Cellular Nanomachines

Bhanu P. Jena

Cellular Nanomachines

From Discovery to Structure-Function and Therapeutic Applications

Springer

Bhanu P. Jena
Department of Physiology
Wayne State University School of Medicine
Detroit, MI, USA

ISBN 978-3-030-44495-2 ISBN 978-3-030-44496-9 (eBook)
https://doi.org/10.1007/978-3-030-44496-9

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To Minakhi and Siddhartha
You are my inspiration

Foreword

Major discoveries and inventions are made by the prepared mind. While human curiosity drives creativity, urging us to explore our natural world, it necessitates and nurtures the invention and development of new tools and approaches to explore. Reciprocally, new discoveries also lead to new inventions. In the past 400 years since the invention of the light microscope leading to the discovery of the unit of life the cell, there has been an explosion in our understanding of the chemistry of life. This new understanding has taught us that Nature is the ultimate architect and engineer, capable of designing and building the most effective and efficient living unit the "Cell" and the precision nanomachines within, to undertake various life functions. Cellular nanomachines are a marvel of Nature, undertaking with great precision the vital cellular tasks like synthesis of new proteins life's building blocks to their proper folding and assembly, their transport, and their secretion from within the cell to the outside. Secretion is required for cell–cell communication such as neurotransmission for coordination, thought, memory, smell, vision, taste, and hearing, for the digestion of food, for endocrine control such as insulin release in response to elevated blood glucose, and for release of immune products in response to a foreign antigen such as a pathogenic bacteria or virus, among others. In this book, the author lucidly takes the reader on a journey of the major paradigm-shifting discoveries made in the past century on key cellular nanomachines, their complex yet precise and elegant design and function, the diseases linked to their dysfunction, and the therapeutic approaches to overcome them. The major focus of this book is on the "porosome" nanomachine, the universal secretory portal in cells. The discovery of the porosome was made using atomic force microscopy at the nanoscale resolution of live cells. Each of these discoveries is pioneering and outstanding scientific contributions of great service to humanity.

Iowa State University
Ames, IA, USA

Lloyd L. Anderson,

Preface

The unit of life is the cell. An average human body contains nearly 38 trillion cells, equal to a linear length that could circle half the globe. The cell is analogous to a well-organized city, with thousands of reactions and millions of interactions occurring at any given moment to sustain life processes. A fundamental understanding of these vital cellular processes therefore is critical to comprehending life and in the detection and treatment of diseases that develop as a consequence of altered states. Since the discovery of the cell in 1665 using the then newly invented light microscope, great progress has been made in our understanding of its structure and function using a wide range of imaging modalities. In the past century, new tools and approaches, especially multimodal imaging, have enabled the discovery of cellular nanomachines, providing a molecular-level understanding of cellular structure–function. The average size of a cell is about 15 μm. In comparison, the thickness of a human hair is 150 μm. Cellular nanomachines, Nature's engineered marvels, are the life-sustaining cellular workhorses. These nanomachines measure just 15–150 nm; hence, 10,000 of 15 nanometer-sized structures could fit in the thickness of a human hair. In this book written for the general readership, the discovery of these life-sustaining cellular nanomachines, a brief description of their structure–function, and their impact on health and disease are discussed. Except for one, most of the cellular nanomachines discussed in this book include schematic illustrations for simplicity and clarity.

Among the key cellular nanomachines discovered are the "*porosome complex*" or "secretory nanomachine" at the cell plasma membrane for energy-dependent transient docking, fusion, and the selective and precise fractional release of intravesicular contents from cells during secretion; the "*nuclear pore complex*" for the precise and selective bidirectional transport of proteins and nucleotides between the nucleus and the cell cytosol; the energy-dependent protein-folding machinery or "*chaperonin*"; "*proteasome*," the energy-dependent garbage disposal in cells; "*ribosome*," the protein synthetic machinery in cells; "*ATP synthase*," the nanomachine that generates cellular energy; and "*myosin*," the cellular molecular motor for movement and transport.

