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ESSENTIALS The cell is a dynamic entity. Cells are not simply building blocks that are linked together to create an organism: each cell comprises a dy- namic network of interacting macromolecules. Just how dynamic has been brought home by recent advances in cell imaging technologies. A host of multisubunit molecular structures must assemble and dis- assemble in a highly coordinated, exquisitely regulated, and beauti- fully choreographed manner to ensure the integrity of the cell and provide its ability to function correctly as a single unit within a large multicellular organism. Introduction The cell is the fundamental unit of all forms of independent life on this planet, from the simplest single-celled prokaryote to the most complex multicellular eukaryote. A limiting membrane, the plasma membrane, encloses the contents of the cell and allows a host of en- zymatic reactions and intermolecular interactions to occur within a confined, and regulated, environment. This raises the question, ‘What is the limiting membrane composed of and what are the con- tents of the cell?’. The major component of the limiting membrane is a lipid bi- layer, and the major components of the lipid bilayer are amphi- pathic phospholipids. Amphipathic molecules have one part that is water soluble (hydrophilic) and one part that is water insoluble (hydrophobic). A property of amphipathic molecules is that, in an aqueous environment, they spontaneously organize them- selves so that the hydrophobic regions face one another (shielding them from the surrounding water) leaving the hydrophilic regions (often referred to as ‘head groups’) exposed. In fact, an appropriate mixture of phospholipids in water will lead to the spontaneous for- mation of lipid vesicles, with water on the inside and water on the outside (Fig. 3.1.1). This gives us the basic template for the limiting membrane of the cell, the contents of which comprise a vast range of biomolecules, from simple building blocks to large macromol- ecular complexes. Prokaryotes compared with eukaryotes Before going further, it is worthwhile clarifying the difference be- tween prokaryotes and eukaryotes. The defining difference is that prokaryotes have no nucleus whereas eukaryotes do. Prokaryotes can be divided into two major divisions, or domains, the eubac- teria and the archaebacteria, which appear to have diverged from a common ancestor at around about the same time that ancestral eukaryotes evolved as the third domain of life on earth. All three domains use DNA as their hereditary material and in- formation store. In prokaryotes this DNA resides within the cell alongside all the other cellular contents; in eukaryotes it is contained within the nucleus and thereby physically separated from the bulk of the contents of the cell. Just as the cell itself is a membrane-bound structure, so is the nucleus within each eukaryotic cell. In fact, the membrane that surrounds the nucleus is a double lipid bilayer and is referred to as the nuclear envelope. 3.1 The cell George Banting and Jean Paul Luzio Phospholipid Hydrophillic headgroup Hydrophobic ‘tails’ Lipid bilayer Lipid vesicle Fig. 3.1.1 Phospholipids spontaneously self-assemble in aqueous environments to form lipid vesicles.

 The nucleus is just one of several membrane-bound structures within eukaryotic cells. The larger of these structures are referred to as organelles and they serve to compartmentalize the cell, allowing specific processes and reactions to occur within defined and con- trolled local environments. Smaller membrane-bound compart- ments are referred to as vesicles and tubules; these are generally involved in transport between organelles or between organelles and the plasma membrane. The contents of a eukaryotic cell are referred to as the cytoplasm; the aqueous part of the cytoplasm outside membrane-bound com- partments is referred to as the cytosol; and the inside of an organelle, vesicle, or tubule is termed the lumen of that compartment. Transcription, translation, and macromolecular crowding In all cells, DNA is transcribed into messenger RNA (mRNA) by the enzyme RNA polymerase; the mRNA is, in turn, translated into protein by ribosomes. In prokaryotes this all occurs within the same space, since there are essentially no intracellular membrane-bound compartments and transcription and translation can be coupled (i.e. an mRNA can be being translated while still being transcribed). However, things are different in eukaryotes, since (in general) the transcribed mRNA leaves the nucleus (via protein-lined channels, nuclear pores, in the nuclear envelope) before being translated in the cytosol. It has been estimated that, on average, each mammalian cell con- tains about 1010 protein molecules of about 10 000 to 20 000 different kinds. On top of this there are multiple copies of a range of other large macromolecules, notably nucleic acids and complex sugars. All of this within a cell that is around 20 μm in diameter (although there is massive variation here). It is, therefore, hardly surprising that there is a very high total concentration of macromolecules within mammalian cells—estimated to be up to 400 g/litre—meaning that anything up to 40% of the cell volume is physically occupied by these molecules. Thus, while the inside of the cell is clearly an aqueous environment, it is a very crowded and highly ordered aqueous en- vironment, and probably has a consistency rather like that of thick soup or porridge. Lipid bilayers and integral membrane proteins A lipid bilayer, be it the plasma membrane at the cell surface or the defining membrane of an intracellular organelle, is—among other things—a permeability barrier. It is permeable to small lipophilic molecules, partially permeable to water, but impermeable to ions and large molecules. It therefore not only retains the contents of the cell or organelle, but also provides a physical barrier between the contents of the cell or organelle and the exterior environment. A cell, however, clearly has to interact with its exterior environ- ment, whether it be a single-celled prokaryote (and most living or- ganisms are single cells) or a complex multicellular organism such as a human being with an estimated 1013 cells assembled in such a way, and communicating with one another, so that they create a living being that is far more than the sum of the individual parts. This interaction with the exterior environment of the cell is de- pendent upon proteins that reside in the lipid bilayer. Many of these proteins actually span the lipid bilayer, with part of the protein res- iding outside the cell, part within the cell, and part within the hydro- phobic core of the lipid bilayer. In the case of eukaryotic cells, the vast majority of these integral membrane proteins have been post- translationally modified by the addition of specific sugar residues to create glycoproteins. In many cases the correct sugar modifications are critical for the correct function of the glycoprotein, particularly for those glycoproteins that are involved in cell–cell or cell–substrate interactions (NB: aberrant glycosylation is frequently observed on glycoproteins at the surface of cells present in tumours). Membrane proteins perform a multitude of roles, just about all of which can be considered to be involved in some way in commu- nication between the inside and the outside of the cell. They act as transporters of ions, sugars, amino acids, peptides, hormones, and other molecules; they act as receptors for extracellular ligands such as hormones and neurotransmitters, and transmit signals to the in- side of the cell; they act to link cells to one another or to the under-

