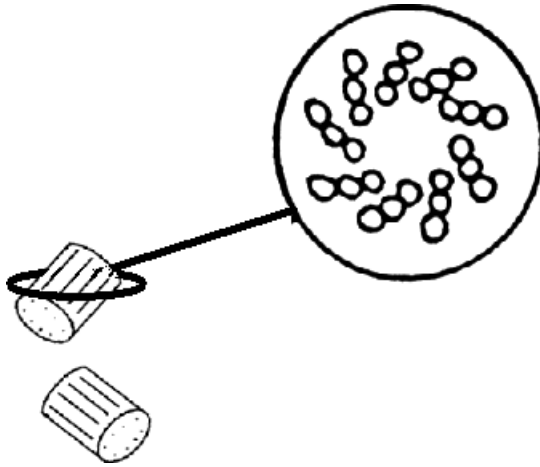
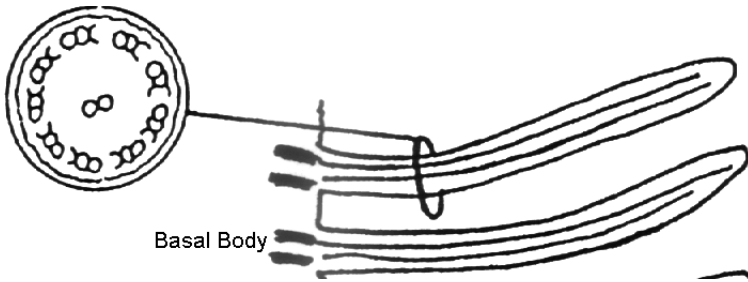


Fig. 1.12. Each barrel-shaped centriole is composed of 9 triplets of microtubules.

In cells that are dividing, each pair of centrioles duplicates once during every cell cycle, so that all daughter cells possess only a single pair of centrioles. Some post-mitotic epithelial cells, however, have the capacity to generate *hundreds* of centrioles in sub-membrane structures called deuterosomes. These centrioles then move towards the plasma membrane to form basal bodies, structures from which cilia grow (Fig. 1.13).<sup>30</sup> Clearly, then, one function of centrioles is to promote cilia formation. Several proteins, such as *SAS-6* and a transcription factor called *Foxj1*, appear to be essential for this function. Cilia are much more complicated than they appear to be. Recent estimates suggest that about 300–500 proteins may be required for full ciliary function.<sup>23</sup>

What do centrioles do in non-ciliated cells? They are a component of the centrosome, and the centrosome is vital for the generation of microtubules that form the mitotic spindle, so it could be that centrioles are needed for the coordination of chromosomal movement. However, a number of cell types, such as mature oocytes, have diminished centrosomes and *absent* centrioles, and nevertheless move chromosomes during meiosis just fine.<sup>21</sup> Thus, centrioles might be dispensable for this function.



**Fig. 1.13.** Diagram showing how basal bodies of centrioles form the origin of the axoneme of microtubules within a cilium.

One investigator, Guenter Albrecht-Buehler, has proposed a novel function for centrioles. He has noted that the dimensions of centrioles are perfect for the refraction of certain wavelengths of light (infrared frequencies). Also, the geometry of the centrioles, located at 90 degrees to one another, could, at least in theory, provide some information as to the direction of a light source. Albrecht-Buehler has shown that cells in culture do orient towards pulses of infrared light, and if they are “blinded” by shining a light on the centrosome, they lose this ability.<sup>4</sup> So, possibly, centrioles may function as some sort of rudimentary “eyes” for cells.

This proposal is not as outlandish as it may seem at first. Cilia have now been shown to have sensory functions in many cells, including kidney cells, neurons, and olfactory cells. In the kidney, single, non-motile cilia appear to monitor the volume of urine flowing through a tubule. If this sensory function is lost by a mutation in specific ciliary proteins, tubule cells react as if urine flow is blocked, and greatly enlarge to form the cysts found in polycystic kidney disease. Non-motile sensory cilia in the brain affect neuronal function; if their function is disturbed, a neurological disorder called Bardet-Biedl syndrome occurs.<sup>28</sup> Most notably, the outer segments of photoreceptor cells of the retina are basically highly modified cilia. Conceivably, a sensory function for centrioles and cilia that is rudimentary in most cells has been adapted and expanded to promote the function of specific sensory cells.

