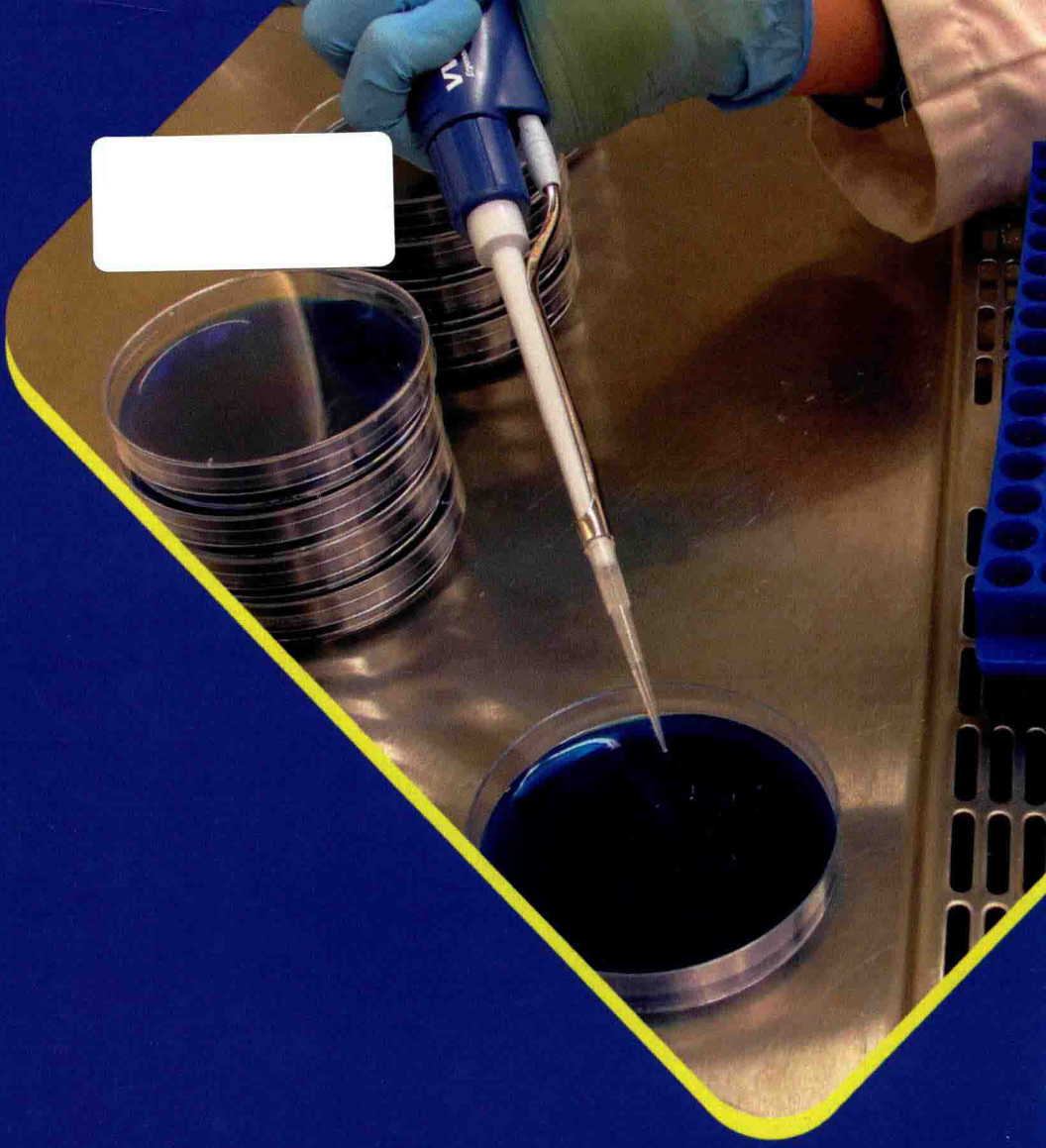

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Preface

In the previous century it was thought that biology and mathematics can't go together. Biology and mathematics are subjects with different domains. It is true. But the fact is that mathematics does the general formulation which can also be implemented in the biological problems. The latest developments in molecular biology lead mathematics to solve the newly created problems. After the completion of the Human Genome Project, the application of other subjects in molecular biology has taken a start. That new field is named as computational molecular biology or bioinformatics. The term, Bioinformatics, was coined by Hwa Lim in the late 1980's, to encompass all forms of computational study and analysis of biological problems. The Central dogma, DNA makes RNA makes Protein, has long been a staple of biology text books. More recently, this paradigm has been extended from individual genes to whole genomes by advances in genomic technologies. In the 21st century Bioinformatics has transformed the discipline of Biology from a purely lab-based science to an information science as well. Many had the vision of establishing bioinformatics in a leadership role over experimental biology, similar to that theoretical physics enjoys over experimental physics. Somewhere along the line, it seems that bioinformatics lost this ambition and became sidetracked onto what physicists would call a 'phenomenological' pathway.