lying substrate; in short, they are the physical link between the inside of the cell and the outside world. It is not surprising, therefore, that it has been estimated that about a third of all the proteins encoded by the human genome are membrane proteins. Organelles The main organelles within eukaryotic cells are the nucleus, the endoplasmic reticulum (ER), the Golgi apparatus, mitochondria, lysosomes, endosomes, and peroxisomes (and chloroplasts in plants), with a range of specialized organelles occurring in different cells of higher eukaryotes (see Fig. 3.1.2). While there remains much speculation about the origin and evo- lution of intracellular organelles, there is little doubt that mitochon- dria are derived from an extinct α-proteobacterium that lived inside another prokaryotic cell (a process called endosymbiosis). One hy- pothesis proposes that the modern eukaryotic cell derives from one ancestral prokaryote phagocytosing another, which evolved into the modern mitochondrion (a second phagocytic event could account for the origin of chloroplasts in plant cells). Other organelles could result from infolding and vesiculation of the prokaryotic plasma membrane. An alternative hypothesis is that membrane-bound blebs were extruded by the ancestral prokaryotic cell, which is homolo- gous to the modern-day nucleus. These blebs are proposed to have expanded around the proto-mitochondria, generated the organelles of the secretory system and fused to form the plasma membrane. Nucleus The nucleus can be considered to be at the heart of the eukaryotic cell. It harbours the vast majority of DNA within the cell (i.e. the nuclear genome). It is also, among other things, the site of gene tran- scription, the site of mRNA processing, and the site of ribosome as- sembly. The control of gene expression, which often occurs at the level of transcriptional regulation, is fundamental to the regulation of cell function and involves a complex interplay between the gen- omic DNA in the nucleus and a host of cellular proteins. Many of these proteins shuttle, in a controlled manner and in response to specific signals, between the nucleus and the cytosol. They are able to do so because there are gaps, termed nuclear pores, in the mem- brane that envelopes the nucleus. As mentioned earlier, the nuclear membrane is a double lipid bilayer. Thus, there is an inner nuclear membrane in contact with the contents of the nucleus and an outer lipid bilayer in contact with the cytosol. The space between the two

 lipid bilayers is the lumen of the nuclear membrane and is a space that is contiguous with the lumen of the endoplasmic reticulum. Nuclear pores are complex multiprotein assemblies that allow certain proteins to pass in and out of the nucleus while excluding others. Fully processed mRNA also leaves the nucleus via nuclear pores before being translated in the cytosol by ribosomes. The dy- namic traffic within, and in and out of, the nucleus is imperative for cell function. Mitochondria The mitochondria are also enveloped in two layers of membrane, an inner one and an outer one. They are involved in the oxidation of molecular fuels, including pyruvate derived from sugars and fatty acids, to generate the adenosine triphosphate (ATP) that is needed as an energy source for the reactions of the cell. Mitochondria, along with chloroplasts in plant cells, are enclosed in a double-layered membrane and are the only nonnuclear organelles to contain DNA, reflecting their endosymbiotic origin. The mitochondrial outer membrane is freely permeable to ions and small molecules but the inner membrane acts as a diffusion barrier only allowing the trans- port of selected ions and metabolites by means of transport proteins. The generation of ATP by mitochondria is fundamental to eukary- otic cell function and it is the inner membrane that contains the mitochondrial ATP synthase as well as the machinery for electron transport (the respiratory chain) that enables oxygen consumption. The experiments that led to an understanding of the chemiosmotic process that couples oxidation energy to ATP production constitute one of the great achievements of late 20th-century biochemistry. The ATP synthase is a