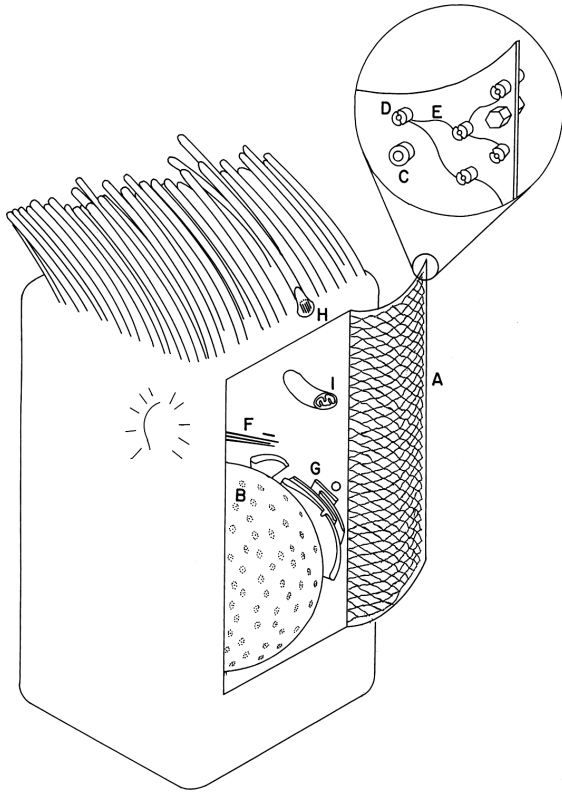
It is possible that centrioles functioned in ancient, ancestral cells as some sort of an intracellular sensor. Subsequent movement of duplicated centrioles to the cell surface could have resulted in a single, sensory cilium, and then, further duplication of cilia and the acquisition of motility in cilia resulted in the type of cilia seen in pseudostratified ciliated columnar epithelia that use cilia to move mucus.

## 5. Control of Overall Cell Shape

All of the material discussed above shows how we are gaining an understanding of how cells control the amounts and shapes of their internal organelles. However, how is the overall shape of the cell itself governed?

The cell membrane alone is too fragile to control cell shape: composed of an equal mix of membrane proteins and phospholipids, the plasma membrane is efficient in forming a watertight film around a cell, but is very fluid and weak as a soap bubble. To corral within the cell all of its components and to regulate cell shape, the cell membrane must be reinforced by attachments to cytoskeletal fibers of *actin*. This is accomplished by a network of filamentous proteins found on the cytoplasmic surface of the plasma membrane. One of these proteins, *spectrin*, forms connections between an anion transporter protein called *Band 3* that provides for the passage of bicarbonate ions through the plasma membrane.

*Spectrin* (illustrated in Fig. 1.14) also forms attachments to *actin* filaments via proteins called *ankyrin* and *tropomodulin*. *Tropomodulin*, in particular, seems essential for the interactions between the cytoskeleton and the plasma membrane. If it is deleted from tall columnar epithelial cells *in vitro*, the layer of *spectrin* on lateral cell membranes fails to form properly, and the cells are converted from tall cells to short cells.<sup>34</sup> Similar types of events must be occurring in most cells to ensure that cells retain an overall shape appropriate for their function.



**Fig. 1.14.** Diagram of a ciliated epithelial cell, showing the network of proteins on the cytoplasmic surface of the plasma membrane (A). D labels the anion transport channels interconnected with each other by strands of spectrin (E). Other transporter proteins (C) permit the passage of water soluble molecules (sugars, amino acids, ions) across the plasma membrane, while still other integral membrane proteins (hexagons) function as receptors for extracellular ligands. The nucleus, with numerous nuclear pores, is seen at B. G and I label the Golgi apparatus and a mitochondrion, respectively. F indicates a bundle of actin filaments that is participating in the deformation of the plasma membrane. H represents a basal body of a cilium.

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