Bioinformatics and computational molecular biology have emerged as new branches of science. These are the results of the inevitable marriage of mathematics, information science and biosciences. Bioinformatics and Computational Biology are rooted into life sciences as well as computer and information sciences and technologies. Both of these interdisciplinary approaches draw from specific disciplines such as mathematics, physics, computer science, engineering, biology and behavioral science. Bioinformatics applies principles of information sciences and technologies to make the vast, diverse and complex life science data more understandable and useful. Computational biology uses mathematical and computational approaches to address theoretical

and experimental questions in biology. Although bioinformatics and computational biology are distinct, there is also significant overlap and activity at there interface. Bioinformatics is the technological aspect and Computational (molecular) biology is the scientific aspect. The relationship between computer science and biology is a natural one for several reasons. First, the phenomenal rate of biological data being produced provides challenges: massive amount of data have to be stored, analysed, and made accessible. Second, the nature of the data is often such that a statistical method, and hence computation, is necessary. This applies in particular to the information on the building plans of proteins and of the temporal and spatial organization of their expression in the cell encoded by the DNA. Third, there is a strong analogy between the DNA sequence and a computer program.

The book is perfectly suited to opening up this subject to the widest range of students, giving insights into this fascinating area of this subject.

—Editor

Contents

Chapter 1

Microbodies: Peroxisomes and Glyoxysomes

A microbody is a cytoplasmic organelle of a more or less globular shape that comprises degradative enzymes bound within a single membrane. Microbodies are specialized as containers for metabolic activity. Types include peroxisomes, glyoxisomes, glycosomes and Woronin bodies. Peroxisomes contain enzymes of b-oxidation (break down fats and produce Acetyl-CoA), as well as enzymes of many other important pathways like amino acid and bile acid metabolism, oxidation/detoxification of various harmful compounds in the liver (ex. alcohol).

Glyoxysomes are found in germinating seeds of plants as well as in filamentous fungi. Glyoxysomes are peroxisomes with additional function - glyoxylate cycle. Glycosomes, besides peroxisomal enzymes, also possess glycolysis enzymes and are found in kinetoplastida like Trypanosomes. Woronin bodies are special organelles found only in filamentous fungi. One established function of Woronin bodies is the plugging of the septal pores after hyphal wounding, which restricts the loss of cytoplasm to the sites of injury.

Peroxisomes are organelles present in almost all eukaryotic cells. They participate in the metabolism of fatty acids and many other metabolites. Peroxisomes harbour enzymes that rid the cell of toxic peroxides. Peroxisomes are bound by a single membrane that separates their contents from the cytosol (the internal fluid of the cell) and contain membrane proteins critical for various functions, such as importing proteins into the organelles and aiding in proliferation. Peroxisomes are formed from the endoplasmic reticulum. Peroxisomes were identified as organelles by the Belgian cytologist Christian de Duve in 1967 after they had been first described in a PhD thesis of Rhodin a decade earlier.

Occurrence and Evolution

Peroxisomes are found in virtually all eukaryotic cells. Peroxisomes contain enzymes for certain oxidative reactions, like the beta-oxidation of very-long-chain fatty acids.

Prokaryotes lack peroxisomes. The enzymatic content of peroxisomes varies across species, but the presence of certain proteins common to many species has been used to suggest an endosymbiotic origin; that is, peroxisomes evolved from bacteria that invaded larger cells as parasites, and very gradually evolved a symbiotic relationship. However, this view has been challenged by recent discoveries. For example, peroxisome-less mutants can restore peroxisomes upon introduction of the wild-type gene.

Two independent evolutionary analyses of the peroxisomal proteome found homologies between the peroxisomal import machinery and the ERAD pathway in the endoplasmic reticulum, along with a number of metabolic enzymes that were likely recruited from the mitochondria. These results indicate that the peroxisome does not have an endosymbiotic origin; instead, it likely originates from the ER, and its proteins were recruited from pools existing within the primitive eukaryote, as quoted in the science textbook Biozone. However, latest research have suggested that the peroxisome may have had an actinobacterial origin, although this is still controversial.

Other organelles of the Microbody family related to peroxisomes include glyoxysomes of plants and filamentous fungi, glycosomes of kinetoplastids and Woronin bodies of filamentous fungi.