remarkable nanomachine, comprising over 20 individual proteins, that functions as a proton driven turbine to produce ATP by a process called rotary catalysis. The rotor stalk in a single ATP synthase can spin at 8000 revolutions per minute, gen- erating 400 ATP molecules per second. Mitochondrial disorders are an important cause of neurological diseases and disorders of muscle, including cardiac muscle. Mitochondrial abnormalities may be inherited or acquired. Those specifically affecting the intrinsic genome of this organelle are transmitted in a matrilinear fashion. Descriptions of important mitochondrial disorders are to be found in Chapter 24.19.5. Peroxisomes Peroxisomes are membrane-bound organelles that contain high concentrations of oxidative enzymes such as catalase and are there- fore important for a range of oxidative processes necessary for the elimination of multiple substances (e.g. in the breakdown of very long chain fatty acids). Several diseases caused by defects in peroxi- somal proteins are described in Chapter 12.9. Endoplasmic reticulum (ER) The membranes of the ER are contiguous with those of the nuclear membrane, thus the lumen of the ER is contiguous with the space between the two membranes of the nuclear envelope. The lumen of the ER serves as a calcium store for the cell, with concentrations of calcium in the ER lumen being around 10–3 M, compared with 10–8 M to 10–6 M in the cytosol. Regulated release of calcium from the ER, in response to extracellular signals detected by membrane proteins at the cell surface and transmitted via specific intracellular second- messenger molecules, leads to changes in the activity of a host of cellular processes. This is because many intracellular proteins bind calcium, and their activities and/or interactions with other proteins are dependent upon whether or not they are calcium bound. In fact, the calcium concentration in the cytosol has to be very carefully controlled because it is an important regulator of many intracel- lular processes including muscle contraction and secretion. Excess calcium within the cytosol can rapidly lead to cell death. There is, therefore, a complex array of transporters operating to ensure that calcium levels remain high in the lumen of the ER and are only tran- siently raised in the cytosol in response to specific stimuli. Some of these transporters are in the ER membrane, others in the plasma membrane, others in the mitochondria. Microtubules Actin Intermediate filaments Endoplasmic reticulum Golgi apparatus Trans Golgi network (TGN) Secretory vesicle Late endosome Recycling endosome Lysosome Nucleus Endocytic vesicle Mitochondrion Peroxisome Fig. 3.1.2 Cartoon showing some of the main components of a higher eukaryotic cell.

 The ER is not, however, simply a calcium store. It is also a major site of lipid biosynthesis within the cell. It is also the site of synthesis for proteins that are destined to be secreted from the cell or to be membrane proteins. Such proteins are synthesized by ribosomes that become attached to the cytosolic face of the ER membrane soon after they have started to translate an mRNA encoding a secretory or integral membrane protein. Before folding up as a secretory pro- tein in the lumen of the ER, or passing laterally from the transloca- tion channel into the ER membrane if it is destined to be an integral membrane protein, the nascent protein is translocated through a protein-lined channel in the ER membrane. This channel opens only when a ribosome synthesizing a secretory or integral membrane protein is bound to its cytosolic face. The ER is thus the start of the secretory pathway in eukaryotic cells. The fact that the translocation channel opens only when a ribosome is bound to it preserves the in- tegrity of the ER membrane as a permeability barrier and ensures no leakage of ions, such as calcium, from the ER lumen. Some of the most abundant proteins in eukaryotic cells reside in the lumen of the ER; these are proteins that are involved in assisting the correct folding (see Box 3.1.1) of newly synthesized proteins in the secretory pathway and include proteins such as the enzyme protein disulphide isomerase

(which ensures that the correct di- sulphide bonds are formed in proteins), calnexin, and calreticulin. The latter two are also calcium-binding proteins. Thus, although most proteins that enter the secretory pathway at the ER are des- tined to be secreted or to become integral membrane proteins in the plasma membrane, certain proteins (both soluble proteins within the lumen of organelles along the secretory pathway and integral membrane proteins within the membranes of organelles along the secretory pathway) are primarily localized to specific compart- ments along the secretory pathway. It has become increasingly clear over recent years that a combin- ation of retention and retrieval signals (often short linear sequences of amino acids) within proteins serve to ensure these localizations, with retention signals serving to hold proteins in place and retrieval signals operating to bring proteins back to their steady-state local- ization from a point further along the secretory pathway. Most diagrams of eukaryotic cells in textbooks, including Fig. 3.1.2 here, show the ER as a membranous organelle linked to the nucleus; this is correct, but it fails to illustrate the extent of the ER since, in most cells, it pervades much of the extranuclear space of the cell and is a highly dynamic organelle. More than half of the total membrane area of a mammalian cell can be ER. Golgi apparatus The step beyond the ER in the secretory pathway is the Golgi ap- paratus. The Golgi apparatus has been likened to a small stack of pitta bread, with each pitta corresponding to a cisterna (segment) of the Golgi. Traffic through the secretory pathway, from the ER to the Golgi and beyond, is mediated by shuttling transport vesicles, which transfer cargo molecules between organelles. The process is called vesicular transport, whereby vesicles bud from a donor compartment in a process that enables protein sorting to allow se- lective incorporation of soluble and integral membrane protein cargo into the forming vesicles, while leaving organelle-resident proteins behind. The vesicles are subsequently targeted to a spe- cific acceptor compartment into which they unload their cargo upon fusion of their limiting membranes. Thus, in the transport step from the ER, vesicles are delivered to the cis face of the Golgi apparatus. The recruitment of specific cargo into the vesicles, the budding of the vesicles, and their fusion with the Golgi are all steps that involve discrete, and transient, assemblies of proteins. The different cisternae of the Golgi apparatus—known as cis, medial, and trans, although there may well be many more than three in certain cell types—are the next steps along the secretory pathway. It now appears that passage through this part of the pathway can be quite complex, with both forward (anterograde) and backward (retrograde) vesicular traffic occurring. The anterograde traffic moves cargo towards the trans side of the Golgi apparatus and the retrograde traffic retrieves material that is required earlier in the secretory pathway (i.e. in the medial or cis cisternae of the Golgi apparatus or in the ER). Vesicular traffic within the Golgi apparatus also seems to be com- plemented by a process that has been termed cisternal maturation. This describes the maturation of a cis cisterna into a medial cisterna by the vesicular retrieval of material that should not be present in a medial cisterna. The retrieved vesicles fuse with newly arrived vesicles from the ER to form a new cis cisterna; meanwhile the medial cisterna matures into a trans cisterna via the same process. As all of this is happening, the proteins that are passing along the secretory pathway are being sequentially post-translationally modi- fied, primarily by the addition of a series of sugar residues to gen- erate glycoproteins, by specific enzymes with discrete steady-state localizations maintained by retention and retrieval signals, within specific cisternae of the Golgi apparatus. Beyond the trans cisterna of the Golgi apparatus lies the trans Golgi network (TGN) from which a range of vesicles and tubules bud to deliver their cargo to its destination. This may be the cell surface, for proteins that are to be secreted or to become integral membrane proteins in the plasma membrane, but may also be an intracellular organelle. Thus, for example, lysosomal enzymes have to be delivered to the lysosome and this is done via the secretory