The earliest of cellular nanomachines discovered was the molecular motor protein *myosin* in 1859 by Wilhelm Kühne [Kühne, W. (1859) *Arch. f. Anat. Physiol. u. wissensch. Med.* 748]. Next was the discovery of the *nuclear pore* complex in 1949 by Harold G. Callan using electron microscopy [Callan H. G., Randall J. T., Tomlin S. G. (1949). An electron microscope study of the nuclear membrane *Nature* 163, 280]. In 1955, the *ribosome* was discovered by George E. Palade also using transmission electron microscopy [Palade, G.E. (January 1955). A small particulate component of the cytoplasm. *J Biophys Biochem Cytol*. 1 (1): 59–68.]. In 1958, Efraim Racker discovered *ATP synthase* [Pullman ME, Penefsky H, Racker E. 1958. A soluble protein fraction required for coupling phosphorylation to oxidation in submitochondrial fragments of beef heart mitochondria. *Arch. Biochem. Biophys.* 76:227] and was the first to identify in the 1960s the F1-ATPase with the knobs seen in electron micrographs of mitochondrial membranes. The concept of the presence of a protein-folding machinery later termed *chaperonin*, distinct from the known ability of proteins to self-fold, was born out of the work of Costa P. Georgopoulos in 1972 [Georgopoulos, C. P., Hendrix, R. W., Kaiser, A. D., Wood, W. B. 1972. Role of the host cell in bacteriophage morphogenesis: effects of a bacterial mutation on T4 head assembly. *Nat. New Biol.* 239:38]. Similarly, the *proteasome* was discovered in 1979 by Avram Hershko, Aaron Ciechanover, and Irwin A. Rose [Hershko, A.; Ciechanover, A.; Rose, I.A. (1979), "Resolution of the ATP-dependent proteolytic system from reticulocytes: a component that interacts with ATP," *Proc. Natl. Acad. Sci. USA*, 76 (7): 3107]. The secretory machinery *porosome* was discovered in my laboratory in 1996 (published online) using atomic force microscopy [Schneider, S.W., Sritharan, K.C., Geibel, J.P., Oberleithner, H., Jena, B.P. 1997. Surface dynamics in living acinar cells imaged by atomic force microscopy: Identification of plasma membrane structures involved in exocytosis. *Proc. Natl. Acad. Sci. USA*. 94:316] and later imaged using electron microscopy and solution X-ray.

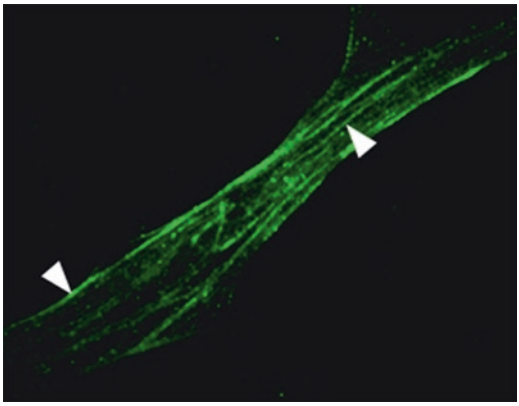
In the subsequent years following their discoveries, each cellular nanomachine has been intensely investigated by many laboratories who have made major contributions to a greater understanding of their structure using multiple imaging modalities, their function and composition, their functional reconstitution, and an understanding of their involvement in a number of diseases associated with their dysfunction. The final chapter explores some of the operating principles such as chirality and enthalpy that may govern the assembly of higher-order structures such as cellular nanomachines.

While a large body of information in the form of in-depth reviews and textbook chapters are available on cellular nanomachines discussed in this book, seldom are readers exposed to the scientific journey leading to their discovery. It is the objective of this book to provide the reader with the background, time-line, and approaches that lead to the discovery and understanding of each of the seven cellular nanomachines. The author intends readers to be critically aware that all discoveries are made possible due to the accumulated body of scientific information, hypothesis, and tools developed and available to the inquisitive and prepared mind. The contents of this book are therefore far from exhaustive, rather it provides the reader

with a brief narration of the scientific journey leading to the discovery of each cellular nanomachines and their structure–function and participation in health and disease. The "porosome" though discussed in various textbooks is the most recently (1996–1997) discovered nanomachine and therefore is discussed in greater detail. The author thanks Dr. Won Jin Cho for the modified schematic illustration presented in Figs. 2.1, 3.1, 4.1, 5.1, 6.1, and 7.1 and cover illustration.