Function

Peroxisomes contain oxidative enzymes, such as catalase, D-amino acid oxidase, and uric acid oxidase. However the last enzyme is absent in humans, explaining the disease known as gout, caused by the accumulation of uric acid. Certain enzymes within the peroxisome, by using molecular oxygen, remove hydrogen atoms from specific organic substrates (labeled as R), in an oxidative reaction, producing hydrogen peroxide (H_2O_2, itself toxic):

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

Catalase, another enzyme in the peroxisome, in turn uses this H_2O_2 to oxidize other substrates, including phenols, formic acid, formaldehyde, and alcohol, by means of the peroxidation reaction:

$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$, thus eliminating the poisonous hydrogen peroxide in the process.

This reaction is important in liver and kidney cells, where the peroxisomes detoxify various toxic substances that enter the blood. About 25% of the ethanol we drink is oxidized to acetaldehyde in this way. In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through this reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

A major function of the peroxisome is the breakdown of fatty acid molecules, in a process called beta-oxidation. In this process, the fatty acids are broken down two carbons at a time, converted to Acetyl-CoA, which is then transported back to the cytosol for further use. In animal cells, beta-oxidation can also occur in the mitochondria. In yeast and plant cells, this process is exclusive for the peroxisome.

The first reactions in the formation of plasmalogen in animal cells also occur in peroxisomes. Plasmalogen is the most abundant phospholipid in myelin. Deficiency of plasmalogens causes profound abnormalities in the myelination of nerve cells, which is one of the reasons that many peroxisomal disorders lead to neurological disease (adrenoleukodystrophy). Peroxisomes also play a role in the production of bile acids and proteins.

In higher plants, peroxisomes contain also a complex battery of antioxidative enzymes such as superoxide dismutase, the components of the ascorbate-glutathione cycle, and the NADP-dehydrogenases of the pentose-phosphate pathway. It has been demonstrated the generation of superoxide (O_2) and nitric oxide ($^{\bullet}NO$) radicals.

The peroxisome of plant cells is polarised when fighting fungal penetration. Infection causes a glucosinolate molecule to play an antifungal role to be made and delivered to the outside of the cell through the action of the peroxisomal proteins (PEN2 and PEN3).

Protein Import

Proteins are selectively imported into peroxisomes. Since the organelles contain no DNA or ribosomes and, thus, have no means of producing proteins, all of their proteins must be imported across the membrane. It is believed that necessary proteins enter through the endoplasmic reticulum during biogenesis as well as through membrane proteins.

A specific protein signal (PTS or peroxisomal targeting signal) of three amino acids at the *C-terminus* of many peroxisomal proteins signals the membrane of the peroxisome to import them into the organelle. Other peroxisomal proteins contain a signal at the *N-terminus*. There are at least 32 known peroxisomal proteins, called peroxins, which participate in the process of importing proteins by means of *ATP hydrolysis*. Proteins do not have to unfold to be imported into the peroxisome. The protein receptors, the peroxins *PEX5* and *PEX7*, accompany their cargoes (containing a PTS1 or a PTS2, respectively) all the way into the peroxisome where they release the cargo and then return to the cytosol - a step named *recycling*. Overall, the import cycle is referred to as the *extended shuttle mechanism*. Evidence now indicates that ATP hydrolysis is required for the recycling of receptors to the cytosol. Also, ubiquitination appears to be crucial for the export of PEX5 from the peroxisome, to the cytosol. Little is known about the import of PEX7, although it has helper proteins that have been shown to be ubiquitinated. It is interesting to note that Pex7 helper proteins share many similarities with Pex5, and, in higher eukaryotes, they are functionally replaced by a long isoform of PEX5 (PEX5L).

Deficiencies

Peroxisomal disorders are a class of conditions that lead to disorders of lipid metabolism and diseases of the nervous system. Well-known examples are X-linked adrenoleukodystrophy, the most frequent, and Zellweger syndrome. Peroxisomes matrix proteins are synthesized on free ribosomes in the cytosol and are imported post-translationally in pre-existing vesicles.

Genes

Genes that encode peroxisomal proteins include:

- PEX1
- PEX2 - PXMP3
- PEX3
- PEX5
- PEX6
- PEX7
- PEX10
- PEX11A, PEX11B, PEX11G

- PEX12
- PEX13
- PEX14
- PEX16
- PEX19
- PEX26
- PEX28
- PEX30
- PEX31

Glyoxysome

Glyoxysomes are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi. As in all peroxisomes, in glyoxysomes the fatty acids are hydrolyzed to acetyl-CoA by peroxisomal α-oxidation enzymes. Besides peroxisomal functions, glyoxysomes possess additionally the key enzymes of glyoxylate cycle (isocitrate lyase and malate synthase) which accomplish the glyoxylate cycle bypass.