pathway. Lysosomes Lysosomes can be considered as the recycling centres of the cell. The cytosolic surface of the lysosome membrane is now recognized as a major site of action of signalling complexes that regulate cellular Box 3.1.1 Proteostasis • Ensuring that proteins are correctly folded is part of the maintenance of proteome homeostasis (known as proteostasis), something which is crucial for cellular and organismal health. • Proteostasis is achieved by an integrated network of several hundred proteins (maybe even a couple of thousand), including most promin- ently (1) molecular chaperones and their regulators, which assist in de novo folding or refolding, and (2) the ubiquitin−proteasome system (UPS) and autophagy system, which mediate the timely removal of irreversibly misfolded and aggregated proteins. • Deficiencies in proteostasis have been linked to the progression of numerous diseases, such as neurodegeneration and dementia, type 2 diabetes, amyloidosis, lysosomal storage disease, cystic fibrosis, cancer, and cardiovascular disease. • Pharmacological approaches to improve the folding, trafficking, and function of misfolded proteins are currently being developed.

3.1 The cell 213 metabolism, including a transcription factor, TFEB, which can translocate to the nucleus and upregulate autophagy (a process of orderly degradation and recycling of cellular components) and lyso- some biogenesis genes when the cell is starved. Macromolecules are delivered to lysosomes to be broken down into their constituent building blocks (e.g. proteins to amino acids, polysaccharides to monosaccharides) by a host of acid hydrolases (proteases, glycosidases, nucleases, lipases, and so on). The building blocks are then exported from the lysosome and used by the cell to make new macromolecules. It is clearly important that the hydrolysis of macromolecules is strictly compartmentalized, otherwise the cell would destroy itself. In fact, the cell not only compartmentalizes lysosomal enzymes within the lysosome, but also ensures that these enzymes only be- come fully active once they have been delivered into the lysosome. The lysosomal enzymes are acid hydrolases, that is to say that they function at low pH. The pH of the lumen of the lysosome is about 4.5. This is in contrast to the pH in the lumen of the Golgi and TGN (approximately 6.5 to 6.7) or the pH of the cytosol (approximately 7.4). Thus, as lysosomal enzymes are delivered from the TGN to the lysosome (by vesicular transport) they become activated because of the lower pH in the lysosome. In fact, lysosomal enzymes are not delivered directly from the TGN into the lysosome, but are delivered to an intermediate compart- ment, the late endosome. This is an interface between the secretory pathway and the endocytic pathway (a pathway carrying material that has been internalized from the cell surface). A late endosome fuses with a mature lysosome, delivering its contents to the lyso- somal hydrolases in a transient hybrid organelle, the endolysosome, in which digestion commences (Fig. 3.1.3). Membrane lipids and integral membrane proteins that should be in the late endosome are then retrieved from the endolysosome and recycled to form a new late endosome and allow regeneration of a mature lysosome. A similar process of delivery to lysosomal hydrolases occurs in the autophagic pathway in which an autophagosome, formed by a double membrane enclosing a region of cytoplasm to be degraded, fuses with a lysosome to form an autolysosome from which a mature lysosome can be regenerated. The luminal pH of the late endosome is intermediate between that of the TGN and that of the lysosome (i.e. between 5 and 6). This reduced pH is generated by the action of Fig. 3.1.3 Correlative light and electron microscopy of endolysosomes. A cultured fibroblastic cell was incubated with a membrane permeable cathepsin substrate called Magic RedTM, which releases red fluorescent cresyl violet dye within the endolysosomes after hydrolysis (upper left). The location of the endolysosomes within the cell can be identified by merging the fluorescence image with a differential interference contrast microscopy image (upper

right). Transmission electron microscopy of the same cell shows the ultrastructure of the individual endolysosomes (lower images), identified at the centre of boxes 1 and 2. Scale bars: upper images, 10 microns; lower images, 0.2 microns.