Detroit, MI, USA Bhanu P. Jena
December 25, 2019

Introduction



Left: Arrangement of myosin IIb fibers in a human skeletal muscle cell in culture (*From: ACS Biomater. Sci. Eng. 2019, 5, 9*). ©Bhanu Jena

Cellular nanomachines are Nature's engineered marvels that perform with great efficiency and precision the life-sustaining cellular activities. *Myosin*, for example, is the universal molecular motor in living organisms and belongs to a superfamily of motor proteins. Analogous to a car engine that utilizes chemical energy in the form of gasoline to perform mechanical work to propel the vehicle forward, the myosin molecular motor uses chemical energy in the form of adenosine triphosphate or ATP for locomotion. Myosin typically has a globular head region (heavy chain) measuring 16.5 nm in length and 6.5 nm in width and a tail (superhelical rod) measuring up to 150 nm in length. The myosin head has ATP binding and ATPase activity that utilizes ATP as fuel to perform mechanical work, leading to a wide range of motions, from transport of subcellular organelles within cells to cellular motion and to the locomotion of an entire organism via skeletal muscle myosin II. Myosin II is a 520 kDa hexamer comprised of two heavy chains and four light chains. Each heavy chain is 220 kDa, composed of a short globular head and a long coiled-coil tail region. The two myosin II light chains modulating force transduction have a

molecular mass of 22 and 19 kDa, respectively, and belong to a large family of calcium-binding proteins.

In contrast, the nanomachine *ATP synthase* is specialized with the task of generating the universal life-sustaining energy currency ATP in all living organisms. ATP synthase measures approximately 10 nm in diameter and 20 nm in height and has two regions F0 and F1, with each region composed of several subunits. The F0 region is embedded in the inner mitochondrial membrane and serves as a proton pore. The F0 subunits are arranged in a ring embedded in the mitochondrial inner membrane, and when an F0 subunit is protonated, it undergoes a conformational change that drives neighboring subunits to rotate, resulting in conformational changes in the F1 subunits and consequent catalysis of adenosine diphosphate (ADP) and Pi to ATP. Analogous to myosin, the other cellular nanomachines like the ribosome, the nuclear pore, porosome, proteasome, and chaperonin, all require ATP to operate, demonstrating the critical role of ATP synthase.

Ribosomes are approximately 30 nm in size protein synthetic nanomachines that are themselves composed of nearly 80 different proteins. The ribosome is composed of two subunits: a small subunit which reads the mRNA and the large subunit which precisely links specific amino acids to form a polypeptide chain. During polypeptide biosynthesis at the endoplasmic reticulum, linear polypeptide chains undergo self-folding to form 3D mature and functional proteins, while some require assistance. This assistance in the folding of nascent linear polypeptides is provided by a large class of cylindrical nanomachines called *chaperonins*, each measuring approximately 15 3nm, where ATP-dependent folding of polypeptides occurs in addition to the prevention of their aggregation.

Misfolded or damaged proteins, and certain proteins that are required to be degraded, are carried out by another unique cellular nanomachine called the *proteasome*. The proteasome is a 15 × 11.5 nm cylindrical structure with a 1.3 nm pore through which only a partially folded or linear polypeptide can enter for ATP-mediated proteolysis. The proteasome has been referred to as the garbage disposal of the cell.

Similarly, communication between cells in a multicellular organism is critical in maintaining homeostasis. This is achieved via chemical messages such as the hormone insulin that is secreted from beta cells of the endocrine pancreas to maintain blood glucose levels, or secretion of neurotransmitters from a brain neuron to communicate with other neurons in the process for thought, learning, or memory. Secretion of digestive enzymes from acinar cells of the exocrine pancreas following food intake is required for digestion. The cellular nanomachine at the cell plasma membrane that accomplishes this vital cellular task of secretion is the *porosome*. Porosomes are unidirectional cup-shaped lipoprotein secretory nanomachines ranging in size from 15 nm at the nerve terminal to 180 nm in the exocrine pancreas.

While porosomes are composed of 30–40 proteins, the 120 nm *nuclear pore complex* is a bidirectional transporting nanomachine, composed of nearly 1000 protein molecules. The nuclear pore complexes are aqueous channels traversing the outer and inner nuclear membrane, serving as gatekeepers for the selective transport

of proteins and nucleotides between the nucleoplasm and the cytoplasm. Although ions can freely move through the nuclear pore, proteins, mRNA, ribosome subunits, and tRNA are actively transported through the complex. *In this Springer Nature book, the porosome nanomachine will be the primary focus.*

Contents