Thus, glyoxysomes (as all peroxisomes) contain enzymes that initiate the breakdown of fatty acids and additionally possess the enzymes to produce intermediate products for the synthesis of sugars by gluconeogenesis. The seedling uses these sugars synthesized from fats until it is mature enough to produce them by photosynthesis.

Ribosomes

Ribosomes are the components of cells that make proteins from all amino acids. One of the central tenets of biology, often referred to as the "central dogma," is that DNA is used to make RNA, which, in turn, is used to make protein. The DNA sequence in genes is copied into a messenger RNA (mRNA). Ribosomes then read the information in this RNA and use it to create proteins. This process is known as translation; i.e., the ribosome "translates" the genetic information from RNA into proteins. Ribosomes do this by binding to an mRNA and using it as a template for the correct sequence of amino acids in a particular protein. The amino acids are attached to transfer RNA (tRNA) molecules, which enter one part of the ribosome and bind to the messenger RNA sequence. The attached amino acids are then joined together by another part of the ribosome. The ribosome moves along the mRNA, "reading" its sequence and producing a chain of

amino acids. Ribosomes are made from complexes of RNAs and proteins. Ribosomes are divided into two subunits, one larger than the other. The smaller subunit binds to the mRNA, while the larger subunit binds to the tRNA and the amino acids. When a ribosome finishes reading a mRNA, these two subunits split apart. Ribosomes have been classified as ribozymes, since the ribosomal RNA seems to be most important for the peptidyl transferase activity that links amino acids together.

Ribosomes from bacteria, archaea and eukaryotes (the three domains of life on Earth), have significantly different structures and RNA sequences. These differences in structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected. The ribosomes in the mitochondria of eukaryotic cells resemble those in bacteria, reflecting the likely evolutionary origin of this organelle. The word ribosome comes from *ribo*nucleic acid and the Greek: *soma* (meaning body).

Description

Archaeal, eubacterial and eukaryotic ribosomes differ in their size, composition and the ratio of protein to RNA. Because they are formed from two subunits of non-equal size, they are slightly longer in the axis than in diameter. Prokaryotic ribosomes are around 20 nm (200 ångströms) in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins (known as a ribonucleoprotein or RNP). Eukaryotic ribosomes are between 25 and 30 nm (250-300 ångströms) in diameter and the ratio of rRNA to protein is close to 1. Ribosomes translate messenger RNA (mRNA) and build polypeptide chains (e.g., proteins) using amino acids delivered by transfer RNA (tRNA). Their active sites are made of RNA, so ribosomes are now classified as "ribozymes".

Ribosomes build proteins from the genetic instructions held within messenger RNA. Free ribosomes are suspended in the cytosol (the semi-fluid portion of the cytoplasm); others are bound to the rough endoplasmic reticulum, giving it the appearance of roughness and thus its name, or to the nuclear envelope. As ribozymes are partly constituted from RNA, it is thought that they might be remnants of the RNA world. Although catalysis of the peptide bond involves the C2 hydroxyl of RNA's P-site adenosine in a protein shuttle mechanism, other steps in protein synthesis (such as translocation) are caused by changes in protein conformations.

Ribosomes are sometimes referred to as organelles, but the use of the term *organelle* is often restricted to describing sub-cellular components that include a phospholipid membrane, which ribosomes, being entirely particulate, do not. For this reason, ribosomes may sometimes be described as "non-membranous organelles".

Ribosomes were first observed in the mid-1950s by Romanian cell biologist George Palade using an electron microscope as dense particles or granules for which he would win the Nobel Prize. The term "ribosome" was proposed by scientist Richard B. Roberts in 1958:

During the course of the symposium a semantic difficulty became apparent. To some of the participants, "microsomes" mean the ribonucleoprotein particles of the microsome fraction contaminated by other protein and lipid material; to others, the microsomes consist of protein and lipid contaminated by particles. The phrase "microsomal particles" does not seem adequate, and "ribonucleoprotein particles of the microsome fraction" is much too awkward. During the meeting, the word "ribosome" was suggested, which has a very satisfactory name and a pleasant sound. The present confusion would be eliminated if "ribosome" were adopted to designate ribonucleoprotein particles in sizes ranging from 35 to 100S.

– *Roberts, R. B., Microsomal Particles and Protein Synthesis*

The structure and function of the ribosomes and associated molecules, known as the *translational apparatus*, has been of research interest since the mid-twentieth century and is a very active field of study today.