 a proton pump (a vacuolar ATPase) in the limiting membrane of the late endosome and lysosome. Many inherited disorders of lysosomal function have been identified. These diseases and their treatments are described in Chapter 12.8. The special capacity of the lysosomal compartment for complementation by internalizing proteins supplied externally has allowed several important therapeutic enzymes to be developed. There are also many disease-causing single gene disorders associated with malformation or malfunction of lysosome-related organelles that exist in a variety of differentiated and specialized cells. These have some properties and proteins in common with lysosomes but also contain cell type-specific proteins, often destined for secretion as a result of the lysosome-related organelle fusing with the plasma membrane. Examples include secretory lysosomes in cytotoxic T lymphocytes and natural killer cells, melanosomes in melanocytes, and a variety of platelet granules. Endocytosis and endosomes The existence of late endosomes implies that there must also be early endosomes. There is clearly a flow of membrane and protein along the secretory pathway culminating in the fusion of vesicles with the plasma membrane. In the absence of any compensatory membrane internalization, the surface area of the cell would therefore continu- ally increase. Such internalization does occur, thereby ensuring that most cells remain relatively constant in size. The internalization of membrane from the cell surface (a process termed endocytosis) occurs via a variety of routes, the best char- acterized being clathrin-mediated endocytosis. Just as with ves- icular transport in the secretory pathway, the process involves the assembly of specific protein machinery at the cytosolic face of the plasma membrane, the invagination of the plasma membrane, and the pinching off of clathrin-coated, membrane-bound vesicles. The protein coat (including clathrin) that has been instrumental in the formation of these vesicles then disassembles and the uncoated vesicles fuse to form early endosomes; the vacuolar ATPase is al- ready active in early endosomes and they have a lumenal pH of ap- proximately 6.5 to 6.8. The endocytic process not only ensures that a balance is maintained between the amount of membrane inserted in the plasma membrane and the amount removed, but also allows the selective recruitment of specific integral membrane proteins (often with extracellularly bound ligand) into the endocytic pathway. The different endocytic mechanisms selectively recruit different cargo and thereby serve as molecular filters, internalizing certain in- tegral membrane proteins while leaving others at the cell surface. The complexity of the endomembrane system (i.e. the membranes of the endocytic compartments) in mammalian cells has become ap- parent in recent years. Thus, for example, further protein machinery is used to sort integral membrane proteins destined for degradation away from the limiting membrane of endosomes into intraluminal vesicles that accumulate in late endosomes, giving them the appear- ance of multivesicular bodies in the electron microscope. In addition to early and late endosomes, there are also recycling endosomes. These are compartments from which material that is to be returned to the plasma membrane is retrieved. Such ma- terial might be receptors that have been internalized along with their ligand, but which need to be returned to the cell surface having released their ligand at the lower pH of the early endosome/recyc- ling endosome. An example of such a receptor is the transferrin re- ceptor which releases the iron that is bound to transferrin in the early endosome/recycling endosome, leaving the transferrin receptor and apotransferrin to be recycled to the cell surface for reuse. Other receptors that are internalized are destined for degradation in the lysosome and are delivered to the late endosome. An example

of such a receptor is the epidermal growth factor receptor. When this receptor binds its ligand at the cell surface it transmits a cascade of signals across the cell which trigger cell growth and cell division. Such signals should only be transient, otherwise unregulated cell growth and cell division occur; the cell ensures that the signals trans- mitted by the receptor are transient by internalizing the receptor and sending it to the lysosome for degradation. Cytoskeleton Movement of vesicles and tubules between compartments does not occur at random but is dependent upon motor proteins and the cytoskeleton. The cytoskeleton is the name given to a framework within the cell which gives the cell its shape and provides a structure to which organelles and proteins can be attached, thus providing an architecture that gives spatial organization to the cell. There are three main components of the cytoskeleton in mamma- lian cells: microtubules, actin filaments, and intermediate filaments (Fig. 3.1.2). Each of these components is a polymer of protein sub- units and all three are dynamic structures with the potential for as- sembly and disassembly according to the needs of the cell. Microtubules are highly dynamic polymers of heterodimers of α- and β-tubulin which assemble to form long hollow tubes ap- proximately 25 nm in diameter. Monomers of globular (G) actin polymerize to form filamentous (F) actin, which is a double- stranded helical polymer with a diameter of 5 to 9 nm. Elongated and fibrous subunits assemble to form intermediate filaments with a diameter of approximately 10 nm (e.g. lamins A, B, and C assemble to form the nuclear lamina that provides the inner lining to the nu- clear envelope). The different components of the cytoskeleton provide comple- mentary features of the cellular architecture. Microtubules serve to localize organelles within the cell and provide the tracks along which many classes of transport vesicles and tubules move, the movement being powered by motor proteins (notably kinesin and dynein) attached to the membranes of the vesicles or tubules. Microtubules also play a critical role during cell division, since they are pivotal in the physical separation of chromosomes during mitosis. Actin filaments can cross the cell and provide the struc- ture that determines the shape of the cell’s surface. These filaments play major roles in protrusions from the cell surface. For example, they run along the length of the microvilli that extend from the apical surface of polarized epithelial cells, and they are absolutely necessary for cell locomotion since concerted rearrangements of the actin cytoskeleton underlie cell movement. Myosin motor proteins also interact with actin, the best characterized such inter- action being between actin and myosin II in skeletal muscle; this interaction is responsible for generating the force that is required for muscle contraction. Both microtubules and actin filaments are highly dynamic structures. Their assembly and disassembly are

3.1 The cell 215 precisely and finely regulated by a host of cellular proteins in re- sponse to a range of extracellular signals. Intermediate filaments are relatively stable by comparison, providing mechanical strength to the cell. The dynamic cell The preceding overview of the secretory and endocytic pathways in mammalian cells highlights their dynamic nature. The elegant car- toons that grace most textbooks in this field indicate the subcellular organelles and other cellular components and their relative posi- tions within the cell, but, because they are two-dimensional static images, cannot give any indication of the complex dynamics that operate within cells. All the different vesicle budding, vesicle trans- port, vesicle targeting, and vesicle fusion steps involve the assembly and disassembly of specific and discrete macromolecular complexes. There is exquisite spatiotemporal control of each of these events. The dynamic nature of microtubules and actin filaments adds to the complexity of the interactions that occur within cells. The dynamic nature of the eukaryotic cell has been made evident over the past 10 years or so following the widespread use of a range of tools and microscopy systems that allow the imaging of specific pro- teins within

live cells. One real breakthrough came with the isolation of the DNA sequence encoding green fluorescent protein (GFP), which is encoded by the genome of the jellyfish Aequorea victoria and naturally fluorescent, emitting green light when it is illumin- ated with blue light. The now standard techniques of molecular gen- etics have allowed researchers to link the DNA sequence encoding GFP to the DNA sequences encoding a range of different proteins. These hybrid DNA sequences can be introduced into eukaryotic cells and the localizations and intracellular movements of the hybrid proteins they encode can be monitored by appropriate microscopy techniques. This has allowed us to see the dynamic instability of microtubules within living cells, the movement of proteins along the secretory pathway, the sorting of proteins in the endocytic pathway, and many other cellular processes. Genetic engineering has also provided us with a suite of spectral variants of GFP, each emitting light of a different wavelength (i.e. a different colour), thereby allowing the imaging of two or more dif- ferent proteins in the same cell at the same time. It is remarkable that in most cases the presence of a fluorescent protein attached to a pro- tein of interest has little if any effect on the function of that protein. Developments in microscopy are also making a huge contribution to our understanding of cell dynamics. Remarkably, it has been dis- covered that there are ways of overcoming Abbe’s diffraction limit, derived from the laws of physics, which dictates that visible light cannot distinguish between objects closer to each other than around 200 nm (about half the wavelength of visible light). This has led to the construction of so-called super-resolution microscopes with greater than an order of magnitude improvement in resolution. The development of lattice light sheet microscopy, with scanning speeds of hundreds of planes per second and exceptionally low photo- bleaching and phototoxicity, has recently enabled extraordinary spatiotemporal resolution in living cells. We may soon see the results of studies on the interplay of a dozen or more proteins in the same cell at the same time, helping us to understand how they orchestrate a complex cellular function such as vesicular traffic. The overall importance of the dynamic nature of the cell is high- lighted by the pathogenesis of the diverse group of genetic disorders that make up the hereditary spastic paraplegias. In these diseases, lower limb spasticity and weakness is caused by a progressive distal axonopathy that mainly involves the longest corticospinal tract axons that can reach one metre in length. The existence of protru- sions of this length from a single cell undoubtedly presents many challenges in cell dynamics, considering that most mammalian cells are only a few tens of microns across at their greatest width. Many of the proteins encoded by hereditary spastic paraplegia genes are now recognized as being involved in intracellular vesicular traffic or shaping of intracellular organelles, with c.60% of cases due to mu- tations in the gene encoding the protein spastin that couples mem- brane modelling to the severing of microtubules. Biological membranes It is over 40 years since Singer and Nicholson proposed the ‘fluid mosaic’ model for biological membranes. This proposed that inte- gral membrane proteins could diffuse freely in the sea of the lipid bilayer. The imaging of populations of GFP-tagged proteins has con- firmed earlier studies which show that this is essentially the case, but more sophisticated single-particle tracking studies have shown that the plasma membrane is partitioned with regard to molecular diffusion in the plane of the lipid bilayer. One major reason for this is specific interactions with the underlying actin cytoskeleton that can create molecular picket fences around two-dimensional domains (incorporating both bilayers). These interactions tend to be between actin and the cytosolic domain of specific integral membrane pro- teins (Fig. 3.1.4). Interactions in biological membranes are often indirect (i.e. via one or more intermediate proteins), thereby providing the oppor- tunity for regulation of the interaction. For example, the cytosolic domain of the cystic fibrosis transmembrane conductance regulator (CFTR) interacts with a cytosolic protein called EBP50 at the apical surface of polarized human airway epithelial cells.

EBP50 in turn binds another protein, ezrin, and ezrin binds the actin cytoskeleton, thereby tethering CFTR to the actin cytoskeleton and keeping it in the right place in the plasma membrane. Similar interactions be- tween the cytosolic domains of specific integral membrane proteins and the actin cytoskeleton most probably also occur in the context of organellar membranes. In addition to the plasma membrane having two-dimensional or- ganization as a result of picket fences, there are also raft domains enriched in cholesterol and glycosphingolipids (together with glycosylphosphatidylinositol-anchored proteins) as a result of the affinities of these lipids for each other. Although there is some dis- agreement about the best practical way to define a raft, it is clear that they exist, at least as microdomains. Interactions between rafts and the actin cytoskeleton have been detected and seem to function as signalling nodes due to the recruitment and concentration of pro- teins required for signalling pathways on the cytosolic side. As with other aspects of cellular organization, the two-dimensional organ- ization of the plasma membrane is dynamic and reversible. Differential gene expression A human body clearly arises from a single cell, the fertilized egg. This single cell eventually gives rise to the multitude of different cell types within the body. For this to occur, cells must grow and divide