Ribosomes consist of two subunits that fit together and work as one to translate the mRNA into a polypeptide chain during protein synthesis. Bacterial subunits consist of one or two and eukaryotic of one or three very large RNA molecules (known as ribosomal RNA or rRNA) and multiple smaller protein molecules. Crystallographic work has shown that there are no ribosomal proteins close to the reaction site for polypeptide synthesis. This suggests that the protein components of ribosomes act as a scaffold that may enhance the ability of rRNA to synthesize protein rather than directly participating in catalysis.

Biogenesis

In bacterial cells, ribosomes are synthesized in the cytoplasm through the transcription of multiple ribosome gene operons. In

eukaryotes, the process takes place both in the cell cytoplasm and in the nucleolus, which is a region within the cell nucleus. The assembly process involves the coordinated function of over 200 proteins in the synthesis and processing of the four rRNAs, as well as assembly of those rRNAs with the ribosomal proteins.

Ribosome Locations

Ribosomes are classified as being either "free" or "membrane-bound".

Free and membrane-bound ribosomes differ only in their spatial distribution; they are identical in structure. Whether the ribosome exists in a free or membrane-bound state depends on the presence of an ER-targeting signal sequence on the protein being synthesized, so an individual ribosome might be membrane-bound when it is making one protein, but free in the cytosol when it makes another protein.

Free Ribosomes

Free ribosomes can move about anywhere in the cytosol, but are excluded from the cell nucleus and other organelles. Proteins that are formed from free ribosomes are released into the cytosol and used within the cell. Since the cytosol contains high concentrations of glutathione and is, therefore, a reducing environment, proteins containing disulfide bonds, which are formed from oxidized cysteine residues, cannot be produced in this compartment.

Membrane-bound Ribosomes

When a ribosome begins to synthesize proteins that are needed in some organelles, the ribosome making this protein can become "membrane-bound". In eukaryotic cells this happens in a region of the endoplasmic reticulum (ER) called the "rough ER". The newly produced polypeptide chains are inserted directly into the ER by the ribosome and are then transported to their destinations, through the secretory pathway. Bound ribosomes usually produce proteins that are used within the plasma membrane or are expelled from the cell via *exocytosis*.

Structure

The ribosomal subunits of prokaryotes and eukaryotes are quite similar. The unit of measurement is the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size and accounts for why fragment names do not add up (70S is made of 50S and 30S). Prokaryotes have 70S ribosomes, each consisting of a small

(30S) and a large (50S) subunit. Their large subunit is composed of a 5S RNA subunit (consisting of 120 nucleotides), a 23S RNA subunit (2900 nucleotides) and 34 proteins. The 30S subunit has a 1540 nucleotide RNA subunit (16S) bound to 21 proteins.

Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit. Their large subunit is composed of a 5S RNA (120 nucleotides), a 28S RNA (4700 nucleotides), a 5.8S subunit (160 nucleotides) and ~49 proteins. The 40S subunit has a 1900 nucleotide (18S) RNA and ~33 proteins.

The ribosomes found in chloroplasts and mitochondria of eukaryotes also consist of large and small subunits bound together with proteins into one 70S particle. These organelles are believed to be descendants of bacteria and as such their ribosomes are similar to those of bacteria.

The various ribosomes share a core structure, which is quite similar despite the large differences in size. Much of the RNA is highly organized into various tertiary structural motifs, for example pseudoknots that exhibit coaxial stacking. The extra RNA in the larger ribosomes is in several long continuous insertions, such that they form loops out of the core structure without disrupting or changing it. All of the catalytic activity of the ribosome is carried out by the RNA; the proteins reside on the surface and seem to stabilize the structure.

The differences between the bacterial and eukaryotic ribosomes are exploited by pharmaceutical chemists to create antibiotics that can destroy a bacterial infection without harming the cells of the infected person. Due to the differences in their structures, the bacterial 70S ribosomes are vulnerable to these antibiotics while the eukaryotic 80S ribosomes are not. Even though mitochondria possess ribosomes similar to the bacterial ones, mitochondria are not affected by these antibiotics because they are surrounded by a double membrane that does not easily admit these antibiotics into the organelle.

High-resolution Structure

The general molecular structure of the ribosome has been known since the early 1970s. In the early 2000s the structure has been achieved at high resolutions, on the order of a few ångströms. The first papers giving the structure of the ribosome at atomic resolution were published in rapid succession in late 2000. First, the 50S (large prokaryotic) subunit from the archaeon *Haloarcula marismortui* was