 (and sometimes die) in a highly regulated manner. Not only do the cells need to grow and divide, different populations of cells must dif- ferentiate along different lineages in order to generate the different cell types required to populate the different tissues and organs of the body. Each of the 1013 or so cells in the human body is a phenomen- ally complex and dynamic entity. Furthermore, different subsets of the approximately 20 000 to 25 000 genes in the human genome are expressed in different cell types, with further variations in gene ex- pression occurring during development and in response to external stimuli. This differential gene expression leads, at least in part, to the diversity of cell types found throughout the body. Thus, for example, a neuron is clearly very different from an epithelial cell lining the gut. However, both have the same fundamental organization described in the preceding paragraphs. They both express a core set of shared genes, providing the fundamental cellular organization, but each ex- presses a different set of specific genes. The specific genes expressed will help to define the phenotype of the cell. The differences between cells can often be quite subtle (e.g. different cell types have different protein subunits making up their intermediate filaments), different motor proteins are expressed in different cell types, and differential glycosylation of glycoproteins and glycolipids occurs in different cell types. Alternative splicing and post-translational modifications The fact that there appear to be only 20 000 to 25 000 genes in the human genome does not mean that only this number of proteins can be encoded by the genome. Many genes are subject to the process of alternative splicing, whereby specific exons are included or excluded as the precursor mRNA is processed (spliced) in the nucleus to re- move the noncoding intron sequences. Thus, one gene can give rise to several related, but different, mRNA transcripts. Furthermore, differential processing and differential post-translational modifica- tion of proteins leads to further variety in the range of protein prod- ucts produced from the genome. The range of proteins in a cell (the proteome) is therefore poten- tially considerably larger than the number of genes in its genome. In the case of cytosolic proteins, and the cytosolic domains of integral membrane proteins, many of the post-translational modifications that occur are transient and reversible. Thus, many such proteins are subject to phosphorylation (the addition of a phosphate group to the side chain of a specific amino acid). This process is catalysed by specific enzymes (kinases) and occurs on specific serine, threo- nine, or tyrosine residues in target proteins. This modification is reversible by the action of members of another family of enzymes (phosphatases) that remove the phosphate.

Phosphorylated proteins have different activities, and often interact with a different subset of proteins, from their nonphosphorylated counterparts; thus, revers- ible phosphorylation is a mechanism whereby the cell can regulate interactions and thereby processes occurring within it. It is not uncommon for a kinase to be activated by phosphoryl- ation and it is not uncommon for one kinase to activate another by phosphorylation, thus establishing a signalling cascade that has built-in amplification of the initial signal—amplification because each kinase is an enzyme capable of acting upon multiple substrate molecules while it is in the active state. The initial signal might be the binding of a ligand to its receptor at the cell surface (e.g. the binding of epidermal growth factor to its receptor at the cell surface). As mentioned earlier, this initiates a cascade of signals across the cell which trigger cell growth and cell division, a cascade which is es- sentially a cascade of phosphorylation events. Such a process clearly needs to be transient or it would lead to unregulated cell growth and cell division. The initial signal is removed by the internalization and degradation of the epidermal growth factor receptor, as previ- ously outlined, but this still leaves an activated kinase cascade per- petuating the ‘grow and divide’ message. It is the action of specific phosphatases removing the phosphate groups from the kinases in the cascade that turn off the signalling pathway. Thus, once again, we have a highly dynamic cellular system with exquisite spatiotemporal control. Reversible phosphorylation is one example of several reversible post-translational modifications that serve to regulate cellular func- tion. The principles relating to phosphorylation as a form of post- translational regulation (i.e. that phosphorylated proteins interact with different proteins compared to their nonphosphorylated coun- terparts, or that phosphorylated enzymes have different activities from their nonhosphorylated counterparts), and that this plays a role in the regulation of cell function, also applies to other forms of reversible post-translational modification. Integral membrane protein tethered to the actin cytoskeleton via intermediate proteins. Integral membrane protein not tethered to the actin cytoskeleton and free to diffuse in the plane of the lipid bilayer, but hindered from doing so by the tethered proteins. Lipid bilayer Actin cytoskeleton Fig. 3.1.4 Integral membrane proteins in the lipid bilayer. Some are tethered to the underlying actin cytoskeleton, keeping them in place and providing barriers to the free diffusion of those integral membrane proteins that are not so tethered.

3.1 The cell 217 Post-transcriptional gene silencing (miRNA) The 20 000 to 25 000 genes in the human genome account for only about 2% of the total DNA in the genome. So, what is the role of all the other DNA? A significant amount of it serves structural pur- poses (e.g. the sequences at the centromeres (middles) and telo- meres (ends) of chromosomes), but recent evidence shows that much of it plays crucial regulatory roles, working by the process of post-transcriptional gene silencing. The phenomenon of post-transcriptional gene silencing was first described in plants but has subsequently been shown to be widespread in eukaryotes. In this process a short (19–23 nucleotides long) double- stranded RNA molecule associates with a target mRNA (the nucleotide sequence of one of the RNA strands in the double-stranded molecule is complementary to the sequence of the target mRNA). This occurs in the context of a multiprotein complex and leads to either a block in translation or the degradation of the target mRNA. This mechanism therefore regulates protein expression post-transcriptionally, hence the designation post-transcriptional gene silencing. The short double-stranded RNA molecules involved in post- transcriptional gene silencing are produced from slightly larger pre- cursor RNA molecules known as micro RNAs (miRNAs) which are themselves produced by transcription of relevant DNA sequences by DNA polymerase in the cell’s nucleus. The number of DNA sequences within the human genome that encode miRNAs has yet to be finalized, but there appear to be at least as many such sequences as there are protein-encoding DNA sequences (i.e. conventional genes). miRNA sequences have been shown to play

critical regulatory roles in a range of processes (e.g. during development and in the immune response to pathogens). They have also been implicated as playing a role in several disease states, such as heart disease and cancer. Future developments The availability of the human genome sequence has given us access to information concerning the basic building blocks of the cell, but it is how those building blocks are modified and used in a multitude of different dynamic interactions that gives organization, function, and life to the cell. A major challenge of the next decade is to integrate the vast amounts of data that are now available, and will continue to be- come available, on the molecular mechanisms that underlie cellular organization, structure, and function. Such a challenge will have to be met if we are to achieve a clearer, and more complete, under- standing of the cell and are to develop the capacity to refine our means of modifying cellular functions that are disturbed in disease. FURTHER READING Alberts B, et al. (2015). Molecular biology of the cell, 6th edition. Garland Science, New York. Baum DA, Baum B (2014). An inside-out origin for the eukaryotic cell. BMC Biology, 12, 76. Berridge MJ (2006). Cell signalling biology. Portland Press, Colchester. http://www.cellsignallingbiology.org/ Blackstone C, et al. (2011). Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat Rev Neurosci, 12, 31–40. Bonifacino JS, Glick BS (2004). The mechanisms of vesicle budding and fusion. Cell, 116, 153–66. Brooks SA, et al. (2008). Altered glycosylation of proteins in cancer: what is the potential for new anti-tumour strategies. Anticancer Agents Med Chem, 8, 2–21. Bushati N, Cohen SM (2007). microRNA functions. Ann Rev Cell Dev Biol, 23, 175–205. Chalfie M, et al. (1994). Green fluorescent protein as a marker for gene expression. Science, 11, 802–5. Chen B-C, et al. (2014). Lattice light sheet microscopy: imaging mol- ecules to embryos at high spatiotemporal resolution. Science, 346, 1257998. Clapham DE (2007). Calcium signaling. Cell, 131, 1047–58. Ellis RJ, Minton AP (2003). Join the crowd. Nature, 425, 27–8. Giepmans BN, et al. (2006). The fluorescent toolbox for assessing pro- tein location and function. Science, 14, 217–24. Goldman RD, et al. (2008). Intermediate filaments: versatile building blocks of cell structure. Curr Opin Cell Biol, 20, 28–34. Huotari J, Helenius A (2011). Endosome maturation. EMBO J, 30, 3481–500. Irannejad R, et al. (2015). Effects of endocytosis on receptor-mediated signaling. Curr Opin Cell Biol, 35, 137–43. Kusumi A, et al. (2012). Dynamic organizing principles of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of Singer and Nicholson’s fluid-mosaic model. Annu Rev Cell Dev Biol, 28, 215–50. Lanzetti L (2007). Actin in membrane trafficking. Curr Opin Cell Biol, 19, 453–8. Levine B, Kroemer G (2019). Biological functions of autophagy genes: a disease perspective. Cell, 176, 11–42. Lewin B, et al. (2007). Cells. Jones and Bartlett, Sudbury, MA. Lippincott-Schwartz, J (2004). Dynamics of secretory membrane traf- ficking. Ann N Y Acad Sci, 1038, 115–24. Luzio JP, et al. (2014). The biogenesis of lysosomes and lysosome- related organelles. Cold Spring Harb Perspect Biol, 6, a016840. Ross JL, Ali MY, Warshaw DM (2008). Cargo transport: molecular motors navigate a complex cytoskeleton. Curr Opin Cell Biol, 20, 41–7. Settembre C, et al. (2013). Signals from the lysosome: a control centre for cellular clearance and energy metabolism. Nat Rev Mol Cell Biol, 14, 283–96. Singer SJ, Nicolson GL (1972). The fluid mosaic model of the structure of cell membranes. Science, 18, 720–31. Stadler BM, Ruohola-Baker H (2008). Small RNAs: keeping stem cells in line. Cell, 132, 563–6. Stagg SM, LaPointe P, Balch WE (2007). Structural design of cage and coat scaffolds that direct membrane traffic. Curr Opin Struct Biol, 17, 221–8. Stefani G, Slack FJ (2008). Small non-coding RNAs in animal develop- ment. Nat Rev Mol Cell Biol, 9, 219–30. Ungewickell EJ, Hinrichsen L (2007). Endocytosis: clathrin-mediated membrane budding. Curr Opin Cell Biol, 19, 417–25. Yang YX, Rastetter RH, Wilhelm D (2016). Non-coding RNAs: an introduction. Adv Exp Med Biol, 866, 13–32. Zhang J, et al. (2002). Creating new fluorescent probes for cell biology. Nat Rev Mol Cell Biol, 3, 906–18.

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